The effects of lithium chloride on cellular interaction behaviors of peripheral embryonic neurons of *Gallus gallus*.

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Introduction

Lithium is a chemical element belonging to the alkali metal group. Due to its mood-stabilizing effects, the exact mechanisms of which are not fully understood, lithium has been employed for many years in the medical field primarily as a treatment for psychiatric conditions such as schizophrenia, bipolar disorder, and depression with suicidal ideations (Malhi et al., 2012). Its usage has also been explored in the treatment of movement disorders, extrapyramidal diseases, asthma, thyroid diseases, cardiac conditions, and sleep disorders (Frost & Messiha, 1983), as well as neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's and severe brain injuries potentially due to its up-regulation of cell survival molecules and down-regulation of pro-apoptotic factors (Wada et al., 2005). Several studies have also found that lithium exposure increases axon growth and elongation in neurons of the central nervous system (CNS) (Dill et al., 2008), growth cone expansion and axonal branching (Lucas et al., 1998), and axonal regeneration in peripheral neurons (Su et al., 2014).

The primary purpose of this study was to examine the effect of lithium on the initiation of cell-to-cell interactions of peripheral neurons dissected from ten-day-old *Gallus gallus* embryos. In this study, dissected neurons in the experimental condition were incubated at 37° C for four hours in a solution containing 10 mM LiCl while neurons in the control condition were incubated for the same amount of time and temperature in a solution containing no LiCl. Observations were conducted to compare the number and percentage of interactions between neurons. Based on

literature examining the pharmacodynamics of lithium therapy, the hypothesis for this study was that cells exposed to lithium would show an increase in cellular interaction behaviors than cells not exposed to lithium.

This study was similar in nature to those of previous Wheaton Neurobiology students Tracy McCann (2014), Bohan Yang (2014), and Juliana Fess (2006), each of whom studied the effects of methyl mercury on the interactions of neurons and glial cells. Their works were referred to in the determination of data analysis methods for this study.

Materials and Methods

Dissection and Plating of Peripheral Neurons: The dissection and plating of ten-day-old *Gallus gallus* peripheral neurons was performed using the materials and protocol included in Primary Culture of Chick Embryonic Peripheral Neurons 1: DISSECTION (Morris, 2015a).

LiCl Perturbation and Incubation: LiCl working solution was created from a 1 mM stock solution of LiCl in H_2O as a vehicle. For the experimental condition, 1 M of LiCl was diluted to 10 mM. In the four experimental dishes, growth medium was removed using the Pasteur pipette method and replaced with 1.5 mL of 10 mM LiCl in serum-free medium (SFM). In the two control dishes, growth medium was again removed using a Pasteur pipette and replaced with 1.5 mL of SFM. All dishes were then incubated for four hours at 37° C.

Removal of LiCl and Washing: Cells were removed from the incubator after four hours. LiCl was removed from the experimental dishes and SFM was removed in the control dishes using a Pasteur pipette. Cells were then washed with Hank's Balanced Salt Solution (HBSS) twice, allowing five minutes between each wash, and 1.5 mL of pure SFM was added. Dishes were then incubated again at 37° C for 10 minutes. Dishes were removed from the incubator,

SFM was removed and set aside, and cells were washed three times with HBSS, again allowing five minutes between each wash. After the washes were completed, the SFM was re-added to the dishes.

Creation of Chip Chambers: Chip chambers were created using the protocol included in Primary Culture of Chick Embryonic Peripheral Neurons 2: OBSERVATION OF LIVE UNLABELED CELLS (Morris, 2015b).

Observation and Imaging: Observation and data collection was done in collaboration with Madeline Parker, Shiqi Liu, Kaitlyn Solano, Ellen Fossett, and Angela Mirabella. Imaging of cells was done according to the protocol in Morris, R.L. (2015b). Observation of cells was conducted using transmitted light microscopy on a Nikon Eclipse E400 microscope at 40x magnification and phase 2 setting with a Spot Insight 2 camera on a 1.0x C mount. Still images were taken using the SPOT software running on a Macintosh iMac with software iOS 10.10.

Measurement and Data Analysis: Seven images of the control and experimental slides each were chosen using those that contained at least one axon-to-axon connection. Seven different images were chosen to observe multiple individual, unique areas of the respective condition slides. The number of interactions between individual neurons was examined. Individual neurons were located and defined by the bright “halo” around the cell bodies. Glial cells were located and defined by their flat, triangular, leaf-life appearance. An axon was defined as any long, thin, branching process extending from the cell body. A cell-to-cell interaction was defined as any physical contact between an axon of one neuron and an axon of another. Interactions between neurons and glia and between the glia themselves were not counted in the observation process. The percentages of cellular interactions between neurons were calculated using the total number of interactions observed in each condition divided by the total number of

neurons in each condition. A comparison of the percentages of interactions between the experimental and control conditions was made.

Results

Three experimental and one control slide were imaged. In the control condition, 31 individual neurons and 35 individual cell-to-cell interactions were observed. In the experimental condition, 26 individual neurons and 12 individual cell-to-cell interactions were observed. Interactions between neurons and glia and between individual glial cells were not examined. A total percentage of neuron-to-neuron interactions of 112.90% was observed in the control condition, compared to 46.15% in the experimental condition.

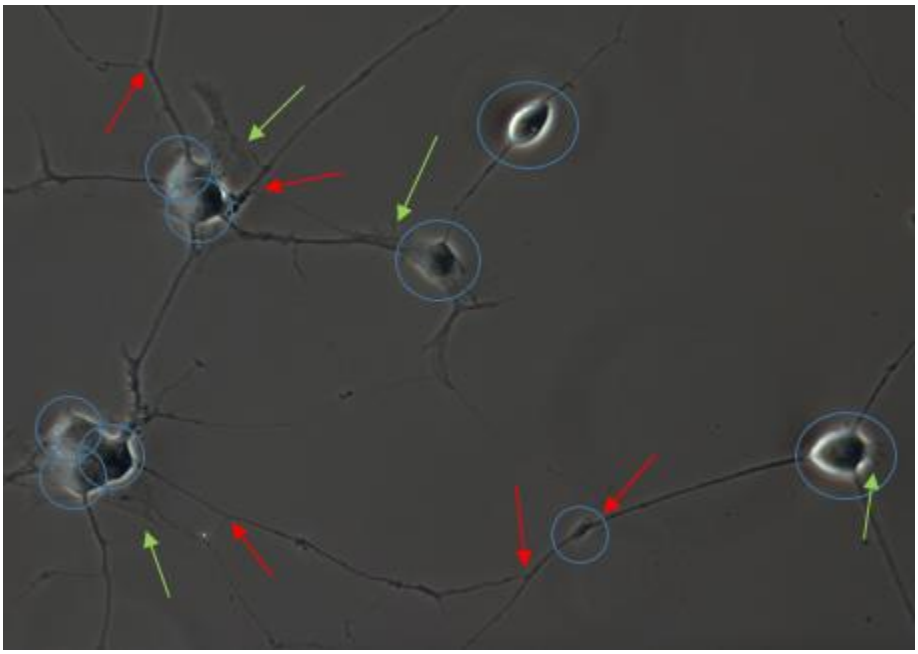

Figure 1: Image from a control slide not exposed to lithium. The red arrows indicate individual interaction points where axons from two separate neurons make contact. The blue circles indicate individual neuron cell bodies. The green arrows indicate glial cells.

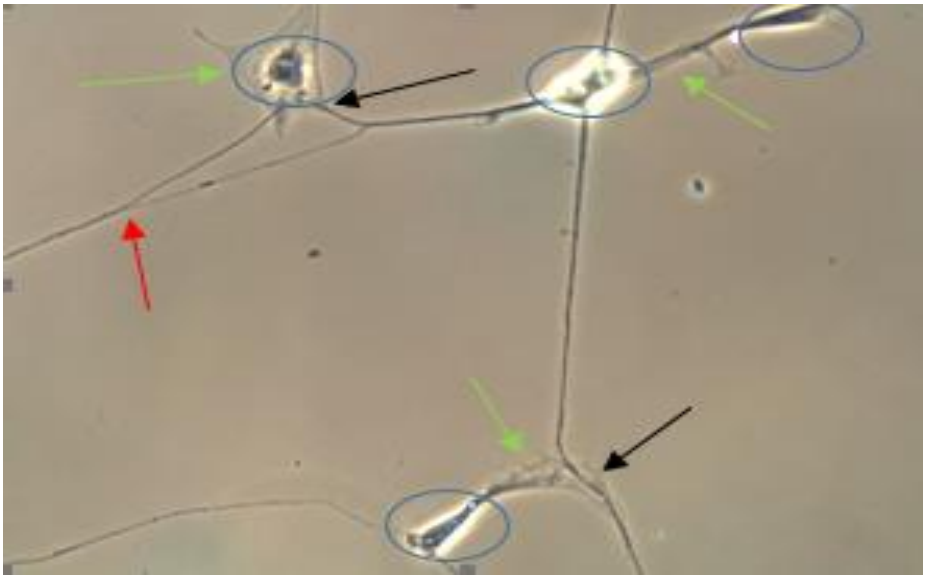

Figure 2: Image of interacting neurons and glia from an experimental slide exposed to lithium. The red arrow indicates an interactions between the axons of two different neurons. The green arrows indicate glial cells. The blue circles highlight neuron cell bodies. Note that the two top left and top middle circles are likely to contain more than one cell body, though they are difficult to distinguish from one another. The black arrows indicate points of contact between axons and glial cells, which were not examined in this study.

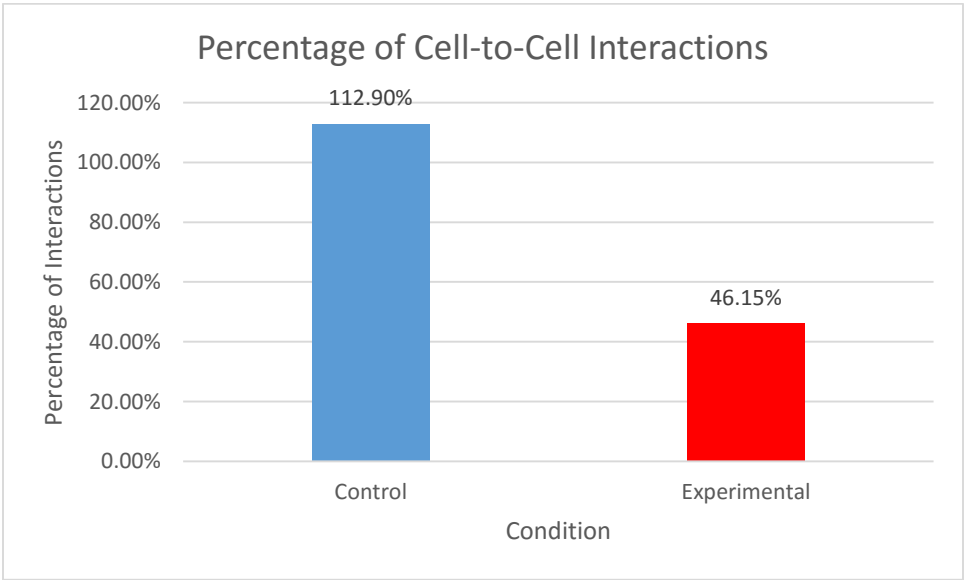

Figure 3: Bar graph displaying the cell-to-cell interactions between neurons in each condition. Neurons in the control group show a much greater percentage of interactions compared to the experimental group.

Discussion

The results of this experiment show that lithium exposure does not increase cell-to-cell interaction behavior in neurons, and therefore the original hypothesis was not supported. However, it does appear that lithium indeed has an effect on the interaction behaviors of cells. Neurons exposed to lithium showed significantly less interactions than neurons not exposed to lithium. From this we can determine that lithium actually decreases the cell's likelihood of and ability to make connections with other cells, the opposite of what the original hypothesis stated would happen.

If this study were to be repeated, the results may be more accurate if time-lapse images or videos were used to actively observe the formation of cell-to-cell connections. Additionally, it would be beneficial to take images of the cells before exposure to lithium and incubation to observe the presence and number of pre-existing connections. Both of these procedures could determine the potential error of this study, which was that we were not able to distinguish which cellular projections and connections were already present or what they looked like before the treatment.

Despite the number of studies, including those previously mentioned, that showed that lithium exposure increased axonal growth, branching, and regeneration, the results of this study were similar to those seen by Shah et al., (2013). They found that while low doses of lithium (0.5 – 2.5 mM) increased the presence of growth cones and the extension of axons in dissociated neurons cultured from the spiral ganglion of the cochlea of mice, high doses of lithium (12.5 mM) slowed axon elongation and caused microtubules to become disarranged and unstable. The authors state that the likely reason for this is that lithium completely inhibits glycogen synthase

kinase 3 (GSK-3), a protein kinase heavily involved in cellular proliferation, migration, and apoptosis. It is possible that this is what occurred in the neurons exposed to lithium in this study.

To further examine the results seen in this study, future studies should seek to determine at what point lithium becomes detrimental to the cell and the exact mechanisms of why this occurs. As exhibited by Shah et al., (2013), there is clearly a threshold at which lithium stops becoming beneficial to the survival and regeneration of the cell, and it seems to be worthwhile to look into this further.

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I have abided by the Wheaton College Honor Code in this work.