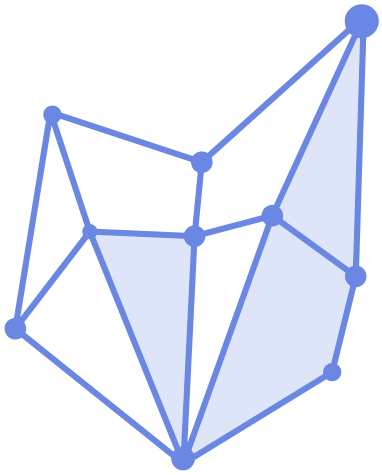

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# The Thermoregulatory Response to Lithium & its Inhibition of Stress-Induced Physiological & Behavioural Changes: A Novel Mechanism of Action & Brain Pathway

Jett Zivkovic

Thesis submitted to Flinders University for the degree of

**Doctor of Philosophy**

College of Medicine & Public Health,
Flinders University of South Australia

16th of October 2024

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## **CHAPTER 1.** INTRODUCING LITHIUM & THERMOREGULATION

# ABSTRACT

Despite lithium being an effective mood stabiliser and the gold standard treatment for bipolar disorder, its mechanism of action remains largely unknown. This thesis investigates the physiological and behavioural responses to lithium administration in guinea pigs and rats, inspired by John Cade's seminal observations in 1949, when he discovered lithium's therapeutic potential by noting lithium-induced lethargy in guinea pigs. I utilised a range of methods, including, but not limited to, telemetric probes and infrared thermography for temperature measurement, automatic tracking software and video analysis for behaviour measurement, and fine electrodes and microsurgical techniques for measurement of brown adipose tissue (BAT) sympathetic nerve activity.

I demonstrated that lithium induces a dose-dependent increase in lethargic behaviour ('cooling posture') and a decrease in body temperature and locomotor activity in guinea pigs. These effects were proposed as a coordinated thermoregulatory response rather than lithium toxicity, challenging conventional views.

In rats, lithium was found to dose-dependently reduce body temperature, BAT thermogenesis, and locomotor activity, as well as inhibit cutaneous vasoconstriction. Importantly, I found that lithium also inhibits stress-induced BAT thermogenesis, emotional hyperthermia, and hyperlocomotion, suggesting a direct counteraction of the physiological and behavioural mechanisms triggered by psychological stress.

I further explored the underlying physiological mechanisms and discovered that lithium dose-dependently inhibits cold exposure-induced increases in BAT sympathetic nerve activity, BAT thermogenesis, expired $CO_2$, heart rate, and mean arterial pressure. These inhibitions suggest that lithium reduces sympathetic outflow from the rostral medullary region (rMR) of the brain. I found that this reduction in sympathetic outflow is not mediated by the vagus nerve but requires the area postrema (AP), as ablating the AP stops lithium from inhibiting each cold-evoked increase in the parameters. I propose two central pathways for lithium's effects, both involving the area postrema (AP) and the hypothalamomedullary thermoregulatory network. The first pathway involves the regions AP/nucleus tractus solitarius (NTS), lateral parabrachial nucleus (LPB), preoptic area (POA), dorsomedial hypothalamus (DMH), and rostral medullary region (rMR). The second pathway involves the regions AP/NTS, intermediate reticular nucleus/parvicellular reticular nucleus (iRt/PCRt), and rMR.

This research provides a fresh perspective on lithium's therapeutic mechanisms and potential clinical applications. My findings implicate specific brain regions involved in psychological stress and stress-related psychiatric conditions, which lithium is used to treat, and suggest potential strategies for mitigating lithium-associated weight gain. Further research is needed to refine the understanding of the proposed pathways and translate these insights into clinical practice, potentially leading to more targeted therapeutic strategies for bipolar disorder and other mood disorders.

# DECLARATION

I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university
2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and
3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Signed: 

Date: 19/09/2024

# ACKNOWLEDGEMENTS

First and foremost, I would like to extend my heartfelt appreciation to my principal supervisor, Associate Professor Yoichiro Otsuka, known to many as YoYo, for overseeing my development as a researcher. His invaluable mentorship in imparting crucial skills, ranging from microsurgical techniques to computer programming and effective scientific writing, has played a pivotal role in the success of my project. I am grateful for the opportunities he provided for my growth, his assistance in difficult portions of experiments such as the dissection of fine nerves, his encouragement, and his friendship.

My sincere thanks go to Emeritus Professor William "Bill" Blessing for sharing his bottomless wisdom, enlightening lessons, engaging stories, and contagious laughter. I deeply appreciate his no-nonsense attitude towards research, which has helped me maintain focus and strive for excellence in my work.

I am grateful to Dr. Lewis Vaughan for his advice on animal welfare and for teaching me advanced surgical techniques, such as aseptic surgery and subcuticular suturing.

Special thanks go to my peers and friends, Dr. Anna Antipov, Dr. Tim Hibberd, Mr. Quinn Martin, and Mr. Radwan Ansaar, for bringing joy and camaraderie to each day of my research journey.

I am indebted to the entire CMPH animal facility staff for providing and caring for the animals I had the privilege of conducting my research on, and to the animals themselves for their sacrifice.

My gratitude extends to the Biomedical Engineering team for their assistance in creating the custom apparatus used in my experiments, and to the microscopy facility for accommodating my histological needs.

I would like to acknowledge Flinders University for granting me the scholarship that enabled me to pursue this research full time.

Lastly, I am eternally grateful to my partner, Naomi Hilton, for her unwavering support and encouragement, ensuring that I saw this endeavour through to the end.

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| | |
|---|---|
| **5-HIIA** | 5-hydroxyindoleacetic acid |
| **5-HT** | 5-hydroxytryptamine or serotonin |
| **AP** | area postrema |
| **apoB** | apolipoprotein B |
| **ATP** | adenosine triphosphate |
| **BAT** | brown adipose tissue |
| **BATSNA** | brown adipose tissue sympathetic nerve activity |
| **BBB** | blood-brain barrier |
| **CCK** | cholecystokinin |
| **CeA** | central nucleus of the amygdala |
| **$Cl^-$** | chlorine anion |
| **CNS** | central nervous system |
| **$CO_2$** | carbon dioxide |
| **CSF** | cerebrospinal fluid |
| **CTA** | conditioned taste aversion |
| **CVO** | circumventricular organ |
| **dDMH** | dorsal part of the dorsomedial hypothalamus |
| **dlPAG** | dorsolateral periaqueductal gray |
| **DMH** | dorsomedial hypothalamus |
| **DMX** | dorsal motor nucleus of the vagus |
| **DP** | dorsal peduncular cortex |
| **DTT** | dorsal taenia tecta |
| **EC** | enterochromaffin |
| **$EtCO_2$** | end-tidal CO2 |
| **FDA** | Federal Drug Administration |
| **FFT** | fast Fourier transform |
| **FMC** | Flinders Medical Centre |
| **GI** | gastrointestinal |
| **GSK-3** | glycogen synthase kinase-3 |
| **HPA** | hypothalamic–pituitary–adrenal |
| **HR** | heart rate |
| **i.p.** | intraperitoneal |
| **i.v.** | intravenous |
| **IML** | intermediolateral cell column |
| **IMPase** | inositol monophosphatase |
| **IP3** | inositol-3-phosphate |
| **iRt** | intermediate reticular nucleus |
| **LDL** | low-density lipoprotein |
| **LHb** | lateral habenula |
| **$Li^+$** | lithium cation |

| | |
|---|---|
| **$Li_2CO_3$** | lithium carbonate |
| **$LiAlSi_2O_6$** | spodumene |
| **$LiAlSi_4O_{10}$** | petalite |
| **LiCl** | lithium chloride |
| **LOB** | lying-on-belly |
| **LPB** | lateral parabrachial nucleus |
| **LPBd** | dorsal lateral subnucleus of the lateral parabrachial nucleus |
| **LPBel** | external lateral subnucleus of the lateral parabrachial nucleus |
| **MAP** | mean arterial pressure |
| **MDMA** | N-methyl-3,4-methylenedioxy-amphetamine or ecstasy |
| **mEq** | milliequivalent |
| **$Mg^{2+}$** | magnesium cation |
| **MLR** | mesencephalic locomotor region |
| **MnPO** | median part of the preoptic area |
| **MPA** | medial preoptic area |
| **NA** | nucleus ambiguous |
| **$Na^+$** | sodium cation |
| **NaCl** | sodium chloride |
| **NTS** | nucleus tractus solitarius |
| **OLM** | oriens-lacunosum-moleculare |
| **PBS** | phosphate buffered saline |
| **PCRt** | parvicellular reticular nucleus |
| **$PGE_2$** | prostaglandin $E_2$ |
| **PI** | phosphatidylinositol |
| **POA** | preoptic area |
| **POMC** | proopiomelanocortin |
| **PVH** | hypothalamic paraventricular nucleus |
| **rMR** | rostral medullary raphe region |
| **RVLM** | rostral ventrolateral medulla |
| **s.c.** | subcutaneous |
| **SEM** | standard error of the mean |
| **SON** | hypothalamic supraoptic nucleus |
| **SPW-R** | sharp wave-ripple |
| **TGA** | Therapeutic Goods Administration |
| **UCP-1** | uncoupling protein-1 |
| **vDMH** | ventral part of the dorsomedial hypothalamus |
| **VLDL** | very low-density lipoprotein |
| **VTA** | ventral tegmental area |
| **WAT** | white adipose tissue |
| **Δ** | delta or change |

# Chapter 1.

# Introducing Lithium & Thermoregulation

## 1.1 Significance & Overarching Goal

The purpose of this chapter is to equip the reader with a foundational understanding of the essential interdisciplinary concepts that underpin this thesis. A broad spectrum of topics will be covered, including mammalian thermoregulation, the autonomic nervous system, relevant brain regions, pharmacological principles, behaviour, and centrally, the lithium atom. Establishing a comprehensive background in these diverse areas will provide the reader with the necessary context to appreciate the complexities and interconnections within the research presented in this thesis.

The overarching goal of this thesis is to investigate the physiological and behavioural effects of lithium across different mammalian species, specifically guinea pigs and rats, and to elucidate the neural mechanisms underlying these effects. This research proposes a novel principle suggesting that the consequential effects of lithium arise from the body's integrated response to the lithium ion, rather than from lithium's direct action within the brain. According to this principle, the peripheral response to lithium can lead to impacts on the brain, without necessitating the crossing of lithium through the blood-brain barrier. This research aims not only to redefine the mechanism of action of lithium, providing a more robust and comprehensive theoretical framework for understanding its therapeutic effects, but also to reassess its acute effects that have traditionally been overlooked or dismissed as side effects or signs of toxicity. This new approach challenges prevailing theories that are primarily molecular-focused and posit an intra-brain origin of lithium's action. It offers a more nuanced perspective of lithium’s effects and strives for better explanatory coverage within the field.

By exploring the interactions between these diverse topics and examining the neural mechanisms responsible for lithium's effects, this research contributes to our understanding of lithium's therapeutic potential in the context of psychiatric disorders. This integrated perspective on lithium's effects on the body and brain will ultimately inform future research and therapeutic strategies, enhancing the overall knowledge in the field and providing a more cohesive understanding of lithium's impact on physiology and behaviour.

## 1.2 Background & Context

### 1.2.1 The Lithium Atom, Its Ion, & Salt

Lithium, a soft, silvery-white alkali metal with an atomic number of 3, is distinguished as the lightest metal, having a molar mass of 6.941 g/mol and the smallest atomic radii among metals (Jeppson et al., 1978). Despite being the least reactive of the alkali metals, lithium still undergoes numerous chemical reactions and is not naturally found in its pure form (Jeppson et al., 1978). Its surface tarnishes in air due to reactions with oxygen and nitrogen, and it reacts readily with water (Schiemann et al., 2016). Owing to its unique electrochemical properties, including its high energy density by weight and electrode potential, lithium serves as an exceptional cathode material in rechargeable batteries (Liu & Azimi, 2021).

Indeed, in a biological context, it is lithium in its charged state, the lithium cation ($Li^+$) that is of interest due to its primary effectiveness in treating manic and depressive episodes in bipolar disorder (Schou, 2004). Hence, $Li^+$ is the primary subject of investigation and application in this study. This ion becomes active when introduced into the body, often given in the form of lithium salts like lithium chloride (LiCl) or lithium carbonate ($Li_2CO_3$) (Schou, 2004). LiCl, for example, readily dissolves in water, forming $Li^+$ and the chlorine anion, $Cl^-$. In modern medicine, $Li_2CO_3$ is typically used mainly due to it being the first and most studied form (Pacholko & Bekar, 2021). However, in animal research, both LiCl and $Li_2CO_3$ are commonly used (O'Donnell & Gould, 2007). Principally, it does not matter which salt is used, as the lithium ion is the active component (Schou, 2004).

These lithium salts can be introduced into the body through various routes. In humans, oral administration is the most common route (Oruch et al., 2014), while in animal research, the route of administration can vary and may include oral, intravenous, subcutaneous, or intraperitoneal routes depending on the study design (O'Donnell & Gould, 2007). Intraperitoneal administration, which involves the injection of a substance into the peritoneal cavity (the space within the abdomen that houses organs such as the intestines, stomach, and liver), is often used in animal research due to its ease of delivery and the rapid absorption of the substance into the bloodstream (Al Shoyaib et al., 2020). This intraperitoneal route is comparable to oral administration in that substances are ultimately absorbed into the mesenteric blood vessels and transported through the portal vein to the liver, whether originating from the peritoneal cavity or the intestines.

The main difference between these routes is the absorption rate, with intravenous administration providing the most rapid absorption directly into the bloodstream, followed by intraperitoneal, subcutaneous, and oral routes. Although the oral route is relatively the slowest, it still results in rapid absorption, with peak blood concentrations occurring within 1 to 2 hours or sooner in both rats and humans (Amdisen, 1977; Morrison et al., 1971).

Lithium is highly bioavailable (close to 100%) regardless of the administration route because it is readily absorbed, not metabolised by the liver, and not bound to plasma proteins (Baer, 1973). This allows lithium ($Li^+$) to move freely in the blood, resulting in the same effects no matter how it was administered once it enters the bloodstream. For example, in rats, lithium is equally effective in producing conditioned taste aversions, an important phenomenon explored in this thesis, when administered orally, intraperitoneally, or subcutaneously (Nachman & Ashe, 1973).

In this thesis, while acknowledging the various lithium salts and routes of administration used in both human medicine and animal research, I will specifically focus on the use of LiCl administered via the intraperitoneal route. The aim is to investigate the role and impact of lithium cations in mammalian thermoregulation, analysing the physiological and behavioural responses of the body to the presence of lithium ions. I will examine how these responses contribute to the overall thermoregulatory processes in mammals and explore the potential links between these thermoregulatory effects and the therapeutic effects of lithium.

### 1.2.2 Lithium Chloride

Lithium chloride (LiCl), the lithium salt utilised in this thesis, appears as a white cubic crystal or hygroscopic powder with a molar mass of 42.394 g/mol. It melts at 610 °C, boils at 1383 °C, and has a density of 2.07 g/cm³. It is remarkably soluble in water, with 84.5 g able to dissolve in 100 g of water at 25 °C (Rumble, 2017). I chose to use LiCl in this investigation due to its excellent dissolvability in water enabling me to deliver a large range of doses in only a small volume of solution to the animals.

LiCl, along with other lithium salts, is typically derived from lithium-containing ores such as spodumene or lithium-rich brines (Meshram et al., 2014). When sourced from ores, the minerals are crushed, roasted at high temperatures, and often further ground. They then undergo processes like acidification, alkalisation, and chlorination to yield lithium salts (Meshram et al., 2014). For instance, calcinated spodumene is treated with a high-temperature concentrated sulphuric acid solution and then leached with water to produce a lithium sulphate solution (Liu & Azimi, 2021). Upon addition of

sodium carbonate to this solution, a double displacement reaction occurs, converting lithium sulphate into lithium carbonate and sodium sulphate (Fosu et al., 2020). The lithium carbonate forms a precipitate, as it is poorly soluble in water (Jeppson et al., 1978), and is purified through a series of steps, which include filtration, washing, drying, and recrystallisation (Liu & Azimi, 2021). It can then be reacted with hydrochloric acid to form LiCl (Gao et al., 2023).

Alternatively, another high-temperature method can directly extract LiCl from spodumene. This involves the use of chlorides such as potassium chloride, sodium chloride, or ammonium chloride to create soluble lithium chloride (Gao et al., 2023).

When LiCl is produced from brines, which are high-concentration solutions of various salts weathered from rocks and dissolved in water, the process typically involves pumping these brines from underground reservoirs into evaporation ponds (Vera et al., 2023). A prime example of this process can be seen in the 'lithium triangle', a region of the Andes mountain range in South America that includes parts of Chile, Argentina, and Bolivia (Heredia et al., 2020). The water in these ponds is evaporated with the aid of heat from the sun and wind, leaving behind crystallised salts, which are then processed to extract lithium, often resulting in LiCl (Berdikulova et al., 2022).

### 1.2.3 A Brief History of Lithium

I will cover where lithium comes from, how it was discovered, how it came to be used in modern psychiatry, and then shift my focus to the experiments that inspired and guided this thesis.

#### *1.2.3.1 Origin & Discovery*

Lithium holds a unique position in the universe's history, having been present since the very beginning. Among the elements, only hydrogen, helium, and a trace amount of lithium were synthesised during the Big Bang, the cataclysmic event that marked the universe's beginning approximately 13.8 billion years ago (Valcin et al., 2021). Few other medicinal substances can lay claim to such cosmic origins, with the same atoms that were present at the dawn of the universe now coursing through the veins of patients being treated with lithium today.

While some lithium was formed in the early universe, much of the lithium we observe in the cosmos today is believed to have been produced by classical novae (Tajitsu et al., 2015). These are thermonuclear explosions that occur on a white dwarf star within a binary system, wherein the white

dwarf accretes hydrogen-rich material from its companion star (José et al., 2006). Once a critical mass of material accumulates on the white dwarf's surface, it instigates an explosive thermonuclear fusion of hydrogen, leading to the formation of elements, including lithium (Gallagher & Starrfield, 1978; Hernanz, 2015).

Leaping forward several billion years to Earth, 1817, Swedish chemist Johan August Arfwedson, under the tutelage of the renowned Swedish chemist Jöns Jakob Berzelius, discovered lithium while analysing the mineral petalite ($LiAlSi_4O_{10}$) (Wietelmann & Klett, 2018), now recognised as a low-grade lithium-bearing ore (Kavanagh et al., 2018).

Initially, the new alkali was named 'lithion' by Berzelius (Berzelius, 1817), derived from the Greek word 'lithos', meaning stone (Wietelmann & Klett, 2018). However, the name was later modified to lithium (Miśkowiec, 2023), likely due to Berzelius's concern that 'lithion' had a peculiarly Swedish termination and might not adapt well to other languages (Johnson, 1984b).

Today, spodumene ($LiAlSi_2O_6$), another lithium-bearing mineral, is preferred to others due to its higher lithium grade, simpler composition, and fewer impurities (H. Li et al., 2019). In 2011, a spodumene deposit in Greenbushes, Western Australia, was responsible for 85% of the world's lithium production from ores (Tadesse et al., 2019). Spodumene is expected to remain the primary mineral source of lithium in the foreseeable future (H. Li et al., 2019).

In 1818, a year after Arfwedson's discovery of lithium, English chemist William Thomas Brande succeeded in isolating lithium into its pure metallic form (Brande, 1848). He achieved this through the electrolysis of lithium oxide, a compound typically derived from lithium carbonate or lithium peroxide (Ktalkherman et al., 2009), accomplishing a feat that had eluded Arfwedson.

In 1854, Robert Wilhelm Bunsen, the German chemist known for inventing the Bunsen burner, and British chemist/physicist Augustus Matthiessen, successfully produced lithium metal in larger quantities (Bunsen & Matthiessen, 1855). They achieved this by employing an electrolytic process on molten lithium chloride. This method was later adopted by the now-defunct Metallgesellschaft AG in Germany, marking the first commercial production of lithium metal in 1923 (Wietelmann & Klett, 2018).

#### *1.2.3.2 Historical Medical Use*

The earliest recorded medical use of lithium can be traced back to Ancient Greece. Approximately 1894 years ago, during the lifetime of Greek physician Galen of Pergamon (129–216 AD), individuals exhibiting symptoms of mania were often treated with waters from certain alkaline springs. This condition was putatively first described by Aretaeus of Cappadocia, another Greek physician from the 2nd century AD (Allbutt, 1921). Contemporary understanding suggests that the therapeutic properties of these springs were likely attributable to the presence of lithium. However, the element itself was not identified or known at the time (Mohandas & Rajmohan, 2007).

The intentional medical use of lithium was first documented in the mid-19th century, beginning with its application in the field of rheumatology. In 1843, Scottish physician and chemist Alexander Ure demonstrated that a solution of lithium carbonate could dissolve uric acid bladder stones (Ure, 1843). This discovery was further popularised in 1859 by English physician Alfred Baring Garrod, who recommended lithium carbonate treatment for gout after finding uric acid in the blood of patients with the condition in 1848 (Garrod, 1848, 1859). Later, in 1868, French internist Armand Trousseau, along with Garrod in 1876, proposed that mania could be a manifestation of a rheumatic condition they termed 'brain gout' (Garrod, 1876; Trousseau, 1865). In 1888, British physician Alexander Haig also linked depression, which he referred to as 'melancholia', to gout (Haig, 1888).

Alongside these rheumatic studies, lithium was also being explored for more direct uses in affective disorders. In 1870, American physician and writer Silas Weir Mitchell suggested the use of lithium bromide as an anticonvulsant and sedative (Mitchell, 1870), later recommending it in 1877 for treating 'general nervousness' (Mitchell, 1877). In 1871, prominent American military physician and neurologist William Hammond became the first physician to use lithium bromide in treating acute mania, and he described a case of acute mania with comorbid depression marked by a strong suicidal tendency (Hammond, 1871).

Meanwhile, from 1886 to 1896, Danish physicians and brothers Carl and Frederik "Fritz" Lange were conducting studies on the use of lithium compounds, specifically lithium carbonate, in treating what they termed 'periodical depression' (Lange, 1886, 1896; Lange, 1894). Carl Lange's theory proposed that an excess of uric acid in the body frequently causes this mental illness, and he treated approximately two thousand patients with lithium. Despite his extensive work, Lange's theory was largely dismissed by his contemporaries, which led to his work being overlooked during his time (Schioldann, 2011).

This early work, up until the turn of the 20th century, represents the first collection of indirect and direct treatments of bipolar disorder, encompassing both manic and depressive phases, with lithium. However, research on lithium fell into obscurity and remained virtually dormant for the next 50 years (Shorter, 2009), until Australian psychiatrist John Cade reintroduced the drug to the field of psychiatry in 1949, highlighting its antimanic effects (Cade, 1949).

### 1.2.4 John Cade: The Pioneer of Lithium Treatment

Groundbreaking discoveries often come from unexpected places. This is aptly illustrated by the journey of an Australian psychiatrist, John Frederick Joseph Cade (de Moore & Westmore, 2016; Johnson, 1984b). His pioneering work, conducted in a makeshift laboratory, led to the discovery of lithium as the first effective treatment for a psychiatric disorder, specifically bipolar disorder. Remarkably, lithium's efficacy has stood the test of time, as it remains the single most effective treatment in all of psychiatry (Ruffalo, 2017). Cade's journey and findings inspired the research that underpins this thesis. Hence, it is fitting that I revisit this man's history and his seminal experiments with lithium.

#### *1.2.4.1 Early Life & Journey into Psychiatry*

Born in the small Victorian town of Murtoa, Australia, on the 18th of January 1912, John Cade spent much of his formative years around mental health patients due to his father's work at Beechworth and Sunbury Mental Hospitals (Cade, 1999). This early exposure sparked his deep empathy for patients and interest in the aetiology of psychiatric illness.

Following his father's footsteps, Cade ventured into the field of medicine. He completed his MB, BS in 1934 from The University of Melbourne and subsequently undertook residencies at St Vincent's and the Royal Children's Hospitals (Ironside, 1993). It was during his residency that he fell ill with pneumococcal pneumonia (Cade, 1999). The care he received from a nurse, Estana Evelyn Jean Charles, would later blossom into a deeper relationship (de Moore & Westmore, 2016). After recovering, Cade decided to follow his father's path into psychiatry. At 24, he joined the Victorian Department of Mental Hygiene as a Medical Officer and began his work at Beechworth Mental Hospital in 1936 (de Moore & Westmore, 2016). In 1937, he returned to Melbourne, married Jean, and took up residence at the Bundoora Repatriation Mental Hospital (Cade, 1999; de Moore &

Westmore, 2016). He furthered his qualifications by acquiring his postgraduate MD in 1938, also from Melbourne University (Ironside, 1993).

#### *1.2.4.2 Experiences from the World at War*

In 1941, Cade departed from Australia to serve in the 2nd/9th Field Ambulance unit during the Second World War, where he eventually rose to the rank of Major (de Moore & Westmore, 2016). However, his service was interrupted when he was captured as a prisoner of war by the Japanese in Singapore, a gruelling experience that lasted three and a half years. During his interment at Changi camp, Cade was tasked with establishing an improvised hospital to care for his fellow prisoners. Amidst this adversity, he closely observed the soldiers' mental states, noting cases of severe melancholia and manic-depressive disorder that stood out from the general distress caused by their environment (de Moore & Westmore, 2016).

Two cases deeply influenced his thinking. The first involved a young soldier in the manic phase of manic-depressive disorder. When this soldier unexpectedly died, an autopsy revealed a significant subdural haematoma, a physical brain injury likely sustained in battle. This finding challenged the prevailing view of mental illnesses as purely psychological. The second case involved a sergeant who initially presented with symptoms such as failing hearing and increased muscle tone, leading to uncoordinated movements. These symptoms were initially interpreted as psychological. However, following the sergeant's sudden death, a post-mortem examination revealed a loss of myelin in his central nervous system, pointing to a physical origin for his symptoms (de Moore & Westmore, 2016).

These experiences, coupled with his observations of soldiers with severe depression, led Cade to theorise a biological basis for severe mental illnesses such as manic-depressive disorder. This shift in perspective marked a significant turning point in his understanding of mental disorders, laying the groundwork for his future research into their physiological origins.

#### *1.2.4.3 Searching for a Biological Basis of Mental Disorders*

In August 1945, following the dropping of atomic bombs on Hiroshima and Nagasaki, the Japanese unconditionally surrendered. Cade was liberated and returned from Singapore to Bundoora in 1946. Immediately, he set out to test his hypothesis of a biological basis for psychiatric illnesses. Drawing inspiration from his observations in Changi camp and his readings on endocrinology, specifically the behaviour-altering effects of thyroid hormones (de Moore & Westmore, 2016), Cade developed a

groundbreaking hypothesis. He posited that mania might be caused by an excess of a naturally occurring substance in the body, and depression by its deficiency (Mitchell & Hadzi-Pavlovic, 2000). Despite the lack of modern imaging devices or concrete evidence, Cade remained undeterred. Resolute in his pursuit, he began examining a readily available and non-invasive source: urine.

Cade set up an unconventional laboratory in the pantry of a newly built, yet unoccupied ward at Bundoora Hospital. Mostly self-funded, he made do with what he had: a bench, sink, chemical containers, basic lab instruments, and cages filled with guinea pigs sourced from Mont Park Mental Hospital. Some of these guinea pigs were cared for back at home, treated as family pets and fed kitchen scraps (de Moore & Westmore, 2016). Perhaps the most distinctive aspect of his research was his burgeoning collection of urine-filled jars. Cade collected samples from depressive, manic, schizophrenic patients, and healthy controls. These jars were stored in the family fridge next to the cheese, much to the dismay of his wife Jean (de Moore & Westmore, 2016).

#### *1.2.4.4 The Lithium Guinea Pigs: The Groundbreaking Discovery*

Cade's investigation began with a basic differential toxicity test, aiming to uncover a potential metabolite behind manic-depressive illness (Cade, 1947). He injected the collected urine samples into the guinea pigs' intraperitoneal cavity. He found that all samples were lethal to the guinea pigs, but urine from manic patients seemed particularly toxic. Cade attributed this toxicity to urea, a nitrogenous constituent common in all samples. However, the urea concentrations were comparable across all samples and significantly below the toxic threshold.

This led Cade to propose the existence of 'quantitative modifiers', substances that could enhance or reduce urea's toxic effects. He identified creatinine, which decreased urea toxicity, and uric acid, which increased it (Cade, 1947). However, neither substance could fully account for the lethal effect of patients' urine. Consequently, Cade suggested a third, unidentified substance that might counteract creatinine's protective effect and synergise with uric acid to enhance urea's toxicity.

However, his investigation took an unexpected turn. Up until this point, Cade had been dissolving these substances in either water or saline (Cade, 1947). Given the poor solubility of uric acid in water, he decided to work with a form of uric acid that would dissolve more easily (Cade, 1949). Uric acid forms salts known as urates when it reacts with a base, but these urates vary in their solubility. The lithium salt, known as lithium urate, is unique because it dissolves in water more readily than any other uric acid salt (Hesse, 1876). This high solubility made it Cade's choice, as it could dissolve into

uric acid and lithium ions in water. In a stroke of serendipity, Cade found that urea, when combined with lithium urate, was less toxic to the guinea pigs than when administered alone, as all the guinea pigs survived the treatment. This suggested that the lithium ion might have a protective role against urea toxicity (Cade, 1949).

To confirm the effects of lithium, Cade replaced lithium urate with lithium carbonate, achieving identical results. He then administered what he referred to as 'large doses' of a 0.5% aqueous lithium carbonate solution to the guinea pigs, without urea (Cade, 1949). The outcome was historically significant. Upon the administration of lithium, Cade noted a transformative effect on the guinea pigs' behaviour. Instead of displaying their usual skittishness, they remained calm yet alert, even when placed on their backs. Cade described this phenomenon, stating, "...the animals, although fully conscious, became extremely lethargic and unresponsive to stimuli for one to two hours before once again becoming normally active and timid" (Cade, 1949). Cade's observations of this lethargy and the guinea pigs' subsequent return to normal behaviour led him to conclude that lithium had a temporary calming effect. Upon discovering that lithium produced "no discernible ill effects" when he administered it to himself, Cade subsequently utilised the drug to successfully treat ten patients suffering from mania (Jefferson, 1998). His findings marked the starting point of my investigation, which will commence in **Chapter 2**.

#### *1.2.4.5 Fears of Toxicity*

The same year that Cade published his groundbreaking findings, the medical use of lithium encountered controversy. Lithium salts, specifically lithium chloride (LiCl), bear a taste similar to regular table salt (NaCl). This similarity led to the brief use of LiCl as a substitute for NaCl in patients required to control their sodium intake (Hanlon et al., 1949). Despite being marketed as 'perfectly safe', the frequent consumption of LiCl with food resulted in uncontrolled intake and consequently dangerous levels of lithium in the blood of consumers (Hanlon et al., 1949). This led to the Food and Drug Administration (FDA) banning the use of lithium as a salt substitute (Johnson, 1984a), inciting a toxicity panic among physicians and a reluctance to use lithium medicinally. However, certain proponents of lithium continued its use, arguing that its anti-manic properties outweighed the potential for toxicity.

In response to these concerns, researchers, particularly Danish psychiatrist Mogens Schou, conducted rigorous clinical trials that were instrumental in establishing lithium's safety and efficacy (Baastrup & Mogens, 1967; Johnson, 1984a; Schou et al., 1954). Schou's work focused on determining the correct

dosage and safe therapeutic blood lithium levels for acute treatment and long-term prevention of mood disorders. These efforts helped to restore confidence in lithium as a therapeutic agent and solidify its role in modern psychopharmacology.

### 1.2.5 Lithium in Modern Psychopharmacology

Following Cade's pioneering work and Schou's significant contributions, lithium has been extensively studied, and its role in psychiatric treatment has been firmly established. It is now recognised as an effective mood stabiliser with antimanic and antidepressant effects, and it is the gold standard treatment for bipolar disorder worldwide (Girardi et al., 2016; Jann et al., 2016; Machado-Vieira et al., 2009; Rybakowski, 2020a, 2020b). Lithium exhibits extensive antimanic effects and is considered one of the most effective agents for long-term prophylaxis in bipolar disorder (Baastrup & Mogens, 1967).

Not only is lithium effective in treating acute mania and preventing recurrent manic episodes, but it is also effective in preventing depressive episodes (Severus et al., 2014). This makes lithium the only substance used in the treatment of bipolar disorder so far that can prevent both manic and depressive episodes (Licht, 2012). It is often used in combination with antidepressants due to its ability to enhance or initiate their therapeutic effects (Haeberle et al., 2012; Johnson & McFarland, 1996; Moret, 2005; Post, 2018), which is especially useful for treatment-resistant depression (Rybakowski, 2020a). This augmentation is also seen when lithium is paired with other psychotropic agents in the treatment of bipolar disorder (Post, 2018). Additionally, lithium has been shown to reduce aggressive behaviour (Jakobsson et al., 2017).

Compared to other mood stabilisers, lithium often has higher efficacy in treating manic episodes and sometimes depressive episodes in bipolar patients (Rybakowski, 2020a). When used as a monotherapy for bipolar disorder, lithium often exhibits superior efficacy compared to other mood stabilisers (Kessing et al., 2018; Rybakowski, 2018). Moreover, due to its affordability and unpatentable nature, lithium presents a cost-effective treatment option (Kline, 1973).

Lithium exhibits substantial neurotropic and neuroprotective properties, enhancing neurogenesis and gliogenesis, promoting increases in hippocampal and cortical volume, and reducing the size of lesions associated with various neurodegenerative and neurological conditions (Ghanaatfar et al., 2023; Machado-Vieira et al., 2009; Post, 2018). In addition to these effects, lithium has been shown to modulate immune responses and reduce inflammation, which may contribute to its neuroprotective capacity (Haupt et al., 2021). It may also confer protective benefits against dementia and cognitive

decline (Post, 2018; Rybakowski, 2020a). Furthermore, studies have found an association between lithium use and longer telomere length (Martinsson et al., 2013), a factor whose shortening is typically associated with aging and reduced longevity.

Notably, lithium has a unique suicide-preventative effect (Cipriani et al., 2013; Del Matto et al., 2020). In fact, it is the only known drug to specifically reduce suicidal ideation (Müller-Oerlinghausen et al., 2005; Smith & Cipriani, 2017). It is important to note that around one million people die from suicide each year globally; it is the second leading cause of death next to accidents among younger people aged 15 to 39 (Lewitzka et al., 2015).

Remarkably, outside of a clinical context, higher trace lithium concentrations in sources such as drinking water, despite being well below therapeutic doses, has been associated with better mood and lower rates of suicide, aggression, violent crime, and even dementia (Kessing et al., 2017; Mauer et al., 2014; Memon et al., 2020). Such a correlation is observed in several countries, including Japan, the United States, Greece, and Denmark. In fact, the implications of these findings are so compelling that some researchers have proposed the fortification of food, water sources, and vitamins with lithium, aiming to harness its beneficial effects through the possible stabilisation of mood (Araya et al., 2022; Naeem et al., 2021).

Unfortunately, despite the numerous benefits of lithium, the extensive research supporting its use, and its gold standard namesake, it is currently underutilised in the treatment of mood disorders. This concern has been raised by notable researchers such as Polish psychiatrist Janusz Rybakowski and American psychiatrist Robert Post, who strongly advocate for its use (Post, 2018; Rybakowski, 2020a). One of the main reasons for this underutilisation may be attributed to apprehensions regarding lithium’s side-effects. These fears of toxicity have persisted since lithium's introduction by Cade in 1949, not only hindering its clinical use but also potentially clouding our understanding of its mechanisms of action.

However, Post argues that the side-effect profile of lithium within the therapeutic range is often exaggerated. He contends that these side-effects are relatively benign and can be effectively managed with appropriate dosing and monitoring strategies (Post, 2018). One of the objectives of this thesis is to reframe certain physiological and behavioural effects of lithium, which are often overlooked and misconstrued as toxic side-effects, as integral components of its therapeutic action.

### 1.2.6 Current Hypotheses on Lithium's Mechanisms (Pharmacodynamics)

Despite its introduction by Cade in Australia in 1949 (Cade, 1949), its approval by the Therapeutic Goods Administration (TGA) in Australia in 1967 (National Health and Medical Research Council's Poisons Schedule Sub-committee, 1967), its widespread use in psychiatric medicine since the 1960s around the world, and its subsequent approval by the FDA in the United States in 1970 (Shorter, 2009), the precise mechanisms by which lithium works in the body to produce its therapeutic, mood-stabilising effects remain unknown (Haupt et al., 2021; Schou, 2004).

#### *1.2.6.1 Lithium Competes with Native Cations*

Prevailing hypotheses are primarily focused on molecular interactions in the brain, with many positing that lithium's therapeutic action stems from its competitive interaction with native ions, particularly sodium ($Na^+$) and magnesium ($Mg^{2+}$) (Dudev et al., 2019). This is a complex phenomenon, and the following section will briefly cover our current understanding of it.

Lithium ($Li^+$), sodium ($Na^+$), and magnesium ($Mg^{2+}$) are ions with distinct physicochemical properties that significantly influence their behaviour in aqueous solutions and biological systems. $Li^+$ and $Na^+$ are monovalent cations with a single positive charge, while $Mg^{2+}$ is a divalent cation with a double positive charge. The ionic radii of these ions, defined by the distance from the nucleus to the outer electron shell in their ionic states, differ from their atomic sizes. In their ionic states, $Mg^{2+}$ is the smallest, closely followed by $Li^+$, and then by $Na^+$ (Dudev et al., 2019).

The charge density of an ion, defined as its charge divided by its volume, is another important factor. Given its smaller ionic radius and double positive charge, $Mg^{2+}$ has a higher charge density than either $Li^+$ or $Na^+$. However, it is noteworthy that $Li^+$ has a higher charge density than $Na^+$ due to its smaller ionic radius (Dudev et al., 2019). High charge density often results in a stronger attraction to water molecules and a more densely packed hydration shell (Guo & Friedman, 2009).

The hydration free energy of an ion, a measure of the energy change when an ion is surrounded by water molecules to form a hydration shell, is influenced by these properties. Among $Na^+$, $Li^+$, and $Mg^{2+}$, the hydration free energy values become increasingly negative in this order (Friedman & Krishnan, 1973). A more negative hydration free energy indicates a stronger attraction to water molecules and a more exothermic (heat-releasing) hydration process. For instance, when an adequate amount of the lithium salt LiCl is dissolved in water in a test tube, it separates into $Li^+$ and $Cl^-$ ions (both having

negative hydration free energies). This process is exothermic and releases heat, which can be felt in the palm of your hand.

These properties not only influence how these ions move through water and interact with other molecules in biological systems, such as in the bodies of humans and other mammals, but also their ability to pass through specific ion channels and bind to particular sites on proteins (Dudev et al., 2019; Jakobsson et al., 2017; Noskov & Roux, 2007).

#### *1.2.6.2 Sodium-Channel Mediated Cellular Entry & Reabsorption of Lithium*

Given its smaller ionic radius and comparable charge (Dudev et al., 2019), $Li^+$ can traverse sodium channels, both voltage-gated and non-voltage-gated epithelial types (Richelson, 1977; Thomsen & Shirley, 2006), penetrating cells with an efficiency similar to that of $Na^+$ (Naylor et al., 2016). This enables $Li^+$ to enter a multitude of cell types in addition to excitable cells such as neurons (Black & Waxman, 2013). Furthermore, $Li^+$ can utilise the sodium-phosphate co-transporter in the nephron proximal tubule for reabsorption in the kidney (Uwai et al., 2014), allowing it to be reabsorbed into the blood.

#### *1.2.6.3 Lithium Inhibits Magnesium-Dependent Enzymes*

In certain enzymes such as inositol monophosphatase (IMPase) and glycogen synthase kinase-3β (GSK-3β), $Li^+$ has been observed to displace $Mg^{2+}$ that is weakly bound (Dudev et al., 2019; Mota de Freitas et al., 2006). This displacement is likely facilitated by a combination of factors. Firstly, although $Mg^{2+}$ is slightly smaller than $Li^+$ in ionic radius, their radii are relatively similar, which may allow $Li^+$ to occupy the same space as $Mg^{2+}$ in the protein. Secondly, the sites where $Mg^{2+}$ binds in these enzymes have a high positive charge density; this density can be reduced when $Li^+$, which carries a single positive charge, substitutes for $Mg^{2+}$, which carries a double positive charge (Dudev & Lim, 2011; Dudev et al., 2019). This reduction in charge density makes the substitution energetically favourable. Lastly, the less negative hydration free energy of $Li^+$, which indicates a weaker interaction with water molecules compared to $Mg^{2+}$, may allow $Li^+$ to shed part of its hydration shell more readily, facilitating its binding to the protein (Noskov & Roux, 2007). Once bound, $Li^+$ can impair the function of the host protein by altering its structural configuration and stability, which in turn reduces the activity of the enzyme. This contributes to the observed inhibitory effects of $Li^+$ on certain $Mg^{2+}$-dependent enzymes.

#### 1.2.6.3.1 Inositol Monophosphatase

The inositol depletion hypothesis, one of the earliest mechanistic hypotheses for lithium's action (Berridge et al., 1989), posits that lithium inhibits several magnesium-dependent enzymes, including inositol monophosphatase (IMPase), involved in inositol phosphate metabolism (Brown & Tracy, 2013; Yu & Greenberg, 2016). IMPase, a critical intracellular enzyme within the phosphatidylinositol (PI) signalling pathway (Brown & Tracy, 2013; Pisanu et al., 2016), is believed to be inhibited by $Li^+$ through the displacement of weakly bound $Mg^{2+}$ from these metalloenzymes (Dudev et al., 2019). This inhibition prevents the conversion of inositol-3-phosphate (IP3) to myo-inositol, leading to a depletion of free myo-inositol concentrations (Brown & Tracy, 2013; Yu & Greenberg, 2016). Myo-inositol, a biologically active sugar-alcohol isomer, plays key roles in cellular functions such as membrane phospholipid composition, osmoregulation, signal transduction, protein phosphorylation, chromatin remodelling, gene expression, and mRNA export from the nucleus (Chhetri, 2019). Consequently, its depletion due to lithium may disrupt a wide range of cellular processes.

Studies have observed brain myo-inositol depletion in rats following the acute and chronic administration of lithium (Allison & Stewart, 1971; O'Donnell et al., 2000). Magnetic resonance imaging studies have reported elevated myo-inositol levels in bipolar patients (Silverstone et al., 2005), leading to the proposal that lithium's therapeutic effect is due to altered cell signalling caused by IMPase inhibition (Haimovich et al., 2012).

However, these findings are not consistently supported throughout the literature (Brown & Tracy, 2013; Yu & Greenberg, 2016). For instance, selectively reducing inositol levels by genetic means in mice did not produce a mood-stabilising outcome comparable to that of lithium (Shaldubina et al., 2007; Shaldubina et al., 2006). This discrepancy suggests that the inositol depletion hypothesis may not fully account for lithium's therapeutic action.

#### 1.2.6.3.2 Glycogen Synthase Kinase-3

The glycogen synthase kinase-3 (GSK-3) hypothesis is another prominent mechanistic hypothesis proposed for lithium's action. GSK-3 is a ubiquitous, magnesium-dependent protein kinase initially identified for its role in inactivating glycogen synthase in response to insulin (Brown & Tracy, 2013; Embi et al., 1980; Yu & Greenberg, 2016). It is now recognised to regulate a myriad of cellular functions, including neuroplasticity, embryonic development, immune responses, signal transduction, and cell survival (Brown & Tracy, 2013; Salcedo-Tello et al., 2011; Yu & Greenberg, 2016). There are

two isoforms of GSK-3: GSK-3α and GSK-3β. Despite their biochemical similarities and wide expression in all mammalian tissues (Salcedo-Tello et al., 2011), much research has focused on GSK-3β due to its abundance in the brain (Brown & Tracy, 2013; Yu & Greenberg, 2016). Over 40 proteins, involved in various cell signalling pathways and implicated in diseases like Alzheimer's and cancer, are regulated by GSK-3β (Yu & Greenberg, 2016).

Lithium, competing with $Mg2^{+}$, binds to and directly inhibits GSK-3, triggering serine phosphorylation (Brown & Tracy, 2013; Yu & Greenberg, 2016). Animal studies, particularly in mice, have observed behavioural and mood-stabilising effects similar to lithium when using selective GSK-3 inhibitors (Brown & Tracy, 2013; Yu & Greenberg, 2016). However, the inhibitor constants (Ki) for lithium against both GSK-3 isoforms are equal to or exceed the upper limit of lithium's therapeutic range in humans (Shaldubina et al., 2001). This suggests that achieving complete inhibition of GSK-3 may necessitate plasma lithium levels that are toxic, and thus, lithium at therapeutic levels may only partially inhibit GSK-3. Furthermore, no direct correlation has been established between lithium's therapeutic action and the partial or complete inhibition of any individual GSK-3-related cascade (Shaldubina et al., 2001). The situation is further complicated by the fact that GSK-3β alone has the potential to interact with hundreds of other proteins (Jakobsson et al., 2017). These complexities indicate that our understanding of the role of GSK-3 in lithium's therapeutic effect is far from complete.

#### *1.2.6.4 The Drawback of Molecular-Focused Hypotheses*

While the prevailing hypotheses provide minute details and valuable insights into lithium's molecular interactions, they do not yet offer a clear and cohesive link between lithium's action on a specific intracellular target and its distinct therapeutic effects. Translating lithium's innumerable molecular interactions to the organism level is indeed challenging. Given that many of lithium's effects are attributed to its ion's competition with other native ions such as $Na^{+}$ and $Mg^{2+}$, and considering that $Mg^{2+}$ alone interacts with over 3000 proteins (Piovesan et al., 2012), the potential scope of lithium's molecular interactions is vast. This is further complicated by observations that lithium modulates the expression of an enormous number of genes (Alda, 2015; Pisanu et al., 2016). This complexity highlights the enormity of the task faced by these molecular-level hypotheses, which do not effectively account for the potential physiological responses or compensatory mechanisms at the whole-body level that may be triggered by these intricate intracellular interactions.

Some researchers have proposed the overarching notion that lithium 're-regulates everything', suggesting its diverse interactions collectively contribute to a specific therapeutic outcome (Post,

2018; Snitow et al., 2021). Others have suggested that lithium reduces neuronal excitability (Dudev et al., 2019). However, both these hypotheses fall short in clearly specifying where in the brain these actions occur, which specific neuronal pathways are affected, or how these changes in such areas and pathways would result in mood stabilisation such as the reduction of mania. This lack of specificity presents a substantial gap in our current comprehension of lithium's therapeutic efficacy.

While I do not discount the possibility that lithium's molecular interactions contribute to its therapeutic effects, I propose a shift in focus towards understanding the physiological responses to lithium at the organism level. This approach, which considers the intricate and integrated nature of mammalian physiology, may provide a more comprehensive explanation of lithium's therapeutic action.

### 1.2.7 Revisiting Lithium's Overlooked Effects: A Novel Thermoregulatory Hypothesis

The key to lithium's mechanism of action, I propose, may be found in the results of John Cade's pioneering experiments with guinea pigs and the distinct behavioural changes he observed (Cade, 1949). It was the observation of lethargy, which made the typically skittish guinea pigs calm and unphased by stimuli that would usually frighten them, that led Cade to use lithium to treat the excited thinking and behaviour in manic patients (Cade, 1949). These behavioural changes, which have not been replicated in subsequent guinea pig studies, have been frequently dismissed among researchers as mere side effects of lithium toxicity precipitating from overdose (de Moore & Westmore, 2016; Mitchell, 1999; Price & Heninger, 1994; Schou, 1992).

Alongside Cade's observation of lethargy, other physiological responses to lithium, such as hypothermia (Hesse, 1876; Jensen, 1974; Perkinson et al., 1969) and nausea, have also been largely dismissed as toxic side effects (Hanlon et al., 1949; Okusa & Crystal, 1994). These effects, though seemingly disparate, may hold significant clues to understanding lithium's mechanism of action.

In this thesis, I propose a novel perspective that challenges the prevailing view of lithium's acute effects as merely toxic. I posit that the observed lethargy, hypothermia, and nausea are not merely toxic side effects, but integral components of the body's natural response to lithium. I further suggest that these seemingly disparate effects are interconnected, representing a unified thermoregulatory response to lithium.

This hypothesis, which I will elaborate on and substantiate in the subsequent chapters, positions these responses as central to understanding lithium's therapeutic efficacy. My primary objective is to investigate and characterise these acute effects in rodents, with a particular emphasis on the broader implications these effects have for mammalian physiology. By examining these responses, I aim to highlight their fundamental role in elucidating the therapeutic action of lithium in humans. In the interest of brevity, I will provide a detailed review of lithium's hypothermic effect, while lithium-induced lethargy and nausea will be thoroughly discussed within the context of relevant studies from **Chapter 2** onwards.

#### *1.2.7.1 Current Literature on Lithium-Induced Hypothermia*

A significant piece of evidence supporting my thermoregulatory hypothesis is the observation that lithium has been observed to induce hypothermia, a reduction in core body temperature, in a variety of small mammals. This effect has been largely ignored in recent literature and historically dismissed as just another toxic side effect (Okusa & Crystal, 1994), much like the lethargy observed in Cade's guinea pigs.

The earliest mention of lithium-induced hypothermia may be the observation by A. Hesse in his 1876 German medical doctoral dissertation. Hesse found that in rabbits, a dose of 2 grams of lithium chloride, which would prove to be a lethal amount for most small mammals, led to a significant drop in body temperature (Hesse, 1876). Specifically, a decrease in temperature of 1 °C was noticeable after 10 minutes post-injection. 20 minutes later, it had fallen by another 1 °C and continued to fall until a decrease of 5 °C was observed shortly before death. However, most importantly, Hesse noted that smaller, non-lethal doses also resulted in slight decreases in temperature, though not much detail was presented on this aspect (Hesse, 1876).

While the lethal doses represent the extreme end of this thermoregulatory response, it is the occurrence of hypothermia at non-lethal, non-toxic doses that is important and integral to my investigations in this thesis.

Nearly a century later, in 1969, Perkinson et al. reported what is, to my knowledge, the first instance of acute lithium-induced hypothermia in rats. This was observed following a small intraperitoneal dose of 0.47 mEq/kg (Perkinson et al., 1969), which is not considered to fall within the toxic range (Cox et al., 1971; O'Donnell & Gould, 2007).

In 1974, Jensen conducted a study in which rats were administered a lithium-infused diet for six days, ensuring the maintenance of therapeutic levels of lithium in the blood serum (Jensen, 1974). Over the course of the study, the rats exhibited both hypothermia and reduced locomotor activity compared to the controls that were not fed lithium. These observations suggest that these effects can occur both in the context of acute and chronic lithium administration, indicating a consistency in lithium's impact across different durations of exposure.

In 1976, Tulunay was the first to demonstrate dose-dependent hypothermia in rats following intraperitoneal lithium injections (Tulunay, 1976). This dose-dependent effect was later confirmed by Ogilvie and Lobb in a similar study conducted on mice (Ogilvie & Lobb, 1981).

While the hypothermic effect of lithium is well-documented in animal models, it remains unexplored in the context of human physiology. An intriguing case study, however, provides some insight into this phenomenon (Follezou & Bleibel, 1985). The case revolves around a 74-year-old patient diagnosed with manic-depression, who had been consistently treated with lithium carbonate and levopromazine for three years.

Following a surgical intervention for a gastric tumour, the patient was admitted for adjuvant chemotherapy. During this period, his core body temperature gradually decreased from 37 °C to 35.7 °C. This occurred despite the absence of any discernible physical or neurological abnormalities, normal urine output, and stable serum electrolyte levels. Importantly, the serum lithium level at the time was recorded at 1.2 mEq/L, a concentration within the therapeutic range (Follezou & Bleibel, 1985).

Following the discontinuation of lithium intake, the patient's body temperature steadily increased to above 37 °C within 48 hours, paralleled by a decrease in serum lithium levels to 0.72 mEq/L. The attending physicians noted the absence of signs typically associated with lithium toxicity. However, the authors of the case study, unsurprisingly, interpreted the lithium-induced hypothermic effect as a potential early warning sign of lithium poisoning, a toxic side-effect (Follezou & Bleibel, 1985).

Given that this is based on a single case study, it is far from definitive. The potential influence of the patient's other medication or the preceding surgical operation cannot be discounted. Nevertheless, this case raises the question of whether this thermoregulatory response had been a consistent, yet unnoticed, feature during the patient's three-year lithium treatment regimen, particularly since it was not associated with any physical or neurological abnormalities. This highlights the need for a thorough

investigation into the potential hypothermic effects of lithium in humans, especially as this thesis will argue that the thermoregulatory response to lithium may be integral to its therapeutic action.

### 1.2.8 Temperature

Given lithium's hypothermic effect, a basic understanding of temperature and thermodynamics is critical for this thesis. Once I have established these fundamentals, I will then delve into the intricacies of mammalian thermoregulation.

In everyday language, temperature is often understood as a measure of 'hotness' or 'coldness' and is initially assessed through our sensory experience, such as touching a warm cup of coffee or feeling a cold winter breeze (Atkins, 2010; Zemansky & Dittman, 1997). However, these subjective measures can vary from person to person, and do not provide a consistent or precise measurement.

To accurately quantify temperature, we use a device known as a thermometer. A thermometer measures temperature by exploiting a physical property that changes predictably with temperature (Atkins, 2010). This could be the thermal expansion of a liquid like mercury in a glass, the change in electrical resistance of a semiconductor material as seen in a thermistor, or the change in voltage across a junction of two different metals as observed in a thermocouple. Another property is radiant exitance, which is the amount of electromagnetic radiation emitted from a surface (Zemansky & Dittman, 1997). In this thesis, I will be using thermistors, thermocouples, and an infrared camera to quantify temperature.

Without a scale, a thermometer can indicate if two systems have the same temperature, a state known as thermal equilibrium, if the physical property the thermometer is based on, such as the volume of mercury, changes the same way when placed into contact with each system (Atkins, 2010). To assign numerical values to these observations and create a temperature scale, we use certain fixed points as standards. These standards are reproducible physical phenomena, such as the freezing and boiling points of water, or the triple point of water, where it exists simultaneously in solid, liquid, and gaseous phases (Zemansky & Dittman, 1997). By calibrating the thermometer to these standards, we can translate the physical changes it observes into precise temperature measurements.

Multiple temperature scales are commonly used today, including Celsius, Fahrenheit, and Kelvin. The Celsius scale, named after the Swedish astronomer Anders Celsius, was originally defined with 100 °C as the freezing point of water and 0 °C as the boiling point. However, this was later reversed to align

with our intuitive understanding of temperature, setting 0 °C as the freezing point and 100 °C as the boiling point of water (Atkins, 2010). The Fahrenheit scale, named after German instrument maker Daniel Fahrenheit, was the first to use mercury in a thermometer, setting 0°F at the lowest temperature achievable with a salt, ice, and water mixture, and 100°F at his body temperature, resulting in water freezing at 32°F and boiling at 212°F (Atkins, 2010).

The Kelvin scale, introduced by William Thomson (Lord Kelvin) in 1848, is a temperature scale that begins at absolute zero, 0 Kelvin, the lowest attainable temperature at which all molecular motion ceases (explained below), equivalent to -273.15 °C (Zemansky & Dittman, 1997). The scale was constructed to maintain consistency with the Celsius scale, such that a change of 1 Kelvin corresponds to a change of 1 degree Celsius. The triple point of water, where all three phases coexist, occurs at 0.01 °C or 273.16 Kelvin (Zemansky & Dittman, 1997). In 2019, the Kelvin was redefined in terms of the Boltzmann constant rather than in terms of the triple point of water, a fundamental constant linking average kinetic energy of particles to temperature (Machin, 2018). It is the most commonly used temperature scale in thermodynamics (Atkins, 2010; Zemansky & Dittman, 1997). However, in this thesis, I will be using Celsius.

In the realm of thermodynamics, temperature takes on a more nuanced meaning. It is a macroscopic property that emerges from the random microscopic movements of a vast number of particles within a system (Atkins, 2010; Lagerspetz, 1987; Zemansky & Dittman, 1997). These movements, which can be translational, rotational, or vibrational, constitute kinetic energy, also referred to as thermal energy (Atkins, 2010).

Temperature acts as a statistical parameter that determines the most probable distribution of particles across the available kinetic energy states in a system (Atkins, 2010; Kittel & Kroemer, 1980). As the temperature increases, the distribution broadens, signifying that more particles are likely to occupy higher kinetic energy states. Thus, temperature is proportional to the average kinetic or thermal energy of particles within a system (Zemansky & Dittman, 1997).

For instance, an increase in temperature amplifies the random movements and collisions of freely moving particles in a gas; it incites more vigorous jostling and sliding of closely-packed, yet mobile particles in a liquid; and it heightens the vibrations of particles in a solid, which are closely arranged in a fixed pattern (Atkins, 2010; Zemansky & Dittman, 1997).

In the case of hypothermia induced by lithium in a guinea pig, a complex biological system containing all three phases of matter (solid, liquid, and gas), there is a decrease in the animal's core body temperature. This indicates that the molecular movements, which correspond to the kinetic energy within the guinea pig at the location where the temperature was measured, have slowed down, affecting the overall physiological state of the guinea pig.

Understanding temperature in this context, beyond our sensory perception, enables us to comprehend how it fundamentally alters the behaviour of matter, including biological matter, as demonstrated in the case of the guinea pig. This understanding is crucial, as it will enable us to better appreciate the implications of the thermoregulatory responses that are central to the experiments discussed in this thesis.

### 1.2.9 Heat

Heat, in the eyes of thermodynamics, refers to the transfer of thermal energy between two systems solely due to a temperature difference. Heat typically flows from the system with a higher temperature to the one with a lower temperature. As this heat transfer occurs, the thermal energy of the hotter system decreases while that of the cooler system increases until both systems reach thermal equilibrium, a state where they share the same temperature (Zemansky & Dittman, 1997).

For instance, a cup of hot tea left in a cooler room will transfer heat to the surrounding air. As this happens, the thermal energy of the tea decreases while the thermal energy of the air increases. Eventually, the tea and the air reach the same temperature. Similarly, a rat, which continuously generates thermal energy due to metabolic processes, maintains a body temperature higher than its surrounding environment. This process persists throughout the rat's lifetime, resulting in persistent heat loss to the environment. Upon death, when metabolic processes cease, the rat's body will no longer generate thermal energy and will eventually reach thermal equilibrium with its surroundings.

Throughout this thesis, I will be observing how mammals generate thermal energy and manage heat loss to regulate their internal temperature.

### 1.2.10 Mammalian Thermoregulation

Building upon our understanding of temperature and heat, I now turn my attention to the process of thermoregulation in mammals. Thermoregulation, the regulation of internal temperature within an organism, is a critical homeostatic process (Morrison, 2016).

This process is essential, as temperature, by affecting the behaviour of particles of virtually all matter, can alter the physicochemical processes upon which life depends. Consequently, it influences biological systems at multiple levels, from the molecular to the organismal (Lagerspetz, 1987; Tattersall et al., 2012). At the molecular level, temperature determines the rate and efficiency of biochemical reactions, including those catalysed by enzymes, which often have optimal temperatures for function (Arcus et al., 2016). At the cellular level, temperature affects the function and integrity of cellular structures, including proteins and membranes (Knapp & Huang, 2022). At the tissue and organ level, temperature can influence the rate of metabolic processes and the functioning of organ systems. Finally, at the organismal level, temperature affects overall physiology, behaviour, and ultimately, the survival of the organism (Morrison, 2016).

The internal temperature of most mammals, including guinea pigs, rats, and humans, is regulated within a narrow range above the surrounding environment. For instance, humans typically maintain an average core body temperature of 36.32–37.76 °C (Geneva et al., 2019), guinea pigs at approximately 37.3–39.9 °C (Levy et al., 2020), and rats at 36–38.7 °C (Meinrath & d'Amato, 1979).

These species are classified as homeotherms, a term derived from the Greek for 'same heat'. Homeotherms maintain a stable core body temperature that is largely independent of the ambient temperature of their surrounding environment (Tattersall et al., 2012). This is in contrast to poikilotherms (Greek for 'varied heat'), such as reptiles, whose core body temperature varies considerably, often conforming to or near the ambient temperature (Romanovsky, 2018; Sokolova, 2008).

While homeothermy is a characteristic of most mammals, it is a universal trait among mammals to be classified as endotherms (Greek: 'within heat'). Endothermy, characterised by the ability to generate heat internally, primarily through metabolic processes, allows organisms to maintain an internal body temperature higher than the ambient temperature (Romanovsky, 2018; Tattersall et al., 2012). Conversely, an ectotherm (Greek: 'outside heat'), such as a lizard, relies entirely on external heat sources in the environment to manage their core body temperature (Crompton et al., 1978).

The homeothermic and endothermic attributes of mammals enable them to remain active into the night, where environmental heat sources are fewer, and to inhabit a diverse range of environments with varying temperatures, circumstances that would be restrictive for ectotherms (Crompton et al., 1978; Tan & Knight, 2018).

Extreme deviations from the normal core body temperature can induce detrimental biophysical changes and impede normal physiological function (Tattersall et al., 2012).

When the body temperature exceeds 42 °C, a state referred to as pathological hyperthermia, a large number of cellular proteins within most mammals are highly susceptible to denaturation (Fuller et al., 1998; Kiyatkin, 2019; Romanovsky, 2018; Rzechorzek et al., 2022). This process alters the shape of the proteins, which subsequently leads to a loss of function. If these proteins play critical roles in cellular function, such as enzymes catalysing metabolic reactions, their denaturation can lead to cell death. This cellular demise can escalate, affecting tissues and organs and, ultimately, the entire physiology of the organism, potentially resulting in the organism's death (Walter & Carraretto, 2016).

Conversely, pathological hypothermia, which can occur at temperatures nearing 23 °C (Gregersen et al., 2013), can lead to similarly harmful effects as hyperthermia, albeit through different mechanisms. As the body's temperature drops, metabolic processes, which are inherently temperature-dependent (Clarke & Fraser, 2004), can decelerate to a point where energy production is insufficient to meet the body's needs. This can disrupt cellular processes that require energy substrates, such as adenosine triphosphate (ATP), impairing cellular function (Hochachka, 1986). The escalating disruption, as in hyperthermia, can affect the tissues these cells constitute, potentially culminating in organ failure and the death of the organism.

Hyperthermia is typically more dangerous for mammals as there are fewer degrees between normal body temperature and pathological hyperthermia than between normal body temperature and pathological hypothermia (Romanovsky, 2018).

In fact, some mammals naturally enter states of decreased body temperature and metabolism. These states are known as torpor, which typically lasts around 3–12 hours, and hibernation, which is usually longer (more than a week on average) and tends to involve a deeper drop in body temperature and metabolism (Ruf & Geiser, 2015). This characteristic is known as heterothermy, whereby an organism

displays aspects of both poikilothermy and homeothermy (Ruf & Geiser, 2015). For example, the body temperature of the striped skunk (*Mephitis mephitis*) falls to 26 °C during daily torpor (Hwang et al., 2007). Many torpid and hibernating mammals can withstand much colder extremes than the 23 °C I discussed above (Ruf & Geiser, 2015).

While a temperature of 0 °C would almost certainly result in death for non-torpid or non-hibernating mammals such as humans, since we cannot sufficiently reduce our energy needs and due to the destructive effects of cellular freezing, there are notable exceptions. For instance, the Arctic ground squirrel (*Spermophilus parryii*) can survive with a body temperature as low as -2.9 °C due to its remarkable ability to supercool its body fluids and reduce its energy requirements sufficiently to support sub-zero temperatures (Barnes, 1989).

This demonstrates the adaptability of mammalian thermoregulation. While the mammals used in the experiments of this thesis, namely rats and guinea pigs, are not typically considered torpid or hibernating species, it is an interesting mechanism to keep in mind. This is particularly relevant since I will be observing hypothermic responses to lithium, a scenario that, while not as extreme as hibernation or torpor, still represents a significant deviation from the normal thermal homeostasis of these organisms.

#### *1.2.10.1 Core & Peripheral Body Temperature Variation & Rhythmicity*

Body temperature in mammals is generally divided into core and peripheral temperatures, with the core body temperature being the defended variable in mammalian thermoregulation (Romanovsky, 2018). The core consists of vital organs such as the central nervous system (including the brain and spinal cord), the heart, liver, and other viscera located in the thoracic, abdominal, and pelvic cavities. These organs operate best at certain temperatures, which vary interspecies and even interindividual, but for most mammals it is around 37 °C (Morrison & Nakamura, 2019; Romanovsky, 2018).

In contrast, the peripheral temperature involves the extremities and skin. The temperature of the periphery can vary substantially, ranging from just under the core temperature to just above the ambient temperature, depending on the volume of blood flow the body allows to the extremities and skin (Morrison & Nakamura, 2019; Romanovsky, 2018).

However, it is important to note that the core body temperature is not uniform and can vary depending on the organ and the location of measurement (Geneva et al., 2019). For example, the

temperature of the brain is often higher than body temperatures measured at other core locations (Kiyatkin, 2019). In healthy humans, brain temperature has been shown to range from 36.1 to 40.9 °C, with the mean brain temperature being 38.5 ± 0.4 °C (Rzechorzek et al., 2022). This temperature varies spatially by 2.41 ± 0.46 °C, with the lowest temperatures found closer to the brain's surface and the highest temperatures detected deeper within the brain, such as in the thalamus. Moreover, the average brain temperature fluctuates by approximately 0.57 °C over time, with deeper regions such as the hypothalamus showing greater temporal fluctuations of 1.21 ± 0.65 °C (Rzechorzek et al., 2022).

During inactive periods (nighttime for diurnal humans and daytime for nocturnal rats), both brain and body temperatures reach their lowest points. This 24-hour rhythm is considered circadian (Refinetti, 2020). However, within this 24-hour period, brain and body temperatures also fluctuate in an ultradian periodicity, irregularly occurring every 1 to 2 hours. In rats, this temperature fluctuation, which can reach up to 1 °C (similar to the range observed in humans), is integral to the basic rest-activity cycle (Blessing et al., 2013). During this cycle, increases in body and brain temperatures and other physiological parameters such as heart rate and arterial pressure coincide with periods of activity, reflecting an integrated physiological response (Blessing & Ootsuka, 2016).

While the focus of this thesis is not centred around the ultradian periodicity of body temperature or the basic rest-activity cycle, these fluctuations will naturally be present in my temperature recordings.

#### *1.2.10.2 Physiological & Behavioural Thermoeffectors*

Mammals utilise a combination of physiological and behavioural mechanisms, also known as thermoeffectors, to maintain core body temperature by balancing thermal energy generation and heat loss to the environment (Morrison & Nakamura, 2019). These thermoeffectors are summarised in **Figure 1.1** below.

Understanding these mechanisms will enable us to elucidate the specific thermoregulatory pathways and processes lithium modifies later in the thesis.

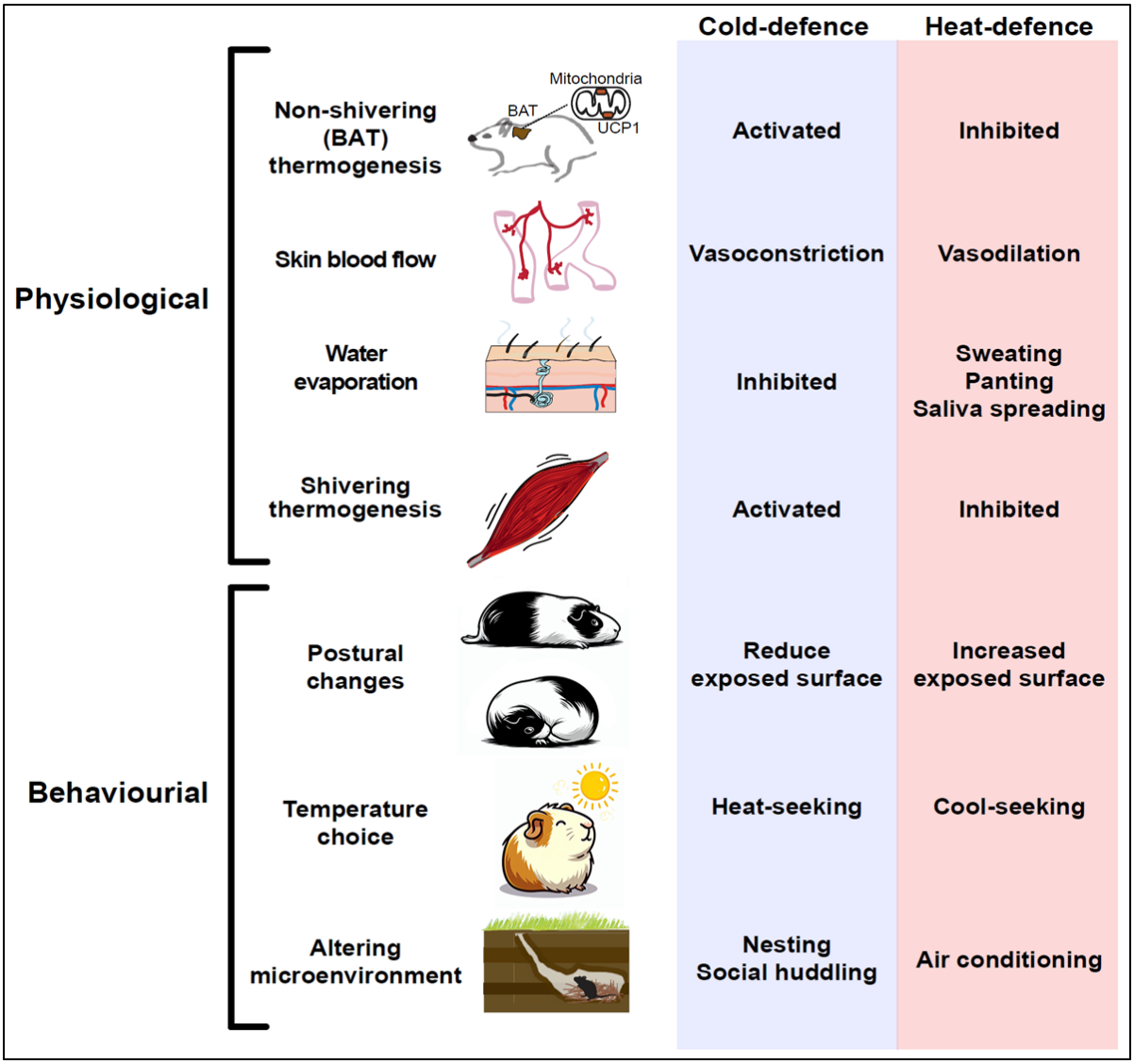


**Figure 1.1.** Examples of physiological and behavioural thermoregulatory mechanisms mammals use to increase body temperature (cold-defence) and decrease body temperature (heat-defence). Adapted from Tan and Knight, with permission from publisher (Tan & Knight, 2018).

#### 1.2.10.2.1 Cold-Defence

In response to cold environments, mammals employ 'cold-defence' mechanisms to increase core body temperature. Physiologically, one such mechanism is shivering thermogenesis, which involves rapid and repeated involuntary contractions of skeletal muscles (Morrison & Nakamura, 2019). These contractions are not coordinated to perform mechanical work, such as movement or load-bearing. Instead, they function to be energetically inefficient, resulting in a large proportion of the chemical energy stored in ATP being converted into thermal energy (Morrison & Nakamura, 2019).

Another physiological mechanism is non-shivering thermogenesis, which primarily occurs in brown adipose tissue (BAT) (Cannon & Nedergaard, 2004), a process referred to as BAT thermogenesis (Ootsuka et al., 2009). In this process, within the mitochondria of BAT cells, uncoupling protein-1 (UCP-1) is activated. This activation results in the conversion of chemical energy derived from fatty acid oxidation into thermal energy, bypassing the usual ATP synthesis pathway that combines ADP and inorganic phosphate into ATP (Chouchani et al., 2019). The generation of thermal energy inside the BAT raises its temperature higher than the surrounding tissues and blood. This temperature difference results in heat transfer from BAT to the surrounding tissues and blood, thereby increasing their temperature. The warmed blood then circulates throughout the body, transferring heat to the rest of the body core and thus raising the overall core body temperature, including the brain (Blessing et al., 2013; Mohammed et al., 2014).

To minimise heat loss, mammals can use cutaneous vasoconstriction, which is the narrowing of skin blood vessels, reducing blood flow to the skin and thus decreasing heat loss from the blood to the environment (Morrison & Nakamura, 2019).

Behaviourally, mammals may resort to strategies such as increasing locomotion, which generates thermal energy due to muscle contractions (Mota-Rojas et al., 2021). They may also seek warmer areas in the environment, like those under direct sunlight, or modify their body posture, such as curling up, to reduce exposed surface area and minimise heat loss to the surroundings (Tan & Knight, 2018).

#### 1.2.10.2.2 Heat-Defence

In response to hot environments, mammals employ ‘heat-defence’ mechanisms to decrease core body temperature. Physiologically, mammals can reduce the generation of thermal energy by inhibiting shivering and non-shivering thermogenesis. They can also increase heat loss through vasodilation, or the widening of skin blood vessels, which increases blood flow to the skin surface, facilitating the transfer of heat from the blood to the environment (Morrison & Nakamura, 2019; Mota-Rojas et al., 2021).

Mammals can also utilise evaporative cooling to increase heat loss. In this process, liquid is introduced to the surface of the skin. When this liquid evaporates, it absorbs heat from the body, thereby reducing the body's temperature. This is a direct application of the principles of thermodynamics, specifically the concept of latent heat, which is the energy required to change a substance to a higher phase of

matter. In this case, the liquid is changed into a gaseous phase, and the energy required for this phase change is absorbed as heat from the skin, thus cooling the skin down (Gurung & Acharya, 2018). For humans, the liquid used in evaporative cooling is sweat, which is produced by sweat glands distributed across the skin. However, mammals such as rats and guinea pigs that lack sweat glands instead spread their saliva across their fur to facilitate evaporative cooling (Morrison & Nakamura, 2019; Tan & Knight, 2018).

Mammals may exhibit behaviours such as decreasing locomotion, which reduces thermal energy generation from muscle contractions (Mota-Rojas et al., 2021), seeking cooler environments like shaded or subterranean spaces, or modifying their body posture, such as splaying out, to increase surface area exposure and enhance heat dissipation (Tan & Knight, 2018).

In my experiments, I will be focusing on the thermoregulatory physiological responses of BAT thermogenesis and skin blood flow, as well as the behavioural responses of postural changes and locomotor activity, specifically in the context of lithium treatment.

#### *1.2.10.3 The Central Command of the Brain*

The brain acts as the central orchestrator in maintaining optimal core body temperature by balancing the physiological and behavioural thermoeffectors discussed above (Nakamura & Morrison, 2011).

The brain primarily regulates physiological thermoeffectors through the sympathetic branch of the autonomic nervous system, or simply the 'sympathetic nervous system'; however, shivering is regulated through the somatic motor system (Nakamura et al., 2022). The level of sympathetic activation from the brain is frequently referred to as 'sympathetic outflow'. The sympathetically-driven physiological thermoeffectors, such as BAT thermogenesis, cutaneous vasoconstriction, and cardiovascular activity, are collectively known as 'sympathetic responses' (Nakamura et al., 2022).

The regulation of core body temperature can be conceptualised as a reflex, with both feedback and feedforward mechanisms playing crucial roles (Morrison & Nakamura, 2019). Feedback mechanisms involve monitoring changes in core body temperature and triggering appropriate thermoeffector responses to restore the temperature to its optimal range. Feedforward mechanisms, on the other hand, primarily respond to changes in both ambient and subcutaneous temperatures, enabling the

body to anticipate and respond to temperature changes in the environment before they significantly impact core body temperature (Morrison & Nakamura, 2019).

The complex network situated deep within the brain, known as the hypothalamomedullary network, functions as the central hub for thermoregulatory control (Nakamura et al., 2022). This network is mainly composed of the preoptic area (POA) and the dorsomedial portion of the hypothalamus (DMH), as well as the rostral medullary raphe (rMR) region located in the medulla oblongata. The POA receives warm and cold temperature-related signals from thermoreceptors in the skin through a distinct pathway via the lateral parabrachial nucleus (LPB) in the pons (Morrison & Nakamura, 2019; Tan & Knight, 2018). This pathway is separate from the spinothalamocortical pathway that transmits temperature sensations that we consciously perceive (Nakamura et al., 2022).

In the POA, cutaneous temperature signals, which signify ambient temperature, are integrated with core body temperature information, primarily monitored by warm-sensitive neurons in the POA. Depending on the predominance of cold or warm signals from the skin, physiological and behavioural cold-defence or heat-defence responses are initiated (Nakamura et al., 2022).

In the case of cold-defence, the DMH is disinhibited, driving the activation of the rMR (Nakamura et al., 2022).

The rMR contains groups of sympathetic premotor neurons, marking the top-down beginning of the sympathetic nervous system, which separately control each physiological thermoeffector (Nakamura et al., 2022). These sympathetic premotor neurons transmit signals to preganglionic neurons primarily located in the intermediolateral cell column (IML) of the thoracolumbar spinal cord (Biaggioni et al., 2022). These neurons synapse onto sympathetic ganglia adjacent to the spinal cord, subsequently activating the thermoeffector organs that drive BAT thermogenesis, cutaneous vasoconstriction, and associated cardiovascular activity (Blessing, 2003; Nakamura & Morrison, 2008).

For heat-defence, the DMH remains inhibited, preventing the activation of the rMR and thus deactivating the physiological thermoeffectors (Nakamura et al., 2022).

The pathways regulating behavioural thermoeffectors are less well-defined but involve the POA and DMH. For a comprehensive overview of this network, please refer to Nakamura and colleagues' definitive review paper (Nakamura et al., 2022).

The complexities and nuances of the hypothalamomedullary network will be discussed in greater detail in **Chapter 4**, where I propose its involvement in the action of lithium.

#### *1.2.10.4 Psychological Stress*

So far, mammalian thermoregulation has mostly been discussed in relation to maintaining a stable core body temperature. However, the brain can use these same thermoregulatory systems to facilitate intentional deviations from the normal body temperature in response to certain types of stress (Nakamura et al., 2022). These deviations are not indicative of a failure in thermoregulation, but rather represent an adaptive response to manage stress.

Stress, in a biological context, is a state of threatened homeostasis or wellbeing caused by certain stimuli known as stressors (Godoy et al., 2018; Schneiderman et al., 2005). Stress/stressors can be categorised into two main types: physical and psychological.

Physical stressors, such as significant temperature variations, directly affect the body's physiological status and its ability to maintain homeostasis (Godoy et al., 2018). The body's cold-defence and heat-defence mechanisms, as I discussed in **Sections 1.2.10.2.1 and 1.2.10.2.2**, are in fact physiological and behavioural responses to the physical stress induced by variations in temperature. Essentially, these mechanisms represent stress responses.

Pathogenic organisms serve as another example of physical stressors. The introduction of a pathogen into the body induces infection stress, triggering a pyrogenic immune response. This response leads to hyperthermia, which we know as 'fever' (Nakamura, 2011). The purpose of infection-induced hyperthermia is to deliberately elevate the body's temperature, thereby creating an environment unsuitable for the survival and function of the invading pathogens (Nakamura, 2011). Simultaneously, this temperature increase is regulated to remain within a range tolerable for the host, aiding in the eradication of the infection.

In contrast, psychological stressors are stimuli that are perceived as threats, eliciting a state of mental strain or tension due to the anticipation of potential endangerment, pain, discomfort, or the failure to satisfy internal drives, without producing actual changes in homeostatic status (Godoy et al., 2018). This mental state is commonly referred to as psychological stress (Nakamura et al., 2022). In humans, such stressors may include uncomfortable social interactions, public speaking, an insurmountable

work deadline, or encountering a grizzly bear. For animals like guinea pigs and rats, stressors may involve sudden changes in their environment, such as a loud noise or the introduction of an unfamiliar animal, or the sight, smell, and sound of a nearby predator (Clark et al., 1997; Wright et al., 2007).

The sympathetic response to psychological stress involves a series of physiological changes, including increased heart rate (tachycardia), elevated blood pressure (hypertension), cutaneous vasoconstriction, and activation of BAT thermogenesis (Nakamura & Morrison, 2022). These changes contribute to 'emotional hyperthermia,' a state of increased body temperature. For a review of the literature on emotional hyperthermia, please refer to the subsection below.

In addition to these physiological responses, psychological stress can also induce behavioural changes. Depending on the type of behaviour elicited, these changes can result in an increase in locomotor activity, such as aggression or avoidance behaviours (Nakamura & Morrison, 2022; Strekalova et al., 2005). This is where the phrase ‘fight or flight’ originates from, coined by American physiologist Walter Bradford Cannon (Cannon, 1915). Conversely, the opposite can occur with behaviours such as attentive freezing and immobilising fright (Bracha et al., 2004).

In **Chapter 3**, I will explore the implications of emotional hyperthermia and its functional role. I will also investigate the impact of lithium on this physiological response and on behavioural changes resulting from psychological stress. To induce psychological stress, I will employ a paradigm known as the resident-intruder test, which is detailed in **Section 1.3.2**.

As you may have noticed, the physiological responses to psychological stress and infection stress are akin to those triggered by cold stress, resulting in hyperthermia. This similarity arises because these stresses all influence the same hypothalamomedullary network, stimulating sympathetic outflow from the rMR to increase core body temperature via sympathetic responses, albeit through unique pathways (Nakamura et al., 2022). In **Chapter 4**, I will delve into the specific pathways activated by cold and psychological stress, and elucidate the role of lithium in modulating these responses.

#### 1.2.10.4.1 Emotional Hyperthermia

Stress-induced hyperthermia, or simply ‘emotional hyperthermia’, is a defining feature of the body’s physiological response to psychological stress in mammals (Britton & Kline, 1939; Hetem et al., 2013; Lkhagvasuren et al., 2011; Meyer et al., 2008), and also occurs in other vertebrates such as birds (Carere & Van Oers, 2004; Gray et al., 2008; Nakamura, 2015; Nord & Folkow, 2019).

Emotional hyperthermia, sometimes termed 'psychogenic fever' in literature (Oka, 2015), is not immune-induced in response to infection like a typical fever, making the term 'fever' potentially misleading. Despite their similar presentations and shared endpoints in their brain pathways, emotional hyperthermia and fever are initiated through distinct neurobiological pathways (Nakamura et al., 2022). This distinction is evidenced by the fact that anti-inflammatory drugs do not mitigate emotional hyperthermia, unlike in fevers, while anxiolytics do have an effect (Vinkers, Groenink, et al., 2009).

Emotional hyperthermia has also been observed in reptiles (Britton & Kline, 1939; Cabanac & Bernieri, 2000; Cabanac & Gosselin, 1993). However, it is not typically seen in amphibians or fish. There is some data suggesting the presence of emotional hyperthermia in zebrafish (Rey et al., 2015), but this is a matter of ongoing debate (Jones et al., 2019; Key et al., 2017; Rey et al., 2017). It is important to note that these ectothermic animals, which are incapable of generating significant internal heat, must seek warmth in their environment to achieve emotional hyperthermia.

Stressors such as restraint, altered social context, hostile encounters, and environmental changes are enough to psychologically trigger emotional hyperthermia (Nord & Folkow, 2019; Oka, 2015). There is a great deal of clinical and experimental evidence that psychological stress causes an acute increase in the body temperature of humans (Hasan & White, 1979; Oka, 2015; Renbourn, 1960) and animals, including rats (Bläsig et al., 1978; Briese, 1970; Briese & Cabanac, 1981, 1991; Gollnick & Ianuzzo, 1968; Kluger et al., 1987; Long et al., 1990; Singer et al., 1986; Stewart & Eikelboom, 1979; York & Regan, 1982), rabbits (Snow & Horita, 1982; Yokoi, 1966), and mice (Cabanac & Briese, 1992).

In humans, emotional hyperthermia, particularly prevalent in women (Oka, 2015; Olivier, 2015), can elevate core body temperatures to low-grade (37–38 °C) and high-grade (up to 41 °C) levels (Oka, 2015). A few studies spanning the 20$^{th}$ century exemplify this phenomenon: Basowitz et al. reported heightened oral temperatures in paratroopers before a jump, compared to controls (Basowitz et al., 1955). Similarly, Renbourn observed increased oral temperatures in adult boxers and young athletes before bouts and contests, respectively (Renbourn, 1960). Furthermore, Briese found that medical students exhibited higher oral temperatures before an examination than before a laboratory demonstration, suggesting that exams act as a stressor, elevating core body temperature (Briese, 1995).

In animal studies, stress-induced hyperthermia is commonly observed during handling of homeotherms like rats, even when handled gently (Briese & Cabanac, 1991; Nord & Folkow, 2019; Stewart & Eikelboom, 1979). Introduction to new environments or group housing can elevate rats' core temperature by up to 2.5 °C (Briese, 1970; Gollnick & Ianuzzo, 1968; Stewart & Eikelboom, 1979; Thompson et al., 2003). York and Regan found that simply moving a rat's cage from storage to the laboratory bench induced emotional hyperthermia (York & Regan, 1982). This response was observed in both naïve and chronically handled rats. The naïve group likely perceived it as a stressful unconditioned stimulus, while the chronically handled group, which exhibited a more severe reaction, likely viewed it as a classically conditioned environmental cue linked to stressful lab bench events (York & Regan, 1982).

In a study by Thompson et al., male rats habituated to a procedure involving intraperitoneal saline injections and rectal temperature measurements over 11 weeks showed no hyperthermia when handled by a familiar person (Thompson et al., 2003). However, they exhibited significant hyperthermia when placed with unfamiliar animals of the same species and sex. This study also found that female rats displayed more emotional hyperthermia and required a longer habituation period than males (Thompson et al., 2003). Furthermore, McGivern et al. found that males had a quicker and more attenuated core body temperature response than females, suggesting greater female sensitivity to emotional hyperthermia or associated stressors (McGivern et al., 2009). These observations intriguingly parallel the heightened sensitivity of women to emotional hyperthermia, indicating a greater prevalence of this response among females in both species (Oka, 2015; Olivier, 2015). Although the experiments in this thesis do not involve females, this phenomenon will be considered and discussed in **Chapter 4**.

Controlling stress-induced hyperthermia in animal experiments, particularly when temperature is a measured variable, presents a significant challenge. It necessitates the habituation of the animal, minimal researcher intervention, and a close approximation of the animal's natural environment to mitigate the physiological impact of artificial laboratory conditions (Gray et al., 2008; Vinkers, de Jong, et al., 2009). These considerations will be incorporated into the conscious experiments discussed in this thesis.

### 1.2.11 Peripheral Targets

My thermoregulatory hypothesis diverges from the prevailing mechanistic hypotheses, which propose that lithium's action begins within the brain (see **Section 1.2.6**). Instead, I suggest that the initiation of lithium's action occurs in the periphery, a perspective underpinned by several key observations.

Firstly, the acute lethargic effect of lithium, as observed by Cade, is reversible and lasts between 1 to 2 hours (Cade, 1949). This duration is shorter than the time it takes for lithium to peak in the brain, given its slow permeation through the blood-brain barrier (Wraae, 1978). The reversibility and duration of this effect suggest that it aligns with a normal physiological process and implies an initial peripheral action of lithium.

Secondly, nausea, a non-painful yet unpleasant sensation of visceral malaise (Andrews, 1992), is often associated with lithium intake and is also correlated with hypothermia (Ngampramuan et al., 2014). This association implies that nausea could be a crucial component of the body's thermoregulatory response to lithium. Notably, lethargy, observed as a reduction in locomotor activity, is intrinsic to the continuum of nausea, further strengthening this association (Graybiel & Knepton, 1976). The absence of nausea when lithium is injected directly into the cerebrospinal fluid of the lateral ventricles in rats further supports the hypothesis of peripheral initiation (Smith, 1980a).

To explore this hypothesis further, my investigation will focus on the vagus nerve and the area postrema (AP) (**Figure 1.2**), two neural structures known to transmit nausea-related signals to the brain (Babic & Browning, 2014). Given their location outside the blood-brain barrier, these structures are hypothesised to be the initial loci for lithium's action in the periphery, triggering a thermoregulatory response that contributes to its therapeutic effects.

A detailed elaboration on this nausea-based concept will commence from **Chapter 2** onwards.

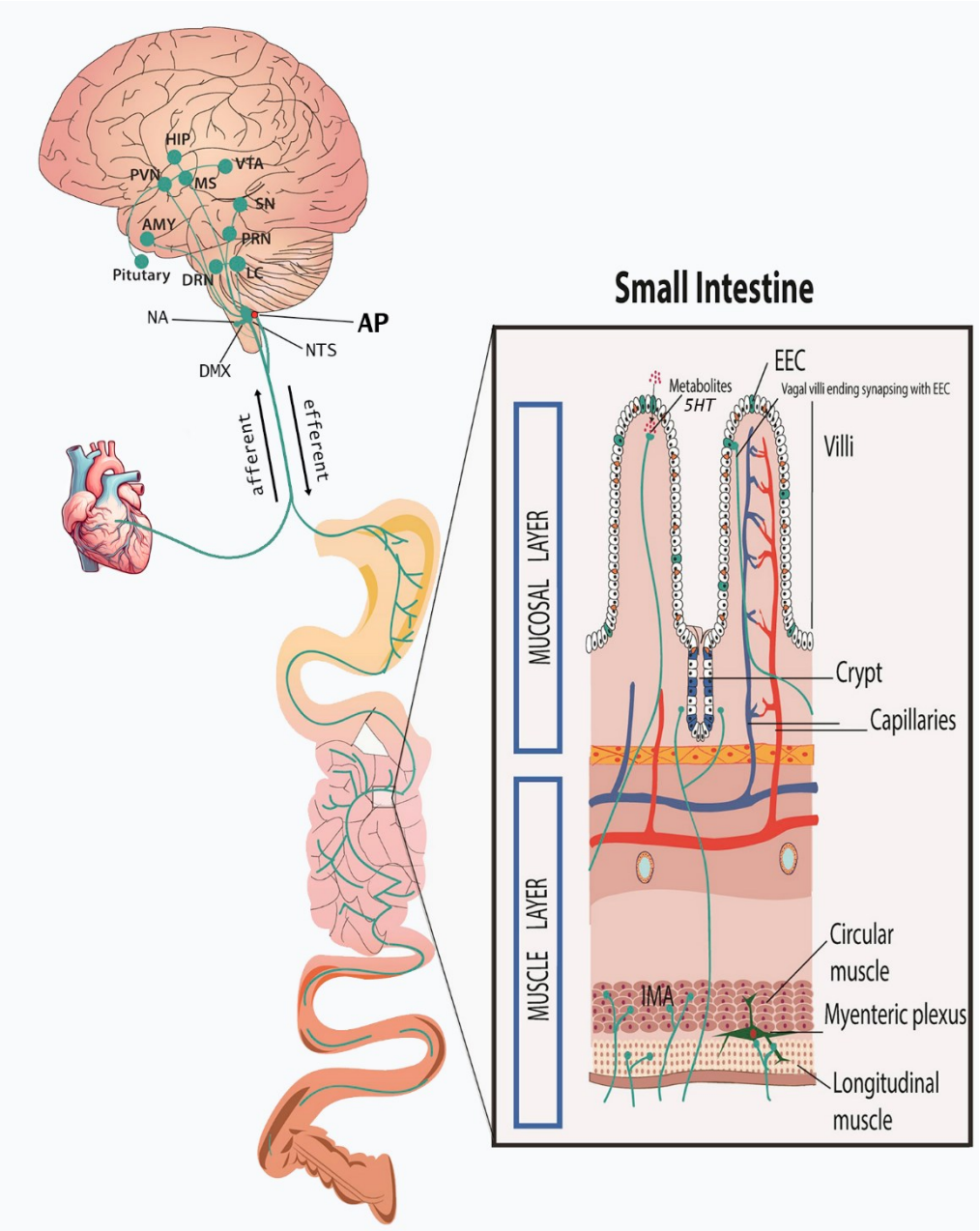


**Figure 1.2.** A schematic representation of the vagus nerve and the area postrema (AP), two peripheral targets of my investigation, in relation to the brain and gastrointestinal tract. **The vagus nerve**, depicted as a green line, originates from the medulla oblongata of the caudal brainstem. The vagus nerve extends into the periphery, innervating numerous organs; however, only the heart and gastrointestinal (GI) tract are shown in the figure. Efferent fibres of the vagus, which carry signals away from the brain, innervate the heart from the nucleus ambiguus (NA) and dorsal motor nucleus of the vagus (DMX). The DMX also provides efferents to the GI tract. **The figure inset** includes a detailed representation of the tissue layers of a portion of the small intestine. Among the epithelial cells of the villi in the mucosal layer are a subtype of enteroendocrine cells called enterochromaffin (EC) cells, highlighted in green. EC cells interact with the contents of the lumen and can respond to different substances, releasing neurohormones such as serotonin (5-HT) or permitting metabolites from gut microbiota to pass through. These neurohormones or metabolites can activate or modulate vagal afferents that terminate near or onto the EC cells. For instance, 5-HT released from EC cells can activate these vagal afferents via 5-$HT_3$ receptors, sending signals through the afferent fibres to the brain primarily via the nucleus tractus solitarius (NTS) and AP. This system, referred to as 'the gut-brain axis', provides a pathway for the brain to receive and process information about the gut's contents. These neurohormones or metabolites can also enter the bloodstream through nearby blood vessels. **The AP**, marked as a red dot, is situated in the floor of the caudal fourth ventricle, superjacent to the NTS, and responds to contents in the peripheral bloodstream. Adapted from Fülling and colleagues, with permission from the publisher (Fülling et al., 2019).

#### *1.2.11.1 The Vagus Nerve*

The vagus nerve, the tenth among twelve cranial nerves, is the longest and most widely distributed nerve in the mammalian body, earning its name from the Latin term for 'wandering' (Ottaviani & Macefield, 2011). It originates from the lower part of the brainstem, the medulla oblongata, descending through and innervating many organs within the head, neck, thorax, and abdomen. The vagus nerve contains both sensory (afferent) fibres, carrying information to the brain, and motor (efferent) fibres, carrying information away from the brain, with the majority being sensory fibres (Breit et al., 2018; Chang et al., 2015).

All vagal afferents have their cell bodies located in the bilateral superior and inferior ganglia, also known as the jugular and nodose ganglia, respectively, just below the skull (Ottaviani & Macefield, 2011). The jugular ganglion projects mostly to the paratrigeminal nucleus of the spinal trigeminal tract in the dorsolateral medulla oblongata (Driessen, 2019). The nodose ganglion projects primarily to the nucleus tractus solitarius (NTS) and area postrema (AP) in the dorsomedial medulla oblongata, with limited projections to the dorsal motor nucleus of the vagus (DMX) (Borgmann et al., 2021; Driessen, 2019; Kim et al., 2020; Wang et al., 2020). The NTS, AP, and DMX are collectively known as the dorsal vagal complex (Bassi et al., 2022). The cell bodies of vagal efferents are found in the DMX in the dorsomedial medulla oblongata (Mussa & Verberne, 2013), and the nucleus ambiguus (NA) in the ventrolateral medulla oblongata (Stuesse & Fish, 1984).

The vagus nerve extends many branches, each serving different functions. However, in the interest of brevity and relevance to this thesis, I will focus on introducing the anatomy related to vagal afferents providing sensory input to the gastrointestinal (GI) tract and their communication with the NTS and AP, as well as the vagal efferents providing parasympathetic innervation to the heart from the DMX and NA (**Figure 1.2**). For a comprehensive review on the intricate anatomy of the vagus nerve in mammals, please refer to the work of Ottaviani and Macefield (Ottaviani & Macefield, 2011).

The roots of the vagus emerge bilaterally from the retro-olivary area posterior to the retro-olivary groove (Duque et al., 2018) in the ventral medulla oblongata, exiting the skull via the jugular foramen, where lie the jugular and nodose ganglia. The vagus continues to descend below the nodose ganglia into the neck region where it becomes the cervical vagus, entering the carotid sheath with the common carotid artery and the internal jugular vein (Ottaviani & Macefield, 2011).

In this cervical region, the vagus gives rise to cardiac branches. The superior cervical cardiac branch originates proximal to the branching site of the recurrent laryngeal nerve. Any branch from the recurrent laryngeal nerve is called an inferior cervical cardiac branch (Zandstra et al., 2021).

The vagus then exits the carotid sheath at the base of the neck and enters the thoracic cavity, becoming the thoracic vagus (Ottaviani & Macefield, 2011). Here, a branch distal to the recurrent laryngeal nerve is called a thoracic cardiac branch (Zandstra et al., 2021).

Throughout its course, preganglionic cardiac parasympathetic fibres originating from the NA and the DMX reach the heart via these cardiac branches (Ottaviani & Macefield, 2011). Unlike preganglionic cardiac sympathetic fibres, which synapse onto postganglionic neurons within the sympathetic trunk adjacent to the spinal cord, these fibres synapse onto postganglionic neurons within ganglionated plexuses in the epicardial fat pads and heart wall (Zandstra et al., 2021).

The primary parasympathetic effect of these cardiac branches of the vagus nerve on the heart is to slow the heart rate (Gordan et al., 2015; Ng et al., 2001). This is achieved by decreasing the firing rate of the sinoatrial node, the pacemaker of the heart, through the action of acetylcholine binding to muscarinic $M_2$ receptors (Wehrwein et al., 2016). In contrast, cardiac sympathetic fibres release norepinephrine onto adrenergic receptors in the heart, which increases the firing rate of the sinoatrial node (Gordan et al., 2015).

As the vagus nerves descend alongside the oesophagus, they transition into the abdominal cavity. This transition occurs through the oesophageal hiatus (Ottaviani & Macefield, 2011), a hole in the diaphragm located at the level of the tenth thoracic vertebra (Oezcelik & DeMeester, 2011). Upon this transition, the positioning of the left and right vagal branches shifts, evolving into the anterior and posterior trunks of the subdiaphragmatic vagus, respectively (Browning & Travagli, 2023).

The anterior trunk subdivides into the anterior gastric, common hepatic, and accessory celiac branches (Browning & Travagli, 2023). The anterior gastric branch primarily innervates the anterior side of the stomach and the proximal part of the duodenum (Berthoud & Neuhuber, 2000). The common hepatic branch extends its reach to the area of the pyloric sphincter of the stomach, the proximal duodenum, and provides some innervation to the liver, pancreas, and gall bladder (Berthoud et al., 1991; Berthoud et al., 1992). The accessory celiac branch densely innervates the intestine, extending from the

duodenum to the splenic flexure, where the transverse colon transitions into the descending colon (Wang & Powley, 2007). Some sparse innervation is also present in the descending colon (Altschuler et al., 1993; Berthoud et al., 1991). Thus, this branch covers the small intestine and the majority of the large intestine.

The posterior trunk contains the posterior gastric and celiac branches (Browning & Travagli, 2023). The posterior gastric branch targets the dorsal aspect of the stomach and the proximal duodenum (Berthoud & Neuhuber, 2000; Berthoud et al., 1991; Tache & Wingate, 1991). The celiac branch, working alongside the accessory celiac branch, extends its innervation to the same regions of the intestines (Altschuler et al., 1993; Berthoud et al., 1991; Wang & Powley, 2007).

Tracing studies have revealed that vagal afferents from various organs in the gastrointestinal tract and thoracic cavity project to the NTS and AP in the dorsal vagal complex of the medulla oblongata. For example, afferents from the stomach fundus, duodenum, cardiac atria fat pads, trachea (Bassi et al., 2022), as well as intestinal afferents from the gastric, coeliac, accessory coeliac, and hepatic branches terminate throughout the NTS and AP (Norgren & Smith, 1988). These terminations are overlapping and do not have clear compartmentalisation or viscerotopic organisation (Bassi et al., 2022). Instead, the central axons of these visceral afferents exhibit substantial divergence, with axons from a single organ branching out to make synaptic connections across multiple subregions of the NTS and AP. This divergent connectivity pattern, leading to an overlap of inputs from diverse organs, suggests the NTS and AP serve as crucial integrative centre for processing a wide range of visceral sensations at the earliest stages of central processing.

The vagus nerve is a crucial component of the 'gut-brain axis', serving a vital role in mediating signals related to nausea and vomiting (Breit et al., 2018). This nerve facilitates bidirectional communication between the gastrointestinal (GI) tract and the brain. Through this axis, vagal afferents convey information about the physiological state of the GI tract to the brain. Based on the information it transmits, the vagus nerve can trigger various responses, which are orchestrated by the brain via brainstem nuclei such as the NTS and AP. For instance, it can induce the sensation of nausea and the act of emesis in response to local irritation in the gut and peritoneum, an absorptive membrane lining the abdominal cavity and viscera, including the gut. Additionally, the nerve can respond to certain chemicals and enterotoxins. It can even instigate nausea-related behaviours such as conditioned taste aversion (CTA) (Andrews & Horn, 2006; Borison, 1989; Zhong et al., 2021).

CTA is a behavioural phenomenon observed in many mammals, including humans. It involves associating a taste, particularly novel ones, with an aversive stimulus like nausea, leading to subsequent avoidance of that taste (Chambers, 2015). This behavioural response is particularly valuable in studies involving animal models that lack the ability to vomit, such as guinea pigs and rats, which are the primary models used in this thesis.

For instance, in rats, when copper sulphate, a mucosal irritant, is introduced to the gut or peritoneum and paired with sweet-tasting sucrose, it induces a CTA towards the sucrose. This response is attenuated following a procedure known as subdiaphragmatic vagotomy, which involves transecting the abdominal vagus, severing vagal communication between the gut and brain (Coil et al., 1978).

In anaesthetised ferrets, chemical, mechanical, and electrical stimulation of the antral mucosa of the stomach leads to emetic responses such as retching, which are abolished after cervical vagotomy (Andrews & Wood, 1988).

Furthermore, electrical stimulation of the vagus nerve itself leads to nausea in humans and vomiting in experimental animals such as ferrets and dogs, solidifying its role in nausea-emetic signalling (Andrews & Horn, 2006; Lewis, 1942).

Of particular relevance to my investigation, lithium has been shown to activate vagal afferents in several ways (Niijima & Yamamoto, 1994). When introduced over the mesenteric surface, lithium stimulates the gastric branches of the vagus nerve. When injected into the portal vein, the primary vessel channelling blood from the gastrointestinal tract to the liver, lithium activates the common hepatic branch. Furthermore, when infused into the duodenum, lithium triggers the celiac branches of the vagus nerve (Niijima & Yamamoto, 1994).

In **Chapter 4**, the role of the cervical and subdiaphragmatic vagus in lithium's thermoregulatory effect will be investigated.

#### *1.2.11.2 The Area Postrema*

The area postrema (AP) is one of seven brain nuclei known as circumventricular organs (CVOs) that lie on the perimeter of the third and fourth ventricles of the brain about the midline (Kiecker, 2018). The AP is located on the dorsal surface of the medulla oblongata. Its name, derived from Latin, aptly

signifies its 'hindmost area' location (De Souza, 2020). The AP is unique from the other CVOs in that it plays a pivotal role in the phenomena of nausea and vomiting, also known as emesis (Leslie, 1986).

The AP is located at the caudal end of the fourth ventricle, where it forms part of the ventricular floor (Longatti et al., 2015). It is positioned just anterior to the obex membrane, in close proximity to the point where the fourth ventricle narrows to form the central canal (Longatti et al., 2015; Milhorat & Miller, 1994). The AP extends rostrally from the obex for approximately 0.8mm in rats and 4mm in humans (Paxinos et al., 2023; Paxinos & Watson, 2007). It overlays the nucleus of the tractus solitarius (NTS), while the gracile tubercle, a slight elevation marking the location of the gracile nucleus, is positioned laterally on either side of the AP (Longatti et al., 2015; Paxinos et al., 2023; Paxinos & Watson, 2007). The funiculus separans, situated anteriolaterally, serves as a diffusion barrier, partitioning the AP from these surrounding structures (Litvin et al., 2020; Price et al., 2008).

Although the AP exhibits gross anatomical differences across mammalian species, such as appearing as a bilateral structure with two convex prominences in humans (Paxinos et al., 2023) and as a midline structure with a single convex prominence in rodents (Paxinos & Watson, 2007), its fine structure or ultrastructure is remarkably similar across all studied mammals (Leslie, 1986).

The AP sets itself apart from other brainstem nuclei due to the absence of a conventional blood-brain barrier, a characteristic shared with most other CVOs (Leslie, 1986). This unique feature is largely attributable to its dense network of fenestrated capillaries and the absence of endothelial tight junctions within them, both of which facilitate the permeation of substances from the bloodstream into the perivascular spaces surrounding the capillaries (Dempsey, 1973; Klara & Brizzee, 1975; Miller & Leslie, 1994). Once these perivascular spaces are filled, substances, depending on their size (Willis et al., 2007), can readily cross into the extracellular space of the AP where they can interact with the parenchyma, including neurons (Torack & Finke, 1971). Furthermore, projections from AP neurons, along with glial cell processes, either contribute to the ensheathment or extend into the perivascular spaces (Dempsey, 1973; Miller & Leslie, 1994), thereby maximising the AP's exposure to circulating factors derived from the blood that might not enter the extracellular space.

This configuration enables the AP to function as an interface between the peripheral circulation and the brain, responding to alterations in blood chemistry and communicating this information to various brain pathways (Price et al., 2008). Hence, within the context of this thesis, the AP is classified as a peripheral target, despite its anatomical location within the brain.

The AP significantly contributes to the induction of vomiting and behavioural indices of nausea, such as loss of appetite and conditioned taste aversion (CTA) in response to specific circulating agents (Borison, 1989; Miller & Leslie, 1994).

In various experimental animals, the AP reacts to a wide range of chemicals introduced into the bloodstream or directly onto its surface, including lithium (Adachi et al., 1991), thereby eliciting nausea-related behaviour and emesis (Zhang et al., 2021). Consequently, the AP is often referred to as the emetic 'chemoreceptor trigger zone' (Borison, 1989). Ablation of the AP has been shown to prevent or attenuate these responses (Borison, 1989; Miller & Leslie, 1994; Ritter et al., 1980). Furthermore, direct electrical stimulation of the AP induces emesis in cats and produces CTA in rats (Gallo et al., 1988; Miller & Leslie, 1994).

In humans, there are clinical cases supporting the AP's role in nausea and vomiting. For example, in cases of severe, intractable vomiting where conventional antiemetic treatments have failed, lesions of the AP have provided relief (Lindstrom & Brizzee, 1962). Moreover, a patient with a haemangioblastoma in the fourth ventricle experienced persistent nausea due to the benign tumour compressing and activating the AP. Following surgical removal of the tumour, the nausea subsided (Abecassis et al., 2013).

In **Chapter 4**, the AP's involvement in lithium's thermoregulatory action will be investigated in detail, and its connections in the brain will be discussed.

#### *1.2.11.3 5-$HT_3$ Receptors*

The vagal afferents, which include endings in the gastrointestinal tract and terminals in brainstem nuclei such as the NTS and AP, along with the AP itself, express 5-$HT_3$ receptors (Browning, 2015; Zhong et al., 2021). The primary ligand for these receptors is serotonin (5-hydroxytryptamine; 5-HT).

The majority of 5-HT in the body, over 90%, is synthesised by enterochromaffin (EC) cells (Bellono et al., 2017). These cells, functioning as chemoceptors, are located in the epithelial lining of the lumen of the small intestine (**Figure 1.2 Inset**). They respond to variations in the chemical composition of luminal contents and to mechanical stimulation (Bellono et al., 2017). In response to these stimuli, EC cells release a variety of neurohormones, including 5-HT (Mawe & Hoffman, 2013; Naylor & Rudd, 1996).

5-$HT_3$ receptors have been identified to partially mediate nausea and emesis in both animals and humans (Costall & Naylor, 2004). For instance, cisplatin, a chemotherapeutic drug known for its potent nausea-inducing properties, triggers the release of 5-HT from EC cells (Minami et al., 1996). This released 5-HT subsequently activates vagal afferents by binding to 5-$HT_3$ receptors located on nearby vagal afferent fibres (Minami et al., 1996), or it may enter the bloodstream and stimulate the AP via 5-$HT_3$ receptors (Mawe & Hoffman, 2013). The use of 5-$HT_3$ antagonists has been shown to reduce the nausea and emesis triggered by cisplatin-induced 5-HT release (Costall & Naylor, 2004).

Given that there is evidence suggesting that lithium not only induces the release of 5-HT from the intestinal mucosa (Iwata & Okamoto, 1973) but also activates both vagal afferents and AP neurons (Adachi et al., 1991; Niijima & Yamamoto, 1994), the 5-$HT_3$ receptor will be a focus of investigation in **Chapters 2 and 3**. Specifically, I will examine whether the blockade of these receptors with 5-$HT_3$ antagonists, namely ondansetron and palonosetron, can inhibit the action of lithium.

## 1.3 Methodological Approach

### 1.3.1 Animal Model

My research methodologies incorporate the use of animal models to investigate physiological and behavioural phenomena. The selected type of animal model plays a significant role in shaping the validity of my research outcomes and their potential for translation to human conditions.

In this thesis, I primarily utilise conscious male guinea pigs and rats, along with anaesthetised rats. Each model offers unique advantages and is selected to meet the specific needs of each experiment. I have chosen to exclude female animals to avoid the complexities introduced by the oestrous cycle, which significantly influences thermoregulation (Matthew et al., 1999). However, I believe that incorporating female animals into subsequent investigations is an important step forward for a more comprehensive understanding of these phenomena.

Guinea pigs and rats, when conscious, serve as effective models for studying naturalistic behavioural responses and physiological processes. Their complex behaviours allow for investigations that yield results applicable to real-world conditions, enhancing the ecological validity of my research (Schmuckler, 2001).

Anaesthetised rats are used in experiments requiring control over variables potentially affected by the animal's conscious state. This approach allows precise manipulation and measurement of physiological parameters, proving especially useful in studies targeting specific physiological processes or responses. For instance, delicate electrophysiological measurements of peripheral nerve discharges, as conducted in **Chapter 4**, are made feasible by this method, which would be impractical in freely moving animals (Fish et al., 2011).

The experiments in **Chapter 4** employ non-recovery procedures using anaesthetised rats, where the animals do not regain consciousness post-anaesthesia. This approach is ethically driven to prevent post-operative pain and complications due to invasive monitoring methods and the anaesthetic combinations required for exceptionally prolonged procedures, as conducted in **Chapter 4** (Fish et al., 2011).

My choice to incorporate conscious guinea pigs in **Chapter 2** draws inspiration from John Cade's seminal lithium experiments conducted on the same animal model (Cade, 1949). Considering the profound impact of his work and the lack of subsequent replications, I deem it crucial to revisit and reproduce his findings. Thus, my research not only acknowledges these foundational experiments but also strives to contribute fresh insights.

The inclusion of rats in this thesis is justified by their prevalent use in both thermoregulatory and lithium-related research (Bernstein et al., 1992; Guimaraes et al., 2015; Nakamura et al., 2022; O'Donnell & Gould, 2007; Tulunay, 1976). This extensive usage provides a substantial comparative foundation, enabling me to contextualise my findings, including those that are novel, within a well-established framework.

Both guinea pigs and rats display thermoregulatory parallels, encompassing physiological and behavioural responses as well as brain control centres (Tan & Knight, 2018). The primary distinction between these rodents and humans is the rodents' use of saliva spreading across their body for evaporative cooling, rather than sweating (Taylor & Gordon, 2019). Furthermore, these rodents share thermal comfort zones that overlap with those of humans. Specifically, guinea pigs have a comfort range of 18 to 24 °C (Fawcett, 2011), rats prefer 19.8 to 24.9 °C (Gordon, 1987), and humans typically find 17 to 26 °C comfortable (Asseng et al., 2021; Cui et al., 2013).

The experiments designed in this thesis take into account the specific ambient temperature preferences of these rodents. This approach allows me to create an environment that promotes optimal physiological functions, upholds animal welfare, and enhances the validity of my experimental data. Given the thermoregulatory similarities, including the shared brain control centres, and overlapping thermal comfort zones between these rodents and humans, my findings hold significant translational potential to human subjects.

Further details on the use of these animal models, including their specific applications and the ethical considerations involved, will be discussed in the subsequent chapters.

### 1.3.2 The Resident-Intruder Paradigm

The resident-intruder paradigm used in **Chapter 3** is a well-established experimental protocol used to study offensive aggression and defensive behaviour in a semi-natural setting (Koolhaas et al., 2013). This paradigm involves the use of territorial male rats, with the 'resident' rat defending its territory against 'intruder' rats. The resident rat's offensive aggression is examined by observing its behavioural displays and initiative towards the intruder. This paradigm also serves as a tool to investigate social stress, which can be induced by recurrently introducing the same animal as an intruder or by placing it within the resident's territory, separated by a wire mesh screen.

In our laboratory, we have developed a novel variant of this protocol (Mohammed et al., 2014). The resident rat is exposed to acute psychological stress by temporarily introducing the 'intruder', who is enclosed in a ventilated box, into its environment. This modification successfully mitigates the risk of physical harm to both the resident and intruder rats, ensuring that the resident's stress response is induced purely by the perceived threat of an invader, rather than actual physical conflict. In other words, the response becomes purely psychogenic, originating in the mind. This method has proven effective in our lab's past work, evidenced by physiological changes that include increases in BAT thermogenesis and locomotor activity.

## 1.4 Aims & Hypotheses

The overarching goal of this thesis is to investigate the physiological and behavioural effects of lithium across different mammalian species and elucidate the neurophysiological mechanisms underlying these effects (see **Section 1.1**). Specific aims and hypotheses are as follows:

### 1.4.1 Chapter 2

#### *1.4.1.1 Aims*

1. Replicate John Cade's observation of lithium-induced lethargy in guinea pigs.
2. Demonstrate the integrated dose-dependent effects of lithium on lethargic behaviour, body temperature, and locomotor activity in guinea pigs.
3. Determine whether the effects of lithium are mediated through peripheral 5-$HT_3$ receptors using the 5-$HT_3$ antagonists ondansetron and palonosetron.

#### *1.4.1.2 Hypotheses*

1. Lithium administration will induce lethargic behaviour in guinea pigs, replicating Cade's initial observation.
2. Lithium will dose-dependently induce an integrated response of lethargic behaviour, reduced body temperature, and decreased locomotor activity in guinea pigs.
3. The peripheral action of ondansetron and palonosetron on 5-$HT_3$ receptors will attenuate the lethargic behaviour, hypothermia, and reduced locomotor activity induced by lithium.

### 1.4.2 Chapter 3

#### *1.4.2.1 Aims*

1. Examine the dose-dependent effects of lithium on body temperature, brown adipose tissue (BAT) thermogenesis, and locomotor activity in rats.
2. Investigate whether repeated exposure to lithium results in habituation or diminished physiological and behavioural responses, compared to initial lithium administration.
3. Explore lithium's impact on physiological (BAT thermogenesis, emotional hyperthermia) and behavioural responses to psychological stress using the resident-intruder paradigm.
4. Determine if 5-$HT_3$ antagonists (ondansetron and palonosetron) can counteract the effects of lithium in rats.
5. Assess the potential role of lithium in inducing tail vasodilation as a mechanism for heat loss.

#### *1.4.2.2 Hypotheses*

1. Lithium will dose-dependently reduce body temperature, inhibit BAT thermogenesis, and decrease locomotor activity in rats.
2. Repeated exposure to lithium will elicit consistent hypothermic and locomotor depressant effects, comparable to the responses observed after initial lithium administration.
3. Lithium will attenuate the physiological responses (increased BAT thermogenesis, emotional hyperthermia) and behavioural changes induced by psychological stress in the resident-intruder paradigm.
4. The 5-$HT_3$ antagonists ondansetron and palonosetron will not counteract lithium's action in rats.
5. Lithium will induce tail vasodilation, facilitating heat loss and contributing to the hypothermic response.

### 1.4.3 Chapter 4

#### *1.4.3.1 Aims*

1. Assess the baseline effects of lithium on the cold-evoked sympathetic response in intact rats, measured by changes in BAT sympathetic nerve discharge, temperature, metabolic activity ($CO_2$ expiration), heart rate, and mean arterial pressure.
2. Determine if the vagus nerve is responsible for detecting lithium in the periphery or mediating aspects of the response to lithium, using subdiaphragmatic and cervical vagotomies.
3. Examine the role of the AP in mediating lithium's effects on the cold-evoked sympathetic response, using AP lesions.

#### *1.4.3.2 Hypotheses*

1. Lithium will dose-dependently reduce the cold-evoked increases in BAT sympathetic nerve discharge, temperature, expired $CO_2$ levels, heart rate, and mean arterial pressure in intact rats.
2. Subdiaphragmatic and cervical vagotomies will attenuate lithium's effects on the cold-evoked sympathetic response parameters.
3. AP lesions will diminish or abolish lithium's effects on the measured physiological parameters.

## 1.5 Organisation of the Thesis

The experimental portion of this thesis begins with **Chapter 2**. Each subsequent chapter builds sequentially upon the previous, culminating in a comprehensive investigation and conclusion in **Chapter 4**.

**Chapter 2** starts the investigation, studying conscious guinea pigs. It replicates Cade's observations of lithium-induced lethargy (Cade, 1949) and further determines lithium's dose-dependent effects on lethargic behaviour, body temperature, and locomotor activity. Additionally, the role of peripheral serotonin receptors in lithium's actions is explored using 5-$HT_3$ antagonists.

**Chapter 3** extends the investigation to conscious rats. It examines whether the dose-dependent effects of lithium on body temperature and locomotor activity, as well as the involvement or lack thereof of 5-$HT_3$ receptors, are consistent between guinea pigs and rats. A significant part of this chapter explores the physiological thermoregulatory mechanisms triggered in response to lithium, namely its dose-dependent impact on BAT thermogenesis, along with lithium's influence on tail vasoconstriction. Additionally, the chapter investigates whether repeated exposure to lithium alters body temperature and behavioural activity. It also assesses lithium's effects on physiological and behavioural responses to psychological stress, measured via emotional hyperthermia, BAT thermogenesis, and hyperlocomotion, within the resident-intruder paradigm.

In **Chapter 4**, the final chapter, conducted on anaesthetised rats, the research elucidates the mechanisms through which lithium alters the sympathetic control of thermoregulatory cold-defence responses to cold stress. This is measured by observing changes in sympathetic nerve activity in the brown adipose tissue (BAT), as well as alterations in metabolic and cardiovascular parameters. It specifically highlights the roles of the vagus nerve and the area postrema in the periphery in mediating lithium's action. The chapter subsequently suggests potential central pathways within the hypothalamomedullary network. This aims to establish a framework for comprehending both the physiological and behavioural impacts of lithium observed throughout the study, and its therapeutic effects.

# Chapter 2.

# Cade's Guinea Pigs Were Hypothermic

## 2.1 Introduction

In 1949, Australian psychiatrist John Cade introduced lithium to modern psychiatric use for the treatment of bipolar disorder (Cade, 1949). Today, it is the 'gold standard' bipolar treatment, but its mechanism of action remains unelucidated. Current theories are centred around complex molecular interactions in the central nervous system, involving targets such as enzymes, neurotransmitters and secondary messengers, each part of incalculable processes (Alda, 2015). Localisation of these molecular interactions in the brain, the associated neural pathways, and how they directly contribute to its therapeutic effects, such as the reduction of manic symptoms, is unclear (Lucini-Paioni et al., 2021; Malhi et al., 2013; Marmol, 2008).

Beyond the molecular interactions, lithium's effects on whole-body physiology and behaviour, such as the lethargy observed by Cade, have been seemingly overlooked in recent literature. In Cade's initial lithium experiment, he observed that guinea pigs intraperitoneally injected with lithium carbonate became lethargic, and this calming effect led him to explore its potential as a treatment for manic patients (Cade, 1949). However, Cade's 1949 publication coincided with reports of fatal lithium poisonings due to the overuse of lithium chloride as a sodium chloride substitute (Corcoran et al., 1949). Consequently, the Food and Drug Administration banned the use of lithium in the same year (Price & Heninger, 1994).

Reports of lithium intoxication due to overdosage pervaded the literature over the next few decades (Hansen & Amdisen, 1978), and research on lithium's therapeutic effect temporarily dwindled (Price & Heninger, 1994). Danish psychiatrist Mogens Schou, who later championed Cade's clinical work (Baastrup & Mogens, 1967; Schou et al., 1954), believed that the observed lethargy in guinea pigs was due to a toxic overdose rather than a specific tranquilising action of lithium (Schou, 1992). As a result, Cade's guinea pig experiment has not been replicated (to the best of my knowledge), and researchers have continued to dismiss lithium's behavioural effects on Cade's guinea pigs and other small mammals as 'toxic' (Price & Heninger, 1994; Schioldann, 2009). However, Cade described a complete reversal of lithium-elicited lethargy, in which the guinea pigs returned to their normal, timid selves

within 1 to 2 hours. This reversal occurs faster than lithium's elimination from serum, cerebrospinal fluid (CSF), and brain (with respective half-lives after maximum: 7, 10, 12 hrs) in rats (Wraae, 1978), suggesting a reversible physiological process coordinated by the brain in response to lithium rather than a toxic one directly attributable to lithium.

After the clinical resurgence of lithium (Baastrup & Mogens, 1967), behavioural studies in rats consistently reported lethargy-like findings, such as reduced locomotor activity and exploratory behaviour, following lithium administration (Hines & Poling, 1983; Johnson & Wormington, 1972; Smith, 1980b). These researchers were aware of the lack of behavioural animal research on lithium despite its clinical use in treating mania. Johnson and Wormington even suggested that this lack of research might have been due to Schou's scepticism that lithium-induced lethargy was related to its therapeutic effect (Johnson & Wormington, 1972). Nonetheless, the behavioural findings of lithium in animals correlate with the psychological and physiological equivalent in humans: when healthy volunteers are given therapeutic levels of lithium, they report lethargy and a loss of interest in interacting with the environment (i.e., reduced exploratory behaviour) (Judd et al., 1977). At slightly higher doses, nausea and vomiting (emesis) are more likely to occur (Schou, 1968; Schou et al., 1968).

Lethargy and a disinclination for physical or mental activity, which are the antithesis of manic thinking and behaviour, have been shown to precede or occur without nausea, depending on the intensity of the eliciting stimulus (Graybiel & Knepton, 1976). Nausea is a remarkably complex continuum of psychological and physiological states (Borison, 1989) and has many different causes (Singh et al., 2016). It is often an antecedent to emesis (Andrews, 1992; Balaban & Yates, 2017).

An underappreciated aspect of nausea is its connection to hypothermia, which can be considered a correlate of nausea in both emetic and non-emetic species (Ngampramuan et al., 2014). In 1874, German physician Hess found that nauseated seasick humans were hypothermic (Hesse, 1874). Hypothermia is also observed in presumably nauseated rats after subjection to rotational motion (Ngampramuan et al., 2014). The association between motion-evoked nausea and hypothermia is well-documented in humans (Nalivaiko et al., 2014; Ogata & Sasaki, 1963), but has yet to be demonstrated in the context of lithium administration. However, in 1976, Turkish scientist Tulunay published the first observation of dose-dependent lithium-induced hypothermia in rats (Tulunay, 1976). A similar hypothermic response was observed in mice five years later (Ogilvie & Lobb, 1981). These observations, along with the lethargy reported by Cade, are only discussed in the context of

toxicity or poisoning (Timmer & Sands, 1999), which may be why lithium's acute thermoregulatory effect has never been tested in humans.

The mechanism of lithium-elicited lethargy, reduction in locomotor activity, and body temperature may involve the detection of lithium in the periphery rather than inside the brain. In non-emetic animals, such as rats and guinea pigs, researchers use behavioural indices to approximate nausea. One notable example is the conditioned taste aversion (CTA) paradigm (Horn, 2014), which is robustly initiated by lithium (Jacobs & Labows, 1979; Nachman, 1963, 1970). The animals associate the nausea elicited by lithium with the taste of recent ingestions and avoid the taste accordingly. Similar behaviour is seen in humans after food poisoning (Garcia et al., 1974). It has been shown in rats that lithium-elicited CTA is produced after intraperitoneal injections of LiCl, but not after intracerebroventricular injections (Smith, 1980a). This suggests that nausea is triggered when lithium is detected in the periphery, outside of the blood-brain barrier, and the information is relayed to and coordinated by the brain. Therefore, since lethargic behaviour and hypothermia are both associated with nausea, it is possible that lithium-elicited lethargy, reduced locomotor activity, and hypothermia result from peripheral detection of lithium, which then influences upstream brain areas responsible for coordinating these responses.

Peripheral serotonin (5-hydroxytryptamine; 5-HT) plays a large role in the signalling of nausea. Most of the 5-HT in both humans and animals is produced and released peripherally by enterochromaffin (EC) cells in the gastrointestinal tract (Mawe & Hoffman, 2013; Naylor & Rudd, 1996). Activation of nearby 5-$HT_3$ receptors on gut vagal afferent terminals is associated with nausea and emesis. This is why 5-$HT_3$ antagonists are used to prevent nausea and vomiting elicited by chemotherapeutic drugs that cause the release of 5-HT from EC cells (Cubeddu, 1996).

In a study by Iwata and Okamoto, rats treated with oral lithium (5 mEq/kg LiCl) showed higher blood levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIIA) and lower 5-HT levels in the duodenum and jejunum of the small intestine, as compared to saline-treated rats (Iwata & Okamoto, 1973). This suggests that lithium stimulates the release of serotonin from intestinal stores (EC cells) and into the blood circulation. I propose that the lithium-induced lethargy, reduced locomotor activity, and hypothermia are mediated by the binding of this 5-HT to vagal afferents, signalling the brain, which then coordinates these physiological and behavioural responses.

Supporting this idea, Guimaraes et al. demonstrated that the 5-$HT_3$ antagonist ondansetron inhibited lithium-elicited hypothermia in rats (Guimaraes et al., 2015). This suggests that the hypothermic and possibly the lethargic effects induced by lithium may indeed begin peripherally, through the action of serotonin on the gut-brain axis.

Although thermoregulatory and behavioural effects of lithium have been reported separately, no study has simultaneously recorded the two parameters, which are fundamentally integrated. In a typical thermoregulatory response, whole-body physiology and behaviour are altered together to achieve a higher or lower body temperature. There is a lack of discussion of this integration in the lithium literature, which has not only treated these integrated parameters as standalone but also labelled them as toxic side effects of lithium. As the lethargy elicited by lithium is antithetical to manic behaviour, the acute thermoregulatory and behavioural effects of lithium, which have not been measured together in the guinea pig, may be an important factor in its therapeutic effect.

In this study, I aim to replicate John Cade's observation of lithium-induced lethargy in guinea pigs, demonstrate the integrated dose-dependent effects of lithium on lethargic behaviour, body temperature and locomotor activity, and determine whether these effects are mediated through 5-$HT_3$ receptors in the periphery using ondansetron and palonosetron. Palonosetron is a second-generation 5-$HT_3$ antagonist with a stronger and longer anti-nausea effect than ondansetron in animals and humans (Eglen et al., 1995; Gralla et al., 2003; Navari, 2015).

In an effort to accurately assess the physiological responses of animals in experimental settings, it is crucial to minimise external stimuli that may confound the results. Particularly in studies involving the measurement of body temperature, traditional methods (e.g., rectal measurement) often necessitate handling the animal (Ogilvie & Lobb, 1981; Tulunay, 1976), an action that can induce stress and emotional hyperthermia (Stewart & Eikelboom, 1979), thereby potentially obscuring the true readings. Just as the mere presence of a researcher entering the room can alter an animal's body temperature, such uncontrollable changes in the environment can significantly impact the validity of the findings (Vinkers, Groenink, et al., 2009; Vinkers, van Oorschot, et al., 2009).

In this study, I employed an innovative method previously developed by our lab (Mohammed et al., 2014) that allows for continuous, non-intrusive monitoring of the animal's temperature throughout the experiment. This method involves the use of an isolated recording chamber, specifically designed to mitigate the effects of environmental changes outside the chamber. Complementing the use of the

isolated chamber, I also utilised a telemetric system to measure core body temperature, which further enhances my ability to accurately capture the natural course of the animal's temperature. By limiting the animal's ability to detect these changes, I can ensure that the data collected are less likely to be affected by extraneous variables and more reflective of the true physiological effects of my experimental manipulations.

I hypothesise that lithium will dose-dependently induce lethargic behaviour, reduce body temperature, decrease locomotor activity, and that both ondansetron and palonosetron's peripheral action will attenuate these effects.

## 2.2 Methods

### 2.2.1 Ethics Approval

With the approval of the Flinders University Animal Welfare Committee, the experiments in this report were conducted at Flinders University (FMC, Bedford Park, SA, Australia) according to the Australian Code for the care and use of animals for scientific purposes (8$^{th}$ edition).

### 2.2.2 Animals

Male tricolour guinea pigs from the Flinders University Animal Facility were utilised in each experiment of this chapter. The guinea pigs had an initial body weight ranging from 385–789 g (n = 6).

Outside of experimentation, all guinea pigs were communally housed within an open-air battery (814 x 610 x 256 mm per cage, minimum 2 animals; GP-SUITE, Tecniplast, Lane Cove West, NSW, Australia), inside the animal facility. The only exception was during the post-surgery recovery period, during which the guinea pigs were singly housed to reduce the risk of physically disturbing the sutures.

Each cage was bedded with sawdust and equipped with cardboard hides for environmental enrichment.

The guinea pigs had unrestricted access to water and food, which included spinach, carrots, capsicum, pellets (Ol' Jacks Rabbit & Guinea Pig Micro Pellets, Lauke Mills, Barossa Valley, SA, Australia), and meadow and lucerne hay. The ambient temperature of the room was maintained at 22.4–22.6 °C, with a relative humidity of 62.1%. A controlled diurnal light cycle was implemented (7am light, 7pm dark) to accommodate their behaviour.

To minimise microbial growth, cage cleaning and bedding replacement were carried out weekly. Food and water supplies were replenished daily.

### 2.2.3 Radiotelemetry Temperature Transmitter Implantation

Guinea pigs were placed under general anaesthesia (isoflurane in 100% oxygen [0.8–1 L/min]: 3–5% for induction, 1–4% for maintenance). Before implantation, animals received one subcutaneous (s.c.) dose of analgesics (4 mg/kg carprofen [Carprieve, Norbrook, Tullamarine, VIC, Australia] + 0.05 mg/kg

buprenorphine [ilium TemVet, Troy Laboratories, Glendenning, NSW, Australia]) and antibiotics (10 mg/kg enrofloxacin [Baytril, Bayer, Pymble, NSW, Australia]). A telemetric temperature transmitter (7.25 g, 3.5 $cm^3$; TA-F40, Data Sciences International [DSI], Transoma Medical, Arden Hills, MN, USA) was then implanted intraperitoneally (i.p.). Once emerged from anaesthesia, the animals were returned to the animal facility for at least one week's recovery period. For the first two days of recovery, animals received one s.c. injection of carprofen (2 mg/kg) daily. To promote healing, the guinea pigs were isolated in separate cages until the end of the recovery period (denoted by a return to pre-surgical body weight) before returning to communal housing.

### 2.2.4 Recording Chamber Setup

Animals were placed inside an acrylic open-top cage (350 x 400 x 450 mm) situated within an isolated climate-controlled chamber (0.32 $m^3$; Biomedical Engineering, Flinders Medical Centre, Bedford Park, SA, Australia). The chamber was maintained at 20–24 °C with 60 air changes per hour and followed a diurnal light cycle (7am light, 7pm dark). As guinea pigs are social creatures, each experimental guinea pig was paired with a companion guinea pig to prevent psychological stress related to social isolation. The open-top cage was divided into two equal sections with a clear, perforated divider, with the experimental guinea pig and the companion guinea pig each occupying one half. The companion guinea pig remained untouched inside the chamber throughout the recording period. This setup allowed the guinea pigs to detect each other without physical contact, ensuring uninterrupted recording of the experimental guinea pig.

### 2.2.5 Core Body Temperature Measurement

Temperature-related AM-band signals from the intraperitoneal (i.p.) TA-F40 implants were relayed by receivers located beneath the acrylic cage to interfacing Matrix 2.0 hardware (MX2, DSI). The processed signals from MX2 were acquired by PowerLab hardware (ADInstruments, Castle Hill, NSW, Australia) and recorded in degrees Celsius (°C) using LabChart software (ADInstruments) and PhysioTel software (DSI) at a sampling rate of 10 Hz.

### 2.2.6 Lethargic Behaviour and Locomotor Activity Measurements

Guinea pigs were recorded inside the recording chamber using a generic video camera module. Video recording was started approximately 1 hour before injecting lithium or control. Footage was obtained

with LabChart software (resolution: 640 x 480, 20 frames per second, bitrate: 1.01 Mbps), H.264-encoded, and stored for post hoc video analysis.

Lethargic behaviour was measured by manually reviewing each second of footage from the time of lithium/control treatment until 5 hours post-injection. Lethargic behaviour was defined as a 'cooling posture', where the guinea pig lay flat on its belly or side with at least one leg splayed outward (example shown in **Figure 2.2 Inset**). Lethargic behaviour was presented as the number of seconds per minute spent in cooling posture or total minutes spent in cooling posture.

Locomotor activity was determined by measuring the total distance moved by the guinea pigs using EthoVision XT software (Noldus, Wageningen, Netherlands). The software automatically tracked the guinea pigs' centre of mass and calculated the total distance moved in 10-minute intervals for approximately 1 hour before injection and 4 hours after injection. Manual frame-by-frame adjustments were made to correct errors in automatic centre of mass detection. Traces were also generated for each 10-minute interval (example shown in **Figure 2.8**). Total distance moved was presented cumulatively relative to the injection timepoint.

### 2.2.7 Lithium Dose-Response Protocol

After habituating inside the recording chamber for at least 24 hours to stabilise body temperature and behaviour, guinea pigs (not the accompanying guinea pigs) were injected i.p. (left lower quadrant) with either the control (4 mEq/kg NaCl [Sigma-Aldrich, St. Louis, MO, USA] in 0.5 mL $H_2O$ [Fresenius Kabi, North Ryde BC, NSW, Australia]) or a dose of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl [Sigma-Aldrich]; 1 mEq = 42.394 mg LiCl). Each animal behaved freely and was recorded for up to 24 hours post-injection before returning to the animal facility with their accompanying guinea pig. A 3-day washout period was ensured before the next dose. The first dose administered to each successive animal was given in rotating order to account for serial effects.

### 2.2.8 5-$HT_3$ Antagonist Protocol

Following a 24-hour habituation in the recording chamber, experimental guinea pigs received i.p. (left lower quadrant) injections of the control (0.9% saline, 0.5 mL [Fresenius Kabi]) or a 5-$HT_3$ antagonist (2 mg/kg ondansetron [Baxter, Old Toongabbie, NSW, Australia] or 0.1 mg/kg palonosetron [Aloxi, Helsinn Healthcare SA, Pazzallo, Switzerland], 0.5 mL). 30 minutes later, 2 mEq/kg LiCl in 0.5 mL $H_2O$

was injected i.p. in the same position. Animals were returned to the animal holding facility after 24 hours of recording for a washout period of 3 days before the next treatment.

### 2.2.9 Data Analysis

Body temperature, lethargic behaviour, and locomotor activity were processed in Igor Pro (WaveMetrics, Portland, OR, USA). When analysing each body temperature recording, notch noise was accounted for by interpolating the two datapoints surrounding the noise; this prevented any gaps in the temperature data caused by notch noise. Body temperature recordings were smoothed by downsampling from 10 Hz to 1 per minute. Delta body temperature values were calculated by subtracting the mean of 5–10 minutes before injection from each data point. In the 5-$HT_3$ experiment, locomotor activity between the pre-treatment and lithium injections was not measured due to visual obstruction during handling. All averaged data were presented with SEM.

### 2.2.10 Statistical Analysis

All statistical analyses were performed using Prism (GraphPad, San Diego, CA, USA). For body temperature, the minimum body temperature between 60 and 120 mins post-lithium injection was taken; for lethargic behaviour, the total time spent in cooling posture was taken over the first 60 minutes post-lithium; for locomotor activity, the cumulative distance moved at 240 mins post-lithium was used.

For the lithium dose-response data, one sample t tests were conducted to compare the minimum body temperature achieved after each dose and the control to a hypothetical mean of 0. Linear regression analyses were performed to assess the relationship between each parameter (minimum body temperature, total minutes spent in cooling posture, and cumulative distance moved) and log-transformed lithium dose. When comparing 4 mEq/kg LiCl and 4 mEq/kg NaCl, paired t tests were used. Additionally, multiple linear regressions were run between log-transformed dose and cumulative distance moved at a single point in time every 10 minutes after lithium injection.

For the 5-$HT_3$ antagonist-lithium data, the control, ondansetron and palonosetron pre-treatment groups were compared using ordinary one-way ANOVA and Tukey's test for multiple comparisons instead of linear regression. A repeated measures mixed-effects model, fitted using restricted maximum likelihood (REML), was used to assess the effect of treatment and its interaction with time on cumulative distance moved, with time as the repeated measure.

## 2.3 Results

### 2.3.1 Lithium Dose-Response

After each dose of LiCl except 0.1 mEq/kg, lethargic behaviour (referred to as 'cooling posture'; see **Figure 2.2 Inset**) was observed within a few minutes (**Figure 2.1**). At higher doses of lithium (2–6 mEq/kg LiCl), most of the time spent in cooling posture occurred in the first 1–2 hours post-injection. The same can be said for 1 mEq/kg LiCl, but at a smaller magnitude. In the hypotonic LiCl (0.1 mEq/kg) group, time spent in cooling posture was sporadic and did not resemble the same pattern as the higher doses. Lethargic behaviour in the hypertonic saline (4 mEq/kg NaCl) group shared both a similar pattern to the higher doses of LiCl and the sporadicity of 0.1 mEq/kg LiCl (**Figure 2.1**).

LiCl dose-dependently increased the amount of time the guinea pigs spent in a lethargic state (cooling posture) within the first 60 minutes after injection (log-linear regression, $R^2 = 0.470$, $P < 0.0001$, **Figure 2.2**). In the first 60 minutes post-injection, guinea pigs spent fewer minutes in cooling posture after hypertonic NaCl when compared to LiCl at equal milliequivalents (18.67 ± 7.15 vs 42.19 ± 5.301 mins, paired t test, $P < 0.05$).

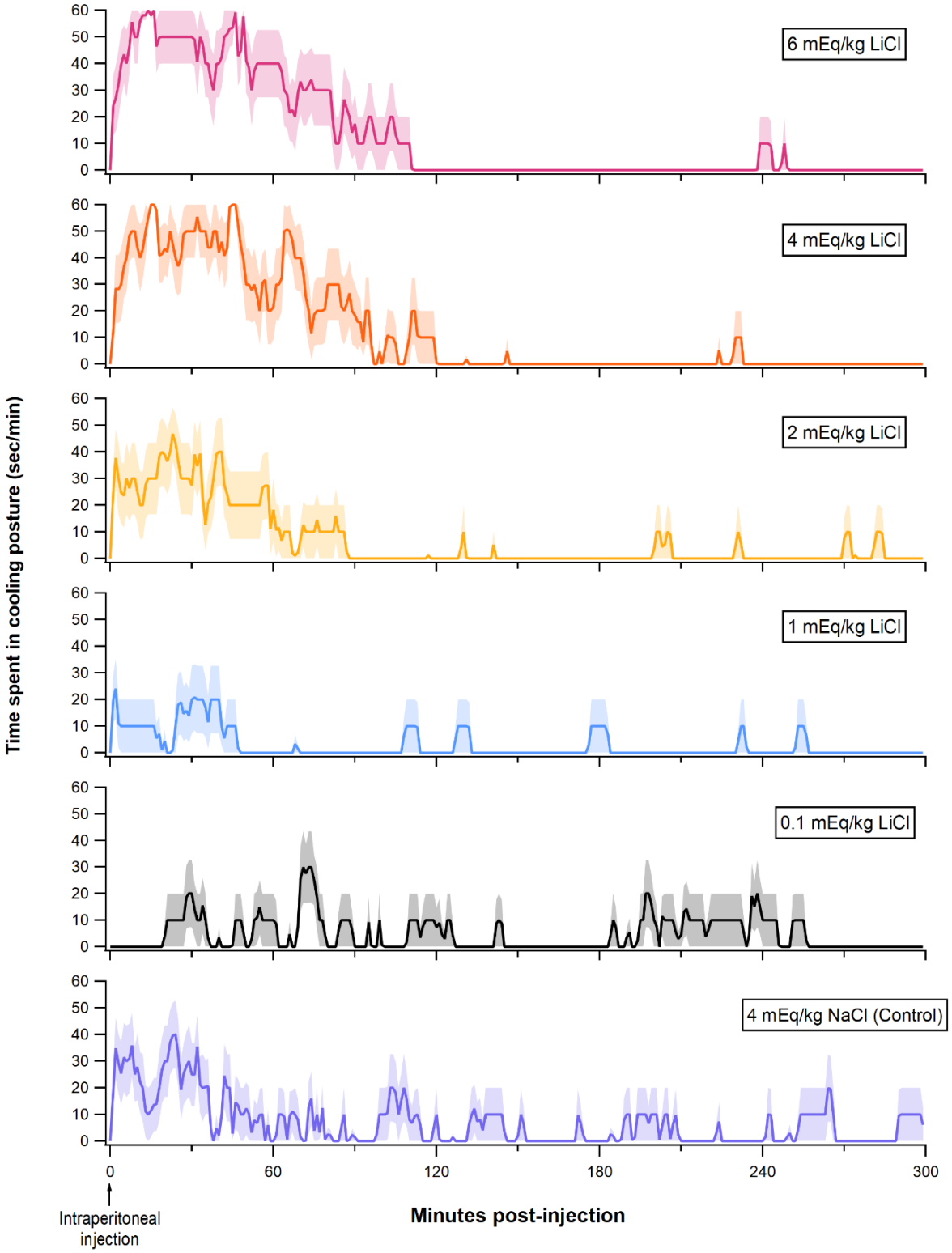


**Figure 2.1.** Seconds spent in cooling posture every minute after intraperitoneal doses of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl) or control (4 mEq/kg NaCl) in guinea pigs. Lines and shadings represent mean ± SEM, n = 6.

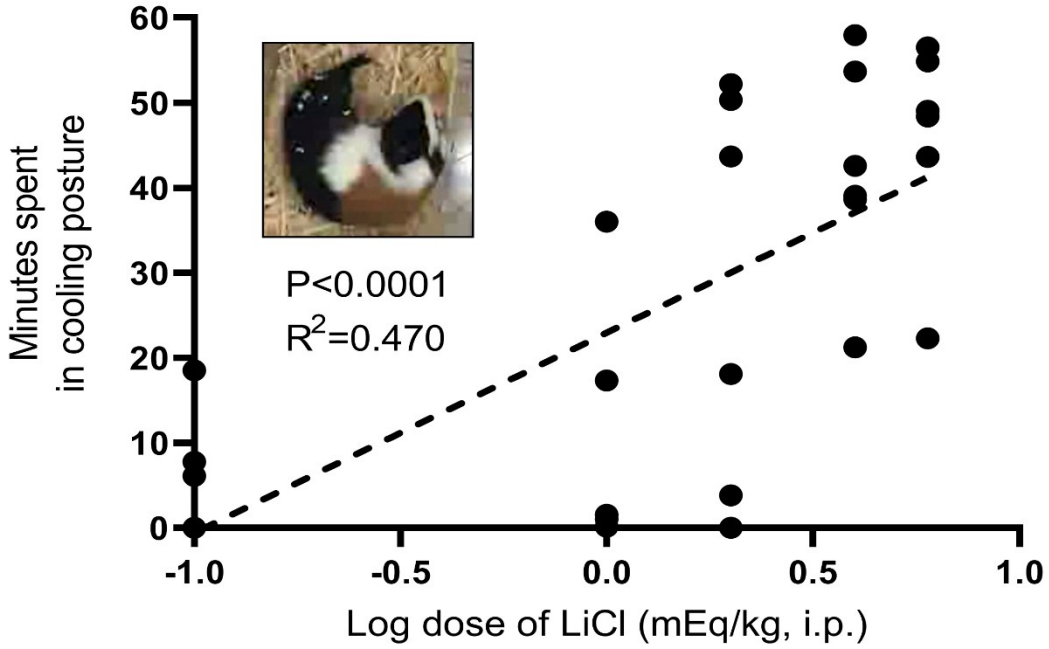


**Figure 2.2.** Linear regression: total number of minutes spent in cooling posture 0–60 minutes post-injection against log-transformed intraperitoneal doses of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl). Datapoints represent individual animals, n = 6 per dose. **Inset:** an example of cooling posture in one guinea pig that is lying still with at least one limb splayed out.

Increasing intraperitoneal doses of lithium chloride led to larger decreases in body temperature over time (**Figure 2.3**). Mean minimum change in body temperature 60–120 minutes after injection for each LiCl dose and the hypertonic saline control are shown in **Table 2.1**. As the dose of lithium increased, the time to minimum body temperature also increased (**Figure 2.3**). All LiCl doses except 0.1 mEq/kg led to a noticeable transient hypothermia; hypertonic NaCl also produced hypothermia, but had a weaker hypothermic effect compared to LiCl at the same number of milliequivalents (paired t test, P = 0.0053).

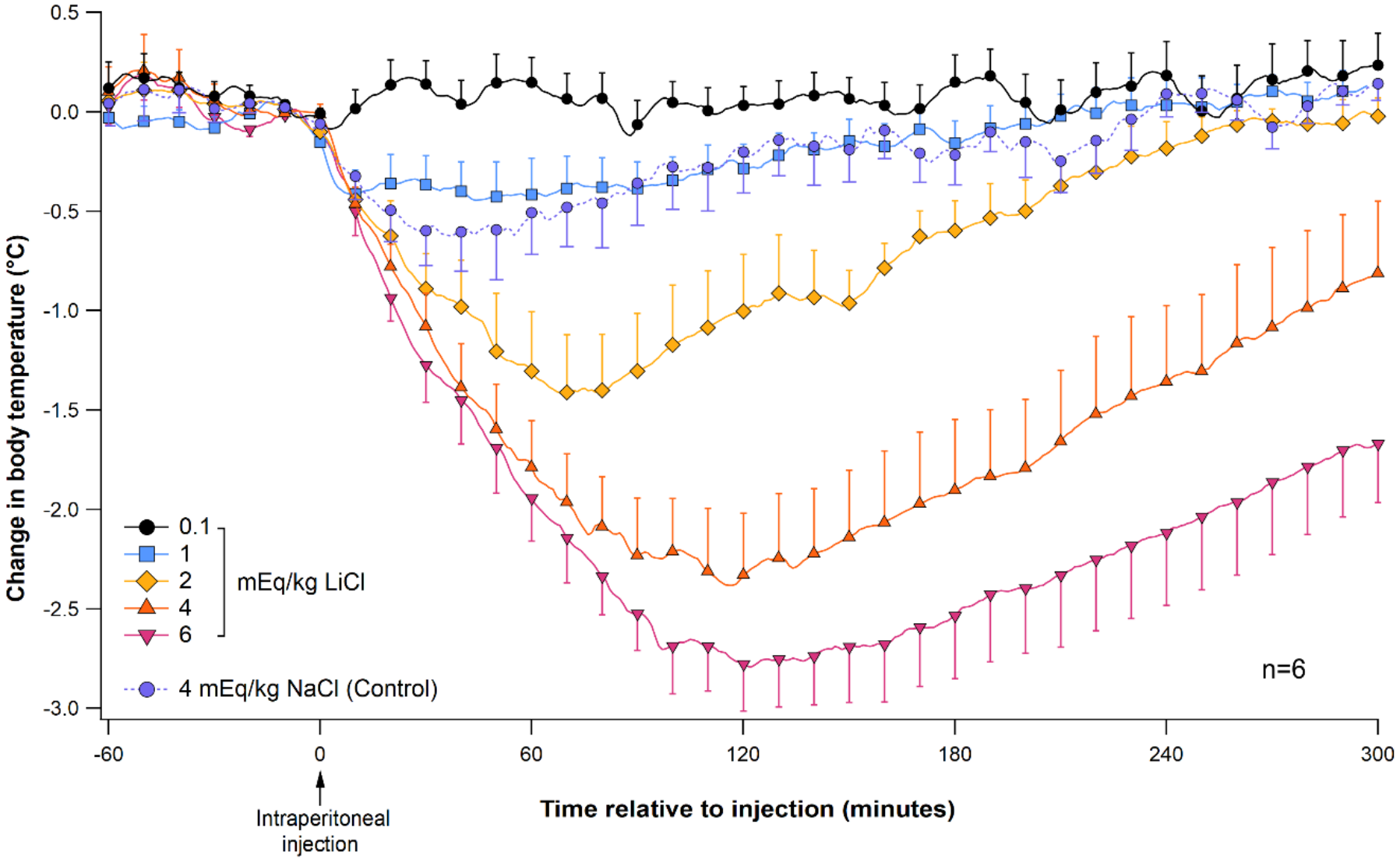


**Figure 2.3.** Change in guinea pig core body temperature over time in response to intraperitoneal doses of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl) or control (4 mEq/kg NaCl) at 0 minutes. 0.1 mEq/kg LiCl also acts as an osmolar control. Datapoints represent mean ± SEM, n = 6.

**Table 2.1.** Minimum delta body temperature value between 60–120 minutes post-injection for each dose of lithium chloride or control (hypertonic sodium chloride). Data represented as mean ± SEM, n = 6. One sample t test results (compared to 0) are included. **Bold P values** indicate statistical significance.

| LiCl dose | Min body temperature (Δ °C) | One sample t test (compared to 0) |
|---|---|---|
| 0.1 mEq/kg | -0.18 ± 0.12 | P = 0.1769 |
| 1 mEq/kg | -0.54 ± 0.17 | **P = 0.0244** |
| 2 mEq/kg | -1.50 ± 0.28 | **P = 0.0033** |
| 4 mEq/kg | -2.50 ± 0.31 | **P = 0.0004** |
| 6 mEq/kg | -2.87 ± 0.27 | **P = 0.0001** |
| Control (4 mEq/kg NaCl) | -0.61 ± 0.21 | **P = 0.0346** |

Linear regression between minimum body temperature and log-transformed dose of LiCl showed a strong negative correlation ($R^2$ = 0.703, P < 0.0001, **Figure 2.4**).

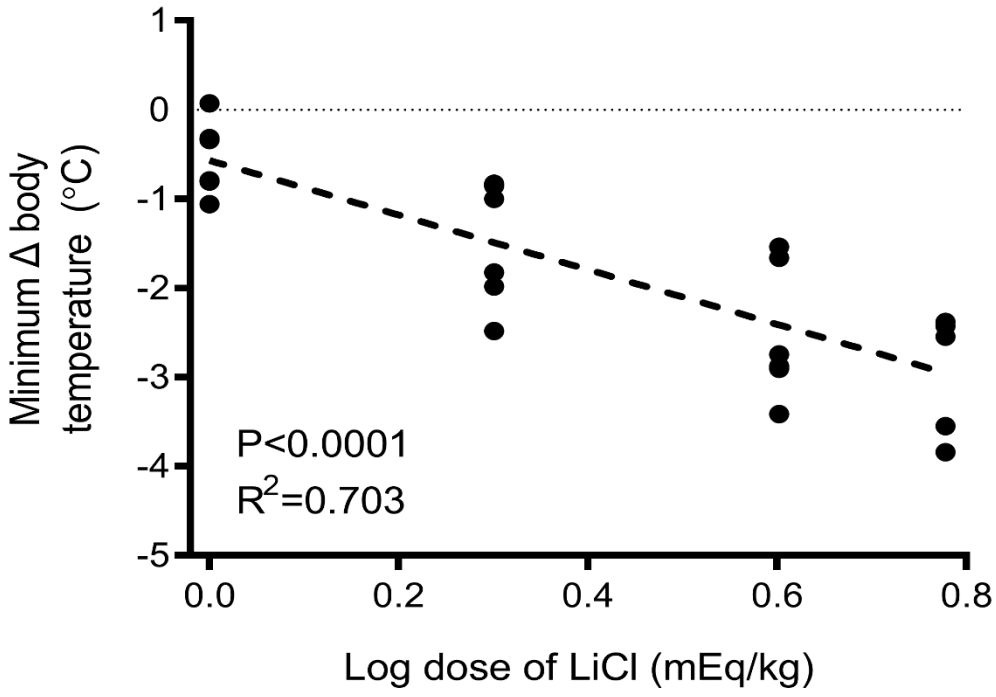


**Figure 2.4.** Linear regression: Minimum values of delta core body temperature between 60–120 minutes post-injection against log-transformed lithium doses (1, 2, 4, 6 mEq/kg LiCl). 0.1 mEq/kg LiCl was excluded as it was not significantly different from 0 (one-sample t-test, shown in **Table 1**). Datapoints represent individual animals, n = 6 per dose.

Minimum body temperature and minutes spent in cooling posture were negatively correlated (linear regression, $R^2 = 0.760$, $P < 0.0001$, **Figure 2.5**): more time spent in cooling posture during the first 60 minutes after injection led to larger decreases in body temperature 60–120 minutes after injection (i.e., stronger hypothermia occurred).

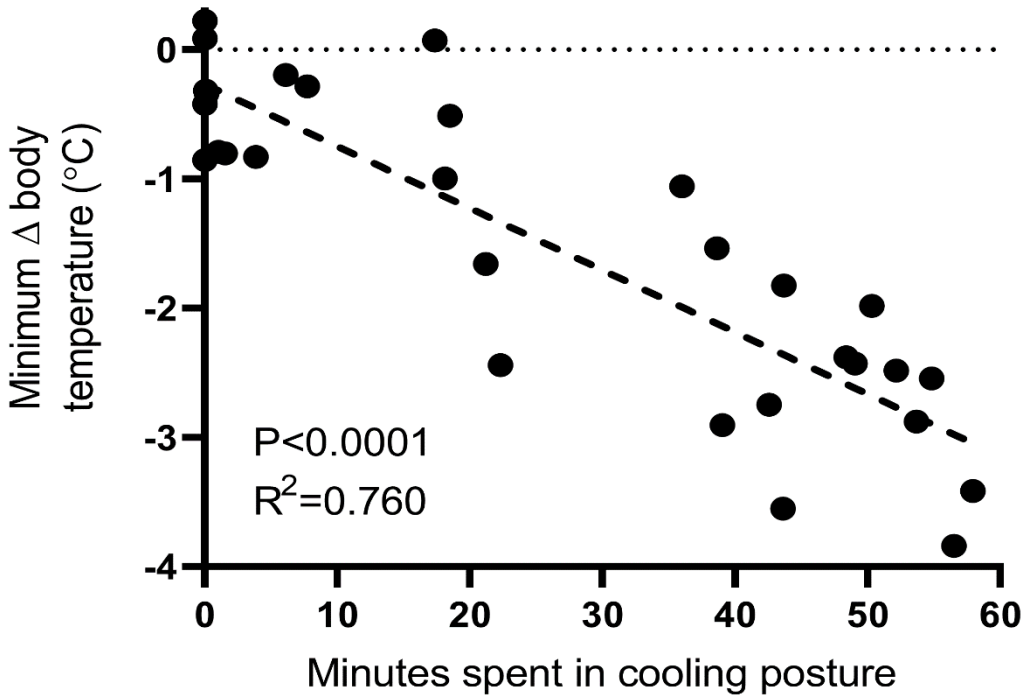


**Figure 2.5.** Linear regression: minimum change in body temperature between 60–120 minutes post-injection against total number of minutes spent in cooling posture 0–60 minutes post-injection. Datapoints represent individual animals that received an intraperitoneal dose of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl), n = 6 per dose.

Locomotor activity of guinea pigs decreased over time as LiCl dose increased (**Figure 2.6**). Multiple linear regressions between cumulative distance moved and log-transformed LiCl dose were run every 10 minutes after injection. From 50 minutes post-injection, the linear regression fits were significantly non-zero (briefly highlighted in **Figure 2.6**); the effect sizes were low but expected due to the highly variable nature of behaviour.

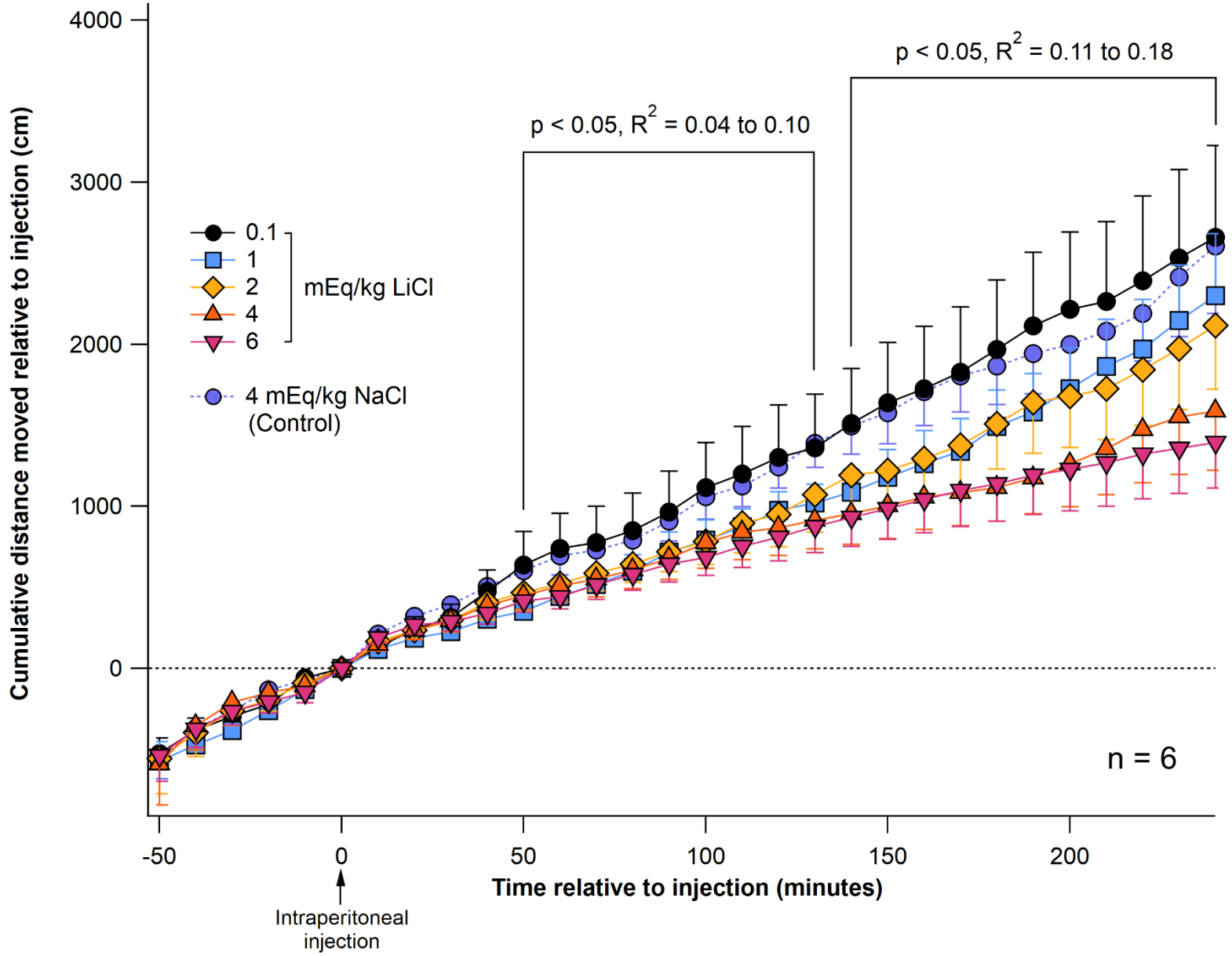


**Figure 2.6.** Cumulative distance moved by guinea pigs over time from intraperitoneal injection point of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl) or control (4 mEq/kg NaCl) at 0 minutes. 0.1 mEq/kg LiCl also acts as an osmolar control. Brackets above 50–130 & 140–240 minutes represent multiple significant linear regressions of cumulative distance moved at each timepoint against log-transformed lithium doses. Datapoints represent mean ± SEM, n = 6.

**Figure 2.7** illustrates lithium's dose-dependent reduction of cumulative distance moved at 240 minutes post-injection (log-linear regression, $R^2$ = 0.178, P = 0.0104). The hypertonic 4 mEq/kg NaCl control group had moved more at 240 minutes since injection than the 4 mEq/kg LiCl group (2605 ± 415.1 vs 1588 ± 364.8 cm, P < 0.05). An example of total distance moved over 10 minutes by one animal 230–240 minutes after each dose of lithium and the control is visualised in **Figure 2.8**.

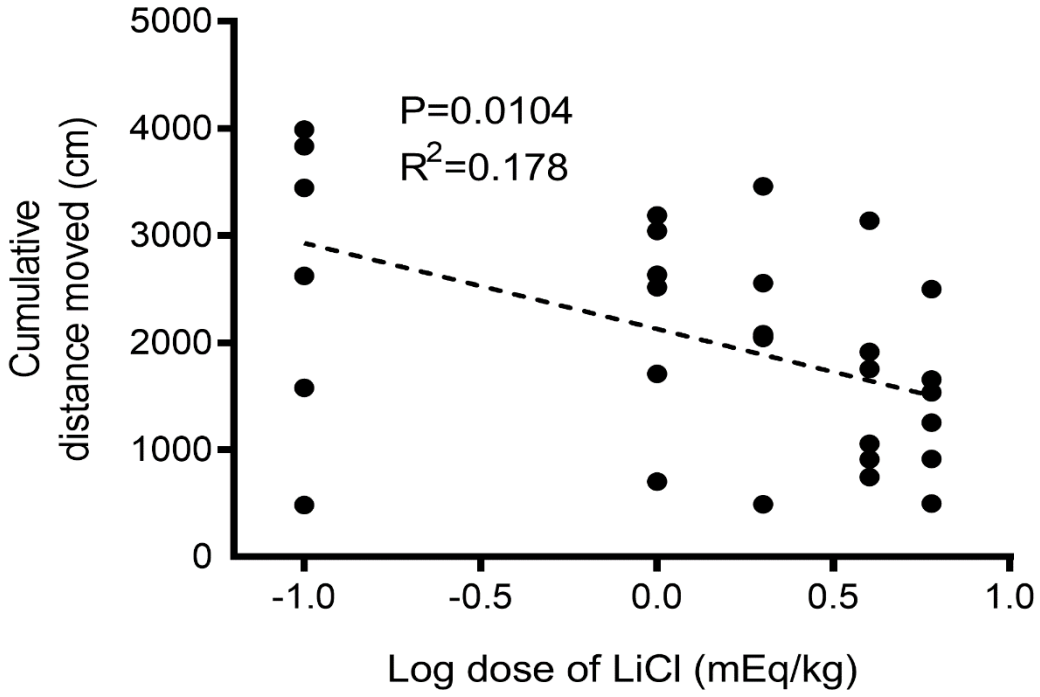


**Figure 2.7.** Cumulative distances moved at 240 minutes post-injection against log-transformed lithium doses (0.1, 1, 2, 4, 6 mEq/kg LiCl). Datapoints represent individual animals, n = 6 per dose.

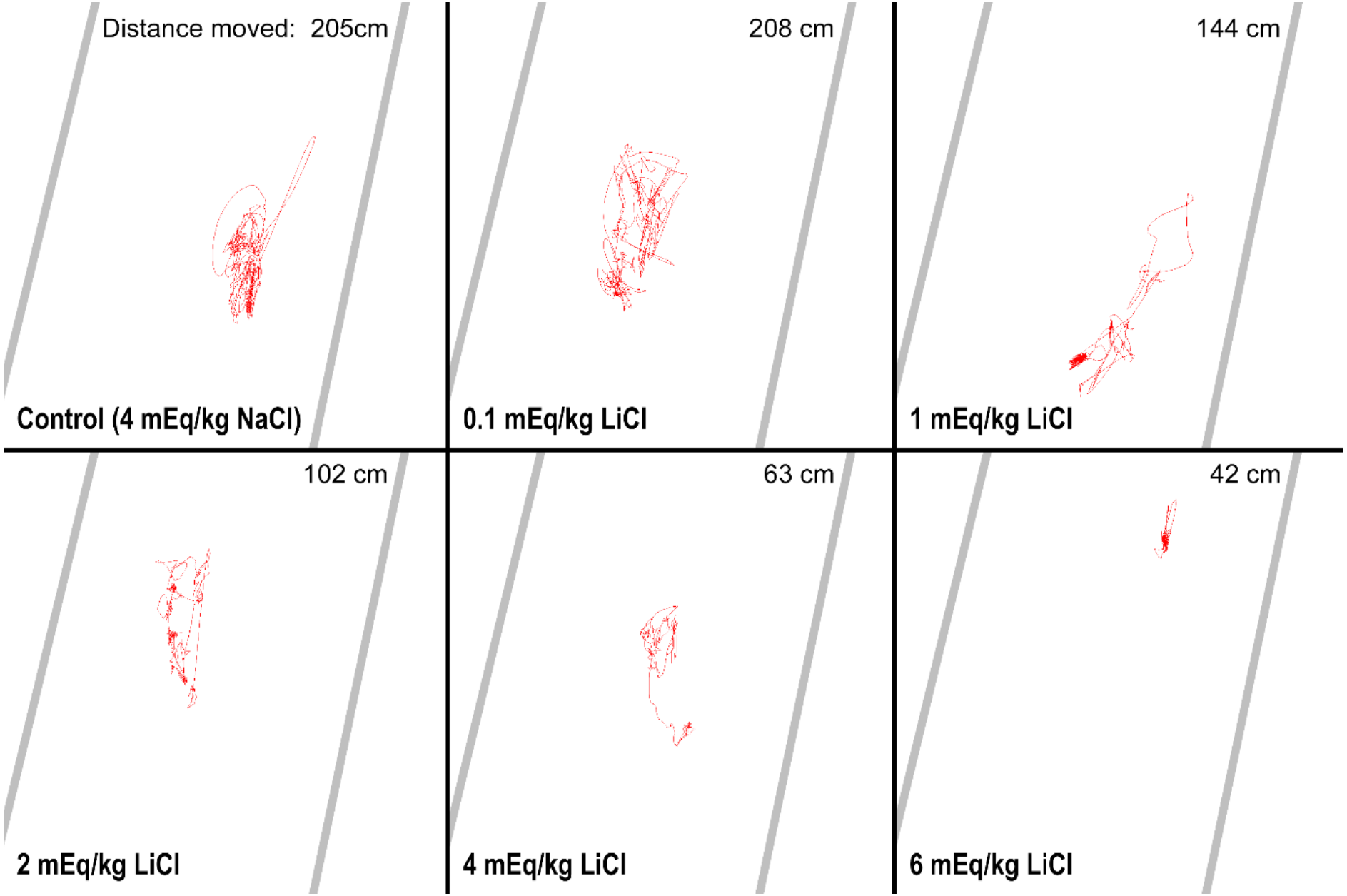


**Figure 2.8.** 10-minute trace of movement by one guinea pig 230–240 minutes after injection of lithium (0.1, 1, 2, 4, 6 mEq/kg LiCl) or control (4 mEq/kg NaCl). Grey lines represent border of chamber.

Upon the administration of intraperitoneal injections, guinea pigs exhibited a series of transient behavioural responses that were not initially anticipated or included in the quantification of behaviour. The initial reaction was intense but short-lived, characterised by acute, spasm-like movements and excited running around that lasted only a few seconds. This acute spasmic reaction then transitioned into intermittent movements suggestive of mild discomfort, akin to the occasional twitching or adjusting of a shoulder. These movements of discomfort, although sporadic, persisted for a duration ranging from approximately 30 seconds to 1 minute.

These responses were observed in animals administered with a dosage of 1 mEq/kg LiCl, with the number of animals exhibiting these behaviours escalating as the dose increased. Similar reactions were also noted in animals following the administration of hypertonic saline (4 mEq/kg NaCl). Despite the initial intensity, the reactions were brief and sporadic, and the subsequent discomfort movements were neither continuous nor long-lasting.

### 2.3.2 Lithium with 5-$HT_3$ Antagonist Pre-Treatment.

Even with 5-$HT_3$ antagonist pre-treatment, the guinea pigs displayed lethargic behaviour within minutes after lithium; the majority of time spent in cooling posture occurred within the first two hours post-lithium (**Figure 2.9**). Between the three groups, there was no significant difference in the number of minutes spent in cooling posture within the first 60 minutes post-lithium (ondansetron: 29.73 ± 5.324, palonosetron: 33.59 ± 5.967, control: 21.28 ± 8.939 minutes, one-way ANOVA, P = 0.4568, **Figure 2.10**).

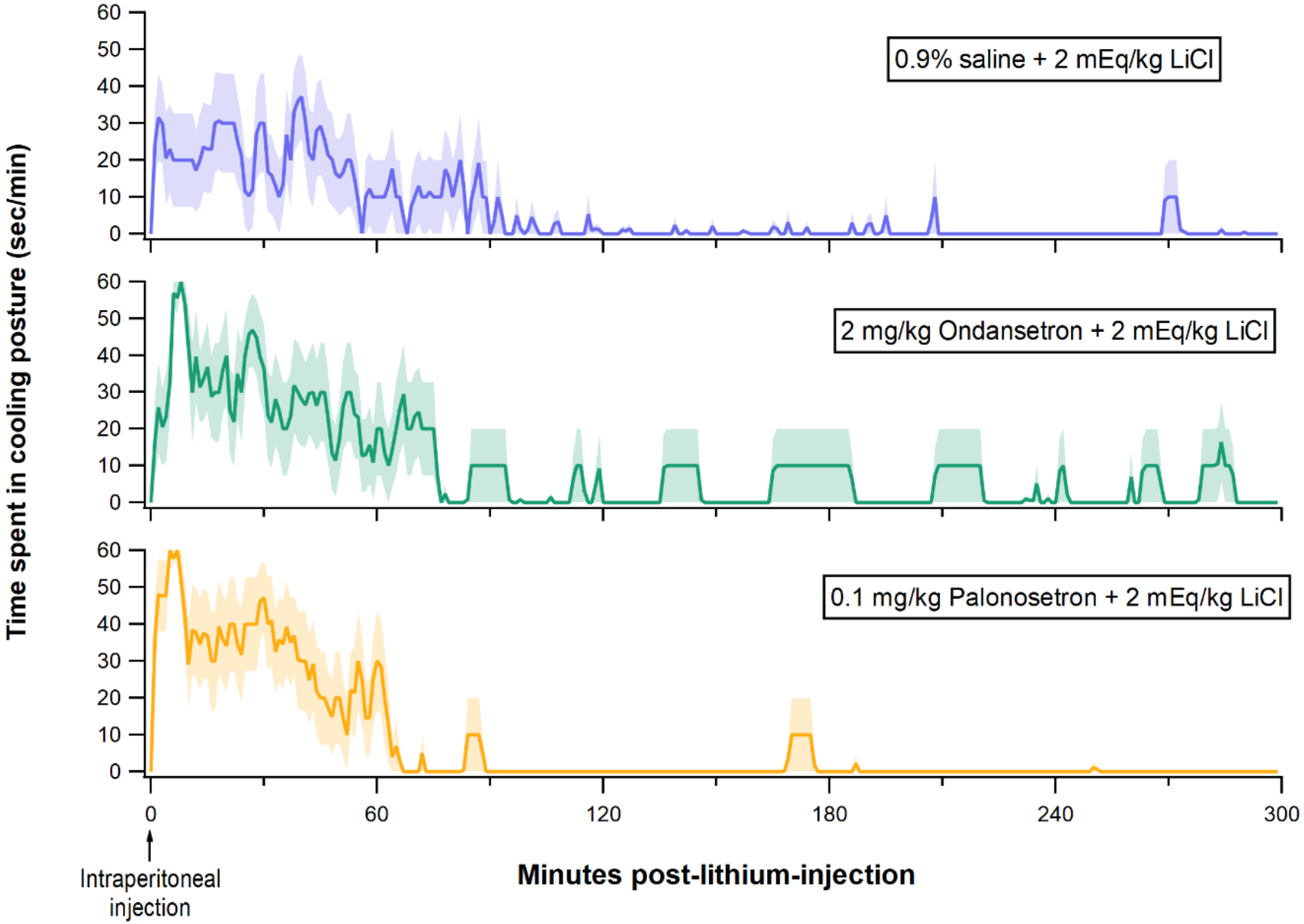


**Figure 2.9.** Seconds spent in cooling posture every minute after an intraperitoneal dose of lithium (2 mEq/kg LiCl) in guinea pigs. Ondansetron (2 mg/kg), palonosetron (0.1 mg/kg) or control (0.9% saline) was given i.p. 30 minutes before lithium. Lines and shadings represent mean ± SEM, n = 6.

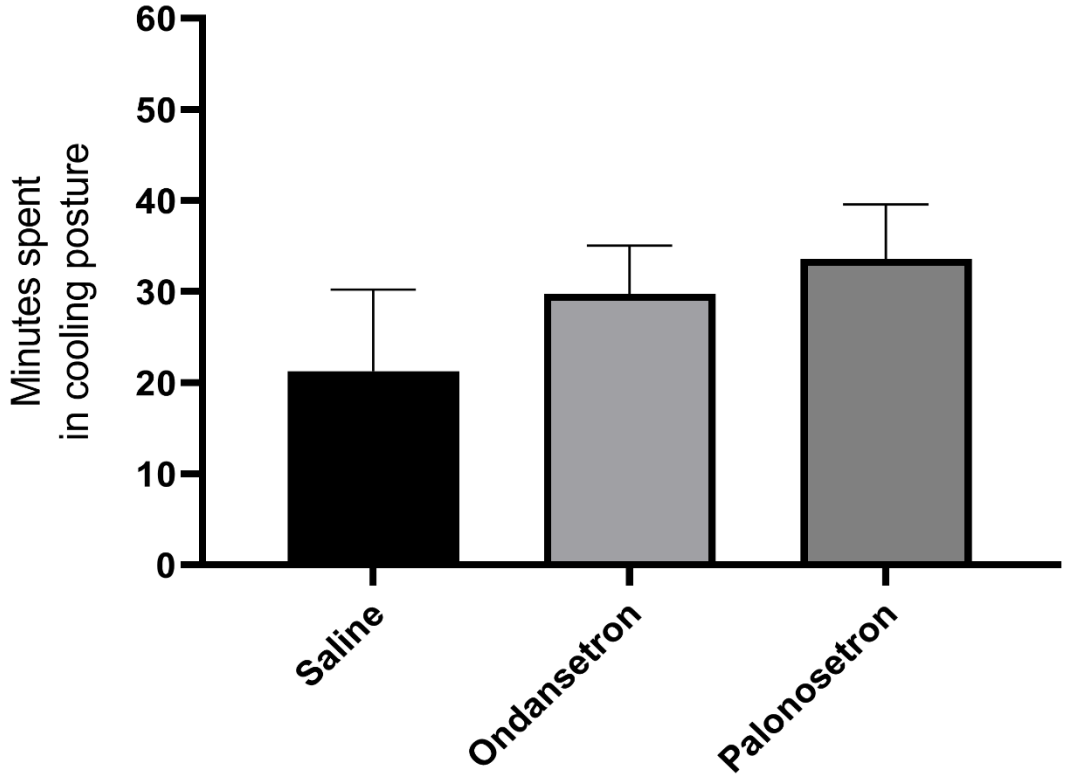


**Figure 2.10.** Total number of minutes spent in cooling posture 0–60 minutes after intraperitoneal lithium (2 mEq/kg LiCl). Each group received control (0.9% saline), ondansetron (2 mg/kg) or palonosetron (0.1 mg/kg) i.p. 30 minutes before lithium. Bars represent mean ± SEM, n = 6.

Ondansetron, palonosetron or saline given 30 minutes prior to lithium led to similar reductions in body temperature over time (**Figure 2.11**). Mean minimum change in body temperature 60–120 minutes post-lithium was not significantly different across all three groups (ondansetron: -1.21 ± 0.21, palonosetron: -1.36 ± 0.29, control: -1.11 ± 0.24 °C, one-way ANOVA, P = 0.7882, **Figure 2.12**).

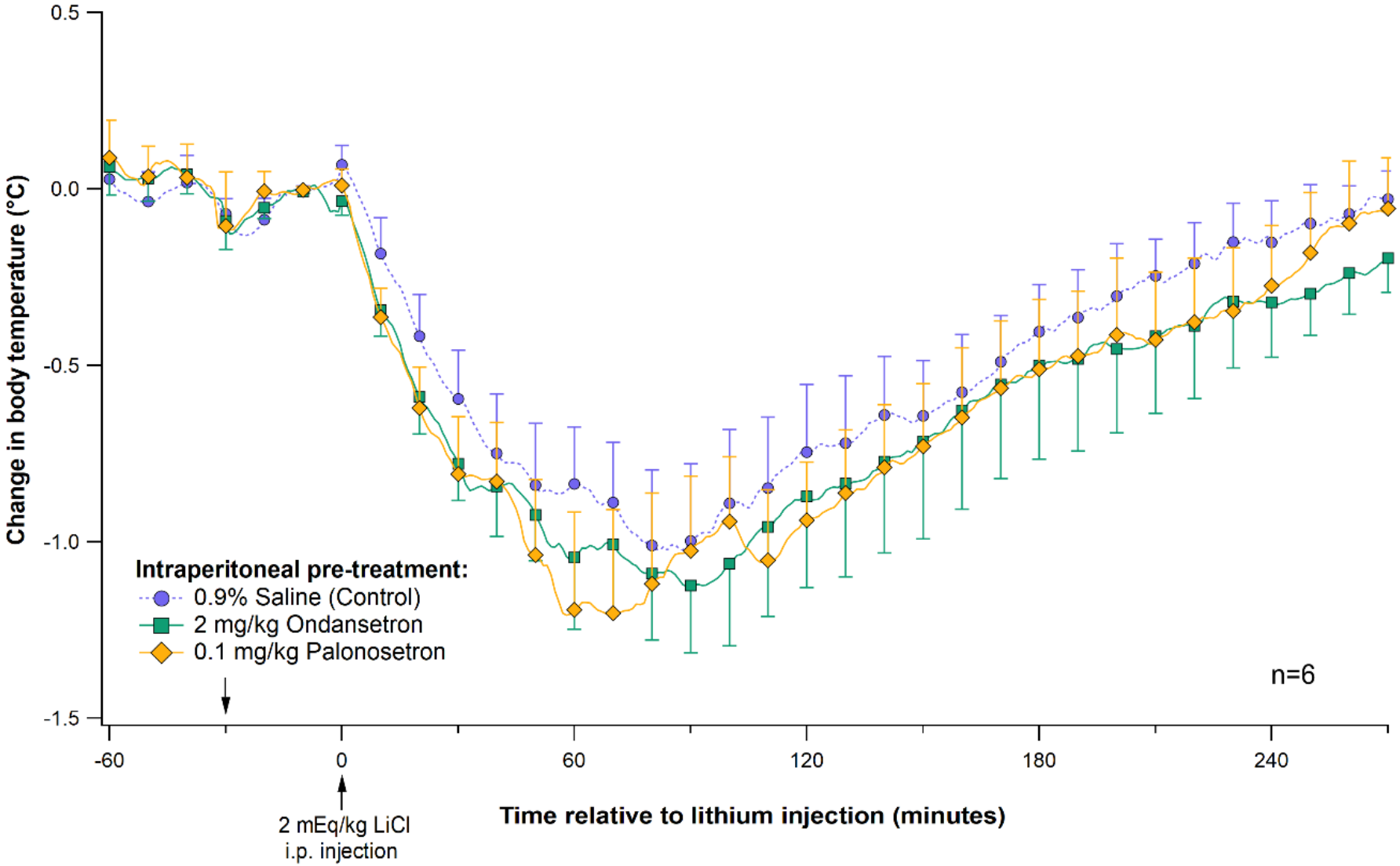


**Figure 2.11.** Change in core body temperature over time in response to intraperitoneal lithium (2 mEq/kg LiCl, at 0 minutes) 30 minutes after intraperitoneal 5-$HT_3$ antagonist pre-treatment (2 mg/kg ondansetron, 0.1 mg/kg palonosetron, or 0.9 % saline control). Datapoints represent mean ± SEM, n = 6.

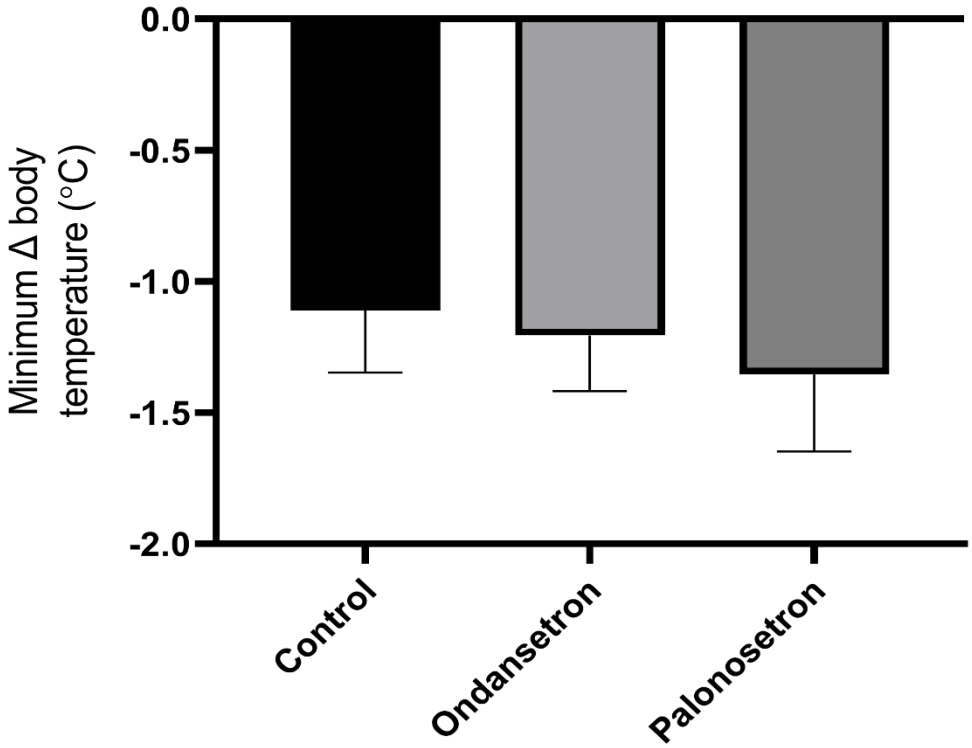


**Figure 2.12.** Minimum value of delta core body temperature between 60–120 minutes post-lithium-injection against 5-$HT_3$ pre-treatment (2 mg/kg ondansetron, 0.1 mg/kg palonosetron, or 0.9 % saline control). Bars represent mean ± SEM, n = 6.

Although it appeared that saline pre-treatment led to less distance moved over time after lithium (**Figure 2.13**), the means at each time point post-injection were not significantly different across the three groups (repeated measures mixed-effects model: treatment, P = 0.4901; time-treatment interaction, P = 0.0890). At 240 minutes post-lithium, mean cumulative distance moved did not significantly differ between the ondansetron, palonosetron and saline groups (ondansetron: 2237 ± 237.8, palonosetron: 1870 ± 381.4, control: 1556 ± 246.8 cm, one-way ANOVA, P = 0.2948, **Figure 2.14**).

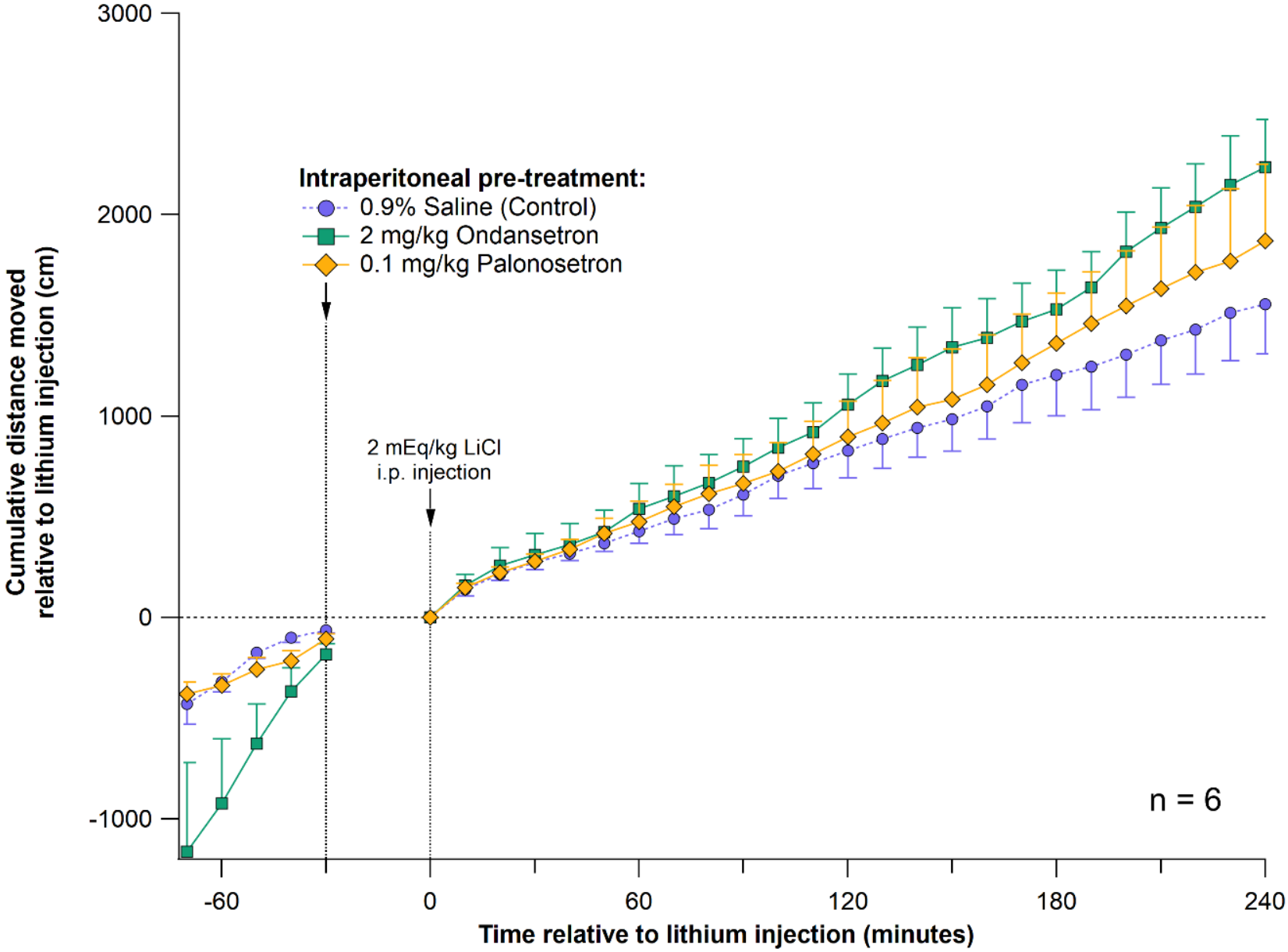


**Figure 2.13.** Cumulative distance moved over time from intraperitoneal injection point of lithium (2 mEq/kg LiCl, at 0 minutes) 30 minutes after intraperitoneal 5-$HT_3$ antagonist pre-treatment (2 mg/kg ondansetron, 0.1 mg/kg palonosetron, or 0.9 % saline control). Datapoints represent mean ± SEM, n = 6. Gap between intraperitoneal pre-treatment and lithium injection from -30–0 minutes was not analysed.

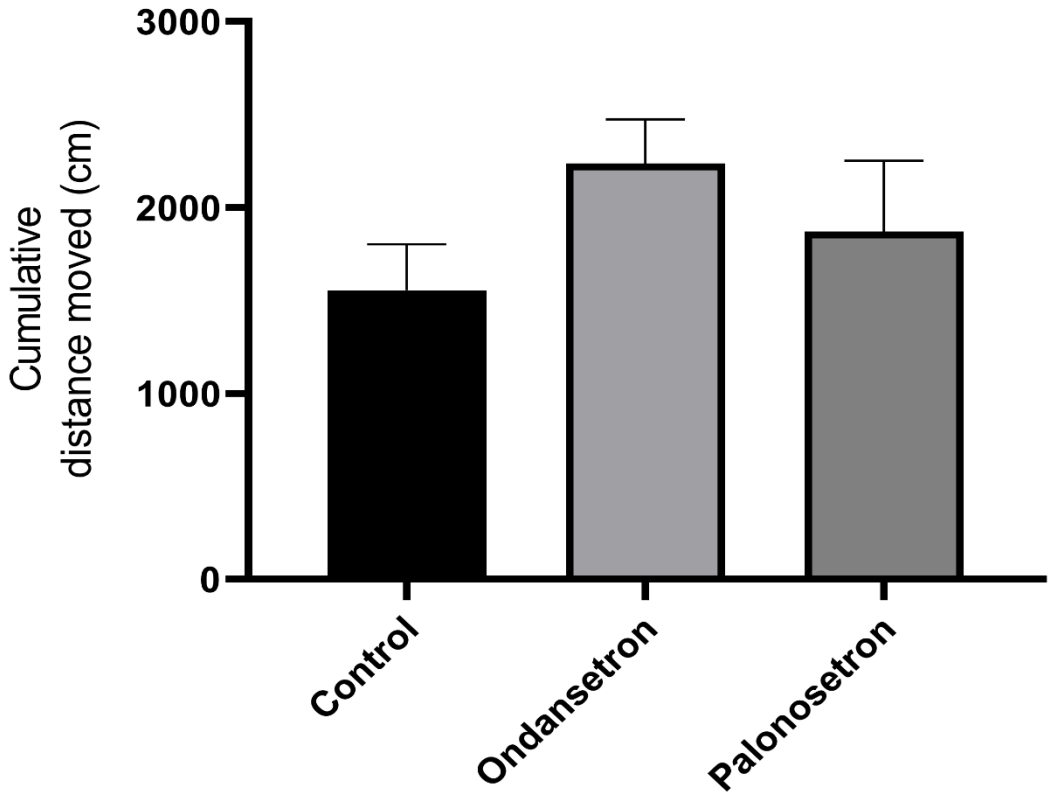


**Figure 2.14.** Cumulative distance moved at 240 minutes post-lithium-injection against 5-$HT_3$ pre-treatment (2 mg/kg ondansetron, 0.1 mg/kg palonosetron, or 0.9 % saline control). Bars represent mean ± SEM, n = 6.

## 2.4 Discussion

In this study, I re-examined lithium-induced lethargy in guinea pigs, first observed by John Cade in 1949, and investigated its relationship with the hypothermic effects of lithium. I introduced a hypothesis that diverges from traditional interpretations of lithium's physiological and behavioural impacts. Historically, lethargy and hypothermia have been viewed as separate toxic effects of lithium; however, my hypothesis posits that these responses are components of a single, integrated physiological-behavioural response, coordinated by the brain when lithium is detected in the periphery, outside the blood-brain barrier. This perspective suggests that the lethargic behaviour and reduction in body temperature serve as adaptive mechanisms in response to the presence of lithium in the periphery. By viewing these effects as a unified response, I challenge the traditional link to toxicity and promote a deeper investigation into the integrated physiological-behavioural response's importance in lithium's therapeutic action, illuminating its largely unknown mechanism in treating mood disorders, such as bipolar disorder.

### 2.4.1 Lithium's Effect on Lethargic Behaviour

I firstly found that lithium dose-dependently increased the presence of lethargic behaviour in guinea pigs, subsiding within 1–2 hours, which supports my hypothesis. This finding is consistent with John Cade's observation of lithium-induced lethargy in guinea pigs (Cade, 1949). The dose Cade used was around 6 mEq/kg, which is considered very high dose for any species. As such, studies strictly refer to lithium-elicited lethargy as a sign of toxicity (Johnson, 1984b; Price & Heninger, 1994; Smith, 1980b).

However, my findings suggest a different interpretation. I found that this behaviour is present at doses as low as 1 mEq/kg, corresponding to the therapeutic serum lithium maintenance level of 1 mEq/L in humans (Malhi et al., 2016). The same can be said for my selected dose of 2 mEq/kg, as higher doses from 1.5 to 3 mEq/kg can also achieve human therapeutic serum levels in rodents (Cox et al., 1971; O'Donnell & Gould, 2007). Supporting my findings in guinea pigs, previous studies in rats using therapeutically relevant doses of lithium also reported similar behaviour. In 1984, Parker et al. described 'lying-on-belly' (LOB) behaviour in Sprague-Dawley rats after 3 mEq/kg of lithium i.p. (Parker et al., 1984). A later study in 1990 by Meachum and Bernstein showed that Long-Evans rats exhibited the same LOB behaviour after about 1 mEq/kg of LiCl i.p. (Meachum & Bernstein, 1990). The researchers of the latter postulated that the LOB behaviour was indicative of the animals 'feeling ill'. It seems that they were unaware that their observation parallels Cade's observation of lethargy in guinea pigs. In both studies, the rats displayed an increase in LOB behaviour within 5–10 minutes after

lithium administration over an observation period of 30–60 minutes. This behaviour is strikingly similar to the 'cooling posture' I observed in my guinea pigs. Together, these findings indicate that lithium-induced lethargy originally observed by Cade is not indicative of a nonspecific toxic or adverse effect, but a normal behavioural response to lithium.

While the cooling posture I observed was profound after doses higher doses of lithium, it was also present after an inert dose of 0.1 mEq/kg LiCl. However, the lethargic behaviour did not occur immediately after injection as in the other dose groups. Notably, the lack of cooling posture coincided with a small increase in body temperature, likely due to emotional hyperthermia in response to handling/injection stress (Dallmann et al., 2006; Oka, 2015). A similar hyperthermic response was observed by Ogilvie and Lobb after a dose of ~0.47 mEq/kg LiCl in mice (Ogilvie & Lobb, 1981). This suggests that the cooling posture is a normal thermoregulatory behaviour that occurs naturally when the animal must cool down. In support of this idea, our lab has previously shown that increases in body temperature coincide with increases in active behavioural activity (environmental exploration), as well as the inverse, as part of the basic rest-activity cycle. In this cycle, body temperature and behavioural activity fluctuates in an ultradian rhythm roughly every 1–2 hours (Blessing, 2018). Therefore, the spontaneous presence of cooling posture throughout the recording after 0.1 mEq/kg LiCl, is likely reflective of this ultradian phenomenon. Overall, lithium appears to dose-dependently initiate normal thermoregulatory behaviour.

### 2.4.2 Lithium's Hypothermic Effect

In further support of my hypothesis, my next finding was that lithium led to a dose-dependent decrease in guinea pig body temperature. This marks the first demonstration of lithium-induced hypothermia in guinea pigs and is in agreement with reports of dose-dependent lithium-elicited hypothermia in rats and mice (Ogilvie & Lobb, 1981; Tulunay, 1976).

It is important to note that lithium partly overrides the cold-defence response to low ambient temperatures. Cunningham and Niehus demonstrated that lithium-induced hypothermia (3 mEq/kg LiCl) occurs in rats even at a near-freezing ambient temperature of 5 °C (Cunningham & Niehus, 1993). However, the extent of lithium-induced hypothermia is similar between rats exposed to 21 °C and 5 °C groups, indicating that there is a temperature threshold below which body temperature must not drop and cold-defence mechanisms must activate. This suggests that the drop in body temperature is the brain's adaptive and calculated response to lithium. In further support of this brain-orchestrated hypothesis, the hypothermic response can be elicited simply through tastes associated with lithium.

Bull et al. demonstrated that rats exhibiting aversions to a taste previously associated with lithium also experienced hypothermia (Bull et al., 1991). This finding indicates that the brain forms an engram, or memory trace, of the lithium-associated taste, which can activate the nausea-related pathway triggered by lithium, subsequently inducing hypothermia without the actual ingestion or injection of lithium. This anticipatory mechanism supports the idea that the hypothermic response to lithium is an innate and adaptive process orchestrated by the brain.

It is well known that rats and mice consistently exhibit acute hypothermia in response to many toxicants such as insecticides, heavy metals and solvents (Gordon, 2010). Moderate hypothermia in rodents is associated with improved survivability from toxicants and other insults such as hypoxia and ischemia. Therefore, this hypothermic response is considered an adaptive defence mechanism (Guimaraes et al., 2015), which likely attenuates temperature-dependent biochemical reactions, lowering metabolic rate and preventing cellular damage (Gordon, 2001). I believe that lithium triggers this protective response without being toxic/damaging. This may, at least partially, explain why lithium aided in the survival of Cade's guinea pigs in the face of urea intoxication (Cade, 1949). Despite this, hypothermia is synonymous with toxicity in the lithium literature. But, my findings, as well as those found by others in rats and mice, show that lithium-induced hypothermia occurs at a therapeutic level of 1 mEq/kg.

### 2.4.3 Lithium's Effect on Locomotor Activity

I also found that lithium dose-dependently reduced locomotor activity, which is consistent with others' observations in rats (Gray et al., 1976; Syme & Syme, 1973; Tomasiewicz et al., 2006). Smith and Smith showed that lithium specifically decreases voluntary action rather than affecting reactivity or muscle strength (Smith & Smith, 1973). The decrease in locomotor activity I observed was partly due to the increase in lethargic behaviour following lithium injection, as the animals were moving less when assuming the cooling posture. Furthermore, once the cooling posture subsided, the animals tended to stand still in a huddled posture that would favour heat conservation. This coincided with body temperature returning to baseline after the hypothermia reached its peak.

### 2.4.4 Lithium's Role in Thermoregulation

The relationship between lethargic behaviour and body temperature is seldom discussed in the literature. The changes I measured in lethargic behaviour and hypothermia were highly correlated. Much like the temporal continuum of nausea, the increase of lethargic behaviour preceded the peak

of hypothermia. In other words, the majority of time spent in cooling posture occurred before body temperature reached its minimum. This suggests that the behaviour I observed was part of a coordinated thermoregulatory response triggered by lithium. Thermoregulation in mammals consists of an integrated suite of behavioural and physiological processes. For example, when a reduction in body temperature is required, mammals can promote heat loss through altering posture, reducing locomotor activity, vasodilating surface blood vessels, and inhibiting shivering and non-shivering thermogenesis (i.e., brown adipose tissue) (Mota-Rojas et al., 2021; Stelzner & Hausfater, 1986; Tansey & Johnson, 2015). Therefore, the guinea pigs were likely assuming a posture that facilitated heat loss, which is why I termed it as 'cooling posture'. The same may be true for LOB behaviour in rats.

### 2.4.5 Sodium as a Control

In my dose-response experiment, I used hypertonic saline to control for a hyperosmolar effect and see if the sodium cation displayed similar effects to lithium. Intragastric hypertonic saline is known to induce nausea and vomiting in humans (Metheny & Krieger, 2020), similar to lithium. Likewise, intragastric and intraperitoneal injections of hypertonic saline can produce CTA in rats (Hargrave & Bolles, 1971; Mediavilla et al., 2005; Revusky & Garcia, 1970). I found a similar effect in guinea pigs whereby sodium chloride at 4 mEq/kg produced nausea-indicative lethargic behaviour (cooling posture) and hypothermia. Nagashima et al. has also reported decreased body temperature and cool-seeking behaviour in crj-Wistar rats injected with hypertonic saline compared to isotonic saline (Nagashima et al., 2001). However, I found that lithium triggered a stronger behavioural and hypothermic response when compared to sodium at the same osmolality. In agreement with this finding, Meachum and Bernstein demonstrated that an equimolar dose of sodium chloride was less effective at producing LOB behaviour than lithium chloride in rats (Meachum & Bernstein, 1990). Further, Ogilvie and Lobb found that NaCl, at a comparable milliequivalent to LiCl, did not produce a significant change in body temperature in mice (Ogilvie & Lobb, 1981). My observations are also consistent with the fact that lithium is far more effective at producing CTAs than sodium (Nachman & Ashe, 1973).

Together, these findings suggest that the body can differentiate between the lithium and sodium cations, and that lithium's effects are specific and independent of osmolality. The same conclusion was arrived at by Ogilvie and Lobb, as well as Nachman and Ashe, in that lithium's hypothermic and nausea-indicative effects are not dependent on volume or concentration of the injected solution of LiCl, but on the absolute quantity of lithium injected (Nachman & Ashe, 1973; Ogilvie & Lobb, 1981).

It is not clear why sodium has similar, yet lesser, nausea-like and hypothermic effects compared to lithium. One possibility is that sodium and lithium ions exhibit different levels of activity on the same nausea-related pathway, which in turn differentially contributes to lethargy and hypothermia. This may be due to the body's ability to differentiate between sodium and lithium ions, resulting in nuanced physiological responses that exhibit different degrees of intensity. Sodium's reduced nausea-like effect compared to lithium might be attributed to its ubiquitous presence in the body as an endogenous ion. Unlike lithium, sodium is an essential component of numerous homeostatic systems and is naturally found within the body. As a result, the body may have a higher tolerance for sodium, requiring a larger quantity in the body before nausea is triggered.

Sodium's role in regulating blood pressure is an important physiological mechanism that may interact with the sodium-induced hypothermic effect. Hypertonic saline has been shown to lead to sustained increases in mean arterial pressure and heart rate when delivered through oral, intravenous, subcutaneous, and intraperitoneal routes (Fortaleza et al., 2020). Recent research suggests the primary mechanism driving this blood pressure increase, independent of changes in blood volume (Gavras & Gavras, 2012), is that elevated sodium levels in the blood are detected by sodium-sensitive neurons in the organum vasculosum of the lamina terminalis (OVLT), a circumventricular organ in the brain (Nomura et al., 2019). This triggers activation of the sympathetic nervous system via the rostral ventrolateral medulla (RVLM), which in turn raises blood pressure (Nomura et al., 2019). A secondary, lesser contribution to the blood pressure elevation involves the release of vasopressin from the pituitary gland into the bloodstream, also postulated to be triggered by the sodium-sensitive OVLT neurons detecting high sodium levels (Nomura et al., 2019). While sympathetic activation via the RVLM leads to systemic blood pressure increases through enhanced cardiac output and vasoconstriction of arterioles throughout the body and its organs (Kumagai et al., 2012; Sugiyama et al., 2011), vasopressin contributes to this effect by causing vasoconstriction through activation of V1a receptors in vascular smooth muscle cells (Henderson & Byron, 2007). These vasoconstrictive properties may be relevant to thermoregulation because output from the RVLM plays a partial role in maintaining cutaneous vasoconstriction (Ootsuka & Tanaka, 2015), and vasopressin has also been observed to dose-dependently induce cutaneous vasoconstriction (Nilsson et al., 1987). Such cutaneous vasoconstriction would inherently limit heat dissipation from the body. Together, these vasoconstricting properties of the two sodium-related blood pressure-increasing mechanisms may interfere with sodium-induced hypothermia. Therefore, in addition to sodium being less potent than lithium in activating the nausea-related pathway, these vasoconstrictive mechanisms may also limit

its hypothermic effect, which could offer some explanation as to why it induced a lesser hypothermic effect compared to lithium.

### 2.4.6 The Role of Visceral Pain

In my experiments, I observed an unexpected acute behavioural reaction to hypertonic lithium and sodium solutions. It is crucial to determine if this affected my results. I used a similar approach to Tulunay (Tulunay, 1976), as well as Ogilvie and Lobb (Ogilvie & Lobb, 1981), by dissolving LiCl in fixed volumes. This led to increasingly hypertonic injected solutions as the dose of LiCl increased. However, these researchers did not report any acute reactions to the injections.

In my observations of the guinea pigs, the animals displayed only transient behavioural responses immediately after receiving injections of hypertonic solutions. The acute reactions of discomfort to hypertonic lithium and sodium are short-lived in comparison to the prolonged and profoundly painful reactions to highly noxious stimuli such as acetic acid and phenylquinone (Collier & Schneider, 1969; Danneman, 1997; Gawade, 2012). Further, the lethargic behaviour or cooling posture I observed throughout the experiment differs characteristically from behavioural signs of pain in guinea pigs (Danneman, 1997).

It is unlikely that the visceral pain caused by hypertonic intraperitoneal injection of LiCl or NaCl is responsible for the behavioural and hypothermic effects. This is evident as isotonic lithium (0.15 M) effectively induces CTA in rats (Nachman & Ashe, 1973) and guinea pigs (Braveman, 1974) and induces hypothermia in rats (Bernstein et al., 1992).

No adverse events occurred during or after the experiment, as confirmed by regular monitoring at the animal facility. Additionally, the removal of the telemetric temperature transmitters revealed no noticeable inflammation, haemorrhage, or tissue damage. Previous studies provide further context for my observations. Johnson observed a "mild post-injection syndrome" after administering a hypertonic injection of 6 mEq/kg LiCl (6 M, i.p.) which was transient, ill-defined in nature, and occurred unreliably (Johnson, 1977). In a separate study, O'Connor et al. demonstrated that i.p. injections of isotonic and hypertonic lithium solutions and 0.9% saline produced discomfort lasting around two minutes (O'Connor et al., 1987), suggesting a general effect from the injection itself. These observations appear to be similar to ours. Further, Johnson's research revealed an absence of behavioural signs indicative of tissue damage (Johnson, 1976, 1979), which is consistent with my own observations upon removal of the telemetric temperature transmitters, where I found no noticeable

inflammation, haemorrhage, or tissue damage. Together, these findings suggest that the potential pain or discomfort related to hypertonic lithium injection is unlikely to be of physiological significance or relevant to the thermoregulatory effects I observed.

### 2.4.7 The Role of Serotonin Receptors

Thus far, my hypothesis has been supported. However, the outcome of the 5-$HT_3$ antagonist pre-treatment experiment refuted the serotonergic aspect of my hypothesis, as neither ondansetron nor palonosetron attenuated lithium's behavioural and hypothermic effects. The guinea pigs continued to exhibit behaviours and postures consistent with nausea. If I were to ignore variability and statistical insignificance, 5-$HT_3$ antagonist pre-treatment led to a stronger lethargic and hypothermic effect after lithium when compared to saline pre-treatment, which again refutes the hypothesis. In support of my finding, which was that 5-$HT_3$ antagonists did not significantly change lithium's effect, Rudd et al. found that 5-$HT_3$ receptors are not involved in conditioned taste aversions elicited by 5-HT as well as ipecacuanha or cisplatin. They also found that the use of 5-$HT_3$ antagonists and agonists did not respectively prevent or elicit conditioned taste aversions in the rat (Rudd et al., 1998). Similarly, Martin et al. found that 5-$HT_3$ agonist 2-M-5HT did not affect body temperature in rats (Martin et al., 2000). This is perplexing as ondansetron prevents serotonin-releasing cisplatin-induced nausea in humans and dogs, of which conditioned taste aversion and lethargy are indices; although, it is not always effective (Cubeddu et al., 1990; Elwood et al., 2010).

In contradiction to my finding, Guimaraes et al. showed that ondansetron (2 mg/kg) given 30 minutes before LiCl (~ 1.5 mEq/kg) prevented hypothermia in Wistar rats (Guimaraes et al., 2015). Further, Tuerke et al. observed a reduction in LOB behaviour in Sprague-Dawley rats when given ondansetron (0.5 mg/kg) 30 minutes before LiCl (3 mEq/kg) (Tuerke et al., 2012). Besides the difference in species, the reason for these conflicting results is unclear. As I observed lithium-induced lethargic behaviour, hypothermia, and reduced locomotor activity in guinea pigs after 0.9% saline, ondansetron and palonosetron (which is much more potent than ondansetron) (Aapro et al., 2006; Park & Cho, 2011; Siddiqui & Scott, 2004), lithium's effects are unlikely to be mediated by 5$HT_3$ receptors in the guinea pig.

### 2.4.8 Peripheral Mediation

However, lithium's effects may still be mediated peripherally. Tulanay suggested that lithium's acute hypothermic action was due to its replacement of sodium ions in the hypothalamus (Tulunay, 1976).

He came to this conclusion in reference to rat studies showing that the lithium ion ($Li^+$) distributed well in the hypothalamus after chronic (4+ weeks) lithium treatment (Edelfors, 1975; Ho et al., 1970). As the hypothalamus is a key brain region in thermoregulation (Boulant, 2000), this conclusion has merit, but it does not explain the fast-acting behavioural effect of lithium I have observed. The responses I observed occurred within a few minutes, which does not correlate with lithium's slow permeation of the blood-brain barrier (BBB). In rats, lithium levels in the CSF and brain can take up to 2 and 24 hours, respectively, to reach a maximum; serum levels peak at around 15 minutes (Wraae, 1978). I saw lithium induce lethargic behaviour in guinea pigs almost immediately, lasting up to 2 hours before reversal, suggesting peripheral action. However, an electrophysiological study by Yamamoto et al. identified neurons within the viscerosensory insular cortex, which is involved in processing interoceptive information (Craig, 2002), that responded to intraperitoneal lithium (0.15 M) with a latency of around 5 minutes (Yamamoto et al., 1989). Since this occurs much faster than lithium can enter the brain, this finding supports the possibility of peripheral detection and subsequent neural signalling to the brain, contributing to the rapid physiological and behavioural responses to lithium. In support of this peripheral mechanism, Smith reported that when LiCl was injected bilaterally into the CSF of the lateral ventricles in rats, it failed to produce CTA and did not affect locomotor activity (Smith, 1980a).

While my findings indicate that the 5-$HT_3$ receptors do not seem to play a significant role in the observed effects of lithium, I cannot discount the involvement of a serotonergic mechanism. This is due to the fact that other serotonin receptor subtypes may be implicated. As such, it is plausible that the gut-brain axis could still be a peripheral target of lithium. Apart from 5-$HT_3$, terminals of vagal afferents contain many other types of receptors, such as CCKa and CCKb. These receptors, which share the ligand cholecystokinin (CCK), have been linked with mediating CTA in rats (Mosher et al., 1996). Furthermore, CCK is associated with nausea in humans (Miaskiewicz et al., 1989) and has been found to induce hypothermia in rats (Martin et al., 2000).

In addition to the aforementioned receptors, the area postrema is another peripheral target that warrants further investigation. This circumventricular organ, which samples blood outside of the blood-brain barrier (BBB) and receives input from vagal afferents (Babic & Browning, 2014), is known as the chemoreceptor trigger zone for nausea and vomiting (Bernstein et al., 1992). It expresses both 5-$HT_3$ and CCK receptors, among many others (Jackson & Yakel, 1995; Sun & Ferguson, 1997; Zhong et al., 2021). Importantly, studies on rats have shown that lesioning the area postrema can affect the lithium-elicited CTA (Kosten & Contreras, 1989), LOB behaviour, and hypothermia (Bernstein et al.,

1992). Although my data does not establish a direct link between 5-$HT_3$ receptors and lithium's effects, the intricate nature of serotonergic mechanisms and the possible roles of other peripheral targets, such as the area postrema and other receptor types, underline the necessity for continued exploration in this research field.

### 2.4.9 Clinical Relevance of Lithium's Effects

As lithium's effects of nausea-lethargy and hypothermia are present at a therapeutic level, I believe that they may be clinically relevant. I acknowledge that while my study investigates lithium's acute effects, its clinical application is usually chronic. Some patients may see changes within days, but full effects generally occur after 6-10 days of treatment (Schou, 1968). This of course warrants investigation of lithium in a chronic context.

However, I have established that lithium's hypothermic response, which is highly correlated with lethargic behaviour, occurs at a therapeutic level of 1 mEq/kg. Furthermore, a study conducted by Jensen demonstrated that rats fed a lithium-infused diet for six days, coinciding with the expected effect onset in humans, exhibited both hypothermia and reduced locomotor activity compared to the controls (Jensen, 1974). This observation not only suggests that lithium's impact remains consistent across different durations of exposure, both acute and chronic, but also enhances the clinical relevancy of the effects I have observed.

Temperature and mood are deeply integrated, which is demonstrated by the hypothalamus' simultaneous involvement in the regulation of temperature, mood and stress (Bao & Swaab, 2019). Dysregulation in body temperature such as chronic emotional hyperthermia (also known as psychogenic fever) is often seen in bipolar patients along with other mood disorders (Oka, 2015). Therefore, I suggest that the acute behavioural and hypothermic effects of lithium, when applied chronically, act to correct dysregulated thermoregulation, resulting in the therapeutic outcome of reduced manic behaviour in humans.

Interestingly, a historical example of potentially similar nausea-related action exists in the literature. In 1864, well before the use of lithium and other modern treatments for mania, Harris used steroidal alkaloids extracted from *Veratrum viride,* known as 'Norwood's Tincture', to alleviate the paroxysms in a patient with acute mania (Harris, 1864). After administration of this substance, the manic patient described feeling nauseous while the doctor observed the patient's behaviour transition to a state of "calm serenity"; in addition, the patient exhibited pallor, a sign of hypothermia (Nalivaiko, 2018).

Veratrum alkaloids were chiefly used as an antihypertensive agent in the 1950s but were discontinued for their narrow therapeutic index (Crummett et al., 1985; Jaffe et al., 1990). The lethargic response and therapeutic outcome seen in the manic patient is similar to that induced by lithium, which is also known for its narrow therapeutic window.

### 2.4.10 Next Steps

In closing, this study successfully replicated John Cade's observation of reversable lithium-induced lethargy in guinea pigs. I found that lithium, in a dose-dependent manner, increased the presence of lethargic behaviour, decreased core body temperature and reduced locomotor activity. These effects constitute a coordinated thermoregulatory physiological response to lithium, which occurred at a therapeutic-equivalent dose. The temperature-affective behavioural changes are antithetical to manic behaviour. Therefore, I propose that the body's thermoregulatory response to lithium is involved in lithium's antimanic therapeutic effect rather than toxicity. This response is fast-acting, which suggests peripheral initiation and communication to the brain (e.g., gut-brain axis and/or area postrema), however it is unlikely to be mediated through 5-$HT_3$ receptors. Further investigation of a peripheral target is required. In the next chapter, I will replicate the main findings here in rats and further characterise lithium's acute thermoregulatory and behavioural effects.

# Chapter 3.

# Lithium Under Stress: Examining Thermoregulatory & Behavioural Adaptations in Rats

## 3.1 Introduction

Building upon the previous investigation into lithium's hypothermic and behavioural responses in guinea pigs, this chapter extends this exploration to rats, with an additional focus on the implications of lithium under acute psychological stress. I aim to examine the dose-dependent effects of lithium on body temperature, brown adipose tissue (BAT) thermogenesis, and locomotor activity in rats, and establish whether these effects persist over time considering the chronic nature of psychiatric lithium use.

One of the primary challenges in thermoregulatory studies, particularly when measuring emotional hyperthermia, is the handling stress imposed on animals during rectal measurements of core body temperature. This technique, predominantly used in existing dose-response lithium studies involving rats and mice (Ogilvie & Lobb, 1981; Tulunay, 1976), can induce hyperthermia due to emotional stress, thereby confounding results and creating shortcomings in the existing literature (Stewart & Eikelboom, 1979). To address these issues, I will employ a tethered method previously designed by our lab (Ootsuka et al., 2009) that simultaneously measures BAT and body temperature, offering a significant improvement over traditional techniques. Additionally, I will utilise the telemetric method established in the previous chapter for a continuous and non-invasive monitoring of core body temperature. To ensure the relevance of my findings to clinical practice, I will use therapeutically relevant doses of lithium in each experiment (Cox et al., 1971; O'Donnell & Gould, 2007). These improved methods will provide a more precise understanding of the thermoregulatory effects of lithium.

The investigation is guided by my hypothesis that lithium induces an integrated physiological response coordinated by the brain. I expect this to manifest as lithium-induced hypothermia, accompanied by

changes in other appropriate thermoregulatory effectors such as the inhibition of BAT thermogenesis and induction of tail vasodilation, responses not previously measured in response to lithium.

The resident-intruder paradigm, a well-established model for evoking social stress (Koolhaas et al., 2013), serves as an activator of BAT thermogenesis in my conscious animal model and is also employed as an animal model for bipolar disorder (Benedetti et al., 2008; Gessa et al., 1995; Valvassori et al., 2017). Psychological stress is a major precipitating factor in the development and exacerbation of bipolar disorder (Post & Leverich, 2006). In our lab's adaptation of the model (Mohammed et al., 2014), the intruding animal induces psychological stress in the resident animal without physical contact, resulting in emotional hyperthermia and altered behaviour. This modification allows me to focus solely on the effects of psychological stress on thermoregulation and behaviour, providing valuable insights into the role of stress in the pathophysiology of bipolar disorder.

Examining lithium's behavioural and thermoregulatory effects in the context of psychological stress is crucial. Psychological stressors not only activate BAT thermogenesis and cause emotional hyperthermia, but they also trigger significant behavioural changes (Nakamura & Morrison, 2022). These physiological and behavioural responses contrast sharply with the hypothermic and tranquilising effects of lithium I observed in guinea pigs. Understanding whether lithium inhibits these stress-induced changes in both physiology and behaviour is insightful for several key reasons. First, it can provide valuable insights into its therapeutic effects, particularly for stress-related psychiatric disorders. Second, it sheds light on lithium's underlying mechanisms of action and its interplay with stress pathways. Third, it can inform the development of novel interventions aimed at enhancing stress resilience and adaptation. Lastly, understanding lithium's impact on stress-induced physiological and behavioural responses also enables me to delve into its potential influence on cognitive processes such as memory, learning, and decision-making, providing further insights into the therapeutic benefits of lithium in treating psychiatric disorders characterised by cognitive impairments (Kim & Kim, 2023).

In addition to these aims, I will investigate whether 5-$HT_3$ antagonists (ondansetron and palonosetron) can counteract the effects of lithium, building upon findings from Guimaraes et al. that ondansetron can prevent lithium-induced hypothermia in rats (Guimaraes et al., 2015). This will help establish if the inability of these 5-$HT_3$ antagonists to prevent lithium's effects in guinea pigs is due to a difference between species.

In my study, I will also consider another finding by Guimaraes et al., who reported a diminished hypothermic response to lithium when a second dose was given 7 days later (Guimaraes et al., 2015). This observation is significant for my study, where animals might receive successive doses of lithium with a minimum of 3 days apart. Although I did not suspect any habituation in my previous chapter exploring dose rotations in guinea pigs, I did not explicitly investigate this. Therefore, in this chapter, I aim to evaluate if this observation holds true in rats by examining whether repeated exposure to lithium influences the lithium-induced reductions in body temperature and behavioural activity. This will allow me to better comprehend if the body's response to lithium changes upon subsequent encounters, and if so, how this might impact my interpretation of lithium's acute effects.

Previous studies addressing lithium's effect on tail temperature are scarce. Jones et al. included tail temperature measurements to enhance their understanding of lithium's effect on REM sleep and secondary messengers in the rat brain (Jones et al., 2008). However, their methods had limitations; they could only measure temperature at a single point along the tail using a thermistor, their temporal resolution was limited (every 10 minutes), and they conducted their temperature measurements during the rats' inactive hours (during the light). Moreover, they used a lithium dose of 5 mEq/kg LiCl, which prompts the question of whether the observed effects would be present at a more therapeutically relevant dose. Therefore, in my study, I will use a lithium dose of 2 mEq/kg LiCl. In contrast to Jones et al., I will use infrared thermography, which allows me to measure tail temperature at any point along the tail and select the maximum temperature. I will conduct measurements during the rats' active hours (during the dark) and with a minute-to-minute resolution. Given the vital role of hairless skin, such as that on a rat's tail, in regulating mammalian body temperature by adjusting blood flow during various physiological responses (Ootsuka & Tanaka, 2015), I hypothesise that lithium might induce vasodilation in the rat tail, thereby facilitating heat loss. This experiment marks the first application of thermal imaging technology to measure rat tail temperature (and by proxy, vasomotion) during active hours at a minute-to-minute resolution in response to intraperitoneal lithium administration.

By the end of this chapter, I aim to provide a comprehensive understanding of the physiological, behavioural, and cognitive impacts of emotional hyperthermia on the body, its significance in adapting to psychological stress, and the potential role of lithium in modulating these stress-induced responses. This investigation will ultimately enhance our understanding of lithium's implications for psychiatric disorders.

## 3.2 Methods

### 3.2.1 Ethics Approval

The experiments detailed in this study were carried out at Flinders University (FMC, Bedford Park, SA, Australia) in adherence to the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition (National Health and Medical Research Council et al., 2013). The conduction of these experiments was sanctioned by the Flinders University Animal Welfare Committee.

### 3.2.2 Animals

Male Sprague-Dawley rats from the Flinders University Animal Facility were used in each experiment of this chapter. The dose-response and resident-intruder experiments involved rats weighing between 417–597 g (n = 6). For the 5-$HT_3$ antagonist experiment, I utilised rats weighing between 377–523 g (n = 6). The habituation experiment used rats weighing between 300–350 g (n = 6), and the tail temperature experiment incorporated rats weighing between 250–500 g (n = 7).

Outside of experimentation, all rats were communally housed under a reversed 12-hour light/dark cycle to accommodate their nocturnal behaviour, with unrestricted access to food and water.

Data with uncorrectable noise or broken recordings were excluded from the sample sizes.

### 3.2.3 Surgical Procedures

General anaesthesia and post-operative care were consistent across all surgical procedures. Rats were placed under general anaesthesia (isoflurane in 100% oxygen [0.8–1 L/min]: 2.5–3% for induction, 1–2% for maintenance) and received a subcutaneous (s.c.) dose of analgesics (5 mg/kg carprofen; Carprieve, Norbrook, Tullamarine, VIC, Australia) and antibiotics (5 mg/kg enrofloxacin; Baytril, Bayer, Pymble, NSW, Australia) prior to any implantation procedure. After emergence from general anaesthesia, the animals were returned to the animal holding facility for a recovery period of at least one week. The rats were housed in communal cages under a reversed light-dark cycle (7 am dark, 7 pm light). For the first two days of recovery, the animals received additional analgesics: ad-libitum drinking water with carprofen (134 µg/ml). The rats were considered fully recovered and ready for experimentation once they reached pre-surgical body weight.

#### 3.2.3.1 Head Socket Implant

In the dose-response and resident-intruder experiments, I utilised a previously established method from our lab to concurrently measure both body and BAT temperatures (Ootsuka et al., 2009).

Temperature probes, manually crafted from thermistors (NTH5G10P, Murata, Kyoto, Japan), were encased within silicone (RTV 3-1744, Dow Corning, Midland, MI, USA). To gauge body temperature, a probe was inserted through a 1.5 cm incision made inferior to the thyroid gland on the ventral side of the neck, then guided along the ventral trachea into the anterior mediastinal region near the heart. Another probe was placed in the interscapular BAT region close to the Sulzer's vein for BAT temperature monitoring. Each probe was connected to insulated wires that were subcutaneously routed to the rat's head.

The ends of these insulated wires, opposite to the thermistors, were soldered to a cranial socket. This socket was cemented onto the skull and reinforced with screws embedded within the skull. The cranial socket served as the relay point for the electrical signals generated by the temperature probes.

#### 3.2.3.2 Implantation of Radiotelemetry Transmitters

In the 5-$HT_3$ antagonist and lithium habituation experiments, I adopted the wireless radiotelemetry technique from **Chapter 2** to monitor body temperature.

A telemetric temperature transmitter (7.25 g, 3.5 $cm^3$; TA-F40, Data Sciences International [DSI], Transoma Medical, Arden Hills, MN, USA) was implanted into the peritoneal cavity of each rat.

### 3.2.4 Experimental Setup

#### 3.2.4.1 Standard Recording Chamber

A recording chamber, similar to the one described in **Chapter 2**, was used for each experiment in this chapter, with the exception of the tail temperature experiment. Rats were singly placed inside an open-top acrylic cage (350 x 400 x 450 mm) with ad-libitum access to food and water. The cage was contained within an isolated climate-controlled chamber (0.32 $m^3$; Biomedical Engineering, Flinders Medical Centre, Bedford Park, SA, Australia). The environment inside the chamber was maintained at 20–24 °C with 60 air changes per hour. Reverse-cycle lighting (7am dark, 7pm light) was utilised so my observations were made during the rats' active hours.

#### *3.2.4.2 Adapted Cage Setup for Tail Temperature Experiment*

For the tail temperature experiment, I had to adapt my setup due to the size of the infrared camera. This equipment was too large to fit inside the recording chamber I used in other experiments. Therefore, I opted to use an isolated darkroom and a similar open-top acrylic cage (350 x 200 x 450 mm; Biomedical Engineering, Flinders Medical Centre, Bedford Park, SA, Australia).

Each rat was housed individually in these cages, which were kept at an ambient temperature of 23 ± 1 °C. I adjusted the room lighting to follow a reverse cycle (7am dark, 7pm light) in consideration of the rats' nocturnal nature. This meant my experiments were conducted during the rats' active hours, in the dark.

Throughout the course of the experiment, I ensured that each rat had ad-libitum access to food and water, maintaining consistency with the conditions from my previous setups.

### 3.2.5 Measurements

#### *3.2.5.1 Body and BAT Temperature (Tethered System)*

A tethered system involving the implanted head socket (**Section 3.2.3.1**) was used to measure body and BAT temperature in the rats of the dose-response and resident-intruder experiments. The implanted head socket was linked to the PowerLab hardware system (ADInstruments, Castle Hill, NSW, Australia) via a flexible cable and a counter-balanced swivel apparatus (SL12C, PlasticOne, Roanoka, VA, USA) positioned above the rat's cage. The electrical signals representing body and BAT temperatures were relayed from the socket to a bridge amplifier (Biomedical Engineering, Flinders University), then digitised at 1 Hz by the PowerLab hardware system. The digitised signals were recorded as degrees Celsius (°C) using the LabChart software (ADInstruments) on a Windows platform.

#### *3.2.5.2 Locomotor Activity and Behavioural Observation*

In each experiment of this chapter, except for the tail temperature experiment, I used a pyroelectric passive infrared sensor (NaPiOn, AMN1111, Panasonic, Osaka, Japan) to quantify the locomotor activity of each rat. The sensor was mounted above the cage within the recording chamber. The voltage signals generated by the sensor were fed into the PowerLab hardware system, where they were digitised at a frequency of 200 Hz.

I qualitatively observed post-injection rat behaviour, including interactions between the resident rat and the intruder during the resident-intruder experiment, using a generic video camera module. The recorded footage was archived for future review, but was not subjected to quantitative analysis.

#### *3.2.5.3 Body Temperature (Telemetric System)*

I measured the core temperatures of rats in the 5-$HT_3$ antagonist and lithium habituation experiments using the implanted telemetric temperature transmitters (**Section 3.2.3.2**). This procedure is identical to the one used for the guinea pigs in **Chapter 2**. Please see **Chapter 2** for measurement details.

#### *3.2.5.4 Tail Temperature*

An infrared camera (ThermoVision A40, FLIR Systems, Wilsonville, Oregon, United States) positioned above the acrylic cage continuously recorded the animal's blackbody radiation. The thermal data was subsequently processed using ThermaCAM Researcher Professional (FLIR Systems) software and saved as thermographic footage for later analysis.

### 3.2.6 Experimental Protocols

#### *3.2.6.1 Dose-Response*

After at least 24 hours of habituation inside the recording chamber, rats' body and BAT temperature, as well as behaviour, were stabilised. They were then injected intraperitoneally (i.p., left lower quadrant) with 0.5 mL of either the vehicle control (Ringer's solution; Fresenius Kabi, North Ryde BC, NSW, Australia) or a dose of lithium (0.47, 2.36, or 4.72 mEq/kg LiCl [Sigma-Aldrich, St. Louis, MO, USA] in Ringer's solution). These lithium doses are equivalent to 20, 100, and 200 mg/kg LiCl.

Post-injection, each rat was free to behave and was recorded for up to 24 hours before being returned to the animal facility. A 3-day washout period was ensured before administering the next dose. To account for serial effects, the first dose administered to each successive rat followed a rotating order (e.g., the first rat received the vehicle control, the second rat received 0.47 mEq/kg LiCl, the third rat received 2.36 mEq/kg LiCl, etc.).

#### 3.2.6.2 5-$HT_3$ Antagonist

The 5-$HT_3$ antagonist experiment conducted in this chapter utilised the same procedures as outlined in **Chapter 2**, including the use of identical doses. However, while the previous chapter focused on guinea pigs, this chapter applies the same protocol to rats.

For a detailed overview of the experimental protocol, including dosage information, please refer to **Section 2.2.8** of **Chapter 2**, bearing in mind the change in experimental subjects.

#### 3.2.6.3 Lithium Habituation

Rats were allowed to habituate inside the recording chamber for 12–24 hours to stabilise body temperature and behaviour. The animals then received an intraperitoneal injection (right lower quadrant) of lithium (2 mEq/kg LiCl [Sigma-Aldrich] in 0.5 mL $H_2O$ [Fresenius Kabi]). The freely behaving rats were recorded for up to 24 hours before returning to the animal facility. This process, beginning at the habituation period, was repeated so that the rats received the same dose of lithium 3 days after the previous injection. Once the rats were injected with lithium on day 1, 4, and 7, a final dose was given 4 days later (on day 11). The experiment concluded after each rat received a total of 4 successive injections.

#### 3.2.6.4 Tail Temperature

After habituating inside the acrylic cage for at least 1 hour to stabilise tail skin temperature, the rats were intraperitoneally (i.p.) injected (lower right quadrant) with either the control (0.9% saline, 0.5 mL; Fresenius Kabi) or lithium (2 mEq/kg LiCl [Sigma-Aldrich] in 0.5 mL $H_2O$ [Fresenius Kabi]). The animals behaved freely and were recorded for up to 6 hours before being returned to the animal facility. A 3-day washout period occurred before the next dose. The first dose in each animal was given in alternating order (i.e., if the first animal received control, the next animal received lithium) to account for serial effects.

#### 3.2.6.5 Resident-Intruder

Each ‘resident’ rat was allowed to habituate inside the recording chamber for at least 24 hours to stabilise body and BAT temperature, as well as behaviour. After this period, the resident rat was injected intraperitoneally with either 0.5 mL of the vehicle control (Ringer's solution; Fresenius Kabi),

or a lithium dose (0.47 or 4.72 mEq/kg LiCl; Sigma-Aldrich) suspended in 0.5 mL Ringer's solution. These lithium doses are equivalent to 20 and 200 mg/kg LiCl.

Thirty minutes post-injection, an 'intruder' rat was introduced into the recording chamber as a salient psychological stressor for the resident rat. This intruder, contained within a ventilated acrylic box, was placed on the opposite side of the cage to the resident rat. They were unable to physically interact but could see and smell each other (Mohammed et al., 2014).

After the intruder had been in the recording chamber for 30 minutes, it was removed. The resident rat continued to be recorded for up to 24 hours. Subsequently, the rat was returned to the animal facility, where a 3-day washout period was ensured before the next dose was administered. To account for serial effects, the first dose given to each rat followed a rotating order.

### 3.2.7 Humane endpoint

Once experiments were finished, animals were humanely euthanised with an i.p. injection of pentobarbitone sodium (180 mg/kg; Virbac Pty Limited, Milperra, NSW, Australia).

### 3.2.8 Data analysis

All data was processed in Igor Pro (WaveMetrics, Portland, OR, USA). Recordings that were either too noisy or broken, due to random occurrences, were excluded from the results.

#### *3.2.8.1 Body and BAT Temperature (Tethered System)*

The tethered body and BAT temperature data, acquired as outlined in **Section 3.2.5.1**, underwent a comprehensive preprocessing phase. This phase was designed to ensure the integrity of the data for subsequent analysis.

Initially, I applied a noise reduction process, specifically targeting sporadic fluctuations such as notch noise. I interpolated datapoints surrounding any identified noise spikes, leveraging the continuity of physiological signals to preserve the underlying trend while eliminating aberrations.

Following noise reduction, I implemented a downsampling procedure, converting the original 1 Hz data frequency to a less granular rate of one data point per minute. This transformation maintained the overall pattern of temperature changes, while reducing computational complexity.

For the evaluation of injection effects, I computed delta values. These represent deviations in temperature post-injection, benchmarked against the average baseline temperature within the 5–10 minutes preceding the injection. This approach allowed me to quantify the relative impact of vehicle or lithium injection on body and BAT temperatures, accounting for individual baseline variations.

#### *3.2.8.2 Body Temperature (Telemetric System)*

Adopting a similar approach as outlined in **Section 3.2.8.1**, I carried out a thorough preprocessing phase for the telemetric body temperature data obtained as detailed in **Section 3.2.5.3**. The data, initially recorded at a frequency of 10 Hz, was downsampled to a frequency of one data point per minute. I then calculated delta values using the same 5–10-minute pre-injection baseline period employed in **Section 3.2.8.1**, enabling me to assess the relative impact of the injections on body temperature.

#### *3.2.8.3 Locomotor Activity*

Building upon the data acquisition detailed in **Section 3.2.5.2**, I transformed the digitised voltage signals, representing periods of movement and rest, into a binary format: '1' for periods when the rat was moving, and '0' for periods of rest. This transformation allowed me to standardise the distinction between periods of activity and inactivity.

The duration of movement was quantified by counting the number of '1's per minute, effectively translating this to seconds of movement per minute (sec/min), providing a precise measure of the rats' locomotor activity.

Finally, these sec/min values were cumulated over time, starting from the point of lithium or vehicle injection. This allowed me to observe the cumulative locomotor activity over the course of the experiment.

#### *3.2.8.4 Tail Temperature*

From the thermographic footage obtained as described in **Section 3.2.5.4**, I extracted the maximum skin temperature along the tail at one-minute intervals. This provided me with a detailed timeline of tail temperature changes.

To assess how tail temperature evolved in response to the injection, I computed delta (change) values. These values represent the deviation of the maximum tail temperature at each time point from the average baseline temperature calculated over the 1–5 minutes prior to injection.

### 3.2.9 Statistical Analysis

Statistical analyses were performed using Prism software (GraphPad, San Diego, CA, USA).

All dose-response analyses incorporated log-transformed lithium doses. This procedure not only satisfies the linearity assumption intrinsic to regression models but also aligns with the logarithmic behaviour commonly observed in many biological systems, including dose-response relationships (Gupta, 2018). The control dose (vehicle) was integrated into the analyses by assigning it a value one log unit lower than the smallest lithium dose, which is equivalent to a tenfold decrease in the original dose. This approach circumvents the mathematical issue associated with the undefined logarithm of zero, and importantly, it ensures the meaningful inclusion of the control data that would otherwise be excluded.

All results are presented as mean ± SEM. The significance level was predetermined at $P < 0.05$. The following subsections detail the statistical methods applied in each experiment.

#### *3.2.9.1 Dose-Response*

The dose-response relationship between lithium administration and the measured variables (BAT and body temperature, and locomotor activity) was assessed using linear regression analyses. The greatest decrease in BAT and body temperature from the injection point, observed within 30–90 minutes post-injection, and the cumulative seconds of locomotor activity at specified time points (e.g., 190 minutes post-injection) were regressed against log-transformed lithium doses.

Correlations between BAT and body temperature, and locomotor activity were evaluated using correlation analysis.

#### *3.2.9.2 5-HT$_3$ Antagonist*

In the 5-HT$_3$ antagonist experiment, a repeated measures one-way ANOVA was employed to compare the mean greatest decrease in body temperature 30–90 minutes post-lithium injection, across different pre-treatment groups (saline control, ondansetron, and palonosetron). A repeated measures two-way ANOVA with Tukey's multiple comparisons test was used to examine the effects of pre-treatment and time on locomotor activity (cumulative seconds of movement), and to assess the interaction between these two factors. Additionally, a repeated measures one-way ANOVA was used to compare cumulative seconds of movement at a single time point (240 minutes post-lithium) among the pre-treatment groups.

#### *3.2.9.3 Lithium Habituation*

In the lithium habituation experiment, linear regression analyses were used to evaluate the relationship between the day of injection (1, 4, 7, and 11) and the greatest decrease in body temperature from the injection point, within 30–90 minutes post-lithium, as well as the cumulative seconds of movement at various time points post-injection. Due to a random recording error resulting in a missing data point, a mixed-effects model (restricted maximum likelihood) was employed instead of a repeated measures ANOVA, which cannot accommodate missing values. This model was used in conjunction with Tukey's multiple comparisons test to compare the cumulative distance moved at 240 minutes post-injection across different days.

#### *3.2.9.4 Tail Temperature*

Linear regression analyses were conducted every minute post-injection to investigate the change in tail skin temperature between the control and lithium groups. A significant non-zero slope at any given minute indicated a significant difference in tail skin temperature between the groups. The $R^2$ values for multiple significant linear regressions were reported as a range instead of individual values.

The maximal change (either positive or negative) in tail skin temperature was recorded within the 0 to 30 minutes post-injection timeframe. A paired t test was used to compare this maximal change in tail skin temperature between the control and lithium groups.

#### *3.2.9.5 Resident-Intruder*

In the resident-intruder experiment, linear regression analyses were conducted to evaluate the relationships between the administered lithium dose and several response variables. These variables included the maximal change in both BAT and body temperature during intruder exposure (occurring 30–60 minutes post-injection), as well as the percentage of time spent moving during intruder exposure.

To assess the presence of emotional hyperthermia in response to intruder exposure, changes in BAT and body temperature were evaluated during the 30-minute window following intruder introduction (30–60 minutes post-injection). For each dose group, including the vehicle (Ringer's solution) group, the temperature values were calculated as deviations from the baseline temperature at the precise moment the intruder was introduced (set to zero). A one-sample t-test was then performed to determine if the maximum temperature change during the intruder exposure window was significantly different from zero. This approach allowed for a direct evaluation of whether significant increases in BAT and body temperature, indicative of emotional hyperthermia, occurred in response to the intruder stimulus.

The effects of differing lithium doses on the subjects' activity were examined using a one-way ANOVA followed by Dunnett's multiple comparisons test. This approach facilitated a comparison of the percentage of time spent moving during intruder exposure between rats injected with vehicle and those treated with various lithium doses.

Additionally, correlation analyses were used to explore the relationships between BAT and body temperature, and between these variables and locomotor activity during the intruder exposure period.

## 3.3 Results

### 3.3.1 Lithium Dose-Response: Effects on Body and BAT Temperature, and Behavioural Activity

Increasing doses of intraperitoneal lithium led to greater reductions in BAT (**Figure 3.1**) and body temperature (**Figure 3.2**) over time. These changes took effect shortly after injection. Minimum values of change in BAT and body temperature 30–90 minutes after injection are shown in **Table 3.1**. Linear regressions showed that lithium dose-dependently reduced BAT ($R^2$ = 0.621, P < 0.0001, **Figure 3.3 Left**) and body temperature ($R^2$ = 0.701, P < 0.0001, **Figure 3.3 Right**). BAT and body temperature showed a significant positive correlation ($R^2$ = 0.583, P < 0.0001, **Figure 3.4**).

**Table 3.1.** Effect of lithium dose on minimum change in BAT and body temperature 30–90 minutes post-injection. Change is relative to 5–10 minutes before injection. Data represents mean ± SEM.

| LiCl dose | Sample number | Min BAT temperature (Δ °C) | Min body temperature (Δ °C) |
|---|---|---|---|
| Ringer's solution | 6 | 0.14 ± 0.14 | 0.02 ± 0.06 |
| 0.47 mEq/kg | 6 | -0.37 ± 0.27 | -0.36 ± 0.13 |
| 2.36 mEq/kg | 4 | -0.69 ± 0.15* | -1.15 ± 0.17** |
| 4.72 mEq/kg | 5 | -1.46 ± 0.05*** | -1.79 ± 0.30*** |

Significantly different from vehicle (Dunnett's): * P < 0.03, ** P < 0.001, *** P < 0.0001.

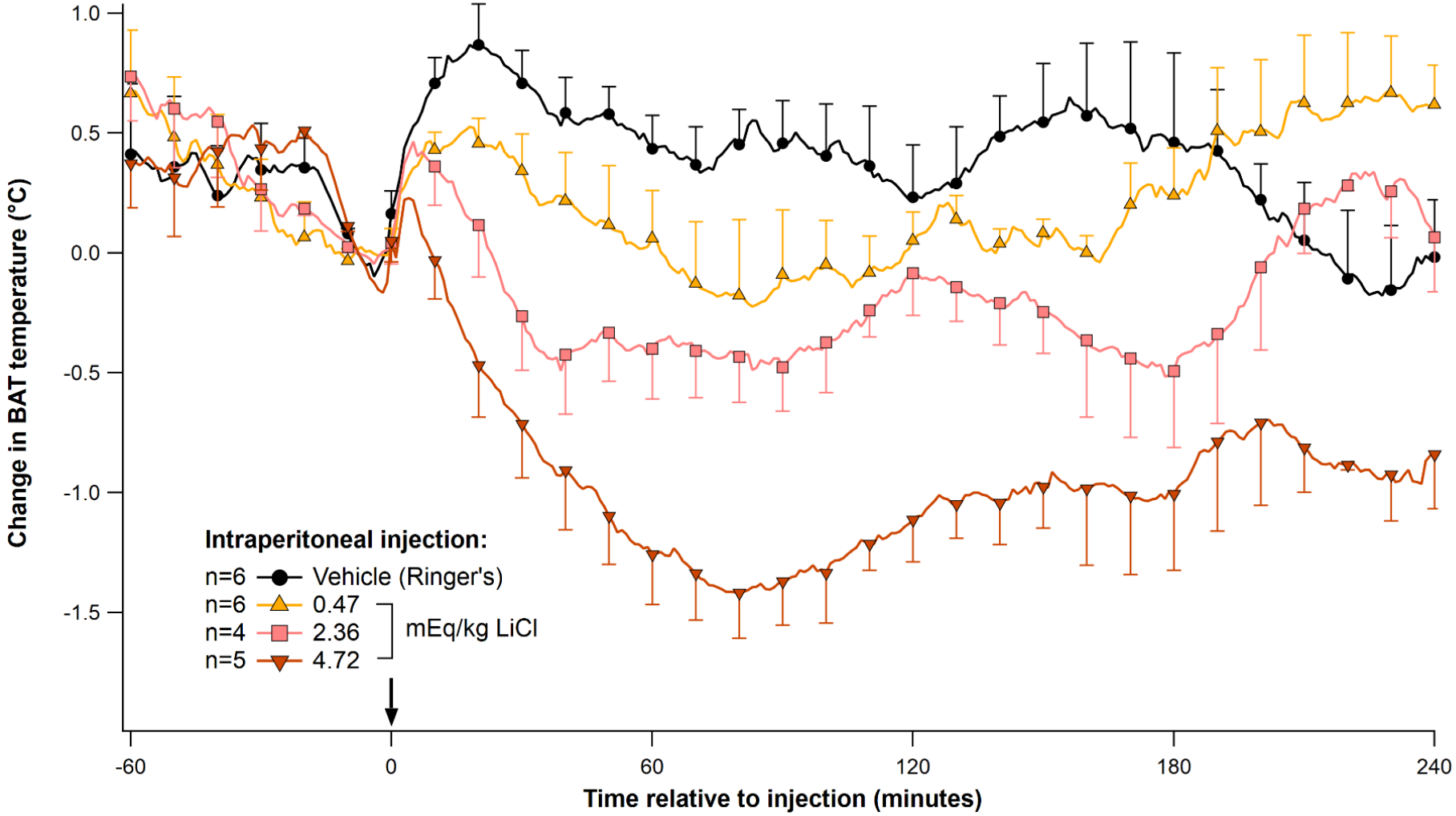


**Figure 3.1.** Change in brown adipose tissue (BAT) temperature of rats over time after intraperitoneal injection of lithium (0.47, 2.36, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution) at 0 minutes. Datapoints represent mean ± SEM. Sample numbers indicated on graph.

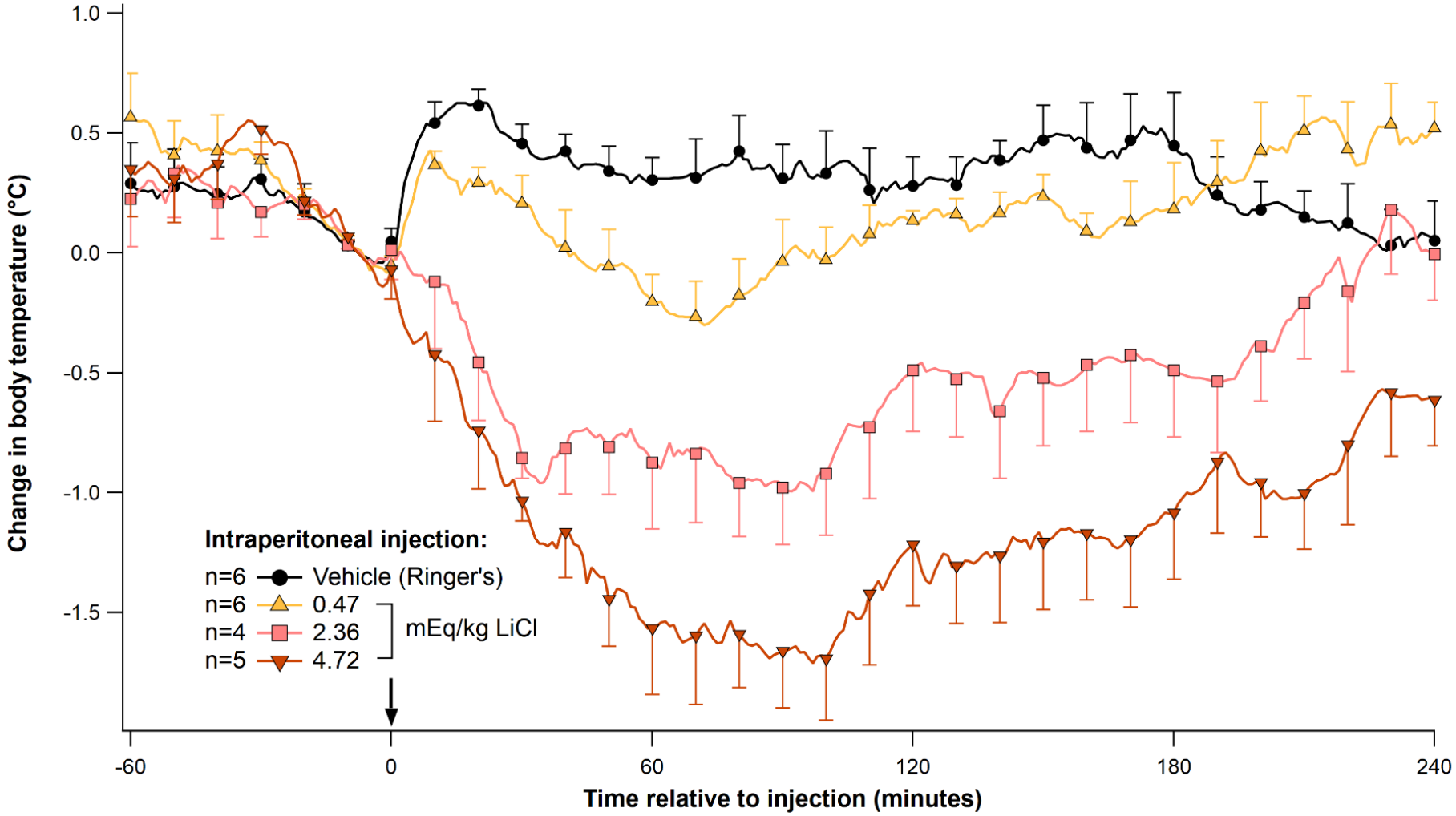


**Figure 3.2.** Change in rat core body temperature over time after intraperitoneal injection of lithium (0.47, 2.36, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution) at 0 minutes. Datapoints represent mean ± SEM. Sample numbers indicated on graph.

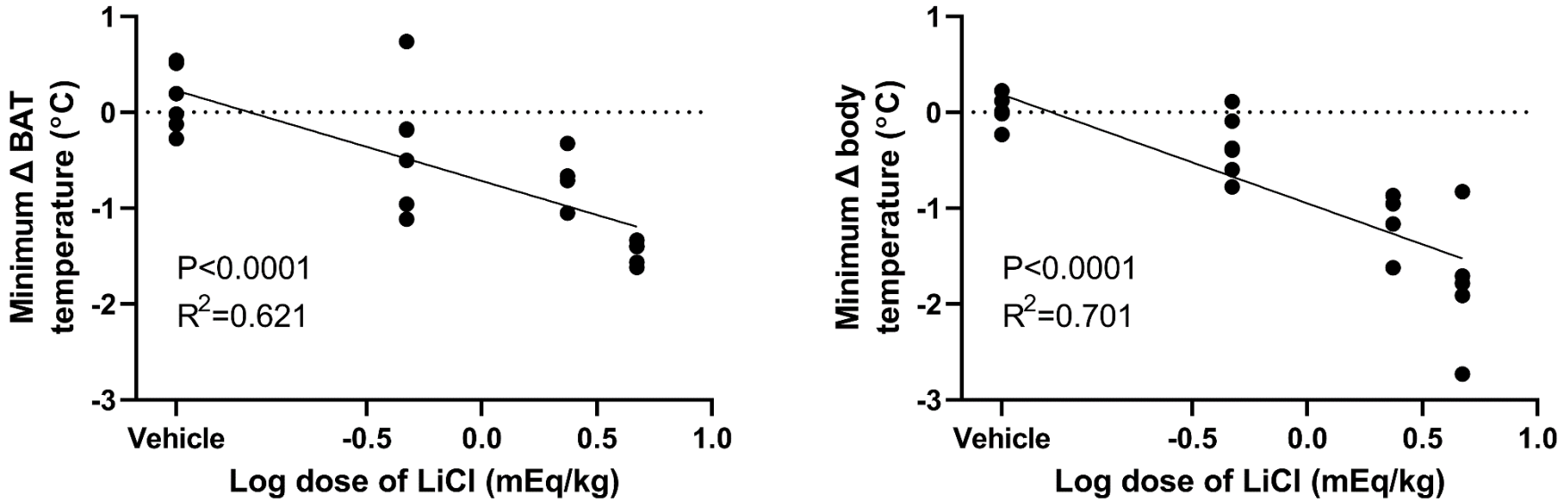


**Figure 3.3. Left:** Linear regression of minimum change in rat brown adipose tissue (BAT) temperature 30–90 minutes post-injection against increasing log-transformed doses of lithium (Ringer's solution vehicle, 0.47, 2.36, and 4.72 mEq/kg LiCl). **Right:** Linear regression of minimum change in rat core body temperature 30–90 minutes post-injection against increasing log-transformed doses of lithium (Ringer's solution vehicle, 0.47, 2.36, and 4.72 mEq/kg LiCl). Datapoints represent individual animals. From left to right on both x-axes, n = 6, 6, 4, and 5.

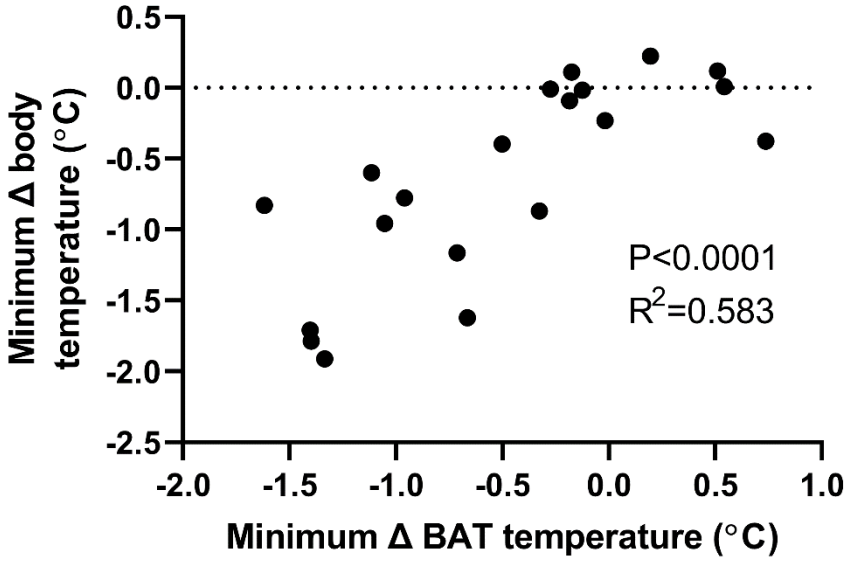


**Figure 3.4.** Correlation between minimum change in rat brown adipose tissue (BAT) and core body temperatures 30–90 minutes post-injection at respective doses of lithium (0.47, 2.36, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution). Datapoints represent individual animals.

Cumulative seconds of rat movement after each intraperitoneal dose of lithium is shown in **Figure 3.5**. As the dose of LiCl increased, rat locomotor activity decreased. Every 10 minutes after the injection point, a linear regression was run between cumulative seconds of movement and log-transformed LiCl dose. From 60 minutes post-injection, each linear regression showed a significant negative relationship (briefly highlighted in **Figure 3.5**). The linear regression at 190 minutes post-injection is highlighted in **Figure 3.6**, showing that lithium dose-dependently decreased time spent moving by rats ($R^2 = 0.431$, $P < 0.002$). At 190 minutes post-injection, cumulative seconds moved showed a significant positive correlation with BAT ($R^2 = 0.229$, $P < 0.04$, **Figure 3.7 Left**) and body temperature ($R^2 = 0.305$, $P < 0.01$, **Figure 3.7 Right**).

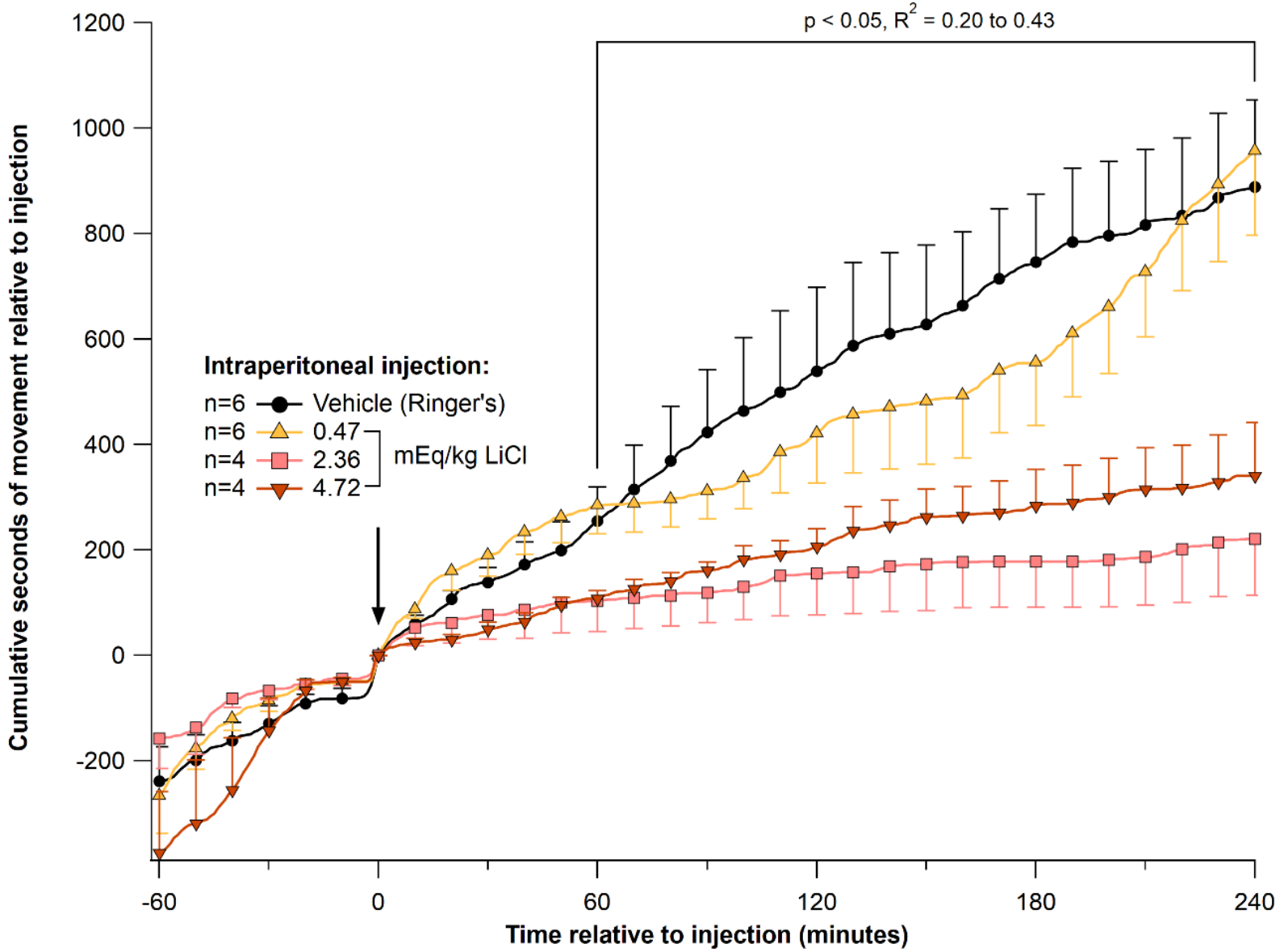


**Figure 3.5.** Cumulative seconds of movement by rats over time from intraperitoneal injection point of lithium (0.47, 2.36, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution) at 0 minutes. Bracket above 60–240 minutes represents multiple significant linear regressions at each timepoint between cumulative distance moved and log-transformed lithium doses. Datapoints represent mean ± SEM. Sample numbers indicated on graph.

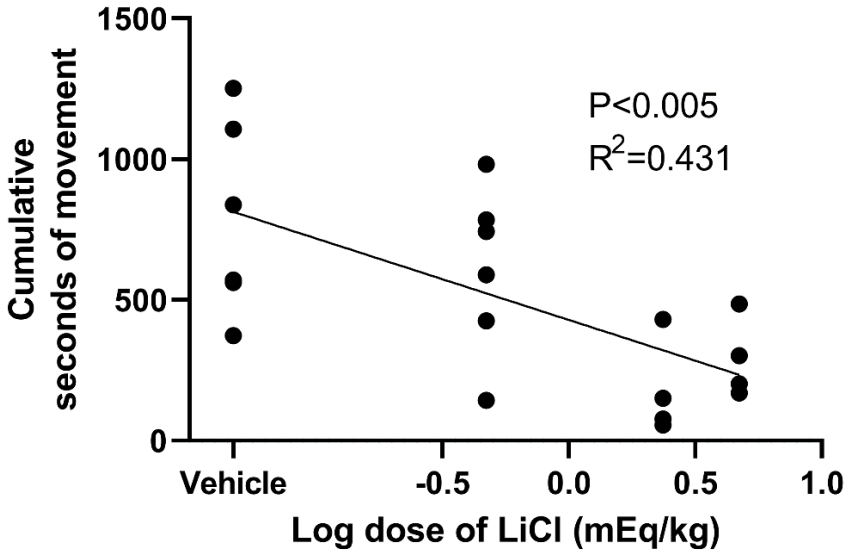


**Figure 3.6.** Linear regression: Cumulative seconds of rat movement at 190 minutes post-injection against increasing log-transformed doses of lithium (Ringer's solution vehicle, 0.47, 2.36, and 4.72 mEq/kg LiCl). Datapoints represent individual animals. From left to right on the x-axis, n = 6, 6, 4, and 4.

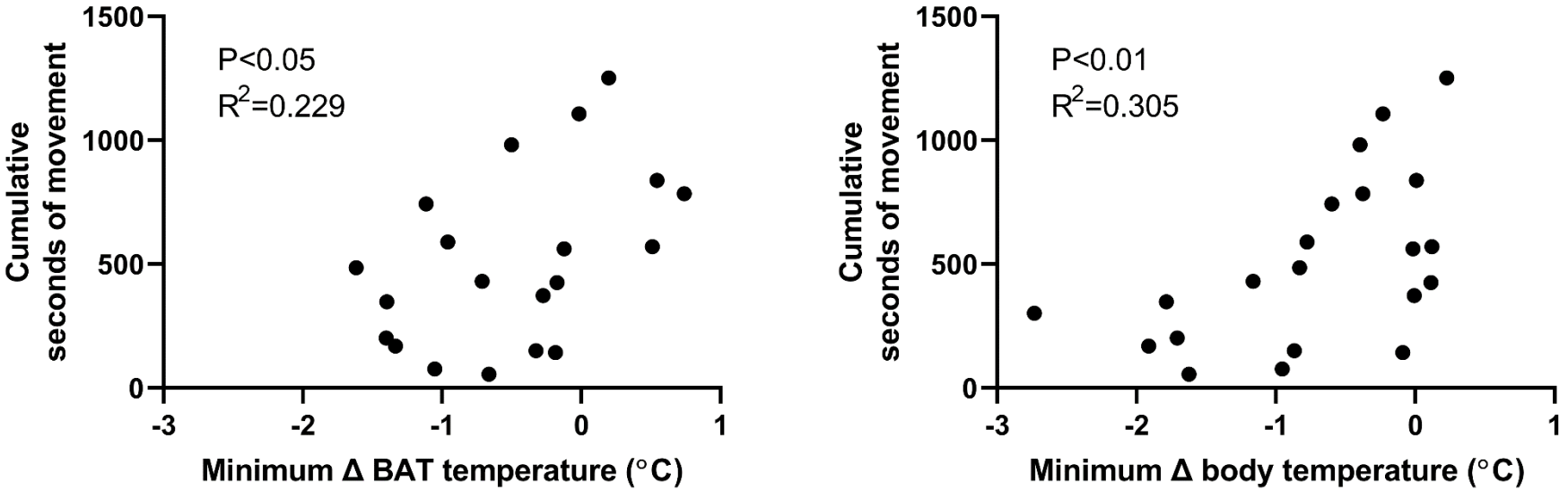


**Figure 3.7. Left:** Correlation between minimum rat delta brown adipose tissue (BAT) temperature 30–90 minutes post-injection and cumulative seconds of rat movement at 190 minutes post-injection. **Right:** Correlation between minimum rat delta core body temperature 30–90 minutes post-injection and cumulative seconds of rat movement at 190 minutes post-injection. Datapoints represent individual animals that received an intraperitoneal dose of vehicle (Ringer's solution) or lithium (0.47, 2.36, 4.72 mEq/kg LiCl).

### 3.3.2 Lithium with 5-$HT_3$ Antagonist Pre-treatment: Influence on Body Temperature and Behavioural Activity

Lithium led to a similar reduction in body temperature over time when given 30 minutes after saline control, ondansetron, or palonosetron (**Figure 3.8**). The mean minimum change in body temperature between 30 and 90 minutes post-lithium was not significantly different across the three groups (Control: -1.37 ± 0.15, Ondansetron: -0.99 ± 0.24, Palonosetron: -1.23 ± 0.21 °C, repeated measures one-way ANOVA, P = 0.4530, **Figure 3.9**).

After injection of lithium, the cumulative seconds of movement were not significantly different over time between the three groups (repeated measures two-way ANOVA: treatment, P = 0.6855; time-treatment interaction, P = 0.9911, **Figure 3.10**). At 240 minutes post-lithium, the mean cumulative seconds of movement were not significantly different between the groups (Control: 1208 ± 128.8, Ondansetron: 1262 ± 154.4, Palonosetron: 1016 ± 125.3 seconds; repeated measures one-way ANOVA, P = 0.4096, **Figure 3.11**).

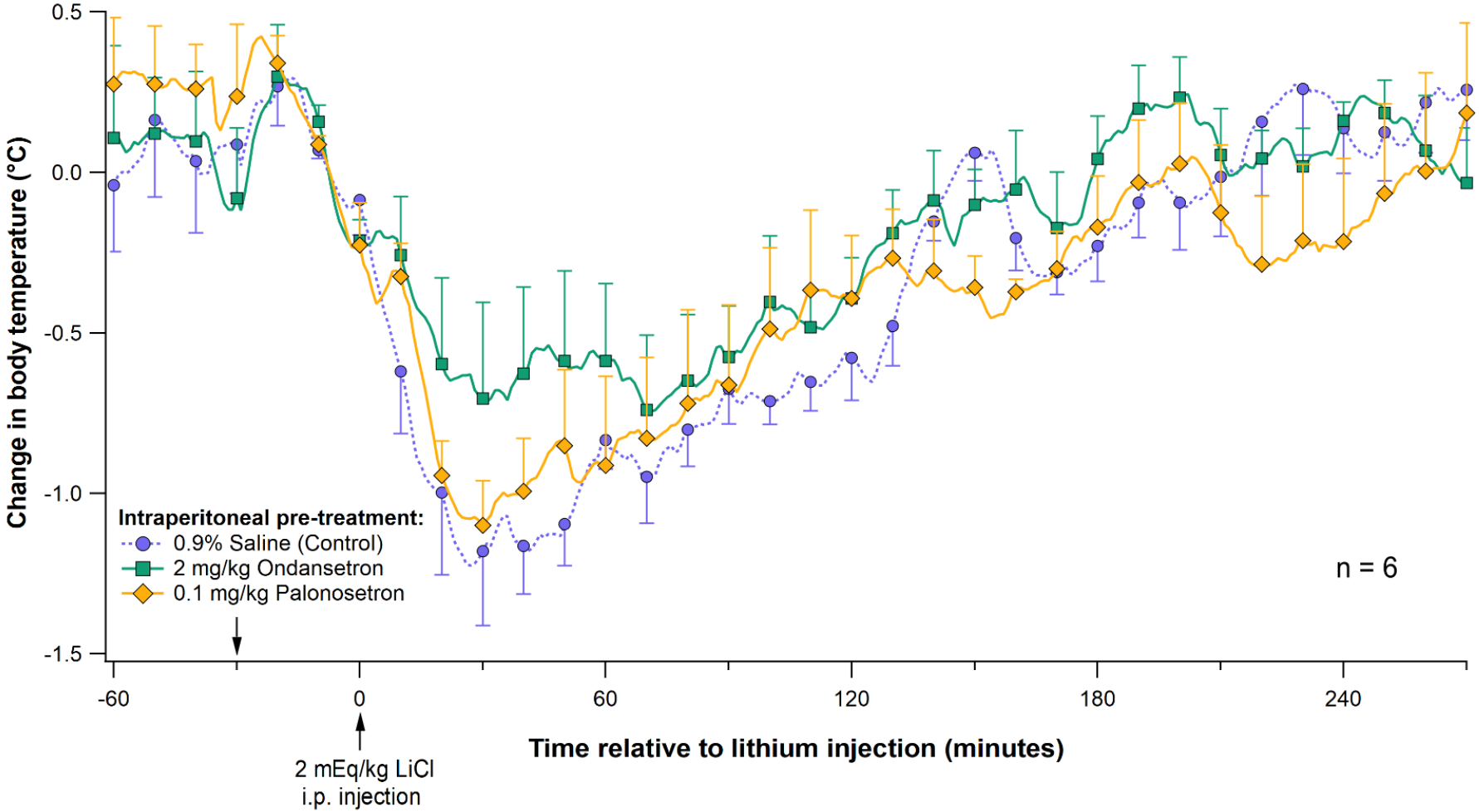


**Figure 3.8.** Change in rat core body temperature over time after intraperitoneal injection of lithium (2 mEq/kg LiCl) at 0 minutes. Groups received intraperitoneal $5HT_3$ antagonist pre-treatment (0.9 % saline control, 2 mg/kg ondansetron, or 0.1 mg/kg palonosetron) 30 minutes before lithium. Datapoints represent mean ± SEM, n = 6.

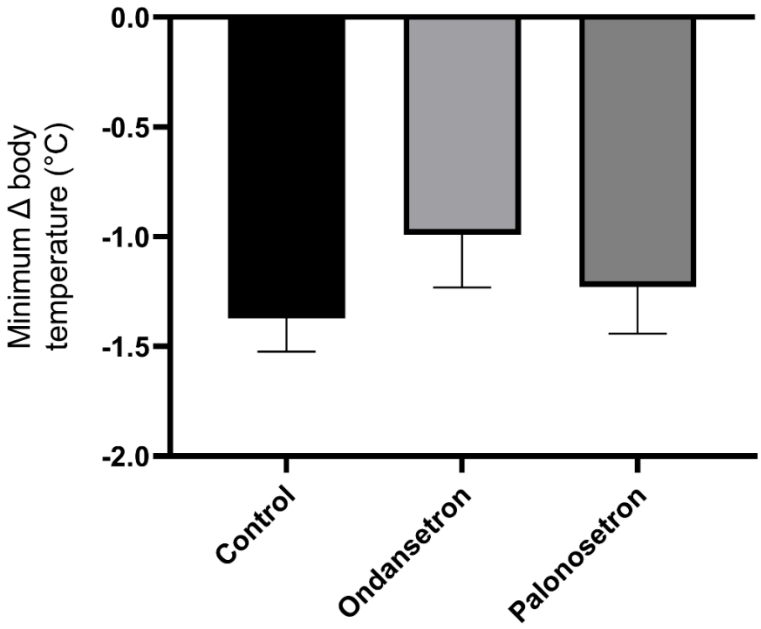


**Figure 3.9.** Minimum delta body temperature value between 30–90 minutes post-lithium (2 mEq/kg LiCl, i.p.) among $5HT_3$ antagonist pre-treatment groups (0.9 % saline control, 2 mg/kg ondansetron, or 0.1 mg/kg palonosetron, i.p.). Bars represent mean ± SEM, n = 6.

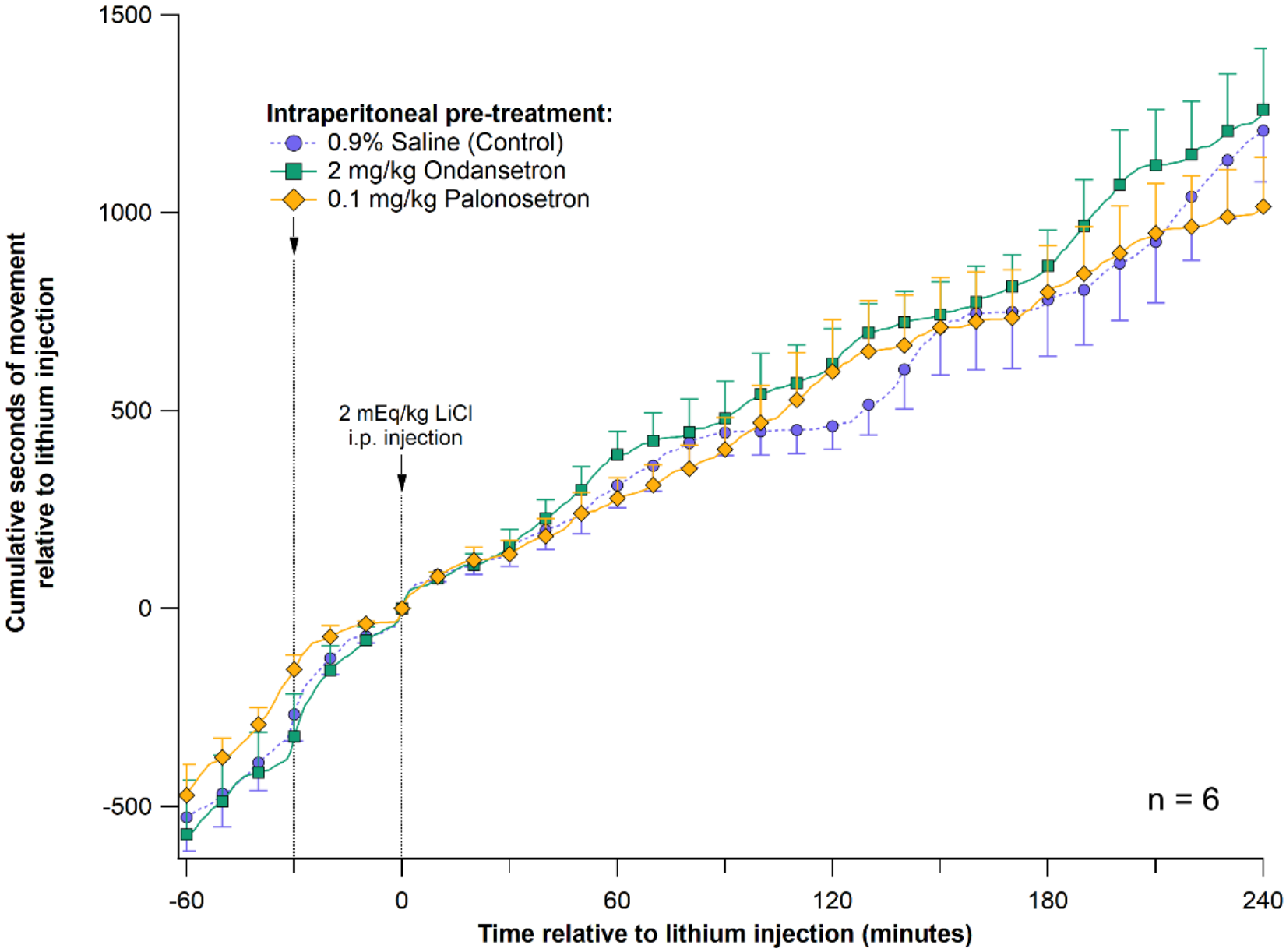


**Figure 3.10.** Cumulative seconds of rat movement over time from lithium (2 mEq/kg LiCl) intraperitoneal injection point at 0 minutes. Groups received intraperitoneal $5HT_3$ antagonist pre-treatment (0.9 % saline control, 2 mg/kg ondansetron, or 0.1 mg/kg palonosetron) 30 minutes before lithium. Datapoints represent mean ± SEM, n = 6.

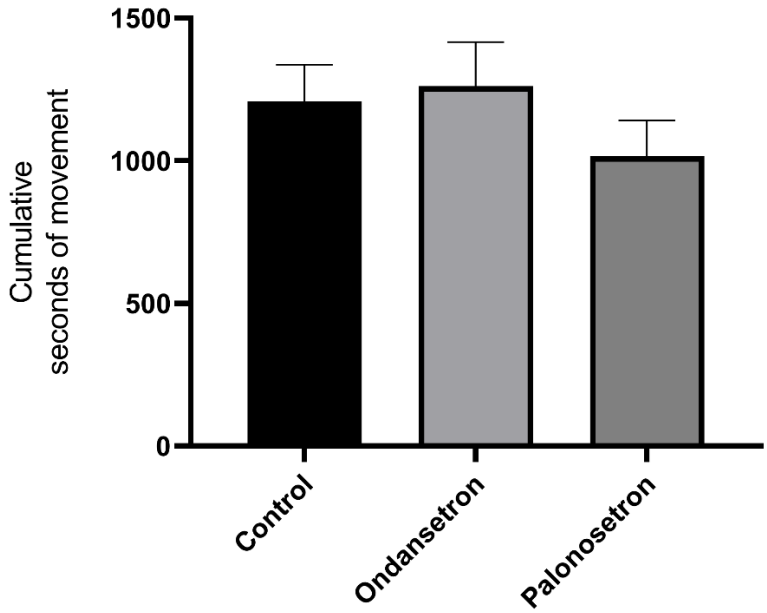


**Figure 3.11.** Cumulative seconds of rat movement at 240 minutes post-lithium (2 mEq/kg LiCl, i.p.) among $5HT_3$ antagonist pre-treatment groups (0.9 % saline control, 2 mg/kg ondansetron, or 0.1 mg/kg palonosetron, i.p.). Bars represent mean ± SEM, n = 6.

### 3.3.3 Repeated Doses of Lithium: Influence on Body Temperature and Locomotor Activity

After repeated intraperitoneal injections of lithium chloride in rats on days 1, 4, 7, and 11, body temperature decreased similarly each time (**Figure 3.12**). Linear regression showed no significant relationship between day of injection and minimum change in body temperature 30–90 minutes post-lithium ($R^2$ = 0.025, P = 0.4577, **Figure 3.13**). If I ignore the temporal continuity and analyse minimum change in body temperature between the days with repeated measures one-way ANOVA with Tukey's multiple comparisons test, none of the groups were significantly different (P > 0.05).

Cumulative seconds of rat movement increased similarly after each lithium injection on days 1, 4, 7, and 11 (**Figure 3.14**). Every 10 minutes post-injection, multiple linear regressions showed no relationship between day of injection and cumulative seconds of movement ($R^2$ = 0.008 to 0.128, P = 0.094 to 0.679). The linear regression between these two parameters at 240 minutes post-injection is shown in **Figure 3.15** ($R^2$ = 0.116, P = 0.1122). Even when breaking temporal continuity and applying a mixed-effects model (restricted maximum likelihood) with Tukey's multiple comparisons test, cumulative distance moved at 240 minutes post-injection was not significantly different between any of the days (P > 0.05).

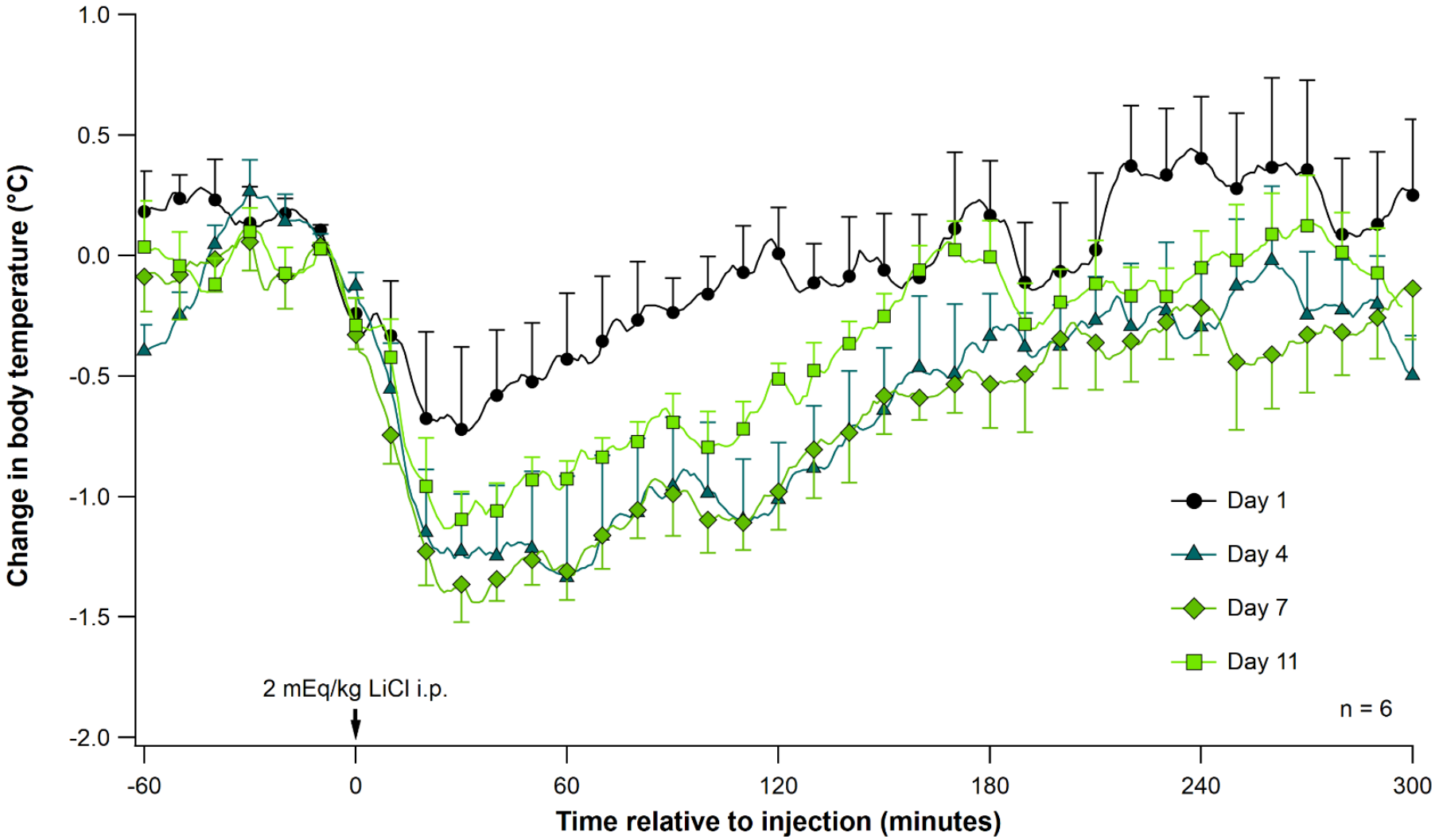


**Figure 3.12.** Rat core body temperature over time in response to successive intraperitoneal injections of lithium (2 mEq/kg LiCl, at 0 minutes) on days 1, 4, 7, and 11. Datapoints represent mean ± SEM, n = 6.

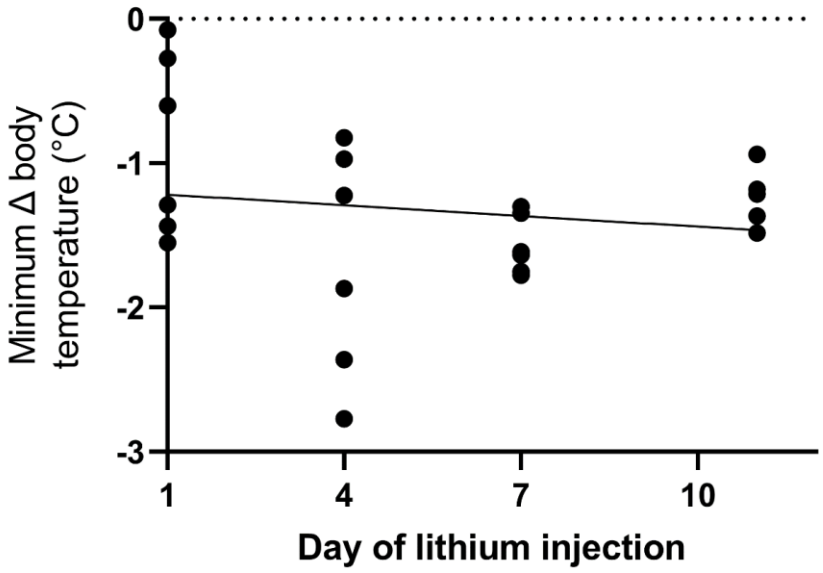


**Figure 3.13.** Linear regression: Minimum values of delta core body temperature between 30–90 minutes post-lithium (2 mEq/kg LiCl, i.p.) on each day of injection (1, 4, 7, & 11). Datapoints represent individual animals, n = 6 per day.

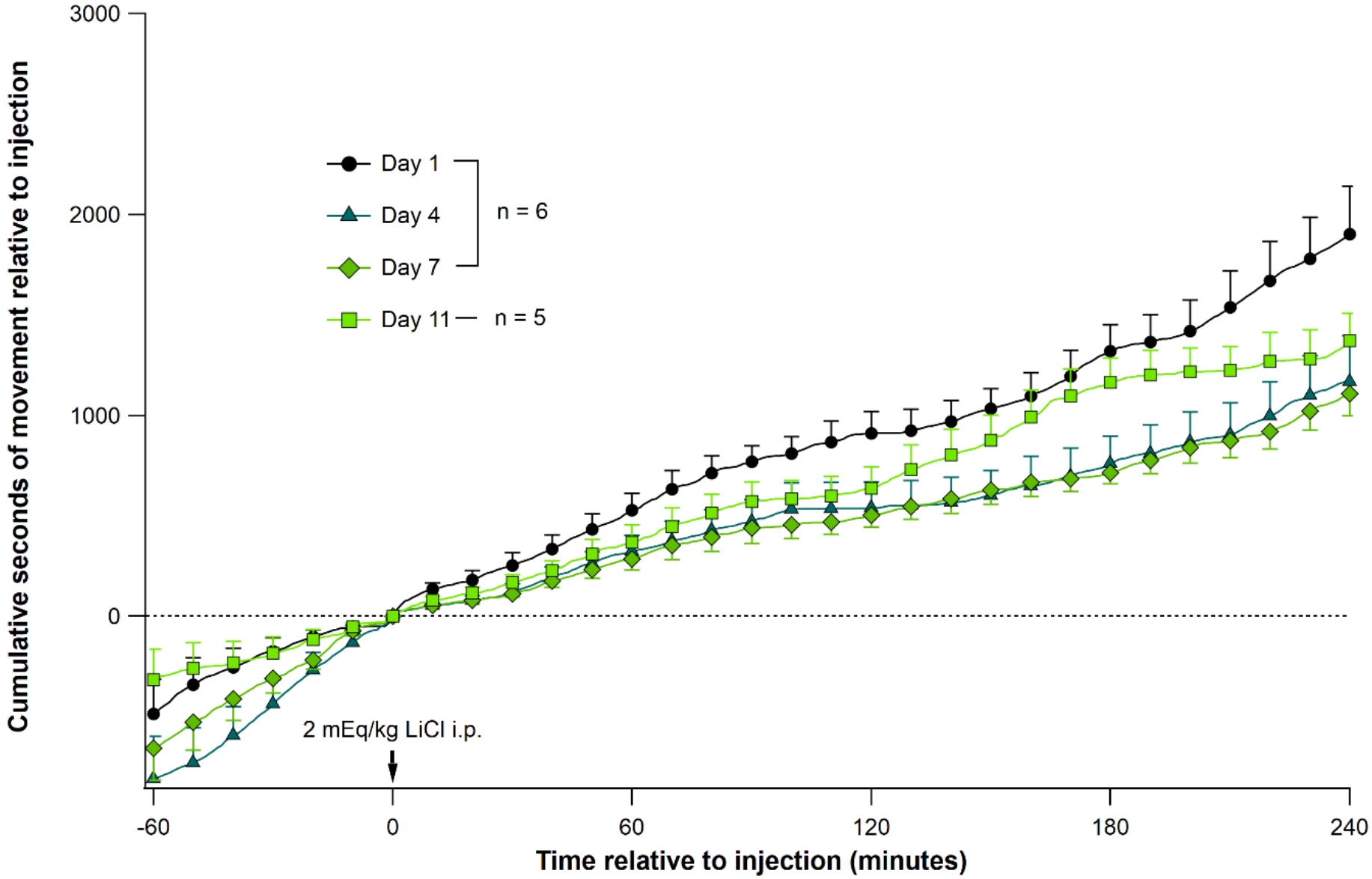


**Figure 3.14.** Cumulative seconds of rat movement over time from intraperitoneal injection point of lithium (2 mEq/kg LiCl, at 0 minutes) on days 1, 4, 7, and 11. Datapoints represent mean ± SEM, n = 6 on days 1, 4, & 7, n = 5 on day 11.

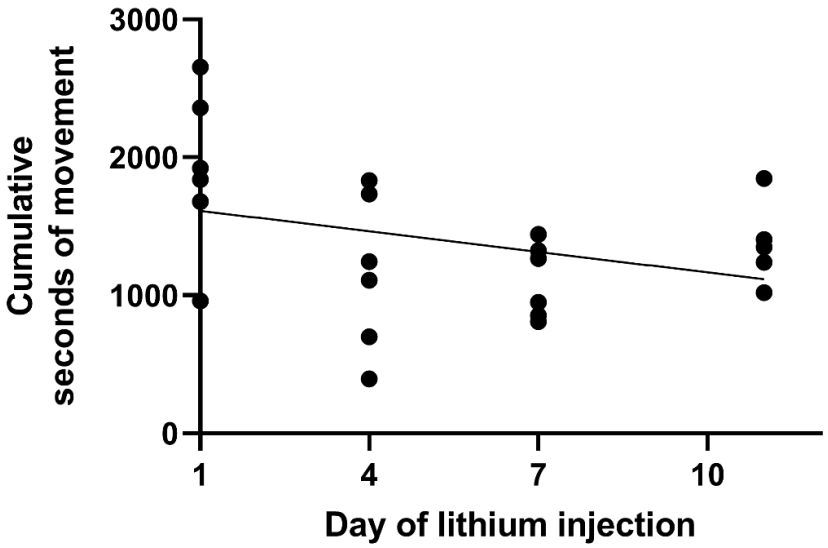


**Figure 3.15.** Linear regression: Cumulative seconds of rat movement at 240 minutes post-lithium (2 mEq/kg LiCl, i.p.) on each day of injection (1, 4, 7, & 11). Datapoints represent individual animals, n = 6 on days 1, 4, & 7, n = 5 on day 11.

### 3.3.4 Effect of Lithium on Tail Skin Temperature: Changes in Response to Intraperitoneal Injection

The intraperitoneal injection of lithium chloride led to an observable increase in tail skin temperature, a contrast to the decrease noticed following the saline control injection (**Figure 3.17**). Note that the value at 0 minutes is the average of tail temperature captured directly preceding and proceeding the injection. A linear regression between tail skin temperature and log-transformed dose was run every minute after injection; from 1 to 29 minutes, the slopes were significantly non-zero (briefly highlighted in **Figure 3.17**).

Please see **Figure 3.16** for representative infrared thermographs from one animal post-injection of control (**left**) and lithium (**right**).

The mean maximal change in tail skin temperature, captured between 0 and 30 minutes post-injection with control and lithium chloride, was -1.49 ± 1.12 and 3.74 ± 1.14, respectively (**Figure 3.18**). These differences were found to be statistically significant (paired t test, $P = 0.0418$, $R^2 = 0.5258$).

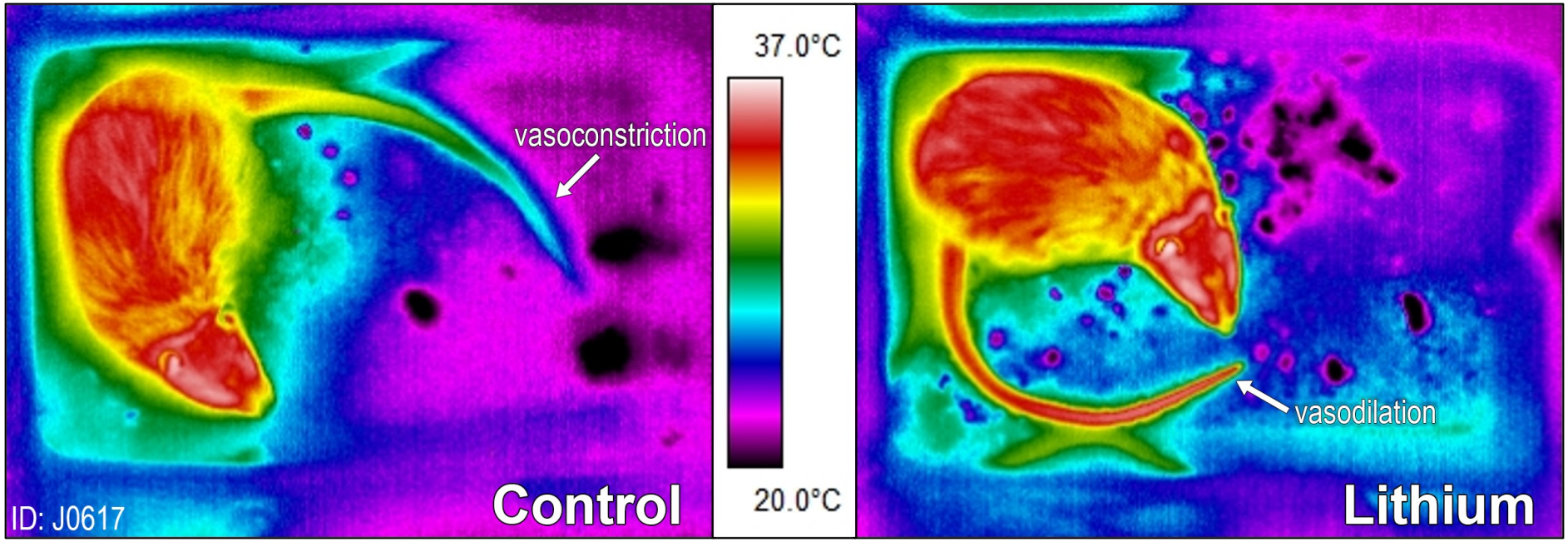


**Figure 3.16.** Example infrared thermographs from one animal illustrating temperature changes post-intraperitoneal injection. The left panel presents the animal 5 minutes and 40 seconds after injection with 0.5 mL of 0.9% saline control, while the right panel shows the same time-point after injection with 2 mEq/kg LiCl in 0.5 mL $H_2O$. A temperature colour scale, placed in the middle, serves as a reference. Arrows at the tail tips signify the observed temperature changes: a colder temperature (indicative of vasoconstriction) is observed following saline injection in the left thermograph, while a warmer temperature (indicative of vasodilation) is noted following lithium injection in the right thermograph.

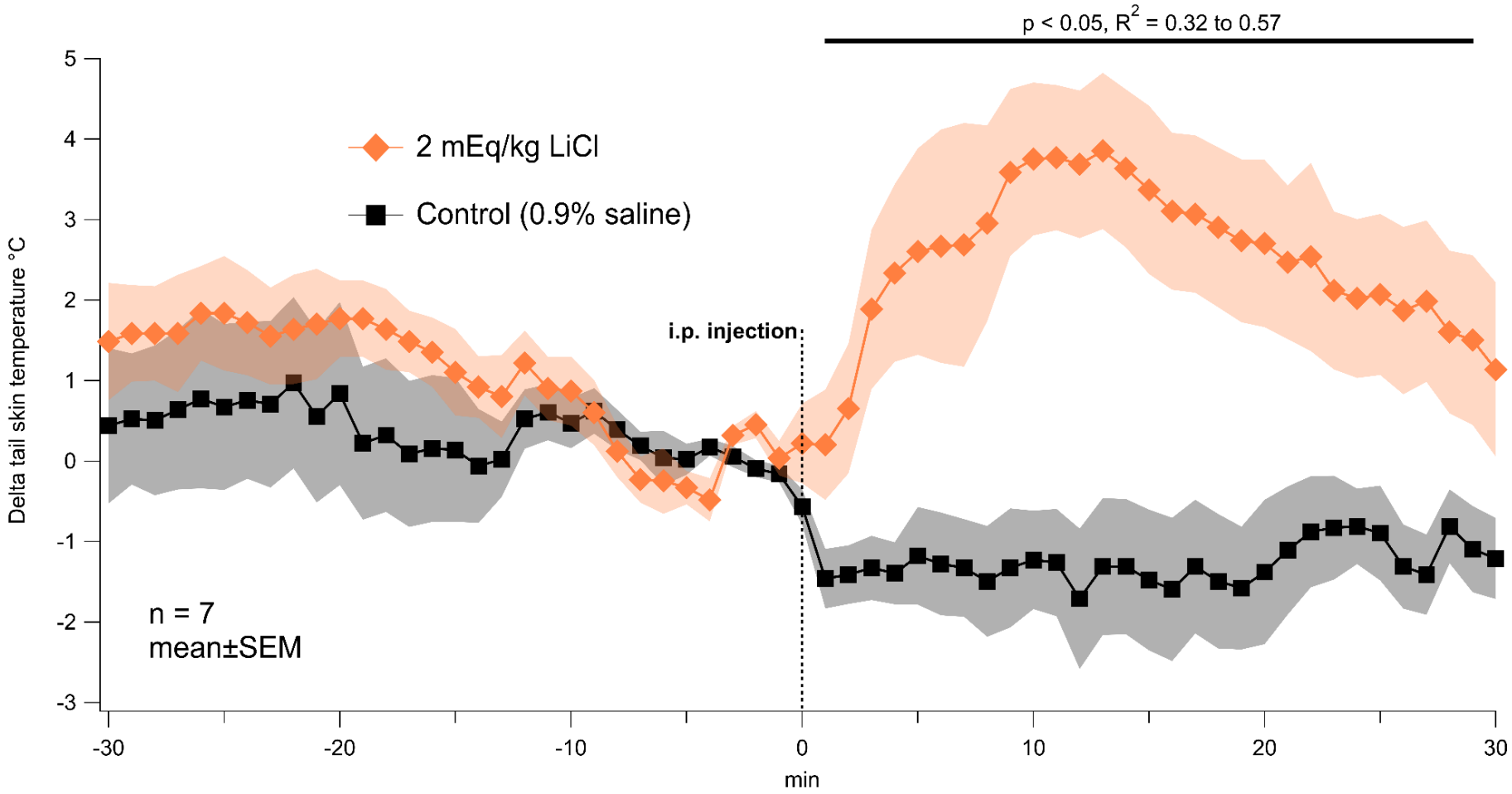


**Figure 3.17.** Change in rat tail skin temperature over time in response to an intraperitoneal dose of control (0.9% saline) or lithium (2 mEq/kg LiCl) given at 0 minutes. Bracket above 1–29 minutes represents multiple significant linear regressions of delta tail skin temperature at each minute between groups. Datapoints and shading represent mean ± SEM, n = 7.

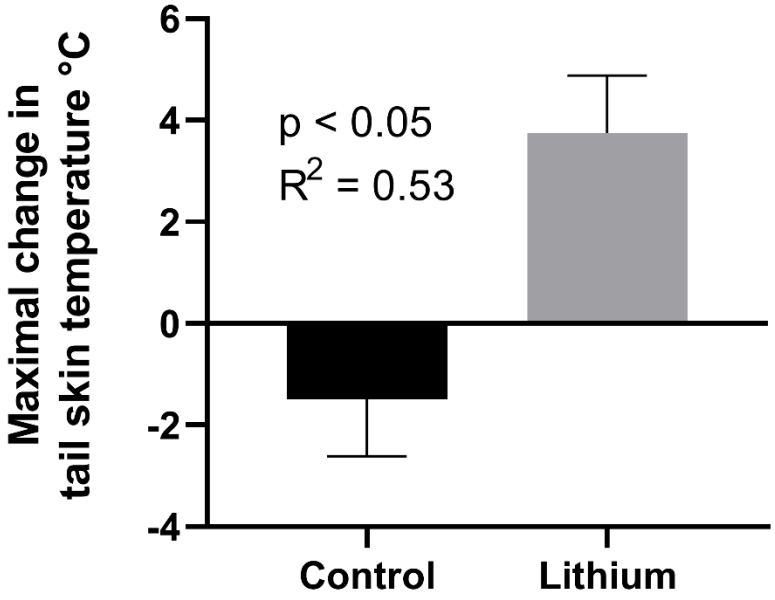


**Figure 3.18.** Maximal change in tail skin temperature within 30 minutes after intraperitoneal injection of control (0.9% saline) or lithium (2 mEq/kg LiCl). Bars represent mean ± SEM, n = 7.

### 3.3.5 Lithium Resident-Intruder Experiment: Impact on BAT Thermogenesis, Emotional Hyperthermia, and Behavioural Activity

After intraperitoneal injection of the vehicle, introduction of the intruder 30 minutes later elicited emotional hyperthermia characterised by increases in BAT (**Figure 3.19**) and body (**Figure 3.20**) temperature. As the dose of lithium increased, the rises in BAT and body temperature during intruder exposure 30–60 minutes after injection were reduced. Note that 0.47 mEq/kg LiCl attenuated these increases without decreasing BAT and body temperature prior to intruder exposure. The maximal changes in BAT and body temperature are shown in **Table 3.2**. Linear regressions of maximal change in BAT ($R^2 = 0.769$, $P < 0.0001$, **Figure 3.21 Left**) and body temperature ($R^2 = 0.512$, $P < 0.003$, **Figure 3.21 Right**) against log-transformed LiCl dose showed significant negative relationships (i.e., lithium dose-dependently reduced both BAT and body temperature during intruder exposure). Further, BAT and body temperature maximal changes were significantly positively correlated ($R^2 = 0.502$, $P < 0.005$, **Figure 3.22**).

**Table 3.2.** Effect of lithium dose on maximal (positive or negative) change in BAT and body temperature during window of intruder exposure (30–60 minutes post-injection). Change is relative to introduction of intruder. Data represents mean ± SEM.

| LiCl dose | Sample number | BAT temperature maximal change (Δ °C) | Body temperature maximal change (Δ °C) |
|---|---|---|---|
| Ringer's solution | 6 | 0.59 ± 0.08* | 0.53 ± 0.07* |
| 0.47 mEq/kg | 4 | 0.15 ± 0.14 | -0.05 ± 0.21 |
| 4.72 mEq/kg | 4 (BAT), 5 (Body) | -0.55 ± 0.19 | -0.34 ± 0.25 |

* Significantly different from 0 (one sample t test, $p < 0.001$).

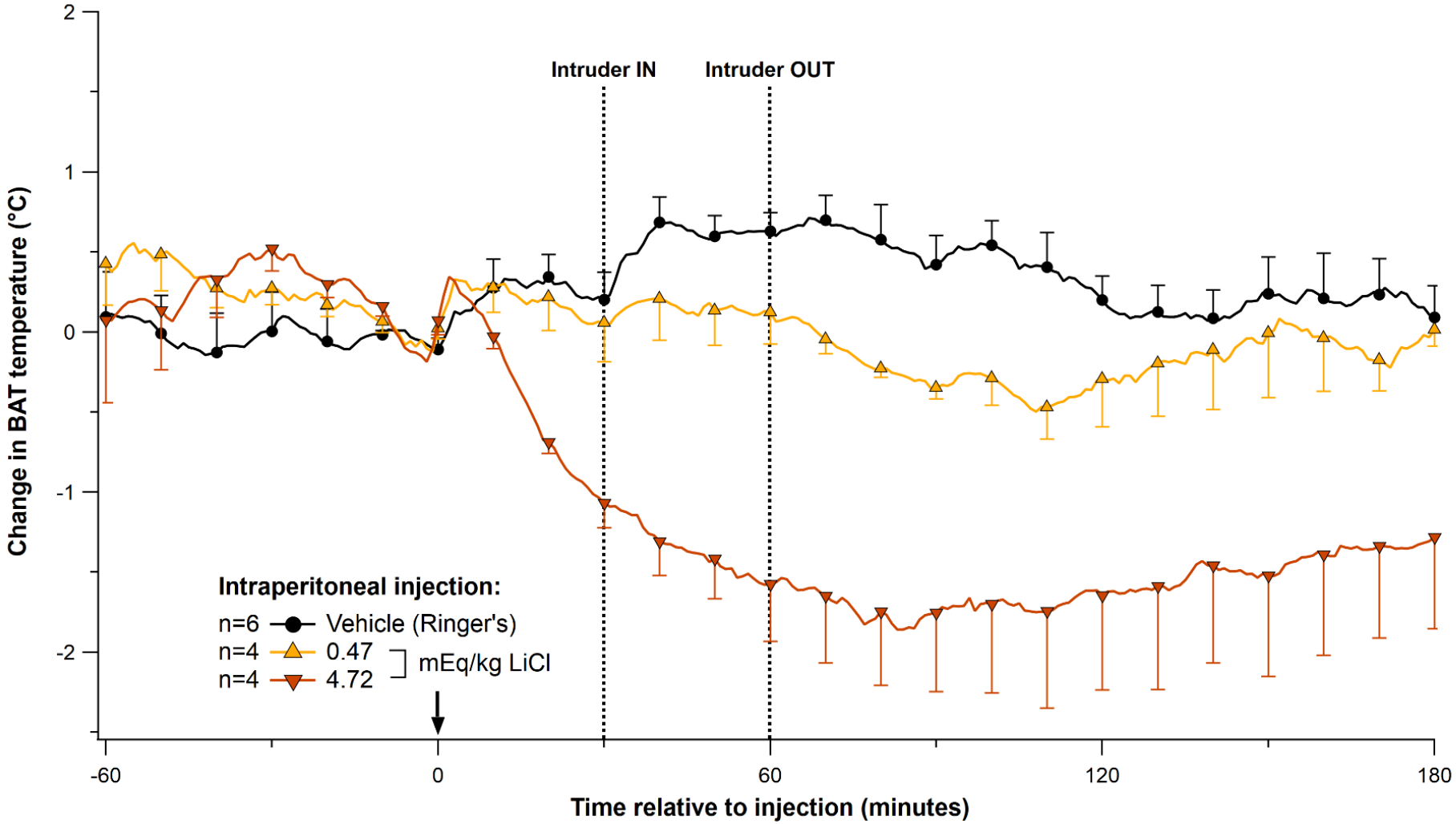


**Figure 3.19.** Change in rat brown adipose tissue (BAT) temperature over time in response to an intraperitoneal injection of lithium (0.47, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution) at 0 minutes. The introduction and removal of an intruder rat is marked at 30- and 60-minutes post-injection, respectively. Datapoints represent mean ± SEM. Sample numbers indicated on graph.

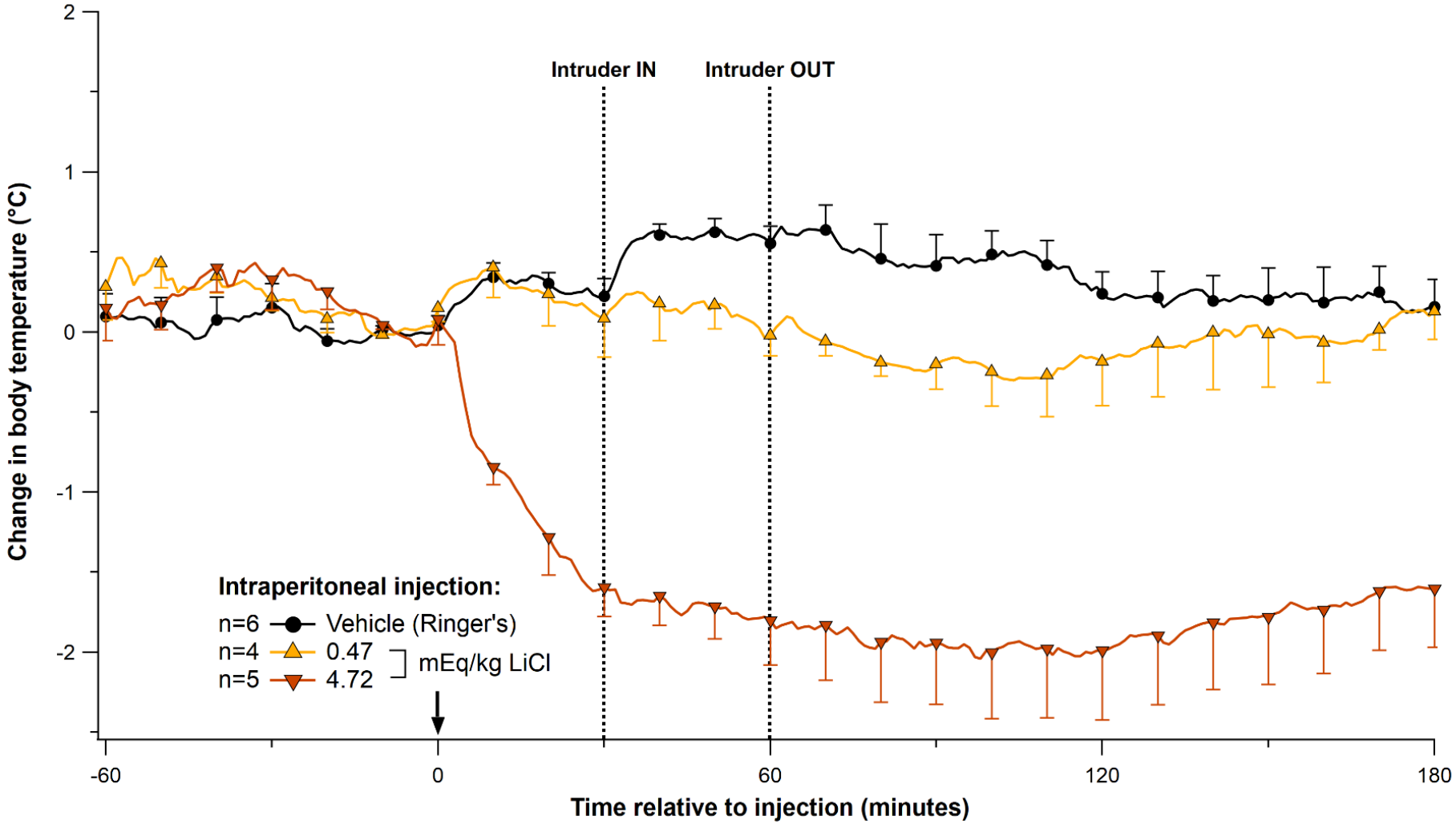


**Figure 3.20.** Change in rat core body temperature over time in response to an intraperitoneal injection of lithium (0.47, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution) at 0 minutes. The introduction and removal of an intruder rat is marked at 30- and 60-minutes post-injection, respectively. Datapoints represent mean ± SEM. Sample numbers indicated on graph.

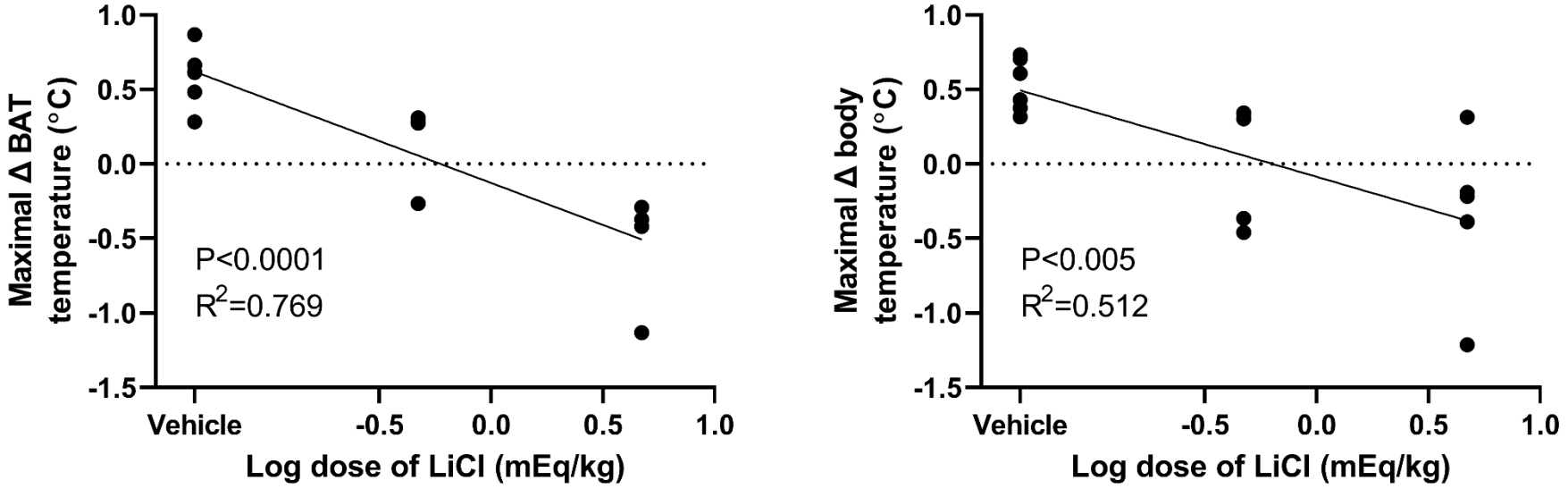


**Figure 3.21. Left:** Linear regression of maximal change in rat brown adipose tissue (BAT) temperature during intruder exposure (30–60 minutes post-injection) against increasing log-transformed doses of lithium (Ringer's solution vehicle, 0.47, and 4.72 mEq/kg LiCl); from left to right on the x-axis, n = 6, 4, and 4. **Right:** Linear regression of maximal change in rat core body temperature during intruder exposure (30–60 minutes post-injection) against increasing log-transformed doses of lithium (Ringer's solution vehicle, 0.47, and 4.72 mEq/kg LiCl); from left to right on the x-axis, n = 6, 4, and 5. Datapoints represent individual animals.

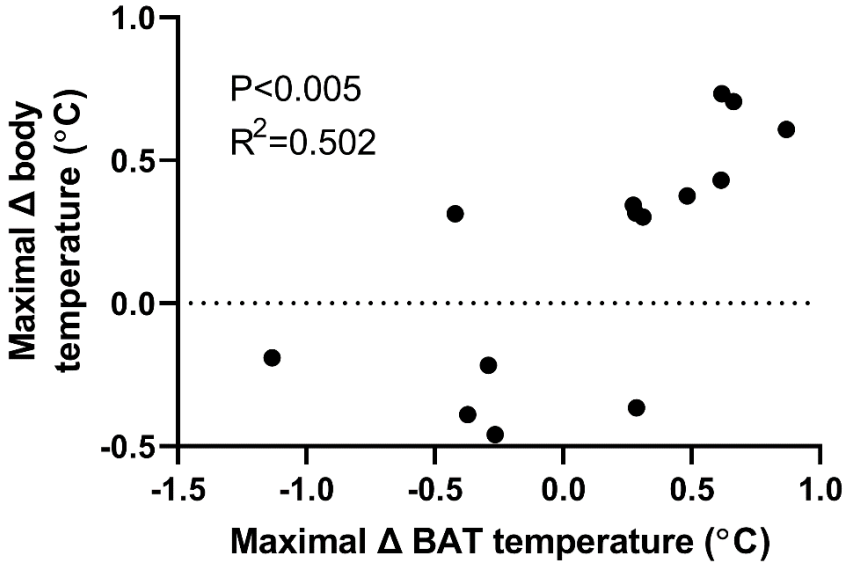


**Figure 3.22.** Correlation between maximal change in rat brown adipose tissue (BAT) and body temperatures during intruder exposure (30–60 minutes post-injection) at respective doses of lithium (0.47, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution). Datapoints represent individual animals.

Upon entry of the intruder, resident rats treated with vehicle became noticeably more active, investigating the perimeter of the intruder's cage and mounting it; those treated with lithium reacted and engaged less with the intruder's cage. Cumulative time spent moving by rats after each intraperitoneal dose of lithium or vehicle, including the window of intruder exposure, is shown in **Figure 3.23**. As LiCl dose increased, locomotor activity of rats decreased. Linear regression analysis showed that lithium dose-dependently decreased the percentage of time spent moving within the window of intruder exposure ($R^2 = 0.606$, $P < 0.001$, **Figure 3.24**). Both BAT ($R^2 = 0.413$, $P < 0.02$, **Figure 3.25 Left**) and body temperature ($R^2 = 0.313$, $P < 0.04$, **Figure 3.25 Right**) were significantly positively correlated with locomotor activity (i.e., body and BAT decreased as time spent moving decreased). One-way ANOVA ($F(2, 15) = 7.078$, $R^2 = 0.486$, $P < 0.01$) followed by Dunnett's multiple comparisons test showed that rats injected with 0.47 mEq/kg LiCl ($P < 0.03$) and 4.72 mEq/kg LiCl ($P < 0.01$) moved significantly less during intruder exposure than those treated with vehicle.

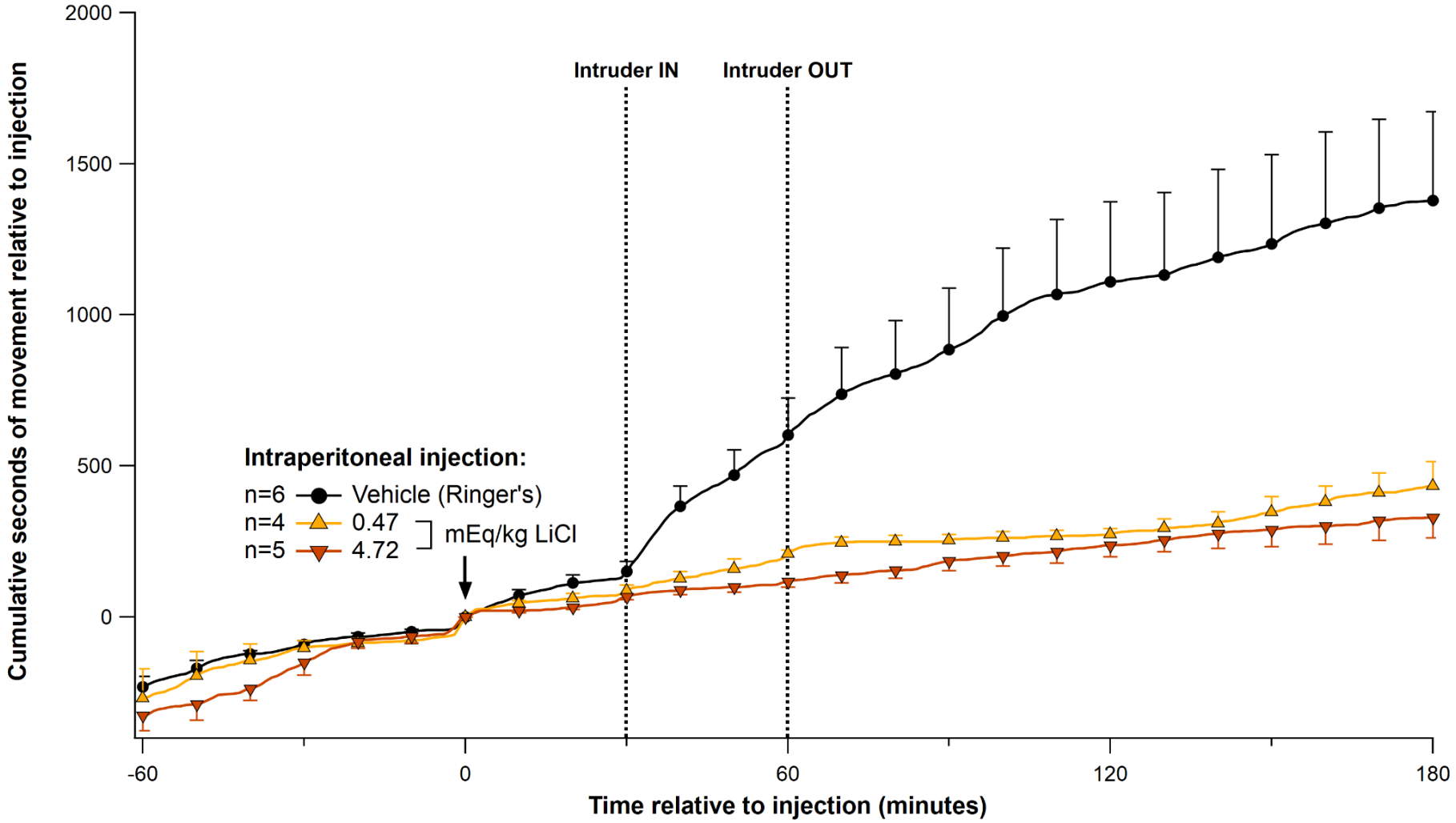


**Figure 3.23.** Cumulative seconds of movement by rats over time from intraperitoneal injection point of lithium (0.47, 4.72 mEq/kg LiCl) or vehicle (Ringer's solution) at 0 minutes. The introduction and removal of an intruder rat is marked at 30- and 60-minutes post-injection, respectively. Datapoints represent mean ± SEM. Sample numbers indicated on graph.

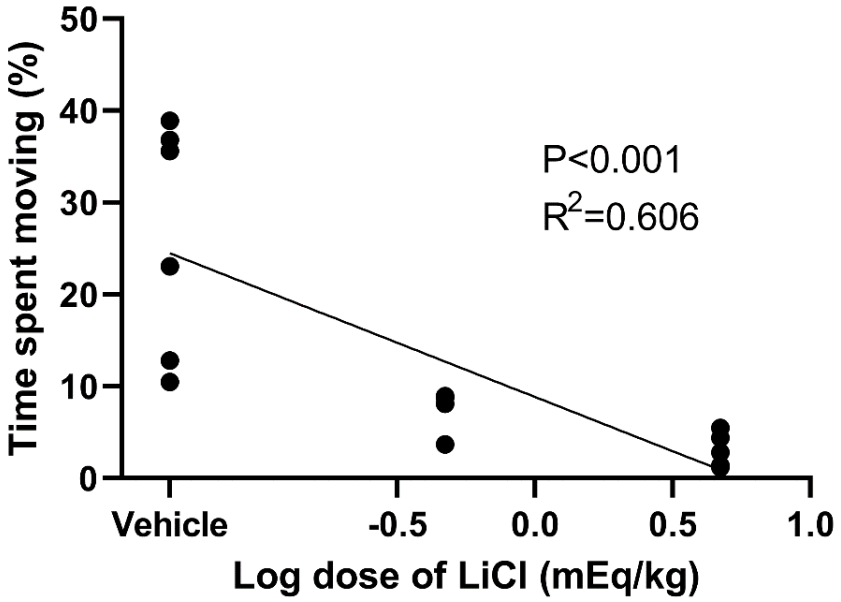


**Figure 3.24.** Linear regression: Percentage of time spent moving during intruder exposure (30–60 minutes post-injection) against increasing log-transformed doses of lithium (Ringer's solution vehicle, 0.47, and 4.72 mEq/kg LiCl). Datapoints represent individual animals. From left to right on the x-axis, n = 6, 4, and 5.

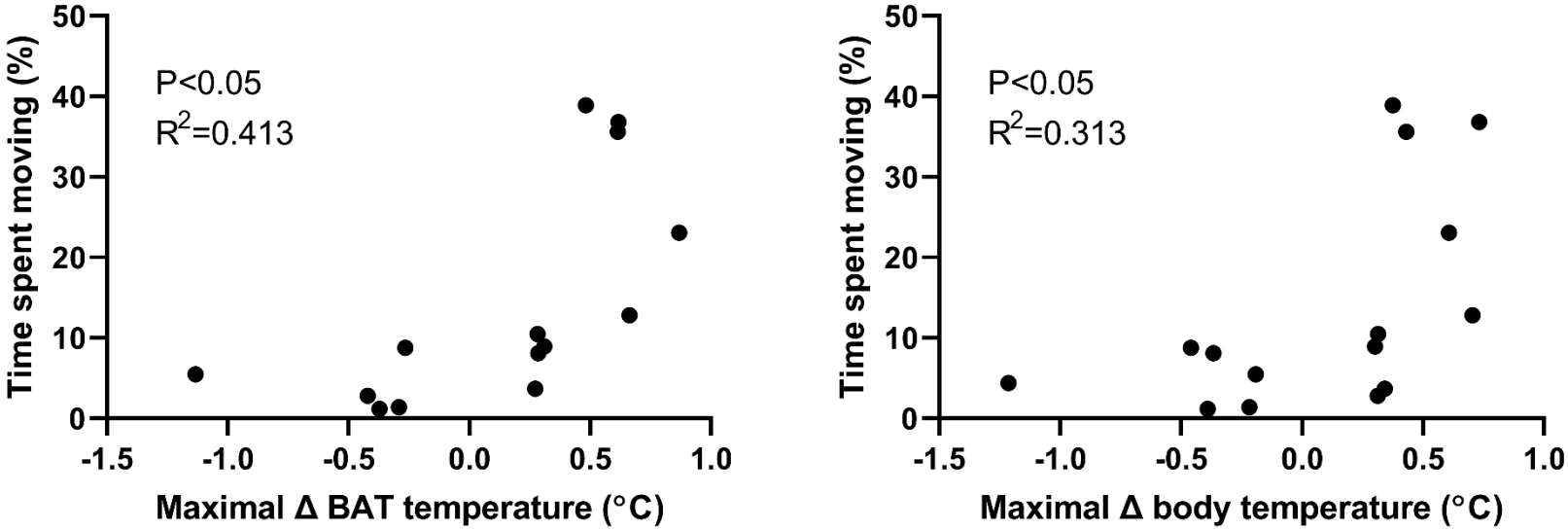


**Figure 3.25. Left:** Correlation between maximal rat delta brown adipose tissue (BAT) temperature and percentage of time spent moving during intruder exposure (30–60 minutes post-injection). **Right:** Correlation between maximal rat delta core body temperature and percentage of time spent moving during intruder exposure (30–60 minutes post-injection). Datapoints represent individual animals that received an intraperitoneal dose of vehicle (Ringer's solution) or lithium (0.47, 4.72 mEq/kg LiCl).

## 3.4 Discussion

### 3.4.1 Lithium's Effects on Body Temperature, BAT Thermogenesis, and Locomotor Activity in Rats

In the previous chapter, I found that lithium dose-dependently reduced body temperature and locomotor activity in conscious guinea pigs in resting conditions. In this chapter, I determined whether these effects are consistent in another rodent species, rats. To enrich this investigation, I included the simultaneous measurement of brown adipose tissue (BAT) temperature to see whether lithium inhibits BAT thermogenesis, a critical factor in regulating hour-to-hour body temperature in small mammals (McAllen et al., 2010; Nakamura et al., 2022; Ootsuka et al., 2009). I firstly hypothesised that lithium would dose-dependently reduce body temperature and locomotor activity in rats, based on both what I observed in guinea pigs and existing evidence in the rat literature. Secondly, I hypothesised that lithium will inhibit BAT thermogenesis, as BAT thermogenesis is typically inhibited when the body naturally cools itself down (Nakamura et al., 2022). Confirming my hypotheses, I found that lithium dose-dependently reduced BAT and body temperature, and locomotor activity. This is consistent with my observations in guinea pigs in the preceding chapter. This supports the idea proposed in **Chapter 2** that lithium's acute effects are a normal physiological response coordinated by the brain. Additionally, my observations suggest that the response to lithium may be a robust and conserved response across mammalian species, thereby increasing the likelihood of its presence in humans.

My findings align with Tulunay's observations of dose-dependent lithium-induced hypothermia in rats (Tulunay, 1976), as well as Ogilvie and Lobb's similar demonstration in mice (Ogilvie & Lobb, 1981). My study improves upon these findings by using a method of continuously measuring body temperature, avoiding body temperature increases associated with handling stress (Stewart & Eikelboom, 1979) during the rectal measurements used by these researchers. Further, this investigation marks the first lithium dose-response on body temperature in rats since Tulunay's study in 1976.

My findings that lithium dose-dependently reduces locomotor activity align with a substantial body of literature describing lithium-induced reductions in rat locomotion and exploratory behaviour, as measured using various methods (O'Donnell & Gould, 2007). For instance, Syme and Syme, employing a displacement-sensitive activity platform, reported that rats treated with 3 mEq/kg isotonic LiCl were less active compared to those treated with an equivalent dose of isotonic saline (Syme & Syme, 1973).

Similarly, Tomasiewicz et al. observed that lithium dose-dependently reduced locomotor activity (as gauged by distance moved), echoing my own findings (Tomasiewicz et al., 2006). Moreover, Gray et al., by tallying how many times a rat crossed lines distributed along the floor of the testing chamber, found that 2 mEq/kg hypertonic LiCl resulted in decreased locomotor activity (Gray et al., 1976).
My study distinguishes itself by being the first to concurrently demonstrate the dose-dependent effects of lithium on body temperature and behavioural activity. Further, I provide the first evidence of lithium-induced BAT thermogenesis reduction in conscious rats. I identified a significant correlation between locomotor activity and both BAT and body temperature, suggesting that the change in behavioural activity is a hypothermic response to lithium where the body intentionally minimises movement to facilitate heat loss to the environment. This is in line with my observations in guinea pigs in the previous chapter wherein lithium dose-dependently reduced body temperature and locomotor activity and increased the occurrence of a 'cooling posture'. However, my study has a limitation: unlike the previous chapter, I did not directly assess the presence of a 'cooling posture', even though reduced movement would suggest a state of stillness similar to the cooling posture. Nonetheless, the lithium-induced 'lying-on-belly' behaviour described in the literature (Meachum & Bernstein, 1990; Parker et al., 1984) parallels the guinea pigs' cooling posture in terms of timing and function, and corroborates my findings.

These findings could potentially be replicated in humans, as the presence of metabolically active BAT in humans is well-defined (Cypess et al., 2009; Nedergaard et al., 2007; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Zingaretti et al., 2009). The only notable challenge is to record BAT temperature accurately and continuously in humans. Once this is achieved, lithium's acute effects may be able to be demonstrated in humans for the first time. This would change the way we interpret lithium's mechanism of action in the treatment of mood disorders.

### 3.4.2 Evaluating the Effect of 5-$HT_3$ Antagonists on Lithium's Thermoregulatory and Behavioural Impacts

In the previous chapter, I found that 5-$HT_3$ antagonists did not inhibit lithium's hypothermic and behavioural effects in guinea pigs. In this chapter, I repeated the experiment in rats to rule out the possibility of a species-dependent effect. In support of my hypothesis, I found that lithium's hypothermic and behavioural effects were unaffected by ondansetron and palonosetron. This is consistent with my findings in guinea pigs and provides further evidence that the serotonergic system via 5-$HT_3$ is not involved in lithium's mechanism of action. However, these findings contradict the observations by Guimaraes and colleagues wherein ondansetron substantially attenuated lithium-

induced hypothermia in rats (Guimaraes et al., 2015). Other than a minor difference in weight range, one obvious factor that might account for the discrepancy in results is their use of the Wistar strain as opposed to my use of Sprague-Dawley. Another notable difference between Guimaraes and colleagues' study and ours is the tonicity of lithium solution administered. However, it is unlikely that the molarity of the solution is responsible for lithium's effects, as confirmed by my guinea pig findings in the previous chapter, and findings by Nachman and Ashe in rats (Nachman & Ashe, 1973) and Ogilvie and Lobb in mice (Ogilvie & Lobb, 1981) (refer to the previous chapter's discussion for more detail). Further, Guimaraes et al. did not provide a quantitative analysis of the behavioural activity, but they did note that the rats were quiescent after lithium, which aligns with my observation of dose-dependent reduced locomotor activity. However, the authors did not mention whether ondansetron reversed the quiescence induced by lithium and relied on their thermoregulatory observations alone to determine the presence of nausea. This makes it difficult to compare my behavioural results with theirs and determine whether ondansetron prevented the rats from entering a nausea-like state. The reason for the discrepancy in the results remains unknown and requires further investigation.

Research into conditioned taste aversion (CTA), a phenomenon potently triggered by lithium's nauseating effect, lends further support to my findings. For instance, Mele et al. found that 5-$HT_3$ receptors do not play a role in lithium's ability to induce CTA in rats. Their conclusion was based on the observation that the 5-$HT_3$ antagonists ondansetron and zacopride did not lessen the acquisition of lithium-induced CTA (Mele et al., 1992). This aligns with my evidence that the 5-$HT_3$ antagonists ondansetron and palonosetron do not inhibit the nausea-indicative reduction in locomotor activity and associated hypothermia induced by lithium.

While lithium can stimulate serotonin release from the gastrointestinal tract, these results suggest that the 5-$HT_3$ receptor does not mediate lithium's effects on behaviour and thermoregulation. This conclusion is further supported by Rudd et al.'s work. They found that the 5-$HT_3$ receptor does not participate in the development of CTAs induced by 5-HT itself, or by 5-HT-releasing agents such as ipecacuanha and cisplatin. They observed that CTAs were not blocked by the 5-$HT_3$ antagonists ondansetron and granisetron, and that the selective 5-$HT_3$ agonist m-chlorophenylbiguanide did not induce CTA (Rudd et al., 1998). Lastly, Xie et al.'s recent study demonstrated that despite genetic modifications in mice preventing serotonin synthesis in their EC cells, which are typically responsible for around 95% of total body serotonin production (Linan-Rico et al., 2016), these mice still developed a CTA to intraperitoneal lithium. Taken together, these findings suggest that not only is 5-$HT_3$ not

involved, but serotonin itself may not be involved in the behavioural-thermoregulatory response to lithium.

However, the literature indicates a species-dependent variation in this response. Unlike in rats, 5-$HT_3$ antagonists are effective in attenuating the emetic reflex induced by cisplatin in species capable of vomiting, such as ferrets and humans (Percie du Sert & Andrews, 2014; Rojas et al., 2014; Rudd & Naylor, 1996). It is important to note, however, that the absence of vomiting does not necessarily mean the absence of nausea. The complete inhibition of nausea in ferrets is unclear, as the primary measures of effect are the number of retches and vomits (Percie du Sert & Andrews, 2014; Percie du Sert et al., 2011). Furthermore, in humans, 5-$HT_3$ antagonists do not always effectively control acute chemotherapy-induced nausea. According to a review by Gregory and Ettinger, many comparative clinical studies of 5-$HT_3$ antagonists reported complete control of nausea in about half or fewer of their patients during the acute period following chemotherapy. Some studies reported complete control rates as low as 15% and others as high as 79% (Gregory & Ettinger, 1998). This variation, together with the other findings, suggests the involvement of other neurohormones and their respective receptors in the signalling of nausea.

Overall, given my findings that lithium-induced hypothermia and locomotor activity reduction are consistent across two mammalian rodent species using not only ondansetron but also palonosetron, which is known to be far more effective than ondansetron (Aapro et al., 2006; Park & Cho, 2011; Siddiqui & Scott, 2004), it is highly likely that the 5-$HT_3$ receptor is not involved in lithium's mechanism of action.

### 3.4.3 Persistence of Lithium's Effects on Physiological and Behavioural Responses with Repeated Exposure

In my habituation experiment, I aimed to determine whether the hypothermic and behavioural response to lithium changed after repeated exposure to acute lithium doses. In line with my hypothesis, I found that successive intraperitoneal injections of lithium consistently reduced body temperature and locomotor activity. There was no statistically significant relationship between day of injection and these parameters. This was expected, as I did not observe serial effects in the dose-response experiments in the preceding and current chapters.

It is important to note that my results represent acute and not chronic lithium treatment. I can be confident that drug accumulation is not a confounding factor in this experiment and that the results

represent a change or lack thereof in the body's response to acute exposure lithium. Drug accumulation is a phenomenon that is typically seen if the repeated dose occurs sooner than 4 half-lives of the given drug (Wadhwa & Cascella, 2023). The terminal plasma half-life of lithium in rats is 6–7 hours (Wood et al., 1986; Wraae, 1978) in comparison to 18–36 hours in humans (Ward et al., 1994). If I take the lower and upper extremes of the rat range, the fourth half-life would amount to 24–28 hours. The dose interval in my experiment was 72 hours between the doses on day 1, 4, and 7; and 96 hours between the doses on day 7 and 11. With this in mind, drug accumulation in the rats was unlikely. This also confirms that the three-day washout period between treatments worked as intended theoretically and was not a limitation to my results. Therefore, as I found no statistically significant trends between the day of injection and both minimum body temperature and cumulative distance moved, this indicates a strong and consistent physiological and behavioural response to acute lithium. If I were to forgo the lack of statistical significance, lithium appeared to be more effective in reducing body temperature on day 4, which would also refute a hypothesis of habituation or decreased response to lithium.

In support of my results, Batson administered eight intraperitoneal doses of lithium 48 hours apart in rats and showed that the temperature-reducing effect of lithium did not diminish over time (Batson, 1983). Batson used 1.8 mEq/kg LiCl, which is comparable to my 2 mEq/kg dose. My study enhances these findings by offering a higher temporal resolution and a longer recording duration (i.e., data analysed every minute for a total duration of 6 hours), as opposed to Batson's 3 measurements over a 1.5-hour period. The author also demonstrated that lithium's reduction in locomotor activity did not diminish over time either, consistent with my observations.

Building upon my results, it is noteworthy to highlight the clinical trend elucidated by Schou (Schou, 2004). Schou underscored that lithium, unlike many medications, does not lose its potency over time. Typically, many drugs necessitate escalating doses or intermittent treatment cessation to maintain their effectiveness. However, lithium consistently bucks this trend. Even after extensive periods of usage, its therapeutic efficacy remains undiminished. This enduring effectiveness is not compromised even if the treatment is briefly interrupted and subsequently resumed. My findings align with this clinical trend, further validating the consistent impact of lithium observed in my study.

However, in contradiction to these findings, Guimaraes et al. found that lithium-induced hypothermia diminished between two doses 168 hours (7 days) apart (Guimaraes et al., 2015). The discrepancy here is difficult to determine. Perhaps it is again a difference in strain, as mentioned in another

contradiction in the 5-$HT_3$ antagonist experiment above (**Section 3.4.2**). This warrants further investigation using the Wistar strain. Besides this, the period in between these doses is considerably longer than those in my own study and Batson's. A two-point trend is far less informative than a 4 or 8-point trend. Further, Guimaraes and colleagues did not analyse differences in lithium's behavioural effects between these two points. Considering my results, together with Batson's, show that the body's response to lithium does not diminish after repeated exposure, it is likely that lithium's acute physiological and behavioural effects are not affected by tolerance or habituation.

### 3.4.4 Effect of Lithium on Tail Temperature and Vasodilation

In my rat tail temperature experiment, I aimed to see whether lithium increased blood flow to the tail via vasodilation. I indirectly measured this using infrared thermography. In support of my hypothesis, I demonstrated that intraperitoneal lithium administration quickly increased tail temperature in less than 5 minutes. Specifically, tail skin temperature increased as soon as 1 minute following intraperitoneal injection of 2 mEq/kg LiCl, whereas the isotonic saline control decreased it.

It is well-established that tail temperature increases as the cutaneous vascular bed of the tail vasodilates, increasing blood flow, and conversely decreases during tail cutaneous vasoconstriction, restricting blood flow (Ootsuka & Tanaka, 2015). Therefore, lithium-induced tail temperature increase is indicative of cutaneous vasodilation in the tail, whereas the temperature decrease in response to the control is indicative of tail cutaneous vasoconstriction. Notably, I provide the first evidence that lithium's vasodilatory action can occur as quickly as one minute. Although I did not simultaneously record BAT and body temperature in this experiment, the result is consistent with the near-immediate behavioural and hypothermic responses to lithium I observed in guinea pigs in the previous chapter, in addition to the hypothermic responses I observed in rats in the current chapter. My observations are in agreement with the finding of Jones et al., who found that an intraperitoneal injection of LiCl (5 mEq/kg) increased tail temperature within 10 minutes (Jones et al., 2008). However, I was able to demonstrate lithium-induced vasodilation at a lower, therapeutically relevant dose of 2 mEq/kg (Cox et al., 1971; O'Donnell & Gould, 2007). These rapid responses support the idea proposed in the previous chapter that lithium initially interacts with a peripheral target, triggering a central response that leads to the observed effects like tail vasodilation. This is likely because it takes much longer for lithium to cross the blood-brain barrier and accumulate in the brain (see **Section 2.4.8**).

In addition, Jones et al. measured brain temperature in the hypothalamic area and found that the temperature of the hypothalamus decreased concurrently with the increase in tail temperature (Jones

et al., 2008). This aligns with my observation of lithium-induced reduction in BAT thermogenesis and body temperature in my dose-response experiment. Collectively, these results suggest that the increase in tail temperature is a sign of vasodilatory action aimed at enhancing heat exchange with the environment, thereby lowering core body temperature. This data strengthens the proposition from **Chapter 2** that the response to lithium is orchestrated by the brain, as cutaneous vasomotion, along with BAT thermogenesis, is controlled by the brain.

The decrease in tail temperature following the isotonic 0.9% saline control indicates tail vasoconstriction, leading to an increase in core body temperature. This was probably due to emotional hyperthermia elicited by handling stress during injection, consistent with the sustained increase in BAT and body temperature following injection of isotonic Ringer's solution vehicle in the dose-response experiment. This also suggests that lithium inhibits stress-induced cutaneous vasoconstriction, as lithium administration did not lead to a decrease in tail temperature. Interestingly, Jones et al. injected isotonic saline (0.15 M) at a dose of 5 mEq/kg NaCl (one milliequivalent higher than my usage in **Chapter 2**) and found that an equal dose of equimolar LiCl resulted in a significantly higher tail temperature (Jones et al., 2008). This observation supports one of my earlier conclusions: the effect of lithium is specific and not merely a consequence of hypertonicity. However, in contrast to the small increase in tail temperature observed by Jones et al. after their injection of 5 mEq/kg isotonic NaCl, my study noted a drop in tail temperature. I administered 0.5 mL of 0.9% saline (approximately 0.15 M) to my animals, which corresponds to a dose of approximately 0.21 mEq/kg isotonic NaCl for an average rat weight of 0.375 kg. It is worth noting that due to variations in individual animal weights, the actual doses ranged between 0.15 and 0.31 mEq/kg. Nonetheless, I attribute this discrepancy to the body's ability to distinguish between lithium and sodium ions. While sodium ions have effects similar to those of lithium, they are less pronounced, as observed and discussed in the previous chapter.

The measurement of lithium's thermoregulatory effects in humans remains an unexplored area, despite its theoretical feasibility. Evidence from animal models indicates that thermoregulatory responses can occur across various hairless body parts, not just in rat tails. In response to psychological stress, for instance, both tail and paw temperatures have been reported to decrease in rats (Vianna & Carrive, 2005). Similar findings have been found in the rabbit ear pinna (Yu & Blessing, 1999). This leads to the proposition that the guinea pig ear, another typically hairless region, might function similarly as a heat exchanger and respond to lithium in a comparable manner. Such observations in animals provide a compelling rationale for analogous investigations in humans. Indeed, Oka et al.

documented a case where emotional hyperthermia resulted in increased core body temperature and decreased fingertip temperature in a human subject, suggesting cutaneous vasoconstriction (Oka et al., 2013). As noted by Schou, the quantitative assessment of lithium's therapeutic effect can be challenging, a statement that remains valid today (Schou, 1968). However, from the observations made in this thesis, I propose a novel, non-invasive method for the quantitative assessment of lithium's therapeutic action: measuring surface skin temperature, particularly in regions such as the fingertips. I anticipate that this method will provide a snapshot of the body's sympathetically controlled thermoregulatory and metabolic responses to lithium. As this approach has not been tested in humans before, it presents a significant opportunity for innovative research. Therefore, conducting a study to investigate whether acute lithium administration leads to cutaneous vasodilation in humans would represent a critical step in translating my findings to human physiology. If successful, this pioneering research has the potential to revolutionise our understanding of lithium's therapeutic effect.

### 3.4.5 Lithium's Role in Modulating Stress-Induced Physiological and Behavioural Responses

Earlier in this chapter, I discovered that lithium reduces BAT and body temperature and locomotor activity in a dose-dependent manner under resting conditions. In my intruder experiment, I aimed to determine if lithium also inhibits BAT thermogenesis, emotional hyperthermia, and hyperlocomotion during acute psychological stress. To accomplish this, I utilised a resident-intruder procedure previously established by our lab (Mohammed et al., 2014) to elicit acute psychological stress in rats treated with two different doses of lithium (0.47 & 4.72 mEq/kg LiCl) and a control group treated with vehicle (Ringer's solution).

#### *3.4.5.1 Physiological and Behavioural Responses to Acute Psychological Stress*

To begin, I will discuss my observations in the vehicle pre-treated group. After intraperitoneal injection of Ringer's solution, I found that a caged intruder triggers an increase in both BAT and body temperature of the resident rat. Therefore, I successfully demonstrated acute psychological stress-induced emotional hyperthermia, which BAT thermogenesis contributed to. This response aligns with previous findings from our lab, reporting a 1 and a 0.7 °C increase in BAT and body temperature, respectively, under similar experimental conditions and vehicle pre-treatment (Mohammed et al., 2014). These temperature increases are marginally higher than my current findings and might be explained by small differences in body weight range, genetic drift within colonies (Bodnar et al., 2015), and surgical/experimental procedure. Further, in rats pre-treated with vehicle, I observed an increase

in locomotor activity during intruder exposure; I also noted that the rats engaged with the intruder's cage during their heightened activity, investigating its perimeter and mounting it. This aligns with our lab's prior studies, which observed an increase in locomotor activity in rats when an intruder was introduced and described the rats becoming active to the point of approaching and climbing onto the intruder's cage (Antipov et al., 2020; Brizuela et al., 2019; Mohammed et al., 2014). These consistent observations indicate that emotional hyperthermia and hyperlocomotion occur together as part of the response to acute psychological stress.

#### *3.4.5.2 Understanding the Functional Significance of Emotional Hyperthermia*

I believe that the emotional hyperthermia I observed in the rat in response to intruder-elicited psychological stress has broad implications on physiology, cognition, and behaviour, beyond what was measured. Previous work in our lab has briefly discussed this, proposing that emotional hyperthermia is a short-term adaptive physiological response (Blessing et al., 2017; Mohammed et al., 2014). I will expand upon this discussion, as there is a considerable amount of complexity to consider.

##### 3.4.5.2.1 Impact of Emotional Hyperthermia on Muscular Performance

Part of this adaptive response may involve muscular performance. During emotional hyperthermia, increased body temperature and muscle blood flow lead to the heating of muscles (Nakamura & Morrison, 2022). Research conducted by Stein et al. and Bennet demonstrate that elevated muscle temperatures increase the speed of muscle contraction and relaxation, thereby boosting maximal power output (Bennett, 1985; Stein et al., 1982). This heightened performance directly enhances rate-dependent behaviours such as running and jumping. Therefore, it can be inferred that emotional hyperthermia, which increases muscle temperature, likely optimises muscle performance, facilitating faster locomotion. This is especially advantageous in situations requiring immediate and swift action, such as encountering an intruder or potential threat, escaping danger, or navigating complex environments. The observed increase in locomotor activity in resident rats upon the introduction of an intruder may represent a practical manifestation of this phenomenon. It suggests that the elevated physiological temperature resulting from emotional hyperthermia enables the expression of specialised behaviours to effectively respond to the intruder, which would otherwise be limited.

#### 3.4.5.2.2 Hippocampal Function and Cognitive Enhancement During Emotional Hyperthermia

Cognitive performance may also be part this adaptive response. Our lab has previously shown that BAT thermogenesis plays a significant role in increasing brain temperature during emotional hyperthermia, in addition to the heat generated by the brain itself. In this process, the temperature of intrascapular BAT is higher than both body and brain temperature, and it is ideally located to supply the head with heated blood (Mohammed et al., 2014). Therefore, in my experiment, it is highly likely that the resident rat's brain temperature was elevated during its exposure to the intruder. The significance of brain temperature is often overlooked in the literature (Kiyatkin, 2019), yet it holds substantial sway over the brain's physicochemical properties and, consequently, its functionality. Temperature is known to have a profound effect on the brain, modulating the function of neurons and their interactions with each other (Karlsson & Blumberg, 2004). For example, as brain temperature increases, neurotransmitter release and reuptake become faster (Kiyatkin, 2010). Additionally, Yu et al. established that higher physiological temperatures (37–42 °C) in mammals result in more energy-efficient brain signalling, which allows for the use of an energy-efficient neural code. (Yu et al., 2012).

Thus, emotional hyperthermia, which is present during periods of heightened arousal and attention (Kiyatkin, 2007, 2010), including salient situations such as when an animal perceives a potential threat (Blessing et al., 2017), may enhance cognitive processes. In my study, this suggests that the resident rat benefited from enhanced cognition during intruder-elicited acute psychological stress. This adaptation likely serves a crucial role in facilitating the organism's survival in salient situations. One example of such thermal cognitive enhancement might be observed in the hippocampus, a brain region that has been linked to memory processing, learning, decision-making, spatial navigation, contextual encoding, and the creation of cognitive maps of experience (Eichenbaum, 2017; Knierim, 2015; Lisman et al., 2017; Maguire & Mullally, 2013; Stachenfeld et al., 2017). In situations anticipating a predatory threat, the hippocampus is postulated to play a key role in driving defensive behaviour. This stems from its essential function in scrutinising the environment and in the identification of environmental cues that signify fear (Zhong et al., 2022). Neurons in the hippocampus have been shown to become more excitable and fire faster during hyperthermia (Kim & Connors, 2012).

Specifically, temperature has been shown to alter two key aspects of mammalian hippocampal neural activity: theta rhythms and sharp wave-ripples (SPW-Rs).

#### 3.4.5.2.2.1 *Theta Rhythm*

Theta rhythms are low-frequency oscillatory patterns of neural activity generated mainly in the hippocampus that play crucial roles in synchronising and coordinating activity among various brain regions and facilitating synaptic plasticity (Drieu & Zugaro, 2019; Nuñez & Buño, 2021). These rhythms have been linked to a myriad of cognitive and behavioural processes such as spatial navigation and planning, episodic memory, learning, and decision-making (Drieu & Zugaro, 2019; Kunz et al., 2019; Nuñez & Buño, 2021; Wikenheiser & Redish, 2015). Theta rhythms are present during active states such as exploration, attentiveness/alertness, and emotional excitement (de Menezes et al., 2009; Liu et al., 2022; Mikulovic et al., 2018; Sainsbury et al., 1987; Sławińska & Kasicki, 1998; Yu & Blessing, 1997).

In previous work conducted by our lab, it was found in conscious rabbits and rats that, in response to a salient stimulus, hippocampal theta rhythms were accompanied by cutaneous vasoconstriction in the rabbit ear and rat tail (de Menezes et al., 2009; Yu & Blessing, 1997). In the context of my research, the intruder in my experiment can be considered no less than a salient stimulus, as I have shown that the intruder elicits emotional hyperthermia in the rat, of which cutaneous vasoconstriction is well known to be a part of (Ootsuka & Tanaka, 2015). Ootsuka et al. established a correlation between hippocampal theta rhythm and BAT thermogenesis, with theta rhythm leading by approximately 5 minutes (Ootsuka et al., 2009). Blessing proposed that, considering the delay between the initiation of BAT thermogenesis and the measurable change in BAT temperature, the brain likely triggers both BAT thermogenesis and theta rhythm concurrently (Blessing, 2018). This dual activation is associated with the animal's attentional shift and engagement with the external environment. This strengthens the possibility that hippocampal theta rhythm was present during my intruder experiment, as BAT thermogenesis and locomotor activity (environmental engagement) increased upon intruder introduction. In support of this, a study conducted by Kim et al. involving rats foraging for food in risky situations showed that theta rhythm was present and specifically increased as rats approached a looming gator-like robot simulating a predatory threat (Kim et al., 2015); additionally, they found that lesioning the amygdala abolished this phenomenon, which highlights the emotional aspect of this response, as the amygdala is a key mediator of psychological stress-induced responses such as cutaneous vasoconstriction, BAT thermogenesis, and behavioural activity (Nakamura & Morrison, 2022). Further, Sławińska and Kasicki observed that higher emotional states, such as those experienced during the fight or flight response, are concurrent with higher theta frequency compared to low emotional states like exploratory behaviour (Sławińska & Kasicki, 1998). Therefore, this

suggests that not only was the theta rhythm likely present in my intruder experiment, but they were potentially enhanced due to intruder-elicited psychological stress.

Whishaw and Vanderwolf observed in rats that theta rhythm, corresponding with voluntary movements like walking, running, and jumping, escalated in frequency right before the initiation of such behaviours (Whishaw & Vanderwolf, 1973). They found theta frequency rose with the complexity of tasks. Others have shown that theta frequency increases in proportion to speed of locomotion (Goyal et al., 2020). This implies heightened hippocampal activity with demanding tasks. Adding to this, Richard et al. demonstrated that higher theta frequency is associated with enhanced spatial memory performance (Richard et al., 2013). Whishaw and Vanderwolf also noted a positive correlation between body temperature and theta frequency (Whishaw & Vanderwolf, 1971), suggesting that increased body temperature might boost hippocampus-dependent cognition and behaviour, thus complementing temperature-induced muscular performance enhancement discussed earlier (Bennett, 1985). They found theta rhythm and voluntary movement diminished at cold body temperatures (20–23 °C), despite functional reflexive movements, highlighting the crucial role of body temperature in producing theta rhythm and facilitating related behaviour.

Considering this collection of evidence, I propose that emotional hyperthermia, through heating the brain, contributes to heightened cognitive ability during acute psychological stress by enhancing hippocampal theta frequency and its associated functions, enabling faster and complex movements to efficiently navigate the environment and rapidly adjust behaviour in response to the stressor/threat, increasing the chances of survival.

#### 3.4.5.2.2.2 Sharp Wave Ripples

On the other hand, hippocampal sharp wave ripples or SPW-Rs are complex oscillatory patterns consisting of a high-frequency ripple oscillation overlaying a slower, large amplitude sharp wave (Buzsáki, 2015; Joo & Frank, 2018; Liu et al., 2022). These patterns are thought to share and complement functions with theta rhythms, including those related to episodic memory, learning, spatial navigation and planning, and decision-making (Buzsáki, 2015; Drieu & Zugaro, 2019). However, SPW-Rs occur during different behavioural states: quiet behavioural states such as pauses in the environment and following complex behavioural tasks (Girardeau et al., 2014). They essentially surround active states that theta would normally dominate (Drieu & Zugaro, 2019). A unique characteristic of SPW-Rs is that they represent temporally compressed forward and reverse spike sequences that occurred during wakeful theta rhythm activity; that is, sets of neurons that fired in a

particular order during a theta rhythm are able to be replayed forward and backward at an accelerated rate during an SPW-R (Diba & Buzsáki, 2007; Foster & Wilson, 2006). This allows the animal to replay past experiences and flexibly recombine them with newly acquired experiences to create SPW-Rs containing potential future scenarios, aiding prospective decision-making (Gupta et al., 2010; Joo & Frank, 2018; Liu et al., 2022; Pfeiffer & Foster, 2013). Importantly, SPW-Rs are embedded in global brain-state changes, highlighting the intricate connection between hippocampal function and the overall activity and functional states of the brain (Nitzan et al., 2022). This interconnectedness significantly impacts the communication between the hippocampus and other brain areas, such as the neocortex and subcortical structures (Liu et al., 2022). The widespread influence of SPW-Rs on these brain regions underscores their critical role in supporting various cognitive functions and coordinating brain-wide communication (Nitzan et al., 2022).

Highlighting the functional role of SPW-Rs, Girardeau established that eliminating SPW-Rs during post-training periods impairs performance in spatial memory tasks (Girardeau et al., 2009). Petersen et al. found that brain temperature fluctuations were significantly correlated with SPW-R parameters. They also found that locally heating the hippocampal CA1 region increased ripple frequency, duration, and occurrence rate (Petersen et al., 2022). Similarly, according to a study by Cheah et al., increased core body temperature enhances SPW-R frequency and rate of occurrence (Cheah et al., 2021); both of these parameters are positively correlated with hippocampal network excitation and known to increase following periods of complex behavioural tasks and learning (Girardeau et al., 2014; Ponomarenko et al., 2008).

In my intruder experiment, the elevated BAT and body temperature in the resident rat persisted for up to an hour after the intruder's removal. This prolonged rise in body temperature, coinciding with the post-active period SPW-Rs are known to occupy, may have facilitated the enhancement of SPW-R parameters, drawing from the other studies.

Taken together, these findings suggest that emotional hyperthermia may contribute to enhanced cognition by enhancing SPW-Rs and related functions, facilitating more efficient replay of experiences that occurred during psychological stress and richer combinations of potential future scenarios. This may promote memory consolidation and learning from the stressful experience. Consequently, the animal may then adapt its behaviour to navigate future stressors more effectively, improving survivability.

Future analyses of the presence and characteristics of theta rhythms and SPW-Rs during intruder-elicited psychological stress will help contribute to our understanding of their role in psychological stress and emotional hyperthermia's functional role in enhancing cognition.

#### 3.4.5.2.2.3 Mechanisms: TRPV4 & TRPV1

One reason for why temperature affects hippocampal excitability may be that hippocampal neurons express heat-sensitive channels. One of which is TRPV4, which is activated at temperatures above 34 °C (Shibasaki et al., 2015; Shibasaki et al., 2007). Shibasaki et al. demonstrated that these channels are crucial for hippocampal neuronal excitability at physiological temperature (37 °C). Neurons lacking TRPV4 exhibited reduced excitability at 37 °C. The temperature response profile of heat-evoked whole-cell currents revealed that, from 34 °C to 37 °C, TRPV4 ion channel activation is proportional to temperature, with higher temperatures leading to increased activation and excitability (Shibasaki et al., 2007). Shibasaki et al. also demonstrated that mice lacking TRPV4 exhibited abnormal behaviour and reduced hippocampal neural activity (theta rhythm) at physiological temperature (Shibasaki et al., 2015). Consequently, hippocampal functions may be augmented at higher physiological temperatures, partly due to the activation of TRPV4 channels.

Hippocampal neurons have also been found to express functional TRPV1 channels, though the expression of TRPV1 in the hippocampus has been controversial (Hurtado-Zavala et al., 2017). Initially, TRPV1 was reported to be absent from the central nervous system entirely (Caterina et al., 1997), while later research suggested broad expression of TRPV1 in the brain, including in hippocampal pyramidal neurons (Cristino et al., 2006). However, a subsequent study using a TRPV1 reporter mouse line found highly restricted expression in a transient population of hippocampal cells hypothesised to be Cajal-Retzius cells (Cavanaugh et al., 2011). More recently, TRPV1 was revealed to be functionally expressed in a small subpopulation of oriens-lacunosum-moleculare (OLM) inhibitory interneurons in the adult hippocampus, where it promotes excitatory innervation onto these interneurons (Hurtado-Zavala et al., 2017). Despite the varied findings on expression patterns, these TRPV1 channels are implicated in plasticity in the hippocampus and hippocampal-dependent behaviours, such as memory formation and spatial information processing (Hurtado-Zavala et al., 2017; Kauer & Gibson, 2009).

Although TRPV1 is typically activated at temperatures exceeding 40 °C in vitro (Caterina et al., 1997), evidence suggests TRPV1 or TRPV1-containing heteromeric channels can be activated at lower, physiologically relevant temperatures in vivo. Factors such as phosphorylation, the presence of

endovanilloids, and changes in pH levels can sensitize and lower the activation threshold of TRPV1 channels in physiological conditions (Romanovsky et al., 2009; Yonghak et al., 2020). Hypothalamic vasopressin neurons expressing TRPV1 have been shown to exhibit temperature-sensitive inward currents at temperatures as low as 35 °C (Jeong, Lee, et al., 2018). Moreover, TRPV1 appears to form heteromeric channel complexes with other TRPV subunits like TRPV3 and TRPV4 in certain neuronal populations, including hypothalamic proopiomelanocortin (POMC) neurons (Jeong, Lee, et al., 2018). These heteromeric TRPV1-like channels retain capsaicin sensitivity but have lowered temperature activation thresholds around 37 °C, allowing them to detect physiological fluctuations in brain temperature.

Importantly, deeper brain regions such as the hypothalamus and hippocampus can physiologically exceed the 40 °C threshold in mammals, including healthy humans (Fuller et al., 1998; Rzechorzek et al., 2022). This raises the possibility that psychological stress-induced increases in thermogenesis and body temperature may contribute to activating TRPV1 or TRPV1-like channels in these brain regions. Once activated in the hippocampus, these channels have been linked to increased synaptic transmission, plasticity, and excitability of hippocampal networks via disinhibition, potentially contributing to enhanced hippocampal function (Hurtado-Zavala et al., 2017; Kauer & Gibson, 2009).

This is further supported by studies demonstrating TRPV1's involvement in hippocampal-dependent behaviours. Mice lacking TRPV1 exhibit impaired fear-based learning (Marsch et al., 2007), while activation of hippocampal TRPV1 in rats enhances spatial memory retrieval during stressful situations (Li et al., 2008). Therefore, the potential activation of TRPV1 or TRPV1-like channels in the hippocampus by stress-induced brain hyperthermia may contribute to enhanced hippocampal function and improved cognitive performance under stressful conditions. Investigating the activity of hippocampal neurons expressing temperature-sensitive channels like TRPV1 and TRPV4 during hyperthermic responses, particularly in the context of lithium treatment, represents a promising area for future research. This could provide insights into lithium's therapeutic mechanisms by elucidating its impact on thermoregulation and thermosensitive neuronal populations implicated in cognitive processes. One approach would be using viral vectors to express fluorescent calcium indicators in TRPV1 or TRPV4-expressing neurons within the hippocampus of transgenic animals (Hu et al., 2022; Spencer et al., 2018). These calcium indicators, such as GCaMP, allow optical readouts of neuronal activity by fluorescently reporting increases in intracellular calcium levels associated with action potentials. The activity of these TRPV1/TRPV4 neuronal populations could then be measured during

stress-induced hyperthermia and in response to lithium using in vivo calcium imaging techniques like fibre photometry (Y. Li et al., 2019).

#### *3.4.5.3 Lithium's Dose-Dependent Inhibition of Stress Responses*

Having discussed my observations in the vehicle pre-treatment group, I will now discuss how lithium alters this response. My data demonstrates that lithium, in a dose-dependent manner, inhibits the increases in BAT and body temperature and locomotor activity in resident rats after an intruder's introduction. Therefore, in support of my hypothesis, lithium dose-dependently inhibits psychological stress-induced BAT thermogenesis, emotional hyperthermia, and hyperlocomotion. This finding is complemented by what I found in the rat tail temperature experiment in which lithium administration prevents cutaneous vasoconstriction elicited by handling stress. This reinforces the idea that lithium inhibits the physiological response to acute stress. This outcome aligns with my earlier dose-response experiment in this chapter, wherein lithium dose-dependently reduced these parameters under uninterrupted, resting conditions. Taken together, these findings show that the physiological and behavioural response to lithium is consistent between resting and stressful conditions. This strongly suggests that the body's detection of lithium may lead to the activation of a brain pathway that overrides inputs from normal thermoregulatory circuits and stress-related circuits. The exploration of this pathway will be a focal discussion point in the subsequent chapter of this thesis.

These results provide the first evidence of lithium-induced reduction of hyperlocomotion in response to acute psychological stress. This finding is similar to the large collection of evidence detailing lithium's ability to consistently decrease amphetamine-induced hyperlocomotion (O'Donnell & Gould, 2007; Poitou et al., 1975). Although these two methods are markedly different, they are united in that they are considered models of manic behaviour. Administration of amphetamines to healthy individuals and bipolar patients elicits mania-like symptoms and exacerbates existing symptoms, respectively (O'Donnell & Gould, 2007; Young et al., 2011). Thus, the amphetamine model of mania is used to assess hyperactivity. On the other hand, variants of the resident-intruder paradigm, such as what I used, are recognised as a model for studying a specific aspect of manic behaviour, namely provocative, intrusive, or aggressive actions (Einat, 2006), which are commonly associated with manic individuals (Young et al., 2011). The intruder-elicited hyperlocomotion observed in my resident-intruder study may generally reflect manic-like behaviour of hyperactivity, as considered by other researchers (Benedetti et al., 2008; Gessa et al., 1995; Valvassori et al., 2017). Thus, it may be a compatible model of a manic episode. Therefore, my finding that lithium inhibits psychological stress-induced hyperlocomotion may be clinically relevant, especially as it is consistent with lithium's

antimanic action in humans (Cade, 1949). In further support of this, lithium has been shown to consistently reduce aggressive behaviour in rodents and humans, both manic and non-manic (Cade, 1949; Müller-Oerlinghausen & Lewitzka, 2010; Shader et al., 1974; Sheard, 1975). Therefore, this implicates lithium's thermoregulatory effects as clinically relevant, as they are deeply connected to its behavioural action, as I have demonstrated throughout this thesis.

#### 3.4.5.3.1 Lithium's Selective Influence on Acute Stress Responses

A notable finding in my intruder experiment was that 0.47 mEq/kg LiCl selectively inhibited the physiological stress responses, including BAT thermogenesis and emotional hyperthermia, as well as the behavioural stress response of hyperlocomotion. This is important, as this same dose did not produce significant reductions in BAT temperature, core body temperature, or locomotor activity compared to vehicle in the previous dose-response experiment under non-stress conditions. Therefore, this low dose, comparable to therapeutic serum levels (Malhi et al., 2016; O'Donnell & Gould, 2007) at the time of intruder exposure (Smith, 1976), selectively inhibits the acute physiological and behavioural responses triggered by psychological stress, without impacting baseline physiology and behaviour. While there is limited research directly examining the effects of lithium on psychological stress responses, my findings align with Gambarana and colleagues' observation that chronic lithium treatment attenuates reactive behaviours in response to physical stressors (Gambarana et al., 1999). This selective action against stress-induced responses gives us further insight into lithium's therapeutic effects in stress-related disorders.

#### 3.4.5.3.2 Potential Cognitive Consequences of Lithium During Stress Responses

As I have discussed the likelihood of emotional hyperthermia contributing to enhanced cognition as part of an adaptive response above, it is important to consider lithium's impact on cognition, as its hypothermic effect likely reduces brain temperature. Cold-activated receptors in the hippocampus may help preserve its function, enabling spatial learning even at reduced brain temperatures (Karlsson & Blumberg, 2004). This suggests that lithium is unlikely to negatively impact baseline hippocampal function, rather prevent its increased-brain-temperature enhancement during emotional hyperthermia. It would be valuable to conduct further experiments to determine whether psychological stress produces the same hippocampal theta rhythm and SPW-R changes that are seen with increased brain temperature and whether lithium selectively prevents these changes without affecting basal levels.

#### 3.4.5.3.3 Lithium's Potential Role in Mitigating Chronic Stress-Induced Damage

Chronic stress detrimentally impacts the hippocampus by suppressing neurogenesis, impairing synaptic plasticity, and inducing neurodegeneration (Kim & Kim, 2023). This results in reduced hippocampal volume and the disruption of hippocampal processes such as memory, learning, and spatial navigation (Lee et al., 2009; Radley et al., 2015; Radley & Morrison, 2005). Similarly, patients with stress-related psychiatric disorders such as depression and bipolar disorder tend to have reduced hippocampal volume, which can be reversed by antidepressant treatment (Brosch et al., 2022; Hajek et al., 2012; Radley & Morrison, 2005; Warner-Schmidt & Duman, 2006). Not surprisingly, deficits in hippocampal-related functions such as learning and memory are seen in patients with these conditions (Bearden et al., 2006; Lee et al., 2012). Importantly, long and short-term lithium has been shown to increase hippocampal volume in patients with bipolar disorder, correlating with memory improvement (Yucel et al., 2007; Yucel et al., 2008). In rats, Wood et al. demonstrated that chronic lithium treatment prevented the chronic stress-induced structural changes of hippocampal neurons (Wood et al., 2004). Further, Vasconcellos et al. found that deficits in spatial memory due to chronic stress were attenuated by chronic lithium treatment (Vasconcellos et al., 2003). Thus, there is a large body of evidence that suggests lithium counters the deleterious effects of chronic stress on hippocampal function. My finding that lithium inhibits the physiological response to acute psychological stress supplements these findings, in that lithium's anti-stress action occurs quickly, at least in rodents. Additionally, this mechanism would partly explain why lithium is effective in a range of psychiatric conditions beyond its gold standard use in bipolar disorder.

#### 3.4.5.3.4 Potential Use of Lithium in Reversing Pathological Hyperthermia

Lithium's ability to inhibit BAT thermogenesis and emotional hyperthermia may be useful in the context of pathological hyperthermia during acute N-methyl-3,4-methylenedioxy-amphetamine (MDMA or 'ecstasy') intoxication. In our lab's prior work, it was discovered that clozapine effectively reversed MDMA-induced hyperthermia in rabbits and rats (Blessing et al., 2003; Blessing et al., 2006). This reversal was attributed to the vasodilation occurring in the heat-exchanging vascular beds of the rabbit ear pinna and rat tail, which facilitated heat loss. Additionally, clozapine was observed to inhibit BAT thermogenesis as another contributing factor to the reversal. Kiyatkin et al. reported similar findings in rats whereby clozapine reversed body and brain hyperthermia (Kiyatkin et al., 2016). Curiously, lithium shares similar hypothermic effects such as inhibition of both BAT thermogenesis and cutaneous vasoconstriction, as I have observed throughout this chapter. However, whether lithium reverses MDMA-induced hyperthermia is yet to be tested. Further experiments designed to answer

this question would be of great clinical value, as lithium could potentially reverse life-threatening hyperthermia due to MDMA toxicity. This life-saving use of lithium may be applicable to other stimulants known to produce pathological hyperthermia in cases of toxicity, such as amphetamine and methamphetamine (Bowyer & Hanig, 2014; Matsumoto et al., 2014).

#### *3.4.5.4 Distinguishing Between Adaptive and Maladaptive Stress Responses*

While psychological stress can result in an adaptive response that enhances functions like cognition and behavioural performance, it can also have deleterious effects. This depends on the type and duration of stress (Dhabhar, 2014; McEwen, 2007, 2008). Notably, Tomar et al. demonstrated in mice that acute stress enhances the coordination of hippocampal neural activity, facilitating spatial information processing, while chronic stress impairs functional connectivity and disrupts the coordination of neural activity in hippocampal circuits (Tomar et al., 2021). Profoundly intense psychological stress can also be physically damaging. For example, it has been shown in animal studies that prolonged forcible immobilisation stress, which induces a sense of helplessness, can precipitate intense autonomic responses leading to physical lesions such as peptic ulcers (Stevens, 1977). Therefore, while my observations of the rat's response to acute psychological stress during the resident-intruder procedure may have reflected a beneficial, adaptive behaviour, it may not be the case for other forms of stress used in other studies.

#### *3.4.5.5 Next Steps*

In the following chapter, I will investigate whether lithium inhibits BAT sympathetic nerve activity and other sympathetic physiological parameters. Furthermore, I will delve into lithium's mechanism of action and attempt to construct a plausible model for the brain pathways it influences.

# Chapter 4.

# Lithium's Peripheral Mechanism: Unveiling Sympathetic Pathways

## 4.1 Introduction

The collective evidence from the preceding chapters of this thesis has served to confirm the hypothermic effects of lithium across different species, specifically guinea pigs and rats. My investigations have shown that lithium dose-dependently decreases body and brown adipose tissue (BAT) temperature and locomotor activity. Notably, lithium also influences thermoregulatory behaviour that contributes to hypothermia, such as cooling posture in guinea pigs and reduced locomotor activity in both guinea pigs and rats. A decrease in BAT temperature offers a mechanistic insight into how lithium induces its hypothermic effects. Moreover, I discovered that lithium initiates cutaneous vasodilation, another mechanism that contributes to lithium-induced hypothermia.

In the previous chapter, I observed that lithium selectively inhibits BAT thermogenesis, emotional hyperthermia, and hyperlocomotion elicited by acute psychological stress in rats. Furthermore, lithium prevents increases in cutaneous vasoconstriction in response to handling stress. These observations led me to postulate that lithium must reduce sympathetic outflow from the brain. One of the prevailing hypotheses of this thesis is that lithium's action is initiated in the periphery, as its effects transpire faster than the ion can reach equilibrium in the brain (Wraae, 1978). Therefore, it is highly likely that the hypothalamomedullary network in the brain is influenced by the presence of lithium in the periphery, thereby reducing sympathetic outflow from the rostral medullary raphe (rMR) that governs thermoregulatory and cardiovascular effectors such as BAT, cutaneous vascular beds, and the heart. For an extensive review of the hypothalamomedullary network, please see Nakamura and colleagues' work (Nakamura et al., 2022). This network also contains brain areas implicated in behavioural thermoregulation and locomotor activity (Nakamura & Morrison, 2022).

Thus, a central concept emerges as the main focus in my exploration: lithium's impact on the sympathetic response, also known as the stress response. This is a suite of physiological responses controlled by the sympathetic branch of the autonomic nervous system that include increased

thermogenesis, heat conservation, and cardiovascular responses. This response can be triggered by various stressors, including psychological stress, cold, and infection (Nakamura et al., 2022). In the specific context of psychological stress, this response is often referred to as the 'fight or flight' response (Nakamura & Morrison, 2022). Despite the potential importance of this concept, the involvement of the sympathetic nervous system in lithium's action is an underexplored area in the literature, evidenced by a lack of discussion in many reviews (Anand et al., 2020; Brown & Tracy, 2013; El-Mallakh, 2004; Jope, 1999; Lenox & Hahn, 2000; Malhi et al., 2013; Phiel & Klein, 2001; Quiroz et al., 2010; Shaldubina et al., 2001; Smith, 1980b; Young, 2009), with only one animal study briefly suggesting its role (Jones et al., 2008).

As for the site of lithium's peripheral action, the afferent fibres of the vagus nerve and the area postrema (AP) stand out because they both mediate nausea (Zhang et al., 2021), a well-known effect produced by lithium administration (Horn, 2014; Schou, 1968; Schou et al., 1968), and are both activated by lithium (Adachi et al., 1991; Niijima & Yamamoto, 1994; Tsukamoto & Adachi, 1994; Ueda et al., 2016). The vagus nerve, in addition to mediating information related to contents in the gastrointestinal tract (Wang et al., 2020), is a major conduit of parasympathetic information, and can reduce heart rate, potentially overriding sympathetic-induced increases (Capilupi et al., 2020). Furthermore, vagal afferents project to the AP region (Ferguson, 1991), suggesting a potential coordinated role in lithium's action. Meanwhile, the AP, a chemoreceptive brain structure outside the blood-brain barrier, can detect changes in the blood composition from the periphery (Price et al., 2008).

In this thesis, I have established that nausea is closely associated with lithium's physiological and behavioural hypothermic effects. Although I have shown that these effects are not mediated by the 5-$HT_3$ receptor, it is noteworthy that these receptors are expressed in both the vagal afferents and the AP (Tyers & Freeman, 1992). However, the roles of these structures in modulating other physiological responses to lithium, such as the reduction in sympathetic activity, and whether they are crucial in detecting and mediating the response to lithium remain substantially underexplored. It is important to note that there is evidence in the literature indicating that lesioning the AP prevents lithium-induced conditioned taste aversion, hypothermia, and lethargic behaviour (Bernstein et al., 1992). However, it remains an open question whether this structure also mediates other physiological responses to lithium, such as the inhibition of sympathetic activity that drives BAT thermogenesis and cardiovascular changes. Given the AP's known involvement in certain lithium effects, it is likely that this structure plays a significant role in these responses as well.

Despite the well-established therapeutic efficacy of lithium in treating psychiatric conditions (Tondo et al., 2019), the current state of the literature reveals a considerable gap in understanding how lithium influences specific brain regions to modify sympathetic outflow and thermoregulatory responses. This lack of knowledge extends to how lithium's physiological effects relate to its therapeutic action. While lithium is known to impact numerous physiological systems through its molecular interactions (Malhi et al., 2013), the resulting effects are varied and complex. This complexity makes it challenging to elucidate a clear and coherent mechanism of action. Most of the current hypotheses focus on lithium's intracellular targets (Agam, 2014; Klein & Melton, 1996; Malhi et al., 2013), notably its inhibition of certain enzymes involved in intracellular signalling pathways. However, these do not provide a comprehensive explanation for the drug's distinctive physiological and behavioural effects. As such, there is a pressing need for research that explores the neural mechanisms underlying lithium's effects in a more integrated manner, rather than focusing solely on its molecular targets.

In this chapter, my primary objectives were twofold: to investigate lithium's impact on the thermoregulatory sympathetic response and to identify the peripheral mechanism through which it acts. To meet these objectives, I designed and executed two distinct yet complementary experiments involving anaesthetised rats. These experiments focused on two peripheral targets: the vagus nerve and the AP.

In both experiments, I specifically examined isolated post-ganglionic sympathetic nerve axons in the interscapular BAT of anaesthetised rats. Each rat was subjected to cold stress, proven to be a potent trigger of the sympathetic response (Nakamura et al., 2022), following the administration of saline, 2 mEq/kg LiCl, or 4 mEq/kg LiCl. I continuously monitored a set of physiological parameters: BAT sympathetic nerve discharge and temperature, indicative of BAT thermogenesis; exhaled $CO_2$ and heart rate, indicative of metabolic activity; and mean arterial pressure, a parameter affected by a combination of cardiac output and peripheral vascular resistance (e.g., cutaneous vasoconstriction). All these measurements were focused on the cold-evoked increases, as they each form part of the overall sympathetic response. This comprehensive monitoring allowed me to assess whether lithium reduces the cold-evoked sympathetic drive, supplied by the rMR to BAT and cardiovascular centres, in a dose-dependent manner.

The first experiment began with a control group of rats that were not subjected to any surgical intervention. This helped me establish the baseline effects of lithium on the sympathetic response, thus achieving my first goal. For my second objective, I performed bilateral vagotomy on two groups of rats at different locations along the vagus nerves to assess any changes in the effects of lithium.

One group of rats underwent vagotomy at the subdiaphragmatic level, which cut off afferent signals coming from the gut. Another group received vagotomy at the cervical level, which terminated both afferent signals from the gut and efferent signals from the brain to areas such as the heart. This surgical design allowed me to determine if the vagus nerve is responsible for either the detection of, or aspects of the response to, lithium. This was particularly important given that efferent signals from the brain carried by the vagus have the ability to slow heart rate (Capilupi et al., 2020).

The second experiment paralleled the first but shifted its focus from the vagus nerve to the AP, a chemoreceptive brain structure that resides outside of the blood-brain barrier. This adjustment furthered my second aim by allowing me to explore the role of the AP, a structure uniquely positioned to monitor the blood in the periphery, in mediating lithium's physiological effects on the cold-evoked sympathetic response. In this experiment, I created lesions in the AP by aspiration in one group of rats, comparing their responses with a sham-operated control group. In the control group, the AP was surgically exposed but left undisturbed. Following exposure to cold stress as in the first experiment, I conducted identical measurements and evaluations. This way, I was able to determine whether the vagus and the AP have roles in modulating lithium's action.

In essence, my primary hypothesis was that lithium dose-dependently reduces each increase in the physiological parameters of the cold-evoked thermoregulatory sympathetic response, including BAT sympathetic nerve discharge and temperature, expired $CO_2$ levels, heart rate, and mean arterial pressure. Building on this, I expected that the vagus nerve and the AP, both of which have been implicated in mediating nausea induced by lithium, play critical roles in detecting lithium in the periphery and transmitting this information to the brain, thereby modulating the sympathetic response and contributing to the observed physiological effects.

In the proceeding sections of this final chapter, I will detail my methods and present my findings. I will discuss the physiological and clinical implications of lithium's impact on the sympathetic response and the respective roles of the vagus nerve and the AP, with an emphasis on the latter. Based on my findings, I will present an integrative perspective of lithium's action, proposing central pathways within

the hypothalamomedullary network that aim to explain the behavioural and physiological responses to lithium and its therapeutic action. This new model will challenge the current molecular-focused hypotheses, which, while valuable, lack specificity. By presenting a comprehensive map of how and where lithium produces its effects, I hope to contribute to a more nuanced understanding of this ion, thereby informing future research and therapeutic strategies for psychiatric conditions.

## 4.2 Methods

### 4.2.1 Ethics Approval

With the approval of the Flinders University Animal Welfare Committee, the experiments reported in this study were conducted at Flinders University (FMC, Bedford Park, SA, Australia) in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition (National Health and Medical Research Council et al., 2013).

### 4.2.2 Animals

Male Sprague-Dawley rats (250–450 g; n = 6 for each group in both experiments of this chapter) were sourced from the Flinders University Animal Facility. Outside of experimental procedures, the rats were communally housed under a reversed 12-hour light/dark cycle to accommodate their nocturnal nature, with ad libitum access to food and water. For the vagotomy experiment, rats were randomly assigned to one of three groups: control, cervical vagotomy, and subdiaphragmatic vagotomy. For the area postrema (AP) lesion experiment, rats were randomly assigned to one of two groups: sham and lesion.

It should be noted that any recordings that were too noisy and could not be corrected, those that were broken, or animals that did not survive during the experiment were excluded from the sample sizes.

### 4.2.3 Cervical Vagotomy

In accordance with methods previously described in the literature (Loomis et al., 1997), the procedure for bilateral cervical vagotomy commenced following tracheotomy. The cervical vagi were identified on either side of the trachea and carefully isolated from the neighbouring carotid arteries and the sympathetic trunk.

Upon identification, silk ligatures were looped around both the left and right vagus nerves. These ligatures were then securely tied, and each vagus nerve was subsequently transected between the ligatures.

Post-transection, the separated ligatures were inspected under the operating microscope to confirm the absence of any bridging nerve fibres between the bisected nerve ends. This step affirmed the complete severance of the vagus nerves, thereby validating the effectiveness of the cervical vagotomy procedure.

### 4.2.4 Subdiaphragmatic Vagotomy

The procedure for subdiaphragmatic vagotomy was initiated during the preliminary surgical stage, following the tracheotomy. As previously described in the literature (Houpt et al., 1997), the thoracic vagal trunks were identified and traced from the stomach to just below the diaphragm.

Once the trunks were isolated, a pair of silk ligatures were securely tied around each trunk. Subsequent to the placement of the ligatures, a controlled and precise transection of each marked vagal trunk was carried out between the silk ligatures.

Following the transection, a verification process was promptly initiated. The bisected segments of the vagal trunks, demarcated by the tied silk ligatures, were thoroughly inspected under the operating microscope for the presence of any residual nerve connections. The absence of identifiable connecting fibres between the bisected segments confirmed the success of the vagotomy. This immediate post-transection verification process was crucial to ensure the effectiveness of each performed vagotomy.

### 4.2.5 Area Postrema Lesion

In line with procedures previously described in the literature (Averill et al., 1996; Curtis et al., 1999; Edwards et al., 1993), the lesion procedure commenced once each rat was securely positioned within the stereotaxic frame. An incision, approximately 3cm in length, was initiated at the occipital crest and extended caudally towards the mid-cervical region. This revealed the underlying muscular tissue, which was subsequently dissected and retracted laterally.

The atlantooccipital ligament was carefully incised, exposing the foramen magnum. The foramen magnum was then dorsomedially enlarged to provide a more extensive view of the underlying meninges. These meninges were subsequently incised to expose the dorsal surface of the medulla.

In certain instances, a gentle elevation of the cerebellum was necessary to achieve an unobstructed view of the AP under an operating microscope.

The AP was then carefully aspirated using a blunted 23-gauge needle attached to a syringe. Utmost care was taken during this aspiration procedure to minimise harm to the surrounding structures.

Following the aspiration, the site was delicately packed with sterile gauze that had been thoroughly soaked in isotonic saline. This packing process served to create a barrier to prevent the exposed tissue from drying out, thereby preserving the integrity of the surgical site.

#### *4.2.5.1 Sham Operation*

For the sham-operated group, the procedure largely mirrored the one described above (4.2.5), up until the point of AP visualisation.

Once the dorsal surface of the medulla was exposed and the AP was clearly visualised under the operating microscope, the procedure was halted. The AP was left untouched, ensuring no aspiration occurred in this group.

Subsequently, the surgical site was delicately packed with sterile gauze soaked in isotonic saline. This helped create a protective barrier, preventing the exposed tissue from drying out.

### 4.2.6 Preparatory Surgery and Experimental Setup

Preparatory surgery for the anaesthetised experiments commenced with the rats being placed under indefinite general anaesthesia (isoflurane in 100% oxygen [0.8–1 L/min]: 2.5–3 % for induction, 1–2 % for maintenance). Core body temperature and end-tidal $CO_2$ were monitored with a rectal thermocouple (TC-2000 thermocouple meter; Sable Systems) and a $CO_2$ monitor (Normocap; Datex, Helsinki, Finland), respectfully. A tracheotomy was performed to facilitate artificial respiratory control at a later stage.

In the vagotomy experiment, rats in the vagotomy groups underwent either cervical (see **Section 4.2.3**) or subdiaphragmatic vagotomy (**Section 4.2.4**). Rats in the control group did not receive this procedure.

Subsequently, the right femoral vein was cannulated for drug and fluid administration. The right femoral artery was also cannulated and connected to a transducer linked to a bridge amplifier (NL108;

Digitimer Ltd., Welwyn Garden City, U.K.) for the measurement of arterial blood pressure (AP). The arterial line was filled with a heparin solution (50 U/ml in 0.9% saline) to prevent coagulation-induced blockage. An intraperitoneal catheter was inserted through the left lumbar region of the abdominal cavity before each rat was mounted in a prone position onto a stereotaxic apparatus. The rats were enveloped in a water jacket to regulate body temperature. Skin temperature was monitored by placing a thermocouple between the water jacket and the ventral body. The isoflurane was gradually tapered off (with oxygen still supplied) and replaced with a cocktail of urethane (400–800 mg/kg) and alpha-chloralose (40–80 mg/kg) as the general anaesthetic agent, which was infused intravenously at a rate of 0.035 ml/min. The rats were suspended in the stereotaxic frame to ensure the vertebral column was horizontally flat.

In the AP lesion experiment, rats in the lesion group had their APs lesioned (see **Section 4.2.5**), while those in the control group underwent a sham operation (**Section 4.2.5.1**).

Once suspended, a brown adipose tissue (BAT) sympathetic nerve was isolated from the right-hand side of the interscapular region, as previously described (Ootsuka & McAllen, 2006). The nerve was placed over a pair of silver electrodes in a pool of paraffin oil to record sympathetic nerve activity. BAT temperature was measured with a small thermocouple inserted contralaterally to the isolated BAT sympathetic nerve. D-tubocurarine dichloride pentahydrate (3 mg/mL in Ringer's solution; Sigma-Aldrich, St. Louis, MO, USA) was administered intravenously every hour to paralyse the rats (preventing the nerve from spontaneously moving due to natural respiration), while their breathing was artificially controlled via a ventilator at a volume of 2–3 mL/cycle and rate of 60–65 cycles/min.

### 4.2.7 Experimental Procedure: Cold-Evoked Sympathetic Response after Lithium

Once the core body temperature of the rats stabilised at 37 °C (± 0.2 °C), cold stress was applied by circulating icy cold water (4–10 °C) through the water jacket (skin temperature Δ-12 °C ± 2 °C) for 3 minutes before reverting to warm water (36–45 °C). This step was implemented to ensure that the isolated nerve was functional. Electrical discharges from the isolated BAT sympathetic nerve were detected by silver electrodes, amplified (gain: x20,000, NL104 amplifier; Digitimer Ltd., Welwyn Garden City, U.K.) and filtered (band-pass: 10–1000 Hz, NL125 filter; Digitimer).

Once the cold-evoked BATSNA returned to baseline, a control solution (0.9% saline, 0.5 mL; Fresenius Kabi, North Ryde BC, NSW, Australia) was administered intraperitoneally. Ten minutes later, cold

exposure was applied again. After BATSNA reached baseline, the process was repeated for 2 mEq/kg and then 4 mEq/kg LiCl (Sigma-Aldrich) in 0.5 mL $H_2O$ (Fresenius Kabi).

BATSNA, AP, heart rate (HR), temperature (BAT, body & skin), and end-tidal $CO_2$ ($EtCO_2$) signals were digitised by PowerLab hardware (ADInstruments, Castle Hill, NSW, Australia) at sampling rates of 1 kHz (BATSNA), 100 Hz (AP), 400 Hz (HR), 10 Hz (temperature), and 40 Hz ($EtCO_2$), and recorded with LabChart computer software (ADInstruments).

Before ending the experiment, the sympathetic identity of the isolated nerve was confirmed with the intravenous administration of hexamethonium bromide (10 mg/kg; Sigma-Aldrich), a ganglionic blocker that attenuates sympathetic activity.

### 4.2.8 Humane Endpoint and Post-Experiment Procedures

At the conclusion of each experiment, animals were humanely euthanised with an intravenous injection of pentobarbitone sodium (180 mg/kg; Virbac Pty Limited, Milperra, NSW, Australia).

In the area postrema experiment, additional steps were taken for subsequent histological analysis. The rats from both groups were initially transcardially perfused with 0.5 M phosphate buffered saline (PBS), followed by sequential formaldehyde fixative solutions. The first solution contained 10% formaldehyde in 0.4 M PBS, and the second comprised 10% formaldehyde with 20% sucrose in 0.4 M PBS.

The following day, the brains were sectioned into serial slices of 50µm thickness using a cryostat (Cryocut 1900; Leica Microsystems Pty Ltd, North Ryde, NSW, Australia). The sections were then stained with a Neutral Red solution (Sigma-Aldrich) to enhance visibility of the neural tissue structures, particularly nuclei.

Finally, the stained sections were examined under light microscopy. This allowed for the confirmation of successful removal of the area postrema in the lesion group, or its intactness in the sham group. This step was crucial for verifying the accuracy and effectiveness of the experimental procedures.

### 4.2.9 Data Analysis

All recorded data were processed in Igor Pro (WaveMetrics, Portland, OR, USA). Data artifacts such as notch noise were accounted for by interpolating the two datapoints surrounding the noise. Digitised BATSNA signals were downsampled from 1 kHz to 400 Hz and divided into 5.12-second segments. The fast Fourier transform (FFT) algorithm was applied to each 5.12-second segment using the Hanning window function with no overlap (Ootsuka & McAllen, 2006). BATSNA amplitude was then expressed as the total power (dBμV) between 10–40 Hz of the spectral power densities calculated from FFT auto-spectra. BATSNA power was then smoothed using binomial (gaussian) smoothing with 51 operations. Note that Power BATSNA appeared to increase before the onset of cooling; this was due to the application of binomial smoothing. As the nerve response data most likely followed a log-normal distribution (Buzsáki & Mizuseki, 2014; Koch, 1966), BATSNA power was log-transformed, as done previously by this lab (Brizuela et al., 2019); this simultaneously prevented skewed data, satisfied the normal distribution requirement of statistical analyses, and compensated for variability between each nerve recording, such as different numbers of filaments in each isolated nerve. $EtCO_2$ was divided into 0.06-second windows and each value within a window was replaced with the window peak; this reduced rapid fluctuations in $EtCO_2$ and ensured that the $EtCO_2$ data represented the true measurement of $CO_2$ during expiration. Mean arterial pressure (MAP) was calculated from AP measurements using a low-pass filter with a cut-off frequency of 1 Hz. $EtCO_2$, HR, MAP, and skin temperature were downsampled to 1 Hz. All delta (Δ, change) values were calculated by subtracting the value of a measurement at the start of cooling from each respective datapoint. All averaged data was presented with SEM.

### 4.2.10 Statistical Analysis

Prism (GraphPad, San Diego, CA, USA) was used for all statistical analyses. Values for statistical analysis were calculated as follows: the area of BATSNA was calculated from the onset of cooling to the end of the response. The end of the response was manually determined by selecting the point in time where log-transformed BATSNA power and its differentiated wave returned to or closest to pre-cooling level (baseline). For HR and MAP, delta values were taken at the time of peak of cooling. For BAT temperature, end-tidal $CO_2$, and body temperature, delta values were taken 2 minutes post-cooling-peak.

In both the vagotomy and area postrema experiments, paired t tests were used within the control group (or sham group for the area postrema experiment) to assess the significance of changes in

measured parameters (BATSNA log power, BAT temperature, end-tidal $CO_2$, HR, MAP, and body temperature) 10 minutes after the administration of 0.9% saline and subsequent cooling (Zar 1999).

In each separate experiment, a one-way ANOVA was performed to compare the cooling-evoked changes following saline administration between the control group and the experimental groups (Zar 1999). In the vagotomy experiment, these groups included the control, cervical vagotomy, and subdiaphragmatic vagotomy groups. In the area postrema experiment, the groups were the sham and area postrema lesion groups.

For the dose-dependent effect of lithium, I performed linear regression analyses within each group of both experiments, using the log-transformed lithium dose as the predictor variable. The slopes of these regression lines represented the dose-dependent changes in each parameter.

These regression slopes for each parameter, in relation to the log-transformed lithium dose, were compared within each experiment between groups using a method functionally equivalent to an analysis of covariance (Zar, 1999). This method involved fitting separate linear regression models to each group, and then testing whether the slopes (and intercepts) of these models were significantly different in each experiment. If the slopes of more than two groups were compared, follow-up pairwise comparisons were made using one-way ANOVA. The null hypothesis was that there were no differences between the slopes of the regression lines of the different groups within each experiment.

In the cervical vagotomy group, two animals were identified as non-responsive to cold exposure. As these animals did not display any change in key physiological parameters in response to cooling following the administration of saline, they were considered outliers and were removed in a secondary analysis.

All statistical analyses were conducted with a significance level set at $P < 0.05$. 'Slope significance' refers to the p-value obtained from testing the null hypothesis that the slope of the regression line is zero. A significant slope ($P < 0.05$) indicates that there is a statistically significant relationship between the predictor variable (in this case, the log-transformed lithium dose) and the dependent variable (the measured parameter). 'Goodness of fit' is quantified by the $R^2$ value, which ranges from 0 to 1. It provides a measure of how well the observed outcomes are replicated by the model, based on the proportion of total variation of outcomes explained by the model. A higher $R^2$ value indicates a better fit of the data to the regression line.

## 4.3 Results

### 4.3.1 The Effect of Lithium on the Sympathetic Response to Cooling in Anaesthetised, Vagotomised Rats

#### *4.3.1.1 Control Group*

In the control group (**Figure 4.2**), I observed significant changes 10 minutes after the intraperitoneal administration of 0.9% saline and subsequent skin cooling. The brown adipose tissue (BAT) sympathetic nerve activity (SNA, BATSNA) log power increased from 3.13 ± 0.13 to 3.46 ± 0.12 dBμV (n = 6, P < 0.05, paired t test). Similarly, the BAT temperature rose from 35.51 ± 0.37 to 35.96 ± 0.31 °C (n = 6, P < 0.05, paired t test). The end-tidal $CO_2$ levels also increased, from 3.58 ± 0.18 to 3.82 ± 0.14 % (n = 6, P < 0.05, paired t test), as did the heart rate (HR), which went from 402.8 ± 21.05 to 441.2 ± 16.86 bpm (n = 6, P < 0.05, paired t test). The mean arterial pressure (MAP) increased from 122 ± 6.41 to 132.4 ± 7.20 mmHg (n = 5, P < 0.05, paired t test). However, the body temperature did not show a significant change, remaining relatively stable at 37.29 ± 0.10 to 37.24 ± 0.11 °C (n = 6, P > 0.05, paired t test).

The relationship between the log-transformed dose of lithium and each parameter was examined using linear regression analysis. The results revealed that intraperitoneal administration of lithium dose-dependently attenuated all parameters, except for body temperature. **Table 4.1** provides the specific values used in this analysis, including the area of log-transformed BATSNA power and the change (delta, Δ) in each parameter. Additionally, the table presents the p values, which indicate the significance of the regression slope (testing the null hypothesis that the slope is zero), and the $R^2$ values, which represent the goodness of fit.

**Figure 4.1** provides a representative recording from one animal in the control group, illustrating the individual changes in BATSNA log power, BAT temperature, end-tidal $CO_2$ levels, HR, and MAP during the cooling period subsequent to each intraperitoneal administration of 0.9% saline, 2 mEq/kg LiCl, and 4 mEq/kg LiCl. This individual data contributes to the aggregated response shown in **Figure 4.2**, which aligns the responses from each control animal according to peak cooling at 0 minutes.

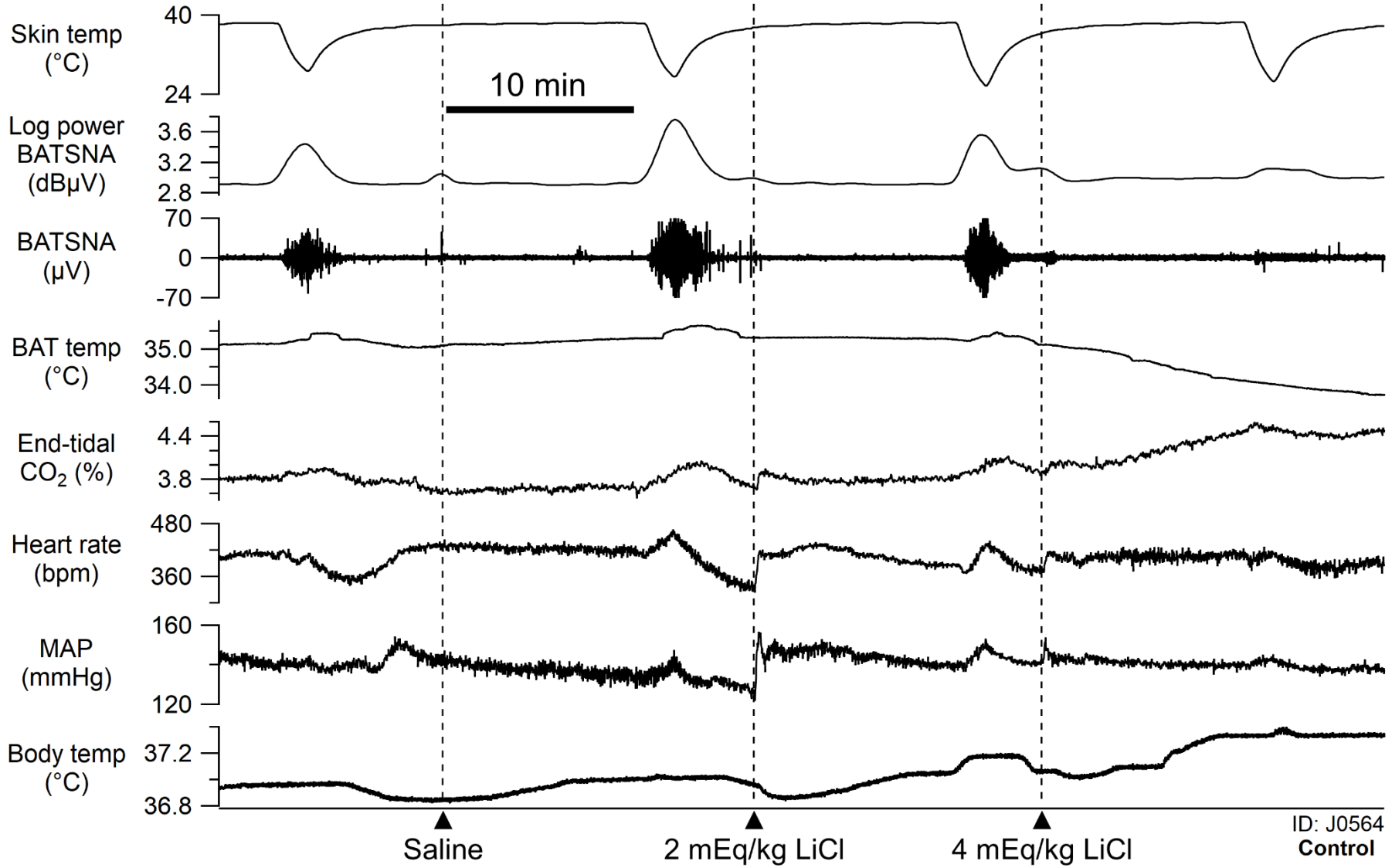


**Figure 4.1.** Example recording from one animal in the control group, demonstrating the physiological responses to cold exposure following intraperitoneal administration of 0.9% saline and lithium doses of 2 mEq/kg and 4 mEq/kg. The cold exposure commenced 10 minutes post-injection and was sustained for 3 minutes. From top to bottom, the traces represent skin temperature (°C), log-transformed BATSNA power (dBμV), raw BATSNA power (μV), BAT temperature (°C), end-tidal $CO_2$ levels (%), heart rate (bpm), mean arterial pressure (mmHg), and body temperature (°C). Note that the magnitude of each cooling-evoked response becomes less pronounced following successive lithium injections.

#### *4.3.1.2 Cervical and Subdiaphragmatic Vagotomy Groups*

At the 10-minute mark following the intraperitoneal injection of 0.9% saline, during the cooling phase, both the cervical (**Figure 4.3**) and subdiaphragmatic (**Figure 4.4**) vagotomy groups exhibited increases in BATSNA log power, BAT temperature, end-tidal $CO_2$, HR, and MAP that were statistically similar to those observed in the control group ($P > 0.05$, one-way ANOVA). Body temperature remained constant in these groups, with no significant difference compared to the control group ($P > 0.05$, one-way ANOVA).

In the cervical vagotomy group, intraperitoneal lithium administration resulted in a dose-dependent attenuation of all parameters, with the exception of end-tidal $CO_2$, MAP, and body temperature. Specific values and linear regression results can be found in **Table 4.1**.

Similarly, in the subdiaphragmatic vagotomy group, intraperitoneal lithium administration led to a dose-dependent attenuation of each parameter, except for MAP and body temperature. Details are provided in **Table 4.1**.

I compared the regression slopes of each parameter and log-transformed dose across each group using an analysis of covariance. The slopes of log-transformed BATNSA power area (**Figure 4.5 A**), and changes in BAT temperature (**Figure 4.5 B**), end-tidal $CO_2$ (**Figure 4.5 C**), HR (**Figure 4.5 D**), MAP (**Figure 4.5 E**), and body temperature (**Figure 4.5 F**) did not significantly differ across the groups ($P > 0.05$).

##### 4.3.1.2.1 Secondary Analysis

There is an immediate reason why end-tidal $CO_2$ was not dose-dependently affected by lithium in the cervical vagotomy group. Two animals were identified as non-responsive to cold exposure. In these animals, more than one physiological parameter did not change in response to cooling after 0.9% saline (BAT temperature, end-tidal $CO_2$, and HR). Therefore, a secondary analysis with these outliers removed was performed. The rationale for this secondary analysis was that, in the two non-responsive animals, it would be impossible to accurately assess the effects of lithium on the sympathetic response to cold exposure. This is because these animals did not initially display any change in key physiological parameters in response to cooling following the administration of saline, which served as my control condition. With the outliers removed, end-tidal $CO_2$ was dose-dependently attenuated by lithium and the goodness of fit for the already-significant slopes were improved (see **Table 4.2**). The removal of non-responsive animals from the cervical group did not result in any significant changes regarding the lack of statistical significance in the comparison of linear regression slopes across the groups. In other words, excluding the non-responsive animals did not alter the overall finding that there were no significant differences ($P > 0.05$) in the linear regression slopes between the groups.

**Table 4.1.** Physiological responses to cooling 10 minutes post-intraperitoneal lithium (2, 4 mEq/kg LiCl) or 0.9% saline in control, cervical, and subdiaphragmatic vagotomy rats. The table includes log-transformed brown adipose tissue sympathetic nerve activity (BATSNA) power area and changes (Δ) in BAT temperature, end-tidal $CO_2$ ($EtCO_2$), heart rate (HR), mean arterial pressure (MAP), and body temperature. Δ values represent changes from the start of cooling to 2 minutes after the peak of cooling for BAT temperature, $EtCO_2$, and body temperature, and to the peak of cooling for HR and MAP. Data are mean ± SEM. Also included are linear regression results; bold italicised ***p values*** indicate statistical significance. See **Figures 4.2**, **4.3**, and **4.4** for sample sizes.

| | LiCl dose | Power area (dBμV·mins) | BAT (Δ °C) | $EtCO_2$ (Δ %) | HR (Δ bpm) | MAP (Δ mmHg) | Body (Δ °C) |
|---|---|---|---|---|---|---|---|
| **Control** | Saline | 33.96 ± 5.12 | 0.45 ± 0.13 | 0.24 ± 0.07 | 38.42 ± 12.10 | 10.35 ± 2.24 | -0.06 ± 0.03 |
| | 2 mEq/kg | 17.44 ± 2.05 | 0.09 ± 0.08 | 0.02 ± 0.09 | 25.05 ± 11.40 | 6.04 ± 2.19 | -0.07 ± 0.03 |
| | 4 mEq/kg | 11.98 ± 0.37 | -0.09 ± 0.03 | -0.10 ± 0.01 | 3.79 ± 2.45 | 2.10 ± 0.91 | -0.04 ± 0.05 |
| Slope significance | | ***P < 0.0001*** | ***P = 0.0005*** | ***P = 0.0024*** | ***P = 0.0351*** | ***P = 0.0100*** | P = 0.9035 |
| Goodness of fit | | $R^2$ = 0.6318 | $R^2$ = 0.5390 | $R^2$ = 0.4471 | $R^2$ = 0.2489 | $R^2$ = 0.4115 | $R^2$ = 0.0009 |
| **Cervical vagotomy** | Saline | 37.28 ± 4.75 | 0.35 ± 0.21 | 0.24 ± 0.18 | 20.40 ± 14.13 | 5.36 ± 3.26 | -0.24 ± 0.11 |
| | 2 mEq/kg | 17.36 ± 1.58 | 0.03 ± 0.16 | 0.06 ± 0.12 | 1.46 ± 8.88 | 1.03 ± 3.25 | -0.23 ± 0.10 |
| | 4 mEq/kg | 15.94 ± 3.64 | -0.08 ± 0.02 | -0.09 ± 0.07 | -9.37 ± 3.76 | -0.34 ± 1.39 | -0.20 ± 0.09 |
| Slope significance | | ***P = 0.0004*** | ***P = 0.0477*** | P = 0.0945 | ***P = 0.0454*** | P = 0.1386 | P = 0.8373 |
| Goodness of fit | | $R^2$ = 0.6329 | $R^2$ = 0.2687 | $R^2$ = 0.2002 | $R^2$ = 0.2736 | $R^2$ = 0.1607 | $R^2$ = 0.0034 |
| **Subdiaphragmatic vagotomy** | Saline | 36.66 ± 6.12 | 0.75 ± 0.22 | 0.55 ± 0.06 | 40.33 ± 7.18 | 2.81 ± 2.42 | -0.33 ± 0.22 |
| | 2 mEq/kg | 15.37 ± 3.79 | 0.17 ± 0.29 | 0.10 ± 0.18 | 27.12 ± 14.42 | 1.66 ± 2.06 | -0.38 ± 0.26 |
| | 4 mEq/kg | 10.88 ± 1.50 | -0.07 ± 0.03 | -0.10 ± 0.03 | 9.15 ± 4.50 | 4.74 ± 3.27 | -0.36 ± 0.24 |
| Slope significance | | ***P = 0.0004*** | ***P = 0.0121*** | ***P = 0.0002*** | ***P = 0.0299*** | P = 0.7740 | P = 0.9117 |
| Goodness of fit | | $R^2$ = 0.6348 | $R^2$ = 0.3952 | $R^2$ = 0.7047 | $R^2$ = 0.3358 | $R^2$ = 0.0078 | $R^2$ = 0.0010 |

**Table 4.2.** Secondary analysis of physiological responses to cooling 10 minutes post-intraperitoneal administration of lithium (2, 4 mEq/kg LiCl) or saline (0.9% saline) in cervical vagotomy rats, with non-responsive animals removed. The table includes log-transformed brown adipose tissue sympathetic nerve activity (BATSNA) power area and changes (Δ) in BAT temperature, end-tidal $CO_2$ ($EtCO_2$), heart rate (HR), mean arterial pressure (MAP), and body temperature. Δ values represent changes from the start of cooling to 2 minutes after the peak of cooling for BAT temperature, $EtCO_2$, and body temperature, and to the peak of cooling for HR and MAP. Data are mean ± SEM. Bold italicised ***p values*** indicate statistical significance. Sample size is n = 3; please refer back to **Table 4.1** for original data and **Figure 4.3** for initial sample size.

| | **LiCl dose** | **Power area (dBμV·mins)** | **BAT (Δ °C)** | **$EtCO_2$ (Δ %)** | **HR (Δ bpm)** | **MAP (Δ mmHg)** | **Body (Δ °C)** |
|---|---|---|---|---|---|---|---|
| **Cervical vagotomy** | Saline | 36.06 ± 5.79 | 0.66 ± 0.17 | 0.48 ± 0.18 | 38.51 ± 12.82 | 6.27 ± 5.28 | -0.07 ± 0.08 |
| | 2 mEq/kg | 16.26 ± 0.90 | 0.12 ± 0.27 | 0.17 ± 0.13 | 13.10 ± 8.09 | 1.47 ± 3.40 | -0.11 ± 0.10 |
| | 4 mEq/kg | 14.09 ± 4.05 | -0.11 ± 0.01 | -0.09 ± 0.02 | -7.16 ± 4.01 | 0.65 ± 1.13 | -0.07 ± 0.07 |
| Slope significance | | ***P = 0.0034*** | ***P = 0.0137*** | ***P = 0.0166*** | ***P = 0.0093*** | P = 0.2510 | P = 0.9414 |
| Goodness of fit | | $R^2$ = 0.7297 | $R^2$ = 0.6042 | $R^2$ = 0.5835 | $R^2$ = 0.6434 | $R^2$ = 0.1828 | $R^2$ = 0.0008 |

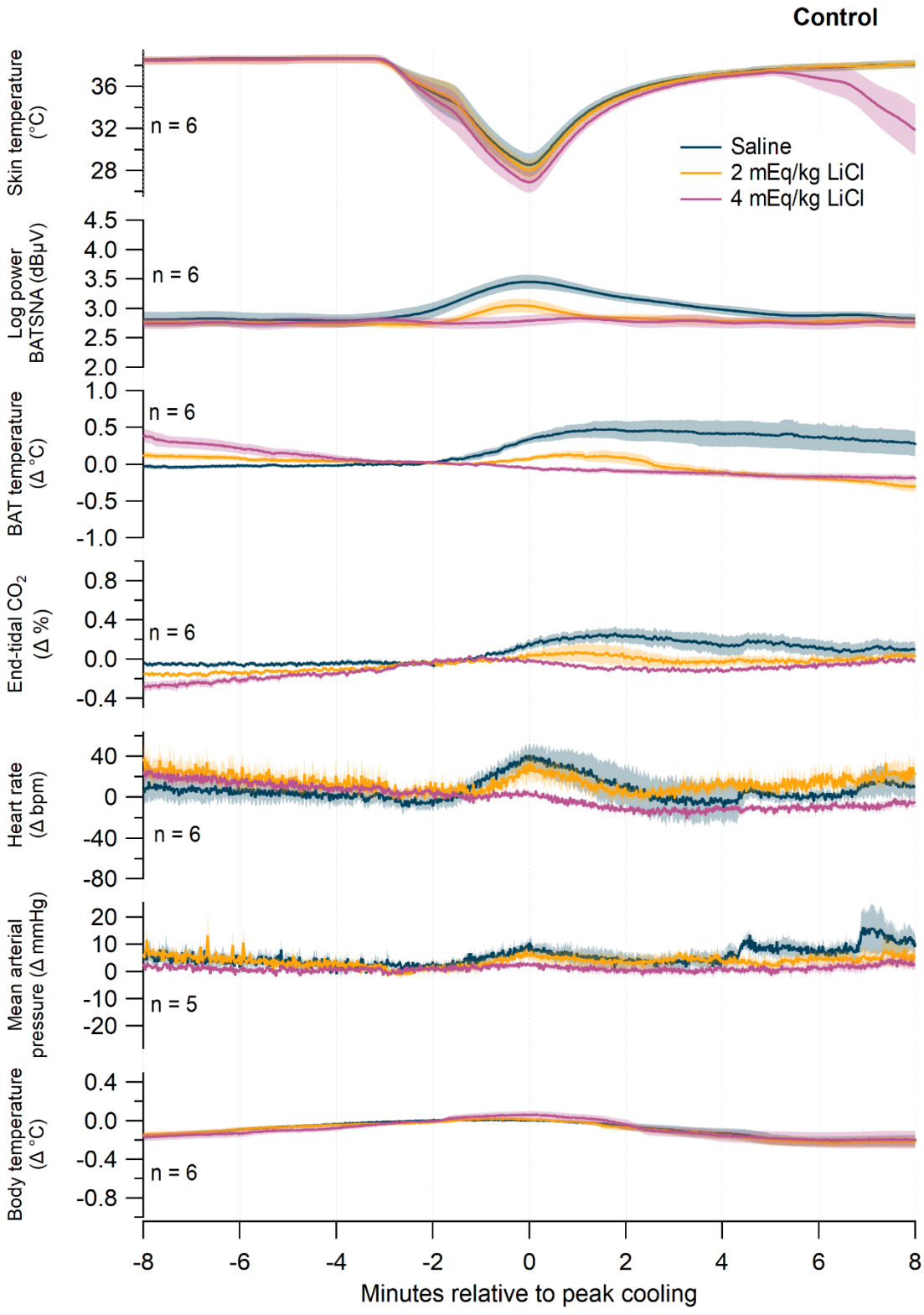


**Figure 4.2.** Control group rats' sympathetic response to cooling exposure initiated 10 minutes after intraperitoneal lithium (2, 4 mEq/kg LiCl) or treatment control (0.9% saline). Measurements of the sympathetic response are, from top to bottom, absolute skin temperature, area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in BAT temperature, end-tidal $CO_2$, heart rate, mean arterial pressure, and body temperature. Traces are aligned to skin temperature nadir ('peak cooling') at 0 minutes. Lines and shading represent mean ± SEM. Sample number for each measurement is displayed next to respective y-axes.

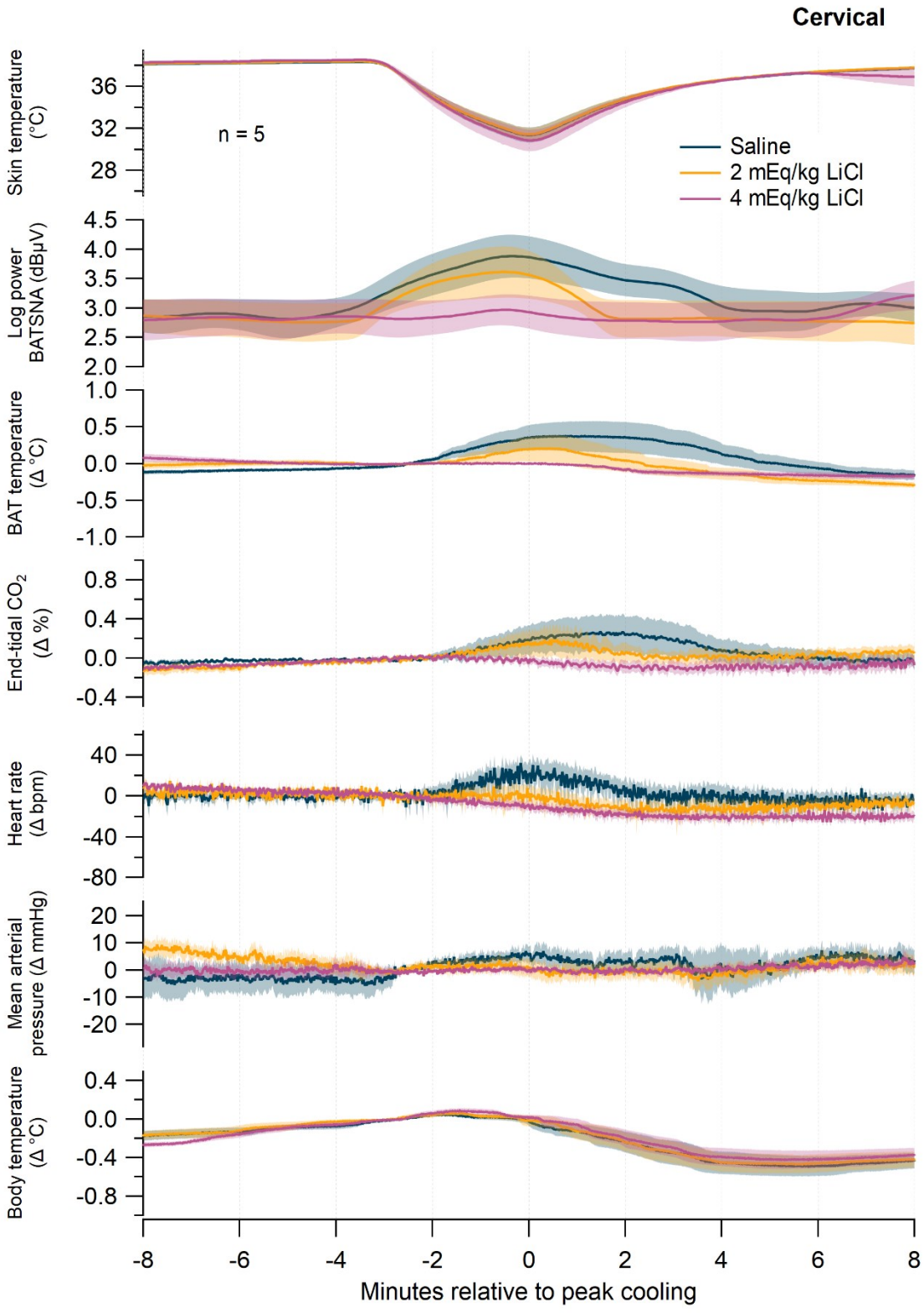


**Figure 4.3.** Sympathetic response of rats with a cervical vagotomy to cooling exposure initiated 10 minutes after intraperitoneal lithium (2, 4 mEq/kg LiCl) or treatment control (0.9% saline). Measurements of the sympathetic response are, from top to bottom, absolute skin temperature, area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in BAT temperature, end-tidal $CO_2$, heart rate, mean arterial pressure, and body temperature. Traces are aligned to skin temperature nadir ('peak cooling') at 0 minutes. Lines and shading represent mean ± SEM, n = 5.

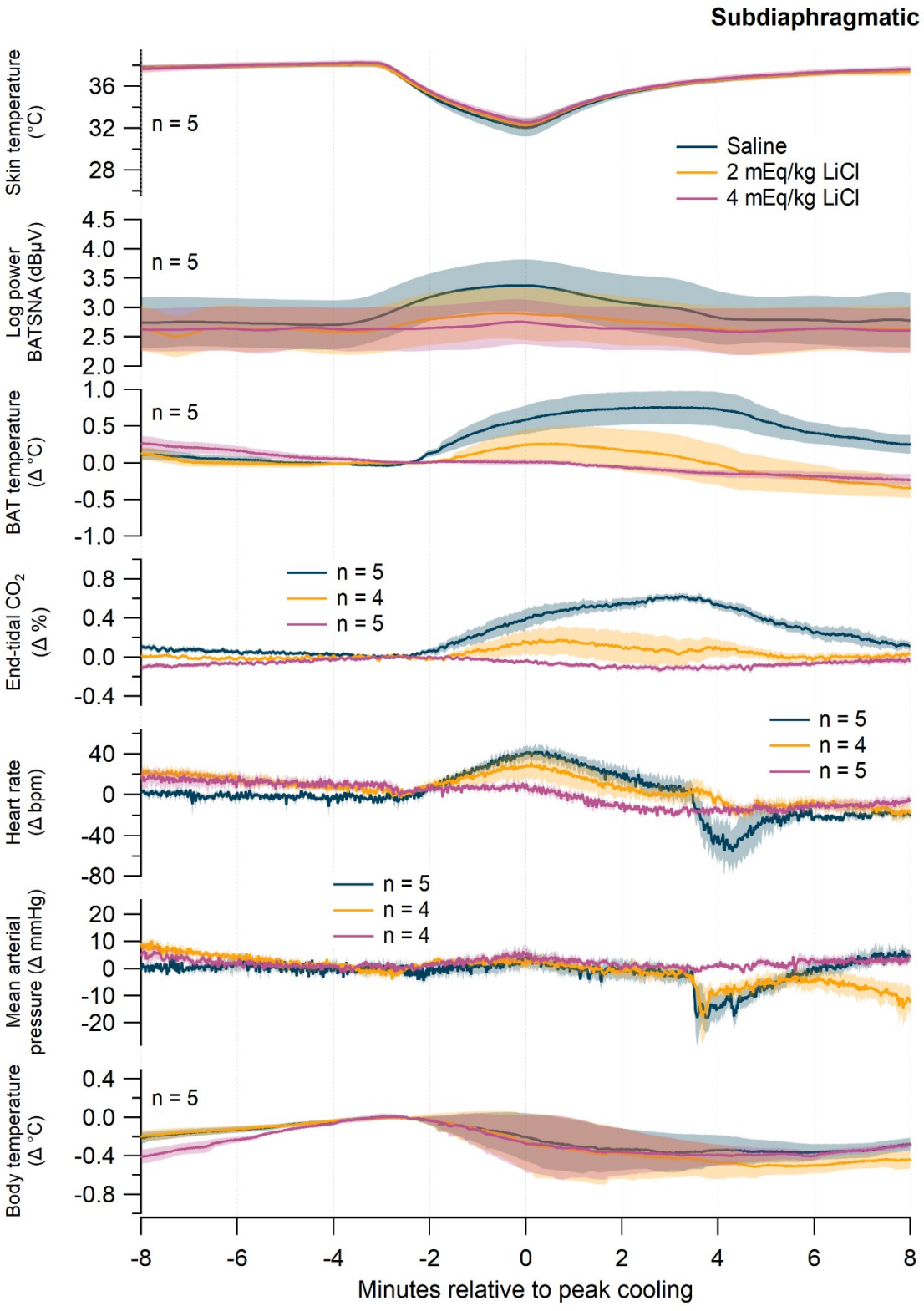


**Figure 4.4.** Sympathetic response of rats with a subdiaphragmatic vagotomy to cooling exposure initiated 10 minutes after intraperitoneal lithium (2, 4 mEq/kg LiCl) or treatment control (0.9% saline). Measurements of the sympathetic response are, from top to bottom, absolute skin temperature, area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in BAT temperature, end-tidal $CO_2$, heart rate, mean arterial pressure, and body temperature. Traces are aligned to skin temperature nadir ('peak cooling') at 0 minutes. Lines and shading represent mean ± SEM. Sample number for each measurement is displayed near each trace.

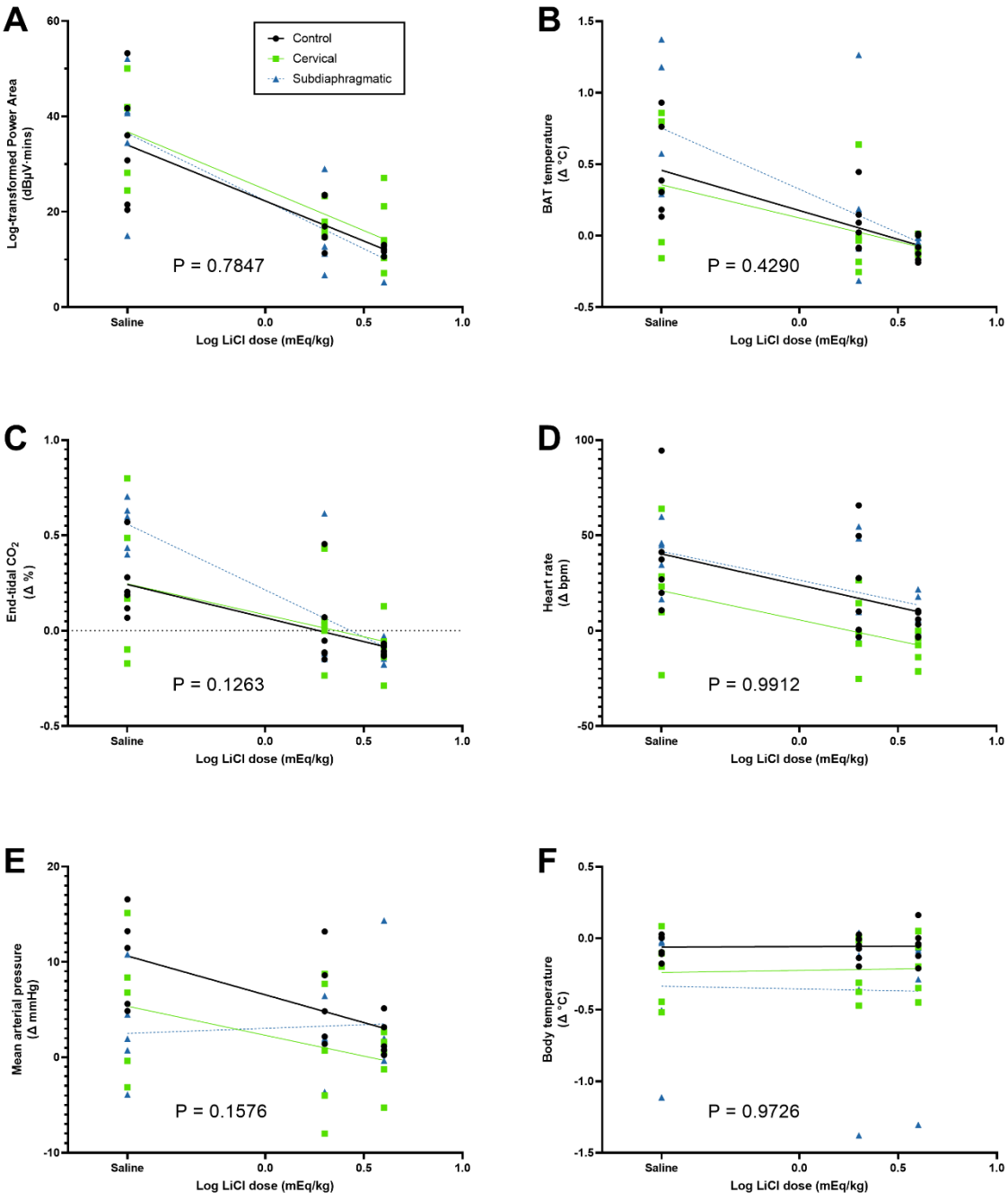


**Figure 4.5.** Comparison of linear regression slopes for each physiological measurement during cold exposure (**A:** area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in **B:** BAT temperature, **C:** end-tidal $CO_2$, **D:** heart rate, **E:** mean arterial pressure, and **F:** body temperature) among the control, cervical vagotomy, and subdiaphragmatic vagotomy groups. The regressions illustrate the relationship between these measurements and the log-transformed dose of intraperitoneal lithium (2, 4 mEq/kg LiCl) or the treatment control (0.9% saline). P values indicate whether there is a significant difference between the respective slopes of a graph. Each data point on a graph represents an individual animal.

### 4.3.2 The Impact of Area Postrema Lesions on Lithium's Ability to Reduce the Sympathetic Response to Cooling

#### *4.3.2.1 Histology Results*

Histological assessment revealed that in the lesion group, the area postrema was completely excised in all rats, with the exception of two cases where it was nearly completely excised. The subpostremal area (SubP), bordering the AP and the NTS, was also largely removed. Along with the removal of the SubP, varying levels of damage were observed to the bordering NTS, although this did not extend far into the lateral, commissural, and medial portions of the NTS. The dorsal motor nucleus of the vagus and the hypoglossal nucleus were completely spared. In the sham group, the area postrema remained intact.

For neuroanatomical reference, I referred to the Rat Brain: In Stereotaxic Coordinates, 6th Edition (Paxinos & Watson, 2006). Please see **Figure 4.6** for two representative photomicrographs of coronal brain sections: one from the lesion group, illustrating a removed area postrema, and another from the sham group, displaying an intact area postrema. Each brain section is approximately at a position -14.04 mm relative to Bregma.

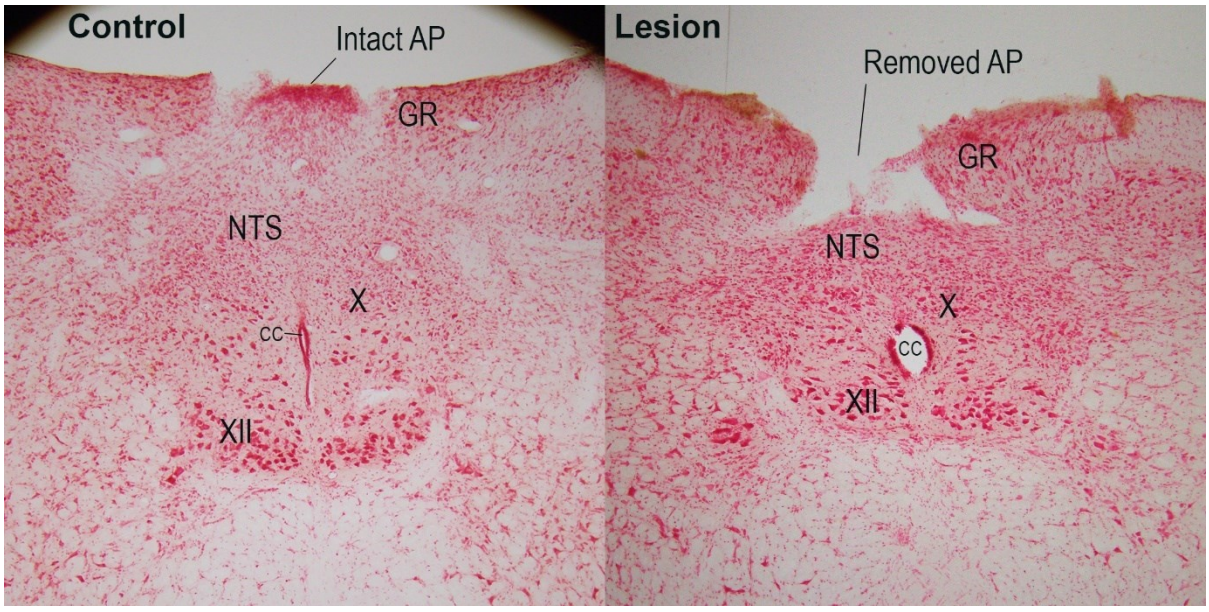


**Figure 4.6.** Representative photomicrographs of coronal brain sections, each approximately located at -14.04 mm relative to Bregma. The left panel depicts a section from a control (sham-operated) rat, highlighting an intact area postrema (AP), while the right panel illustrates a section from a rat in the lesion group, showing a completely removed AP. In both panels, the following anatomical structures are labelled: AP: area postrema, CC: central canal, GR: gracile nucleus, NTS: nucleus of the solitary tract, X: dorsal motor nucleus of the vagus, and XII: hypoglossal nucleus. These images serve as clear anatomical references, underscoring the effectiveness of the lesion procedure.

#### *4.3.2.2 Sham Group*

In the sham group (**Figure 4.8**), significant changes were observed 10 minutes after the intraperitoneal administration of 0.9% saline and subsequent cooling. Specifically, BATSNA log power (7.19 ± 0.19 to 7.69 ± 0.20 dBμV), BAT temperature (35.89 ± 0.28 to 36.84 ± 0.40 °C), end-tidal $CO_2$ levels (4.36 ± 0.18 to 5.24 ± 0.22 %), HR (390.7 ± 19.73 to 449.5 ± 25.60 bpm), and MAP (111.3 ± 4.10 to 118.00 ± 3.94 mmHg) all increased ($n = 6$, $P < 0.05$, paired t test). Body temperature remained relatively stable from 37.33 ± 0.17 to 37.31 ± 0.23 °C ($n = 6$, $P > 0.05$, paired t test).

Linear regression analysis was performed between the log-transformed dose of lithium and each parameter. This analysis revealed a dose-dependent reduction in all parameters following lithium administration, with the exception of MAP. **Table 4.3** contains the linear regression results, including slope significance and goodness of fit, calculated with log-transformed BATSNA power area and the delta of each parameter.

To further illustrate these findings, I present in **Figure 4.7** a detailed recording from a single animal in the sham group. This figure captures each change in BATSNA log power, BAT temperature, end-tidal $CO_2$ levels, HR, and MAP during the cooling period following each intraperitoneal administration of 0.9% saline, 2 mEq/kg LiCl, and 4 mEq/kg LiCl. This individual data point is part of the collective response depicted in **Figure 4.8**, where responses are synchronised according to the peak cooling moment at 0 minutes.

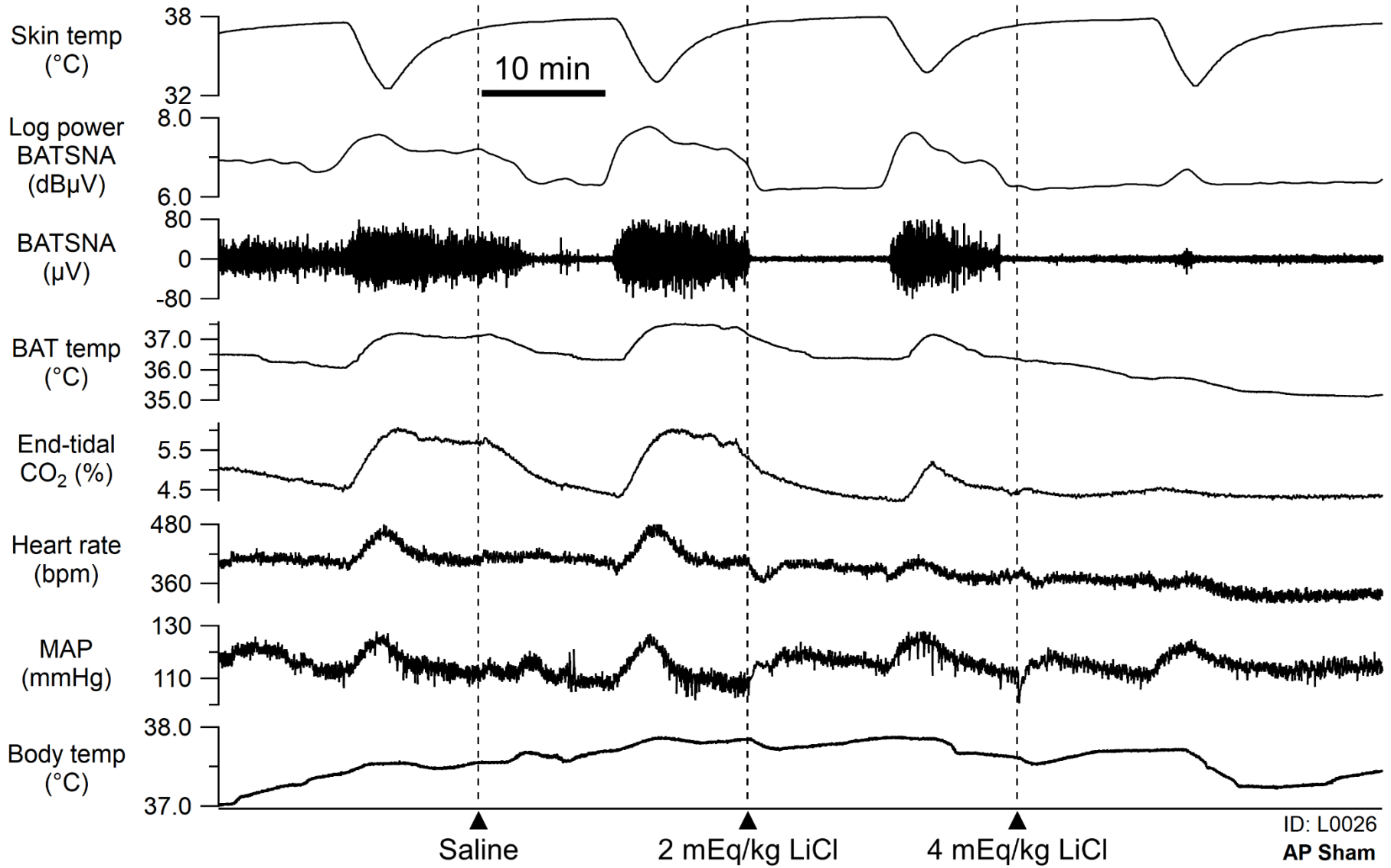


**Figure 4.7.** Example recording from one rat in the sham (control) group, illustrating the physiological responses to cold exposure after intraperitoneal administration of 0.9% saline and lithium doses of 2 mEq/kg and 4 mEq/kg. The cold exposure began 10 minutes post-injection and lasted for 3 minutes. From top to bottom, the traces display skin temperature (°C), log-transformed BATSNA power (dBμV), raw BATSNA power (μV), BAT temperature (°C), end-tidal $CO_2$ levels (%), heart rate (bpm), mean arterial pressure (mmHg), and body temperature (°C). Observe that the magnitude of each cooling-evoked response, aside from MAP, diminishes following successive lithium injections in this sham rat.

#### *4.3.2.3 Area Postrema Lesion Group*

In the area postrema lesion group (**Figure 4.9**), the cooling-evoked increases in BATSNA log power, BAT temperature, end-tidal $CO_2$, HR, and MAP after intraperitoneal injection of 0.9% saline were not statistically different from those seen in the sham group ($P > 0.05$, one-way ANOVA). Additionally, the body temperature was maintained during cooling post-saline, showing no significant difference compared to the control group ($P > 0.05$, one-way ANOVA).

None of the physiological parameters showed a dose-dependent relationship with lithium administration. **Table 4.3** provides the related results, including the significance of the slope and the goodness of fit, derived from log-transformed BATSNA power area and parameter deltas.

Using an analysis of covariance, the regression slopes of each parameter with respect to the log-transformed lithium dose were compared between the sham and area postrema lesion groups. The slopes of log-transformed BATNSA power area (**Figure 4.10 A**), and changes in BAT temperature (**Figure 4.10 B**), end-tidal $CO_2$ (**Figure 4.10 C**), and HR (**Figure 4.10 D**) were significantly different between the groups ($P < 0.05$). The linear regression slopes of MAP (**Figure 4.10 E**) and body temperature (**Figure 4.10 F**) were not significantly different ($P > 0.05$).

**Table 4.3.** Physiological parameters, including log-transformed brown adipose tissue sympathetic nerve activity (BATSNA) power area and changes (Δ) in BAT temperature, end-tidal $CO_2$, heart rate (HR), mean arterial pressure (MAP), and body temperature, were measured in control (sham) rats and rats with area postrema lesions. These measurements were taken during cooling, 10 minutes after intraperitoneal administration of either lithium (2, 4 mEq/kg LiCl) or saline (0.9% saline). The Δ values indicate changes from the start of cooling to 2 minutes after the cooling peak for BAT temperature, end-tidal $CO_2$, and body temperature, and to the cooling peak for HR and MAP. The data is presented as mean ± SEM, and significant ***p values*** are indicated in bold italics. The sample numbers can be found in **Figures 4.8** and **4.9**.

| | **LiCl dose** | **Power area (dBμV·mins)** | **BAT (Δ °C)** | **$EtCO_2$ (Δ %)** | **HR (Δ bpm)** | **MAP (Δ mmHg)** | **Body (Δ °C)** |
|---|---|---|---|---|---|---|---|
| **Control (sham)** | Saline | 113.8 ± 11.85 | 0.95 ± 0.27 | 0.88 ± 0.24 | 58.83 ± 11.05 | 6.71 ± 1.12 | -0.02 ± 0.08 |
| | 2 mEq/kg | 85.95 ± 8.22 | 0.33 ± 0.18 | 0.26 ± 0.16 | 28.70 ± 11.98 | 5.42 ± 1.28 | -0.13 ± 0.05 |
| | 4 mEq/kg | 35.75 ± 3.16 | -0.07 ± 0.05 | -0.10 ± 0.04 | 4.42 ± 5.86 | 4.91 ± 0.86 | -0.21 ± 0.06 |
| Slope significance | | ***P = 0.0002*** | ***P = 0.0015*** | ***P = 0.0006*** | ***P = 0.0017*** | P = 0.2368 | ***P = 0.0497*** |
| Goodness of fit | | $R^2 = 0.5931$ | $R^2 = 0.4791$ | $R^2 = 0.5284$ | $R^2 = 0.4699$ | $R^2 = 0.0863$ | $R^2 = 0.2198$ |
| **Area postrema lesion** | Saline | 97.61 ± 10.00 | 0.59 ± 0.17 | 0.72 ± 0.17 | 51.12 ± 11.84 | 8.28 ± 2.13 | -0.14 ± 0.07 |
| | 2 mEq/kg | 92.35 ± 8.73 | 0.59 ± 0.21 | 0.64 ± 0.18 | 48.65 ± 11.66 | 7.59 ± 1.59 | -0.06 ± 0.07 |
| | 4 mEq/kg | 87.38 ± 9.01 | 0.73 ± 0.26 | 0.45 ± 0.15 | 39.65 ± 9.55 | 3.38 ± 1.28 | -0.14 ± 0.05 |
| Slope significance | | P = 0.4504 | P = 0.7250 | P = 0.3083 | P = 0.5279 | P = 0.1195 | P = 0.7564 |
| Goodness of fit | | $R^2 = 0.0361$ | $R^2 = 0.0079$ | $R^2 = 0.0647$ | $R^2 = 0.0254$ | $R^2 = 0.1446$ | $R^2 = 0.0062$ |

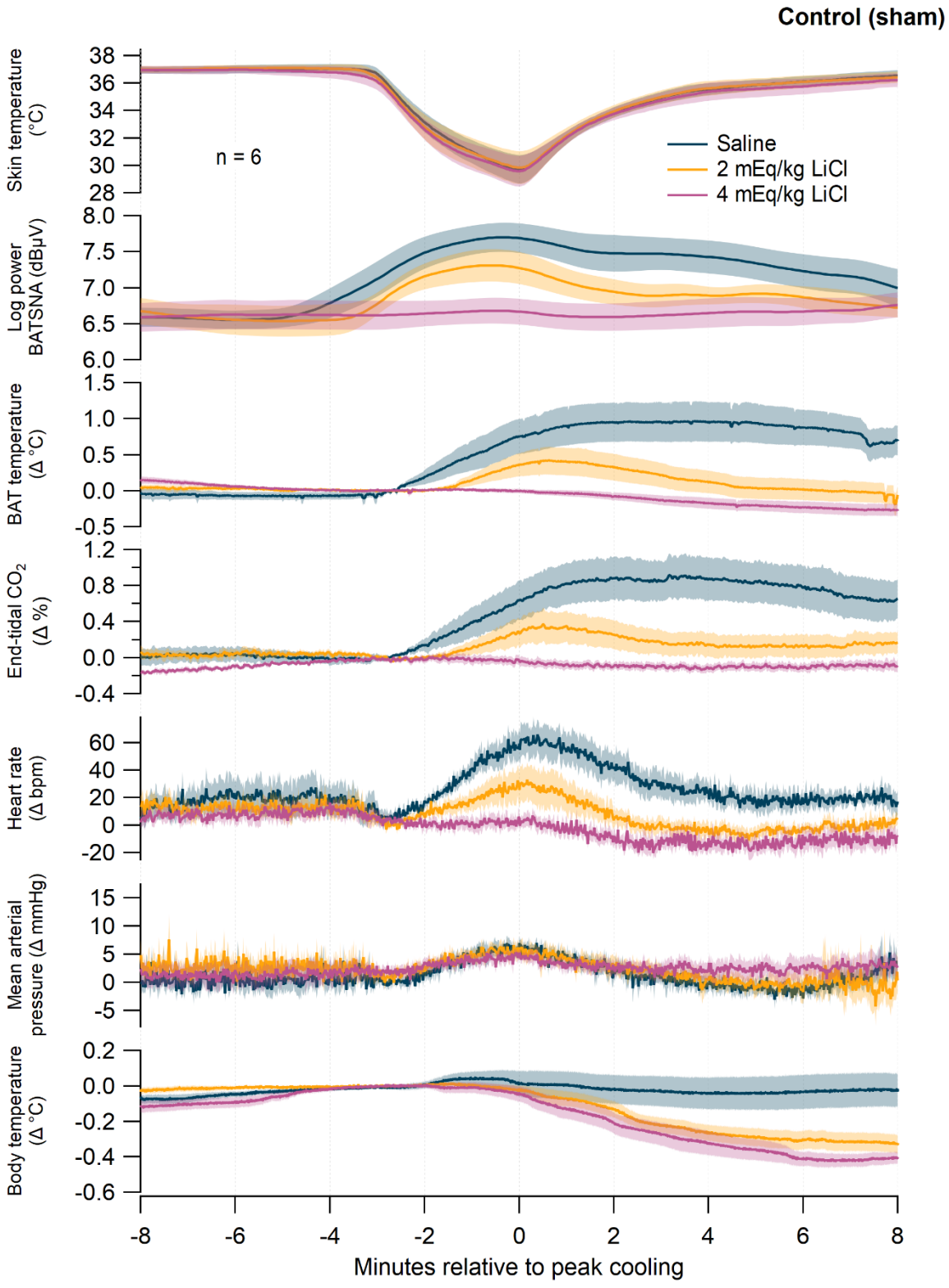


**Figure 4.8.** Sympathetic response of rats without an area postrema lesion (sham procedure) to cooling exposure initiated 10 minutes after intraperitoneal lithium (2, 4 mEq/kg LiCl) or treatment control (0.9% saline). Measurements of the sympathetic response are, from top to bottom, absolute skin temperature, area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in BAT temperature, end-tidal $CO_2$, heart rate, mean arterial pressure, and body temperature. Traces are aligned to skin temperature nadir ('peak cooling') at 0 minutes. Lines and shading represent mean ± SEM, n = 6.

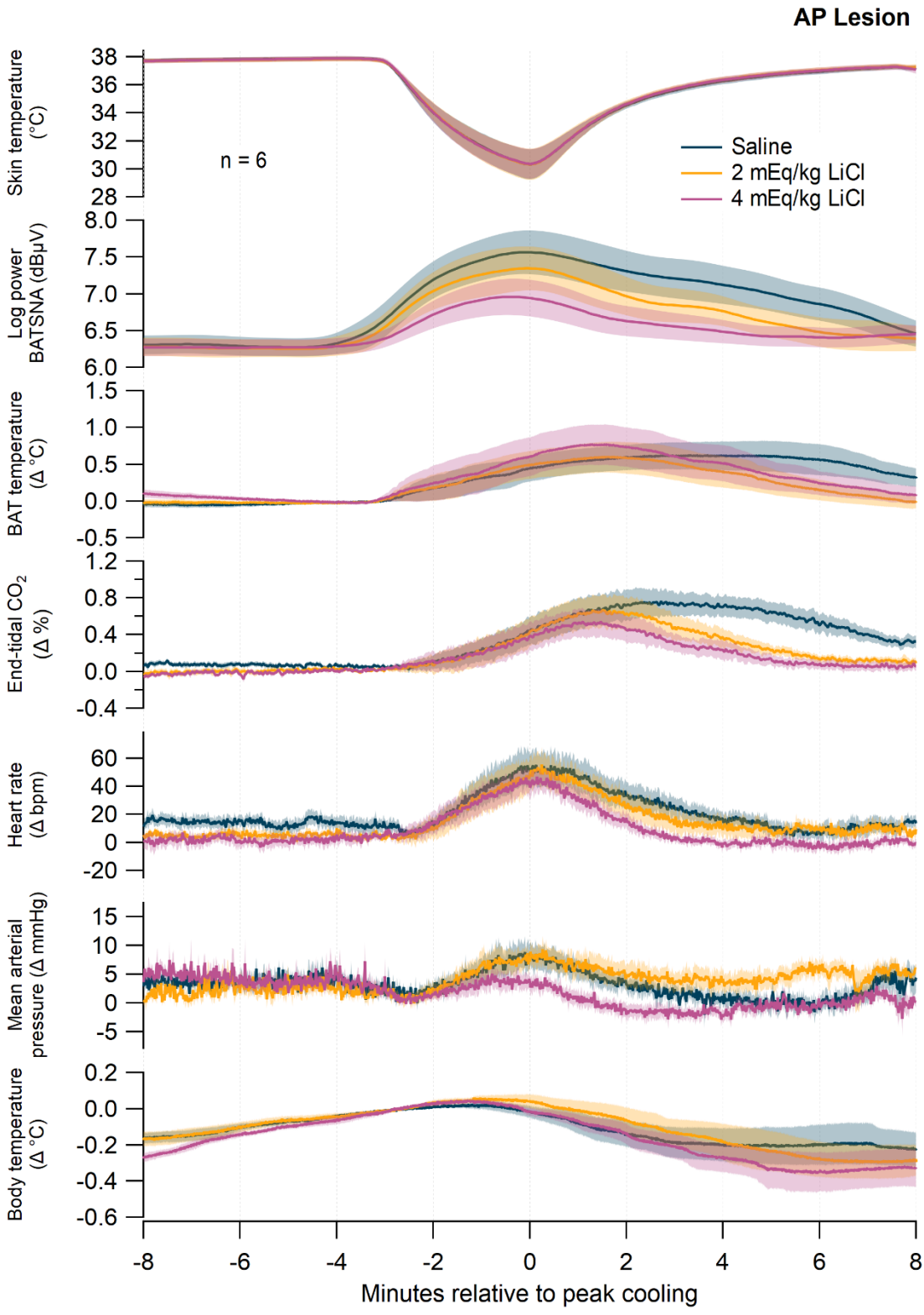


**Figure 4.9.** Sympathetic response of rats with an area postrema lesion to cooling exposure initiated 10 minutes after intraperitoneal lithium (2, 4 mEq/kg LiCl) or treatment control (0.9% saline). Measurements of the sympathetic response are, from top to bottom, absolute skin temperature, area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in BAT temperature, end-tidal $CO_2$, heart rate, mean arterial pressure, and body temperature. Traces are aligned to skin temperature nadir ('peak cooling') at 0 minutes. Lines and shading represent mean ± SEM, n = 6.

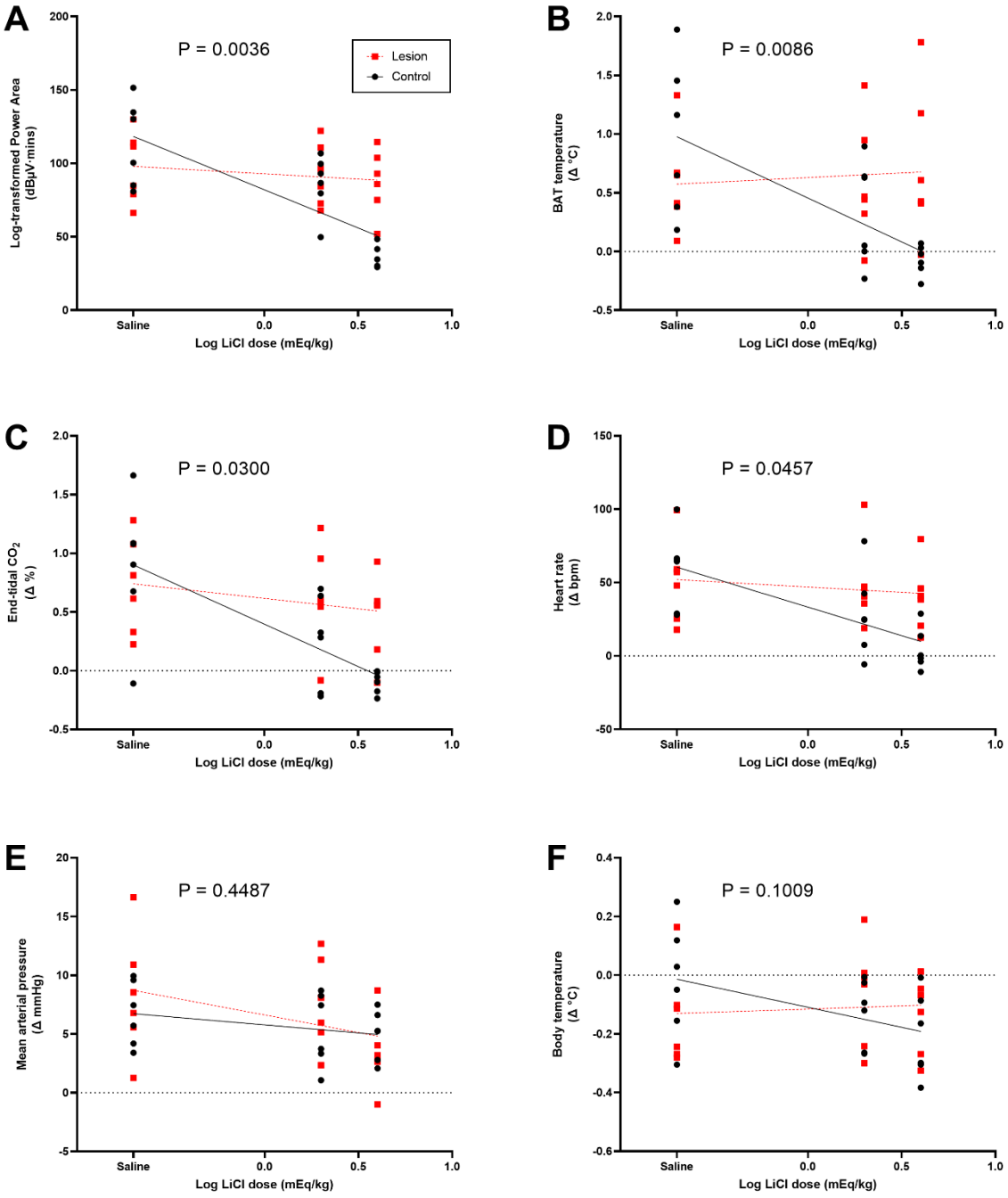


**Figure 4.10.** Comparison of linear regression slopes for each physiological measurement (**A:** area of log-transformed brown adipose tissue (BAT) sympathetic nerve activity power, and changes in **B:** BAT temperature, **C:** end-tidal $CO_2$, **D:** heart rate, **E:** mean arterial pressure, and **F:** body temperature) between the control (sham) group and the area postrema lesion group. The regressions depict the relationship between the measurements and the log-transformed dose of intraperitoneal lithium (2, 4 mEq/kg LiCl) or the treatment control (0.9% saline). P values indicate whether there is a significant difference between the respective slopes of a graph. Each data point represents an individual animal.

## 4.4 Discussion

In this final chapter, I present the first study to demonstrate that lithium can alter brown adipose tissue (BAT) thermogenesis and sympathetic activation in rats, as well as the associated changes in metabolism and cardiovascular function. While there are no previous studies that can be directly compared to my findings, my results have significant value in increasing our understanding of lithium's effects on mammalian physiology and have potential clinical implications. These findings must be carefully considered and discussed in the scientific community.

To provide context for my results, I will begin by comparing the sympathetic response to cooling to previous studies and explaining the physiology behind it. I will then interpret how lithium alters this response and the body's physiology by referring to metabolic and thermoregulatory research. Using this knowledge, I will explore the clinical significance of my findings and discuss lithium's therapeutic and side effects in psychiatric patients. Finally, I will examine the involvement of the vagus nerve and area postrema in my findings.

This discussion is the culmination of my investigation into the integrated nature of lithium's effect, and will often reference and incorporate the findings from the previous chapters. As a result, it is the largest section of this thesis.

### 4.4.1 The Sympathetic Response to Cold Stress

The sympathetic response to cooling consisted of increases in brown adipose tissue (BAT) sympathetic nerve discharge and temperature, and associated increases in heart rate (HR), expired carbon dioxide ($CO_2$), and mean arterial pressure (MAP). These observations mirror previous findings of the cold-defence response in the same species of rat (Nakamura & Morrison, 2007; Ootsuka & McAllen, 2006). The fall in skin temperature stimulated BAT thermogenesis, which was driven by sympathetic efferents innervating the BAT (Bartness et al., 2010; Ootsuka et al., 2009). As the sympathetic drive of BAT thermogenesis increased, so did the metabolic demand. A higher metabolic rate was reflected by increased HR and carbon dioxide production, which suggest greater oxygen delivery and consumption, respectively (Hicks & Bennett, 2004). This makes sense, as BAT activation results in substantial energy mobilisation and expenditure for thermogenic uncoupled mitochondrial oxidative respiration, fuelled by fatty acids and glucose, in mammals, including humans (Jeong, Chang, et al., 2018; Shinde et al., 2021; Townsend & Tseng, 2014). Our lab has previously discussed the significant contribution of BAT metabolism to whole body metabolic rate, despite BAT tissue comprising only 1% of body weight

(Ootsuka et al., 2009). The increase in MAP was likely a result of the sympathetic nervous system increasing cardiac output (HR and contractility) and initiating cutaneous vasoconstriction, particularly in the tail, to minimise heat loss (Blessing et al., 2011; Dampney, 2016; Gordan et al., 2015; Johnson et al., 2016). Additionally, increased MAP and HR likely contributed to the distribution of heat to metabolically important organs, preserving their function in the face of cold stress (Ootsuka et al., 2009), or perhaps enhancing their function, as discussed in **Chapter 3**. Coinciding with this core distribution of heat, the increased HR could potentially contribute to 'cardiac thermogenesis', a phenomenon which results in the generation of heat within the heart itself (Morrison & Nakamura, 2011).

### 4.4.2 Lithium's Effect on the Sympathetic Response to Cold Stress

In support of my hypothesis, I found that lithium dose-dependently reduced each thermogenic (BAT sympathetic nerve discharge & temperature), metabolic ($CO_2$), and cardiovascular (HR & MAP) parameter of the sympathetic response to cooling in anaesthetised rats. As the dose of lithium increased, decreasing skin temperature was unable to stimulate physiological heat-defence mechanisms against cold, which corresponds to lithium-induced hypothermia in conscious guinea pigs (**Chapter 2**) and rats (**Chapter 3**). Lithium's reduction of cold-evoked BAT sympathetic nerve discharge, which inhibited BAT thermogenesis, suggests downregulation of sympathetic outflow from the central nervous system rather than direct interference of lithium with BAT function. This simultaneously agrees with and provides a neurological explanation for my finding in **Chapter 3**, wherein lithium inhibited psychological stress-induced BAT thermogenesis and emotional hyperthermia in conscious rats.

I now know that lithium is dose-dependently effective in inhibiting BAT thermogenesis in resting, neutral ambient temperature conditions (**Chapter 3**) and after stimulation by cold (**current chapter**) and psychological stress (**Chapter 3**). Additionally, Tulunay demonstrated that lithium inhibits pyrogen-induced hyperthermia in rats (Tulunay, 1976). However, it would be worth confirming that lithium specifically inhibits BAT thermogenesis in response to infection.

To the best of my knowledge, this study represents the first report of lithium's suppressive effects on BAT sympathetic nerve activity and BAT thermogenesis induced by cold exposure. Furthermore, my research is the first to demonstrate that lithium inhibits increases in $CO_2$ production, heart rate, and mean arterial pressure under these conditions. Therefore, it is not possible to compare my findings with the existing literature. I encourage additional studies to help fill this gap in knowledge.

#### *4.4.2.1 Physiological Implications*

Since lithium inhibited the increase in sympathetic BAT activation during cold exposure, it consequently suppressed the escalation of energy mobilisation and expenditure required for non-shivering thermogenesis. As a result, the metabolic rate was likely maintained at a lower level, as indicated by the lithium-elicited reduction of HR (less oxygen delivery) and carbon dioxide production (less oxygen consumption) increases that would typically occur during cold exposure. Multiple anaesthetised rat studies similarly demonstrate that metabolic indices such as HR and expired $CO_2$ decrease with the inhibition of BAT thermogenesis (Cao et al., 2010; da Conceição et al., 2020; Nakamura & Morrison, 2007; Nakamura et al., 2005).

Several studies have investigated the effects of lithium on resting heart rate. In conscious female rats, Wilkin and Cunningham demonstrated that lithium reduced resting heart rate (Wilkin et al., 1982). Similarly, Jones et al. found that a single i.p. injection of LiCl (5 mEq/kg) reduced resting heart rate in conscious male rats (Jones et al., 2008). There is also some evidence that lithium therapy reduces heart rate (sinus bradycardia) in humans (Mehta & Vannozzi, 2017). It is worth noting that Linakis et al. found that lithium does not directly affect cardiac function, including HR, in isolated perfused rat hearts (Linakis et al., 2000), suggesting action from the brain. In conjunction with my findings on lithium's suppression of heart rate increases during cold exposure, these results suggest that lithium not only reduces resting heart rate but also prevents increases in heart rate under environmental stress.

This is significant, as resting heart rate and heart rate in response to stress are governed by separate brain areas, the rostral ventrolateral medulla (RVLM) and medullary raphe region (rMR), respectively, which implies that lithium's pathway of action involves these two areas (discussed further in **Section 4.4.6.1.6**).

Mean arterial pressure (MAP) is the product of cardiac output and vascular resistance (Wehrwein & Joyner, 2013). Cardiac output is determined by heart rate and stroke volume (Vincent, 2008), while vascular resistance is influenced mostly by the diameter of blood vessels (Intengan & Schiffrin, 2000). An increase in cutaneous vasoconstriction, which reduces the diameter of skin blood vessels and increases peripheral vascular resistance, coupled with an increase in heart rate, which contributes to an elevation in cardiac output, leads to a higher MAP. Therefore, lithium's dose-dependent attenuation of elevated MAP during cold exposure is likely attributable to a combination of its ability

to suppress cold-evoked increases in heart rate and cutaneous vasoconstriction. Although I did not measure cutaneous vasoconstriction in this chapter, this explanation is consistent with my observation in **Chapter 3**, wherein lithium induced tail vasodilation.

Cutaneous vasoconstriction, BAT thermogenesis, and cardiac output during environmental stresses are mutually driven by sympathetic outflow from the rostral medullary raphe region (rMR), which contains the rostral raphe pallidus and raphe magnus nuclei (Nakamura et al., 2022). Sympathetic premotor neurons in this region provide oligosynaptic input to cutaneous blood vessels, BAT tissue, and the heart. Hence, the rMR is the interface between the central command of the brain and the peripheral sympathetic network.

Excitation of the rMR with optogenetic photostimulation (Kataoka et al., 2014) or injection of neuroexcitatory drugs (Blessing & Nalivaiko, 2001; Cao & Morrison, 2003; Luong & Carrive, 2012; Morrison et al., 1999) results in powerful activation of sympathetic premotor neurons, producing cutaneous vasoconstriction, BAT thermogenesis, tachycardia, and hypertension. Many studies have shown that cold exposure activates the rMR (Nakamura et al., 2022), leading to the responses observed in this chapter.

Similarly, psychological stress activates the rMR and produces comparable responses (Nakamura & Morrison, 2022; Nakamura et al., 2022), which corresponds to my findings of psychological stress-induced BAT thermogenesis in **Chapter 3**. On the other hand, experimental inhibition of premotor neurons in the rMR abolishes or reduces the responses evoked by cold or psychological stress (Nakamura, 2015; Nakamura & Morrison, 2007). Furthermore, in the body's defence against heat, rMR premotor neurons are also inhibited, promoting heat loss through cutaneous vasodilation and the inhibition of BAT thermogenesis (Nakamura & Morrison, 2010). I have shown that lithium has a similar effect, reducing sympathetically mediated cutaneous vasoconstriction (**Chapter 3**), BAT thermogenesis, and cardiac output. This suggests that lithium leads to downregulation of sympathetic outflow from the rMR, which is directly confirmed by my recording of reduced discharges from sympathetic postganglionic axons within isolated BAT nerves. Lithium elicits a response similar to the body's defence against elevated temperature, in which physiology and behaviour (as described in **Chapters 2 and 3**) are altered to favour heat loss. This suggests that lithium activates a normal physiological response coordinated by the brain. Possible brain pathways of this effect will be discussed in the area postrema subsection.

#### *4.4.2.2 Clinical Significance*

##### 4.4.2.2.1 Autonomic Balance

The reduction of sympathetic output from the rMR in response to lithium is clinically significant in the treatment of various psychiatric conditions, such as bipolar disorder, major depressive disorder, anxiety disorders, and borderline personality disorder. Lithium is often utilised as a treatment for these conditions (Belli et al., 2012; Camara, 1990; Jones et al., 2022; Kitchner & Greenstein, 1985; Post, 2018). The aetiology of these disorders is complex, and it is crucial not to oversimplify it. That being said, psychological stress, particularly when chronic, is believed to significantly contribute to their development (Davis et al., 2017). As discussed in the previous chapter, the neurophysiological and behavioural changes occurring during an acute stress response to a perceived threat to one's homeostasis are generally considered adaptive and beneficial for an organism's survival. However, repeated or prolonged exposure to stressors can render this response maladaptive, leading to psychosomatic disorders (Brzózka et al., 2016; Nisenbaum et al., 1991; Schneiderman et al., 2005). Individuals predisposed to developing psychiatric conditions are thought to have low resilience and a maladaptive response to psychological stress (Kim et al., 2018; Shrivastava & Desousa, 2016; Wu et al., 2013). Sympathetic activity from the rMR drives most of the body's physiological responses to stressors (Nakamura & Morrison, 2022; Ulrich-Lai & Herman, 2009).

Several studies have demonstrated that sympathetic activity, as indicated by increased heart rate and decreased heart rate variability, is higher in psychiatric patients than in healthy controls, in both restful and stressful situations (Alvares et al., 2016; Lehofer et al., 1997; Singla et al., 2020; Weinberg et al., 2009). Emotional hyperthermia, a hallmark of sympathetic activation in response to psychological stress (as discussed in **Chapter 3**), has also been observed in patients with psychiatric disorders (Bohorfoush et al., 1965; Duras, 1942; Nozu & Uehara, 2005; O'Toole & Dyck, 1977; Timmerman et al., 1992; Weinstein, 1985). In healthy individuals, parasympathetic activity typically predominates during restful and low arousal situations (McCorry, 2007; Olshansky et al., 2008), but in these patients, tonic sympathetic activation may be disproportionately active regardless of the environmental context. This may create an abnormally persistent 'fight or flight' state that negatively impacts physiological processes such as cognition, emotion, cardiovascular function, metabolism, and hormonal balance (De Kloet et al., 2005; Godoy et al., 2018; Kozlowska et al., 2015; Schneiderman et al., 2005).

The activation of vagal afferents may help reduce depressive, bipolar, and anxiety symptoms (Bottomley et al., 2020; George et al., 2008; Marangell et al., 2008; Nierenberg et al., 2008) by counterbalancing the abnormal drive of the sympathetic nervous system (Berntson et al., 1994; Gurel et al., 2020). This counterbalancing effect can be achieved through two primary mechanisms. Firstly, the activation of vagal afferents can stimulate vagal efferent fibres, thereby increasing parasympathetic inhibitory activity on the heart (Van Weperen & Vaseghi, 2023). This increase in parasympathetic activity may counteract sympathetic excitatory activity on the heart, promoting a balanced autonomic cardiovascular response. Secondly, the activation of vagal afferents can modulate or reduce sympathetic output through upstream brain pathways (Gurel et al., 2020; Van Weperen & Vaseghi, 2023). Gut microbiota may also influence mood via the gut-brain axis in a similar manner (Breit et al., 2018; Foster & Neufeld, 2013). Therefore, lithium's therapeutic efficacy may stem from its ability to impact upstream brain areas and downregulate overactive sympathetic outflow, restore the balance between the sympathetic and parasympathetic branches of the autonomic nervous system, and reduce the maladapted stress response and its negative symptoms.

#### 4.4.2.2.2 Metabolism and Weight Gain

My observation of lithium-induced reduction of brown adipose tissue (BAT) thermogenesis and energy metabolism is clinically important, as it has implications for weight gain and the associated metabolism of lipids and glucose. Lithium's effect on body weight was first noted in 1970 (Kerry et al., 1970), two decades after it was introduced to modern psychiatric medicine (Cade, 1949). Since then, a large body of evidence including multiple reviews (Ackerman & Nolan, 1998; Baptista et al., 1995; Dunner, 2000; Gitlin, 2016; Livingstone & Rampes, 2006) and a large meta-analysis (McKnight et al., 2012) has consistently supported the idea that lithium can cause weight gain. It is one of the most concerning for patients and a significant deterring factor in treatment compliance (Gitlin, 2016). However, the mechanism behind lithium-associated weight gain is still not well understood (Sachs & Guille, 1999). Recent evidence suggests that BAT activity is inversely correlated with fat accumulation, weight gain, and obesity in mammals, including humans (Vijgen et al., 2012; Vijgen et al., 2011; Wang et al., 2015; Worku et al., 2020; Yoneshiro et al., 2013). In obese rats, the sympathetic efferent nerve impulses to the interscapular BAT occur less frequently than in lean rats (Holt & York, 1989). Therefore, it is a promising target for combatting weight gain and obesity (Kuryłowicz & Puzianowska-Kuźnicka, 2020). This is likely due to its role in regulating whole body metabolism and energy expenditure (Chondronikola et al., 2014; Chondronikola et al., 2016), as mentioned previously.

When BAT is activated by the release of norepinephrine from sympathetic axons, intracellular lipolysis is triggered within brown adipocytes. In this process, triacylglycerols from small multilocular lipid droplets are hydrolysed to release fatty acids that then enter the mitochondria to activate uncoupling protein-1 (UCP-1), the protein that enables non-shivering thermogenesis in the electron transport chain (Heeren & Scheja, 2018). The fatty acids also undergo beta-oxidation for entry into the Krebs cycle, where they are broken down further, producing carbon dioxide and electron carriers. Exhaled $CO_2$ accounts for the majority of weight loss (Meerman & Brown, 2014), while the electron carriers enter the electron transport chain for heat generation (Nolfi-Donegan et al., 2020).

Adjacent white adipose tissue (WAT) is also sympathetically stimulated, resulting in the lipolysis of triacylglycerols from large unilocular lipid droplets, creating nonesterified or free fatty acids for use by BAT. These free fatty acids are released from WAT and are directly taken up by BAT or bound to albumin for uptake by the liver where they are packaged as triacylglycerols in very low-density lipoproteins (VLDLs) (Heeren & Scheja, 2018).

Sympathetic stimulation of BAT results in increased uptake of these WAT-derived fatty acids or triacylglycerol-derived fatty acids from circulating lipoproteins to replenish intracellular triacylglycerol stores for oxidation in BAT thermogenesis (Festuccia et al., 2011). This mobilisation and oxidation of lipids from peripheral stores for BAT thermogenesis has been demonstrated in humans (Chondronikola et al., 2016). Further, in rodents, BAT activation decreases circulating triacylglycerol and cholesterol levels (Bartelt et al., 2011; Berbée et al., 2015).

As I have shown that lithium dose-dependently reduces BAT sympathetic nerve discharge and BAT thermogenesis, I can infer that lithium may inhibit norepinephrine release from BAT sympathetic terminals, reducing lipolysis in both BAT and white adipose tissue (WAT). Reduced lipolysis in adipocytes increases the risk of weight gain (Arner & Rydén, 2022). My observations of reduced exhaled $CO_2$ and BAT thermogenesis support this conclusion, as the decrease in fatty acid production and oxidation by BAT mitochondria would result in reduced $CO_2$ and heat production. Since weight loss is primarily due to the exhalation of $CO_2$ (and partly water) from cellular respiration (Meerman & Brown, 2014), the limited $CO_2$ and heat production suggests a decreased amount of weight loss during cold exposure.

The inhibition of BAT thermogenesis/activity by lithium may reduce fatty acid uptake by BAT. Additionally, as I have observed that lithium reduces heart rate associated with increased BAT activity,

I can expect a reduction in myocardial consumption of these fatty acids (Zechner et al., 2009). Together, this may lead to higher levels of circulating fatty acids that are albumin-bound or contained in triacylglycerol-rich lipoproteins (Bartelt et al., 2011; Bond & Ntambi, 2018; Hamann et al., 1996). These circulating fatty acids, unable to be oxidised by BAT, may be stored and accumulate in WAT lipid droplets (Goldberg, 1996; Thompson et al., 2010), inducing hyperplasia or adipogenesis, as measured by an increase in adipocyte number (Longo et al., 2019). Increased fat cell count confers to increased fat mass, which, in turn, contributes to body weight (Arner & Rydén, 2022; Tchoukalova et al., 2010). In support of this, Vendsborg et al. in 1976 showed that weight gain is correlated with fat cell number in patients on long-term lithium (Vendsborg et al., 1976). A study from 2015 by Wang et al. found higher serum triacylglycerol levels in patients with low BAT activity (Wang et al., 2015). High serum triglyceride often coincides with fat accumulation and weight gain (Packard et al., 2020; Yuan et al., 2007). In 2016, Hoeke et al. proposed that inactivated BAT reduces the uptake of triacylglycerols from TG-rich lipoproteins, leading to the slow formation of low-density lipoproteins (LDLs) that acquire fewer apolipoprotein B (apoB) proteins, impairing their clearance by the liver (Hoeke et al., 2016). In support of this, patients treated with lithium have been shown to have elevated levels of triacylglycerols and LDL (Aliyazicioglu et al., 2007).

BAT, also known as a ‘glucose sink’, plays a key role in glucose clearance (Peirce & Vidal-Puig, 2013; Wang et al., 2021) by using the energy substrate partially for non-shivering thermogenesis and for other processes such as de novo lipogenesis to create more fatty acid thermogenic fuel (Hankir & Klingenspor, 2018; McNeill et al., 2020). Hence, low BAT activity is associated with higher fasting glucose levels (Wang et al., 2015). Inactivation of BAT by lithium may further decrease glucose uptake and disposal (Lee et al., 2016), leading to increased blood glucose levels. This is concerning, as patients on lithium have reported increased cravings for carbohydrate-rich foods (Kalucy, 1980). In line with this, serum glucose levels after an intravenous infusion of glucose are higher in rats treated with lithium compared to controls (Shah & Pishdad, 1980); insulin release is also attenuated, potentially slowing glucose storage. On the other hand, BAT activation is thought to reduce appetite and improve insulin secretion (Kelsey, 2019; Peirce & Vidal-Puig, 2013). However, high circulating glucose due to impaired BAT uptake may nonetheless stimulate the release of insulin, which, when glycogen stores are full, promotes fatty acid synthesis through conversion of excess glucose into fatty acids in the liver or white adipose tissue (Czech et al., 2013). These fatty acids are then stored in WAT as triacylglycerols in lipid droplets, contributing to fat accumulation and weight gain (Luo & Liu, 2016; Rosen & Spiegelman, 2006). Therefore, I propose that lithium contributes to weight gain as a consequence of

inhibiting BAT thermogenesis, decreasing total fatty acid and glucose consumption and promoting the storage of these energy substrates in lipid droplets, increasing fat accumulation and WAT mass.

My findings provide new insights into the mechanisms behind lithium-induced weight gain and suggest that targeting brown adipose tissue may be a promising approach for preventing or mitigating this adverse effect of lithium treatment.

One potential strategy would be to pharmaceutically target BAT thermogenesis without increasing the sympathetic drive of other components of the sympathetic response. A case report by Praharaj in 2016 demonstrated that metformin reversed weight gain in an individual treated with lithium (Praharaj, 2016). Metformin, an anti-diabetic drug, has recently become popular for its ability to stimulate weight loss (Seifarth et al., 2013; Yerevanian & Soukas, 2019). In mice, it has been shown to increase BAT mass and stimulate BAT thermogenesis (Karise et al., 2019), which, as discussed, may promote weight loss in humans (Wang et al., 2015).

Metformin has also been shown to acutely act on the central nervous system, decreasing heart rate and blood pressure in anaesthetised spontaneously hypertensive rats (Petersen & DiBona, 1996). This suggests that metformin does not stimulate BAT thermogenesis as powerfully as cold and psychological stress, but enough to mitigate weight gain associated with BAT inactivation without eliciting increases in heart rate. This means that metformin may therefore not antagonise lithium-elicited reduction of cold-induced elevations in heart rate and blood pressure. In addition, metformin is often used to treat weight gain associated with antipsychotic drugs (Bak et al., 2014; Hakami et al., 2022).

Antipsychotics are commonly used to treat schizophrenia and, increasingly, bipolar disorder (Garcia et al., 2016; Geddes & Miklowitz, 2013; Wilkowska & Cubała, 2019). In comparison to lithium, they lead to more weight gain (Müller-Oerlinghausen et al., 2012). This could be because antipsychotics and lithium affect sympathetic output through similar mechanisms, and antipsychotics have a more powerful influence on these mechanisms. Clozapine, chlorpromazine, olanzapine, and risperidone antipsychotics have been shown to exhibit similar effects to lithium in rats, inhibiting cold and psychological stress-elicited BAT thermogenesis, emotional hyperthermia, and cutaneous vasoconstriction (Blessing, 2004; Blessing et al., 2017; Blessing et al., 2006; Stefanidis et al., 2009). Therefore, metformin may reverse lithium-induced weight gain by specifically counteracting lithium-elicited inhibition of BAT activity.

This pharmaceutical approach to selectively targeting and rectifying lithium-induced BAT inhibition may be more effective and convenient than caloric-restrictive, dose-reducing, and treatment-switching approaches (Dempsey et al., 1976; Sachs & Guille, 1999). Establishing a clear guideline for treating lithium-related weight gain, which does not currently exist (Praharaj, 2016), may improve patient treatment adherence and outcomes.

#### 4.4.2.2.3 Sex Differences

Heterogeneity of BAT activity in individuals may explain the differences in weight gain susceptibility and potentially therapeutic effect during lithium treatment. For instance, research suggests that lithium leads to weight gain in women more frequently compared to men (Cypess et al., 2009; Pfannenberg et al., 2010). The same has been suggested for antipsychotics (Castellani et al., 2019). Women have higher BAT prevalence and function than men (Cypess et al., 2009; Pfannenberg et al., 2010). This could be the reason why, among healthy adults, women are more likely to be within a normal BMI compared to men (Chiriboga et al., 2008). A large part of this may be due to the oestrous cycle, as oestrogen has been shown to activate BAT thermogenesis, increase energy expenditure, and decrease food intake in female rats when injected peripherally and centrally (de Morentin et al., 2014). In male rats, BAT thermogenesis is activated when oestrogen is injected centrally (Sievers et al., 2022). As such, lithium-induced suppression of BAT thermogenesis may lead to greater metabolic change in individuals with high levels of BAT activity, such as women.

In 2012, Partonen hypothesised that overactivated BAT may negatively affect mood and mood-related behaviours through its disruption of thermoregulation, the ultradian basic rest-activity cycle, and circadian synchrony (Partonen, 2012). Consistent with this, emotional hyperthermia, which BAT activity contributes significantly to, is more prominent in women and often seen in patients with psychiatric disorders (Oka, 2015). The prevalence of psychiatric conditions such as major depressive disorder and anxiety disorders is consistently higher in women than men (Albert, 2015; McLean et al., 2011). Recently, the same trend in bipolar disorder has been elucidated (Dell'Osso et al., 2021); in bipolar and anxiety and disorders, women may experience more severe symptoms.

The oestrous cycle, which can enhance BAT thermogenesis and therefore emotional hyperthermia, is a crucial factor in contributing to psychiatric conditions (Hwang et al., 2020). This is likely due to the capacity of the hypothalamus and medullary regions to respond to oestrogen through oestrogen steroid receptors (Liu & Shi, 2015; Roepke, 2009; Xu & López, 2018; Ye et al., 2022). These are key

areas in the central circuitry that controls the stress response including sympathetic outflow to BAT and cardiovascular centres, and stress hormone release (Nakamura & Morrison, 2022). I believe lithium acts upon the same pathway through the area postrema (discussed later).

Furthermore, in a study by Atmaca et al. in 2002, weight gain was found to be positively correlated with improved manic symptoms in patients treated with lithium (Atmaca et al., 2002). A similar correlation between weight gain and therapeutic effectiveness has been shown for antipsychotics (Wetterling & MuBigbrodt, 1999). This suggests that patients who exhibit greater metabolic impacts from lithium treatment may experience more favourable therapeutic outcomes. This may be due, at least in part, to their high activity of BAT, which reflects a dysregulated stress response characterised by excessive sympathetic nervous system activity. This may contribute to negative mood and high metabolism. Thus, the administration of lithium in these individuals may inhibit BAT activity, potentially improving symptoms by correcting their constant state of fight or flight, but it may also reduce their metabolism, leading to weight gain.

Ultimately, BAT activity may be a diagnostic and prognostic marker for psychiatric disorders. In support of this, a recent paper by Chen et al. showed that schizophrenic patients treated with antipsychotics had a reduced marker of BAT activation and that clozapine, one of the most obesogenic of the agents, led to the highest decrease in BAT activity (Chen et al., 2022). Considering these implications, it would be pertinent to conduct future animal research investigating lithium's differential impact on female and male thermoregulation.

### 4.4.3 Lithium's Effect After Cervical and Subdiaphragmatic Vagotomy

In my second experiment, I aimed to investigate whether the vagus nerve mediated lithium's inhibitory effect on the sympathetic response discussed above. To do so, I performed vagotomy at either the cervical or subdiaphragmatic level and examined the effect of lithium on the sympathetic response to cold exposure. My hypothesis was that severing the vagus nerve would alter lithium's effect on the sympathetic response.

My findings indicated that the effect of lithium on parameters such as BAT sympathetic nerve activity, BAT and body temperature, expired $CO_2$, heart rate, and mean arterial pressure did not show significant differences compared to intact rats, regardless of whether the vagotomy was performed at the cervical or subdiaphragmatic level. This result counters my initial hypothesis and confirms that severing the vagus nerve does not modify lithium's effect on the sympathetic response to cold stress.

Importantly, this study provides the first evidence for the involvement of non-vagal mechanisms in the effect of lithium on the sympathetic response, expanding our understanding of how the body recognises and responds to lithium.

In line with my observations, Martin et al. demonstrated that rats retained the ability to acquire lithium-induced CTAs following a bilateral subdiaphragmatic vagotomy (Martin et al., 1978). Consistent with these findings, Arnedo et al. demonstrated that subdiaphragmatic vagotomy did not interfere with the induction of CTA in rats following intragastric administration of hypertonic NaCl (Arnedo et al., 1991). This finding is particularly relevant since, although I did not specifically test a high dose of hypertonic NaCl in the current experiment, my previous study in **Chapter 2** indicated its similar yet less potent effects compared to LiCl, suggesting a shared pathway. These findings suggest that lithium's nauseating effect, which produces CTA and the behavioural-thermoregulatory effects I have observed throughout this thesis, persists in the absence of vagal afferent signals from the gastrointestinal tract. My findings will benefit from future studies aimed at investigating the role of the vagus in lithium's effect on the sympathetic response, as it is considerably unexplored in the literature.

#### *4.4.3.1 Physiological Implications*

My findings in this experiment rule out the involvement of the parasympathetic nervous system in the physiological response to lithium, particularly heart rate reduction. The sympathetic and parasympathetic branches of the autonomic nervous system, both innervating the heart, are known to have opposite effects on heart rate. The vagus nerve is a major carrier of parasympathetic information, with its efferents conducting signals from vagal nuclei to the heart, reducing heart rate (Capilupi et al., 2020) and counterbalancing sympathetic cardiac activation in a phenomenon known as 'accentuated antagonism' (Levy & Martin, 1981; Uijtdehaage & Thayer, 2000). However, upon transection of the vagus at the cervical level, its efferent innervation to the heart would have been severed, preventing signals from vagal nuclei from reaching the heart. As lithium was still able to reduce cold-evoked tachycardia in the absence of a functional vagus nerve, it appears that the response to lithium is largely due to the withdrawal of sympathetic outflow from the rMR, independent of parasympathetic activity.

Although the vagus nerve may not be the primary mediator of the physiological response to lithium, it could serve a secondary role. For example, rats with an ablated area postrema (the focus of the next experiment) are still able to distinguish and consume differing amounts of equimolar LiCl and NaCl salt

solutions (Ossenkopp et al., 1997). If the distinction between the two salts relied solely on the area postrema, there would not be differential consumption. This discrepancy is not due to the rats' ability to taste the difference between the two salts (Loy & Hall, 2002; Nachman, 1963), but likely originates from an internal signal or cue following ingestion. This indicates that vagal afferents may detect and relay the presence of lithium in the gut to the brain through a pathway that is distinct from the one associated with the physiological effects I observed. This reduction in consumption of lithium would complement the body's efforts of minimising lithium's impact on cellular processes via hypothermia (discussed in **Chapter 2**). This secondary role of the vagus nerve might be supported by the findings of Qian et al. in mice, which demonstrate that vagotomy abolishes lithium-induced c-Fos expression in the insular cortex, a brain region involved in visceral sensation (Qian et al., 2021).

#### *4.4.3.2 Limitations and Considerations*

A limitation of this experiment worth considering is that vagal afferent discharge was not measured, so I cannot be certain that lithium activated the vagus nerve. However, it is established in the literature that lithium activates vagal afferents. Ueda et al. showed that intraperitoneal lithium in anaesthetised rats increases vagal afferent discharge (Ueda et al., 2016). Further, Niijima and Yamamoto measured the activity of subdiaphragmatic vagal afferents in response to lithium and found that vagal afferent activity increased when lithium was introduced to the mesenteric surface of the visceral peritoneum, which mirrors my method of intraperitoneal lithium delivery (Niijima & Yamamoto, 1994). Therefore, it is likely that lithium was able to activate intact vagal afferents in my anaesthetised experiments. Whether this is a direct effect or involves a mediator is uncertain, but **Chapters 2 and 3** suggest that it is not via 5-$HT_3$ as an intermediary.

Another limitation is that I did not take an extra step beyond visual verification under a surgical microscope to confirm the completeness of vagotomy. For example, I could have intraperitoneally injected a retrograde tracer to see if it was effectively transported to vagal nuclei upon histological examination. This would improve the robustness of future studies.

Lastly, in my analysis I found that the overall pattern of the relationship between lithium dose and every parameter (BATSNA, BAT temperature, $EtCO_2$, heart rate, MAP, and body temperature) is similar across the control, cervical, and subdiaphragmatic groups, as evidenced by the non-significant differences in slopes. This led me to suggest that the vagotomies did not alter lithium's impact on these parameters. However, it is important to acknowledge that in my initial analysis, the dose-dependent relationship between lithium and $EtCO_2$ was significant for the control and

subdiaphragmatic groups but not for the cervical group. In addition, the relationship between lithium dose and MAP reached statistical significance for the control group, but it did not reach statistical significance for either vagotomy groups.

Given the discrepancy in the significance of the relationship between lithium dose and $EtCO_2$ in the cervical group, I conducted a secondary analysis, wherein two non-responsive animals from this group were identified and removed. These outliers were not displaying the expected physiological changes to cooling after saline administration, which could potentially have confounded my analysis of lithium's effects. With these outliers removed, a dose-dependent relationship between lithium and $EtCO_2$ emerged for the cervical group, corroborating the findings in the other groups and strengthening the validity of my results. This result is consistent with the dose-dependent reduction of BAT thermogenesis and heart rate, which suggest a corresponding decrease in metabolic activity that manifests as reduced expired $CO_2$. Importantly, however, the removal of these non-responsive animals did not significantly change the slopes across the groups, reiterating my initial finding of similarity in response patterns across the control, cervical, and subdiaphragmatic groups. Further, the exclusion also enhanced the accuracy of the linear regression model for lithium dose in relation to end-tidal $CO_2$, as well as for BAT sympathetic nerve activity, BAT temperature, and heart rate, compared to the initial analysis. This increase in the goodness of fits indicates that my model's predictive power was enhanced by removing these non-responsive animals. It lends further confidence to my decision to treat these animals as outliers or confounding factors in my data set.

One reason behind the increased rate of unresponsiveness to cold exposure may be the physiological impact of bilaterally transecting the vagus nerve at the cervical level. For example, Fukuda and Honda demonstrated that cervical vagotomy in rats significantly alters respiratory parameters, including end-tidal $CO_2$, underscoring the vagus nerve's critical role in maintaining alveolar ventilation and blood gas homeostasis (Fukuda & Honda, 1982). This profound impact of cervical vagotomy on respiratory parameters could have interfered with the animals' ability to effectively respond to cooling, and it was reflected most by the confounding of the $EtCO_2$ dose-response.

The secondary analysis did not alter the statistical outcome of the non-significant relationship between lithium dose and MAP within the cervical and subdiaphragmatic groups. As a result, it appears that the reduction in MAP is not solely attributable to lithium and the relationship becomes more complex in the presence of vagotomy. Vagotomy has been demonstrated to interfere with MAP regulation in previous studies. For instance, Alcayaga et al. observed that bilateral cervical vagotomy

altered blood pressure parameters, such as systolic and diastolic pressure, MAP, and pulse pressure in control rabbits (Alcayaga et al., 2018). Similarly, Reed and Layman found that bilateral cervical vagotomy in dogs led to blood pressure alterations independent of changes in heart rate (Reed, 1925; Reed & Layman, 1930). Reyes also showed that bilateral subdiaphragmatic vagotomy affects MAP in rats (Reyes et al., 1992). This evidence supports the idea that the MAP response to lithium may be obscured in the vagotomy groups, potentially due to increased variability caused by the disruption of blood pressure regulation or a disrupted sympathetic balance between projections to the heart and vessels. While lithium might still influence MAP, as suggested by the consistent trends across all groups, the current within-group analyses of the vagotomised rats may lack sufficient statistical power to discern this effect amidst the variability introduced by vagotomy. Further investigation is necessary to fully elucidate the interaction between lithium treatment and blood pressure regulation in the context of a severed vagus nerve.

In conclusion, my second experiment showed that the vagus nerve is not necessary for the detection and response to lithium. While vagal afferents are known to play a significant role in transmitting nausea-related signals, my results suggest that lithium's ability to trigger nausea-related responses is mediated through a different pathway. In the next subsection, I will discuss my findings on the area postrema lesion experiment and the area postrema's key role in mediating lithium's effect.

### 4.4.4 The Role of the Area Postrema in Lithium's Effect

In the previous experiment, I established that the vagus nerve does not play a significant role in lithium's action, which led me to investigate other peripheral mechanisms that might be responsible for detecting lithium and communicating its effects to the central nervous system. In this context, I focused on the area postrema, a brain region situated outside the blood-brain barrier that monitors the bloodstream and carries information to other parts of the brain. In the final experiment of this thesis, I compared the effect of intraperitoneal saline and two doses of lithium on the cold stress-elicited sympathetic response between rats with and without area postrema lesions. I hypothesised that ablation of the area postrema would eliminate the dose-dependent effects of lithium on each physiological parameter of the sympathetic response.

Confirming my hypothesis, I discovered that lesioning the area postrema prevented lithium's dose-dependent reduction of cold-evoked increases in BAT sympathetic nerve discharge, BAT thermogenesis, expired $CO_2$, and heart rate. These observations suggest that lithium's administration leads to a significant reduction in sympathetic activity, as evidenced by the dose-dependent decrease

in these physiological parameters. These findings provide the first evidence that the area postrema, as a peripheral detection mechanism, mediates lithium's impact on these physiological sympathetic responses. However, lithium's effect or lack thereof on mean arterial pressure was the same in the presence of a lesioned area postrema; this will be discussed further down. Taken together, these findings strongly indicate that the area postrema plays a significant role in detecting and responding to lithium, resulting in a downregulation of sympathetic outflow from the brain. Consequently, my observations provide robust evidence supporting the notion that a peripheral mechanism, such as the area postrema, detects lithium and triggers a normal centrally coordinated response, thus endorsing my overarching hypothesis throughout this thesis.

My findings are supported by consistent evidence that ablating the area postrema in rats prevents the acquisition of lithium-induced conditioned taste aversions, indicating the absence of nausea (Eckel & Ossenkopp, 1996; Ladowsky & Ossenkopp, 1986; McGlone et al., 1980; Rabin et al., 1983). As established in **Chapter 2**, this nausea is closely associated with the body's hypothermic response to lithium. Notably, Bernstein et al. found that lesioning the area postrema in rats eliminates nausea-indicative 'lying-on-belly' behaviour and hypothermia in response to intraperitoneal lithium administration (Bernstein et al., 1992), further supporting my findings.

As discussed in the second chapter, lithium-induced 'lying-on-belly' behaviour is similar to the lithium-induced 'cooling posture' I observed in guinea pigs. I argued that this behaviour was part of the thermoregulatory response to lithium, as well as the reduction in locomotor activity. In further support of this, Ladowsky and Ossenkopp discovered that rats with a lesioned area postrema also showed no decrease in locomotor activity upon receiving intraperitoneal or intragastric LiCl (Ladowsky & Ossenkopp, 1986). This parallels the consistently observed reduction in locomotor activity induced by lithium in guinea pigs and rats throughout the preceding chapters.

Additionally, McGlone et al. found that lesioning the area postrema in rats prevented lithium from reducing aggressive behaviour (McGlone et al., 1980). This lithium-induced reduction in aggressive behaviour is similar to the lithium-induced inhibition of hyperlocomotion in response to intruder-elicited psychological stress in **Chapter 3**.

Taken together, these findings solidify the role of the area postrema as the primary peripheral mediator responsible for the observed physiological and behavioural effects of lithium throughout this thesis. These observations further support the idea that the effects of lithium on behaviour are

intricately linked to its impact on thermoregulation, indicating a shared pathway for both responses to the ion.

#### *4.4.4.1 Considerations*

##### 4.4.4.1.1 Mean Arterial Pressure (MAP)

The unexpected lack of a dose-dependent reduction in cold-evoked MAP increase in the sham group contradicts my findings from the control group of the vagotomy experiment, where I observed a dose-dependent reduction in MAP response to cold exposure with lithium administration. The reason for this discrepancy remains unclear. The sham operation, intended simply to expose the area postrema without altering its function, is unlikely to be responsible for this discrepancy. Nevertheless, several factors could potentially explain the inconsistent results, such as variations in the baseline response to cold exposure, differences in experimental conditions, and the timing of saline administration after general anaesthetic infusion.

The baseline MAP response to cold exposure, as indicated by the MAP increase after saline administration, was higher in the control group of the vagotomy experiment compared to the sham group of the area postrema lesion experiment. This finding aligns with inconsistencies in cold-induced MAP increases reported in the literature (Nakamura & Morrison, 2007, 2011; Nakamura et al., 2022; Owens et al., 2002). Factors such as pre-experiment fluid intake, flushing frequency of the arterial line with heparinised Ringer solution, and postural variations among experimental animals might be contributing to these inconsistencies, making it challenging to clearly identify the cold-evoked changes in MAP and their relationship to blood pressure control during cold stress.

Another potential reason for the observed differences between the two control groups might be the variation in the timing of administering the 0.9% saline and subsequent cooling following the general anaesthetic infusion. On average, the vagotomy control group received the saline 4.25 hours after the infusion of the general anaesthetic, which is 1 hour later than the sham group (not formally included in the results). The anaesthetic mixture of chloralose-urethane has been documented to influence cardiovascular properties, including blood pressure (Claassen, 1994). It is possible that the longer time gap for the vagotomy control group allowed for the anaesthetic mixture's influence on blood pressure to decrease by the time saline was administered. This could potentially account for the greater increase in MAP observed in response to cooling and lithium's dose-dependent reduction of this response in the vagotomy control group compared to the sham group.

The disparity in baseline MAP response could have implications for understanding the effects of lithium on MAP. If the baseline response in the area postrema control group had been more similar to that of the vagotomy control group, it is possible that the lithium-induced reduction in MAP observed in the vagotomy control group would have also been seen in the area postrema control group. The differential effects of lithium on MAP might be attributed to the variations in baseline responses, or to other factors not yet explored in the current study. In addition, the area postrema control group displayed greater cold-evoked increases in other physiological parameters, such as BATSNA, BAT temperature, $EtCO_2$, and heart rate. This observation suggests that lower-responding animals' MAP might be more sensitive to lithium's effects.

Taken together, the observed discrepancies in the two control groups could be attributed to various contributing factors, including baseline response variations, the influence of the anaesthesia, and differences in experimental conditions. To provide a definitive answer, further investigation is needed to carefully examine and control these factors and elucidate the precise relationship between cold exposure, MAP response, and lithium's effects.

#### 4.4.4.1.2 Body Temperature

Another point of this experiment worth considering is my expectation of a consistent decrease in body temperature, primarily driven by whatever temperature the water blanket was at, irrespective of lithium's ability to produce hypothermia. However, rats in the intact group defied these expectations by maintaining near-stable body temperatures during cold exposure post-saline control. This observation contrasts with control results from the previous experiment. Therefore, lithium appeared to dose-dependently reduce body temperature. This indicates that the ablation of the area postrema prevents lithium-induced hypothermia, which is consistent with the existing literature. Yet, the reason behind the intact group's superior thermoregulation following saline administration, a phenomenon absent in the previous experiment's rats, remains ambiguous.

One speculative explanation could be that the exposure of the caudal brain during the sham operation may have affected brain temperature, thereby impacting thermoregulatory responses. Another factor to consider is the timing of the saline administration and subsequent cooling following the general anaesthetic infusion, as noted in my discussion above regarding discrepancies in MAP responses. In the area postrema experiment, the sham group had the saline injection and subsequent cooling about

an hour later than the control group in the vagotomy experiment. Further investigations are needed to validate these hypotheses.

In sum, my body of research strongly suggests that the area postrema plays a crucial role in detecting lithium in the bloodstream and communicating its effects to the central nervous system. This results in a downregulation of sympathetic activity, which underscores the physiological impact of lithium administration I have consistently observed throughout this thesis. Based on these findings, I will explore the brain regions necessary for producing the physiological and behavioural effects of lithium. Additionally, I will propose two central pathways through which lithium might exert its effects, building upon the insights gained from these observations.

### 4.4.5 Brain Areas Implicated in Lithium's Effects

I will start by reviewing which brain regions are known to be activated by lithium. Several studies using c-Fos immunoreactivity, a technique that reveals neuronal activation, has shown that intraperitoneal and intragastric administration of lithium leads to c-Fos expression in the rodent brain. These findings generally converge on brain regions such as the AP (in line with my functional observations), NTS, LPB, hypothalamus, and amygdala, while some studies have also noted c-Fos expression in additional areas such as the insular cortex (Andre et al., 2007; Gu et al., 1993; Lamprecht & Dudai, 1995; Portillo et al., 1998; Sakai & Yamamoto, 1997; Soto et al., 2017; Yamamoto & Sawa, 2000; Yamamoto et al., 1992).

For instance, Yamamoto et al. found that lithium induces c-Fos expression in various brain areas, including the area postrema (AP), nucleus tractus solitarius (NTS), lateral parabrachial nucleus (LPB), and preoptic area (POA) of the hypothalamus (Yamamoto et al., 1992). These areas are particularly relevant to lithium's effects because they play integral roles in thermoregulatory and physiological responses, as I will discuss in more detail later. Similarly, Sakai and Yamamoto demonstrated that intraperitoneal lithium administration induced c-Fos expression in the AP, NTS, and LPB (Sakai & Yamamoto, 1997). However, they also observed c-Fos expression in these areas following intraperitoneal hypertonic saline administration. This finding is further supported by Kobashi et al., who reported c-Fos expression in similar areas in response to intragastric hypertonic saline (Kobashi et al., 1993). Notably, Yamamoto et al. showed that sodium chloride induces less c-Fos expression in these areas compared to lithium chloride (Yamamoto et al., 1992). The co-localisation of c-Fos expression in these areas by lithium, and to a lesser extent sodium, aligns with my findings from the second chapter where these ions had similar behavioural and physiological effects, but lithium

demonstrated markedly greater potency. Additionally, it aligns with the fact that lithium is more effective at inducing conditioned taste aversions (Nachman & Ashe, 1973).

Importantly, Spencer et al. and Yamamoto et al. demonstrated that ablation of the AP reduces lithium-induced c-Fos expression in areas including the NTS, LPB, hypothalamus, and amygdala (Spencer et al., 2012; Yamamoto et al., 1992). However, Spencer et al. observed that the central nucleus of the amygdala (CeA) and hypothalamic supraoptic nucleus (SON) remained partially responsive to lithium after ablating the area postrema. This suggests peripheral action independent of the area postrema.

Residual activation of these brain areas could be due to vagal afferents transmitting lithium-activated signals to the NTS (Sawchenko, 1983), which directly or indirectly, via the LPB, projects to the CeA and SON (Day & Sibbald, 1988; Krukoff et al., 1993). The activation of splanchnic afferents by lithium may also explain this phenomenon, as these afferents have been found to stimulate comparable brain regions when there is a disturbance in viscerosensory perception during abdominal surgery (Barquist et al., 1996; Bonaz et al., 1994). According to Zhang and Huang, there is evidence to suggest that both abdominal vagal afferents and greater splanchnic nerve afferents not only converge on the AP/NTS region (Lutz et al., 1998; Muir, 2003; Sawchenko, 1983) but also exhibit interactions with each other (Zhang & Huang, 1990). This highlights the complexity involved in determining the non-AP action of lithium and supports the suggestion in the previous experiment that the vagus nerve might mediate other functions related to lithium.

Recently, Nakamura et al. defined a hypothalamomedullary network, which integrates decades of research on multiple brain regions involved in autonomic and behavioural thermoregulation during cold, heat, psychological and infection stress (Nakamura et al., 2022). This network mainly comprises the LPB, POA, DMH, and rMR, and serves as a key framework for understanding the intricate mechanisms of thermoregulation. The hypothalamomedullary network governs the activation of thermoregulatory and cardiac responses, regulating BAT thermogenesis, cutaneous vasoconstriction, body temperature, heart rate, and blood pressure. Additionally, this network plays a role in regulating behavioural responses. Using this model, I have identified a central neural circuit that appears to be responsible for the wide-ranging effects of lithium in my experiments.

Throughout this thesis, I have observed the diverse effects of lithium, such as lethargic behaviour, reduced locomotor activity, and hypothermia in **Chapters 2 and 3**; BAT thermogenesis inhibition and cutaneous vasodilation in **Chapter 3**; and heart rate reduction and possibly blood pressure in the

current chapter. These effects suggest that lithium acts on the hypothalamomedullary network. In support of this idea, the c-Fos studies I reviewed indicate that lithium activates several brain regions that directly or indirectly influence the hypothalamomedullary network. As indicated in this chapter, the area postrema is a major mediator. Consequently, I propose that lithium, through the area postrema, acts on the hypothalamomedullary network to downregulate sympathetic outflow from the rMR, which is the central endpoint of this network.

### 4.4.6 Proposed Pathways of Lithium's Action

Based on my insights, I have formulated two potential pathways that could illuminate the physiological and behavioural impacts of lithium, including its reduction of sympathetic activity, as diagrammatically represented in **Figure 4.11**. My primary pathway suggests a sequential interplay between the area postrema/nucleus of the solitary tract (AP/NTS), the lateral parabrachial nucleus (LPB), the preoptic area (POA), the dorsomedial hypothalamus (DMH), culminating in the rostral medullary raphe region (rMR). Alternatively, the secondary pathway proposes a route from the AP/NTS, through the intermediate and parvicellular reticular nuclei (iRt/PCRt), leading to the rMR. In the subsequent subsections, I aim to delve deeper into the specifics of each region and the proposed pathways. My goal is to offer a comprehensive understanding of lithium's interaction with these neurological circuits, shedding light on its behavioural and physiological effects, and elucidating its therapeutic potential in the context of stress-related psychiatric conditions.

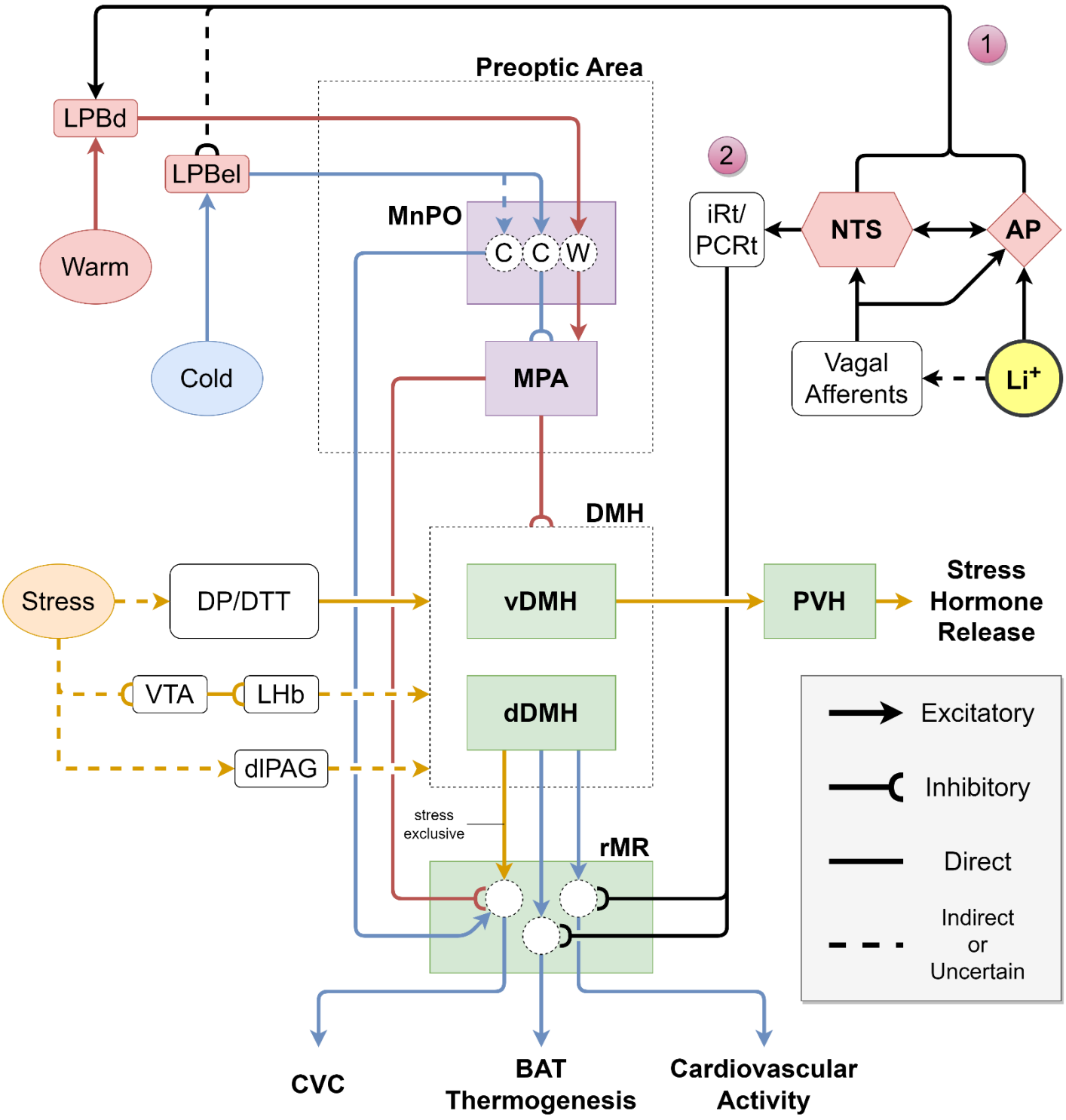


**Figure 4.11.** This diagram illustrates the proposed pathways of lithium's action on brain pathways. **The first pathway**, labelled **(1)**, starts at the Area Postrema (AP), which bidirectionally connects with the Nucleus Tractus Solitarius (NTS). It then proceeds from the AP/NTS to the Lateral Parabrachial Nucleus (LPB). Within the LPB, there are two distinct neuron populations: the LPBd (dorsal lateral subnucleus) responding to warm skin signals and the LPBel (external lateral subnucleus) responding to cold skin signals. As lithium ($L^+$ cation) circulates in the peripheral circulation, it is directly detected by the AP and activates the AP/NTS, which in turn excites the LPBd, functioning as a 'pharmacological warmth' by stimulating the heat-defence mechanism typically initiated by warm skin signals. Concurrently, the LPBel may also be inhibited, either directly or indirectly. Additionally, lithium could activate vagal afferents in the gut, either directly or through the release of mediators, supplying supplementary

information to the AP/NTS. The activation of the LPBd excites warm-sensitive neurons in the preoptic area (POA). This excitation triggers the direct inhibition of cutaneous vasoconstrictor sympathetic premotor neurons in the rostral medullary raphe region (rMR) by the medial preoptic area (MPA). As a result, cutaneous vasoconstriction (CVC) is inhibited, thereby inducing cutaneous vasodilation. It also enhances the tonic inhibition of the dorsomedial hypothalamus (DMH) by the MPA. This amplified inhibition prevents the dorsal part of the DMH (dDMH) from exciting BAT and cardiovascular sympathetic premotor neurons in the rMR, thereby inhibiting BAT thermogenesis and cardiac responses. This offers an explanation for how lithium induces hypothermia. In addition to their physiological functions, the LPB and the DMH are involved in modulating behaviour and locomotor activity. Thus, lithium's influence on these regions in this pathway could lead to the observed effects of lethargy and reduced locomotor activity. The diagram also highlights the stress pathway, which includes the dorsal peduncular cortex and dorsal taenia tecta (DP/DTT), ventral tegmental area (VTA), lateral habenula (LHb), and dorsolateral periaqueductal gray (dlPAG). When stress signals pass through these regions, they activate the DMH, resulting in the excitation of BAT and cardiovascular sympathetic premotor neurons by the dDMH. Moreover, a stress-specific excitatory connection from the dDMH to the CVC sympathetic premotor neurons also exists. The stress pathway activates the ventral part of the DMH (vDMH) as well, which subsequently activates the paraventricular nucleus of the hypothalamus (PVH), leading to the release of stress hormones via the hypothalamic-pituitary-adrenal (HPA) axis. This activation of the DMH by stress can also induce hyperlocomotion and specific behaviours. The diagram illustrates how lithium's influence on the LPBd and potential inhibition of the LPBel can counteract these stress-induced effects on the DMH by augmenting the inhibition of the DMH. **The second pathway**, labelled **(2)**, also begins at the AP/NTS, but diverges from the NTS to the intermediate and parvicellular reticular nuclei (iRt/PCRt). These nuclei project directly to and inhibit the BAT and cardiovascular sympathetic premotor neurons in the rMR. Thus, lithium, through the activation of the AP/NTS, leads to the excitation of inhibitory neurons in the iRt/PCRt, resulting in the inhibition of BAT thermogenesis and cardiac responses. It is possible that this pathway is activated together with the first, as the second pathway alone does not fully cover each effect of lithium. Triangular arrowheads denote excitatory connections, while arc arrowheads denote inhibitory connections. Solid lines indicate direct connections. Dashed lines indicate either indirect/multisynpatic or uncertain connections, please refer to the main discussion of each pathway for details.

#### *4.4.6.1 Pathway 1: AP/NTS → LPB → POA → DMH → rMR*

##### 4.4.6.1.1 Area Postrema (AP)

So far, I have established that the area postrema is a key mediator of the body's response to lithium, while vagal afferents do not play a significant role. The speed at which lithium exerts its effects is notably fast; as evidenced in **Chapter 3's** tail temperature experiment, the impact can be seen within a minute. In 1971, Lukas et al. demonstrated that small molecules (significantly larger than the lithium ion) such as atropine, caffeine, glucose, glycine, and progesterone, were detectable in the systemic circulation of rats as early as 10 seconds post-intraperitoneal injection (Lukas et al., 1971). The peritoneum, a well-perfused membrane with a large surface area, facilitates rapid absorption of drugs, especially small molecules or atoms like lithium ions (Al Shoyaib et al., 2020). Therefore, it is unsurprising that lithium quickly reaches and is detected by the area postrema, which lies outside the blood-brain barrier. This rapid absorption and detection align with the observed speed at which lithium exerts its effects.

I postulated in **Chapter 2** that lithium might trigger the release of neurohormonal mediators, such as serotonin (5-HT), from enterochromaffin (EC) cells in the gastrointestinal tract, which might explain its effects. However, I determined in **Chapters 2 and 3** that the $5\text{-HT}_3$ receptor is not involved. Although I did not specifically investigate other circulating agents that might be released by EC cells such as CCK in this chapter, the literature suggests that lithium may be detected directly by the area postrema, the same way sodium ions are directly detected.

For example, in the area postrema of rats, Tsukamoto and Adachi identified two subpopulations of lithium-sensitive neurons that either increased or decreased firing rate in response to LiCl superfused over its surface (Tsukamoto & Adachi, 1994). They also showed that the neurons responsive to lithium were not responsive to hypertonic saline, meaning that there are specialised lithium-sensing neurons within the area postrema. Similarly, Adachi et al. demonstrated in rats that superfusion of LiCl over the area postrema increased the discharge rate of a nausea-related neuron within the AP (Adachi et al., 1991). Unless mediators are released in direct proximity of the area postrema, these studies suggest that the area postrema directly detects lithium.

Additionally, Adachi et al. also identified sodium-responsive neurons that increased discharge rate in response to sodium (Adachi & Kobashi, 1985). This provides even more insight into why sodium exerts similar but weaker affects to lithium in **Chapter 2**, acting on a similar pathway.

Research has shown that low frequency electrical stimulation of the area postrema decreases heart rate and blood pressure (Ferguson 1991). Further, Gallo et al. demonstrated that electrical stimulation of the area postrema is sufficiently nauseating to be used to develop conditioned taste aversions (Gallo, Arnedo et al. 1988). Both of these findings resemble my observations of lithium's action. However, at higher frequencies, blood pressure increases. This highlights the complexity of the area postrema's function whereby different subpopulations of area postrema neurons achieve different functions. It would appear that lithium might be activating subclasses of neurons within the area postrema that are responsive to low-frequency electrical stimulation.

The area postrema projects to many areas, but primarily to the nucleus of the solitary tract (NTS) and LPB. The area postrema receives input from vagal afferents, the NTS, LPB, DMH, and PVH (Ferguson, 1991). As it is highly likely that lithium directly activates the AP, its effect begins at this level (**Figure 4.11**).

#### 4.4.6.1.2 Nucleus of the Solitary Tract (NTS)

The NTS physically abuts and bidirectionally connects with the area postrema (Shapiro & Miselis, 1985; Wang et al., 2008). It also receives input from vagal afferents (Babic & Browning, 2014). Cai et al. demonstrated that inputs to the NTS from the area postrema are mostly excitatory (Cai et al., 1996). Zhang et al. later confirmed this (Zhang et al., 2021). Thus, in response to lithium, the area postrema may forward this information to the NTS, exciting neurons within the NTS (**Figure 4.11**).

Although the majority of the NTS has a definable blood-brain barrier, it may partially share the area postrema's chemoreceptive ability. In 1990, Gross et al. observed the ultrastructure of the NTS and identified fenestrated blood vessels in the dorsomedial and lateral commissural NTS, including the subpostremal region that abuts the area postrema (Gross et al., 1990). These blood vessels were permeable to tracers and also had large Virchow-Robin spaces that allowed for the interstitial distribution of blood-derived molecules into NTS subregions. Therefore, it may be possible for lithium to reach the NTS this way and for the NTS to respond to lithium. However, it is not known if the NTS can directly detect lithium as the area postrema does.

It is important to note that it is not possible for me to rule out the involvement of the NTS. As other researchers have noted (Averill et al., 1996; Borison, 1989; Miller & Leslie, 1994; Zhang et al., 2021), it is notoriously difficult to precisely remove the area postrema without affecting the neighbouring

NTS border, which sends some of its dendrites into the area postrema (Morest, 1960). As such, when the area postrema was lesioned in my experiment, collateral damage to the NTS was unavoidable. This makes it difficult to precisely evaluate the area postrema's role in the response to lithium, which is a limitation of this experiment. Therefore, it is possible that the NTS is involved in lithium's action, but to what extent is unknown.

The NTS sends projections, along with the area postrema, to the LPB (Menani et al., 2014; Roman et al., 2016), where the onset of lithium's thermoregulatory and behavioural effects may occur (**Figure 4.11**).

#### 4.4.6.1.3 Lateral Parabrachial Nucleus (LPB)

The LPB is a major recipient of viscerosensory information from the NTS and AP (Andre et al., 2007; Craig, 2002; Cunningham Jr et al., 1994), including bloodborne information from the AP (van der Kooy & Koda, 1983). Hence, many studies have shown that lesioning the LPB prevents lithium-induced conditioned taste aversions (Mungarndee et al., 2006; Reilly & Trifunovic, 2000; Wang & Chambers, 2002). This suggests that the LPB is a critical region that mediates lithium-induced nausea and possibly the nausea-indicative lethargic behaviour observed in guinea pigs in **Chapter 2**.

In addition to processing nausea-related signals, the LPB serves as a key relay for thermoregulatory and cardiovascular responses. It receives cutaneous thermosensory signals from the skin, and transmits these signals to the preoptic area (POA), which is responsible for coordinating involuntary thermoregulatory responses in resting, temperature challenge, and infection stress conditions (Morrison & Nakamura, 2011). The LPB contains two separate populations of neurons: one that responds to cool temperatures, and one that responds to warm temperatures. The combined function of these populations is essential for initiating prompt autonomic and behavioural thermoregulatory responses aimed at maintaining body temperature under diverse thermal conditions (Morrison & Nakamura, 2011).

Blocking the excitation of LPB neurons inhibits the ability of rats to physiologically defend body temperature by altering BAT thermogenesis, cutaneous vasoconstriction, and cardiac responses during warming and cooling (Nakamura & Morrison, 2008, 2010). Further, Yahiro et al. demonstrated that neurochemical inhibition of the LPB resulted in rats being unable to behaviourally thermoregulate (Yahiro et al., 2017); in other words, the rats could not move to a cooler location when the environment was too warm, and vice versa. Although not tested, I may infer that this might also apply

to postural changes such as the guinea pig 'cooling posture' behaviour in **Chapter 2**, which ties the function of the LPB together with mediating behavioural nausea responses. Therefore, the LPB is a functionally critical point that has the capacity to mediate both lithium's behavioural and physiological effects.

In terms of behavioural function, the LPB sends extensive projections to the central amygdala (CeA), a key region involved in coding aversive emotions that drive avoidance behaviours (Yahiro et al., 2023). This LPB → CeA pathway may engage neural circuits that generate thermal discomfort emotions, rather than overt warmth sensations mediated by the spinothalamocortical pathway (Nakamura & Morrison, 2010; Yahiro et al., 2017). Therefore, lithium's activation of the LPB could produce an aversive thermal discomfort signal in the CeA that motivates the observed behavioural adjustments to cool down, like the cooling posture or preference for cooler environments, even without conscious perception of ambient warmth.

Within the LPB itself, there are two distinct thermoregulatory populations of neurons contained in the dorsal lateral subnucleus (LPBd) and the external lateral subnucleus (LPBel). The LPBd is activated by cutaneous warm signals, whereas the LPBel is activated by cold signals. When the skin is warmed, LPBd discharge increases and LPBel activity decreases, activating the heat-defence response. On the other hand, skin cooling increases LPBel activity and decreases LPBd discharge, activating the cold-defence response.

Nakamura and Morrison have shown that inhibition of the LPBel reverses cooling-evoked increases in BAT sympathetic nerve discharge and temperature, heart rate, and end-tidal $CO_2$ (Nakamura & Morrison, 2008). Further, Nakamura and Morrison also showed that LPBd activation reversed the cooling-evoked increases in these parameters (Nakamura & Morrison, 2010). Interestingly, Chamberlin and Saper found that electrical and glutamate stimulation of the LPBel in rats caused an increase in blood pressure and heart rate, while electrical stimulation of the LPBd led to a decrease in blood pressure and heart rate (Chamberlin & Saper, 1992). Therefore, as lithium similarly attenuated each increase in these parameters during cooling, it is possible that lithium's pathway involves an excitatory input to the LPBd with an optional inhibitory connection to the LPBel (**Figure 4.11**).

In support of this, there is neuroanatomical evidence that both the AP and NTS project to the external lateral and dorsal lateral subnuclei of the lateral parabrachial nucleus (Herbert et al., 1990). Moreover, Zhang et al. found that excitatory AP neurons send a major branch into the LPBd (Zhang et al., 2021).

Richard et al. also observed excitatory projections from the NTS to the LPBd (Richard et al., 2014). However, there is no evidence at present of inhibitory inputs from the NTS or AP to the LPBel. This suggests that activation of the LPB in response to lithium likely occurs through excitatory projections from the AP and NTS to the LPBd. This fits with the observation that lithium activates areas like the AP, NTS and LPB, as shown in c-Fos studies.

This notion is further supported by a study conducted by Yang et al. wherein they activated excitatory glutamatergic neurons in the NTS that project to the LPB (Yang et al., 2020). Although the method employed did not specifically target the LPBd, it successfully induced hypothermia, which is consistently observed in response to lithium (**Chapters 2 and 3**).

Therefore, taken together, the available neuroanatomical and functional evidence suggests that lithium likely activates the AP/NTS → LPB pathway through excitatory input, primarily from the AP/NTS to the LPBd, likely mediated by glutamate release. This activates pathways from the LPB that produce the observed physiological and behavioural effects (**Figure 4.11**).

The skin-warming-activated LPBd and skin-cooling-activated LPBel both provide excitatory input into the median part of the preoptic area (MnPO), where their opposite effects are processed.

#### 4.4.6.1.4 Preoptic Area (POA)

The POA plays a central role in coordinating heat and cold-defensive responses, informed by distinct sensory inputs from the LPBd and the LPBel, which convey warmth and cold-related signals from the skin, respectively (Nakamura et al., 2022).

Warmth signals from the LPBd augment the tonic activity of warm-sensitive neurons in the median preoptic nucleus (MnPO), which in turn stimulate the medial preoptic area (MPA). The MPA exerts an inhibitory influence on the dorsomedial hypothalamus (DMH), a region that typically provides excitatory drive to specific groups of rMR premotor neurons responsible for driving BAT thermogenesis and cardiovascular activity, including tachycardia and hypertension (Nakamura et al., 2022). This inhibition leads to a reduction in BAT thermogenesis and cardiovascular activity, effects that are mirrored by the administration of lithium. This has led to the proposition that lithium may act as 'pharmacological warmth'.

Simultaneously, warmth signals from the MnPO prompt the MPA to directly inhibit the specific rMR premotor neurons involved in cutaneous vasoconstriction, inducing vasodilation (Nakamura et al., 2022). This process bypasses the DMH, and strikingly resembles the vasodilatory response induced by lithium under resting conditions in **Chapter 3**.

The combined effect of reduced BAT thermogenesis, cardiovascular activity, and the induction of vasodilation, in response to the warmth signals, is the lowering of body temperature (Nakamura et al., 2022). Intriguingly, throughout this thesis, I have observed lithium to exert a similar temperature-lowering effect, reinforcing the concept of lithium acting as a 'pharmacological warmth'.

In contrast, cold-sensitive neurons in the MnPO are excited by LPBel signals, leading to the deactivation of the inhibitory MPA → DMH pathway and thus disinhibition of the DMH (Nakamura et al., 2022). This results in an increase in BAT thermogenesis and cardiovascular activity, as observed during cold exposure in this chapter. These MnPO neurons also directly activate the rMR premotor neurons involved in cutaneous vasoconstriction. The combined effect of increased BAT thermogenesis, cardiovascular activity, and cutaneous vasoconstriction results in an increase in body temperature (Nakamura et al., 2022).

Please refer to **Figure 4.11** for a visual representation of these interactions.

Apart from managing responses to cold and warmth, the POA also plays a crucial role in initiating fever during instances of infection stress (Nakamura et al., 2022). The pyrogenic mediator prostaglandin $E_2$ ($PGE_2$), produced in response to immune signals, inactivates warm-sensitive neurons in the MnPO and MPA. This removes inhibitory control over the rMR and DMH. $PGE_2$ also activates an excitatory input from the MnPO to the rMR cutaneous vasoconstrictor premotor neurons, but it is not known if this is the same input activated by cold skin signals (Tanaka et al., 2013). Consequently, BAT thermogenesis, cardiovascular activity, and cutaneous vasoconstriction are increased, leading to the onset of hyperthermia or fever (Nakamura et al., 2022). Notably, Tulunay's observations revealed that lithium inhibits hyperthermia in response to yeast infection (Tulunay, 1976), which may be due to its capacity to maintain the activation of warm-sensitive neurons in the POA via the LPBd, even during a pyrogenic response. Whether lithium also inhibits the cutaneous vasoconstriction elicited during fever would confirm this hypothesis.

The complex interactions between the POA and the DMH, and their roles in thermoregulation, provide a compelling context for understanding the effects of lithium, which intriguingly parallel the body's physiological responses to warmth. Lithium's impact on BAT thermogenesis, cardiovascular activity, and vasodilation, as well as its potential to lower body temperature, align with the POA's responses to warmth signals. Furthermore, lithium's potential to inhibit fever during infection stress, as observed by Tulunay, suggests it might help sustain the activation of warm-sensitive neurons in the POA. Given these interactions and the significant influence of the POA on the DMH, a more in-depth exploration of the DMH's role within these thermoregulatory pathways becomes a compelling next step.

#### 4.4.6.1.5 Dorsomedial Hypothalamus (DMH)

In the thermoregulatory network, the DMH operates under the control of the POA. It is the primary excitor of rMR sympathetic premotor neurons driving BAT thermogenesis and cardiovascular activity during cold and infection stresses. However, the DMH does not activate cutaneous vasoconstriction in response to these conditions (Nakamura et al., 2022). Instead, as previously noted, the MnPO bypasses the DMH, directly exciting cutaneous vasoconstrictor sympathetic premotor neurons in the rMR.

In contrast, stress-induced cutaneous vasoconstriction is driven solely by the DMH (**Figure 4.11**). This distinction is crucial considering lithium's ability to inhibit cutaneous vasoconstriction under resting conditions and its capacity to prevent transient increases in vasoconstriction elicited by handling stress, as discussed in **Chapter 3**. Given these findings, it is unlikely that lithium exerts its effects by directly inhibiting the DMH from the AP/NTS. Such a mechanism would only prevent stress-induced vasoconstriction while leaving the cold-stress, MnPO-mediated excitatory bypass to the rMR unaffected. Therefore, considering lithium's effectiveness in mitigating psychological stress-induced increases in vasoconstriction and inducing vasodilation under resting conditions, its mechanism of action must encompass both the POA and DMH.

The DMH is a main receiver of stress-related signals from the corticolimbic circuit involving the amygdala, which are consolidated by the dorsal peduncular cortex and dorsal taenia tecta (DP/DTT) in the ventromedial prefrontal cortex as a ‘master signal’ that chiefly excites the DMH’s input to the rMR (Kataoka et al., 2020; Nakamura & Morrison, 2022). The DMH also receives stress-related signals through other regions such as the dorsolateral periaqueductal gray (dlPAG) in the midbrain and the lateral habenula (LHb) in the diencephalon above the thalamus; notably, the activity of the LHb is regulated by the ventral tegmental area (VTA), also located in the midbrain (Brizuela & Ootsuka, 2021;

Brizuela et al., 2018; Nakamura & Morrison, 2022). Through these psychological stress-related pathways alone, the DMH excites all three groups of rMR sympathetic premotor neurons responsible for cutaneous vasoconstriction, BAT thermogenesis, and cardiovascular activity, producing emotional hyperthermia (**Figure 4.11**). I have observed the effects of the former two groups in **Chapter 3** in rats wherein handling stress during saline injection led to a decrease in tail temperature, implying cutaneous vasoconstriction, and the intruder elicited BAT thermogenesis and emotional hyperthermia in the resident rat.

The DMH also plays a neuroendocrine role in the response to stress. While the dorsal part of the DMH (dDMH) mainly projects to the rMR, the ventral part (vDMH) is pivotal in activating the hypothalamic–pituitary–adrenal (HPA) neuroendocrine axis via stimulation of the hypothalamic paraventricular nucleus (PVH). This initiates a cascade that culminates in the secretion of stress hormones, which augment the physiological responses to stress driven by the dDMH (Nakamura & Morrison, 2022). It has been shown that inhibiting DMH neurons reduces the activation of the PVH and resultant release of stress hormones (Morin et al., 2001; Stotz-Potter et al., 1996). Therefore, it is plausible that lithium could attenuate the release of stress hormones induced by psychological stress via its inhibitory effect on the vDMH through the LPBd. In support of this hypothesis, Valvassori et al. demonstrated that lithium prevented HPA axis activation (ACTH and cortisol release) by paradoxical sleep deprivation in mice (Valvassori et al., 2017), a method considered stressful that produces manic-like behaviour (increased locomotor activity, aggression), and a neuroendocrine response similar to that produced by a psychological stressor (Moraes et al., 2022). Further, Haj-Mirzaian et al. found that lithium attenuated HPA hyperactivity, as well as depressive and anxiety-like behaviours produced by social isolation stress in mice (Haj-Mirzaian et al., 2016). This holds clinical relevance, as mood and anxiety disorders are often associated with HPA dysregulation (Juruena et al., 2020; Keller et al., 2017; Tafet & Nemeroff, 2020). For instance, it has been shown that individuals diagnosed with bipolar disorder tend to display increased activity in the HPA axis (Murri et al., 2016). Hence, our understanding of lithium's effects on these pathways provides potential insight into the pathogenesis of psychiatric disorders characterised by such dysregulation. This presents a worthwhile avenue for further exploration in future experiments.

Besides its autonomic and endocrine involvement, there is a large body of evidence suggesting that the DMH is also involved in modulating behaviour and locomotor activity. Electrical activation of the medial hypothalamus, which invokes c-Fos activation in the DMH among other areas, evokes defensive behaviour such as running and jumping (Jordan, 1998). Specific activation of the DMH via

disinhibition has been shown to increase locomotor activity (Hunt et al., 2010). Similarly, Rezai-Zadeh et al. found that selectively activating and inhibiting a subpopulation of DMH neurons resulted in increased and decreased locomotor activity, respectively (Rezai-Zadeh et al., 2014). Other researchers reported similar hyperlocomotive results, along with autonomic increases such as BAT thermogenesis, hyperthermia, and cardiovascular activity (Houtz et al., 2021; Machado et al., 2018; Zaretsky et al., 2018; Zhao et al., 2017). Upstream from the DMH, activation of heat-defence neurons in the LPBd and POA results in a reduction of locomotor activity, indicating inhibition of the DMH (Yu et al., 2016; Zhao et al., 2017). As I suspect that lithium activates the LPBd via the AP/NTS, this mechanism would explain lithium's dose-dependent reduction in locomotor activity in **Chapters 2 and 3**, and that it must involve the DMH.

One explanation for this is the DMH's connection to the mesencephalic reticular nucleus (Thompson et al., 1996), considered part of the mesencephalic locomotor region (MLR), which projects to medullary reticulospinal neurons (Jordan, 1998). The lateral hypothalamus, which the DMH projects strongly to (Thompson et al., 1996), also projects to the medullary reticulospinal neurons. These reticulospinal neurons then descend and directly activate the locomotor central pattern generator throughout the spinal cord, producing rhythmic output to motoneurons, resulting in locomotion (Jordan et al., 2008). Lithium may therefore reduce stress-evoked increases in locomotion by attenuating excitatory input from the DMH to the MLR, reducing reticulospinal motor output.

**Chapter 3** provided insightful discoveries about the effects of lithium on stress-induced responses. Lithium, at a low dose, was found to selectively inhibit acute psychological stress-induced increases in BAT thermogenesis, hyperthermia, and locomotor activity without inducing hypothermia. Additionally, it prevented handling stress-induced cutaneous vasoconstriction. These findings led me to propose that lithium could be attenuating the excitation of the DMH from stress-related corticolimbic inputs, specifically through the DP/DTT and other regions.

Should my hypothesis hold true, which suggests that lithium mimics the physiological response to a warm environment by activating the LPBd through the AP/NTS, it will provide an explanation for my observations. This mechanism would activate the heat-defence response, directly inhibit rMR cutaneous vasoconstrictor neurons from the MPA, and augment the tonic inhibition of the DMH from the MPA. These actions could collectively counteract psychological stress-induced increases in BAT thermogenesis, cutaneous vasoconstriction, emotional hyperthermia, and increased locomotor activity.

Furthermore, under this assumption, I would anticipate emotional hyperthermia to be diminished in a hot environment, as the activation of the LPBd by warm skin signals would counteract the emotional hyperthermia. This notion is supported by a study by Briese, which demonstrated that stress-induced increases in body temperature are significantly lower when the ambient temperature is hot (31 °C) compared to room temperature (23 °C) or cold (8°C) (Briese, 1992). This finding aligns with my proposed pathway activated by lithium, thereby lending further credence to my hypothesis.

Research indicates that reducing activity within the DMH lessens MDMA-induced hyperlocomotion and hyperthermia (Zaretsky et al., 2014). My hypothesis that lithium inhibits DMH activity through the AP/NTS → LPB → POA → DMH pathway provides a possible mechanism for these observations. If lithium indeed suppresses DMH activity, it may well reverse the hyperlocomotion and hyperthermia induced by MDMA, along with similar effects induced by other stimulants like methamphetamine. In support of this, studies have shown that lithium reduces methamphetamine-induced hyperlocomotion in mice (Ago et al., 2012; Furukawa et al., 1975).

My proposed mechanism underscores the pivotal role of the DMH in response to stimulants and aligns with the assertion made in **Chapter 3** about lithium's potential to counteract life-threatening hyperthermia caused by MDMA and similar stimulants. This comprehensive understanding of lithium's action within the neurophysiological pathways could open up new avenues for therapeutic interventions, providing a more targeted approach to managing stimulant-induced hyperthermia and hyperlocomotion. Thus, lithium's role goes beyond mood stabilisation, potentially expanding its utility in the realm of neuropsychiatric therapeutics.

In sum, it is highly plausible that the DMH is a crucial node in the pathway influenced by lithium. Inhibition of this region appears to align with most physiological and locomotor effects observed following lithium administration, including attenuation of the stress-exclusive pathway that drives cutaneous vasoconstriction. However, the fact that lithium maintains potential effectiveness under various stress conditions such as cold, warmth, and infection, where the role of DMH in driving cutaneous vasoconstriction is bypassed by the POA, strongly suggests that the action of lithium likely encompasses a pathway that involves both the POA and DMH. This concept aligns with my proposed pathway and underscores the intricate interplay and regulation of thermoregulatory mechanisms in the body.

#### 4.4.6.1.6 Rostral Medullary Raphe Region (rMR)

The rMR, the final segment in my proposed pathway, represents the critical interface between the central command of the brain and the peripheral sympathetic network. It is postulated that sympathetic premotor neurons within the rMR can be categorised into distinct subgroups (**Figure 4.11**), with each subgroup selectively activating a specific thermoregulatory effector (Nakamura et al., 2022). These subgroups are likely to be regulated by separate yet parallel pathways originating from the hypothalamus, as discussed above (Nakamura et al., 2022).

Upon activation, sympathetic premotor neurons transmit signals to preganglionic neurons primarily located in the intermediolateral cell column (IML) of the thoracolumbar spinal cord (Biaggioni et al., 2022). These neurons are synapsed onto sympathetic ganglia adjacent to the spinal cord, subsequently activating thermogenic and cardiovascular effector organs in the periphery such as BAT, the heart, and arteries (Blessing, 2003; Nakamura & Morrison, 2008). Importantly, the discharge of postganglionic axons in BAT, as I have measured, represents a direct reflection of the signals transmitted from the corresponding sympathetic ganglia that activate BAT.

My data indicates that the brain primarily detects lithium in the periphery through the AP/NTS pathway and coordinates a response, which results in a reduction of sympathetic output from the rMR and decreased activation of each effector organ. However, the rMR is unlikely to mediate hyperlocomotion, as both inhibition of this region and its direct stimulation do not substantially affect locomotor activity (Ikoma et al., 2018; Liu & Jordan, 2005; Zaretsky et al., 2003). This suggests that upstream regions, such as the LPB and DMH, are the main contributors to locomotion reduction in response to lithium. Future work can clarify the rMR's precise role by selectively inhibiting this region and determining whether lithium's behavioural and locomotor effects persist.

In addition to the rMR, the rostral ventrolateral medulla (RVLM) may also play a role in the physiological response to lithium, but to a lesser extent. The RVLM primarily contributes to the maintenance of resting and reflexive cardiovascular function (Biaggioni et al., 2022; Nakamura & Morrison, 2022; Xue et al., 2019). Lithium's reductive influence on basal heart rate in conscious rats (Jones et al., 2008) may partially be explained by a decrease in RVLM activity, as it receives input from the dDMH similarly to the rMR (Nakamura & Morrison, 2022).

In a study by Cao and Morrison, disinhibition of premotor neurons in the rMR led to increased cardiac sympathetic nerve activity, heart rate, and blood pressure (Cao & Morrison, 2003). Notably, even after

inhibition of the RVLM, which lowered these parameters, rMR disinhibition still significantly increased cardiac sympathetic nerve activity and heart rate. This observation highlights the rMR's critical role in increasing cardiac output during cold, psychological, and infection stresses, independent of the RVLM's contribution.

In contrast, it is important to mention that the RVLM, rather than the rMR, may mediate an increase in heart rate during the heat-defence response. It is postulated that the RVLM receives excitatory inputs directly from the LPBd, increasing heart rate, which enhances circulation and promotes heat loss under heat stress (Agarwal & Calaresu, 1993; Nakamura & Morrison, 2010). Despite lithium's effects sharing similarities with the heat-defence response, it does not seem to increase heart rate. Perhaps this subpopulation of RVLM-projecting neurons within the LPBd is not activated by the AP/NTS. This observation supports the earlier suggestion that lithium may reduce RVLM activity. Understanding the role of the RVLM in these processes and its interaction with lithium are significant next steps to fully elucidate the physiological effects of lithium.

In summary, my proposed AP/NTS → LPB → POA → DMH → rMR pathway provides an innovative and integrated framework for understanding how lithium produces the multitude of effects observed throughout this thesis. This is significant as it shifts the understanding of these effects from being seen merely as side effects to recognising them as essential components of the drug's therapeutic action, implicating specific brain regions that are also critically involved in stress-related psychiatric conditions that lithium effectively treats. Moreover, this integrated approach offers a considerably superior alternative to current hypotheses about lithium's mechanism of action, which tend to focus on molecular interactions that occur across numerous physiological systems and provide an unclear explanation for lithium's distinctive effects. My proposed pathway, on the other hand, posits a coherent and focused explanation for lithium's actions. While additional research is required to further refine and test this pathway, it sets the stage for a more comprehensive understanding of lithium's pharmacological effects. This understanding is pivotal for enhancing the therapeutic use of lithium in conditions like bipolar disorder and may pave the way for the development of more targeted and effective treatments for such disorders in the future.

#### *4.4.6.2 Pathway 2: AP/NTS → iRt/PCRt → rMR*

In the second proposed pathway, the NTS provides excitatory projections to GABAergic neurons located in the intermediate and parvicellular reticular nuclei (iRt/PCRt), which subsequently innervate

the rMR (**Figure 4.11**). The iRt/PCRt exerts inhibitory control over select sympathetic premotor neurons in the rMR that are specifically responsible for promoting BAT thermogenesis and cardiovascular responses (Nakamura et al., 2022; Nakamura et al., 2017). Consequently, stimulation of rostral NTS neurons can result in the inhibition of these specific premotor neurons, thereby suppressing BAT thermogenesis and cardiovascular responses (Nakamura et al., 2017). Given the significance of this pathway in regulating these physiological responses, it is plausible that this pathway represents one of the mechanisms potentially activated by lithium through the AP/NTS.

Thus, lithium's partial inhibition of the rMR through the AP/NTS → iRt/PCRt → rMR pathway would counteract the excitation of rMR neurons responsible for BAT thermogenesis and cardiovascular activity during psychological and infection stress. This pathway would work in synergy with the first pathway (AP/NTS → LPB → POA → DMH → rMR) to prevent emotional hyperthermia and fever, as well as promoting hypothermia in resting conditions.

However, the role of this pathway in influencing cutaneous vasoconstriction, thermoregulatory behaviour, and locomotor activity remains unclear. The iRt/PCRt's demonstrated inhibitory effects are predominantly on the premotor neurons in the rMR responsible for BAT thermogenesis and cardiovascular activity, but no evidence thus far extends this influence to modifying cutaneous vasoconstriction. Furthermore, this pathway does not appear to involve the LPB and DMH regions, which are implicated in behaviour and locomotor activity, thereby suggesting that it alone would not lead to modifications in these parameters. Additionally, neither inhibition nor activation of the rMR significantly alters locomotion (Ikoma et al., 2018; Liu & Jordan, 2005; Zaretsky et al., 2003). For these reasons, it is unlikely that this pathway alone accounts for the effects observed with lithium administration. This insufficiency argues for the combined influence of both the first and second proposed pathways. Such a comprehensive approach to both pathways would provide the most robust understanding of the potential mechanisms in the brain's response to lithium.

In personal correspondence, Nakamura suggested a potential method to investigate the hypothesis of lithium's impact on the AP/NTS → iRt/PCRt pathway (K. Nakamura, personal communication, November 10, 2022). This suggestion builds upon his prior work in which his group discovered that the iRt/PCRt inhibits sympathetic premotor neurons in the rMR during starvation (Nakamura et al., 2017). The proposed method begins by stimulating BAT thermogenesis via disinhibition of the DMH through a bicuculline injection. This is followed by the administration of lithium either in the peritoneal cavity or directly into the AP/NTS. Should lithium effectively inhibit BAT thermogenesis, it would

suggest that lithium signalling impedes BAT sympathetic premotor neurons in the rMR. Given this result, I could further examine if lithium, when injected into the AP/NTS, can inhibit BAT thermogenesis triggered by bicuculline or NMDA injection into the rMR. If indeed lithium signalling from the AP/NTS inhibits sympathetic premotor neurons via GABAergic inputs to the rMR, I would anticipate NMDA-induced BAT thermogenesis being inhibited by lithium, while bicuculline-induced BAT thermogenesis would likely remain unaffected.

#### *4.4.6.3 Considerations*

While the proposed pathways provide a comprehensive framework for understanding the effects of lithium on the brain, it is important to note that these representations are likely a simplification of a much more complex and interconnected neuronal network. The pathways in the diagram do not capture all potential connections.

The NTS, for instance, has extensive bidirectional connections with various brain areas, including the hypothalamus and amygdala (Blessing, 1997). The LPB also projects to the insular cortex, which may explain c-Fos activation in that region following lithium administration (Qian et al., 2021). In addition, the LPB and extended amygdala are reciprocally connected (Jaramillo et al., 2021). The extended amygdala region plays a role in modulating acute and long-term responses to stressors (Jaramillo et al., 2021). Therefore, the activation of the LPB by lithium, through the AP/NTS pathway, may alter the extended amygdala's processing of stress-related behaviour. This provides another potential pathway through which lithium inhibits psychological stress-induced increases in behavioural activity, as shown in **Chapter 3**.

The NTS also receives projections from the rMR (Barraco et al., 1992). Although the AP has very few central inputs, it receives significant projections from the LPB, DMH, and PVH (Ferguson, 1991; Shapiro & Miselis, 1985). These connections may serve as a negative feedback loop, silencing the AP to control extreme physiological changes and maintain homeostasis, which might be how the body begins to reverse its response to lithium in **Chapters 2 and 3**.

In summary, while the proposed pathways offer a robust framework for understanding lithium's actions, it is important to consider the complexities and potential additional connections within this network. Future studies should aim to refine our understanding of this intricate circuitry.

## 4.5 Conclusion

In this thesis, I have sought to illuminate the complex physiological and behavioural responses to lithium administration in two rodent species, guinea pigs and rats. My exploration was motivated by the seminal observations of John Cade, who first discerned lithium's therapeutic potential in 1949, when he noted lithium-induced lethargy in guinea pigs. My investigations have led to the discovery of potential central pathways mediating lithium's effects, offering a fresh perspective on the drug's mechanism of action and therapeutic benefits.

The journey began with replicating Cade's observations in guinea pigs. I discovered that lithium elicits a dose-dependent increase in lethargic behaviour, which I termed 'cooling posture', along with a dose-dependent decrease in body temperature, and a reduction in locomotor activity. The cooling posture in guinea pigs, indicative of nausea, was linked temporally and functionally to the hypothermic response.

I proposed that these responses, observed at therapeutically relevant doses, were not indicative of lithium toxicity but were part of a coordinated thermoregulatory response. These physiological and behavioural changes were dose-dependent, quick to initiate, and reversible, suggesting a coordinated physiological response rather than nonspecific toxic effects. My findings challenge the conventional view that these effects are indicative of lithium toxicity and instead propose that they are integral to lithium's therapeutic action.

In the third chapter, I expanded my investigation to rats. I observed that lithium dose-dependently reduces body temperature, BAT thermogenesis, and locomotor activity. Moreover, lithium was found to inhibit cutaneous vasoconstriction, reinforcing my hypothesis of a thermoregulatory response coordinated by the brain, consistent across species. Notably, lithium also dose-dependently inhibits BAT thermogenesis, emotional hyperthermia, and hyperlocomotion elicited by acute psychological stress. My discovery that lithium inhibits the stress response represents a significant advancement in our understanding of its therapeutic action. This is particularly relevant as mood disorders, which lithium is commonly used to treat, are frequently precipitated by psychological stress. This suggests that lithium may directly counteract the physiological and behavioural mechanisms triggered by psychological stress, thereby potentially alleviating the symptoms of mood disorders. Furthermore, my findings indicate the potential use of lithium in reversing life-threatening hyperthermia elicited by drugs such as MDMA.

In the fourth chapter, I delved into the underlying physiological mechanisms of lithium's effects. I discovered that lithium dose-dependently inhibits the increases in BAT sympathetic nerve activity, BAT thermogenesis, expired $CO_2$, heart rate, and mean arterial pressure that are typically triggered by cold exposure. This finding indicates a reduction in sympathetic outflow from the brain, complementing my earlier observation that lithium inhibits the stress response. Importantly, lithium's effects are not mediated by the vagus nerve. Instead, they require the area postrema, a region of the brain outside the blood-brain barrier that monitors the bloodstream and carries information to other parts of the brain. This dependency provides compelling evidence that lithium's action begins in the periphery and that the physiological responses to lithium are coordinated by the brain.

Moreover, my findings suggest a potential mechanism of lithium-induced weight gain, a common side effect of lithium therapy, through its inhibition of BAT thermogenesis and energy metabolism. I propose that targeting BAT thermogenesis may be a promising approach to mitigate this adverse effect of lithium treatment.

I propose two central pathways through which lithium might exert its effects: one involving the sequence of regions AP/NTS, LPB, POA, DMH, and rMR, and another involving the sequence of regions AP/NTS, iRt/PCRt, and rMR. These pathways, centred around the area postrema and the hypothalamomedullary network, provide a comprehensive framework for understanding the physiological and behavioural effects of lithium. Furthermore, they implicate specific brain regions that are critically involved in psychological stress and stress-related psychiatric conditions, which lithium is used to treat.

This thesis illuminates the intricate physiological and behavioural responses elicited by lithium, offering fresh perspectives on its therapeutic mechanisms and potential clinical applications. While my findings provide a solid foundation, further research is essential to refine our understanding of the proposed pathways and translate these insights into clinical practice. Future investigations should also consider lithium's potential in treating conditions such as pathological hyperthermia induced by certain stimulants, as well as strategies to mitigate lithium-associated weight gain. I anticipate that my work will catalyse further exploration into the multifaceted effects of lithium, ultimately contributing to the development of more refined and targeted therapeutic strategies for bipolar disorder and other mood disorders.

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