Lithium carbonate in a post-stroke population: preliminary analyses of neuroanatomical and neuropsychiatric outcomes, and associations with BDNF from a pilot study

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A thesis submitted in conformity with the requirements for the degree of Master of Science (MSc)

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2017

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Abstract

Neuronal atrophy following stroke can lead to changes in grey matter volumes and associated neuropsychiatric impairments. This study aimed to investigate the therapeutic potential of lithium, a putative neurotrophic agent, in the stroke recovery process within a year of stroke incidence. Stroke patients recruited to the study were prescribed lithium for 60 days. Grey matter volumes, cognitive and mood outcomes, and brain-derived neurotrophic factor (BDNF) levels were assessed at baseline and end of treatment course. There was no difference in global grey matter volume between baseline and termination (t=1.977, p=0.074). There was a significant interaction between lithium dose and global grey matter volume (F=14.25, p=0.004), and a correlation between lithium dose and verbal memory (r=0.576, p=0.05), where higher dose was associated with more positive changes in global grey matter volume and verbal memory. There was no difference in serum BDNF levels between baseline and termination (t=-0.357, p=0.728). Lithium pharmacotherapy may be associated with grey matter volume change and verbal memory improvement in stroke patients, suggesting potential therapeutic applications of lithium in a post-stroke population.
Acknowledgements

The work presented herein is a culmination of both the academic and personal growth I’ve undergone in the last two years—work that would not have been possible without the support I’ve received along the way.

To my supervisor, Dr. Krista Lanctôt: Thank you for this opportunity. Thank you for taking a leap of faith when I came to the lab with no clinical experience of which to speak. Thank you for your patience in guiding me through the humdrum of degree requirements, the intricacies of clinical research, and the challenges of scientific inquiry. Thank you for pushing me to be more ambitious and confident in my work, to seize opportunities as they present themselves and not to shy away from novel experiences. These couple of years have been invaluable in my development as a scientist, and the lessons I’ve learned at the lab will undoubtedly serve me for years to come. Thank you for providing the tools and guidance that made this experience possible.

To my advisor, Dr. Nathan Herrmann: Thank you for your mentorship and invaluable clinical insight. Thank you for pushing me to consider perspectives outside of the basic science paradigm in which I’ve trained, and broadening my scientific horizons. Thank you for being a steadfast example of a tirelessly dedicated and compassionate clinician, as well as a relentlessly inquisitive and driven scientist; your example inspires me to demand more of myself in my career.

To Nadia Reider and Janelle Bradley: Thank you both for helping me coordinate and manage all the moving pieces in this study. Thank you for being there for me throughout the study, whether it was to offer advice and support in difficult situations, or to celebrate the small victories along the way. I could not have done it without you!

To Dr. Sandra Black, Chris Scott, Austyn Roseborough, Manu Sharma, and everyone at the L.C. Campbell Cognitive Neurology Research Unit: Thank you for analyzing all the MRI images and generating the brain volumetrics. And especially, thank you for going above and beyond in walking me through the imaging pipeline and providing me with all the resources I needed to understand the process; I truly learned a lot through our discussions.

To Drs. Karl Boyle, Rick Swartz, David Gladstone, Mark Boulos, Julia Hopyan at Sunnybrook, to Dr. Murray Waldman at St John’s Rehab, to Dr. Susan Marzolini and Danielle Lawrence at TRI: Thank you for allowing me to screen for eligible participants at your clinics/units/classes, and taking the time out of your busy schedules to talk to me about your patients.

Dr. Ana Andreazza and Wendy Horsfall: Thank you for graciously opening your lab to me and allowing me the learning opportunity of running my own assays.

To everyone at the Neuropsychopharmacology Research Group: Thank you for making my time at the lab such a fantastic experience! It’s been a pleasure working alongside everyone and getting to know all of you. Myuri, Mahwesh—thank you so much for all your help; you’re both so knowledgeable and such great teachers, and I’ve learned so much from the both of you. Celina, Sarah, Maisha—thank you for dragging me to the gym and encouraging me to be healthier, more active; you literally helped me become a better person! I am truly fortunate to have shared the last two years with the amazing people here.

Lastly, I would also like to extend my sincerest thanks to my family and friends who have supported me throughout my MSc. As the saying goes: it takes a village.
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# Imaging Glossary

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1. Introduction

1.1 Statement of problem

Stroke is a leading cause of death and disability in Canada\(^1,2\). Every year, there are an estimated 62,000 strokes\(^3\), and upwards of 400,000 Canadians are currently living with post-stroke conditions\(^4-6\). Of the different types of stroke, ischemic stroke is by far the most common, accounting for 80-87\% of stroke cases depending on the population\(^7,8\). Immediately following the ischemic event, excitotoxicity, oxidative stress, and inflammation all contribute to neuronal damage and atrophy\(^9-11\). These apoptotic processes culminate in observable changes in grey matter volume following stroke\(^12-14\). Diminishing grey matter volume is associated with cognitive impairment\(^15-17\), from which a large number of stroke patients suffer\(^18,19\), and presence of cerebral infarcts increases risk of dementia\(^20-24\). Post-stroke depression is another common clinical outcome that leads to a poorer quality of life and increased risk of mortality\(^25-27\). Again, there is potentially a link between grey matter volume and clinical outcomes\(^28,29\). Although there is some spontaneous recovery after stroke\(^30-32\), overall prevalence of post-stroke cognitive improvement is low\(^33\). Therefore, there is a need to develop better strategies to address post-stroke sequelae. Currently, pharmacotherapy following stroke is limited to acute thrombolysis\(^34\) and prophylaxis addressing underlying risk factors of stroke (e.g. hypertension, dyslipidemia, atrial fibrillation)\(^8,35-39\). Cognitive and functional impairment have largely been left to rehabilitation programs. Although rehabilitation is associated with better functional outcomes, there is limited evidence on its benefits for cognition\(^40,41\). Therefore, it is prudent to develop new strategies to complement and strengthen current post-stroke treatments.
1.2 Purpose of study and objective

As more and more individuals are living with post-stroke sequelae, it becomes increasingly important to develop effective strategies to treat these neuropsychiatric outcomes. Given that these clinical outcomes are attributable to neuroanatomical changes\textsuperscript{21}, pharmacological agents that target neuroanatomical remodeling are a promising avenue of study. To that end, this study aims to evaluate neuroanatomical, neuropsychiatric, and BDNF changes in a post-stroke population, following administration of a neurotrophic agent, lithium carbonate. Lithium has been shown to protect against neuronal apoptosis, promote factors associated with neurogenesis, and increase global grey matter volumes, thus making it an attractive candidate to study in post-stroke neuronal atrophy\textsuperscript{42-45}. Given lithium’s neurotrophic and neuroprotective profile, our primary objective is to assess changes in grey matter volume in this population using structural imaging tools. Our secondary objective is to identify changes in clinical outcomes that can be associated with lithium treatment or neuroanatomical markers. While valuable to characterize anatomical changes in the brain for its own sake, practical application of our findings will be limited if we cannot identify associations between drug treatment, physiological changes and clinically-relevant changes in function and behavior. Finally, as an exploratory objective, we aim to correlate our neuroanatomical and neuropsychiatric outcomes with a common neurotrophic factor in order to posit a potential molecular pathway that mediates both physiological and behavioral outcomes. We have selected brain-derived neurotrophic factor (BDNF) as our molecule of interest as it has been correlated with grey matter volumes, associated with cognitive and mood outcomes, and is known to be upregulated by lithium therapy\textsuperscript{46-53}. In summary, our goal is to characterize grey matter volume change, identify changes in clinical outcomes and correlate it to neuroanatomical findings, and conduct exploratory investigations on BDNF as a molecular mediator between physiological and behavioral outcomes.
1.3 Statement of research hypotheses and rationale for hypotheses

1.3.1 Primary hypothesis

*Lithium treatment will be associated with changes in global grey matter volume.*

Meta-analyses of cross-sectional data in bipolar populations have found correlations between prevalence of lithium use and grey matter volume, where greater prevalence of lithium usage was associated with greater grey matter volumes\(^{54,55}\). Furthermore, a longitudinal study from Moore et al. demonstrated that increases in global grey matter volume were observed after only 4 weeks of lithium treatment in a fairly small group of bipolar patients, suggesting a large detectable effect of lithium on grey matter volume\(^ {43,45}\). Given that lithium acts upon several neurogenesis and remodeling pathways involved in spontaneous post-stroke recovery (e.g. MARCKS upregulation, modulation of excitatory and inhibitory signaling, BDNF upregulation), there is some basis to expect associations between lithium treatment and grey matter volume in a post-stroke population\(^ {42,49-53,56-62}\).

1.3.2 Secondary hypothesis

*Lithium treatment will be associated with changes in cognitive and mood outcomes. Changes in these outcomes will in turn correlate with changes in grey matter volume.*

While lithium may be a mainstay in treatment of mood disorder, studies have also suggested potential benefits of lithium on cognitive outcomes—be it better executive function or reduced rates of dementia\(^ {63-65}\). Furthermore, considering neuronal atrophy, as reflected by decreased grey matter volume, is correlated with cognitive impairment and loosely associated with depression\(^ {15-17,28,29,66}\). If that were to be the case, a correlation between grey matter volume and cognitive outcomes would be expected.
1.3.3 Exploratory hypothesis

*Lithium treatment will be associated with changes in serum BDNF levels. Changes in serum BDNF levels will be associated with changes in grey matter volume and clinical outcomes.*

Lithium has been shown to increase BDNF in both preclinical models and clinical populations\textsuperscript{49-53}. Given BDNF promotes neurogenesis and survival of neurons\textsuperscript{59, 75, 76}, it is fair to postulate that it may be associated with grey matter volume, especially considering its role in spontaneous post-stroke recovery\textsuperscript{59}. Moreover, we know that BDNF correlates with hippocampal volume in a healthy aging population\textsuperscript{46}. Clinically, serum BDNF has shown some correlations with cognition, and has shown consistent correlations in post-stroke depression\textsuperscript{47, 48, 77, 78}. Thus, it is likely that serum BDNF levels will have some interaction with grey matter volume and clinical outcomes.

1.4 Review of literature

1.4.1 Overview of stroke

Stroke is a leading cause of death and disability in Canada\textsuperscript{1, 2}. Every year, there are an estimated 62,000 strokes, which amounts to a stroke every 8 minutes\textsuperscript{3}. Although stroke can occur in any age group, the risk of stroke sharply increases after the age of 55 and approximately three-quarters of all strokes occur in individuals over 65\textsuperscript{5}. It is estimated that more than 13,000 Canadians die from stroke every year, and that somewhere between 400,000 and 700,000 Canadians are currently living with post-stroke conditions\textsuperscript{4-6}. Stroke and its sequelae cost the Canadian economy $1.3 billion annually in healthcare expenses and lost productivity\textsuperscript{1}.

With acute interventions improving survival following stroke and a population that is aging, the number of individuals living with post-stroke conditions is expected to increase\textsuperscript{4, 8, 79}. Thus, effective management of post-stroke conditions becomes increasingly important.
There are three subtypes of stroke: ischemic, intracerebral hemorrhage, and subarachnoid hemorrhage. Of the three, ischemic stroke is by far the most common, accounting for 80-87% of stroke cases depending on the population\textsuperscript{7,8}. Ischemic stroke is characterized by the occlusion of cerebral blood vessels, which impedes blood flow in affected regions. The source of the occlusion varies: it can stem from embolisms in the heart (i.e. cardioembolic), plaque in arteries (i.e. artery-to-artery), or develop in situ in small vessels of the brain\textsuperscript{79,80}. Brain injury following occlusion depends on severity of impairment to the blood flow in a region, where regions with very low cerebral blood flow become irreversibly damaged (i.e. consolidates into the stroke core). Areas surrounding the core that suffer less severe blood flow impediment are categorized as the ischemic penumbra, and are thought to be structurally intact, if not functionally sound\textsuperscript{81,82}. Regions within the penumbra may evolve along different trajectories over time: some may proceed to spontaneous recovery, others may consolidate into an established infarct\textsuperscript{82}.

Immediately following the ischemic event, excitotoxicity is one of the main processes contributing to neuronal damage and atrophy\textsuperscript{9}. Neurons consume a lot of energy and require oxygen for adenosine triphosphate (ATP) production\textsuperscript{10,83}. In the event of ischemia, oxygen is no longer available, thus no new ATP can be generated and ATP stores are rapidly depleted. ATP is required for the function of the Na\textsuperscript{+}/K\textsuperscript{+}-ATPase, an important transporter involved in maintenance of the ionic gradient across cell membranes. As there is rapid influx of Na\textsuperscript{+} and Ca\textsuperscript{2+} into the cell and simultaneous efflux of K\textsuperscript{+} in the acute phase of ischemia, proper function of this transporter is required to prevent unwanted depolarization\textsuperscript{83}. However, because ATP stores are limited in ischemic conditions, the transporter is not able to maintain the ionic gradient, leading to depolarization. Depolarization of neurons leads to release of excitatory neurotransmitters (e.g. glutamate). Coupled with impaired neurotransmitter reuptake (due to ATP requirement of reuptake mechanism), there is an excess of glutamate in the synaptic cleft, which leads to over-activation
of post-synaptic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Over-activation of these receptors leads to excessive Ca$^{2+}$ influx, which triggers downstream apoptotic cascade via calcium-dependent protein kinases and proteases that degrade cell structure$^{10,83}$. Influx of Na$^+$ and Ca$^{2+}$ can also cause cytotoxic edema, which precipitates necrosis$^{11,83}$. While this may be the major process of neuronal damage in the acute phase, its role in the evolution of infarcts is less clear$^{83}$.

During stages of reperfusion, neuronal damage can occur through oxidative stress$^{11}$. Oxidative stress occurs when endogenous free radical scavengers get overwhelmed by the excess production of free radicals following ischemia$^{10,11}$. One such free radical, nitric oxide, is produced by nitric oxide synthase (NOS). Two calcium-sensitive forms of NOS are commonly found in neurons; they are activated in the presence of high Ca$^{2+}$ concentrations$^{84}$. Given the Ca$^{2+}$ influx following ischemic insult, activity of these neuronal NOS is upregulated, leading to more nitric oxide, superoxide and peroxide$^{84}$. At the same time, superoxide is also being formed by a variety of ischemia-induced processes, including aberrant xanthine dehydrogenase (oxidized to xanthine oxidase under ischemic conditions) and mitochondrial electron transport chain activity$^{11,84}$. Nitric oxide reacts with superoxide to form peroxynitrite, which has been shown to induce DNA damage in the form of base modifications strand breaks$^{85}$. The presence of strand breaks triggers DNA repair mechanisms$^{11,85}$, which appear to be involved in post-ischemia apoptosis in preclinical models$^{86,87}$. Furthermore, reactive oxygen species disrupt the proton gradient across mitochondrial membranes, triggering the release of mitochondrial signals (e.g. cytochrome c) for cell apoptosis$^{11}$.

Oxidative stress also activates astrocytes and microglia, which initiates an inflammatory cascade$^{10}$. Within the parenchyma, astrocytes, microglia and neurons secrete inflammatory mediators (e.g. cytokines, chemokines, NOS) in response to ischemic injury$^{10,11}$. Of these, interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α) have garnered arguably the greatest research interest. IL-
1 is a proinflammatory cytokine that has been implicated in neuronal damage in the reperfusion phase following ischemia: knocking out or inhibiting IL-1 activity leads to decreased neuronal injury in preclinical studies. In vitro data also suggest that IL-1 may induce apoptosis via nitric-oxide mediated processes. The role of TNF-α in post-stroke neuronal atrophy is less clear. Tumor necrosis factor is thought to regulate apoptotic caspase activity following ischemic insult, with preclinical models demonstrating improved neuronal survival in TNF-knockout animals and in presence of TNF inhibitors. However, other studies have suggested that TNF is involved in neuroprotective and restructuring processes following stroke, though the mechanisms through which these actions occur remain contested. Beyond triggering apoptotic processes, inflammation increases permeability of the blood-brain barrier and the release of inflammatory mediators in the parenchyma recruits peripheral leukocytes, leading to more inflammation—which is thought to exacerbate ischemic injury.

Apoptosis is mediated through caspase-dependent and independent pathways. In neuronal cells, caspase-dependent pathways can be triggered by TNF binding to its receptor, Ca\(^{2+}\) influx-activated dependent kinase, and mitochondrial release of cytochrome c—which lead to activation of caspase-3. Activated caspase-3, through interaction with other effectors, catalyzes the breakdown of structural proteins, resulting in cell death. The mechanisms of caspase-independent activity are less well-defined: it is thought that activation of c-Jun N-terminal protein kinase (JNK) and apoptosis inducing factor (AIF) leads to DNA and structural damage that ultimately culminates in cell death. Apoptotic processes are kept in check by proteins like b-cell lymphoma 2 (Bcl-2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which inhibit parts of the apoptotic pathways.
Figure 1 Simplified neuronal caspase-dependent apoptotic cascade adapted from Yakovlev et al.\textsuperscript{96} and D’Amelio et al.\textsuperscript{97}. TNF = tumor necrosis factor, Cas = caspase, Bid/Bax = pro-apoptotic members of Bcl-2 family, Bcl-2 = b cell lymphoma 2 (anti-apoptotic family member), cyt c = cytochrome c, Apaf1 = Apoptotic protease activating factor 1.
These apoptotic processes in neuronal cells culminate in observable changes in grey matter volume following stroke. In the Framingham study, presence of cerebral infarction was associated with decreased grey matter volume; other large cross-sectional studies also report a negative correlation between white matter lesions and global grey matter volume \(^{12-14}\). In a longitudinal study, a significant decrease in posterior cingulate cortex volume was observed over 6 months following ischemic stroke \(^{100}\). Several small studies have also demonstrated that grey matter volume changes occur even in cases of subcortical lesions \(^{101,102}\). Changes in grey matter volumes are of interest as they appear to correlate with cognitive outcome. Vascular cognitive impairment has been shown to associate with decreased grey matter volume \(^{15}\). In one study of ischemic stroke, cognitive function was found to be attributable to global (and frontal) grey matter volume \(^{16}\). In another study comparing stroke patients with and without cognitive impairment, Stebbins et al. found that individuals with cognitive impairment had decreased grey matter volumes \(^{17}\).

Given the association between grey matter volume and cognitive outcome, it is unsurprising that stroke is associated with a myriad of neurological outcomes, including cognitive impairment (which contributes to functional impairment) \(^{18}\) and increased risk for dementia \(^{103}\).

### 1.4.1.1 Cognitive outcomes

Cognitive impairment is one major sequela of ischemic strokes. In one study, 35.2% of stroke patients presented with cognitive impairment compared to just 3.8% of age-matched stroke-free participants \(^{18}\). The authors noted that impairments most commonly occurred in the domains of memory, orientation, language, and attention. A more recent study reported that 64% of stroke survivors had some cognitive impairment, as evidenced by their clinical diagnosis or cognitive assessment score \(^{19}\). In a longitudinal study in Singapore, investigators reported post-stroke deterioration from cognitively intact status to cognitively impaired without dementia at 10% annually; similarly they reported conversion from cognitively impaired without dementia to
dementia status at 11%. However, it was unclear if these patients were cognitively impaired prior to their strokes, thus interpretation of these findings is challenging. In another longitudinal study in an European population, 27.9% of patients showed some cognitive impairment (including dementia) at 3 months post-stroke and 14% of patients were noted to have cognitively declined over a 2-year period; patients who experienced decline were older, less educated, and more cognitively impaired pre-stroke. In a 3-year study, Sachdev et al. compared stroke patients who were cognitively intact before stroke with cognitively-intact stroke-free controls. Overall, patients diagnosed with mild cognitive impairment (MCI) following stroke showed greater decline in logical memory compared to stroke-free controls over a 3-year period. Interestingly, a lower proportion of cognitively-intact stroke patients developed MCI compared to cognitively-intact stroke-free controls. However, 8.5% of cognitively intact and 24.4% of cognitively impaired patients without dementia developed dementia at 3-year follow-up; no stroke-free controls were diagnosed with dementia.

Indeed, cognitive impairment can be a precursor to vascular dementia, which occurs in an estimated 5% to 10% of the aging population with the prevalence doubling every 5.3 years. In examining post-mortem brains, it was found that while presence of one infarct increased the odds of dementia by 69%, presence of multiple infarcts increased odds of dementia two-fold. Population-based post-mortem studies also indicate an association between presence of infarcts and dementia. In the Canadian Study of Health and Aging, individuals with vascular cognitive impairment without dementia were followed for 5 years; 58 out of 72 (81%) surviving participants had developed dementia, with cognition deteriorating in another seven. In a longitudinal study, it was shown that there were more grey matter infarcts and cerebral atrophy in MCI patients who progressed to dementia compared to those who did not. A systematic review of available neuropathological evidence noted that strokes in the parietal cortex, thalamus, and basal ganglia
may be especially likely to lead to post-stroke cognitive impairment\textsuperscript{20}. The review also highlighted that both cortical and subcortical strokes have been linked to dementia.

\subsection*{1.4.1.2 Post-stroke depression}

Another common clinical outcome post-stroke is the onset of depression. From meta-analysis data, the prevalence of depression holds steady at 29\% over the course of 10 years following stroke, with a high of 33\% at 6 months post-stroke\textsuperscript{25}. For comparison, the population lifetime prevalence of depression has been estimated at 17.1\%\textsuperscript{109}. The meta-analysis data also showed a large range for recovery rate, spanning 15\% to 57\% at the 1 year time point. Post-stroke depression has been associated with poorer functional outcomes, more disability, and fewer benefits from rehabilitation\textsuperscript{110-113}. Overall, this leads to poorer quality of life and may ultimately increase risk of mortality\textsuperscript{26, 27}.

Studies in the past have postulated that mood changes are associated with lesions in certain neuroanatomical regions, particularly the left frontal lobe and basal ganglia\textsuperscript{114-117}. The authors argued that lesions in these areas disrupt monoaminergic (noradrenergic and serotonergic) neural circuits involved in the regulation of mood. However, data from meta-analyses suggest that the relationship between lesion location and depression may not be robust, or are at least more temporally nuanced\textsuperscript{118, 119}. Other stroke characteristics may also be important, as one reported that patients with moderate to severe depressive symptoms had larger lesion volumes than patients with mild or no symptoms\textsuperscript{120}. Another study reported that lesion volume was associated with incidence of depressive episodes\textsuperscript{121}. Furthermore, Vataja et al. reported that both volume and frequency of infarct were correlated with post-stroke depression\textsuperscript{66}.

Although meta-analysis data in major depressive disorder have demonstrated a reduction in grey matter volumes in depressed patients\textsuperscript{28}, the relationship between grey matter volume and post-stroke depression is less clear. In one study, investigators reported that severe frontal lobe atrophy
predicted post-stroke depression\textsuperscript{29}. However, lobular atrophy may not reflect grey matter atrophy, as another study noted no significant differences in cortical (and subcortical grey matter) atrophy between depressed and non-depressed patients\textsuperscript{66}. In line with the inflammation and neurodegeneration hypothesis of depression, several reviews of literature have also highlighted inflammatory mediators (e.g. cytokines), which are elevated in post-stroke conditions, as potential actors in post-stroke depression\textsuperscript{122-125}.

1.4.1.3 Recovery

Many stroke survivors experience some spontaneous recovery\textsuperscript{30-32}, though recovery in this case does not necessarily indicate recovery of pre-stroke functions, but rather improvements from initial post-stroke impairment\textsuperscript{126}. This distinction is important because post-stroke functional recovery can be mediated through recovery of baseline functions and compensatory behaviors\textsuperscript{127-132}. Improvements in cognition can be seen as early as 6-months post-stroke and can continue to show improvements at 12 months\textsuperscript{133, 134}. In another study, recovery was noted up to 3 years following stroke\textsuperscript{135}. Nys et al. reported that in a 6- to 10-month period, recovery in visual perception and memory was most common (83% and 78%, respectively), and recovery in abstract reasoning and language was least common (41% and 54%, respectively)\textsuperscript{136}. Another study that compared cognitive function between 3 months and 24 months reported significant improvements across all domains, including executive function, verbal learning and fluency\textsuperscript{137}. However, overall prevalence of post-stroke cognitive improvement appears to be low\textsuperscript{33}.

Several mechanisms are thought to underlie the recovery process, including axonal sprouting in peri-infarct and connected cortical regions\textsuperscript{56, 126}. One potential player in this process is growth associated protein 43 (GAP-43), which has been linked to axonal sprouting and long-term potentiation\textsuperscript{138}. Post-mortem examination of adult brains reveals elevated expressions of GAP-43 mRNA in the presence of ischemic injury\textsuperscript{139}. In pre-clinical models of cerebral ischemia, elevated
GAP-43 gene and protein expression were accompanied by detectable axonal sprouting and functional recovery\textsuperscript{140, 141}. Other pro-growth factors (e.g. CAP23, MARCKS, SPRR1) are also upregulated in the post-stroke milieu, with concurrent downregulation of growth-inhibiting factors in the penumbra\textsuperscript{56}. Glia-secreted TNF-α upregulates insertion of AMPA receptors into synaptic terminals and promotes endocytosis of γ-aminobutyric acid (GABA\textsubscript{A}) receptors\textsuperscript{142, 143}. Furthermore, preclinical studies in stroke models demonstrated increased NMDA receptor binding and decreased expression of GABA\textsubscript{A} receptors\textsuperscript{57, 58}. Changes in receptor density at the synapse is thought to mediate neuron plasticity, and an increase in excitatory signaling could suggest more excitability and potentially long-term potentiation of intact pathways\textsuperscript{126, 143}.

This structural change may be reflected in the changes in activity following stroke. It has been reported that stroke in one cerebral region can affect activity in other regions, proximal or distal, that are connected to the same neural network; activation of these related regions is associated with better performance in clinical outcomes\textsuperscript{144-148}. For example, grey matter hypertrophy in contralesional temporoparietal regions is associated with language production\textsuperscript{149}. Contralesional increases in activity is commonly observed, but the mechanisms underlying these changes remain unclear (e.g. recruitment of associated neural networks, reduced interhemispheric inhibition).

Another possible mechanism is the advent of neurogenesis\textsuperscript{56}. In preclinical models, stroke induced neurogenesis in the dentate gyrus and subventricular zone (SVZ)\textsuperscript{150}. Although neurogenesis in the SVZ is normally specific to genesis of olfactory bulb neurons, in stroke conditions, neuroblasts from the SVZ have been shown to migrate to areas of post-stroke injury, and differentiate into mature neurons that synapse with surrounding neurons\textsuperscript{151, 152}. However, there are suggestions that a good proportion of cells that migrate to the site of injury do not survive to undergo maturation\textsuperscript{152}. Thus, it is prudent to consider factors that promote neuronal survival. In one preclinical study, overexpression of brain derived neurotrophic factor (BDNF) led to recruitment of neurons to the
striatum; newly recruited neurons survived at least 5-8 weeks following overexpression. Infusion of BDNF also produced similar effects, with noted increases of newly generated cells in not only the striatum, but also the thalamus. In preclinical models, microglia activity has been shown to reduce neuronal damage following stroke, possibly through secretion of IGF-1 and BDNF. Factors released/produced in post-stroke conditions (e.g. inducible NOS, erythropoietin) are also implicated in neurogenesis. Inhibition of NOS inhibits post-stroke neurogenesis in the dentate gyrus, whereas treatment with erythropoietin increases BDNF levels and number of new neurons in peri-infarct regions. Although the association between neurogenesis and function is not well-established, findings from pre-clinical and clinical studies provide some preliminary support for a potential relationship.

1.4.1.4 Current therapies

Recombinant tissue plasminogen activator (rtPA) is a standard therapy for acute treatment of ischemic stroke. As its name suggests, rtPA activates enzymatic activity of plasminogen by converting it into fibrin-cleaving plasmin, which dissolves clots occluding cerebral blood vessels and allows for reperfusion of peri-infarct regions. If rtPA is administered within three hours of stroke onset, the number needed to treat to avoid death or disability is 18. Pooled analysis demonstrated that, if administered within a 4.5-hour window, rtPA improves odds of favorable clinical outcomes at 3-6 months. Although very effective, thrombolysis carries the risk of intracerebral hemorrhage, which is seen in 6-7% of cases. Research is ongoing to improve efficacy of thrombolysis through development of new thrombolytics and assessment of different routes of delivery.

Furthermore, preclinical evidence has suggested that thrombolysis without neuroprotection is suboptimal; combination therapy has the potential to extend the therapeutic window of rtPA, reduce reperfusion injury, and inhibit downstream apoptotic cascades. Due to a myriad of
factors, translation of effective neuroprotective pharmacotherapies from preclinical to clinical settings has proven difficult\textsuperscript{164}. Erythropoietin, which has been implicated in neurogenesis and recruitment of new cells to the peri-infarct region\textsuperscript{56}, has been tested in clinical populations, with mixed results: an initial study showed benefits that were not seen in follow-up, despite lower levels of markers of neuronal damage\textsuperscript{165-167}. Magnesium sulfate is another agent that has been shown to be neuroprotective in preclinical models\textsuperscript{168}. In one clinical trial, magnesium sulfate was administered pre-hospitalization with the goal of delaying onset of neurocognitive impairments while the patients waited for thrombolytic intervention, but outcomes at 90 days showed no difference in disability and mortality between treatment and placebo groups\textsuperscript{169, 170}. In another trial, magnesium sulfate was concurrently administered with standard of care (unspecified); again, no difference in clinical outcomes between treatment and control\textsuperscript{171}. Considering the pharmacokinetics of magnesium sulfate, the lack of efficacy may be attributable to low permeability across the blood-brain barrier\textsuperscript{172}. Citicoline, a precursor that is metabolized into choline, has also been investigated in post-stroke contexts\textsuperscript{162, 173, 174}. Again, results are mixed: two large clinical trials reported no effects of citicoline on post-stroke functional improvements\textsuperscript{175, 176}, while a smaller trial and a meta-analysis of pooled study data suggested improvements in cognition and functional recovery\textsuperscript{177, 178}. Albumin, as a binding agent of pro-inflammatory lipids, has also been studied in a clinical population with no discernable efficacy\textsuperscript{162, 179}. Thus, further studies into potentially neuroprotective pharmacotherapy are warranted.

Aside from addressing immediate neuropsychiatric sequelae of stroke, it is also important to prevent recurrent stroke. Cumulative risk of recurrent stroke in a 5-year period has been estimated at 16.6-22.5\%\textsuperscript{180, 181}. Thus, strategies are needed to manage underlying risk factors of stroke, such as hypertension, dyslipidemia, and atrial fibrillation\textsuperscript{8, 35}. Management of hypertension can be achieved through diuretics and renin-angiotensin-aldosterone system (RAAS) modulators (e.g.
angiotensin converting enzyme (ACE) inhibitors, angiotensin II receptor blocker); combination of indapamide (thiazide-diuretic) and perindopril (ACE inhibitor) reduces risk of stroke by 43%\(^36\).

Statins are a class of lipid-lowering drugs that reduce circulating cholesterol levels through inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. High-dose atorvastatin following stroke has been shown to decrease low-density lipoprotein (LDL) levels and reduce relative risk of recurrent stroke by 18%\(^37\). In an observational study, it was reported that stroke patients who were on statin demonstrated better functional outcomes at 3 months compared to those were not\(^182\). Comorbidities such as atrial fibrillation can increase the risk of clot formation, thus anti-platelet and anti-coagulant therapies are necessary. Anti-platelet drugs, as its class suggests, decreases platelet aggregation and clot formation. Data from separate trials indicated reduced risk of recurrent stroke in both aspirin monotherapy and dual platelet therapy with aspirin\(^38,\,39\). On the other hand, anti-coagulants block the formation of vitamin K-dependent clotting factors. Anti-coagulants increase risk of bleeding while offering comparable efficacy (in terms of functional outcome) to aspirin\(^162\).

As there currently is no pharmacotherapy for cognitive and functional impairment, rehabilitation is the main strategy through which post-stroke recovery is facilitated. Post-stroke rehabilitation has been shown to decrease odds of dependency, institutionalization, and mortality\(^40\). There is some clinical trial evidence showing that post-stroke cognitive training improves alertness and attention, but its effect on functional independence remains unclear\(^183\). In terms of visuospatial deficits, there is some evidence of training benefits but overall results across multiple training techniques appear to be mixed\(^184\). Cochrane review of clinical trials of cognitive rehabilitation reported a significant effect of treatment on self-rated assessments of memory in the short term (moderate quality of evidence), but not the long term (low quality of evidence); there was no difference in objective memory assessments, mood, functional abilities, or quality of life\(^41\). While
review of clinical trials on rehabilitation benefits on executive function suggests some efficacy of executive function training in the chronic phase of recovery, trial data on acute phase rehabilitation is not as readily available\textsuperscript{185}. Evidence suggests that both time to rehabilitation and rehabilitation intensity contribute to rehabilitation efficacy\textsuperscript{186}. Given that rehabilitation may not be efficacious in all contexts, investigations into adjunctive therapies may prove fruitful.

1.4.2 Overview of lithium

Lithium is traditionally used as a mood stabilizer for the treatment of bipolar mood disorder. While lithium is effective in the treatment of mania (comparable effect size to other pharmacotherapies for this indication)\textsuperscript{187}, evidence for its efficacy in acute depression is more ambivalent\textsuperscript{42}. Nonetheless, lithium continues to be recommended as a first-line option when it comes to prophylaxis of mood irregularities and maintenance of euthymia\textsuperscript{188}. Lithium has also been reported to be associated with cognitive outcomes: meta-analysis of cross-sectional studies in lithium-medicated and lithium-free subjects demonstrated small impairments in verbal memory and learning, which the authors attributed to prolonged lithium exposure\textsuperscript{189}. However, longitudinal studies did not report memory impairment or a difference between long- and short-term lithium users in memory test scores\textsuperscript{190, 191}. Moreover, one study showed that lithium responders performed better on the Wisconsin Card Sorting Test (a measure of executive function) compared to non-responders\textsuperscript{63}. Another study reported that lithium was not associated with effects on cognitive functioning within a two-year period, but did positively predict improvement in verbal learning\textsuperscript{64}. Furthermore, a population-based study evaluated rates of dementia following initial and follow-up prescriptions of lithium and found that dementia rates were reduced following subsequent prescriptions, suggests that continued lithium treatment is associated with decreased rates of dementia\textsuperscript{65}. 
The pharmacokinetic properties of lithium are reasonably well-characterized\textsuperscript{192, 193}. Lithium is orally administered and is 80-100% bioavailable, depending on formulation; it reaches peak serum concentration in 2-4 hours. Lithium is water-soluble, not protein-bound, and has a volume of distribution of 0.5-1.2L/kg. Notably, lithium crosses the blood-brain barrier. Although plasma and serum levels of lithium are found to be 2-3.6 times higher than those in the cerebrospinal fluid (CSF), lithium levels in the brain have been found to be higher than those in both plasma and serum. Lithium is not metabolized, and is eliminated via the kidney. The clearance rate of lithium is 0.6-2.4L/h and the elimination half-life is 16-30 hours.

In contrast, lithium’s mechanisms of action have yet to be fully elucidated. Following the monoamine hypothesis of depression\textsuperscript{194}, research has been undertaken to elucidate potential actions of lithium on dopaminergic signaling pathways. Application of lithium to rat nucleus accumbens resulted in a decrease in dopamine release; lithium alone had no effect on dopamine release in the medial prefrontal cortex (mPFC), but in the presence of serotonin antagonism, mPFC dopamine release was increased\textsuperscript{195}. In another preclinical study, chronic lithium treatment appeared to reduce potassium-mediated dopamine release, an effect which the authors suggested might explain lithium’s mood-stabilizing effect\textsuperscript{196, 197}. Glutamate is another signaling pathway through which lithium is thought to exert its effect. Pre-treatment with lithium in cell culture inhibited NMDA-dependent $\text{Ca}^{2+}$-influx, possibly through downregulation of subunit phosphorylation, and protected against excitotoxicity\textsuperscript{67}. Looking further downstream, lithium treatment also reduces activation of calpain\textsuperscript{68}, a $\text{Ca}^{2+}$-dependent protease involved in the apoptotic cascade (see Figure 1). Given its putative neuroprotective effects against neurotoxicity, researchers have also examined the effects of lithium pre-treatment in a rodent model of ischemia: they found improved functional outcomes and reduced infarct size in rats that received chronic lithium treatment prior to middle cerebral artery occlusion\textsuperscript{198}. Again in preclinical models, concentrations
of lithium above the therapeutic range increased glutamate release, suggesting excitotoxicity as one mechanism for observed lithium toxicity. On the flip-side, GABAergic signaling, which modulates dopamine and glutamate signaling, is also affected by lithium. In rodent brains, chronic lithium treatment upregulated glutamic acid decarboxylase (GAD) and GABA concentrations, while decreasing dopamine concentrations. Acute lithium treatment has also been shown to increase GABA in the CSF of rodents.

In downstream signaling, lithium alters adenylyl cyclase signal transduction, which is thought to regulate cAMP response element binding (CREB) protein, a transcription factor involved in regulating expression of BDNF and anti-apoptotic Bcl-2. Long-term lithium treatment has been shown to decrease activity of PKC, and expression of PKC and myristoylated alanine-rich C-kinase substrate (MARCKS, see Section 1.4.1.3). Lithium-induced downregulation of PKC activity is thought to underlie lithium’s effects in treatment of mania. Lithium is also implicated in the inhibition of glycogen synthase kinase 3 (GSK-3) activity, either via direct competition with Mg$^{2+}$ binding or upstream activation of protein kinase B (Akt). In a mouse model of neuronal aging, lithium activation of Akt decreased GSK-3 activity and protected against apoptosis. Chronic lithium treatment has also been seen to decrease mRNA expression of pro-apoptotic factors (e.g. p53, Bax) and increase mRNA expression of anti-apoptotic Bcl-2, which could explain decreased cytochrome c release following lithium therapy (see Figure 1). To corroborate these mRNA findings, it has also been shown that protein levels of Bcl-2 are elevated following chronic lithium treatment, and that higher Bcl-2 levels are associated with decreased caspase activity and DNA damage. Lithium treatment increased BDNF in both preclinical models and clinical populations, some in which BDNF levels could be associated with functional outcome. Putative mechanisms and physiological correlates of BDNF are reviewed in Section 1.4.3.
1.4.2.1 Grey matter volume

Given lithium’s putative neuroprotective effects, multiple studies have examined grey matter volumes in lithium-treated bipolar patients. Bearden et al. reported greater grey matter density across various cortical regions, including the cingulate and paralimbic cortices, in lithium-medicated patients compared to lithium-free patients\textsuperscript{204}. Germana et al. reported greater grey matter in the subgenual anterior cingulate gyrus, postcentral gyrus, the hippocampal-amygdala complex and the insula in bipolar patients on lithium treatment compared to those on anticonvulsants or antipsychotics\textsuperscript{205}. Studies by Hajek et al. showed larger hippocampal volumes in lithium-treated bipolar patients compared to lithium-free patients\textsuperscript{206, 207}; larger hippocampal volumes were also associated with lithium treatment in older bipolar patients\textsuperscript{208}. Benedetti et al. demonstrated the association between lithium treatment and a less-active GSK-3β gene promoter genotype with increased grey matter volumes in the frontal lobe\textsuperscript{209}. Meta-analyses have found correlations between prevalence of lithium use and grey matter volume in bipolar patients\textsuperscript{54, 55}. In the meta-analysis by Bora et al., greater prevalence of lithium users in a population was linked to greater grey matter volume in the rostral anterior cingulate cortex\textsuperscript{55}. Similarly, Kempton et al. also reported that greater grey matter volume was correlated with a higher proportion of lithium users in a population\textsuperscript{54}. Furthermore, our recent meta-analysis of cross-sectional data demonstrated that patients taking lithium had greater global grey matter volume compared to lithium-free controls (submitted; see Appendix S2). Likewise, longitudinal studies from Moore et al. and Lyoo et al. have also reported increases in grey matter volume associated with lithium treatment, with Lyoo et al. reporting grey matter volume as a neuro-correlate of clinical improvement\textsuperscript{43-45}. Regional grey matter increases following lithium have also be observed in healthy individuals\textsuperscript{210}. 
1.4.3 Brain-derived neurotrophic factor (BDNF)

BDNF is a protein that plays a role in neuronal proliferation, differentiation, and survival\(^{75}\). In one preclinical study, overexpression of brain derived neurotrophic factor (BDNF) and infusion of exogenous BDNF both led to recruitment of newly-generated neurons to peri-infarct regions and promoted survival of the newly-formed cells\(^{59, 76}\). BDNF also appears to regulate differentiation of neurons born in the adult brain: mice with truncated BDNF had decreased production of GABA synthase and impaired differentiation of de novo cells; similar impairments were seen when TrkB (the receptor for BDNF) was selectively knocked out\(^{211}\). Not only is BDNF important for neuronal development, BDNF can also regulate neuroplasticity and maintain cell survival in the central nervous system (CNS)\(^{212-216}\). Neuronal cultures showed that knockdown of BDNF was deleterious to cell survival\(^{217}\), which may lead to neuro-structural changes. Clinically, a decrease in BDNF has been shown to correlate with decreased hippocampal volume in an aging population\(^{46}\). Preclinical data also support the necessity (and sufficiency) of hippocampal BDNF expression in long-term memory formation\(^{218}\).

A range of preclinical studies have been conducted to examine the role of BDNF in a post-ischemic context. Following ischemic conditions, gene and protein expression of BDNF are increased in the hippocampus\(^{219-223}\). These changes are potentially mediated through activity-dependent mechanisms (i.e. excitatory signaling via NMDA increases BDNF expression, while inhibitory signaling via GABA decreases BDNF expression), and may suggest a role of BDNF in synaptic plasticity\(^{221, 224}\). Furthermore, in preclinical models, microglial secretion of BDNF is thought to reduce neuronal damage\(^{154}\), treatment with BDNF is thought to decrease infarct volume\(^{225}\), and changes in BDNF by exogenous factors are implicated in post-stroke recovery\(^{214, 226-229}\).

Clinically, elevated serum BDNF levels was associated with cognitive impairment in an aging population\(^{77}\). In another aging study, lower serum BDNF was associated with poorer performance
on verbal memory and executive tasks, and a serum BDNF level below 1.5 standard deviations of the mean was associated with MCI\textsuperscript{78}. Serum BDNF levels were also positively correlated to cognition scores in a Parkinson’s cohort\textsuperscript{230}. Furthermore, serum BDNF levels have been reported to be lower in individuals with Alzheimer’s than individuals with mild or no cognitive impairment; this study also demonstrated an association between serum BDNF and cognitive impairment\textsuperscript{231}. However, another study in an Alzheimer’s population reported increased serum BDNF levels in Alzheimer’s and MCI patients\textsuperscript{232}. Longitudinal assessment of Alzheimer’s patients showed limited efficacy of serum BDNF level as a predictor of cognitive decline\textsuperscript{233}. In a smaller stroke study, aerobic exercise following ischemic stroke is associated with higher serum BDNF levels; increased BDNF level positively correlated with better cognitive outcomes\textsuperscript{47}. In another relatively small study, there was no difference in CSF levels of BDNF between ischemic cerebrovascular disease patients and healthy controls; furthermore, BDNF was not correlated with cognitive outcomes\textsuperscript{234}. Considering the heterogeneity of these reports, and the limited clinical evidence for possible associations between BDNF and cognition in a post-stroke context, exploratory studies in this area are very much warranted. In terms of post-stroke depression, there is appreciably more data. One study showed lower serum BDNF levels in depressed patients compared to non-depressed; serum BDNF level also correlated with depression scores\textsuperscript{235}. Another study reported lower serum BDNF levels in depressed group 3-6 months post-stroke, compared to non-depressed stroke patients and healthy controls\textsuperscript{236}. Another study noted that serum BDNF levels at hospital admission was lower in patients that developed post-stroke depression than those who did not, and suggested that serum BDNF levels at admission may be useful for predicting development of post-stroke depression\textsuperscript{237}. Meta-analysis data also supports the finding that patients with post-stroke depression have lower serum BDNF levels\textsuperscript{48}. Taken together, these findings show quite a robust BDNF profile for post-stroke depression.
2. Methods

This study was approved by the Research Ethics Board at Sunnybrook Health Sciences Centre. We received written informed consent from all patients enrolled in the study (see Appendix S1).

Figure 2 Study overview. MRI = magnetic resonance imaging, AE = adverse events.

2.1 Participants

Patients were screened from Sunnybrook Health Sciences Centre, Toronto Rehab Institute, and St John’s Rehab. Patients who met the World Health Organization MONICA Project and National Institute of Neurological Disorders and Stroke (WHO-NINDS) criteria for a recent (<1 year) ischemic cortical stroke (evidenced by MRI report from stroke neurologist) were recruited. Our full list of inclusion and exclusion criteria was as follows:

Inclusion

- ≥40 years old
- speaks and understands English
- MRI or CT evidence of acute cortical ischemic stroke (<12 months)
- Clinical diagnosis of stroke according to WHO-MONICA and WHO-NINDS criteria

Exclusion

- Subarachnoid or intracerebral hemorrhage (which have different etiologies and mechanisms compared to cerebrovascular ischemia\(^ {238-241}\))
- Severe aphasia or dysphasia
- Significant acute medical illness: drug overdose, alcohol abuse, uncontrolled diabetes, untreated hypothyroidism, anemia, severely disturbed liver, kidney, lung, or heart function
• Significant acute neurologic illness: impaired level of consciousness, subdural hematoma, hydrocephalus, brain tumor, Huntington’s chorea, Binswanger’s disease, multiple sclerosis, Parkinson’s disease, progressive supranuclear paralysis
• Psychiatric illnesses: schizophrenia, bipolar disorder, anxiety disorder, dementia
• Initiation of diuretic treatment
• Have not been stable on the same dose of antidepressant for a minimum of 3 months
• Conditions that may contraindicate lithium treatment (including renal dysfunction; >106µM creatinine level; hypocalcemia at <2.2mM) or put subject at risk from MRI procedure

Patients who met the inclusion criteria but had contraindications to lithium treatment or had recently initiated diuretic treatment were also included in the study, but were not prescribed lithium.

Demographic data—including age, sex, marital status, level of education, living situation, employment, and history of depression—were collected at screening. Date of stroke, current medications, and past medical history were extracted from patient charts once written informed consent was received from patients.

2.2 Treatment

After obtaining informed consent from eligible patients, lithium carbonate (Carbolith®, Valeant Canada Ltd) was administered open-label for 60 days, with target serum lithium concentrations of 0.4-0.8mmol/L. Patients were monitored over course of treatment for adverse events using the Udvalg for Kliniske Undersogelser (UKU) Side Effects Rating Scale242; in the event of intolerable adverse events, patients discontinued lithium pharmacotherapy.

2.3 Imaging

2.3.1 Magnetic resonance imaging

Enrolled patients underwent magnetic resonance imaging (MRI) under direction of research MRI technologists at Sunnybrook Health Sciences Centre during their baseline and termination visits.
Images were acquired using a 3.0T General Electric MR750 scanner (GE Healthcare) with an 8-channel head coil. After a 3-plane localizer scan, T1-weighted images were obtained using a 3D fast spoiled gradient echo (fSPGR) protocol (TR=8.1ms, TE=MinFull, flip angle=8, FOV=22cm, slice thickness=1.0mm; see Imaging Glossary for abbreviations). T2/PD images were subsequently obtained using a dual-echo 2D fast spin echo (fSE-XL) protocol (TR=2500ms, TE=11.1 and 90ms, FOV=22cm, slice thickness=3.0mm). Images were sent to the LC Campbell Cognitive Neurology Research Unit for analysis.

2.3.2 Image processing

MRI images were assessed through Semi-Automatic Brain Region Explorer (SABRE)\textsuperscript{243}. In brief, total intracranial volume was extracted from T1 images using Total Intracranial Vault Extraction (TIV-E), which used a T2/PD-derived mask and was supplemented by manual editing of the subarachnoid parenchyma and CSF. Extracted T1 images were then used to divide the brain into different tissue types (e.g. grey matter, white matter, CSF) based on signal intensity; segmentation masks were manually edited to ensure fidelity to image. The brain was also divided into different regions after the images were mapped to Talaraich space using manually annotated anatomical landmarks (e.g. anterior commissure, posterior commissure, posterior edge, central canal, pre-occipital notch, central sulcus, occipital-parietal sulcus, Sylvian fissure). Hippocampal volumes were separately obtained using the Sunnybrook Hippocampal Volumetry tool, a fully-automated segmentation method based on multiple template libraries\textsuperscript{244}. Stroke lesions were visually determined by a trained image analyst; stroke core volume was traced using T1 images while penumbra was traced using T2 images. Lesion Explorer\textsuperscript{245} was used to evaluate subcortical and periventricular hyperintensities in T2/PD images. All imaging analyses were conducted by research staff at the LC Campbell Cognitive Neurology Research Unit and verified by an experienced neuroradiologist.
Figure 3 Image processing pipeline using T1 images (A), which were segmented into white matter, grey matter, CSF (B), and divided into SABRE regions using anatomical landmarks (C).

2.4 Stroke severity

Using the National Institutes of Health Stroke Scale (NIHSS)\textsuperscript{246}, stroke severity was assessed at patient’s baseline visit by trained study staff. The NIHSS quantitatively measures stroke-related neurological deficits such as impaired consciousness, facial palsy, language deficits and motor impairments. It is quick to administer, has good inter-rater reliability and has been shown to strongly predict stroke outcome\textsuperscript{246, 247}. The NIHSS has previously been used by the National Institute of Neurological Disorders and Stroke (NINDS) in stroke-related clinical trials\textsuperscript{34, 248}.

2.5 Clinical evaluations

To assess cognitive outcomes, we used a modified version of NINDS-CSN 30-Minute Neuropsychological Protocol\textsuperscript{249}. This protocol was selected as it was quick to conduct, and was a standardized clinical battery for the post-stroke population. In this study, all components of the protocol were performed once at baseline and once at termination. The protocol’s components are described in brief below.
2.5.1 Category fluency

Category fluency was assessed by “Animal Naming,” a test in which patients were asked to name as many animals as they could within one minute. Greater number of animals named reflected better performance on this task. This test is widely used in elderly clinical populations, and has normative data available.

2.5.2 Phonemic fluency

Phonemic fluency was assessed by the Controlled Oral Word Association Test (COWAT). Patients were given a letter from a set of 3 (e.g. FAS) and asked to name as many words as they could within one minute that began with that letter; this process was repeated until all letters in the set have been tested. Greater number of words correctly generated reflected better performance on this task. As with Animal Naming, COWAT has normative data available.

2.5.3 Non-verbal executive function

Executive function was also evaluated using Digit Symbol-Coding from the Wechsler Adult Intelligence Scale, as Digit Symbol-Coding provides a measure of processing speed and activation. The patient is given a set of 9 symbols, each corresponding with a number from 1-9 (Figure 4). Below the paired set, there are rows of paired boxes, where the boxes on the top of each pair contain a number from 1-9, and the boxes on the bottom are blank. Patients are asked to fill in as many empty boxes as they can with symbols corresponding to the displayed numbers, without skipping, within 2 minutes. Greater number of correctly filled boxes reflected better performance on this task. As with the previous tests, COWAT has normative data available.
We also elected to include the Trail-Making Test, Part B (TMT-B) in our assessment of executive function, as it was a useful measure of processing speed and set shifting. The Trail-Making Test was recommended as a supplemental assessment to the 30-Minute Neuropsychological Protocol. In this task, patients are asked to connect a series of numbers and letters in alternating, ascending order (e.g. 1-A-2-B-3-C). Shorter amount of time taken to complete this task reflected better performance. As with the previous tests, TMT-B has normative data available.

2.5.4 Verbal learning and memory

The revised Hopkins Verbal Learning Test (HVLT-R) Form 1 was used to assess verbal learning and memory. The HVLT-R is a list-learning test consisting of 12 words that can be grouped into 3 categories (e.g. animal, place, precious stone). For the learning component, the list of 12 words was read aloud, and immediately thereafter, the patient was asked to orally recall as many of the listed words as they could. This process was repeated twice more for a total of 3 learning trials. At the end of the 3 learning trials, the patient was set onto other (non-verbal) tasks for 20 minutes.

After 20 minutes, the patient was asked to recall as many words as they could from the list. This was the delayed recall component of the task. After the delayed recall component, a list of 24 words, containing words from the initial list as well as distractors, was read aloud. As each word from the list was read, the patient was asked to identify whether or not the word appeared on the original list. The patient’s ability to recognize words from the initial list was evaluated by subtracting the number of false positives (i.e. words that the patient identified as being from the
original list but were actually distractors) from the number of true positives (i.e. words correctly identified as being from the original list). This cued recall component may be sensitive to vascular cognitive impairment.

In both the learning and delayed recall components, greater number of words recalled reflected better performance in the task. The HVLT-R is relatively quick to administer and has normative data available.

**2.5.5 Depressive symptoms**

The 30-Minute Neuropsychological Protocol recommended the Center for Epidemiological Studies-Depression Scale (CES-D) for the evaluation of depressive symptoms. However, we have selected the Hamilton Depression Rating Scale (HAM-D) for our assessment. The HAM-D has the advantage of being observer-rated, which may mitigate some of the age, mood, and personality influences on self-rated scales\(^{250}\). It has also shown good sensitivity and specificity in a post-stroke population\(^{251}\). It consists of a 17-item questionnaire, where items are either scored out of 2 or 4. A higher score on this test indicated more depressive symptoms.

**2.5.6 Supplemental assessments**

We also conducted supplemental assessments suggested for the 30-Minute Neuropsychological Protocol, namely the standardized Mini Mental State Examination (sMMSE)\(^{252}\), and the Montreal Cognitive Assessment (MoCA)\(^{253}\). Both these assessments were used to evaluate global cognition, whereby a higher score indicated better performance.

**2.6 Serum analyses**

Blood was drawn from patients during their baseline, one-week interim, one-month interim, and termination visits. Blood was either drawn by trained study staff or phlebotomists at Sunnybrook
Specimen Collection Centre. 35mL of blood was collected at baseline and termination; 10mL of blood was collected at each of the interim visits.

2.6.1 Serum lithium and creatinine

For each visit, whole blood was sent to the Department of Clinical Pathology for analysis of serum lithium and creatinine levels. The target range for serum lithium levels was 0.4-0.8mM, which was the recommended concentration range for elderly patients\(^{254}\). Serum lithium levels outside this range prompted adjustments of the patient’s lithium dose as per study physician’s discretion. Serum creatinine was monitored to ensure there was no renal impairment over the course of the study; renal impairment was indicated by creatinine levels exceeding 106µM.

2.6.2 Serum brain-derived neurotrophic factor

Blood collected from patients during baseline and termination visits was used to determine serum BDNF levels. Whole blood samples were left to settle for half an hour before fractionating via centrifugation (10 minutes). Serum was aliquoted for BDNF assays; aliquoted samples were stored at -80°C until time of assay.

Aliquoted serum was thawed on ice, then vortexed briefly to redistribute any sediment that may have formed during storage. BDNF in serum was assessed using the ChemiKine™ Brain Derived Neurotrophic Factor (BDNF) Sandwich ELISA Kit (Millepore). For this assay, serum samples were diluted 1:400 in a sample diluent provided by the manufacturer. The assay was run per manufacturer’s protocol. Briefly, standards and diluted samples were incubated overnight on the ELISA plate at 4°C. An in-house wash buffer (0.05% TWEEN 20 in DPBS) and automatic plate washer was used to wash the plate. Biotinylated mouse anti-BDNF monoclonal antibody was added to the wells and the assay was incubated on a plate shaker at room temperature for 3 hours. The plate was then again washed as described above. Streptavidin-HRP conjugate was added to
each well and the assay was incubated on a plate shaker for 1 hour. TMB was added to the wells and the reaction was incubated for 15 minutes, at which time a stop solution was added. The ELISA plate was analyzed using a microplate reader (Synergy H1, BioTek Instruments, Inc.) and Gen5 software (BioTek Instruments, Inc.). All standards and samples were measured in triplicate; the average reading between triplicated samples was used to calculate concentration on the standard curve. BDNF concentration was expressed as pictogram per milliliter.

2.7 Data analysis

All data analyses were conducted in IBM SPSS Statistics 24. Using descriptive statistics, demographics such as age and sex were characterized; baseline values for days since stroke, NIHSS score, stroke volume, sMMSE and MoCA, HAM-D were also calculated for the entire group. Other clinical characteristics such as body mass index, number of concomitant medications and diabetes status were also noted. Stroke locations across patients were also tabulated: in cases where one patient had multiple infarcts, a tally was marked in each region where an infarct was observed.

Average cumulative lithium dose (i.e. sum of lithium doses across 60 days, presented as lithium dose per day), cumulative serum lithium levels (i.e. area under the curve of serum lithium level over 60 days, presented as maintained/average day-to-day serum lithium level), and number of treatment completers were also calculated using descriptive statistics. Furthermore, we stratified patients by lithium dose per day (henceforth referred to as “dose group”) or maintained serum lithium levels (henceforth referred to as “serum level group”). As it has been previously recommended that elderly bipolar patients should receive 300-600mg of lithium per day to achieve therapeutic effects\textsuperscript{193}, we selected 300mg per day as the cutoff value between our two dose groups: <300mg per day, \geq300 per day. Similarly, as it has been recommended that serum lithium levels
in elderly patients be maintained between 0.4-0.8mM therapeutically\textsuperscript{254}, we selected 0.4mM as the cutoff value between our two serum level groups: <0.4mM, 0.4-0.8mM. We were concerned that patients who fell below these cutoffs might not have experienced therapeutic effects, and thus we elected to analyze them separately from those who met the cutoffs. Demographic data between dose groups were compared using a two-tailed independent T-test for continuous variables, and a one-tailed Fisher’s Exact Test for discrete variables. The same statistical methods were used to compare between serum level groups.

2.7.1 Primary hypothesis

*Lithium treatment will be associated with changes in global grey matter volume.*

Change in global grey matter volume was determined using a two-tailed paired T-test comparing global grey matter volumes at baseline and termination.

We postulated that potential changes in global grey matter volume over time might be obfuscated by the heterogeneity in lithium dosage across patients. Thus, we conducted further analyses on the relationship between lithium dose and global grey matter volume. As both variables were normally distributed (Shapiro-Wilk, p>0.05), correlational analysis was conducted on cumulative lithium dose and percent change in global grey matter volume using Pearson’s correlation. Furthermore, interaction between dose group (<300mg per day vs. ≥300 per day) and change in global grey matter volume was assessed using a repeated-measures analysis of variance (ANOVA), with dose group as the between-subjects factor. Should there be an interaction, the direction of the interaction would be evaluated by comparing mean percent global grey matter change between dose groups using a two-tailed independent T-test. A repeated-measures analysis of covariance (ANCOVA) was conducted to adjust for effects of age and baseline cognition.
Similarly, Pearson’s correlation was used to assess associations between cumulative serum levels of lithium and percent change in global grey matter volume. Again, interaction between serum level group (<0.4mM vs. 0.4-0.8mM) and change in global grey matter volume was assessed using a repeated-measures ANOVA, with serum level group as the between-subjects factor.

2.7.2 Secondary hypothesis

*Lithium treatment will be associated with changes in cognitive and mood outcomes. Changes in these outcomes will in turn correlate with changes in grey matter volume.*

Raw scores obtained from Animal Naming, COWAT, Digit Symbol-Coding, TMT-B, and HVLT-R components were converted into z-scores using sample-appropriate normative data.

Changes in global cognition were assessed using raw scores from sMMSE and MoCA, where scores from baseline were compared with scores from termination using a two-tailed paired T-test. The interaction between cumulative lithium dose or serum lithium levels and changes in these measures was analyzed using a repeated-measures ANCOVA, with cumulative lithium dose or serum lithium levels as a covariate. The interaction between dose group or serum level group and changes in these measures was assessed using a repeated-measures ANOVA, with dose group or serum level group as a covariate.

Changes in executive function were evaluated using the z-scores obtained from Animal Naming, COWAT, Digit Symbol-Coding, and TMT-B. For each assessment, z-scores were compared between baseline and termination using a two-tailed paired T-test. The interaction between cumulative lithium dose or serum lithium levels and these scores was analyzed using a repeated-measures ANCOVA, with cumulative lithium dose or serum lithium levels as a covariate. The interaction between dose group or serum level group and changes in these scores was assessed
using a repeated-measures ANOVA, with dose group or serum level group as the between-groups factor.

Changes in verbal learning and memory were evaluated using the z-scores obtained from components of the HVLT-R. Z-scores for the learning component were compared between baseline and termination using a two-tailed paired T-test. The interaction between cumulative lithium dose or serum lithium levels and changes in this score was analyzed using a repeated-measures ANCOVA, with cumulative lithium dose or serum lithium levels as a covariate. The interaction between dose group or serum level group and change in this score was assessed using a repeated-measures ANOVA, with dose group or serum level group as the between-groups factor. The same statistical methods were employed to assess z-scores from the delayed recall and recognition components.

Changes in depressive mood symptoms were assessed using raw scores from HAM-D, where scores from baseline were compared with scores from termination using a two-tailed paired T-test. The interaction between cumulative lithium dose or serum lithium levels and changes in HAM-D score was analyzed using a repeated-measures ANCOVA, with cumulative lithium dose or serum lithium levels as a covariate. The interaction between dose group or serum level group and changes in this measure was assessed using a repeated-measures ANOVA, with dose group or serum level group as the between-groups factor.

2.7.3 Exploratory hypothesis

Lithium treatment will be associated with changes in serum BDNF levels. Changes in serum BDNF levels will be associated with changes in grey matter volume and clinical outcomes.
Change in serum BDNF levels was determined using a two-tailed paired T-test comparing BDNF levels at baseline and termination. Further analyses were conducted to examine the interaction between change in serum BDNF levels and cumulative lithium dose or cumulative serum lithium level using repeated-measures ANCOVA, with cumulative lithium dose or serum lithium level as a covariate. We also investigated the interaction between dose group or serum level group with change in serum BDNF levels using a repeated-measures ANOVA, with dose group or serum level group as the between-subject factor.
3. Results

3.1 Study demographics

12 ischemic stroke patients were recruited to the study. Of these patients, 8 patients completed 60 days of lithium treatment, 3 patients discontinued pharmacotherapy early due to adverse events (Table 2), and 1 patient was screen dropped due to contraindication to lithium therapy (impaired renal function). Demographics of the population are presented in Tables 1-6.

Table 1 Characteristics of study population (n=12)

<table>
<thead>
<tr>
<th>Population characteristics</th>
<th>Mean (SD) or % (n) or Median (inter-quartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>71.1±11.9</td>
</tr>
<tr>
<td>Males, %</td>
<td>50.0 (6)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.7 (2.4)</td>
</tr>
<tr>
<td>Concomitant medications, n</td>
<td>5.8 (2.3)</td>
</tr>
<tr>
<td>Comorbid diabetes, %</td>
<td>25 (3)</td>
</tr>
<tr>
<td><strong>Stroke characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Time between stroke and study enrolment, days</td>
<td>90.2±65.3</td>
</tr>
<tr>
<td>Median NIHSS score at enrolment¹</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>Stroke volume at enrolment, mL</td>
<td>6.2±8.8</td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Median baseline sMMSE</td>
<td>28 (25-29)</td>
</tr>
<tr>
<td>Median baseline MoCA</td>
<td>22.5 (16-25.5)</td>
</tr>
<tr>
<td>Median baseline HAM-D</td>
<td>2 (0-5)</td>
</tr>
</tbody>
</table>

¹Baseline NIHSS data was only available for 11 patients.

Table 2 Adverse events in study population (n=12)

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Frequency (n)</th>
<th>Number of discontinuations (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accommodation disturbances</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Concentration difficulties</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Decreased salivation</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Decreased sexual desire</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Gait disturbances</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Symptom</td>
<td>Occurrence</td>
<td>Frequency</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Hypokinesia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Increased salivation</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lethargy</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Mood changes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Muscle cramp</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Orthostatic dizziness</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Physical dependence (not sustained)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Polyuria</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Restlessness</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sleep changes</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Tremor</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Weight gain</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Weight loss</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3 Stroke locations across patients (n=12)

<table>
<thead>
<tr>
<th>Location of infarct</th>
<th>Number of patients who had an infarct in each location, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left cerebral hemisphere</strong></td>
<td></td>
</tr>
<tr>
<td>Basal ganglia and thalamus</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Frontal</td>
<td>4 (33.3)</td>
</tr>
<tr>
<td>Parietal</td>
<td>8 (66.7)</td>
</tr>
<tr>
<td>Occipital</td>
<td>1 (8.33)</td>
</tr>
<tr>
<td>Temporal</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td><strong>Right cerebral hemisphere</strong></td>
<td></td>
</tr>
<tr>
<td>Basal ganglia and thalamus</td>
<td>1 (8.33)</td>
</tr>
<tr>
<td>Frontal</td>
<td>5 (41.7)</td>
</tr>
<tr>
<td>Parietal</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Occipital</td>
<td>2 (16.7)</td>
</tr>
<tr>
<td>Temporal</td>
<td>3 (25.0)</td>
</tr>
</tbody>
</table>

NB: Several patients had multiple infarcts.

Table 4 Lithium treatment received over study duration (n=12)

<table>
<thead>
<tr>
<th>Treatment characteristics</th>
<th>Mean (SD) or % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose per day, mg</td>
<td>231.0±150.5</td>
</tr>
<tr>
<td>Maintained serum lithium level, mM</td>
<td>0.275±0.190</td>
</tr>
<tr>
<td>Completed full course of lithium treatment, %</td>
<td>66.7 (8)</td>
</tr>
</tbody>
</table>

Table 5 Demographics across lithium dose groups (<300mg/day vs. ≥300mg/day)
<table>
<thead>
<tr>
<th></th>
<th>Lithium &lt;300mg/day (n=8) Mean±SD or % (n)</th>
<th>Lithium ≥300mg/day (n=4) Mean±SD or % (n)</th>
<th>Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>76.0±7.5</td>
<td>61.3±13.9</td>
<td>t=2.45</td>
<td>p=0.035*</td>
</tr>
<tr>
<td>Males, %</td>
<td>50.0 (4)</td>
<td>50.0 (2)</td>
<td>p=1.00</td>
<td></td>
</tr>
<tr>
<td>Time between stroke and study enrolment, days</td>
<td>103.4±73.0</td>
<td>63.75±42.7</td>
<td>t=0.99</td>
<td>p=0.346</td>
</tr>
<tr>
<td>Patients with NIHSS symptom at enrolment¹, %</td>
<td>42.9 (3)</td>
<td>75.0 (3)</td>
<td>p=0.545</td>
<td></td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>7.12±10.2</td>
<td>4.30±6.3</td>
<td>t=0.50</td>
<td>p=0.626</td>
</tr>
<tr>
<td>Baseline sMMSE</td>
<td>26.0±3.7</td>
<td>28.8±1.0</td>
<td>t=-1.43</td>
<td>p=0.183</td>
</tr>
<tr>
<td>Baseline MoCA</td>
<td>18.9±4.0</td>
<td>26.5±2.1</td>
<td>t=-4.33</td>
<td>p=0.002*</td>
</tr>
<tr>
<td>Dose per day, mg</td>
<td>152.8±117.9</td>
<td>387.5±40.1</td>
<td>t=5.07</td>
<td>p=0.001*</td>
</tr>
<tr>
<td>Maintained serum lithium level, mM</td>
<td>0.23±0.22</td>
<td>0.37±0.09</td>
<td>t=-1.21</td>
<td>p=0.253</td>
</tr>
<tr>
<td>Did not start/complete lithium treatment, %</td>
<td>50.0 (4)</td>
<td>0.00 (0)</td>
<td>p=0.208</td>
<td></td>
</tr>
</tbody>
</table>

¹Baseline NIHSS data was only available for 11 patients.

*Two-tailed significance (p≤0.05) in Student’s T-test of differences between dose groups (<300mg/day vs. ≥300mg/day). Discrete characteristics assessed via one-tailed Fisher’s Exact Test.

Table 6 Demographics across serum lithium level groups (<0.4mM vs. 0.4-0.8mM)

<table>
<thead>
<tr>
<th></th>
<th>Serum lithium levels &lt;0.4mM (n=8) Mean±SD or % (n)</th>
<th>Serum lithium levels 0.4-0.8mM (n=4) Mean±SD or % (n)</th>
<th>Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>74.0±8.0</td>
<td>65.3±17.3</td>
<td>t=0.96</td>
<td>p=0.395</td>
</tr>
<tr>
<td>Males, %</td>
<td>62.5 (5)</td>
<td>25.0 (1)</td>
<td>p=0.545</td>
<td></td>
</tr>
<tr>
<td>Time between stroke and study enrolment, days</td>
<td>118.5±62.7</td>
<td>33.5±7.6</td>
<td>t=3.78</td>
<td>p=0.006*</td>
</tr>
<tr>
<td>Patients with NIHSS symptom at enrolment¹, %</td>
<td>42.9 (3)</td>
<td>75.0 (3)</td>
<td>p=0.545</td>
<td></td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>8.57±10.1</td>
<td>1.40±1.5</td>
<td>t=1.96</td>
<td>p=0.087</td>
</tr>
<tr>
<td>Baseline sMMSE</td>
<td>26.1±3.5</td>
<td>28.5±2.4</td>
<td>t=1.20</td>
<td>p=0.257</td>
</tr>
<tr>
<td>Baseline MoCA</td>
<td>19.8±5.2</td>
<td>24.8±3.0</td>
<td>t=1.76</td>
<td>p=0.108</td>
</tr>
<tr>
<td>Dose per day, mg</td>
<td>185.9±165.7</td>
<td>321.3±52.1</td>
<td>t=1.56</td>
<td>p=0.149</td>
</tr>
<tr>
<td>Maintained serum lithium level, mM</td>
<td>0.18±0.14</td>
<td>0.48±0.09</td>
<td>t=-3.90</td>
<td>p=0.003*</td>
</tr>
<tr>
<td>Did not start/complete lithium treatment, %</td>
<td>50.0 (4)</td>
<td>0.00 (0)</td>
<td>p=0.208</td>
<td></td>
</tr>
</tbody>
</table>

¹Baseline NIHSS data was only available for 11 patients.

*Two-tailed significance (p≤0.05) in Student's T-test of differences between serum level groups (<0.4mM vs. 0.4-0.8mM). Discrete characteristics assessed via one-tailed Fisher’s Exact Test.
3.2 Primary analysis

3.2.1 Global grey matter volume

We compared global grey matter volumes at baseline with volumes after 60 days of lithium treatment. There was no significant difference in grey matter volumes between baseline and termination ($t=1.977$, $p=0.074$).
**Figure 5** T1 MRI images at baseline and termination for patient who received ≥300mg lithium per day (A) and patient who received <300mg lithium per day (B). Colored areas on T1 images indicate stroke cores.

### 3.2.1.1 Interaction with lithium dose

Bivariate correlation analysis revealed that there was no correlation between cumulative lithium dose and percent change in grey matter volume (r=0.447, p=0.145). Comparing change in global grey matter volume over time between dose groups, we saw a significant interaction between time and dose group (F=14.25, p=0.004). This interaction remains significant after adjusting for age (F=10.72, p=0.01) or baseline MoCA scores (F=16.13, p=0.003). Mean percent change in global grey matter volume was significantly different between the ≥300mg per day group (0.63±0.61) and <300mg per day group (-1.52±1.03) (t=−3.80, p=0.003).
Figure 6 Percent change in global grey matter volume was significantly different between the \( \geq 300 \text{mg per day} \) group and \(< 300 \text{mg per day} \) group \((t=-3.80, p=0.003)\).

Bivariate analysis on cumulative serum lithium levels and percent change in global grey matter volume did not demonstrate a significant correlation \((r=-0.11, p=0.974)\). Additionally, we found no significant interaction between change in grey matter volume and serum lithium level group \((F=0.194, P=0.669)\).

3.2.2 Post-hoc analyses

Given that there was an interaction between dose group and global grey matter volume, we set out to determine if this overall interaction was driven by interactions in specific neuroanatomical
regions. We did not specify a particular neuroanatomical region a priori, as bipolar literature has shown many different regions associated with lithium therapy. We conducted post-hoc analyses of regional grey matter volume change over time using repeated-measures ANOVA, with lithium dose group as the between-subjects factor. Within both the left and right parietal regions, there was a significant interaction between dose group and grey matter volume; there were no significant interactions in any other region. These results are presented in (Table 7).

**Table 7** Interaction between dose group (<300mg/day vs. ≥300mg/day) and regional grey matter volume change across time

<table>
<thead>
<tr>
<th>Neuroanatomical region</th>
<th>Lithium &lt;300mg/day (n=8)</th>
<th>Lithium ≥300mg/day (n=4)</th>
<th>Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (mL±SD)</td>
<td>Termination (mL±SD)</td>
<td>Baseline (mL±SD)</td>
<td>Termination (mL±SD)</td>
</tr>
<tr>
<td><strong>Left cerebral hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal ganglia and thalamus</td>
<td>10.64±1.44</td>
<td>11.10±1.82</td>
<td>11.46±1.36</td>
<td>11.91±0.53</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.75±0.37</td>
<td>2.73±0.39</td>
<td>2.86±0.50</td>
<td>2.85±0.51</td>
</tr>
<tr>
<td>Parietal</td>
<td>87.38±8.99</td>
<td>86.70±8.76</td>
<td>93.40±7.06</td>
<td>94.65±5.90</td>
</tr>
<tr>
<td>Occipital</td>
<td>58.35±8.23</td>
<td>57.49±9.11</td>
<td>64.17±9.05</td>
<td>64.74±9.53</td>
</tr>
<tr>
<td>Temporal</td>
<td>66.79±10.30</td>
<td>65.74±10.45</td>
<td>77.20±6.42</td>
<td>76.98±7.45</td>
</tr>
<tr>
<td><strong>Right cerebral hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal ganglia and thalamus</td>
<td>10.63±1.74</td>
<td>11.11±2.03</td>
<td>12.02±2.11</td>
<td>12.92±1.43</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.90±0.32</td>
<td>2.88±0.34</td>
<td>3.02±0.51</td>
<td>3.05±0.48</td>
</tr>
<tr>
<td>Frontal</td>
<td>87.69±7.67</td>
<td>86.62±6.49</td>
<td>94.60±12.48</td>
<td>96.24±9.58</td>
</tr>
<tr>
<td>Parietal</td>
<td>59.30±6.44</td>
<td>58.18±6.60</td>
<td>63.61±10.42</td>
<td>64.07±11.16</td>
</tr>
<tr>
<td>Occipital</td>
<td>29.61±4.36</td>
<td>28.55±3.69</td>
<td>34.37±5.77</td>
<td>33.45±5.96</td>
</tr>
<tr>
<td>Temporal</td>
<td>72.27±10.48</td>
<td>70.91±10.67</td>
<td>79.31±6.43</td>
<td>79.13±6.08</td>
</tr>
</tbody>
</table>

*Significant interaction within repeated measures analyses of variance (ANOVA) between dose group and change in grey matter volume over time (p<0.05).

### 3.2.2.1 Stroke volumes

Given that there was only a significant interaction between dose and parietal grey matter volumes, it was prudent to explore if the presence of a parietal stroke might have influenced this interaction. Of 12 patients, 6 had evidence of left parietal stroke, 1 had evidence of right parietal stroke, 2 had
evidence of bilateral parietal strokes, and 3 had no evidence of a parietal stroke. Interaction between change in parietal grey matter volumes and dose group were evaluated with repeated measures ANCOVA, with stroke presence (binary) or stroke volume as a covariate. In the left parietal lobe, the interaction between dose group and change in grey matter volume remained significant after adjusting for presence of left parietal stroke (F=5.47, p=0.044); it also remained significant after adjusting for change in left parietal stroke volume (F=5.68, p=0.041). In the right parietal lobe, interaction between dose group and change in grey matter volume remained significant after adjusting for presence of right parietal stroke (F=6.08, p=0.036) but was not significant after adjusting for change in right parietal stroke volume (F=3.35, p=0.10).

3.3 Secondary analyses

3.3.1 Global cognition

There was no significant difference in sMMSE scores between baseline and termination (t=0.252, p=0.806), even after adjusting for cumulative lithium dose (F=0.098, p=0.760) or comparing between dose groups (F=0.029, p=0.868). Likewise, there was no significant difference in MoCA scores between baseline and termination (t=0.688, p=0.506), even after adjusting for cumulative lithium dose (F=0.091, p=0.769) or comparing between dose groups (F=0.283, p=0.606).

Adjusting for cumulative serum levels of lithium (F=0.409, p=0.537) or comparing between serum level groups (F=0.065, p=0.804) also did not reveal significant differences between baseline and termination sMMSE scores. Likewise, no significant differences in MoCA scores were found after adjusting for cumulative serum levels of lithium (F=0.023, p=0.883) or comparing between serum level groups (F=0.537, p=0.480).

3.3.2 Executive function

Table 8 Z-scores from clinical assessments of executive function at baseline and termination
## Clinical assessment

<table>
<thead>
<tr>
<th>Category</th>
<th>Baseline (score±SD)</th>
<th>Termination (score±SD)</th>
<th>Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category fluency</td>
<td>-0.59±0.76</td>
<td>-0.37±0.97</td>
<td>t=-1.75</td>
<td>p=0.107</td>
</tr>
<tr>
<td>Phonemic fluency</td>
<td>-0.82±1.09</td>
<td>-0.49±0.84</td>
<td>t=-1.34</td>
<td>p=0.207</td>
</tr>
<tr>
<td>Digit Symbol-Coding</td>
<td>-0.38±1.14</td>
<td>-0.11±0.85</td>
<td>t=-1.39</td>
<td>p=0.193</td>
</tr>
<tr>
<td>Trailmaking Test, Part B</td>
<td>-0.71±1.22</td>
<td>-0.58±1.11</td>
<td>t=-0.66</td>
<td>p=0.526</td>
</tr>
</tbody>
</table>

Statistics derived from paired Student’s T-test of differences between baseline and termination.

Although there was no significant difference in category fluency between baseline and termination (Table 8), this difference became significant after adjusting for cumulative lithium dose (F=5.26, p=0.045); score at termination (-0.37±0.97) was greater than score at termination (-0.59±0.76). It was also significant after adjusting for cumulative serum lithium level (F=8.54, p=0.015). However, there were no interactions between change in category fluency and either cumulative lithium dose (F=2.38, p=0.154) or cumulative serum lithium level (F=4.68, p=0.056). There was no significant difference in category fluency between baseline and termination when comparing dose groups (F=2.15, p=0.173) or serum level groups (F=1.63, p=0.231).

There was no significant difference in phonemic fluency between baseline and termination (Table 8), even after adjusting for cumulative lithium dose (F=0.412, p=0.535) or cumulative serum lithium level (F=2.41, p=0.152). Furthermore, there was no significant difference when comparing between dose groups (F=2.41, p=0.152) or serum level groups (F=1.11, p=0.317).

There was no significant difference in Digit Symbol-Coding between baseline and termination (Table 8). Adjusting for cumulative lithium dose (F=5.73, p=0.038) or cumulative serum lithium level (F=6.23, p=0.032) revealed a significant difference in Digit Symbol-Coding between baseline and termination. However, there were no interactions between change in Digit Symbol-Coding and either cumulative lithium dose (F=3.48, p=0.092) or cumulative serum lithium level (p=3.86, p=0.078). There was no difference when comparing between dose groups (F=1.10, p=0.319) or serum level groups (F=0.830, p=0.384).
Ten out of twelve patients were able to complete the TMT-B. Of these patients, there was no significant difference in the TMT-B between baseline and termination, even after adjusting for cumulative lithium dose (F=0.064, p=0.807) or cumulative serum lithium level (F=0.044, p=0.838). Furthermore, there was no significant difference when comparing between dose groups (F=0.175, p=0.687) or serum level groups (F=0.407, p=0.541).

3.3.3 Verbal learning and memory

Table 9 Z-scores from HVLT-R components at baseline and termination

<table>
<thead>
<tr>
<th>HVLT-R component</th>
<th>Baseline (score±SD)</th>
<th>Termination (score±SD)</th>
<th>Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning</td>
<td>-1.44±0.61</td>
<td>-0.73±0.88</td>
<td>t=-3.19</td>
<td>p=0.009*</td>
</tr>
<tr>
<td>Delayed Recall</td>
<td>-1.32±0.97</td>
<td>-0.88±0.73</td>
<td>t=-1.26</td>
<td>p=0.236</td>
</tr>
<tr>
<td>Recognition</td>
<td>-1.23±1.14</td>
<td>-0.68±1.21</td>
<td>t=-1.80</td>
<td>p=0.100</td>
</tr>
</tbody>
</table>

*Two-tailed significance (p≤0.05) in Student’s T-test of differences between baseline and termination.

Scores from HVLT-R’s learning component were significantly higher at termination than baseline. However, there was no interaction between this difference and cumulative lithium dose (F=2.98, p=0.115), dose group (F=2.43, p=0.150), cumulative serum lithium level (F=0.051, p=0.827) or serum level group (F=0.117, p=0.740).

There was no significant difference in delayed recall scores between baseline and termination. However, when adjusting for cumulative lithium dose, there was an interaction between cumulative dose and change in delayed recall scores (F=4.97, p=0.050); using a bivariate analysis, we saw that greater cumulative lithium dose was positively correlated with a more positive change in delayed recall score (r=0.576, p=0.05). There was no significant interaction between change in delayed recall score and dose group (F=0.917, p=0.361), cumulative serum lithium level (F=1.53, p=0.244), or serum level group (F=0.202, p=0.663).
Greater cumulative lithium dose was positively correlated with a more positive change in delayed recall score ($r=0.576$, $p=0.05$).

There was no significant difference in recognition discrimination index between baseline and termination. However, the difference became significant after adjusting for cumulative serum lithium level ($F=6.11$, $p=0.033$). There was no difference after adjusting for cumulative lithium dose ($F=1.20$, $p=0.300$); there was also no difference when comparing between dose groups ($F=3.18$, $p=0.105$) or serum level groups ($F=1.62$, $p=0.233$). There were no interactions between change in recognition discrimination index and any of the covariates.
3.3.3.1 Post-hoc analyses

Since lithium dose interacted with both change in delayed recall scores and change in global grey matter volume, further analysis was conducted to determine if there is an association between delayed recall scores and global grey matter volume. Given that both variables were normally distributed (Shapiro-Wilk, p>0.05), correlation between change in delayed recall z-scores and percent change in global grey matter volume was analyzed using Pearson’s correlation. There was no association between change in delayed recall scores and percent change in global grey matter volume (r=−0.132, p=0.683).

3.3.4 Post-stroke depression

Of 12 patients, 9 patients presented with depressive symptoms at some point in the study (as assessed by the HAM-D; HAM-D≥1). In these patients, there was no significant change in depression from baseline to termination (t=1.33, p=0.222), even after adjusting for cumulative lithium dose (F=0.310, p=0.595) or cumulative serum lithium levels (F=0.393, p=0.551). Similarly, there was no significant change in depression score when comparing between dose groups (F=2.30, p=0.173) or serum level groups (F=2.30, p=0.173). There were no interactions between change in HAM-D score and any of the covariates.

3.4 Exploratory hypothesis

There was no significant difference in serum BDNF concentrations between baseline and termination (t=−0.357, p=0.728), even adjusting for cumulative lithium dose (F=0.440, p=0.522) or cumulative serum lithium levels (F=0.037, p=0.851). Additionally, there was no difference in change in serum BDNF levels when comparing between dose groups (F=0.026, p=0.875) and serum level groups (F=0.926, p=0.359).
However, there was one statistical outlier (i.e. data point falls outside of the range specified by Outlier Labeling Rule\textsuperscript{256}) for serum BDNF levels; as such all subsequent BDNF analyses were conducted with data from 11 patients. For the 11 patients, again there was no significant difference in serum BDNF concentrations between baseline and termination (t=0.067, p=0.948), even adjusting for cumulative lithium dose (F=0.352, p=0.568) or cumulative serum lithium levels (F=0.032, p=0.862). Additionally, there was no difference in change in serum BDNF levels when comparing between dose groups (F=0.014, p=0.907) and serum level groups (F=0.246, p=0.632).

**Table 10** BDNF levels in patients at baseline and termination

<table>
<thead>
<tr>
<th></th>
<th>Baseline [BDNF] (ng/mL±SD)</th>
<th>Termination [BDNF] (ng/mL±SD)</th>
<th>Statistic</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>With outlier (n=12)</td>
<td>22.45±10.47</td>
<td>23.56±12.73</td>
<td>t=0.357</td>
<td>0.728</td>
</tr>
<tr>
<td>Without outlier (n=11)</td>
<td>24.23±8.87</td>
<td>24.03±13.24</td>
<td>t=0.067</td>
<td>0.948</td>
</tr>
</tbody>
</table>

Statistics derived from paired Student’s T-test of differences between baseline and termination.

### 3.4.1 Post-hoc analyses

To explore if serum BDNF levels affected or interacted with our imaging and clinical outcomes, we conducted post-hoc analyses wherein we added percent change in serum BDNF as a covariate into previous analyses (Sections 3.2 and 3.3) that demonstrated significant change from baseline to termination or significant interaction with lithium.

Repeated-measures ANCOVA was used to model change in global grey matter volume and its interaction with dose group, with percent change in BDNF as a covariate and dose group as the between-subjects factor. Percent change in BDNF did not interact with change in global grey matter volume (F=2.98, p=0.123) and did not seem to affect the interaction between change in global grey matter volume and dose (time-by-dose group: F=16.0, p=0.004).

Similarly, interaction between serum BDNF and clinical outcomes demonstrating significant change in previous analyses (Section 3.4) was explored by repeated-measures ANCOVA, with
percent change in serum BDNF level as a covariate. Percent change in BDNF did not interact with change in category fluency (F=0.211, p=0.657), Digit Symbol-Coding (F=0.189, p=0.674), HVLT-R’s learning component (F=0.126, p=0.731) or HVLT-R’s recognition discrimination index (F=1.52, p=0.249).
4. Discussion

Our study provided a first-look into the interactions between lithium and physiological and clinical outcomes in a post-stroke population. Through MRI data, we saw an interaction between change in global grey matter and lithium dose group; patients who received the target daily dose of lithium (i.e. 300mg) showed positive change in global grey matter volume and those who received less than the target daily dose showed negative change in global grey matter volume. Through clinical assessments, we identified significant improvements in domains of executive function and verbal learning, although these improvements did not interact with measures of lithium dose or serum level. We did identify a positive association between cumulative lithium dose and verbal memory, but verbal memory was not correlated with change in global grey matter volume. We did not detect any interactions between BDNF and neuroimaging or clinical outcomes. Findings from this study provided novel insight on the possible relationship between lithium and neuroanatomical and clinical changes in a post-stroke population, which could be used to inform future studies on new pharmacotherapies for stroke recovery.

4.1 Lithium and global grey matter volume

Evidence from the bipolar disease literature suggested that lithium pharmacotherapy might be associated with greater grey matter volume. Previous meta-analyses have reported that greater grey matter volume was correlated with a higher proportion of lithium users in a population\textsuperscript{55, 257}. Furthermore, our recent meta-analysis of cross-sectional data found that patients taking lithium had greater global grey matter volume compared to lithium-free controls (submitted; see Appendix S2). Likewise, longitudinal studies have also reported increases in grey matter volume associated with lithium treatment\textsuperscript{43, 44}. 
While we hypothesized that we would see a similar increase in grey matter volume in our post-stroke population, we found no significant difference in global grey matter volume between baseline and termination. However, our effect size of 0.56 is comparable to the effect size found in the Moore et al. study (0.55)43. One explanation for the lack of observed differences could be due to the age of our study population. The mean age of this population was much older than those reported in previous studies (33±11 and 31.3±9.3, respectively)43, 44. We were unable to find longitudinal studies that looked at grey matter volume changes following lithium treatment in bipolar patients of a comparable age to our stroke patients. Comparing our effect size to one reported for hippocampal volumes in older bipolar patients taking lithium, our detected effect size is much smaller (0.55 vs 2.23). Nonetheless, caveats must be taken in interpreting this comparison, as 1) the cited study was cross-sectional in nature and length of lithium exposure for those older bipolar patients is unknown, 2) the cited study reported hippocampal volumes, not global grey matter volumes, and 3) the older bipolar patients (mean age=58.2) were still younger than our stroke patients (mean age=71.1).208 Aging is associated with neuronal loss and grey matter volume decreases258, which is exacerbated by neuronal atrophy following stroke10. It could be argued that, for there to be an observed increase in grey matter volume in this population, any neurotrophic actions of lithium would have to be greater than the expected neuronal atrophy in this population; perhaps it would be more fruitful to investigate attenuation of loss rather than stimulation of gains.

Another explanation for the lack of detectable difference was the low daily dose and maintained serum lithium levels in our study population compared to those in previous longitudinal studies. The mean serum lithium level in our study was 0.28±0.19mM, whereas it was 0.9±0.2mM and 0.65±0.19mM in the respective longitudinal studies43, 44. Thus, we adjusted our statistical model with measures of lithium dose or serum lithium level. When we analyzed by dose group, we found that changes in global grey matter volume varied depending on whether patients received at least
300mg of lithium daily: patients who received at least 300mg of lithium daily showed positive change in grey matter volume on average, whereas patients who did not showed negative change. As 300mg is the lower end of the lithium dosage range recommended for older individuals\(^{193}\), this difference could possibly reflect the difference between the effects of therapeutic and sub-therapeutic doses. However, there are caveats to that interpretation. For one, serum lithium levels between dose groups were not significantly different. This proves problematic since the 300mg threshold was determined based on dosing recommendations that target an optimal, therapeutic serum lithium level\(^{193}\); our observed mismatch between dose and serum level casts some doubt as to whether these dose groups accurately captured the patients’ therapeutic status. Furthermore, patients receiving more than 300mg of lithium were significantly younger than those who did not. As age is associated with changes in grey matter volume\(^{258}\), it is uncertain whether the observed difference in global grey matter volume changes was a product of age rather than dose. However, when age was added as a covariate to our analysis, the interaction between dose and change in grey matter volume remained significant, suggesting that age was not driving this interaction. In a similar vein, patients receiving more than 300mg of lithium had significantly higher MoCA scores than those who did not. Baseline cognition is associated with rate of change of grey matter volume\(^{259}\), and thus could be the major contributor to the observed differences between dose group. However, when MoCA score was added as a covariate to our analysis, the interaction between dose and change in grey matter volume remained significant, suggesting that baseline cognition was not driving this interaction. Furthermore, it is important to note that even in the group that received 300mg or more of lithium daily, the mean percent change in grey matter volume was 0.63%, which is appreciably less than the 1.7% reported in data from bipolar patients\(^{43}\).
4.1.1 Lithium and parietal grey matter volume

Our post-hoc analysis also found that the interaction between dose and grey matter volume appeared to have been limited to the parietal regions of the brain. Although literature on the association between lithium treatment and parietal grey matter volumes is scarce, a few cross-sectional studies have reported these associations in the bipolar population\textsuperscript{204, 205, 209}. However, it is important to note that all of these analyses were also exploratory or post-hoc, thus it would be premature to conclude that lithium may preferentially act upon the parietal region. Indeed, studies have reported associations between lithium and grey matter volumes across a variety of regions\textsuperscript{206, 207, 255, 260, 261}. Taken together, it is difficult to determine if the detection of parietal-specific interactions is spurious. However, should there be a parietal-specific effect, lithium could benefit post-stroke recovery in domain deficits associated with parietal lesions, such as spatial attention, non-verbal cued recall, and motor function\textsuperscript{262-265}.

In a stroke context, we also considered if changes in the parietal regions alone could be an artefact of stroke localization to the parietal lobes. The ischemic penumbra (i.e. damaged tissues surrounding the core of an ischemic stroke) is considered viable and can either undergo spontaneous recovery or consolidate into an established infarct\textsuperscript{82}. Thus, changes in the parietal lobe may be influenced by changes in stroke characteristics in that region. However, upon further analysis with stroke localization as a covariate, the interaction between dose and parietal grey matter volumes remained significant. Furthermore, this interaction was also preserved after adjusting for percent change in penumbra volume. Although our results suggest that the interaction between lithium dose and parietal grey matter volume operate independently of stroke-localized changes, further research is necessary to more fully characterize the relationship between lithium treatment and changes in grey matter volume.
4.2 Lithium and clinical outcomes

Following a stroke, patients commonly experience changes in their cognition and mood, namely vascular cognitive impairment and post-stroke depression\textsuperscript{19, 266}. Lithium is widely recognized as an effective mood stabilizer\textsuperscript{267}, however its effects on cognition are more difficult to quantify. Although studies in the past have shown that lithium treatment is associated with cognitive impairment in domains such as verbal learning\textsuperscript{189, 268}, these effects appear transient and may be confounded with underlying cognitive changes due to disease state\textsuperscript{269}. Therefore, while we hypothesized that lithium treatment would improve symptoms of post-stroke depression, the potential effects of lithium on post-stroke cognitive changes were less clear.

4.2.1 Lithium and cognition

Our results showed that there were improvements in category fluency, non-verbal processing speed and activation, and verbal learning after adjustments for cumulative lithium dose or serum lithium level. At a glance, these results suggest that lithium treatment may be associated with cognitive improvements. However, when we examined the interaction between lithium dose or level and change in these domains, there was no significance. This, along with the lack of significance in unadjusted comparative analyses, may indicate that the detected difference may be an artefact of an overloaded statistical model. Conversely, the lack of consistent detection may also result from low power (see Section 4.4.1).

Another explanation for the lack of interaction between lithium treatment and change in cognitive outcomes is the heterogeneous presence of concomitant medication and medical conditions. For example, many of the patients were taking statins for management of dyslipidemia, and statins have been implicated in cognitive changes\textsuperscript{270}. Likewise, several study participants presented with comorbid diabetes, which has been linked to cognitive impairment\textsuperscript{271}. Taken together, the cognitive effects of these factors may influence interactions between lithium treatment and
cognition. Further analyses, with a larger sample size, is necessary to determine if covariates such as medication and diabetes status modify the interaction between lithium treatment and cognition.

Alternatively, given that there was no interaction between lithium levels and cognition, change in cognitive test scores over time could be associated with repeated testing. The animal naming task is susceptible to practice effects due to repeated testing\textsuperscript{272, 273}, as is Digit Symbol-Coding\textsuperscript{274, 275}. And although alternate forms exist for HVLT-R\textsuperscript{276}, only one form was used in the current study. Thus, there could be practice effects on learning and memory\textsuperscript{277}. The potential effects of repeated testing on these measures can be teased out with the introduction of a suitable control group (Section 4.4.3).

Lastly, as mentioned in Section 1.4.3.1, patients can experience spontaneous recovery following stroke. Improvements in cognition can be seen as early as 6-months post-stroke and patients can continue to show improvements up to 3 years\textsuperscript{133-135}, including improvements in executive function, verbal learning and fluency\textsuperscript{137}. Therefore, detected differences between baseline and termination in our study may be due to endogenous recovery processes, especially given that there was no interaction between cognition and measures of dose. Again, whether observed differences were intervention-related or spontaneous can better be determined with the inclusion of a suitable control group (Section 4.4.3).

4.2.1.1 Clinical outcomes and grey matter volume

Since decreased grey matter volume has been associated with impaired cognition\textsuperscript{17, 278, 279}, we elected to explore possible correlations between our observed cognitive and structural imaging outcomes. Given that we did not detect significant change in grey matter volumes but did detect significant interaction between lithium dose and grey matter volume change, we conducted our analysis on domains of cognition that also interacted with dose—to this end, only verbal memory demonstrated such an interaction. Since grey matter volume interacted with lithium dose, and
verbal memory interacted with lithium dose, we postulated that perhaps lithium dose mediates common factors affecting both measures, and that these measures would correlate with one another.

However, when we analyzed grey matter volume and verbal memory, we found no correlation between change in global grey matter volume and change in verbal memory. This may be due to the fact that individually, neither global grey matter volume nor verbal memory exhibited significant change over time. Furthermore, previous studies in different populations have suggested that verbal memory decline is associated with certain neuroanatomical regions (e.g. hippocampus, thalamus)\textsuperscript{280,281}, thus our crude correlation using global grey matter volume might be missing nuances of region-specific correlations. However, our attempts to correlate these regions with verbal memory yielded no significant results—unsurprising, considering there was no significant change in verbal memory nor any of these regions.

Given that there are active processes driving grey matter atrophy and cognitive decline post-stroke\textsuperscript{9}, a longer time of treatment may be needed to see clinical benefits of lithium in this population. Alternatively, although cognitive improvements can be detected as late as 3 years post-stroke\textsuperscript{135}, there may be a specific timeframe post-stroke when lithium would be most efficacious; further studies exploring lithium therapy at various post-stroke time-points (e.g. 3 months, 6 months) may be of interest.

4.2.2 Lithium and mood

Given that lithium is a well-established mood stabilizer, we hypothesized that lithium may alleviate post-stroke depression. However, our results suggest that there is no change in depressive symptoms following lithium treatment. Nevertheless, it is important to note that only 9 of our patients displayed depressive symptoms at any point in the study. And of these 9, only 2 patients presented with potentially clinically significant depression (i.e. HAM-D>7\textsuperscript{282}); both these patients
saw a reduction in HAM-D scores over time. Therefore, it can be argued that the lack of effect observed in this study may be driven by arbitrary fluctuations in HAM-D scores of patients who were below threshold for mild depression. As there is limited literature on lithium in post-stroke depression\textsuperscript{283}, future studies with a sufficiently large population of depressed stroke patients is needed to elucidate the effects of lithium on post-stroke depression.

4.3 Lithium and BDNF

Since lithium has been associated with increased serum BDNF levels in clinical populations\textsuperscript{50, 284}, we conducted exploratory analysis on the association between lithium and BDNF levels in our post-stroke cohort. We did not detect a change in serum BDNF over time, nor a correlation between serum BDNF and any measure of lithium level. Similarly, there was a lack significant correlation between serum lithium levels and BDNF in a study that showed significant increase in BDNF after lithium treatment\textsuperscript{50}.

It is of note that this analysis only included serum BDNF data from 11 patients. One patient was a major outlier, due to an extreme change (i.e. over 5-fold) in BDNF levels from baseline to termination. Not only was this a statistical outlier in our dataset, but also outside the magnitude of change reported in literature\textsuperscript{50, 284}. Upon re-examination of neuroimaging and clinical data from this patient, there were no unusual or unexpected data detected. This suggests that an error may have occurred at the assay level, especially considering the detected baseline BDNF level for this patient was much lower than average (i.e. approximately 13% of the average) whereas termination BDNF level for the same patient was comparable to our sample average. A possible assay-specific error could have been due to poor homogenization of the sample prior to pipetting, leaving protein sediments in the aliquot tube and minimizing the amount of BDNF detectable in the ELISA.
Furthermore, our methodology did not distinguish between proBDNF and mature BDNF. ProBDNF is a precursor of the BDNF protein that acts via its own receptor (p75<sup>NTR</sup>) to facilitate downstream effects unique from those of mature BDNF<sup>285, 286</sup>. Of particular relevance to our primary and secondary outcomes, proBDNF is thought to mediate pro-apoptotic processes in neurons<sup>285</sup> whereas mature BDNF is thought to be neuroprotective<sup>75</sup>, and the interplay between these two molecules is thought to mediate hippocampal plasticity<sup>286-288</sup>. Our methods were only suitable for measuring changes in total BDNF, which would not capture the nuanced relationships between different forms of BDNF. Thus, evaluation of the ratio between pro- and mature BDNF may be warranted in order to elucidate BDNF changes after administration of a putatively neuroprotective agent such as lithium.

One possible explanation for the lack of detectable differences or correlations could be the presence of endogenous protective processes upregulating neurotrophic factors following ischemic insult<sup>9</sup>. Should the endogenous processes have already fully-activated BDNF transcription, lithium therapy would not be expected to change BDNF levels. Genetic makeup of our study population could also affect detectability of BDNF changes: it has been shown, in vitro, that the Val66Met BDNF polymorphism affects BDNF secretion<sup>289</sup>. Although the frequency of this polymorphism varies greatly between populations, data suggest that the allelic frequency of the Val66Met polymorphism is approximately 19.9% in individuals of European descent; this number is in line with reported frequency in an American population (18%)<sup>290, 291</sup>. Therefore, genetic testing for this population for the presence of Val66Met polymorphisms will help elucidate if the polymorphism mediates change in serum BDNF levels following lithium treatment.

Moreover, although we endeavored to elucidate mechanistic underpinnings of change by evaluating serum BDNF, it is important to note that peripheral BDNF levels may not accurately reflect centrally circulating BDNF. While there have been studies demonstrating that BDNF
readily passes the blood-brain barrier\textsuperscript{292, 293}, and that blood and CSF levels correlate across various animal models\textsuperscript{294}, clinical evidence is less clear. Although it was shown that there was correlation between plasma and CSF levels of BDNF in a schizophrenic population\textsuperscript{295}, a study in Alzheimer’s patients showed no correlation between serum and CSF levels\textsuperscript{296}. One possible explanation for the lack of correlation between serum and CSF levels could be peripheral sources of BDNF that are absent in CSF, namely platelets\textsuperscript{297, 298}. The lack of correlation between serum BDNF and neuroanatomical or clinical outcomes (see below) may partially be explained by the discrepancy between peripheral and central levels of BDNF.

4.3.1 BDNF and grey matter volume

As BDNF has been shown to correlate with grey matter volume in an aging population\textsuperscript{46}, we also explored the possibility that serum BDNF levels may influence change in grey matter volume. However, our results showed no interaction between global grey matter volume and BDNF or alteration of the interaction between lithium dose and grey matter change. This may be due to the fact that the model was not sufficiently powered to handle the addition of a covariate. Another possible explanation for the lack of an observable correlation could be the genetic makeup of our study population, as the Val66Met polymorphism in BDNF has been shown to affect grey matter structures\textsuperscript{299}. Again, genetic testing would be helpful in elucidating potential influence of the polymorphism on a potential relationship between changes in grey matter volume and serum BDNF levels.

4.3.2 BDNF and clinical outcomes

Since serum BDNF level is associated with cognitive function\textsuperscript{78} and post-stroke depression\textsuperscript{125}, we investigated the possible interaction between serum BDNF levels and change in cognition scores. There was no interaction between serum BDNF levels and cognition scores. Again, genetic makeup of the population might have influenced this outcome\textsuperscript{300, 301}, and further testing should be
done to elucidate possible effects of the Val66Met BDNF polymorphism on the interaction between serum BDNF and cognition.

4.4 Limitations

4.4.1 Power

Upon inception of the study, required sample size was estimated based on previously published data. Moore et al. reported a significant increase in grey matter volume after 4 weeks of treatment lithium in 27 bipolar patients (t=2.838, p=0.004). Thus, assuming similar effect sizes in our population, for a 2-tailed paired comparison with $\alpha=0.05$, we would need 26 patients to detect an effect with 80% power:

$$n = \frac{(Z_\alpha + Z_\beta)^2}{\left(\frac{\bar{x}_d}{s_d}\right)^2} = \frac{(1.96 + 0.842)^2}{\left(\frac{12}{22.0}\right)^2} = 26.4 \approx 26$$

$Z_\alpha$ is the $z$-value for a given $\alpha$ error, $Z_\beta$ is the $z$-value for a given power, and $\bar{x}_d$ and $s_d$ are respectively mean difference and standard deviation of the difference in means. Mean difference was extracted from a figure, as no numerical values were reported; standard deviation was calculated from the provided t-statistic.

With only 12 patients, our sample was underpowered (53% power) to detect change, especially considering the heterogeneity of lithium treatment status across patients (e.g. screen-drop, early discontinuation, sub-therapeutic lithium levels). The lack of detectable difference in global grey matter volume between baseline and termination in our sample may be a false negative. Moreover, with a small sample size, we were unable to add multiple covariates into our analyses, which prevented us from creating models that encompassed multiple factors that have previously been associated with our outcomes of interest. A larger sample is required to draw more definitive
conclusions about the effects of lithium treatment on grey matter volume changes in a post-stroke population.

4.4.2 Dose and serum levels

Although dose and serum levels were identified as potentially important mediators of outcomes (i.e. potential dose-response interaction), there were methodological short-comings of using either measure as a covariate. As there is high inter-individual variability in serum lithium level for any given dose, dose may not be reflective of the amount of lithium present in a patient’s system\textsuperscript{193}. However, due to methodological oversight, serum lithium level measurements were not fully reliable in this study. Even at steady-state, serum lithium levels fluctuate over the day with peak concentration reached shortly after administration\textsuperscript{304, 305}. Since there was no data collected on time since last lithium dose for the majority of our patients, we cannot ascertain if the serum lithium levels measured were peak or trough values for each patient, limiting the comparability of these values across the sample.

Stratification using cut-off values presented its own set of caveats. For one, dose and serum groups did not perfectly overlap: there were two patients who met the serum level cutoff (0.4mM) but not the dose cutoff (300mg) and two patients who met the dose cutoff but not the serum cutoff. Furthermore, age and baseline MoCA scores were significantly different between dose groups; time since stroke was significantly different between serum level groups. Given that age, cognition, and time since stroke\textsuperscript{12, 15-17, 82, 306} are all thought to associate with grey matter volume, the interaction seen between change in grey matter volume and dose group may be driven by differences in these factors. Multivariate analysis is necessary to tease out contribution of each of these factors to change in global grey matter volume.
4.4.3 Study design

Given that this was a pilot study, we aimed to reduce number of participants required and thus did not include a placebo control arm. Moreover, lithium was prescribed open-label, which can introduce bias in neuropsychological testing. Considering these two limitations, interpretation of results becomes difficult as it is unclear whether detected effects are lithium-specific, or an artefact of naturalistic processes or testing bias. However, our pilot data can be used to inform design of a larger randomized, double-blind placebo-control trial that would be required to validate the findings from this preliminary study. Based on the observed effect size in our primary outcome of change in global grey matter volume, we estimate that 24 patients will be needed to detect a statistically significant global grey matter volume change in a post-stroke population (see 4.4.1 for assumptions and formula). Furthermore, correlation between cumulative dose and verbal memory suggests that improvements in verbal memory may be an outcome of interest for future studies on lithium in stroke recovery. Considering the lack of detectable BDNF changes in our study, future studies that aim to explore BDNF changes in this context may be better served by more specificity in their method of BDNF analysis (e.g. measuring proBDNF and mature BDNF separately).

4.5 Recommendations

Considering the increase in stroke prevalence over time and impact of stroke on daily function, it is imperative to develop new strategies to improve post-stroke outcomes. Lithium looks to be a promising therapeutic strategy as it has been previously shown to increase grey matter volume in clinical populations\textsuperscript{43, 44}, and has been shown to interact with grey matter volume change in the current study. Furthermore, these patients showed improvements in several cognitive outcomes following lithium treatment. And although we were unable to detect treatment effects on depressive symptoms, lithium’s history as a mood stabilizer makes it an attractive candidate for future clinical trials in post-stroke depression\textsuperscript{125}. Lithium was generally well-tolerated by our
patients. Most adverse outcomes were mild and only 3 patients were discontinued due to intolerable adverse events: 1 due to constipation, 2 due to nausea and vomiting (see Table 2). Interestingly, both patients who discontinued due to nausea and vomiting started experiencing these symptoms after initiation of antibiotics, suggesting that increased monitoring of concomitant medications may be helpful in preventing these adverse outcomes. Nevertheless, with these promising preliminary signals and the adequate tolerability of lithium in this population, larger trials investigating the effects of lithium in a stroke recovery context are worthwhile to pursue.

Lessons with respect to feasibility from our pilot study could be useful in designing larger studies. Recruitment for a clinical trial with lithium in a post-stroke population is challenging: many patients decline to participate due to scheduling conflicts with stroke rehabilitation, reluctance to add to existing post-stroke pharmacotherapy, reservations about the primary indications for lithium, and conflicting opinions of their healthcare providers. Some of these concerns may be mitigated by having study physicians who are also directly involved in their post-stroke care: study visits can be scheduled to better coincide with clinical follow-up dates, recommendation to the study would be made by a physician whom the patient feels understands their situation, and communication between healthcare providers would be facilitated.

A concerted effort should also be made to investigate therapeutic potentials of other brain-targeting drugs in the context of stroke recovery. De novo drug development is expensive and time-consuming\textsuperscript{307}, thus investigations of available compounds for new indications can facilitate the discovery of new therapeutic approaches for existing problems. In vascular cognitive impairment, several studies have examined the therapeutic potential of cognitive enhancers such as donepezil and memantine. Donepezil, a cholinesterase inhibitor, has been shown to be efficacious in promoting cognitive improvement in vascular dementia and is generally well-tolerated\textsuperscript{308-310}. Preliminary data from a small open-label trial in stroke patients suggests that donepezil could be
efficacious for cognitive recovery post-stroke\textsuperscript{311, 312}. Memantine is a non-competitive NMDA receptor antagonist; while trials have shown cognitive improvements following treatment, meta-analysis of this data concluded that the change was not clinically distinguishable\textsuperscript{313}. Nonetheless, some preclinical data suggest memantine treatment decreases lesions, and thus could be efficacious in a stroke context\textsuperscript{174}. Furthermore, there is evidence to suggest that mood modifiers can also confer benefits to stroke recovery. Stroke patients treated with escitalopram in a randomized, placebo-controlled trial saw improvement in global cognitive functioning at 12 months\textsuperscript{314}. Considering that other antidepressants have also been associated with morphological change in the brain and/or improvements in cognitive function\textsuperscript{315, 316}, further investigation within this drug class might be warranted.

4.6 Conclusion

Our study demonstrated an interaction between change in global grey matter and lithium dose. Furthermore, we identified significant improvements in domains of executive function and verbal learning, and a positive association between lithium dose and verbal memory. As more and more individuals are living with post-stroke sequelae, it becomes increasingly necessary to develop effective strategies to treat post-stroke cognitive and mood outcomes. Findings from this study provide a preliminary signal suggesting potential therapeutic applications of lithium in a post-stroke population and invite further research in this area.
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List of Publications and Abstracts

Publications

Yue Ran Sun, Nathan Herrmann, Christopher Scott, Sandra Black, Maisha Khan, Krista Lanctot. Global grey matter volume in adult bipolar patients with and without lithium treatment: a meta-analysis. Submitted.

Abstracts

Magali Millecamps, Emerson Krock, Yue Ran Sun, Axel Mathieu, Lois Kehl, Kathleen M. Anderson, Jean Ouellet, Lisbet Haglund, Laura S. Stone. Nerve Growth Factor (NGF) is more abundant in moderately than in severely degenerating intervertebral discs in both human and mice. IASP World Congress on Pain September 2016. Yokohama. Poster presented by Dr. Magali Millecamps.


Yue Ran Sun, Nathan Herrmann, Nadia Reider, Austyn Roseborough, Sandra Black, Alexander Kiss, Rick Swartz, Susan Marzolini, Murray Waldman, Krista Lanctot. Lithium carbonate and


Appendix S1 Informed consent form
INFORMED CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Full Study Title: The Neurotrophic Effects of Lithium Carbonate Following Stroke: A Feasibility Study

Investigators: K.L. Lanctôt, PhD  Sunnybrook Health Sciences Centre
              N. Herrmann, MD FRCPC Sunnybrook Health Sciences Centre
              S.E. Black, MD FRCPC Sunnybrook Health Sciences Centre
              F. Gao, MD Sunnybrook Health Sciences Centre
              M. Bayley, MD FRCPC Toronto Rehabilitation Institute

Sponsor: This pilot study is funded by the Heart and Stroke Foundation of Ontario

INFORMED CONSENT

You are being asked to consider participating in a research study. A research study is a way of gathering information on a treatment, procedure or medical device or to answer a question about something that is not well understood.

This form explains the purpose of this research study, provides information about the study drug, the tests and procedures involved, possible risks and benefits, and the rights of participants.

Please read this form carefully and ask any questions you may have. You may take as much time as you wish to decide whether or not to participate. Please ask the study staff or one of the investigator(s) to clarify anything you do not understand or would like to know more about. Make sure all your questions are answered to your satisfaction before deciding whether to participate in this research study.

INTRODUCTION

You are being asked to consider participating in this study because you have recently had a stroke.

After a stroke, many patients experience difficulties in cognition (the process of thought, memory and concentration). This may be a result of damage to the brain (gray matter loss) that occurs after a stroke. Lithium is a drug that is known to help in brain growth and protect against further gray matter loss in patients who have been diagnosed with bipolar disorder. It is not known whether or not lithium has the same activity in patients who have recently had a stroke. The purpose of this study is to examine the effects of lithium on total gray matter and cognition in post-stroke patients. This study will tell us if lithium can decrease the amount of brain cell loss after a stroke, and enhance recovery in patients who have suffered a stroke.

WHY IS THIS STUDY BEING DONE?
The purpose of this study is to see what effects (good and bad) lithium (Carbolith®) has on your condition. Health Canada has approved lithium for use in the treatment of bipolar disorders, but not for post-stroke cognitive difficulties. Health Canada has, however, approved the use of lithium in post-stroke patients for this research study.

WHAT WILL HAPPEN DURING THIS STUDY?

If you agree to participate in this study, you will have three separate visits with our study staff at Sunnybrook Health Sciences Centre. The first visit will occur at least 4 weeks after your stroke has occurred. At this first visit, you will be asked to undergo an initial assessment with a trained researcher. This will include a review of your demographic data (age, gender and diagnoses) and medical history to determine your eligibility to enter the study.

Once it has been determined that you are eligible to participate in this study, you will be prescribed a low dose of lithium (150-300 mg) which you will be asked to take for 60 days until your last visit. If you are not a candidate for lithium, you may be offered to undergo study procedures without receiving lithium.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?

It is anticipated that about 35 people will participate in this study at Sunnybrook. The length of this study for participants is 60 days. The entire study is expected to take about one year to complete and the results should be known within two years.

WHAT ARE THE RESPONSIBILITIES OF STUDY PARTICIPANTS?

If you decide to participate in this study you will be asked to do the following:

**Screening/Baseline Visit:**

This visit will occur at least 4 weeks after your stroke and will take approximately 3 hours.

- **Demographic Questions:** You will be asked to give personal information about yourself, such as your name, date of birth, race, etc.
- **Health and Medication Questions:** You will be asked to answer questions about your health, your medication history and the medications you take.
- **Height, Weight:** We will measure how tall you are and see how much you weigh.
- **Mood Testing:** You will be asked questions to determine your mood.
- **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.
- **Independence Survey:** You will be asked questions regarding your quality of life and independence.
- **Blood Testing:** A blood draw (36.0mL) will be taken to examine blood levels of lithium, calcium and certain proteins.
- **Cheek Swab:** A sample of skin cells will be taken from the inside of your cheek using a sterile swab. The DNA from these cells will be used to help us determine which forms of certain genes you have. This information may be related to the effectiveness of lithium treatment.
- **Magnetic Resonance Imaging (MRI):** MRI is a method of making pictures of the brain using magnetic waves. This gives us information about the structure of the
brain, including gray matter volume. The scan will take less than one hour. The scan will involve lying still with a simple device placed around your head/neck.

**One-Week Visit:**

5-7 days after baseline visit, this visit will take approximately 20 minutes.

- **Blood Testing:** A blood draw (5.0 ml) will be done to analyze the levels of lithium, calcium, and a protein called creatinine, in your blood. This analysis is done to ensure that you are receiving a safe and effective dose of lithium throughout the study.
- **Side Effects:** You will be asked if you are experiencing any problems with the medication.

**Four-Week Visit:**

30 days after baseline visit, this visit will take approximately 20 minutes.

- **Blood Testing:** A blood draw (5.0 ml) will be done to analyze the levels of lithium, calcium, and a protein called creatinine, in your blood. This analysis is done to ensure that you are receiving a safe and effective dose of lithium throughout the study.
- **Side Effects:** You will be asked if you are experiencing any problems with the medication.

**Termination Visit:**

60 days after baseline visit, this visit will take approximately 2.5 hours.

- **Mood Testing:** You will be asked questions to determine your mood.
- **Memory and Thinking Testing:** You will be asked a series of questions to test your memory and thinking.
- **Independence Survey:** You will be asked questions regarding your quality of life and independence.
- **Blood Testing:** A blood draw (36.0 mL) will be taken to examine blood levels of lithium, calcium and certain proteins.
- **Magnetic Resonance Imaging (MRI):** This scan will take less than one hour.
- **Side Effects:** You will be asked if you are experiencing any problems with the medication.

**Telephone Calls:**

Two telephone calls from study staff, calls will take approximately 10 minutes.

- **Side Effects:** You will be asked if you are experiencing any problems with the medication.

The collection of blood is a necessary part of this research study. The blood sample will be discarded or destroyed once it has been used for the purposes described in this form. **Any of your samples** that are sent outside of the hospital for analysis will have a code and will not contain your name or address, or any information that directly identifies you. The samples will be used for research purposes only and will not be sold. The research done with your sample will not help develop commercial (for profit) products or tests.
WHAT ARE THE RISKS OR HARMS OF PARTICIPATING IN THIS STUDY?
Lithium has been approved in Canada for the treatment of bipolar disorders. You may, however, experience side effects from participating in this study. Some side effects are known and are listed below, but there may be other side effects that are not expected. If you decide to take part in this study and experience severe side effects or study-related injuries, you should contact the study co-ordinator – Nadia Reider (416-480-6100 x3185), Dr. Krista L. Lanctôt (416-480-6100 x2241) or Dr. Nathan Herrmann (416-480-6133).

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Frequency</th>
<th>Severity</th>
<th>Long Term Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expected</td>
<td>Likely</td>
<td>Less Likely</td>
</tr>
<tr>
<td></td>
<td>(30-100%)</td>
<td>(10-30%)</td>
<td>(1-10%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
</tr>
<tr>
<td>Reduced kidney function</td>
<td>X</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Dizziness and Vomiting</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Irregular heartbeat</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Fainting</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>High blood calcium</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Frequent urination</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Skin Rash</td>
<td></td>
<td>x</td>
<td></td>
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<tr>
<td>Bloated feeling in stomach</td>
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<td>x</td>
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<tr>
<td>Hand Tremor</td>
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<td>x</td>
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<tr>
<td>Diarrhea</td>
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<td>x</td>
<td></td>
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<tr>
<td>Nausea</td>
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<td>x</td>
<td></td>
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<tr>
<td>Unusual tiredness</td>
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<td>x</td>
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<tr>
<td>Confusion/Slurred Speech</td>
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<td>x</td>
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</tr>
<tr>
<td>Weight gain</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Painful joints or rigidity</td>
<td></td>
<td>x</td>
<td></td>
</tr>
</tbody>
</table>
Thirst | X | X | X
Blue colour and pain in fingers/toes | X | X | X
Drying thinning of hair | X | X | X

In case of an adverse event or to reach the study physician for urgent matters, please contact the hospital locating number 416-480-4244 and ask for Dr. Herrmann to be paged. This is a 24 hr emergency contact number. In case of medical emergency, proceed to the nearest emergency room.

Lithium therapy has rarely been associated with the development of reduced kidney function. This may result in you experiencing excessive thirst; more frequent urination; and weight gain. Reduced kidney function due to lithium therapy may be only partially reversible once lithium therapy is stopped, particularly in those on long-term therapy and higher doses. Reduced kidney function and other side effects are related to dose of lithium that you take. In order to decrease your risk of developing side effects, you will be treated with a low dose of lithium, and the amount of lithium in your blood will be monitored one and four weeks after initiation of the treatment to ensure that there is a low dose in your body.

When your blood is drawn, there may be some discomfort and/or bruising, however these are expected to be very mild.

During the MRI procedure, you may be bothered by feelings of confinement (claustrophobia), and by clicking and banging noises made by the magnet. Because MRI uses strong magnetic fields, you may not participate in MRI scans if you have a pacemaker, an implanted defibrillator or certain other implanted electronic or metallic devices. An interview will be conducted prior to your MRI to advise the MRI staff if you have had brain surgery for a cerebral aneurysm, or if you have implanted medical or metallic devices, shrapnel, or other metal, such as metal in your eye. There is a small chance that we may observe something abnormal on your MRI. If this is the case, we will inform you, which may cause you anxiety, and suggest the need for further tests.

Lithium may cause birth defects; therefore you should not take part in this study if you are pregnant or planning pregnancy. If you are a woman who may become pregnant, it is important that you practice an acceptable method of birth control to prevent pregnancy.

Lithium can interact with other drugs. It is important that you do not start any new medications, including over the counter medications, without discussing changes with the study physician, or telling the prescriber you are taking lithium. If you do start a new medication, please contact the study co-ordinator, Nadia Reider (416-480-6100 x3185).

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?
You may or may not benefit directly from participating in this study. Your participation may or may not help other people with strokes in the future.

**CAN PARTICIPATION IN THIS STUDY END EARLY?**

The investigators may decide to remove you from this study without your consent for any of the following reasons:

- The investigators decide that continuing in this study would be harmful to you.
- You plan to become pregnant, become pregnant, think you may become pregnant, or plan to discontinue acceptable birth control.
- You are unable or unwilling to follow the study procedures.
- You experience severe side effects as a result of lithium treatment.

If you are removed from this study, the investigator(s) will discuss the reasons with you. You can also choose to end your participation at any time. If you withdraw voluntarily from the study, or at the request of your family doctor, you are encouraged to contact the study co-ordinator, Nadia Reider (416-480-6100 x3185), immediately.

If you leave the study, the information about you that was collected before you left the study will still be used. No new information about you will be collected without your permission.

**ARE STUDY PARTICIPANTS PAID TO PARTICIPATE IN THIS STUDY?**

You will be given a $23.00 honorarium each time you visit Sunnybrook for parking or transportation expenses, plus a $40.00 honorarium for participation in both MRIs. You will incur no costs as a result of participation in this study.

**WHAT OTHER CHOICES ARE THERE?**

You are eligible to receive treatment for your stroke and any cognitive/depressive symptoms you may have even if you choose not to participate in this study. Participation in this study will not affect your treatment in any way.

**DO THE INVESTIGATORS HAVE ANY CONFLICTS OF INTEREST?**

There are no conflicts of interest to declare related to this study.

**COMMUNICATION WITH YOUR FAMILY DOCTOR**

We recommend notifying your family doctor that you are taking lithium. If you have a family doctor and if you agree, we will inform your family doctor about your participation in the trial. Initial 1 choice below:

☐ Yes, contact my family doctor ☐ No, do not contact my family doctor
Family doctor name: __________________________
Contact number: ___________________________
WHAT ARE THE RIGHTS OF PARTICIPANTS IN A RESEARCH STUDY?

All participants in a research study have the following rights:

1. You have the right to have this form and all information concerning this study explained to you and if you wish translated into your preferred language.

2. Participating in this study is your choice (voluntary). You have the right to choose not to participate, or to stop participating in this study at any time without having to provide a reason. If you choose to withdraw, your choice will not have any effect on your current or future medical treatment or health care OR your employment status. Should you choose to withdraw from the study you are encouraged to contact Nadia Reider (416-480-6100 x3185) immediately.

3. You have the right to receive all significant information that could help you make a decision about participating in this study. You also have the right to ask questions about this study and your rights as a research participant, and to have them answered to your satisfaction, before you make any decision. You also have the right to ask questions and to receive answers throughout this study. If you have any questions about this study you may contact the person in charge of this study (Principal Investigator) Dr. Krista L. Lanctôt (416-480-6100 x2241). If you have questions about your rights as a research participant or any ethical issues related to this study that you wish to discuss with someone not directly involved with the study, you may call Dr. Brian Murray, Chair of the Sunnybrook Research Ethics Board at (416) 480-4276.

4. By signing this consent form, you do not give up any of your legal rights.

5. You have the right to receive a copy of this signed and dated informed consent form before participating in this study.

6. You have the right to be told about any new information that might reasonably affect your willingness to continue to participate in this study as soon as the information becomes available to the study staff. This may include new information about the risks and benefits of being a participant in this study.

7. If you become sick or injured as a direct result of your participation in this study, your medical care will be provided.

8. Any of your personal information (information about you and your health that identifies you as an individual) collected or obtained, whether you choose to participate or not, will be kept confidential and protected to the fullest extent of the law. All personal information collected will be kept in a secure location. The study staff, the Sunnybrook Research Ethics Board, and the regulatory authority(ies) (Health Canada and/or FDA) will have access to your personal information for purposes associated with the study, but will only be allowed to access your records under the supervision of the Principal Investigator and will be obligated to protect your privacy and not disclose your personal information. None of your personal information will be given to anyone without your permission unless required by law. When the results of this study are published, your identity will not be disclosed. The data for this study will be retained for 25 years.

9. If, as a result of your participation in this study, any new clinically important medical information about your health is obtained, you will be given the opportunity to decide whether you wish to be made aware of that information.

10. You have the right to access, review and request changes to your personal health information.

11. You have the right to be informed of the results of this study once the entire study is complete.
DOCUMENTATION OF INFORMED CONSENT

Full Study Title: The Neurotrophic Effects of Lithium Carbonate Following Stroke: A Feasibility Study

Name of participant: __________________ Contact number 1: ______________
Contact number 2: ______________

Participant

By signing this form, I confirm that:

• This research study has been fully explained to me and all of my questions answered to my satisfaction
• I understand the requirements of participating in this research study
• I have been informed of the risks and benefits, if any, of participating in this research study
• I have been informed of any alternatives to participating in this research study
• I have been informed of the rights of research participants
• I have read each page of this form
• I authorize access to my personal health information, medical record and research study data as explained in this form
• I have agreed to participate in this study

____________________________  ______________________________  ___________________
Name of participant (print)  Signature  Date

Person obtaining consent

By signing this form, I confirm that:

• This study and its purpose has been explained to the participant named above
• All questions asked by the participant have been answered
• I will give a copy of this signed and dated document to the participant

____________________________  ______________________________  ___________________
Name of Person obtaining consent (print)  Signature  Date

Statement of Investigator

I acknowledge my responsibility for the care and well-being of the above participant, to respect the rights and wishes of the participant as described in this informed consent document, and to conduct this study according to all applicable laws, regulations and guidelines relating to the ethical and legal conduct of research.

____________________________  ______________________________  ___________________
Name of Investigator (print)  Signature  Date
**Appendix S2** Meta-analysis of global grey matter volume in lithium-treated and lithium-free bipolar patients. Diamond indicates SMD and 95% CI. Significance of overall effect: $z=2.11$, $p=0.035$

<table>
<thead>
<tr>
<th>Study</th>
<th>SMD (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bearden 2007</td>
<td>0.37 (-0.45, 1.20)</td>
<td>3.52</td>
</tr>
<tr>
<td>Benedetti 2011</td>
<td>-0.09 (-0.65, 0.46)</td>
<td>7.16</td>
</tr>
<tr>
<td>Benedetti 2015</td>
<td>0.27 (-0.07, 0.60)</td>
<td>16.13</td>
</tr>
<tr>
<td>Chen 2007</td>
<td>-0.32 (-1.13, 0.48)</td>
<td>3.68</td>
</tr>
<tr>
<td>Eker 2014</td>
<td>-0.14 (-1.20, 0.92)</td>
<td>2.20</td>
</tr>
<tr>
<td>Germana 2010</td>
<td>0.01 (-0.46, 0.48)</td>
<td>9.62</td>
</tr>
<tr>
<td>Ha 2009</td>
<td>-0.09 (-0.71, 0.53)</td>
<td>6.01</td>
</tr>
<tr>
<td>Hajek 2012</td>
<td>0.28 (-0.34, 0.90)</td>
<td>5.97</td>
</tr>
<tr>
<td>Hajek 2014</td>
<td>0.32 (-0.24, 0.88)</td>
<td>7.21</td>
</tr>
<tr>
<td>Ivleva 2013</td>
<td>0.35 (0.01, 0.69)</td>
<td>15.80</td>
</tr>
<tr>
<td>Radenchbach 2010</td>
<td>-0.08 (-0.72, 0.57)</td>
<td>5.55</td>
</tr>
<tr>
<td>Sassi 2002</td>
<td>1.33 (0.51, 2.16)</td>
<td>3.52</td>
</tr>
<tr>
<td>Takahashi 2010</td>
<td>0.29 (-0.48, 1.07)</td>
<td>3.96</td>
</tr>
<tr>
<td>van der Schot 2009</td>
<td>0.09 (-0.48, 0.66)</td>
<td>6.93</td>
</tr>
<tr>
<td>Wijeratne 2013</td>
<td>-0.48 (-1.42, 0.46)</td>
<td>2.75</td>
</tr>
<tr>
<td>Overall (I-squared = 12.2%, $p = 0.317$)</td>
<td>0.17 (0.01, 0.33)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**NOTE:** Weights are from random effects analysis