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Mice with ablated adult brain neurogenesis are not impaired in antidepressant response to chronic fluoxetine

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ABSTRACT

The neurogenesis hypothesis of major depression has two main facets. One states that the illness results from decreased neurogenesis while the other claims that the very functioning of antidepressants depends on increased neurogenesis. In order to verify the latter, we have used cyclin D2 knockout mice (cD2 KO mice), known to have virtually no adult brain neurogenesis, and we demonstrate that these mice successfully respond to chronic fluoxetine. After unpredictable chronic mild stress, mutant mice showed depression-like behavior in forced swim test, which was eliminated with chronic fluoxetine treatment, despite its lack of impact on adult hippocampal neurogenesis in cD2 KO mice. Our results suggest that new neurons are not indispensable for the action of antidepressants such as fluoxetine. Using forced swim test and tail suspension test, we also did not observe depression-like behavior in control cD2 KO mice, which argues against the link between decreased adult brain neurogenesis and major depression.

1. Introduction

Major depression is a common mental disorder, however, its pathophysiology remains poorly understood. Stress, the main cause of depression, most severely affects hippocampal formation (McEwen et al., 2012), while hippocampal subgranular zone (SGZ) of the dentate gyrus (DG) is a region where new neurons originate throughout mammalian adulthood. Moreover, stress leads to significant decrease of adult neurogenesis (for review see Warner-Schmidt and Duman, 2006), implicating a role of this decrease in the onset of depression-like symptoms, however, this notion remains controversial (for reviews see Sahay and Hen, 2007; Balu and Lucki, 2009; Eisch and Petrik, 2012).

Hippocampal neurogenesis has also been proposed as a crucial process involved in the therapeutic efficacy of chronic antidepressants (ADs) treatment (*e.g.*, Santarelli et al., 2003). This hypothesis is supported by the fact that recovery from depression requires several weeks – the timescale that overlaps with the time-course of AD-stimulated neurogenesis and is also paralleled with the time needed for differentiation and incorporation of newborn neurons into existing neuronal hippocampal networks (Sahay and Hen, 2007; Balu and Lucki, 2009). Moreover, majority of the antidepressant approaches elevate neurogenesis by increasing proliferation rate and/or by enhancing newborn cells survival (for review see Samuels and Hen, 2011). Indeed, it was shown that animals with blocked adult neurogenesis do not recover from depression-like behavior when chronically administered with ADs (Santarelli et al., 2003; Surget et al., 2008; David et al., 2009; Onksen et al., 2011; Perera et al., 2011). However, recent studies suggest both neurogenesis-dependent and independent mechanisms underlying ADs action, as more studies show none or only partial effect of reducing neurogenesis on restoration of behavioral homeostasis by ADs (Meshi et al., 2006; David et al., 2007; Holick et al., 2008; Surget et al., 2008; Bessa et al., 2009a; David et al., 2009; Singer et al., 2009; Nollet et al., 2012).

Herein, we have employed cyclin D2 knockout (cD2 KO) mice showing lack of adult brain neurogenesis. We showed before that mice with mutated cyclin D2 gene display largely impaired

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proliferation of neuronal precursors in SGZ (Kowalczyk et al., 2004; Jaholkowski et al., 2009; Jedynak et al., 2012). Notably, cyclin D2 mutant mice show deficits in some hippocampal-dependent behaviors (Jedynak et al., 2012), but not in learning in general (Jaholkowski et al., 2009; Jedynak et al., 2012; Urbach et al., 2013), while selected cognitive functions are impaired (Ben Abdallah et al., 2013; Garthe et al., 2014).

In the present study, we set out to test the reaction of cD2 KO mice to chronic fluoxetine administration following chronic stress. We have chosen unpredictable chronic mild stress (UCMS) as known to cause ADs-reversible behavioral changes in rodents that parallel symptoms of major depression (Willner, 2005). This paradigm was also used in the original study suggesting a causal link between ADs efficacy and adult neurogenesis (Santarelli et al., 2003). Also, we have chosen forced swim test (FST) as one of the most widely used test of ADs action (Petit-Demouliere et al., 2005; Krishnan and Nestler, 2011), which results in reduced immobility following their acute (Porsolt et al., 1977a, 1977b) and chronic delivery (*e.g.*, Detke et al., 1997; Dulawa et al., 2004; Holick et al., 2008; Lin and Wang, 2014) as well as in reversal of increased immobility following UCMS (Willner, 2005). Finally, we have used fluoxetine as most commonly used AD in adult neurogenesis-depression studies, *e.g.*, in the original Santarelli et al. (2003) publication.

2. Materials and methods

2.1. General experimental design

A cohort of animals (cD2 KO, $n = 30$; WT, $n = 30$) was subjected to unpredictable chronic mild stress (UCMS) and chronically administered with fluoxetine. When stress and fluoxetine administration ended, all animals were tested behaviorally in the forced swim test (FST) and cell proliferation was assessed in DG using bromodeoxyuridine (BrdU) injection and immunocytochemistry. There was also an open field control test performed 24 h after the last fluoxetine administration. The overall design of experimental procedures is shown in Fig. 1.

2.2. Animals

Cyclin D2 mutant mice (Kowalczyk et al., 2004) were backcrossed into C57BL/6 background over 10 generations and kept as cyclin D2 heterozygotes (+/−). Their homozygous progeny, cD2 KO (−/−) and WT (+/+) littermates, were used. If not stated otherwise, the animals were kept under a natural light/dark cycle in Plexiglas cages with water and food provided *ad libitum*. To ensure proper care and use of laboratory animals, the national rules according to the Animal Protection Act, ensured by the permission from the First Warsaw Local Ethics Committee for Animal Experimentation, were strictly followed. The animals were males, 2–4 months old at the beginning of the experiments, with their age carefully matched between WT and cD2 KO mice. Experimenters were always unaware of the genotype of the mice.

2.3. Unpredictable chronic mild stress (UCMS) and chronic fluoxetine treatment

The mice (cD2 KO, $n = 29$; WT, $n = 29$) were divided into three age-matched groups (non-stressed, $n = 10$; stressed-vehicle, $n = 10$; stressed-fluoxetine, $n = 9$). UCMS protocol was described before (Bisaz et al., 2011; Bisaz and Sandi, 2012) and used with some modifications. Non-stressed and stressed groups were housed in separated and closed housing racks located in the same room during the duration of the stress procedure. As the experiment started, non-stressed groups were left undisturbed. Stressed groups were exposed to UCMS procedure which consisted of different kinds of stressors: cage tilting, damp sawdust, housing in an empty cage, pairing with another stressed animal, cold room, water or food deprivation, inversion of the light/dark cycle, lights on for a short period of time during the dark phase and switching cages amongst stressed animals. One or two of these stressors were applied daily at different times and following a semi-random schedule. Starting from the beginning of the 4th week of UCMS, stressed mice were given either 10 mg/kg/day (comp. Santarelli et al., 2003; Bessa et al., 2009a) fluoxetine hydrochloride (Sigma, PL), dissolved in water and prepared freshly before use, or water. The treatment lasted for 3 weeks and was provided *via* oral gavage (Fine Science Tools Inc., USA). The drug concentration was adjusted weekly from the average body weight of mice to achieve the desired doses.

2.4. Forced swim test (FST)

In order to eliminate the effects of acute fluoxetine injections, FST was performed 3 days after the last delivery of the drug. The test was conducted as described by Porsolt et al. (1977a, 1977b) by placing mice into a glass cylinder (25 cm height, 10 cm diameter) containing 1250 ml of water maintained at 24–25 °C. The test lasted for 6 min. Starting from the 3rd minute of the test, mice were rated for immobility defined as the absence of active, escape-oriented behaviors such as swimming, jumping, climbing, or diving. Two animals were tested simultaneously.

2.5. Tail suspension test (TST)

TST procedure was previously described (Steru et al., 1985). Mice were suspended by the tail temporarily attached to a metal bar using adhesive scotch tape. Total duration of immobility was counted during a 6 min test. Three animals were tested simultaneously. One WT animal climbing its tail was excluded from the experiment.

2.6. Open field

The test was performed 24 h after the termination of UCMS and fluoxetine treatment, it was done as described previously (Jedynak et al., 2012). The apparatus was a wooden floor (50 cm × 50 cm) surrounded by 34 cm high walls. Gray walls and floor as well as

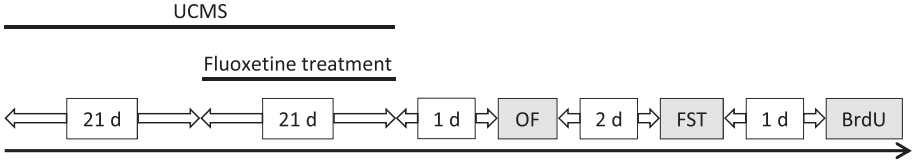

Fig. 1. Schematic plan of experimental procedures and timetable. Mice were subjected to unpredictable chronic mild stress (UCMS) for 6 weeks. Fluoxetine or water was given during the 4th–6th week of UCMS. After 3 days interruption, depression-like behavior was evaluated using forced swim test (FST), then the animals were injected with BrdU and perfused for immunocytochemistry. Control open field (OF) was performed 24 h after the last fluoxetine treatment.