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Repeated forced swimming impairs prepulse inhibition and alters brain-derived neurotrophic factor and astroglial parameters in rats

Milene Borsoi [a,*], Camila Boque Antonio [b], Liz Girardi Müller [b], Alice Fialho Viana [b], Vivian Hertzfeldt [b], Paula Santana Lunardi [c], Caroline Zanotto [c], Patrícia Nardin [c], Ana Paula Ravazzolo [d], Stela Maris Kuze Rates [a,b], Carlos-Alberto Gonçalves [a,c]

[a] Graduate Studies Program in Neurosciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[b] Graduate Studies Program in Pharmaceutical Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[c] Graduate Studies Program in Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[d] College of Veterinary Medicine, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

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ABSTRACT

Glutamate perturbations and altered neurotrophin levels have been strongly associated with the neurobiology of neuropsychiatric disorders. Environmental stress is a risk factor for mood disorders, disrupting glutamatergic activity in astrocytes in addition to cognitive behaviours. Despite the negative impact of stress-induced neuropsychiatric disorders on public health, the molecular mechanisms underlying the response of the brain to stress has yet to be fully elucidated. Exposure to repeated swimming has proven useful for evaluating the loss of cognitive function after pharmacological and behavioural interventions, but its effect on glutamate function has yet to be fully explored. In the present study, rats previously exposed to repeated forced swimming were evaluated using the novel object recognition test, object location test and prepulse inhibition (PPI) test. In addition, quantification of brain-derived neurotrophic factor (BDNF) mRNA expression and protein levels, glutamate uptake, glutathione, S100B, GluN1 subunit of N-methyl-D-aspartate receptor and calmodulin were evaluated in the frontal cortex and hippocampus after various swimming time points. We found that swimming stress selectively impaired PPI but did not affect memory recognition. Swimming stress altered the frontal cortical and hippocampal BDNF expression and the activity of hippocampal astrocytes by reducing hippocampal glutamate uptake and enhancing glutathione content in a time-dependent manner. In conclusion, these data support the assumption that astrocytes may regulate the activity of brain structures related to cognition in a manner that alters complex behaviours. Moreover, they provide new insight regarding the dynamics immediately after an aversive experience, such as after behavioural despair induction, and suggest that forced swimming can be employed to study altered glutamatergic activity and PPI disruption in rodents.

1. Introduction

Altered glutamate levels and proteins associated with cellular survival participate in the cognitive impairment observed in patients with various neuropsychiatric disorders, including major depression (Duman, 2014; Krystal et al., 2002; Nudmamud-Thanoi and Reynolds, 2004; Tokita et al., 2012). Supporting this assumption, studies have been reported changes in several subunits of glutamate receptors in depressed patients (Nudmamud-Thanoi and Reynolds, 2004; Beneyto et al., 2007) and rapid antidepressant-like effects of various N-methyl-D-aspartate receptor (NMDAR) antagonists (Browne and Lucki, 2013; Tokita et al., 2012). In addition, astrocyte alterations have been associated with depression because altered parameters of astrocyte function are observed in depressed patients (Czéh and Di Benedetto, 2013; Popoli et al., 2012; Sanacora and Banasr, 2013), and rodents show depressive-like behaviours after pharmacological blockade of astrocytic glutamate uptake in the amygdala (Lee et al., 2007), after glial ablation (Banasr and Duman, 2008) and in response to glutamine deficiency (Lee et al., 2013) in the prefrontal cortex. Along with these glutamate perturbations, brain-derived neurotrophic factor (BDNF), which is among several signalling pathways underlying synaptic transmission and plasticity, has been strongly implicated in depression neurobiology (Brunoni et al., 2008) and in the cognitive impairment observed in depressed patients (Castrén and Rantamäki, 2010; Duman and Monteggia, 2006).

It is widely accepted that environmental stress is a risk factor for mood disorders and that glutamatergic synapses are highly responsive

* Corresponding author at: Graduate Studies Program in Neurosciences, Federal University of Rio Grande do Sul (UFRGS), 500 Sarmento Leite Street, zip code 90050-170, Porto Alegre, RS, Brazil. Tel.: +55 51 3308 5455; fax: +55 51 3308 5243.
E-mail address: mileborsoi@gmail.com (M. Borsoi).

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to environmental stimuli (Popoli et al., 2012). Both glutamate transmission and BDNF levels can be significantly impacted by exposure to stress (Bath et al., 2013; Duman and Monteggia, 2006, Popoli et al., 2012). Despite the negative impact of stress-induced neuropsychiatric disorders on public health, the molecular mechanisms underlying the response of the brain to stress have not yet been fully elucidated. Exposure to repeated swimming has been proven as an effective tool to evaluate the loss of cognitive function after various pharmacological and behavioural interventions (Abel and Hannigan, 1992; Naudon and Jay, 2005; Porsolt et al., 2010). This test has been widely used to predict the antidepressant effects of several compounds (Porsolt et al., 1978a; Porsolt et al., 1978b; Cryan et al., 2005) and can induce various neurochemical changes observed both in neuropsychiatric diseases (Briones-Aranda et al., 2005; Rada et al., 2003; Sequeira-Cordero et al., 2014; Zucker et al., 2005) and in animal models of neuropsychiatric diseases based on environmental stress exposure (Anisman and Matheson, 2005; Krishnan and Nestler, 2011; Andolina et al., 2013). However, the effect of repeated swimming exposure on glutamatergic function has not been fully explored.

In the present study, we investigate the effect of repeated forced swimming on cognitive behavioural tests and its role in glutamatergic regulation and neurotrophin levels in rats. Rats were exposed to repeated forced swimming before being subjected to behavioural tests putatively associated with neuropsychiatric disorders: novel object recognition, object location test, and prepulse inhibition (PPI). We measured BDNF and the GluN1 subunit of NMDAR in the frontal cortex and hippocampus of rats at various time points after the swimming session, in addition to glutamate uptake. Moreover, we evaluated other parameters associated with astrocyte function (glutathione content and S100B) in the aforementioned brain regions.

2. Results

2.1. Behavioural tests

2.1.1. Immobility behaviour increases during repeated forced swimming, but spontaneous locomotion is not affected

Fig. 1A shows the immobility duration of repeated forced swimming performed for three consecutive days. One-way repeated measures ANOVA showed a significant *day* effect ($F_{9,29} = 14.561$, $p < 0.001$). Post hoc Student-Newman-Keuls analysis revealed an increased immobility duration according to the day (day 1 vs. day 2, $p = 0.017$; day 1 vs. day 3, $p < 0.001$ and day 2 vs. day 3, $p = 0.013$).

To verify whether repeated forced swimming affected exploratory parameters, the number of crossings and rearings were evaluated during the habituation phases (absence of objects) of both the novel object recognition (NOR) task and object location test (OLT), which were administered 24 h after the last swimming exposure. Because the habituation phase of both the NOR task and OLT were performed identically, the data were grouped by condition (rats submitted to repeated forced swimming and those that were not). No changes in the number of crossings (Student's *t* test, $t = 1.565$, $df = 46$, $p = 0.124$) or rearings (Student's *t* test, $t = 0.799$, $df = 46$, $p = 0.428$) (Fig. 1B and C, respectively) were detected.

2.1.2. Novel object recognition test results are not altered by repeated forced swimming

Two-way repeated measures ANOVA showed neither a *group* effect ($F_{1,39} = 0$; $p = 1.00$) nor interaction ($F_{1,39} = 1.010$, $p = 0.328$) but did show a significant *object* effect ($F_{1,39} = 18.221$, $p < 0.001$) on short-term memory (STM). Post hoc analysis revealed that both the non-stressed ($p = 0.002$) and repeated forced swimming ($p = 0.033$) groups spent more time exploring the novel object than the familiar one during the STM retention trial (Fig. 2A), indicating an absence of cognitive impairment. The investigation ratio for the novel object was also not affected by repeated forced swimming (Student's *t* test: $t = 0.013$, $df = 17$, n.s.) (Fig. 2B). Likewise, the STM and long-term memory (LTM) were unaffected by repeated forced swimming. Two-way repeated measures ANOVA showed no *group* effect ($F_{1,39} = 0$; $p = 1.000$) and a significant *object* effect ($F_{1,39} = 36.880$; $p < 0.001$). Student-Newman-Keuls test indicated that both the non-stressed ($p = 0.004$) and repeated forced swimming ($p < 0.001$) groups spent more time investigating the novel object (Fig. 2C). No significant difference was observed in the investigation ratio of LTM (Student's *t* test: $t = 1.403$, $df = 18$; n.s.) (Fig. 2D). No significant difference in time spent exploring the sample objects during the acquisition phase of STM and LTM in either group was detected (data not shown).

2.1.3. Object location test results are not affected by repeated forced swimming

The results of the object location test were not affected by repeated forced swimming for any retention memory evaluated. Two-way repeated measures ANOVA revealed no significant *group* effect in either STM ($F_{1,27} = 0$, $p = 1.00$) or LTM ($F_{1,31} = 0$, $p = 1.00$) (Fig. 4A and 4C, respectively). Student-Newman-Keuls analysis revealed that for both STM ($p < 0.001$) (Fig. 3A) and LTM ($p = 0.003$), rats exposed to forced swimming spent more time exploring the novel object than the familiar one, indicating an absence of cognitive impairment (Fig. 3C). The preference index for the relocated object was not affected during both the STM ($t = 0.718$, $df = 12$, $p = 0.486$) and LTM ($t = 0.526$, $df = 14$, $p = 0.607$) acquisition trials (Fig. 3B and D, respectively). As expected, similar amounts of time were spent exploring the sample objects during the acquisition trial in both the STM and LTM trials of the non-stressed and repeated forced swimming groups (data not shown).

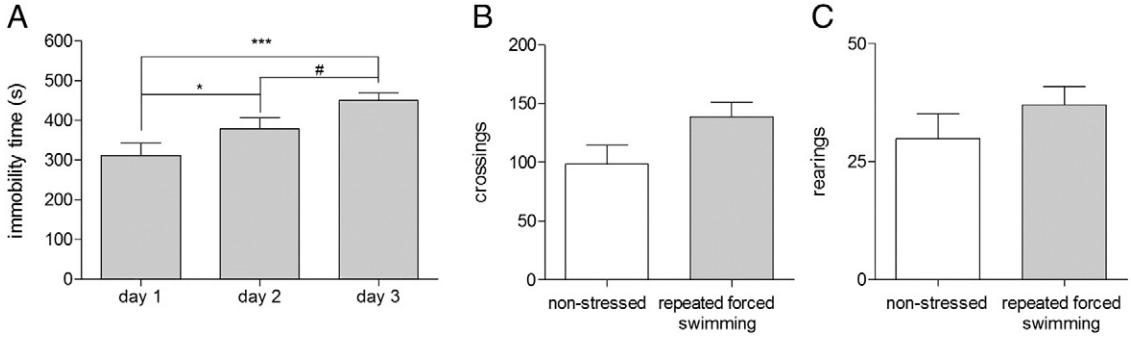

Fig. 1. Immobility acquisition and spontaneous locomotion during repeated forced swimming. (A) Rats were exposed to 10 min of swimming on three consecutive days. One-way repeated measures ANOVA followed by Student-Newman-Keuls analysis: $^{*}p < 0.05$; $^{\#}p < 0.05$, and $^{***}p < 0.001$. Twenty-four hours after the last swimming session, spontaneous locomotion was evaluated during the habituation phase of memory tasks. Data were grouped by condition (exposure to repeated forced swimming or no exposure). (B) Number of crossings. Student's *t* test: n.s. (C) Number of rearings. Student's *t* test: n.s. Data are expressed as the mean ± SEM ($n = 10$ per group).