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Lithium and glutamine synthetase: Protective effects following stress

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ABSTRACT

Even though lithium is widely used as treatment for mood disorders, the exact mechanisms of lithium in the brain remain unknown. A potential mechanism affects the downstream target of the Wnt/β-catenin signaling pathway, specifically glutamine synthetase (GS). Here, we investigate the effect of lithium on GS-promoter activity in the brain. Over seven days, B6C3H-Glultm$^{(T2A\text{-}LacZ)}$ mice that carry LacZ as a reporter gene fused to the GS-promotor received either daily intraperitoneal injections of lithium carbonate (25 mg/kg) or NaCl, or no treatment. Following histochemical staining of β-galactosidase relative GS-promotor activity was measured by analyzing the intensity of the staining. Furthermore cell counts were conducted. GS-promotor activity was significantly decreased in female compared to male mice. Treatment group differences were only found in male hippocampi, with increased activity after NaCl treatment compared to both the lithium treatment and no treatment. Lithium treatment increased the overall number of cells in the CA1 region in males. Daily injections of NaCl might have been sufficient to induce stress-related GS-promotor activity changes in male mice; however, lithium was able to reverse the effect. Taken together, the current study indicates that lithium acts to prevent stress, rather affecting general GS-promoter activity.

GABA	Gamma-Aminobutyric acid
GS	Glutamine synthetase
GSK-3β	Glycogen synthase kinase 3β
MANOVA	Multivariate analysis of variance
NaCl	Natrium Chloride
X-gal	Beta-galactosidase

1. Introduction

The number of people diagnosed with mental disorders is increasing worldwide. Even in high-income countries, between 35% and 50% of people diagnosed with a mental illness go untreated (World Health Organization, 2018). These individuals, especially with mood disorders such as unipolar or bipolar depression, have a higher risk of attempting suicide (Tondo and Baldessarini, 2016). Multiple studies have shown that lithium treatment decreases the risk of suicide in patients with major mood disorders (Tondo and Baldessarini, 2016). The exact mechanism of action of lithium in the brain however, is still unclear.

The therapeutic range of lithium is narrow and there is a high risk of lithium intoxication during treatment. There are also various side effects of long-term lithium use including reduced urinary concentrating ability, hypothyroidism, hyperparathyroidism, weight gain, and with high doses, potential neurotoxicity (McKnight et al., 2012; Simard et al., 1989; Suraya and Yoong, 2001). Understanding the exact mechanisms of lithium in the brain is crucial for the development of medication targeting specific signaling pathways. This ultimately would reduce the side effects of lithium treatment. To date, the different mechanisms of action of lithium in the brain and the whole organism (Oruch et al., 2014) include influencing the gene regulation process (Lenox and Wang, 2003), modulating serotonin release in specific cells (Scheuch et al., 2010), and potentially altering the circadian rhythm by inhibiting the β-glycogen synthase kinase 3 (GSK-3β) (Yin et al., 2006). The inhibition of the GSK-3β might be the answer, as it is an enzyme that participates in the Wnt/β-catenin signaling pathway (Klein and Melton, 1996; Lenox and Wang, 2003; Oruch et al., 2014; Yin et al., 2006). The Wnt/β-catenin pathway is involved in various cellular processes including cell proliferation, cell development, cell survival and motility, as well as postnatal and adult brain plasticity and adult neurogenesis (Klein and Melton, 1996; Maguschak and Ressler, 2012). Alterations in this signaling pathway can cause changes

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in behavior and cognition (Jin et al., 2017; Maguschak and Ressler, 2012). One downstream target of the Wnt/β-catenin signaling pathway is glutamine synthetase (GS) (Cadoret et al., 2002; Kordes et al., 2008). GS is crucial for the maintenance of the glutamate-glutamine-cycle and neurotransmission. GS recycles the neurotransmitter glutamate (Rose et al., 2013), as neurons are incapable of de novo synthesis of glutamate. Given the important role of glial GS in several metabolic pathways, such as the glutamate-glutamine-cycle and metabolism of ammonia (Rose et al., 2013), it is no surprise that alterations in GS expression are associated with several neurological disorders including depression, schizophrenia, epilepsy, Parkinson's, and Alzheimer's disease (Bruneau et al., 2005; Gruenbaum et al., 2015; Hensley et al., 1995; Kalkman, 2011; Kulijewicz-Nawrot et al., 2013; Rose et al., 2013; Yu et al., 2012).

In a study in rats, researchers found an increase of GS activity in the brain after 7 days of lithium treatment (Marcus et al., 1986). Such a finding is supported by the reduced GS activity found in the postmortem brains of depressed patients, as well as suicide cases of schizophrenic patients (Kalkman, 2011). Given the protective effect of lithium against suicide (Tondo and Baldessarini, 2016), and reduced GS activity in postmortem brains in suicide cases, the question arises whether lithium acts by elevating GS activity.

In the current study, we analyzed the effect of intraperitoneal 25 mg/kg lithium carbonate injections on GS-reporter activity in the brain of a GS-reporter mouse model. Reporter genes such as the LacZ gene can be fused to the promotor of a target gene due to transgenic techniques to facilitate the analysis of gene expression of a particular promoter. This model was used to determine the actual effect of lithium treatment on GS-promoter activity as a follow up to the findings by Marcus and colleagues (1986). Due to the fact that injection stress can affect the animal (Freund et al., 2013), a naïve control group with no prior treatment was included (no treatment group). Both male and female mice were analyzed in this study.

2. Material and methods

2.1. Animals

A GS-reporter mouse model, B6C3H-Glultm$^{(T2A\text{-}LacZ\text{-}loxP\text{-}T2A\text{-}Tk\text{-}1\text{-}FRT\text{-}loxP\text{-}T2A\text{-}Fluc\text{-}FRT)Arte}$, obtained from the Institute of Experimental and Clinical Pharmacology and Toxicology at the University of Tübingen, Germany (originally prepared by Taconic Artemis, Cologne, Germany) was chosen. Rapid changes in promoter activity can be detected using a reporter mouse. In this study, one out of the three reporters present in the transgene was utilized, namely the LacZ reporter, which allows quantification of its substrate beta-galactosidase. The LacZ reporter includes a nuclear localization signal in the LacZ open reading frame (SV40 monopartite nuclear localization signal PKKKRKV (cctaagaagaagaggaaggtt)) facilitating the quantification of beta-galactosidase-positive cells. Counting positives is more accurate as staining can easily be seen concentrated in the nucleus.

All animals were housed in a 12/12-hour dark-light cycle and under constant conditions (22 ± 2 °C). The animals had free access to food and water. For the first five generations (F1–F5), heterozygous GS-reporter mice were bred with C3H mice (Charles River, Germany). Generations F6 and F7 were bred within the heterozygous GS-reporter mice. For this study, only heterozygous GS-reporter mice of the F8 generation (F7 generation bred with C3H) were used. All procedures were approved by the local Animal Welfare and Ethics committee of the Country Commission Tübingen, Germany.

2.2. Drug treatment

Following the genotyping for the reporter gene, 23 three-month old female GS-reporter mice, and 19 three-month old male GS-reporter mice, were randomly assigned to one of three experimental groups. The first group was given daily intraperitoneal 25 mg/kg lithium carbonate in 0.1 ml 0.9% natrium chloride (NaCl) injections (100 mg Li_2CO_3 dissolved in 15 ml NaCl) for seven consecutive days (females $n = 8$, males $n = 6$). The chosen dose of 25 mg/kg results in a lithium blood level comparable to 0.8 meq/l in patients (Smithberg and Dixit, 1982). The second group received daily 0.1 ml NaCl injections (weight-independent) and served as an injection/stress control (females $n = 9$, males $n = 6$). The third group remained untreated and served as a no stress control (females $n = 6$, males $n = 7$).

2.3. Staining

Twenty-four hours after the last injection, all animals were anesthetized with ketamine hydrochloride and xylazine hydrochloride (100 mg/kg and 10 mg/kg, intraperitoneal). Transcranial perfusions were performed using 0.12 M sodium phosphate buffer (pH 7.4) followed by 4% paraformaldehyde, as a fixative. Perfusion surgeries were performed as previously described (Gage et al., 2012). Brains were coronally cryosectioned into 40 µm slices from rostral to caudal. To detect LacZ gene activity, beta-galactosidase (X-gal) staining was used. The staining protocol of Kokubu and Lim (2014) was followed, with the modifications outlined below. In brief, free-floating sections were incubated with 1 mg/ml X-gal staining solution for 3 h at 37 °C. The slices were washed and coverslipped with Mowiol.

For general cell counting in the hippocampus, Nissl staining was conducted with a different set of slices to stain DNA and RNA in the cell nucleus by cresyl violet. After X-gal staining, the slices were dehydrated in 70% ethanol and incubated with 1% cresyl violet (Sigma) solution for 3 min. Sections were differentiated in 96% ethanol mixed with a few drops of acetic acid. After dehydration, the slices were treated with isopropanol followed by xylene and coverslipped with DPX (Fluka).

Double staining of beta-galactosidase and GS-protein were also performed. Staining for GS-protein was performed according to standard free-floating immunohistochemistry staining protocols (Bachman, 2013). Slices were briefly incubated with the primary antibody anti-GS from rabbit by GenTex (1:500, GTX109121, overnight 4 °C) in 0.12 M PBS with 0.3% triton (Triton-X100, Sigma-Aldrich) and 1% normal goat serum (Sigma–Aldrich) and the secondary antibody anti-rabbit from goat (1:300, Sigma-Aldrich) for one hour. This was followed by incubation with an avidin-biotin-complex (Vector Laboratories, Vectastain ® ABC Kit) for one hour at room temperature in 1% A + 1% B in PBST. Final staining was by a peroxidase-catalyzed diaminobenzidine reaction (Vector Laboratories, DAB-Kit). Slices were washed in PBS and then stained with X-gal (see above).

2.4. Analysis

Thirteen coronal sections, each 40 µm in thickness between approximately 2.96 mm and −2.14 mm of bregma position, were investigated. The mouse brain atlas by Paxinos and Franklin (2001) was used to determine the level of sections across all animals. X-gal stained slices were imaged with the highest resolution (12.800 dpi) by using a transmitted light scanner (Epson Perfection V600 Photo). Images from single slices were transformed to gray scale, and were further analyzed with Matlab. The gray intensity of the background was first determined. Next the gray intensity of every pixel with a threshold higher than the background was measured. Finally, the mean gray intensity was calculated for each slide.

The hippocampi (at bregma ~ −1.92 mm), of the male animals were cropped with photoshop and converted into greyscale, before mean gray intensities were calculated. In the same layer of hippocampus (bregma ~ −1.92 mm), X-gal and cresyl violet stained cells in the CA1 and CA3 area were photographed with a transmitted light microscope (Olympus BX51) and the software CellD. Cells were counted manually by an investigator blinded to the terms of experimental conditions.

2.5. Statistics

Statistics were performed with SPSS 25. For analysis of gray intensities, Treatment (3: lithium vs NaCl vs no treatment) and Sex (2: male vs female) were set as factors to conduct a multivariate analysis of variance (MANOVA), with the different Brain Sections as variables. Student's *t*-test was applied for post-hoc comparison of Sex for all brain sections. Due to the main effect of Sex, the analysis was also conducted separately for male and female mice. After the MANOVA with Treatment (3: lithium vs NaCl vs no treatment) as the factor and Brain Sections as variables, a one-way ANOVA was conducted for each brain section separately. Gray intensities in the male hippocampus were compared with a one-way ANOVA using Treatment (3: lithium vs NaCl vs no treatment) as the factor. A MANOVA with Treatment as a factor (3: lithium vs NaCl vs no treatment) and Brain Region (CA1, CA3) as a variable was performed separately for each of the cell counts (X-gal positive cells and total cell number). A one-way ANOVA with Treatment as factor was followed for each region. Due to homogeneity in variance and a small difference in sample size, the Gabriel test was chosen as post-hoc test for treatment.

3. Results

3.1. Staining

The X-gal stained cells showed mostly nuclear staining of the GS-reporter. This results from a nuclear localization signal in the LacZ open reading frame in the GS promoter-driven transgene of the GS-reporter mouse. Double staining for X-gal and GS showed co-localization of staining for the GS-reporter and GS-protein, demonstrating the specificity of the GS-reporter (Fig. 1).

3.2. Lower GS-reporter activity in females

Using Pillai's trace, no significant differences between Treatment groups ($V = 0.68$; $F(26, 50) = 0.99$, $p = 0.49$) or an interaction between Treatment and Sex ($V = 0.76$; $F(26, 50) = 1.18$, $p = 0.29$) were evident, but a significant Sex difference ($V = 0.59$; $F(13, 24) = 2.714$, $p = 0.017$) was found. The measured relative gray intensity was significantly lower in females compared to males (Student's *t*-test $t(26) = -3.464$, $p = 0.002$) indicating less GS-reporter activity in females. When comparing the treatment groups separately, only the NaCl treatment group showed a significant difference (Student's *t*-test $t(13) = -4.207$, $p = 0.001$; not corrected for multiple testing, Fig. 2A).

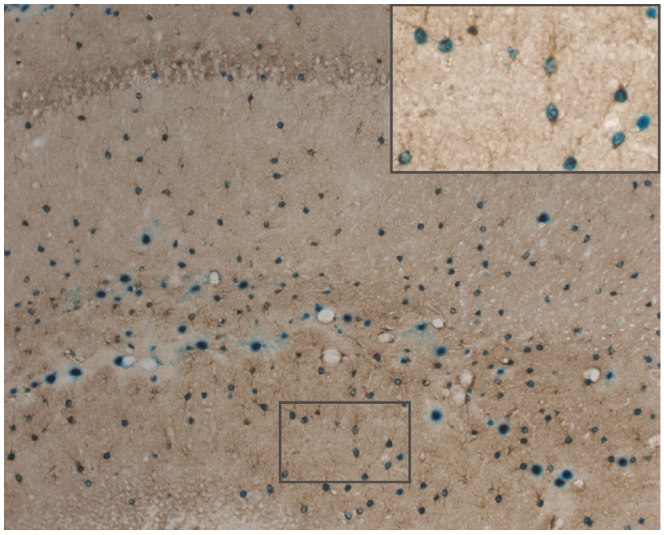

Fig. 1. Histochemically stained cells of a male mouse hippocampus. Beta-galactosidase representing the GS-reporter is stained blue, and glutamine synthetase protein is stained in brown. Nearly all cells with the reporter gene present also contained GS-protein and vice versa. Magnification in 20x and 40x for the right upper corner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Treatment-Related changes in GS-reporter activity in male mice

Due to the main effect of Sex, we conducted statistical analysis separately for both male and female mice. No significant difference between groups were found in females ($V = 1.26$; $F(26, 18) = 1.18$, $p = 0.36$) and males ($V = 1.68$; $F(26, 10) = 2.08$, $p = 0.11$). However, when conducting the one-way ANOVA for each section level, section level 12 in males (at bregma ~ −1.92 mm) showed a significant effect of treatment ($F(2, 16) = 3.651$, $p = 0.049$; not corrected for multiple testing). The post-hoc test revealed a difference that approached significance between the NaCl and lithium groups (Gabriel, $p = 0.075$).

3.4. GS-reporter activity differences in the hippocampus

The one-way ANOVA showed a significant Treatment effect, $F(2, 16) = 4.466$, $p = 0.029$ in the hippocampus at bregma ~ −1.92 mm. The post-hoc Gabriel test revealed an increased gray intensity for the NaCl group compared to the lithium ($p = 0.051$) and no treatment groups ($p = 0.059$). The relative gray intensity did not differ between lithium and no treatment group ($p = 0.997$) (Fig. 2B).

3.5. Overall cell number in hippocampal region CA1 increased in lithium-treated males

After detecting effects in the hippocampus, X-gal stained cells and all Nissl stained cells were counted in discrete regions (Fig. 3A). The number of X-gal positive cells showed no significant differences for Treatment ($V = 0.17$; $F(4, 72) = 0.15$, $p = 0.96$) or Sex ($V = 0.12$; $F(2, 35) = 2.58$, $p = 0.09$) using Pillai's trace. The total cell numbers revealed differences in Sex ($V = 0.4$; $F(2, 35) = 11.86$, $p < 0.001$) and an interaction of Treatment and Sex ($V = 0.28$, $F(4, 72) = 3.02$, $p = 0.023$).

There was no main Treatment effect in males ($V = 0.44$, $F(4, 329) = 2.312$, $p = 0.079$), however there was an individual effect for the CA1 region ($F(2, 16) = 3.783$, $p = 0.045$; Fig. 3B). The Gabriel post-hoc analysis indicated differences between lithium-NaCl treatment ($p = 0.077$) and no treatment ($p = 0.087$) but not for NaCl treatment and no treatment ($p = 1.00$) in the CA1 region. No differences were detected in CA3 ($F(2, 16) = 0.199$, $p = 0.8$; Fig. 3B). In addition, there was no Treatment main effect in females ($V = 0.3$, $F(4, 40) = 1.81$, $p = 0.14$) nor was there an individual effect of region (CA1: $F(2, 20) = 0.742$, $p = 0.489$; CA3: $F(2, 20) = 1.743$, $p = 0.20$).

4. Discussion

GS is a key enzyme in neurotransmitter homeostasis in neuronal cells. The enzyme is relatively stable showing a long half-life (13–22 h) after synthesis (Suárez et al., 2002). To monitor rapid responses following exogenous stimuli, measurements of enzyme content or activity are of limited value only. We therefore investigated the activity of a GS-reporter in a transgenic mouse model, as it enabled us to detect rapid changes in the activation of the promoter-driven reporter following its activation. Double staining confirmed that GS-reporter activity was only detected in cells also expressing GS-protein (Fig. 1).

In this study, a significant effect of treatment on the GS-reporter activity in male mice was shown (Fig. 2B). In the hippocampus of males, GS-reporter activity increased in the NaCl group compared to the lithium and no treatment groups (Fig. 2B). Cell counting following X-gal and Nissl staining of cells in the hippocampus CA1 and CA3 regions (Fig. 3A), revealed significantly more Nissl stained cells in lithium-

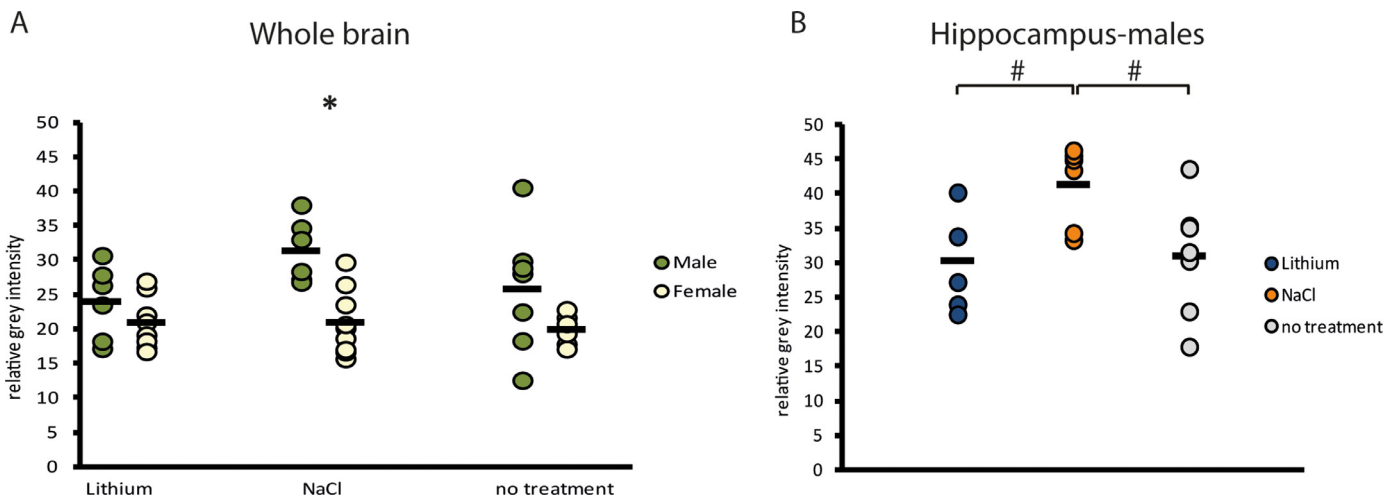

Fig. 2. GS-reporter activity. Data is presented as data points. The black bar represents the mean. A: Females specifically in the NaCl group show less GS-reporter activity (* $p < 0.05$). B: In the male hippocampus (at Bregma −1.92 mm) NaCl treatment resulted in increased activity of the GS-reporter (# $p < 0.06$).

treated males in the CA1 region, compared to NaCl and no treatment groups (Fig. 3B). There was no difference in total number of cells found in the CA3 region for all groups. In female mice, we found no differences in cell numbers between groups for both regions.

The increased GS-reporter activity found in the NaCl group compared to the lithium and no treatment groups was at first surprising. However, repeated daily intraperitoneal injections, even if only saline solution, must be regarded as a mild stressor (Deutsch-Feldman et al., 2015; Freund et al., 2013; Izumi et al., 1997), and have previously been shown to induce depressive-like behavior in rats (Izumi et al., 1997). Therefore, the increase of GS-reporter activity after daily NaCl injection could be the result of stress caused by the injections.

The influence of stress on the glutamate-glutamine-cycle, in which GS plays an important part, has previously been studied. Stress exposure in rodents led to increased prefrontal cortex glutamate (Popoli et al., 2011), and generally decreased gamma-aminobutyric acid (GABA) (synthesized from glutamate) levels in the brain (Skilbeck et al., 2010; Ulrich-Lai and Herman, 2009). Furthermore, hippocampal GABA levels increased when rats were given a stimulating stressor, and decreased when the animals were experiencing a possible negatively perceived stressor (de Groote and Linthorst, 2007). Given that the GS is part of the glutamate-glutamine-cycle and thereby influences the GABA deposit in synapses, it is likely that stress affects GS-expression. Our results show that an increase in GS-reporter activity in the NaCl treated male mice may indicate that the male mice experienced the daily injection as a negative stressor.

Interestingly, lithium-treated male mice significantly differed from the NaCl treated group, and showed GS-reporter activity levels comparable to the no treatment group. This finding indicates that lithium injections prevented an increase in GS-reporter activity as a stress response (as seen in the NaCl injected male mice). The results of the male mice indicate a protective role of lithium on the stress response as no differences in GS-reporter activity was found between lithium-treated and no treatment male mice, but a significantly increase in GS-reporter activity was seen in NaCl treated mice. Given the positive effect of lithium on cell proliferation and neuronal differentiation (Dong et al., 2015; Su et al., 2007; Zanni et al., 2015), a protective effect is likely. At first, this seems to contrast with the results of Marcus and colleges (1986) who reported an increase in the GS activity after lithium treatment. However, they investigated GS activity which might differ from the direct promoter activity we measured by the reporter activity. Moreover, Marcus and colleagues only observed an increase in activity in the brain stem (Marcus et al., 1986), which may not reflect changes in other brain regions.

In female mice, no difference in GS-reporter activity were found between all three treatment groups. This finding might indicate that female mice did not show a stress response to the injections. The fact, that male and female rodents respond differently to stress is already well known (Freund et al., 2013; Leussis et al., 2012). Male rats respond differently to a severe stressor, such as inescapable shocks, compared to females (Leussis et al., 2012). When given a mild stressor, such as NaCl injections, female but not male rats show beneficial effects (Freund et al., 2013). The beneficial effect of stress for female rats in contrast to male rats, has also been reported in a spatial memory task after restraint stress, regardless of the estrus (Bouma et al., 2009). Therefore, it is likely, that females did not react to the given injection procedure as did males in this experiment. The fact that a significant sex difference in GS-reporter activity was only seen in the NaCl treatment group (Fig. 2A), further demonstrates that only males may have reacted to the stressor. In this study, female mice were not categorized based upon their estrus cycle phase, therefore a potential hormonal effect on the stress response cannot be excluded.

A recently published study found significantly higher GS-protein levels in female than in male juvenile rats (Al-Suwailem et al., 2018). The study by Al-Suwailem and colleagues (2018) also reported lower levels of glutamate, as well as other factors, in the glutamate signaling pathway in females. The sex differences in the glutamate signaling pathway is assumed to have a neuroprotective effect in females compared to males, as high glutamate levels can have a neuroexcitotoxic effect (Al-Suwailem et al., 2018). This finding contrasts with our findings where we report less GS-reporter activity in female mice compared to males. However, measured GS-protein levels do not have to correspond to measured GS promoter activity measured by GS-reporter activity. Van Straaten and colleagues (2006) claimed a difference (up to 20-fold) in the murine GS-protein:mRNA ratio indicating a strong transcriptional and posttranscriptional regulation of the GS, whereas posttranscriptional regulation may not have a high impact (van Straaten et al., 2006). Moreover, higher GS levels in juvenile female rats found by Al-Suwailem and colleagues may not necessarily stabilize until adulthood. Given the increase of GS activity in juvenile female rats compared to male juvenile rats, it seems likely that brains prime differently during development based on sex. Furthermore, even though we did not see an interaction of sex and treatment, the main sex effect might be driven by the NaCl treatment group, and therefore reflect differences in stress response rather that a general increase in GS promoter activity in males.

The significantly higher number of cells in male mice CA1 region after lithium carbonate injection, in contrast to NaCl treatment or no

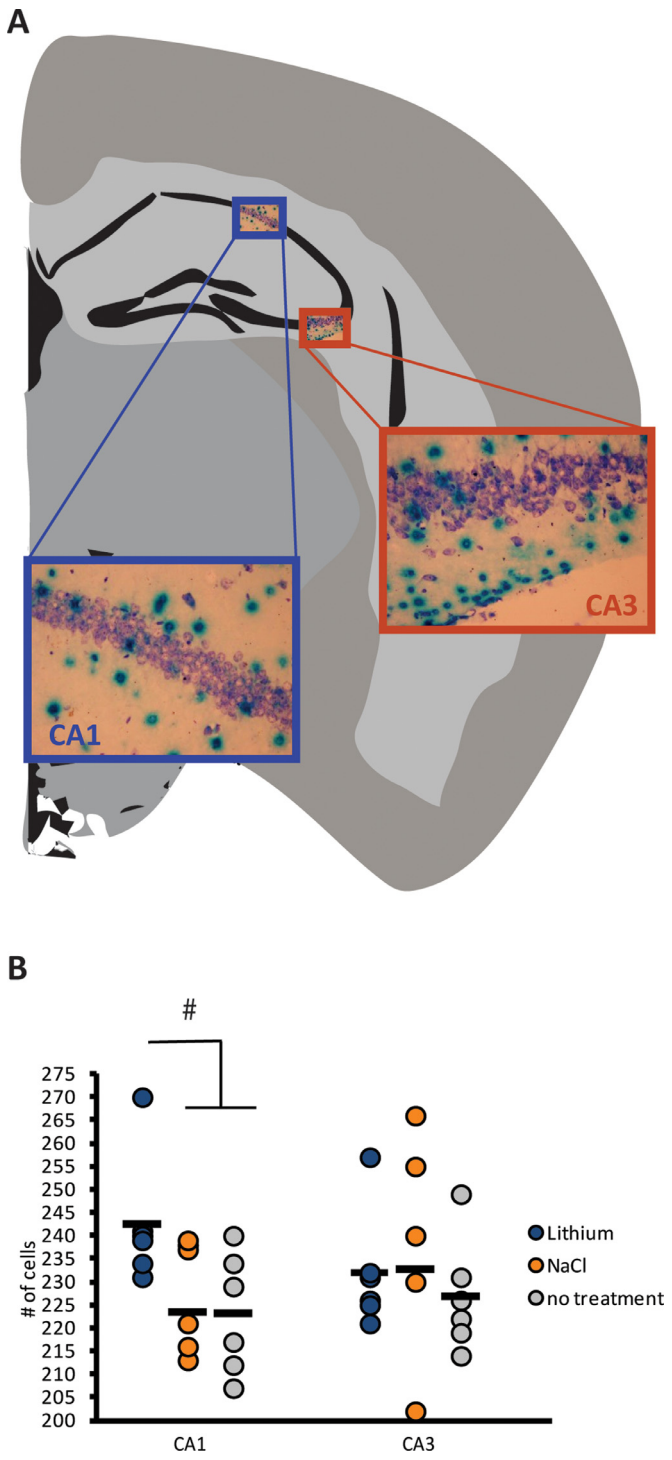

Fig. 3. Effect of treatment on cell numbers in male hippocampus. **A**: Nissl (violet) and GS-reporter positive cells (blue) were counted in the CA1 and the CA3 region of the hippocampus (bregma −1.92 mm). Nissl stained cells containing the GS-reporter appear predominantly blue as X-gal staining concealed Nissl staining; **B**: Total number of counted X-gal and Nissl stained cells in the CA1 and CA3. Total number of cells was increased in lithium-treated mice in CA1 ($\# p < 0.1$). Data is presented as data points. The black bar represents the mean. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

treatment, is in line with other studies that identify an increase in CA1 cells after lithium treatment (Schaeffer et al., 2017). In a study examining the neuroprotective effects of lithium for hippocampal cell loss in a mouse model for Alzheimer's disease, male mice were treated with subtherapeutic and therapeutic doses of lithium at 3 months of age until 11 months of age, by lithium-supplemented chow (to exclude the stress of injection), where the subtherapeutic doses led to a significant increase of CA1 neurons in wild-type mice (Schaeffer et al., 2017).

The results of our study may indicate that lithium has a positive effect on cell proliferation in the CA1 region of male mice after 1 week with a therapeutic dose of lithium carbonate of 25 mg/kg per day. Therefore, it seems likely that to have positive effects on neuronal growth, the dose and length of lithium treatment depend on environmental influences such as stress. To our knowledge, studies on the effects of lithium on cell proliferation in the CA3 region are still rare. However, our study reports no difference in cell numbers in the CA3 region in all three treatment groups for both sexes. One possible explanation for increased cell proliferation in male CA1 but not in CA3 region could be the effect of stress. In a study from Watanabe and colleagues (1992), rats were restrained for 21 consecutive days. Afterwards, the researchers found a significant decrease of apical dendrites of CA3 neurons, but no change in CA1 or dentate gyrus neurons (Watanabe et al., 1992). If lithium acts as a protector against cell atrophy, this could be the reason why we found neither a decrease in cell numbers in the CA3 due to stress, nor an increase in cells compared to the CA1 region.

The not altered cell number in the CA3 after stress would be in line with the finding of Silva and colleagues (2008) who confirmed in a rat study the influence of lithium on both, neurogenesis and apoptosis in the hippocampus by regulating the activity of the GSK-3β. In their study, chronic-mild-stressed rats showed a decrease in hippocampal dentate cell turnover, however the decrease could be prevented with an accompanied lithium treatment. More interestingly, it seemed that control animals with lithium treatment showed an increase in cell proliferation and apoptosis in the hippocampus (Silva et al., 2008). These results confirm the controversial mechanisms of lithium found in therapeutic usage where lithium elevates depressive mood (Tondo and Baldessarini, 2016) while also attenuating a manic state (Beyer and Freund, 2017).

Given the protective effect of lithium treatment on cell turnover found in the rat hippocampus, lithium treatment to prevent suicide in patients might protect the brain against acute negative stress. In this case, GS may play a pivotal role in stress coping mechanisms of the brain. For future patient treatment, it would be interesting to investigate whether GS levels in blood or spinal fluid could predict the lithium dose needed for a protective effect. Moreover, it would be helpful to investigate whether stress assessment of patients before or during lithium treatment can be correlated to the lithium dose needed for a protective effect. Finally, it would be interesting to further investigate inflammatory pathways and activity of the GS-reporter, as GS plays a major role in regulating inflammatory processes in the brain (Palmieri et al., 2017). Lithium, on the other hand, might reduce inflammatory mediators and enzymes involved by inhibiting the GSK-3β signaling pathway (Nassar and Azab, 2014).

The results of our study indicate that lithium may act as a protective factor against stress by inhibiting an upregulation of GS expression particularly in the brain of male mice. Moreover, it seems that lithium might not only protect against stress, but also stimulate adult cell growth in males.

CRediT authorship contribution statement

Annakarina Mundorf: Data curation, Formal analysis, Writing - original draft. **Alexandra Knorr:** Data curation, Formal analysis. **Charlotte Mezö:** Data curation, Formal analysis. **Christina Klein:** Data curation, Formal analysis. **Dominik KE Beyer:** Data curation, Formal analysis. **Andreas J Fallgatter:** Conceptualization. **Michael Schwarz:** Conceptualization, Data curation, Formal analysis. **Nadja Freund:** Conceptualization, Writing - original draft.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.112544.

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