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IMPAIRED STRUCTURAL HIPPOCAMPAL PLASTICITY IS ASSOCIATED WITH EMOTIONAL AND MEMORY DEFICITS IN THE OLFACTORY BULBECTOMIZED RAT

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Abstract—Disturbances in olfactory circuitry have been associated with depression in humans. The olfactory bulbectomized (OBX lesion) has been largely used as a model of depression-like behavior in the rat. However, quantitative neuronal rearrangements in key brain regions in this animal model have not been evaluated yet. Accordingly, we investigated changes in hippocampal plasticity as well as behavioral deficits in this animal model. OBX-induced behavioral deficits were studied in a battery of tests, namely the open field test (OFT), forced swim test (FST), and spatial memory disturbances in the Morris water maze (MWM). To characterize the neuronal remodeling, neuroanatomical rearrangements were investigated in the CA1 hippocampus and piriform cortex (PirC), brain regions receiving inputs from the olfactory bulbs and associated with emotional or olfactory processes. Additionally, cell proliferation and survival of newborn cells in the adult dentate gyrus (DG) of the hippocampus were also determined. OBX induced hyperlocomotion and enhanced rearing and grooming in the OFT, increased immobility in the FST as well as required a longer time to find the hidden platform in the MWM. OBX also induced dendritic atrophy in the hippocampus and PirC. In addition, cell proliferation was decreased while the survival remained unchanged in the DG of these animals. These various features are also observed in depressed subjects, adding further support to the validity and usefulness of this model to evaluate potential novel antidepressants.

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Abbreviations: BrdU, bromodeoxyuridine; DG, dentate gyrus; FST, forced swim test; IHC, immunohistochemical; MWM, Morris water maze; OBX, olfactory bulbectomized; OFT, open field test; PBS, phosphate-buffered saline; PirC, piriform cortex; TDL, total dendritic length.

Key words: adult neurogenesis, animal model, depression-like behavior, dendritic morphology, Golgi–Cox stain, OBX rat.

INTRODUCTION

Major depression has been associated with disrupted neuronal plasticity (Manji et al., 2001) and deregulated hippocampal neurogenesis (Boldrini et al., 2009). In depressed subjects, neuronal rearrangement has been observed in the hippocampus, prefrontal cortex (PFC), and amygdala (Rajkowska et al., 1999; Stockmeier et al., 2004; Cotter et al., 2005; Hercher et al., 2009), and various animal models of depression-like behavior mimic those neuronal rearrangements. For instance, stress-induced depression-like behaviors are accompanied by hippocampal neuronal hypotrophy (Watanabe et al., 1992; Magarinos et al., 1998; Conrad et al., 2007; Morales-Medina et al., 2009). Hippocampal hypotrophy is also observed in the learned helplessness rat (Hajszan et al., 2009) as well as adult offspring having received low maternal care early in their development (Champagne et al., 2008; Bagot et al., 2009). In animals as well as humans, antidepressant treatment increases adult hippocampal neurogenesis (Boldrini et al., 2009) compromised in various animal models of depression (Jaako-Movits and Zharkovsky, 2005; David et al., 2009). Thus, previous findings have consistently suggested that alterations in hippocampal plasticity are key contributors of depression.

The olfactory bulbectomized (OBX) model of depression produces a wide spectrum of behavioral, neurochemical, endocrine, and immunological changes similar to those observed in humans (Kelly et al., 1997; Song and Leonard, 2005). Interestingly, OBX animals show sensitivity to antidepressant treatments only after repeated administration, similar to the human condition, a feature that increases the validity of this animal model (Mar et al., 2002; Wang et al., 2007). The OBX surgical procedure consistently induces depression-like behaviors in diverse behavioral tests (Song et al., 1996; Wang et al., 2007; Tasset et al., 2008; Han et al., 2009). However, a comparative analysis of various behavioral tests that measure emotionality in the same cohort of OBX animals remains to be conducted. In addition, attention deficits and memory loss have been reported in depressed subjects (Castaneda et al., 2008).

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OBX animals show deficits in passive-avoidance behavior (Sieck, 1972; Kelly et al., 1997; Nakagawasai et al., 2003), a condition associated with learning deficits. However, the role of OBX in the Morris water maze (MWM), a well-known paradigm used to evaluate hippocampus-dependent spatial memory in rodents (Morris, 1984; Doggui et al., 2010) is rather controversial (Redmond et al., 1994; Nesterova et al., 2008).

Despite the relevant role of compromised neuronal plasticity in depressed subjects and animal models of depression-like behavior, data are scarce with respect to neuronal morphology in OBX animals. In this regard, Nesterova et al. (2008) found abnormalities (pyknosis, karyolysis, and vacuolysis) in the hippocampus and temporal cortex neurons (Bobkova et al., 2004) as well as cell death in the primary olfactory cortex (Heimer and Kalil, 1978) of OBX rodents. These neurodegenerative changes (Yamamoto et al., 1997) and most of the behavioral deficits are observed only after at least two weeks post lesion (Song and Leonard, 2005), however, there are changes documented during the first week post-lesion as well (Vinkers et al., 2009; Prins et al., 2010). Therefore, we could hypothesize that OBX induces changes in hippocampal plasticity.

In the present study, we assessed a battery of behavioral tests including the open field test (OFT) and forced swim test (FST) in the same cohort of animals, and MWM in a second cohort of animals using the OBX model. Additionally, dendritic arborization and spine density in CA1 hippocampus and piriform cortex (PirC) as well as adult hippocampal neurogenesis were quantitatively evaluated in a separated cohort of animals.

EXPERIMENTAL PROCEDURES

A series of behavioral, neuroanatomical, and immunohistochemical (IHC) studies were carried out to evaluate the role of OBX in the rat. After OBX surgery, animals were allowed to recover for two weeks, and since generally antidepressant drugs are administered for two weeks, behavioral, neuroanatomical, and IHC studies were performed four weeks after the removal of olfactory bulbs. Four animal cohorts were studied: the first was used for the OFT and FST, the second for the MWM, the third for neuroanatomical analyses, and the fourth for IHC studies of dentate gyrus (DG) proliferation and survival of newborn cells of the hippocampus.

Animals

Male Sprague–Dawley rats (Charles River Canada, Montréal, QC, Canada) weighing 150–170 g at the beginning of the treatment were housed two per cage and maintained on a 12-h light/dark cycle with *ad libitum* access to food (Purina Lab Chow) and water. All procedures were approved by the McGill Animal Care Committee and according to the guidelines of the Canadian Council on Animal Care.

OBX surgery

Bilateral olfactory ablation was performed similar to the procedure described in earlier studies (Morales-Medina et al., 2012a,b). Briefly, 5% isoflurane was used to induce anesthesia, and subsequently maintained at 2.5% during the surgical procedure. A cranial window, 5.2 mm anterior to the bregma was created in the frontal bone. The olfactory bulbs were cut and aspirated out. Sham operations were performed in the same manner, but the bulbs were left intact. The prevention of blood loss from the cranial window was achieved by filling the open space with a hemostatic sponge. Following surgery, rats were administered with carprofen and a saline solution (0.9% NaCl) and left in pairs in their cages to recover for two weeks. The olfactory bulbs are completely removed and this transection did not damage the medial frontal cortex (as determined by an examination following brain removal). Only those animals were included in data analysis.

Behavioral tests

The OFT was followed by the FST in 2 consecutive days, as reported in another model of depression-related behavior (Kalynchuk et al., 2004). The OFT was carried out on day 27 post-surgery, while the FST was performed on day 28. The MWM was performed on 5 consecutive days and the probe test was done on day 28 post OBX. The behavioral tests were carried out during the light phase of the light–dark cycle (9:00–13:00). Rats were maintained in similar housing conditions throughout all the tests. Ten to sixteen animals per group were assessed.

OFT. This test was performed under bright light in an OF apparatus (100 × 100 × 40 cm) made of a black wooden box with a gray floor and no top, with a field divided into 64 equal-sized squares. The dimensions of this arena were similar as used previously (Keilhoff et al., 2006). The locomotor activity was observed and recorded for a total of 10 min, including locomotion (horizontal behavior) and the frequency of rearing and grooming (vertical behaviors), by an observer blind to the treatment. After each trial, the testing apparatus was cleaned with Peroxigard solution (Bayer Healthcare, Toronto, ON, Canada).

FST. The FST is a well-documented tool to screen potential antidepressants (Porsolt et al., 1977; Lucki, 1997). For this behavioral test, rats were placed for a 10-min period in a white cylindrical tank (29 cm wide × 43 cm high) with no top, filled with water (25 ± 2 °C). A camera was mounted 1 m above the tank, and an observer blind to the experimental conditions evaluated three behaviors; swimming, struggling, and immobility. Following the tests, the rats were removed from the cylinder, cleaned with a towel, and placed under a red lamp until their fur dried.

Swimming time was regarded as the time when an animal performed an active swimming activity beyond the necessary movements to keep itself floating. Time spent struggling was recorded when the front paws of a rat broke through the surface of the water, and it tried to get out of the tank by making quick strong movements. Finally, immobility time was noted when an animal made the minimum necessary movement to keep its body floating. Depression-related behavior was inferred from an increase in the time the rat spent immobile, which is thought to represent a lack of motivation to escape from the water.

MWM. The MWM protocol used was as has been described previously (Morris, 1984; Benoit et al., 2010; Doggui et al., 2010). We evaluated hippocampal-dependent spatial memory four weeks after the removal of olfactory bulbs or sham surgery. The animals had to find a hidden platform (14 cm in diameter) located 2 cm below the surface of white-colored water at 24 °C in a pool (diameter 1.4 m). For each trial, animals were pseudo-randomly started from a different position. Rats used spatial cues to find the platform that remained in the center of the same quadrant throughout all the training days