LITHIUM PREFERENTIALLY INCREASES NEUROGENESIS IN THE VENTRAL BUT NOT DORSAL HIPPOCAMPUS OF STRESSED BALB/C MICE

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INTRODUCTION

- Stress is a precipitating factor of many psychiatric disorders including depression. Therefore, chronic exposure to stress is increasingly being employed in rodents to model stress-related psychiatric disorders and to test both the behavioural and neurobiological effects of psychotropic drugs.
- Lithium, the major pharmacotherapy for bipolar disorder, is also an effective add-on agent in antidepressant-refractory depression. While the precise molecular mechanisms underlying the antidepressant effects of lithium remain unresolved, it was recently reported that similarly to antidepressant drugs[1], chronic lithium treatment prevents stress-induced changes in behaviour and hippocampal neurogenesis in rats[2]. However, comparable studies investigating the effects of chronic stress and lithium treatment in adult mice are lacking. Characterisation of their effects in mice is important because phenotyping of genetically-modified mice in such models could identify novel targets of antidepressant activity.
- Neuroimaging studies suggest that altered structure and function of the hippocampus is characteristic of some stress-related disorders such as depression. Moreover, animal studies suggest that the hippocampus is anatomically and functionally divided into dorsal (dHi) and ventral (vHi) regions and that the vHi preferentially regulates emotionality and the stress response while the dHi is primarily involved in cognitive function[3] (Fig. 1). Therefore, in the present study we investigated whether chronic immobilisation stress and/or chronic treatment with lithium would alter cell proliferation and survival along the septo-temporal axis of the hippocampus in a stress-susceptible mouse strain, the BALB/c mouse. Finally, since the neurotrophic factors VEGF and BDNF can regulate antidepressant-like behaviour and hippocampal neurogenesis[4], the effects of CIS and lithium treatment on hippocampal mRNA levels of these neurotrophic factors was also investigated.

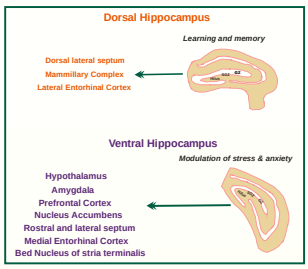
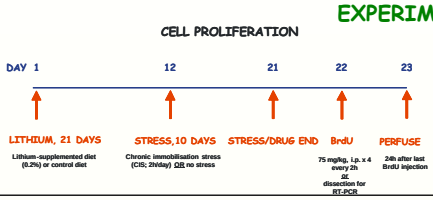
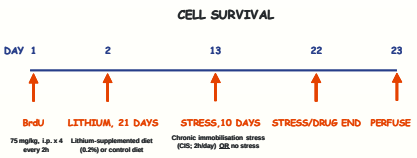


Figure 1. Differences in efferent connections of the hippocampus along the longitudinal axis. The ventral hippocampus projects to many brain areas involved the stress response and that are affected in stress-related disorders such as depression and anxiety disorders. GZ = granular zone/granular cell layer; SGZ = Subgranular zone

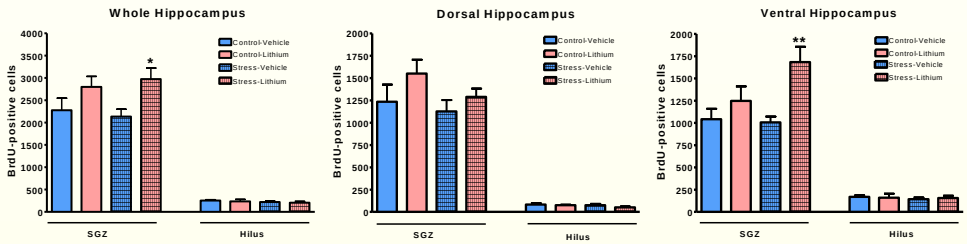
EXPERIMENTAL DESIGN & METHODS

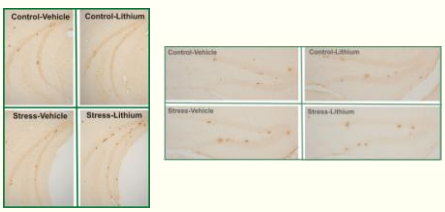




Experimental Design: *Mice received either a lithium-supplemented diet or control diet for 21 days and were given access to a second drinking bottle containing 0.89% NaCl to compensate for lithium-induced ion loss. Twelve days after starting drug treatment, mice were stressed daily, 2 hours per day for 10 days. Chronic immobilisation stress (CIS) was employed as the stressor. Non-stressed control mice were left undisturbed in their homecage. To assess the effects of lithium and CIS on cell proliferation, mice were injected with BrdU at the end of the experiment and were perfused 24 hours later. To assess the effects of lithium and CIS on cell survival, mice were injected with BrdU one day prior to starting drug treatment. A separate cohort of mice did not receive BrdU and their tissue was used in RT-PCR experiments.* ***Animals:*** *Male BALB/cOLaHsd mice aged 8 weeks were employed (Harlan, UK). Immobilisation stress was conducted by placing mice into individual 50ml falcon tubes with an air hole at either end. The immobilised mice were then placed into clean individual cages (2 hours daily for 10 days) and were returned to their home cage following each daily stress session. All animals were housed in groups of 3-4 in their homecage.* ***Cell proliferation*** *and the* ***survival of newly-born cells*** *were analysed using BrdU immunohistochemistry (n = 6 animals per group; 11-12 sections (180 µm apart) analysed per animal). The dHi was defined as AP: -0.94 - AP -2.30 (6-7 sections per animal) and the vHi as AP: -2.46 - AP: -3.80 (5-6 sections per animal).* ***mRNA levels*** *of neurotrophic factors in the hippocampus were measured using semi-quantitative real-time PCR. Results were normalised to β-actin expression followed by comparison to the respective non-stressed vehicle-treated group.* ***Statistical Analysis:*** Data was analysed using Two-way ANOVA followed by posthoc analysis with Fisher's LSD. Posthoc tests were only conducted if one of the main effects of the two-way ANOVA reached a significance level of $p < 0.05$.

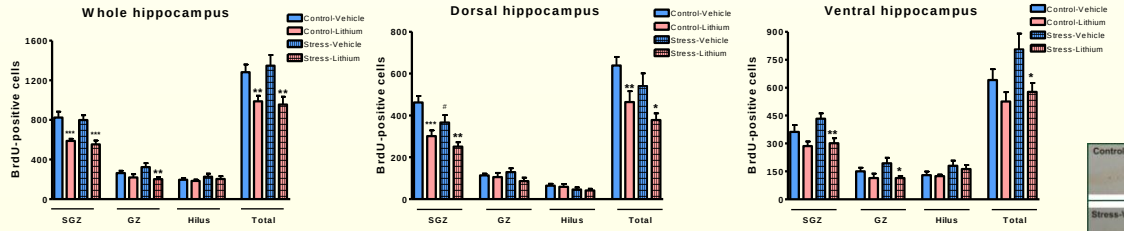
1. LITHIUM SIGNIFICANTLY INCREASES CELL PROLIFERATION IN THE vHi BUT NOT THE dHi OF STRESSED MICE





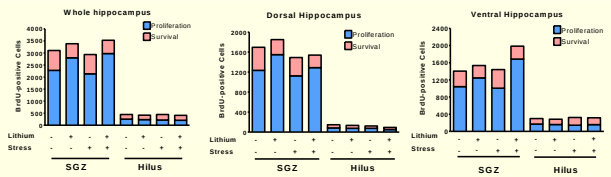
*Significantly different to the stress-vehicle group, *p = 0.018; ** p = 0.002

2. LITHIUM-INDUCED INCREASES IN CELL PROLIFERATION ARE SECONDARY TO REDUCTIONS IN CELL SURVIVAL

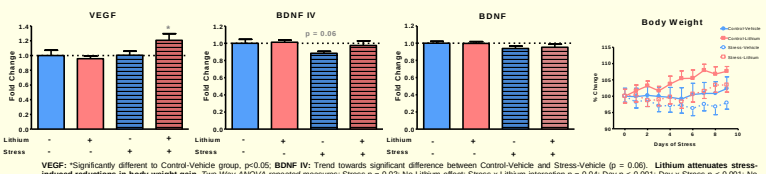


* Significantly different to its respective vehicle-treated group, *P < 0.05; **p < 0.01,***p < 0.001. #Stress significantly decreases cell survival in the subgranular zone compared with the non-stressed vehicle group, p < 0.05.

3. SUMMARY: EFFECTS OF LITHIUM AND STRESS ON CELL PROLIFERATION AND SURVIVAL



4. EFFECTS OF STRESS AND LITHIUM ON NEUROTROPHIC FACTOR mRNA LEVELS IN THE HIPPOCAMPUS AND ON BODY WEIGHT GAIN



VEGF: *Significantly different to Control-Vehicle group, p<0.05; **BDNF IV:** Trend towards significant difference between Control-Vehicle and Stress-Vehicle (p = 0.06). **Lithium attenuates stress-induced reductions in body weight gain.** *Two-Way ANOVA repeated measures:* Stress p = 0.03; No Lithium effect; Stress x Lithium interaction p = 0.04; Day p < 0.001; Day x Stress p < 0.001; No effect of Day x Lithium; Day x Stress x Lithium interaction p < 0.001; n = 7.

CONCLUSIONS

- Lithium increased cell proliferation in the subgranular zone of the hippocampus but this effect was only statistically significant in stressed animals. Moreover, these lithium-induced increases in cell proliferation were localized to the ventral region of the hippocampus. Such effects suggest that lithium-induced increases in hippocampal cell proliferation might only occur or become apparent when hippocampal function is confronted with challenges, such as stress.
- In addition to increasing cell proliferation in stressed animals, lithium also reduced the survival of cells that were generated prior to experimental treatment. This lithium-induced reduction in cell survival was observed in both stressed and non-stressed mice. Specifically, lithium decreased cell survival in the dHi of both stressed and non-stressed mice. In the vHi, lithium significantly reduced cell survival in stressed animals only.
- The lithium-induced increase in cell proliferation suggests a compensatory response to decreases in the survival of newly-born cells. However, upon summation of the total number of surviving BrdU-labelled cells with the total number of proliferating BrdU cells, it appears that lithium selectively increases the total number of BrdU-labelled cells in the ventral hippocampus of stressed animals only. These effects correlate with the lithium-induced increase in VEGF which was only observed in the hippocampus of stressed mice.
- Finally, lithium treatment also attenuated stress-induced reductions in body weight.
- The localization of lithium-induced cell proliferation to the vHi of stressed animals supports the hypothesis that the vHi plays a preferential role in processes relevant to stress-related disorders. Current studies are investigating the functional role of neurogenesis in the vHi and dHi in various behavioural and physiological responses to chronic stress and antidepressant treatments.

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1. Malberg JE and Duman RS (2003) Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology* 28(9):1562-71; **2.** Silva R *et al.*, (2008) Lithium blocks stress-induced changes in depressive-like behavior and hippocampal cell fate: the role of glycogen-synthase-kinase-3β. *Neuroscience* 152(3):656-69.; **3.** Bannerman D *et al.*, (2004) Regional dissociations within the hippocampus - memory and anxiety. *Neurosci Biobehav Rev.* 28(3):273-83.; **4.** O'Leary OF and Castren E. (In Press). Neurotrophic factors and antidepressant action: Recent advances. In: Advances in antidepressant research, Eds. Leonard BE & Cryan JF; *Modern Trends in Pharmacopsychiatry*, Vol. 27