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Increasing adult hippocampal neurogenesis in mice after exposure to unpredictable chronic mild stress may counteract some of the effects of stress

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ABSTRACT

Major depression is hypothesized to be associated with dysregulations of the hypothalamic–pituitary–adrenal (HPA) axis and impairments in adult hippocampal neurogenesis. Adult-born hippocampal neurons are required for several effects of antidepressants and increasing the rate of adult hippocampal neurogenesis (AHN) before exposure to chronic corticosterone is sufficient to protect against its harmful effects on behavior. However, it is an open question if increasing AHN *after* the onset of chronic stress exposure would be able to rescue behavioral deficits and which mechanisms might be involved in recovery. We investigated this question by using a 10-week unpredictable chronic mild stress (UCMS) model on a transgenic mouse line (*iBax* mice), in which the pro-apoptotic gene *Bax* can be inducibly ablated in neural stem cells following Tamoxifen injection, therefore enhancing the survival of newborn neurons in the adult brain. We did not observe any effect of our treatment in non-stress conditions, but we did find that increasing AHN after 2 weeks of UCMS is sufficient to counteract the effects of UCMS on certain behaviors (splash test and changes in coat state) and endocrine levels and thus to display some antidepressant-like effects. We observed that increasing AHN lowered the elevated basal corticosterone levels in mice exposed to UCMS. This was accompanied by a tamoxifen-induced reversal of the lack of stress-induced decrease in neuronal activation in the anteromedial division of the bed nucleus of the stria terminalis (BSTMA) after intrahippocampal dexamethasone infusion, pointing to a possible mechanism through which adult-born neurons might have exerted their effects. Our results contribute to the neurogenesis hypothesis of depression by suggesting that increasing AHN may be beneficial not just before, but also after exposure to stress by counteracting several of its effects, in part through regulating the HPA axis.

1. Introduction

Major depressive disorder (MDD) is one of the main contributors of the Global Burden of Disease (Ledford, 2014). According to the World Health Organization, it has already become the second most prevalent cause of illness-induced disability (Ferrari et al., 2013). Many antidepressant classes are used to treat the disorder, including selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (NRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). SSRIs are the most commonly prescribed antidepressant class, but their downstream

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mechanisms of action are not fully understood (Bauer et al., 2008). Notably, most antidepressants including SSRIs exert their pharmacological action in hours (Celada et al., 2004), but a therapeutic effect is usually observed only after around 3 weeks of chronic treatment (Frazer and Benmansour, 2001). The mechanism of this delay in the onset of action is under investigation, and many experimental findings point in the direction of the hippocampus; more specifically, the involvement of adult-born hippocampal neurons.

The subgranular zone of the hippocampal dentate gyrus (DG) is one of the few areas where the birth of new neurons (*i.e.* neurogenesis) occurs throughout life (Altman and Das, 1965; Snyder and Cameron, 2012; Spalding et al., 2013). The findings that exposure to stress and stress hormones decreases adult neurogenesis (Cameron and Gould, 1994; Gould et al., 1998; Schoenfeld and Gould, 2012) while antidepressant treatments increase it (Malberg et al., 2000) and that adult-born neurons establish synaptic connections after 3–4 weeks (Toni et al., 2007; van Praag et al., 2002), corresponding to the lag in the onset of antidepressant action, all contributed to the hypothesis that antidepressants might exert their action by increasing neurogenesis. This view was extended by studies showing that adult hippocampal neurogenesis is necessary for some (but not all) effects of antidepressants (David et al., 2009; Santarelli et al., 2003; Surget et al., 2008). Human postmortem studies in clinically depressed subjects have found that the level of adult hippocampal neurogenesis and/or the number neural progenitor cells are decreased in patients with MDD, which may be reversed with antidepressant treatment (Boldrini et al., 2009, 2012; Lucassen et al., 2010). More recently, studies that explore behavioral changes in mice after selectively increasing neurogenesis have been carried out in order to elucidate the role of adult-born hippocampal neurons. These studies show that while an increase in adult neurogenesis does not seem to modulate depression-related behaviors under baseline conditions (Sahay et al., 2011), it is sufficient to protect against the behavioral effects of chronic corticosterone (CORT) treatment, representing challenged conditions (Hill et al., 2015). However, since the experimental design utilized consisted of increasing neurogenesis before putting the animals in challenged conditions, it is an open question if increasing neurogenesis once the insult has already started having deleterious effects would be able to rescue behavioral deficits and, if it would, which mechanism would be involved. Exploration of that paradigm might be valuable in the clinical sense, as antidepressants are usually not prescribed before, but after the onset of depression.

In order to investigate the role of increasing adult hippocampal neurogenesis after an insult relevant for major depression, we used a transgenic mouse line (*iBax* mice), in which the pro-apoptotic gene Bax can be selectively ablated in neural stem cells following tamoxifen injections, therefore inducibly enhancing the survival and the functional integration of newly born neurons in the adult brain (Sahay et al., 2011). Before inducing an increase in neurogenesis, the animals were exposed to an insult in the form of unpredictable chronic mild stress (UCMS), a paradigm that consistently produces a naturalistic model of a depression (Nollet et al., 2012). The effects of our treatments were assessed on physical and behavioral levels in order to examine anxiety- and depression-like behaviors, and on endocrine and immunohistochemical levels to assess HPA (hypothalamic–pituitary–adrenal) axis activity and neuronal activation in the BSTMA (bed nucleus of the stria terminalis, medial division, anterior part), an important relay area between the hippocampus and the hypothalamic paraventricular nucleus (PVN) (Herman and Cullinan, 1997), in order to investigate the potential mechanisms involved in the behavioral effects observed. After behavioral testing, the animals were stereotaxically implanted with bilateral guide cannulas in order to assess the reactivity of the DG neurons to a dexamethasone infusion.

We explored the HPA axis as it is a plausible candidate for a mechanism of action since depression is often associated with HPA axis dysfunction (Heuser, 1998; Vreeburg et al., 2013) and since the hippocampus is involved in the regulation of the HPA axis via inhibitory feedback, which is attenuated by chronic stress (Mizoguchi et al., 2003; Surget et al., 2011). It has been shown that adult-born neurons are critical for the hippocampal regulation of the HPA axis (Schloesser et al., 2009; Snyder et al., 2011) and it has been suggested that antidepressants might exert their effects on the HPA axis by increasing the rate of neurogenesis (Surget et al., 2011). A recent study investigated the effect of specifically increasing neurogenesis on the HPA axis and found that increasing neurogenesis does not affect the HPA axis response after acute stress (Hill et al., 2015). However, baseline CORT levels in animals exposed to chronic CORT treatment were not assessed in that study, and increasing neurogenesis could have an effect on the HPA axis under conditions where the endogenous HPA axis is active, for instance UCMS. To investigate the mechanism of altered hippocampal regulation of the HPA axis, we assessed neuronal activation in the BSTMA, which has a critical role in regulating the neuroendocrine responses and receives projections from the hippocampus (Crestani et al., 2013).

2. Methods

2.1. Animals

Male *iBax* mice aged 2–4 months (n = 88) at the beginning of the experiment were used. Animals were generated by interbreeding *Nestin CreER*^{T2}; *Bax* $^{f/f}$ and *Bax* $^{f/f}$ mice as previously described (Sahay et al., 2011), and were maintained on a mixed C57BL/6 and 129/SvEv genetic background. To induce CreER^{T2} mediated recombination of *Bax* in neural stem cells in the adult brain, mice were treated with approximately 54.5 mg tamoxifen/kg body weight, once a day, intraperitoneally for 5 consecutive days. 20 mg/ml tamoxifen (Sigma, T-5648) stock solution was prepared in corn oil and on each day of treatment dissolved to make a 5.5 mg/ml fresh solution. For vehicle (VEH), 10 ml/kg body weight was injected intraperitoneally, once a day for 5 consecutive days. Even though repeated daily injections might have acted as a stressor, we chose this method of delivery due to the fact that it has been used successfully in previous experiments with *iBax* mice (Hill et al., 2015; Sahay et al., 2011). This is also the most commonly used method of tamoxifen administration in which the amount of administered compound can be better controlled (Whitfield et al., 2015), which is not possible by administering the compound in chow or drinking water – methods where dosage depends on eating/drinking behavior and has other limitations (low solubility of tamoxifen in drinking water, palatability etc.). All animals were group housed and kept under standard laboratory conditions (12/12 h light-dark cycle with lights on at 8:30 p.m., room temperature 22 ± 2 °C, food and water *ad libitum*) in standard cages (42 cm × 27 cm × 16 cm) with shelter for 1 week prior to the start of the experiment. Animal care and treatment was in accordance with the European Union Directive 2010/63/EU.

2.2. Experimental design

Animals were divided into four groups: non-stress vehicle (NS-VEH), non-stress tamoxifen (NS-TAM), UCMS Vehicle (UCMS-VEH) and UCMS Tamoxifen (UCMS-TAM) (n = 17–26 per group). At the start of the experiment, UCMS mice were isolated in individual cages, which is an integral part of the UCMS protocol that prevents