



OPEN Lithium salts alter the size and morphology of the Madagascar hissing cockroach's neuroendocrine system

Rafał W. Wojciak^{1✉}, Paweł Marciniak² & Malgorzata Slocinska²

Lithium salts have been used in psychiatry for decades as normothymics. The full mechanism of action of these drugs is not yet known. The limited work on lithium suggests that it may affect brain structure and size in humans and animals. This study aimed to determine the effect of lithium citrate and carbonate on the neuroendocrine system of Madagascar cockroaches. The animals were fed 10 weeks with the control and test diets (enriched with lithium citrate and carbonate at 0.1 and 0.01%, respectively). After anaesthesia, the insects were decapitated, and the brain and retrocerebral complex were removed. The study showed significant changes in the morphology of the examined elements of the neuroendocrine system of insects consuming lithium in the diet, mainly consisting of the enlargement of the examined structures, with increased doses of lithium and the preparation administered in the form of citrate having a greater effect. The morphotic changes in the endocrine system of the Madagascar cockroach exposed to lithium, as shown in this study, may suggest that a similar phenomenon occurs in humans and animals exposed to lithium from the environment, food, or drugs. Further research on this topic may shed more light on the phenomenon.

Keywords Madagascar hissing cockroach, Lithium citrate, Lithium carbonate, Neuroendocrine system, *Corpora cardiaca*, *Corpora Allata*

Lithium, especially in the form of carbonate, is a very effective drug used in psychiatry. It has normothymic effects, i.e., it can normalize the emotional states and behaviors of patients suffering from schizophrenia, bipolar disorder, or depression^{1,2}. Lithium salts offer the advantage of oral administration due to their higher water solubility, which facilitates efficient dissolution and absorption in the human intestine³. The mechanism of action of lithium in this regard is not fully known. Presumably, this element acts as an inhibitor of enzymes that are key to the functioning of neurotransmitter systems inside neurons⁴. For example, lithium is a strong inhibitor of glycogen synthase kinase 3 beta (GSK3B), which is an enzyme that plays a key role in diseases of the central nervous system and influences the density and function of synapses in people suffering from affective disorders^{5,6}. Glycogen synthase kinase 3-beta regulates gene transcription and affects apoptosis, cell structure, stress resistance, synaptic plasticity, and, ultimately, biological rhythms. The inhibitory effect of lithium on GSK-3B is probably important for the mechanism of therapeutic action of lithium in affective and neurodegenerative diseases because this enzyme also plays a role in the metabolism of the amyloid precursor protein and is also important in the phosphorylation of tau protein. On the other hand, Rybakowski⁷ suggested that the effect of lithium on GSK-3B may cause side effects, including disruption of the function of the thyroid gland and kidneys. Lithium also affects the metabolism of magnesium, and according to Birch⁸ its urinary excretion significantly increases in people treated with this element. Lithium also inhibits inositol monophosphatase-1, which causes the depletion of inositol stores and, according to one hypothesis, may be a therapeutic factor in affective disorders⁹. Moreover, the inhibition of protein C kinase and adenylyl cyclase, which converts adenosine triphosphate into cyclic adenosine monophosphate, also plays a role in the mechanism of action of lithium. As described by Rybakowski⁷ an important element of this system is the CREB protein activated by lithium (cellular transcription factor, protein binding the C-AMP response element, i.e., protein binding to CRE), which is a specific transcription factor and a regulator of the expression of many genes.

¹Department of Clinical Psychology, Faculty of Medicine, Poznan University of Medical Sciences, 70 Bukowska Str, Poznan PL-60-812, Poland. ²Department of Animal Physiology and Developmental Biology, Institute of Experimental Biology, Faculty of Biology, Adam Mickiewicz University in Poznan, 6 Uniwersytetu Poznańskiego Str, Poznan PL-61-614, Poland. ✉email: rafwoj@ump.edu.pl

Lithium probably also stimulates brain-derived neurotrophic factor (BDNF), which is necessary for the proper functioning and survival of neurons. Brain trophic factors modulate the activity of many neurotransmitters, including glutamate, gamma-aminobutyric acid, dopamine, and serotonin; hence, abnormalities in the neurotrophin system are important in the pathogenesis and treatment of affective diseases. Experimental studies using experimental animals have shown that lithium increases the expression of the BDNF gene in the brain, and one of the mechanisms responsible for this phenomenon is the activation of the CREB protein¹⁰. Clinical studies have shown that the concentration of BDNF in serum is reduced during manic and depressive episodes and increases after the use of lithium¹¹.

One might assume that the described effects of lithium would be reflected in the structure of the brain. There are few data on this subject. Observations of people treated with lithium, as well as studies on rodents, have shown that lithium increases the weight of the total brain and gray matter, the thickness of the cerebral cortex, and the intensification of neurotransmitter activity^{12–14}. It is assumed that the enlargement of brain structures may be related to the intensification of brain plasticization processes under the influence of lithium compounds¹⁵. There are no clear works on this subject.

Therefore, the aim of this study was to determine the effect of lithium compounds (citrate and carbonate) on the morphology of the neuroendocrine system of the Madagascar hissing cockroach, *Gromphadorhina coquereliana*. The size and dimensions of the brain and retrocerebral complex were assessed.

Results

Figures 1, 2 and 3 show the influence of the tested experimental factors on selected morphological elements of the neuroendocrine system of cockroaches. Tables 1, 2, 3, 4, 5 and 6 present the variable statistical analysis of the obtained results.

The average size of the insect brain divided by hemisphere and the ratio of the length to the width of both hemispheres of the cockroach brain are shown in Fig. 1. The ratio of length to width for all studied groups,

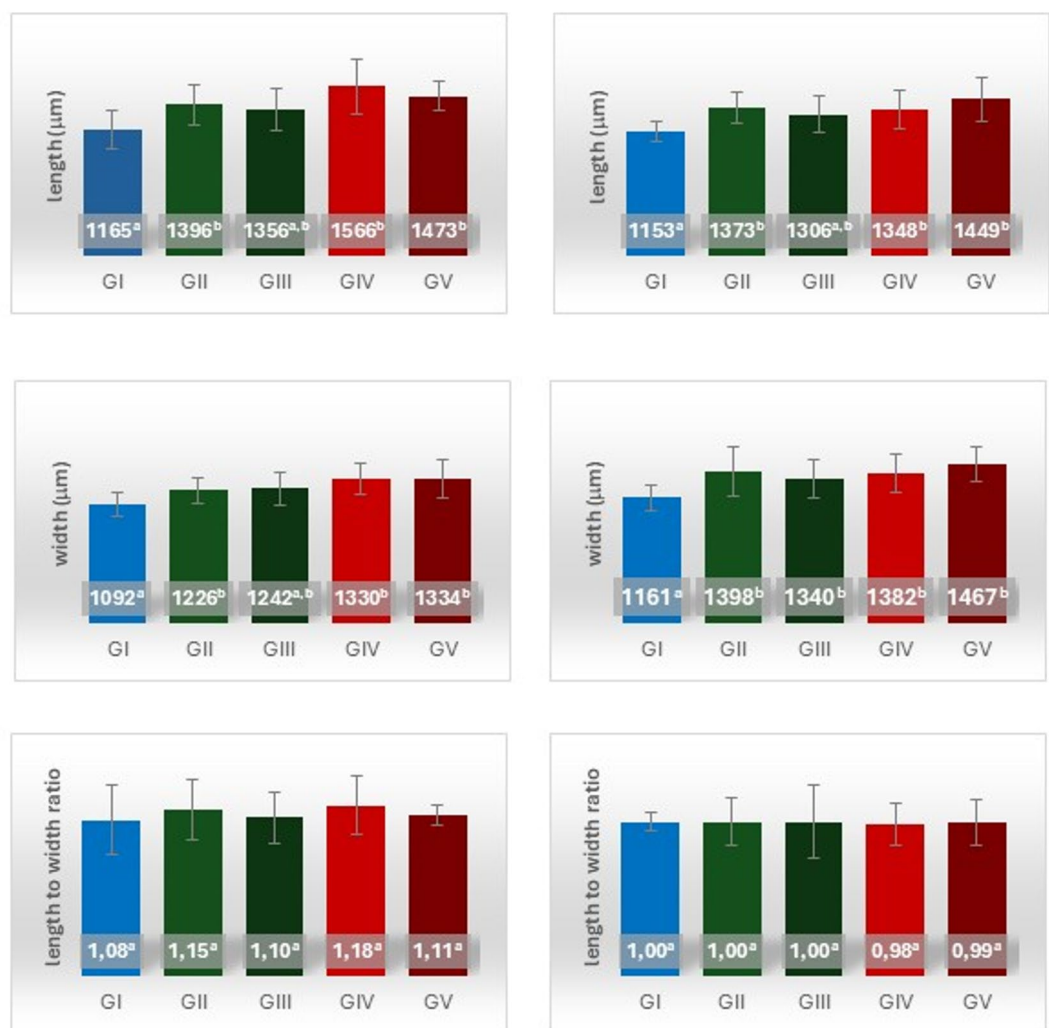


Fig. 1. Average dimensions of the cerebral hemispheres in individual groups of studied insects (R-right, L-left; a,b – statistically significant differences at $p < 0.05$)

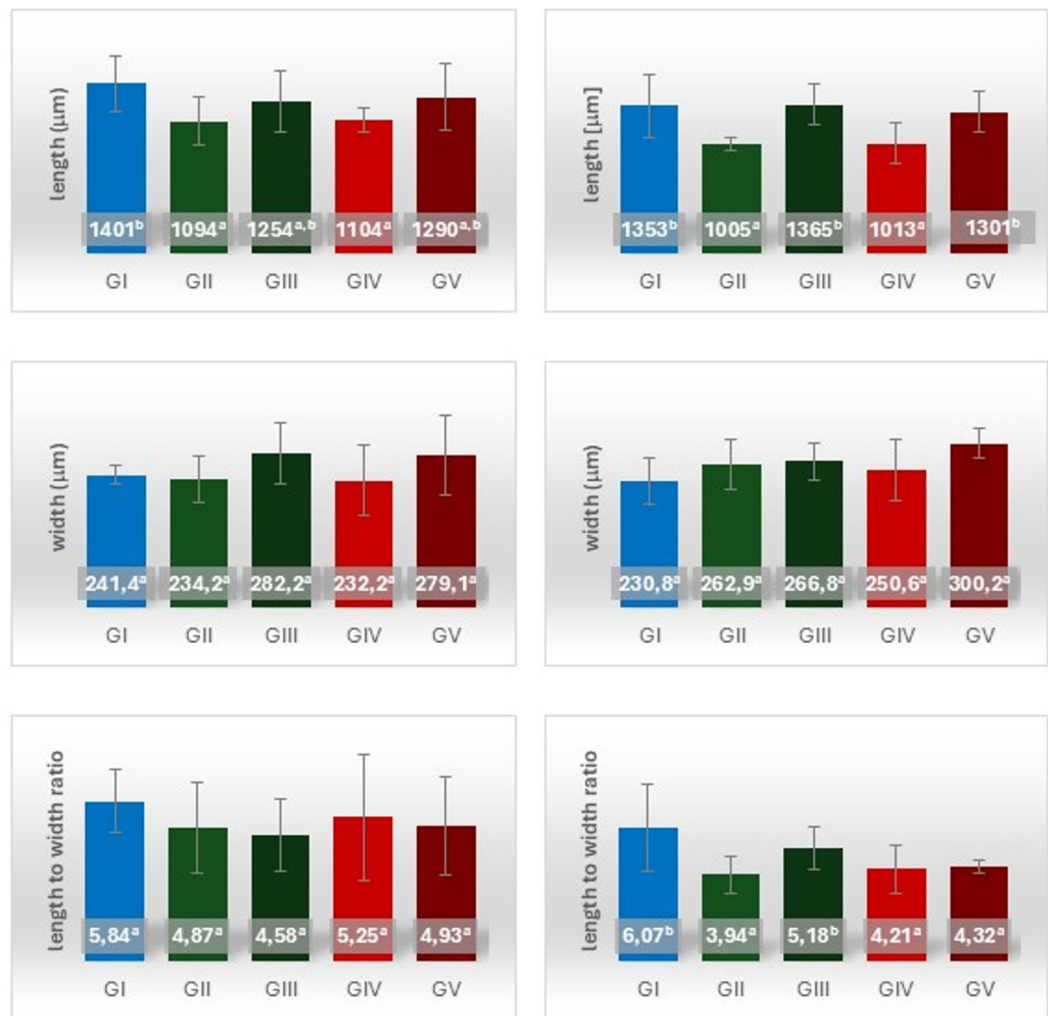


Fig. 2. Average dimensions of Corpora Allata in individual groups of studied insects (R-right, L-left; a,b – statistically significant differences at $p < 0.05$)

measured for both hemispheres, was close to 1.0, which means that the brain hemispheres were similar to the spherical figures and did not significantly differ among the groups. In this case, neither the type of lithium salt nor its content in the diet nor the combined effect of these treatments on this parameter was demonstrated (Tables 1 and 2). However, a significant impact of the tested lithium salts on the sizes of the insects' cerebral hemispheres was observed. The average length and width of the right hemisphere were significantly smaller in the control group ($1165 \pm 111 \mu\text{m}$ and $1092 \pm 78 \mu\text{m}$, respectively) than in the study groups (approx. 1350 ± 121 – $1550 \pm 102 \mu\text{m}$ and 1220 ± 104 – $1330 \pm 128 \mu\text{m}$, respectively). The size of the right hemisphere did not differ significantly between the exposed and control groups. Similar observations have also been made concerning the left hemisphere of the insect brain. A statistically significant effect of the type and content of lithium in the diet on the length and width of both cerebral hemispheres was demonstrated (Tables 1 and 2). Although the average sizes of these structures were significantly larger in the groups treated with lithium than in the control group, there was no combined effect of the studied lithium salts and their doses. However, the type of lithium salt had an influence on the length of the right and left hemispheres ($p < 0.01$ and $p < 0.05$, respectively) as well as on their width ($p < 0.05$ and $p < 0.05$, respectively). A similar effect on the length and width of both hemispheres was observed as a result of exposure to lithium (all $p < 0.05$).

Figure 2; Tables 3 and 4 present the average results and their statistical analysis in relation to measurements of the length and width of *Corpora Cardiac* (CC) in the studied insects. A statistically significant effect was observed on the shortening of the average length of the right and left CC in the groups taking higher concentrations of lithium, regardless of its type (GII and GIV), from approximately $1400 \pm 121 \mu\text{m}$ in the GI tract to approximately $1000 \pm 93 \mu\text{m}$ in the remaining groups. No significant effect on the width of this structure was observed.

Although the average length-to-width ratio of the right CC did not differ significantly and ranged from 4.6 to 5.8, statistically significant differences were observed in the left. The average length-to-width ratio of the left CC was the highest in the control group and the group treated with lower concentrations of lithium carbonate (approx. 5.5) and differed significantly from that in the other groups (approx. 4.0).

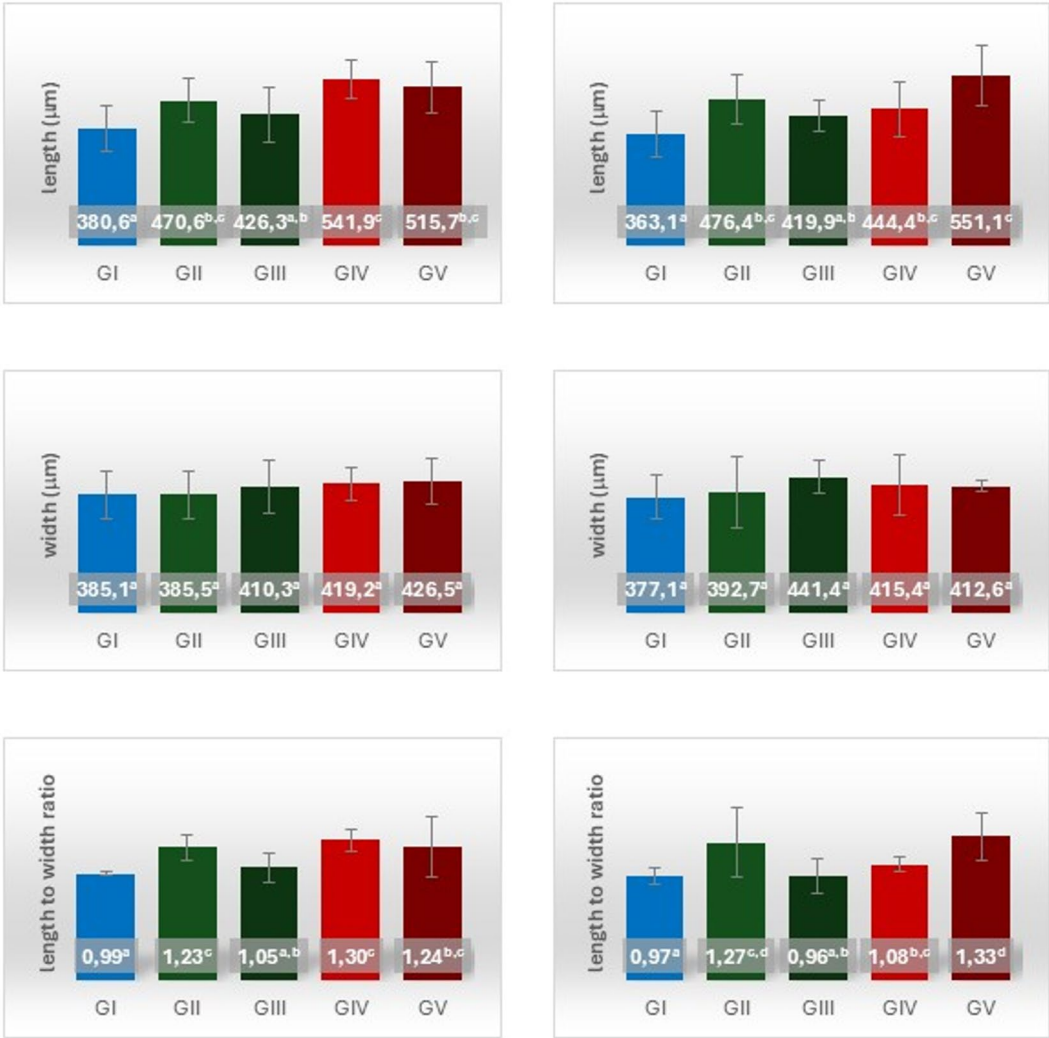


Fig. 3. Average dimensions of Corpora Cardiaci in individual groups of studied insects (R-right, L-left; a,b – statistically significant differences at $p < 0.05$)

	GI control	II-III carbonate	IV-V citrate	II i GIV Li 0,1	III i GV Li 0,01	II-V lithium addition
length (μm)	1165.3 ± 181.3 (897.3-1369.9)	1375.8* ± 179.3 (1090.8-1637.1)	1520.0** ± 197.8 (1254.8-1862.8)	1481.2* ± 227.7 (1234.3-1862.8)	1414.5* ± 168.9 (1090.9-1607.2)	1447.9** ± 198.1 (1090.9-1862.8)
variance analysis		p < 0.01		p < 0.05		NS
width (μm)	1091.9 ± 109.2 (906.4-1195.0)	1234.2* ± 130.4 (1103.7-1521.1)	1332.2** ± 154.5 (1104.9-1500.3)	1278.4* ± 136.8 (1103.7-1500.3)	1288.1* ± 165.7 (1104.9-1521.1)	1283.2** ± 148.0 (1103.7-1521.1)
variance analysis		p < 0.05		p < 0.05		NS
length-to-width ratio	1.08 ± 0.24 (0.79-1.42)	1.13 ± 0.19 (0.93-1.40)	1.15 ± 0.15 (0.99-1.51)	1.17 ± 0.20 (0.93-1.51)	1.11 ± 0.13 (0.93-1.38)	1.14 ± 0.17 (0.93-1.51)
variance analysis		NS		NS		NS

Table 1. Statistical analysis of the influence of main effects - the type and concentration of lithium in food on the dimensions of the right hemisphere of the insect brain. (*, **, ***) – statistically significant differences between means in relation to GI at $p < (0.05, 0.01, 0.001)$ respectively. NS – no significance.

	GI control	GII-III carbonate	GIV-V citrate	GII i GIV Li 0,1	GIII i GV Li 0,01	GII-V lithium addition
length (μm)	1153.4 ± 96.0 (1023.3–1278.4)	1339.2* ± 151.5 (1150.4–1554.7)	1398.6** ± 190.0 (1171.7–1695.0)	1360.2* ± 153.6 (1152.7–1634.6)	1377.5** ± 193.1 (1150.4–1695.0)	1368.9** ± 170.0 (1150.4–1695.0)
variance analysis		$p < 0.05$		$p < 0.05$		NS
width (μm)	1161.0 ± 118.6 (975.8–1257.7)	1369.1* ± 192.4 (1128.3–1747.2)	1424.7** ± 166.1 (1175.3–1666.9)	1390.2* ± 191.3 (1128.3–1747.2)	1403.5* ± 172.1 (1157.0–1660.2)	1396.9** ± 177.2 (1128.3–1747.2)
variance analysis		$p < 0.05$		$p < 0.05$		NS
length-to-width ratio	1.00 ± 0.06 (0.91–1.05)	1.00 ± 0.19 (0.75–1.34)	0.99 ± 0.13 (0.80–1.19)	0.99 ± 0.14 (0.83–1.25)	0.99 ± 0.18 (0.75–1.34)	0.99 ± 0.16 (0.75–1.34)
variance analysis		NS		NS		NS

Table 2. Statistical analysis of the influence of main effects - the type and concentration of lithium in food on the dimensions of the left hemisphere of the insect brain. (*, **, ***) – statistically significant differences between means in relation to GI at $p < (0.05, 0.01, 0.001)$ respectively. NS – no significance.

	GI control	GII-III carbonate	GIV-V citrate	GII i GIV Li 0,1	GIII i GV Li 0,01	GII-V lithium addition
length (μm)	1401.0 ± 222.7 (1137.8–1649.9)	1174.2* ± 228.0 (846.8–1575.6)	1198.8 ± 215.1 (945.0–1561.5)	1099.2** ± 143.2 (846.8–1355.2)	1271.8 ± 248.0 (916.1–1575.6)	1185.5* ± 216.0 (846.8–1575.6)
variance analysis		NI		$p < 0.05$		NI
width (μm)	241.4 ± 16.8 (230.2–271.1)	258.2 ± 53.2 (178.4–357.4)	255.6 ± 69.4 (126.8–389.0)	233.2 ± 52.0 (126.8–298.9)	280.6 ± 60.8 (202.1–389.0)	256.9 ± 60.2 (126.8–389.0)
variance analysis		NI		NI		NI
length-to-width ratio	5.84 ± 1.14 (4.82–7.17)	4.73 ± 1.41 (3.25–7.60)	5.09 ± 0.1.94 (2.84–9.14)	5.06 ± 1.88 (3.40–9.14)	4.76 ± 1.50 (2.84–7.33)	4.91 ± 1.66 (2.84–9.14)
variance analysis		NI		NI		NI

Table 3. Statistical analysis of the influence of main effects - type and concentration of lithium in the food on the dimensions of the right corpora cardiaca of insects. (*, **, ***) – statistically significant differences between means in relation to GI at $p < (0.05, 0.01, 0.001)$ respectively. NS – no significance.

This finding was confirmed by factor analysis, which revealed the influence of the type ($p < 0.05$) and lithium content in the diet ($p < 0.05$) on the length-to-width ratio of the left, but not the right, CC. The combined effect of these exposures was not demonstrated. However, the lithium content in the diet had a statistically significant effect on the length of the right ($p < 0.05$) and left *Corpora Cardiaca* ($p < 0.01$) bones.

Although the observed effect of lithium salts on CC mostly concerned the shortening of the length of this structure, in the case of *Corpora Allata* (CA), this effect was different (Fig. 3). The use of lithium in the diet of experimental insects resulted in a statistically significant increase in the average length of this structure in both the right and left CA, from $381 \pm 72 \mu\text{m}$ and $363 \pm 68 \mu\text{m}$ in GI, respectively, to approximately 450 ± 42 – $550 \pm 58 \mu\text{m}$ in the remaining groups, except for the group that received lithium carbonate at a lower concentration (approx. $420 \pm 38 \mu\text{m}$), in which the average length of the tested structure did not differ significantly compared to the averages of the control group and other groups. No statistically significant differences were observed in the average width of CA (approximately $400 \mu\text{m}$ on average) between the GI tract and the other groups or within the study groups.

However, the differences in the average length influenced the occurrence of significant changes in the length-to-width ratio, which is the smallest parameter, close to 1.0, regardless of the location of the structure, and was observed to be significantly lower in the control group than in the groups receiving a high content of lithium in the form of carbonate and citrate regardless of the concentration (approx. 1.2). No such effect was observed in the group with lower dietary lithium carbonate content. The observed differences in means were reflected in the analysis of the main and total effects of the examined factors on the structure of CA (Tables 5 and 6). The addition of lithium, regardless of the type and content of this element in the diet, increased the average length of

	GI control	GII-III carbonate	GIV-V citrate	GII i GIV Li 0,1	GIII i GV Li 0,01	GII-V lithium addition
length (μm)	1352.8 ± 295.5 (1129.8–1870.7)	1185.2 ± 231.5 (945.1–1549.0)	1156.8 ± 232.4 (760.4–1553.4)	1009.2** ± 130.2 (760.4–1216.8)	1332.8 ± 181.5 (1055.17–1553.4)	1171.0 ± 226.2 (760.4–1553.4)
variance analysis		NI		$p < 0.01$		NI
width (μm)	230.8 ± 41.0 (195.0–297.8)	264.9 ± 38.6 (212.1–326.3)	275.4 ± 49.3 (160.4–330.4)	256.7 ± 49.0 (160.4–326.3)	283.5** ± 34.4 (229.1–330.4)	270.1* ± 43.4 (160.4–330.4)
variance analysis		NI		NI		NI
length-to-width ratio	6.07 ± 1.99 (4.24–9.26)	4.56* ± 1.09 (2.90–6.70)	4.27* ± 0.0.77 (2.64–5.57)	4.08** ± 0.95 (2.64–5.57)	4.75* ± 0.82 (3.98–6.70)	4.41** ± 0.93 (2.64–6.70)
variance analysis		$p < 0.05$		$p < 0.05$		NI

Table 4. Statistical analysis of the influence of main effects - type and concentration of lithium in the food on the dimensions of the left corpora cardiaca of insects. (*, **, ***) – statistically significant differences between means in relation to GI at $p < (0.05, 0.01, 0.001)$ respectively. NS – no significance.

	GI control	GII-III carbonate	GIV-V citrate	GII i GIV Li 0,1	GIII i GV Li 0,01	GII-V lithium addition
length (μm)	380.6 ± 74.4 (259.0–461.1)	448.5 ± 79.1 (352.8–568.6)	528.8** ± 69.9 (415.8–634.8)	506.29** ± 73.6 (395.6–634.8)	471.00* ± 92.8 (352.8–611.1)	488.6** ± 83.5 (352.8–634.8)
variance analysis		$p < 0.01$		$p < 0.05$		NI
width (μm)	385.1 ± 76.3 (261.2–471.1)	397.9 ± 78.9 (308.4–565.2)	422.8 ± 62.2 (327.5–508.2)	402.3 ± 65.6 (308.4–508.2)	418.4 ± 77.4 (327.5–565.2)	410.4 ± 70.3 (308.4–565.2)
variance analysis		NI		NI		NI
length-to-width ratio	0.99 ± 0.02 (0.96–1.02)	1.14* ± 0.15 (0.90–1.34)	1.27** ± 0.20 (0.92–1.67)	1.27*** ± 0.11 (1.06–1.38)	1.14 ± 0.23 (0.90–1.67)	1.20** ± 0.18 (0.90–1.67)
variance analysis		$p < 0.05$		$p < 0.05$		NI

Table 5. Statistical analysis of the influence of main effects - type and concentration of lithium in the food on the dimensions of the right corpora Allata of insects. (*, **, ***) – statistically significant differences between means in relation to GI at $p < (0.05, 0.01, 0.001)$ respectively. NS – no significance.

the right CA, but there was no combined effect of the type and content of lithium on this parameter (Table 4). Considering the examined factors separately, a statistically significant effect of the type of lithium salt given to the animals ($p < 0.01$) and the lithium content in the diet ($p < 0.05$) was shown on the length of the right CA and the ratio of the length to the width of this structure ($p < 0.05$). There was no significant effect of the tested factors on the width of CA. In the case of the left CA (Table 6), there was a significant effect of both the type of lithium salt given ($p < 0.05$) and the lithium content in the diet ($p < 0.05$), as well as the combined effect of both: lithium salts and their doses ($p < 0.05$), on the length of this structure. There was no influence of the examined factors on the width of the left CA. In the case of the average length-to-width ratio of the left CA, although a significant effect of lithium salts on increasing this parameter was observed, there was no effect of either the type or content of lithium. However, a statistically significant ($p < 0.01$) effect of the combined effect of both factors on the ratio of length to width of the left CA was demonstrated.

Discussion

It could be assumed that the influence of lithium compounds on changes in the behavior of experimental animals, as observed in many studies, would be reflected in the differences observed at the neuroendocrine and morphological levels. The present work did not assess physiological mechanisms at the neuronal level but only differences in biometric parameters of the brain and retrocerebral complex of the studied insects. Regardless of the form, lithium increased both the length and width of both brain hemispheres by approximately 20–30%. However, this increase, in the case of the lower dose of lithium carbonate, was by far the smallest compared to the use of the higher dose, and in the case of citrate. Interestingly, the changes in brain size in the tested cockroaches

	GI control	GII-III carbonate	GIV-V citrate	GII i GIV Li 0,1	GIII i GV Li 0,01	GII-V lithium addition
length (µm)	363.1 ± 72.7 (247.0-430.6)	448.1* ± 68.9 (365.2-611.6)	497.7* ± 103.7 (305.6-670.1)	460.4* ± 80.4 (305.6-611.6)	485.5* ± 100.3 (365.2-670.1)	472.9** ± 89.4 (305.6-670.1)
variance analysis		p < 0.05		p < 0.05		p < 0.05
width (µm)	377.1 ± 72.8 (247.1-417.5)	417.1 ± 87.0 (240.2-542.5)	414.0 ± 64.8 (291.4-550.6)	404.1 ± 99.6 (240.2-550.6)	427.0 ± 39.6 (362.8-494.4)	415.5 ± 74.7 (240.2-550.6)
variance analysis		NI		NI		NI
length-to-width ratio	0.97 ± 0.07 (0.85-1.03)	1.12 ± 0.29 (0.81-1.81)	1.21* ± 0.20 (0.99-1.58)	1.17* ± 0.24 (0.99-1.81)	1.15 ± 0.27 (0.81-1.58)	1.16* ± 0.25 (0.81-1.81)
variance analysis		NI		NI		p < 0.01

Table 6. Statistical analysis of the influence of main effects - type and concentration of lithium in the food on the dimensions of the left *Corpora Allata* of insects (*, **, ***) – statistically significant differences between means in relation to GI at p < (0.05, 0.01, 0.001) respectively. NS – no significance.

were proportional, so the ratio of length to width in both hemispheres did not change. Due to improvements in imaging techniques in recent years, it has become possible to observe brain structures in real life, both in humans and animals, which has led to many interesting observations. Using magnetic resonance imaging, Vernon et al.¹⁴ compared the effects of two drugs, haloperidol (an antipsychotic drug) and lithium, on the brain structures of rats. Chronic haloperidol treatment induced a reduction in the volume of the whole brain (4%) and cerebral cortical gray matter (6%), accompanied by an increase in the corpus striatum (14%). In contrast, chronic Li treatment induced increases in whole brain (5%) and cortical gray matter (3%) volumes without significant effects on striatal volume. By 8 weeks after drug discontinuation, haloperidol-induced changes in brain volume had normalized, whereas Li-treated animals retained significantly greater total brain volume, as confirmed postmortem. Similar observations are made in people treated with lithium. Anand et al.¹³ showed that in patients who were introduced to lithium monotherapy, after 2 weeks, an increase in the gray matter of the brain of 0.2% (statistically nonsignificant increase) was detected, and after 8 weeks, an increase of 0.4% (statistically significant value) was detected. At the same time, a significant decrease in the ventricular volume of the brain was observed (−2.4 and −3.6%), while an increase in structures such as total thickness of the cerebral cortex (0.8 and 1.1%, respectively), thickness of the frontal cortex (1.0 and 1.5%, respectively) and parietal cortex (0.9 and 1.3%, respectively) were observed. Similar results on the effect of lithium on the human brain were also obtained by Abramovic et al.¹² who compared 266 patients with 171 people in the control group. Moreover, in patients, these authors examined the relationship between the use of lithium and other antipsychotic drugs and measures of the total brain and various brain structures. Patients without lithium had significantly smaller total brain, thalamus, putamen, corpus striatum, hippocampus, and nucleus accumbens volumes than patients treated with lithium. According to Gray & McEwen¹⁵ the increase in brain volume observed in animal and human studies is related to the initiation and maintenance of enhanced neuroplasticity processes in the brain by lithium. Therefore, lithium pharmacotherapy is often accompanied by an increase in the concentration of trophic factors or stabilization of the levels of neuropeptides, neurohormones, and neurotransmitters. Another element of this effect is the protective effect of lithium on neurons under stress. Evidence for this effect of lithium salts is provided not only by changes observed in the structure of cortical structures in the brain but also by an increase in the volume of subcortical structures, primarily those responsible for generating emotions (hippocampus, nucleus accumbens, part of the lenticular nucleus, thalamus, hypothalamus, and pituitary). Observations of an increase in the volume of these structures in patients during a short period of lithium treatment were described by the previously cited Anand et al.¹³ and other authors^{15–18}.

Although the brain, which is responsible for synchronizing and coordinating endocrine processes, plays a key role in the neuroendocrine system of the Madagascar cockroach, as well as in other insects, the retrocerebral complex, composed of the *Corpora Cardiaca* (Latin: *Corpora Cardiaca*, CC) and the adjacent body (Latin: *Corpora Allata*, CA), is also an important element of the functioning of the system, constituting a neurohemal organ^{19–21}. Although neurohormones secreted in the brain are transported and stored in the retrocerebral complex, in addition to their storage function, the CC is also capable of producing and secreting hormones^{19,20}. The CC neurosecretory lobe produces neuropeptides that exhibit adipokinetic/hypertrehalosemic (regulating the metabolism of sugars, including trehalose) or cardiotropic effects. In contrast, CA produces juvenile hormones (responsible for the control of larval development and reproduction)^{19–21}. The present work showed that lithium at high doses caused a significant shortening of the CC length without significantly affecting the width of this structure. The release of hormones into the CC from intercerebral cells occurs via two routes: axons concentrated in NCCI (Latin: *nervi corporis cardiaci* I) and axons of lateral cells concentrated in NCCII (Latin: *nervi corporis cardiaci* II)^{20,21}. Assuming that this flow is not disturbed, even assuming any increased secretion of hormones in the enlarged brain, keeping them in the CC store is probably shortened, and therefore, they reach the body faster, affecting metabolism and heart function. This may increase the metabolic rate and activity of the

cardiovascular system (faster-targeted delivery of oxygen and nutrients to tissues) in animals taking lithium and thus improve food utilization. This could explain why the animals that consumed lithium consumed less food but gained a similar body weight to the insects in the control group (data not included to the presented work).

A similar effect of lithium was observed when analysing the size of CA in the tested insects. Both lithium carbonate and citrate at high doses significantly influenced the length of this structure, and in the case of citrate, such an effect was also observed when a lower dose was used. There was no effect of either compound on CA width. Due to their partially analogous action (the role of CA in oogenesis and vitellogenesis, CC in metabolism and heart function), the entire retrocerebral complex can be compared with the mammalian pituitary gland, whose endocrine action, although much broader, also involves the processes of reproduction and metabolism. The insect model, however, has its limitations; despite existing structural and functional analogies²² the complexity of the human nervous and neuroendocrine system is much greater than in invertebrates, but for preliminary biomedical research, as a model organism, it allows for understanding the mechanisms of action of various pharmacological compounds *in vivo*²³. In humans, the action of pituitary hormones results in emotional states, triggering the need for closeness and attachment, resulting from, among other factors, the action of oxytocin through euphoric states resulting from the production of endorphins to depressive states related to deficiencies of thyroid-stimulating hormones^{24,25}. The pituitary gland is strongly connected with the hypothalamus and cerebellum^{24,25}. The latter structure, although traditionally considered in the field of motor functions, has recently been described in the context of strong connections with emotional memory²⁶. All these elements may be responsible for the tendencies observed in insects to group together or become less nervous as a result of exposure to stress, as well as the lack of caution in exploring the environment. These observations were also made in the insects studied (unpublished results).

Summary

The morphotic changes in the endocrine system of the Madagascar cockroach, shown in the study, resulting from the intake of lithium in the diet, are consistent with the changes in the size of some brain structures of people and animals administered lithium preparations, as indicated in the publications. At the same time, many studies indicate that patients who use lithium exhibit metabolic changes resulting from changes in the activity of many enzymes described above. This is particularly related to the changes observed in the structures of the retrocerebral complex of insects, which is an analogue of the human pituitary gland. In summary, it seems that changes in the structures of the brain and retrocerebral complex result from the modification of enzymatic activity by lithium. The mechanism of this process is unknown and requires further, more detailed work in various models, including the use of molecular biology techniques. Additionally, whether the effect of structural changes is a temporary or permanent effect of lithium on the structure of the brain and nervous system is unclear. These considerations are becoming increasingly important, especially because of, on the one hand, the increase in the incidence of mental illnesses in industrial societies that require the use of lithium preparations. On the other hand, the increasing level of lithium in the environment results from the increased use of this metal in the electrical industry.

Materials and research methods

Experimental animals and the course of research

The study was carried out using Madagascar cockroaches (*Gromphadorhina coquereliana*). Due to its large size (6–8 cm) and omnivorous nature, this insect is increasingly becoming a good model for nutritional and toxicological research, as well as in neurobiology, where its simpler nervous system than that of vertebrates is utilized^{23,27}. The use of insects also contributes to the principles of 3R in laboratory animal testing, which aims to reduce the suffering of animals used in research through replacement, reduction, and refinement (3R)²⁸. The study involved 75 males from a breeding facility run at the Department of Animal Physiology and Developmental Biology, Faculty of Biology, Adam Mickiewicz University in Poznań. At the beginning of the study, the animals were divided in terms of size and body weight into five test groups (15 animals each) so that the dimensions and body weight of the animals were similar on average in each group. The research was conducted under constant, monitored conditions. Insectaria (350 × 210 × 150 mm), made of PP, were placed in a room at a constant temperature of 27 °C, a relative humidity of 50–60%, and a light cycle of 6/18 hours. During the 10 weeks of the study, the animals' condition was checked once a week. Only three insects from different groups were lost during the study. Dead animals were not taken into account in the analysis of the results. Dead animals were removed. The diet was administered once a week, *ad libitum*, and 5 g of the diet was weighed. The animals had free access to distilled water in the PP containers, and the wall of the insectarium was sprinkled with distilled water. Each week, the insectaria were disinfected with an aqueous solution of 70% ethyl alcohol, during which time the animals were isolated in a weighing container. Each insect and any uneaten food were weighed weekly. Diet intake and body weight gain were determined based on these measurements. The results were used to assess the insects' condition throughout the experiment. Sterile cellulose filter paper was used as a substrate in the insectariums. The elements of the insectarium also included sterile cellulose egg packaging. Before use, the containers were disinfected with a 1% aqueous solution of Virkon S (Bayer) by spraying the surface and disinfected for 0.5 h using a UV-C lamp (Philips).

At the beginning of the study, after the adaptation period (7 days, basic diet), the animals were assigned to 5 test groups: GI-control (fed a test diet), GII – GIII: which were given a test diet enriched with lithium carbonate at a level of 0.1 and 0.01%, respectively, based on the elements in the diet, and GIV – GV, which were fed a test diet enriched with lithium citrate at the level of 0.1 and 0.01%, respectively, based on the element in the diet. The source of lithium was:

Salt	Concentration in solution (mM)
Sodium chloride (NaCl)	274.0
Potassium chloride (KCl)	19.0
Calcium chloride (CaCl ₂)	9.0

Table 7. Composition of physiological saline solution (pH = 7.0).

- lithium carbonate (Li₂CO₃, Warchem, p.a.) – molar mass 73.89; 19% Li in the molecule; 100 mg Li – 527.8 mg Li₂CO₃.
- lithium citrate tetrahydrate (Li₃C₆H₅O₇ × 4H₂O, Warchem, part d a.) – molar mass of 281.98; 7.5% Li in the molecule, 100 mg Li – 1342.8 mg Li₃C₆H₅O₇ × 4H₂O.

The basic research period lasted 10 weeks, after which the insects were subjected to low-temperature anaesthesia (30–60 min at 5 °C). After decapitation, microsurgery was performed on the neuroendocrine system (brain and retrocerebral complex) of 10 randomly selected insects from each group.

Test diets

To achieve the assumed objectives of the work, an experiment was designed in a two-factor system (2 × 2), in which the experimental factors were two lithium salts administered in the diet – inorganic (carbonate) and organic (citrate) – in two doses in the food (0.1 and 0.01%, respectively, calculated as the element). The basis of the experimental diet was commercial, complete food intended for feeding German Shepherd Puppy Dogs (Royal Canin).

Before starting the research, the food was ground using an electric burr grinder. The obtained loose food constituted 98% of the test diet. The remaining 2% was potato starch, which was also the carrier of the tested additives. In the prepared test diets, the dry matter content was measured using the weighing-drying method, and the contents of Ca, Mg, Zn, Cu, Fe, and Li were measured using flame atomic absorption spectrophotometry after prior wet mineralization of the sample. The diets averaged approximately 94% dry matter. When analysing the mineral components, the test diets met the assumptions of the study in terms of the planned Li content (0.1 and 0.01% of the element in the diet). The average content of other elements in the test diets was similar regardless of the diet and averaged approximately 5.8 mg/g diet weight for Ca, 0.7 for Mg, 0.2 for Zn, 0.02 for Cu, and 0.37 for Fe.

Methods

The study of the neuroendocrine system of the cockroach *Gromphadorhina coquereliana* was based on biometry of the brain and retrocerebral complex of insects, in which these structures were isolated by microsurgery²³. During the procedure, and later during microscopic measurements, the extracted and collected structures of the neuroendocrine system were placed in a physiological saline solution, which is typical for the species (Table 7), prepared in deionized water. After low-temperature anaesthesia, the cockroaches were decapitated, and the heads were subjected to microsurgery. The cuticles on the head are cut on both sides and from above with microscissors so that the cuts form a triangle shape connecting the mouth on both sides with the eye line. After removing the triangular cuticle, the preparation was placed under a stereoscopic microscope on a Petri dish covered with silicone elastomer. The preparation is stabilized by entomological pins, and immersion in a saline solution prevents the tissues from sticking together. Then, using microtools, the fat bodies are carefully removed, the nerves that run around the brain are severed, and the retrocerebral complex located above the esophagus, between the cerebral hemispheres, is carefully isolated.

The isolated structures were measured using a Discovery 40 stereomicroscope (Delta Optical) equipped with a DLT-Cam Basic 5Mp microscope camera (Delta Optical) with micro measurement software. Figure 4 shows the measurement planes of the brain hemispheres and the structures of the retrocerebral complex (both in the illustrative drawing and microscopic photos).

Statistical evaluation of results

The results obtained in the course of the work are presented in the form of tables and figures, in which descriptive statistics were used, taking into account the arithmetic mean, standard deviation, range (minimum-maximum), and percentage of detailed data measured for all elements in the group. After confirming the normality of individual distributions (Shapiro–Wilk test), the Student's t-test was used to assess differences between means independently. The analysis of the influence of experimental factors was carried out based on the analysis of variance in a one-way system for both factors separately – the type of lithium preparation used (carbonate and citrate) and the lithium content in the test diet (0.1 and 0.01% of the elements in the diet). To determine the combined effect of both experimental factors, a multivariate analysis of variance (in the 2 × 2 model) was used. All analyses were performed at the level of α = 0.05 (*p* < 0.05) using the Microsoft 365 Office Excel spreadsheet and Statistica ver. 13.3.

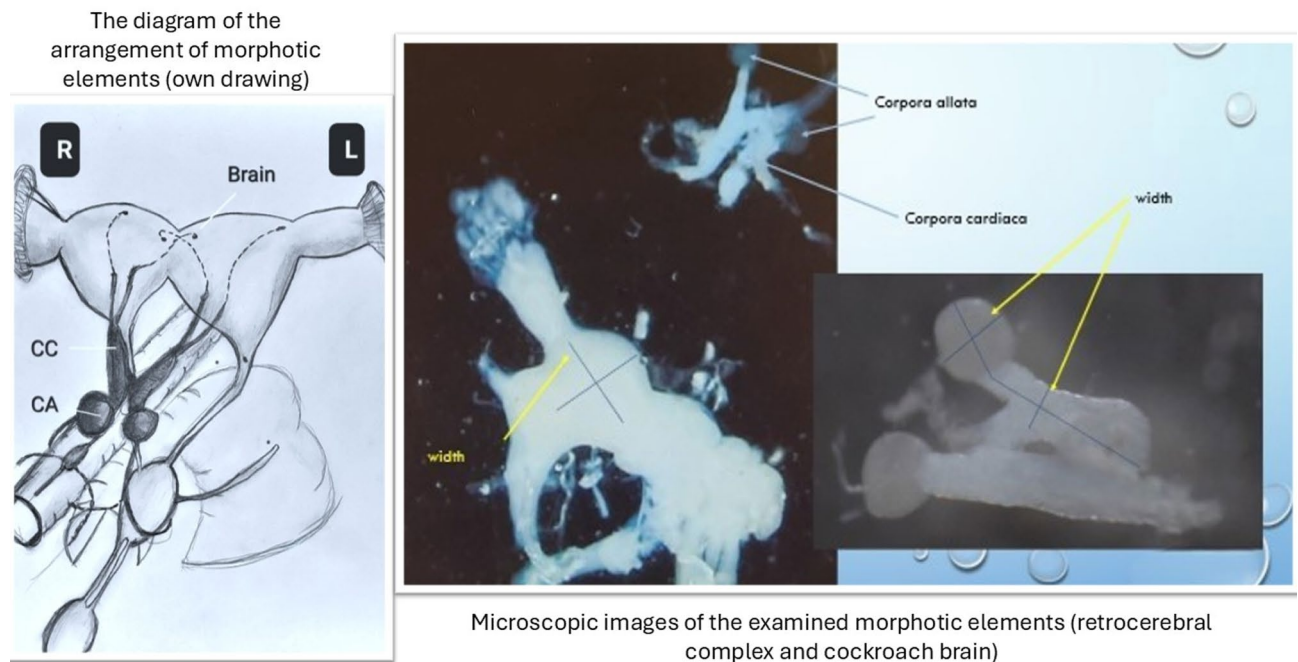


Fig. 4. The diagram of the arrangement of morphotic elements (own drawing) (the left side of the figure) and microscopic images of the examined morphotic elements (retrocerebral complex and cockroach brain) (right side the figure)

Data availability

“The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.”

Received: 6 May 2024; Accepted: 1 September 2025

Published online: 26 September 2025

References

- Kabzinska, K., Cisek-Wozniak, A., Czajeczny, D., Mruczyk, K. & Wojciak, R. W. The influence of Li⁺ ions on Pepsin and trypsin activity in vitro. *J. Trace Elem. Med. Biol.* **66**, 126763. <https://doi.org/10.1016/j.jtemb.2021.126763> (2021).
- Kabzinska, K., Czajeczny, D. & Wojciak, R. W. The controversy around a mood stabilizer – the history of lithium. *Acta Med. Pol.* **9**, 36–51. <https://doi.org/10.20883/amp.2018/18> (2018).
- Makara-Studzińska, M., Koślak, A., Moryłowska-Topolska, J. & Urbańska, A. Lithium therapy – the effectiveness of the medicine, side symptoms, complications and their influence on the quality of the life in affective diseases. *J. Elementol.* **15**, 2, 393–403. <https://doi.org/10.5601/jelem.2010.15.2.393-403> (2010).
- Nivoli, A., Murru, A. & Vieta, E. Lithium: still a cornerstone in the long-term treatment in bipolar disorder? *Neuropsychobiology* **62** (1), 27–35. <https://doi.org/10.1159/000314307> (2010).
- Nowak, J. K. The role of glycogen synthase kinase 3 β in diseases of the central nervous system. *Neuropsychiatry I Neuropsychologia*. **6** (1), 25–35 (2011). <https://www.termedia.pl/Streszczenie-pogladowy-Rola-kinazy-syntazy-glikogenu-3-w-chorobach-osrodkowo-ukladu-nerwowego> 46,17091,0,0.html.
- Forlenza, O. V., de Paula, V. J., Machado-Vieira, R., Diniz, B. S. & Gattaz, W. F. Does lithium prevent alzheimer's disease? *Drugs Aging*. **29** (5), 335–342. <https://doi.org/10.2165/11599180-000000000-00000> (2012).
- Rybakowski, J. K. Antiviral, immunomodulatory, and neuroprotective effect of lithium. *J. Integr. Neurosci.* **21**, 68. <https://doi.org/10.31083/j.jin2102068> (2022).
- Birch, N. J. Inorganic Pharmacology of lithium. *Chem. Rev.* **99**, 9, 2659–2682. <https://doi.org/10.1021/cr9804240> (1999).
- Berridge, M. J., Downes, C. P. & Hanley, M. R. Neural and developmental actions of lithium: a unifying hypothesis. *Cell* **59**, 411–419. [https://doi.org/10.1016/0092-8674\(89\)90026-3](https://doi.org/10.1016/0092-8674(89)90026-3) (1989).
- Hammonds, M. D., Shim, S. S., Feng, P. & Calabrese, J. R. Effects of subchronic lithium treatment on levels of BDNF, Bcl-2 and phospho-CREB in the rat hippocampus. *Basic. Clin. Pharmacol. Toxicol.* **100**, 5, 356–359. <https://doi.org/10.1111/j.1742-7843.2007.00058.x> (2007).
- Rybakowski, J. K. Response to lithium in bipolar disorder: clinical and genetic findings. *ACS Chem. Neurosci.* **5**, 413–421. <https://doi.org/10.1021/cn5000277> (2014).
- Abramovic, L. et al. The association of antipsychotic medication and lithium with brain measures in patients with bipolar disorder. *Eur. Neuropsychopharmacol.* **26**, 1741–1751. <https://doi.org/10.1016/j.euroneuro.2016.09.371> (2016).
- Anand, A. et al. Integrative analysis of lithium treatment associated effects on brain structure and peripheral gene expression reveals novel molecular insights into mechanism of action. *Transl Psychiatry*. **10**, 103–113. <https://doi.org/10.1038/s41398-020-0784-z> (2020).
- Vernon, A. C. et al. Contrasting effects of haloperidol and lithium on rodent brain structure: a magnetic resonance imaging study with postmortem confirmation. *Biol. Psychiatry*. **71**, 855–863. <https://doi.org/10.1016/j.biopsych.2011.12.004> (2012).
- Gray, J. D. & McEwen, B. S. Lithium's role in neural plasticity and its implications for mood disorders. *Acta Psychiatr Scand.* **128**, 5, 347–361. <https://doi.org/10.1111/acps.12139> (2013).

16. Selek, S. et al. A longitudinal study of fronto-limbic brain structures in patients with bipolar I disorder during lithium treatment. *J. Affect. Disor.* **150**, 629–633. <https://doi.org/10.1016/j.jad.2013.04.020> (2013).
17. Chiu, C. T. & Chuang, D. M. Neuroprotective action of lithium in disorders of central nervous system. *Zhong Nan Da Xue Xue Bao Yi Xue Ban.* **36** (6). <https://doi.org/10.3969/j.issn.1672-7347.2011.06.001> (2011). 461–76.
18. Germana, C. et al. The effects of lithium and anticonvulsants on brain structure in bipolar disorder. *Acta Psychiatr Scand.* **122**, 481–487. <https://doi.org/10.1111/j.1600-0447.2010.01582.x> (2010).
19. Marciniak, P., Szymczak, M. & Rosinski, G. Insect peptides hormones – a review of major families. *Postępy Biol. Komórki.* **38**, 43–63 (2011). https://www.pbkom.eu/sites/default/files/artykulydo2012/38_1_43.pdf
20. Adamski, Z. et al. Rosiński, G. Beetles as model organisms in physiological, biomedical and environmental Studies - A review. *Front. Physiol.* **28**, 10319. <https://doi.org/10.3389/fphys.2019.00319> (2019).
21. Nowicki, P., Szymczak, M., Rosiński, G. & Marciniak, P. Neuropeptidomics of the insect neuroendocrine system. *Kosmos* **597–611** <https://kosmos.ptpk.org/index.php/Kosmos/article/download/2447/2375/2587> (67).
22. De Loof, A., Lindemans, M., Liu, F., De Groef, B. & Schoofs, L. Endocrine archeology: do insects retain ancestrally inherited counterparts of the vertebrate releasing hormones gnRH, GHRH, TRH, and CRF? *Gen. Comp. Endocrinol.* **177** (1), 18–27. <https://doi.org/10.1016/j.ygcen.2012.02.002> (2012).
23. Adamski, Z., Nikolaou, P. & Marciniak, P. α -Solanine and α -Tomatine affect the retrocerebral complexes of tenebrio molitor and zophobas atratus beetles. *Arch. Insect Biochem. Physiol.* **117** (3), e70003. <https://doi.org/10.1002/arch.70003> (2024).
24. Bochenek, A. & Reicher, M. General anatomy. Bones, joints and ligaments, muscles. in *Human anatomy. Volume I.* 13th Edn. (PZWL, 2022). (2010).
25. Bochenek, A. & Reicher, M. (eds) Viscera. in *Human anatomy. Volume II.* 10th Edn. (PZWL, 2022). (1999).
26. Fastenrath, M. et al. Human cerebellum and corticocerebellar connections involved in emotional memory enhancement. *PNAS* **119**, 41, e2204900119. <https://doi.org/10.1073/pnas.2204900119> (2022).
27. Chowański, S. et al. Metabolism dynamics in tropical cockroach during a cold-induced recovery period. *Biol. Res.* **58**, 40, 1–20. <https://doi.org/10.1186/s40659-025-00621-6> (2025).
28. Critser, R. & Locke, P. How should the 3 r's be revised and why?? *AMA J. Ethics.* **26**, 9, E724–729. <https://doi.org/10.1001/amajethics.2024.724> (2024).

Author contributions

R.W.W., designed and conducted the study, performed analyses, including anatomical measurements, wrote the article, organized references, and was the editor of the article. P.M. supervised microsurgery. M.S., she supervised all the work. All authors discussed the results and commented on the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to R.W.W.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2025