Long-Term Behavioral Changes After Cessation of Chronic Antidepressant Treatment in Olfactory Bulbectomized Rats

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Background: Olfactory bulbectomy (OBX) in rats causes several behavioral and neurochemical central nervous system changes, reminiscent of symptoms of human depression. Moreover, depression-like behavior after OBX can be reversed with antidepressant drugs. However, the lasting effects of these antidepressant drugs on behavior after cessation of treatment have never been studied.

Methods: Male rats received OBX or sham surgery. After recovery, animals received 14 consecutive daily doses of imipramine (20 mg/kg), escitalopram (5 and 10 mg/kg), or vehicle. Animals were tested in an open field after acute, sub-chronic, and chronic injections, as well as 1, 2, 6, and 10 weeks after cessation of treatment.

Results: The OBX-induced hyperactivity was normalized after sub-chronic administration of imipramine and escitalopram. Two weeks after treatment, activity of OBX animals was comparable to sham-treated animals, but after 6 weeks, OBX animals treated with both doses of escitalopram had returned to pre-treatment hyperactivity levels. The OBX animals treated with the high imipramine dose (20 mg/kg) retained activity levels comparable to sham-treated animals until 10 weeks after cessation of treatment.

Conclusions: Chronic but not acute administration of imipramine and escitalopram normalizes OBX-induced hyperactivity. This effect continues for up to 10 weeks after cessation of treatment in a dose dependant manner.

Key Words: Cessation of treatment, depression, escitalopram, imipramine, olfactory bulbectomy, open field

Major depressive disorder (MDD) is a serious psychiatric condition afflicting a large proportion of the general population. Despite unprecedented advances in our knowledge of the neurobiology, the underlying causes of depression remain poorly understood (Halbreich 2006). Depression is believed to occur as a result of environmental influences (i.e. early-life adversity) in genetically predisposed individuals in whom neuroendocrine and neurochemical abnormalities, including changes in neurotrophic factors, have been identified. Many animal models of depression have been based, therefore, on adverse life events. These models include the learned helplessness (Maier 2001; Porsolt 1979; Porsolt *et al.* 1978; Seligman *et al.* 1980), chronic stress (McNish and Davis 1997; Richardson 1991; Willner *et al.* 1987), and early life trauma models (Ladd *et al.* 1996). The rat olfactory bulbectomy (OBX) model is an animal model of depression that results in similarities to brain chemistry seen in depressed humans (Lumia *et al.* 1992; Slotkin *et al.* 2005; Song and Leonard 2005), such as altered dopamine (Masini *et al.* 2004) and serotonin concentrations in the brain (Van der Stelt *et al.* 2005). Jancsar and Leonard (1984) hypothesized that OBX-induced structural alterations in the locus coeruleus and raphe nuclei might account for these catecholaminergic and serotonergic dysfunctions. Olfactory bulb ablation also leads to enlarged lateral and 3rd ventricles as well as decreased hippocampal volume, which can also be observed in depressed humans (McEwen and Olie 2005; Sheline 2003). Ablation of the bulbs also results in several behavioral changes in rats, including increased hyperactivity in a novel environment, deficits in passive-avoidance learning, and anhedonia (Kelly *et al.* 1997; Wieronska *et al.* 2001).

Olfactory bulbectomy is probably one of the best available models to predict antidepressant activity, because as in humans, chronic but not acute antidepressant treatment is effective (Cryan *et al.* 2002; Frazer and Morilak 2005; Grecksch *et al.* 1997; Leonard and Tuite 1981; Uzunova *et al.* 2004). The exact mechanism for this delay in action is as of yet unknown, but it has been hypothesized that it might be due to down-regulation of certain neurotransmitter receptors in the brain (Lima *et al.* 2002; Lloyd *et al.* 1987). Another theory, according to research using chronic restraint stress by Chen *et al.* (2005), is that this delay is due to the time it takes for the promotion of neurogenesis in the hippocampus, which they observed after 21 days of antidepressant treatment.

The aim of the current experiment was three-fold. First, we wanted to examine the duration of time for the onset of action of imipramine, to further increase the validity of the OBX model as a model for depression; and second, we wanted to examine the effects of new antidepressant drugs on OBX-induced hyperactivity in the open field. Finally, and most importantly, we wanted to observe the duration of these antidepressant-induced effects after cessation of treatment. It is known that after cessation of treatment in patients, it might be months or even years before the patient might show signs of recurring depression. A relapse in depressive symptoms might be related to the depletion of the monoamines thought to be responsible for causing depression, serotonin and noradrenaline, or a significant decrease in tryptophan (Elhwuegi 2003). However, recurrent depression has never

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before been studied in an animal model, and this is an exciting new finding that further strengthens the validity of the OBX model in rats. Because the mechanisms of action of antidepressant drugs are, at this point, relatively unknown, little is also known as to how long the effects of antidepressant administration on the brain might last after cessation of treatment. This has never before been examined in the OBX model, and it might be necessary to further investigate this phenomenon if the mechanisms of antidepressant drugs are to be fully understood.

Methods and Materials

Animals

One hundred twenty-four male albino Sprague Dawley rats (Harlan, Zeist, The Netherlands) weighing between 220 and 250 g upon arrival were used for this experiment (40 for experiment I, and 84 for experiment II). Animals were on a 12-hour light/dark cycle, with lights going off at 6.00 PM and coming on at 6.00 AM. Food and water were available ad libitum. Average temperature was 22°–24°C, with humidity between 30% and 60%. Animals were allowed to acclimate to their surroundings for 1 week and were then run in a preliminary pre-surgical open field test to determine their randomization into surgical and treatment groups. Animals were housed four/cage, with two sham-treated animals and two OBX animals/cage. There was little to no effect of housing OBX and sham animals together, and there was no evidence that the OBX animals were more aggressive toward their cage-mates.

After surgery, animals were allowed to recover for 2 weeks before the rest of the tests were performed. Animals were killed via decapitation, and the brains were examined for verification of complete olfactory bulb ablation. Those animals with partial bulbectomies or damaged prefrontal cortices were excluded from the data analysis. One animal was excluded from the first experiment for damage to the prefrontal cortex. No animals were excluded from the second experiment. Body weights were taken every week. All experiments were performed in accordance with the governmental guidelines for care and use of laboratory animals and were approved by the Ethical Committee for Animal Research of the Faculties of Veterinary Medicine, Pharmaceutical Sciences, Chemistry and Biology at Utrecht University.

Surgical Procedure

Animals were anesthetized with isofluorane gas anesthetic (3%–4%), mixed with oxygen and nitrous oxide. The animals were then placed in the stereotaxic instrument (Kopf), and eye ointment was placed in both eyes to prevent them from drying out during surgery. After the incision was made, Lidocaine (5%) was applied to the wound as a local anesthetic, and iodine was applied as an antiseptic. Two burr holes were then drilled on either side of the skull (taking care not to damage the prefrontal cortex), 2 mm in diameter, 8 mm anterior to bregma, and 2 mm from the midline of the frontal bone overlying the olfactory bulbs. After the burr holes were drilled, the dura mater of the sham-operated animals was pricked with a small needle. For the bulbectomized animals, the tissue was removed with a blunt hypodermic needle and a vacuum pump, and the burr holes were packed with hemostatic sponge to prevent blood loss. Animals receiving sham surgery went through a similar procedure but did not have their olfactory bulbs removed. All incisions were then closed with 4-0 vicryl suture material (resorbable). After surgery, all animals received 5 mL of saline (subcutaneously) and Rimadyl (5 mg/kg, subcutaneously) to reduce pain. When all animals were awake and moving, they were returned to the colony room. Animals were monitored closely for the following 2 days for signs of discomfort or infection and allowed to recover for 2 weeks.

Behavioral Testing

Experiment I: Onset of Action of Imipramine. Two weeks after surgery, the first group of 38 animals (two animals died after surgery) went through a post-surgical open field test. The animals were then assigned, evenly balanced by the amount of distance traveled in the post-surgical open field, to treatment groups of water vehicle or imipramine at 20 mg/kg (Sigma Aldrich, Zwijndrecht, The Netherlands), with a dose volume of 5 mL/kg, given intraperitoneally. One week after the post-surgical open field, animals received an acute injection of imipramine or vehicle and were tested 30 min later in the open field. Injections continued for 14 consecutive days, and on days 7 (sub-chronic) and 14 (chronic) all animals were again tested in the open field to observe the onset of action of imipramine. The open field was lit at normal room illumination (420 lux at floor level), and the boxes measured 72 × 72 cm. Each box was painted a light gray color for ease of observation. Animals were tracked with Noldus EthoVision (Noldus Information Technology, Leesburg, Virginia). Each animal was placed in the center of the open field and allowed to explore for 15 min, after which they were returned to their home cage. Data were analyzed in 15 1-min time bins. At the end of the experiment the animals were killed by decapitation, and olfactory bulb ablation was verified.

Experiment II: Escitalopram and Imipramine. Previous testing showed that chronic treatment with imipramine resulted in altered behavior after cessation of treatment and that these alterations remained apparent up to 6 weeks after cessation of treatment. To better examine this phenomenon and see whether the effect was replicable with a selective serotonin reuptake inhibitor (SSRI), animals received OBX or sham surgery and underwent a testing schedule similar to experiment I but also examining the effect of drug on behavior after cessation of treatment.

Two weeks after surgery, 79 animals (5 animals died after surgery) went through a post-surgical open field test and were assigned to treatment groups: escitalopram at 5 or 10 mg/kg (obtained from Apotheek Koster, Lelystad, The Netherlands), imipramine at 20 mg/kg (Sigma Aldrich), or water vehicle (all administered orally), with a dose volume of 5 mL/kg. Escitalo-

Table 1. Experiment I: Acute Imipramine Treatment

	Vehicle $n = 10$ sham, $n = 9$ OBX		Imipramine 20 mg/kg (IP) $n = 10$ sham, $n = 9$ OBX	
	Sham	OBX	Sham	OBX
Distance Traveled (cm)	4332.32 ± 341.8	6228.57 ± 431.8*	3240.61 ± 305.52	6218.47 ± 332.91*

OBX, olfactory bulbectomy.
*$p < .005$ comparing OBX animals to sham operated animals, in both vehicle and imipramine 20 mg/kg groups.

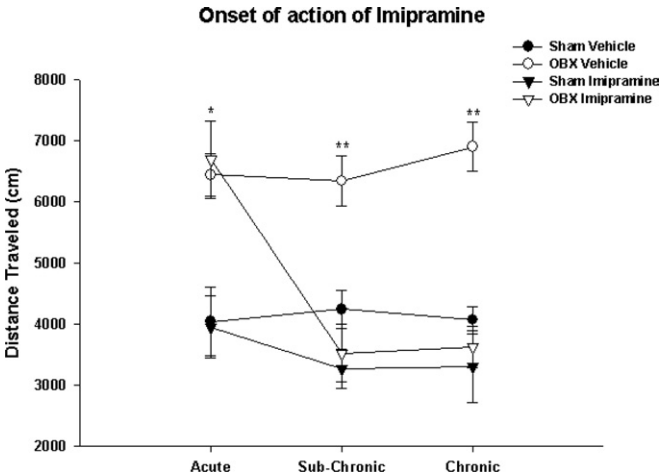


Figure 1. Onset of action of imipramine. After acute antidepressant treatment, olfactory bulbectomy (OBX) animals in both treatment groups remained significantly more active compared with sham-treated animals. After 7 and 14 consecutive days of treatment, OBX animals in the vehicle group were significantly more active than all sham-treated animals. The OBX animals in the imipramine 20-mg/kg group had normalized activity compared with sham-operated animals. Data are expressed as mean distance traveled (cm) ± SEM during the 15-min test session. *$p < .005$ compared with all sham-treated animals. **$p < .001$ compared with all sham-treated animals and OBX animals in the imipramine 20-mg/kg group.

pram was in pill form and, therefore, had to be pulverized and suspended before use. Drugs were given orally for this experiment owing to the fact that the 5- and 10-mg/kg escitalopram doses were made in suspension. Imipramine was also given orally but as a solution. The testing and drug administration schedule, the same as for experiment I, was then performed for this group, with further open field testing at weeks 1, 2, 6, and 10 after cessation of drug treatment.

Statistics

All data are expressed as mean ± SEM. All statistical analyses were carried out with SPSS version 11.0 (SPSS, Chicago, Illinois). Analysis of the open field data for each experiment was done first with a repeated measures analysis of variance (ANOVA), with surgery (OBX and sham) and drug treatment as the main factors and mean distance traveled as a repeated measure. Initial analysis for the first experiment compared pre- and post-surgical open field tests as well as acute, sub-chronic, and chronic tests. For experiment two, a repeated measures analysis was done comparing chronic treatment and tests done after cessation of treatment. If the initial repeated measures analyses revealed significant interactions, these interactions were further analyzed with Bonferroni post hoc analyses. Analysis of body weights was analyzed with a one-way ANOVA (data not shown).

Results

Experiment I: Onset of Action of Imipramine

Pre-operative open field testing showed no differences in activity (mean distance traveled) between groups, and post-surgical testing showed that OBX animals were significantly more active than sham-treated animals in the open field. Repeated measures ANOVA revealed a significant interaction between surgical treatment and distance traveled over time [$F(1,36) = 5.677$, $p = .02$] when comparing pre- and post-surgical open field results. When comparing acute, sub-chronic, and chronic open field results, there was an overall significant interaction between surgical treatment and antidepressant treatment [$F(1,34) = 4.206$, $p = .04$] and a significant interaction of treatment and time [$F(1,34) = 9.88$, $p = .003$]. Analysis of the acute data showed that there was an overall effect of surgical treatment [$F(1,34) = 24.02$, $p < .001$] but that there was no effect of drug treatment on activity [$F < 1.0$, ns]. After 7 days of treatment (sub-chronic), the activity of the OBX animals was significantly decreased compared with sham-treated animals, whereas the activity of the sham-treated animals remained the same [interaction drug treatment and surgery; $F(1,34) = 5.94$, $p = .02$]. Also, after 14 days of drug treatment (chronic), the activity of the OBX animals was still significantly decreased compared with sham-treated animals [interaction drug treatment and surgery; $F(1,34) = 9.16$, $p = .005$].

Post hoc analyses revealed that after acute treatment, OBX-lesioned animals in all groups remained significantly more active when compared with sham-treated animals in all groups (Table 1).

After 7 (sub-chronic) and 14 (chronic) days of imipramine treatment, open field-testing revealed that OBX animals had normalized their behavior, comparable to sham-treated animals. The OBX vehicle animals were significantly more active than OBX and sham animals in all treatment groups, and sham vehicles were not significantly more active compared with imipramine-treated sham-treated animals (Figure 1).

Experiment II: Escitalopram and Imipramine

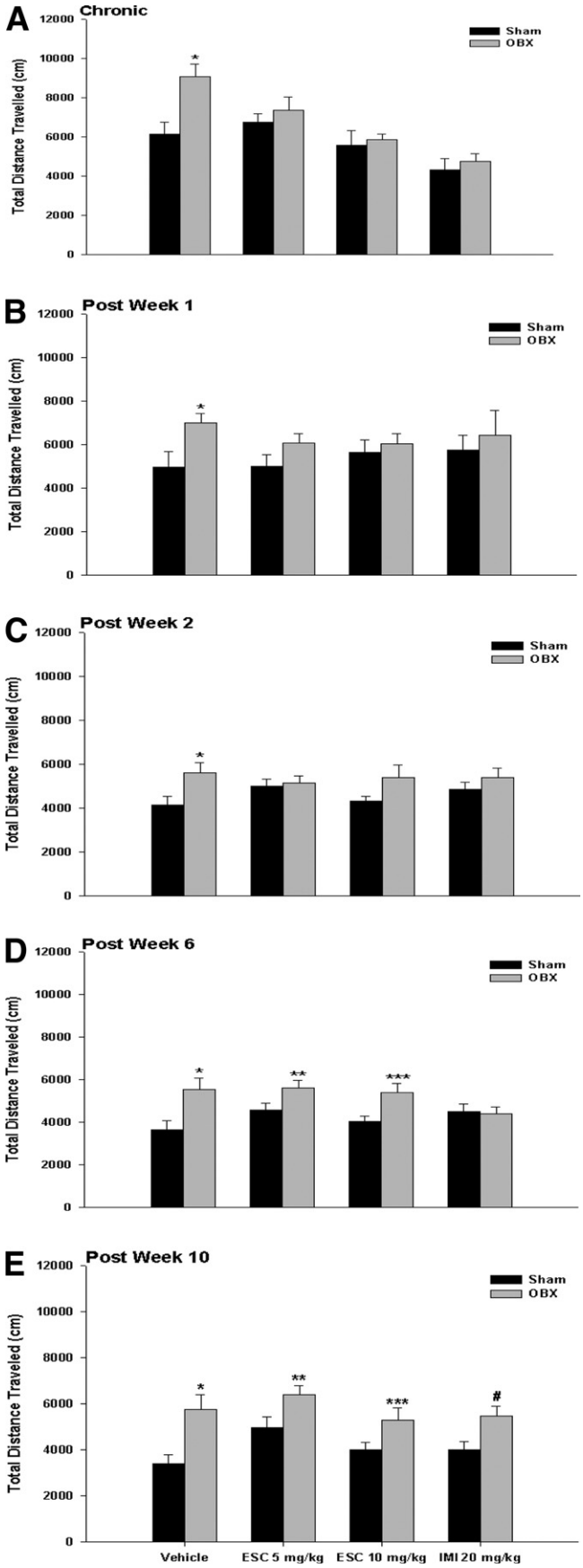
Initial pre-operative open field testing showed no activity differences between groups, as with experiment I. Post-surgical testing revealed a significant increase in activity in OBX compared with sham-operated animals. Acute treatment with escitalopram and imipramine did not alter OBX induced hyperactivity compared with sham animals (Table 2).

Initial ANOVA analysis examined mean distance traveled in the chronic and post-treatment open field tests. The analysis revealed that there was a significant interaction between the surgical treatment and antidepressant treatment [$F(3,71) = 8.43$, $p < .001$] as well as antidepressant treatment and distance traveled over time [$F(3,71) = 5.96$, $p = .001$].

Table 2. Experiment II: Acute Imipramine and Escitalopram Treatment

	Vehicle (Acute) (n = 11 Sham and OBX)		Escitalopram 5 mg/kg (oral) (n = 10 Sham, n = 8 OBX)		Escitalopram 10 mg/kg (oral) (n = 10 Sham, n = 9 OBX)		Imipramine 20 mg/kg (oral) (n = 10 Sham and OBX)	
	Sham	OBX	Sham	OBX	Sham	OBX	Sham	OBX
Distance Traveled (cm)	3038.3 ± 491.9	5972.3 ± 827.1*	4034.8 ± 310.5	5798.3 ± 640.9*	3869.0 ± 480.1	5603.4 ± 626.2*	3318.4 ± 567.4	4675.6 ± 567.4*

OBX, olfactory bulbectomy.
*$p < .009$ comparing OBX animals to sham operated animals, in all treatment groups.



After 14 days of drug treatment, there was a significant interaction between surgery and drug treatment [$F(3,71) = 3.79$, $p = .01$]. The OBX-operated animals in all treatment groups showed activity comparable to sham-treated animals in the open field. Vehicle-treated OBX animals were still significantly more active than all animals, OBX and sham, in all treatment groups.

One week after cessation of treatment, there was a significant interaction between surgery and drug treatment [$F(3,71) = 2.99$, $p = .03$]. The OBX animals in all the treatment groups still exhibited activity levels comparable to sham-treated animals, whereas vehicle OBX animals were still significantly more active than sham-treated animals in the vehicle and escitalopram 10-mg/kg groups. Two weeks after cessation of treatment, there was a significant interaction between surgery and drug treatment [$F(3,71) = 6.65$, $p = .001$]. Activity of all drug-treated OBX animals was still comparable to sham-treated animals. However, OBX vehicle animals remained significantly more active than sham-treated animals in the vehicle and escitalopram 5-mg/kg groups.

Six weeks after cessation of treatment, there was a significant interaction between surgery and drug treatment [$F(3,71) = 11.154$, $p < .001$]. The OBX animals in both escitalopram groups returned to their previous activity levels. The OBX animals in the imipramine 20-mg/kg group remained comparable to sham-treated animals. Vehicle OBX animals remained significantly more active compared with sham-treated animals in the imipramine 20-mg/kg, escitalopram 5-mg/kg, and vehicle groups. After 10 weeks, there was a significant interaction between surgery and drug treatment [$F(3,71) = 3.07$, $p = .03$]. However, there was no main effect of drug at this time point, suggesting that drug treatment still had a subtle but significant effect but only in conjunction with surgical treatment. All OBX animals in all groups were significantly more active than their sham counterparts. The OBX vehicle animals were significantly more active than sham-treated animals in the imipramine 20-mg/kg, escitalopram 5-mg/kg, and vehicle groups (Figure 2).

In both experiments, both sham- and OBX-operated animals showed acclimatization to the open field environment, such that 10 weeks after cessation of treatment, the activity in the open field was lower than the levels 2 weeks after surgery. However, OBX-operated animals continued to exhibit heightened activity levels after acclimatization compared with their sham control couterparts.

Figure 2. Activity levels during and after escitalopram (ESC) and imipramine (IMI) treatment. (**A**) Activity levels after (14 days) of escitalopram/imipramine treatment. Data are expressed as mean distance traveled (cm) ± SEM during the 15-min test session. Olfactory bulbectomy (OBX) and sham post-hoc comparisons: vehicle: $p = .004$; escitalopram 5 mg/kg: NS; escitalopram 10 mg/kg: NS; imipramine: NS. *$p < .05$ compared to all animals (OBX and sham) in all treatment groups. (**B**): Activity 1 week after cessation of treatment. *$p < .05$ compared to sham operated escitalopram 5-mg/kg and vehicle animals. (**C**): Activity after 2 weeks cessation of treatment. *$p < .05$ compared to sham-operated escitalopram 5-mg/kg and vehicle animals. (**D**): Activity after 6 weeks cessation of treatment. **$p < .05$ compared to sham operated escitalopram 5-mg/kg animals. ***$p < .02$ compared to sham operated escitalopram 10-mg/kg animals. (**E**): Activity after 10 weeks cessation of treatment. The OBX and sham post-hoc comparisons: vehicle: $p = .005$; escitalopram 5 mg/kg: $p = .038$; escitalopram 10 mg/kg: $p = .046$; imipramine 20 mg/kg: $p = .020$. *$p < .007$ compared with sham-operated animals in the escitalopram 10-mg/kg and imipramine 20-mg/kg groups as well as sham-operated vehicle animals. **$p < .04$ compared with sham-operated escitalopram 5-mg/kg animals. ***$p < .05$ compared with sham-operated escitalopram 10-mg/kg animals. #$p < .02$ compared with sham-operated imipramine 20-mg/kg animals.

Body weights were measured every week in all experiments, but comparisons revealed no significant difference between OBX and sham animals (data not shown).

Discussion

In this study, we examined the long-lasting effects of antidepressant drugs on OBX-induced hyperactivity in the open field. After 14 days' administration of imipramine or escitalopram, we saw marked reductions in hyperactivity in the OBX animals that lasted for up to 10 weeks after cessation of treatment. It can be reasonably assumed that this altered behavior is a result of long-lasting changes in the brain, because any drug left in the system would be washed out after a few days of cessation (Schatzberg *et al.* 1997).

It is commonly known that removal of the olfactory bulbs in rats might cause several behavioral and neurochemical changes in the brain, reminiscent of those seen in patients suffering from major depression. It is also known that chronic but not acute antidepressant administration can reverse these OBX-induced alterations, but the present study shows that there was already a clear effect of imipramine at a dose of 20 mg/kg on OBX-induced hyperactivity in the open field after 7 days of treatment. This would suggest that the onset of action of imipramine in high doses occurs already after 7 days of treatment, if not sooner. The onset of action of antidepressant drugs is a variable phenomenon in patients. Generally, there is a delay of at least 2 weeks between initiation of treatment and a beneficial effect, but some studies show a sustained response within 1 week of treatment in some but not all patients (Montgomery 1997). A recent study has attributed the early onset of action primarily to pharmacological and pharmacokinetic effects, because no patient- or disease-related characteristic could explain the difference in time to onset among patients (Rojo *et al.* 2005). In line with this observation, Norman and Olver (2004), who studied the effects of new formulations of existing antidepressant drugs, also found that mirtazapine reported a faster onset of action compared with other treatments. The present data suggest that OBX might be a model to investigate whether drugs or formulation of drugs might have a faster onset of action. However, more studies must be done to support the validity of this aspect of the OBX model.

The long-term effects after the cessation of chronic antidepressant administration on OBX-induced hyperactivity have never been described. This study shows that, in particular, chronic imipramine administration might cause long-lasting changes in the OBX rat brain, keeping hyperactivity reduced for 6 weeks or more after cessation of chronic administration. The data show that rats in the escitalopram 5- and 10-mg/kg groups eventually returned to their previous hyperactive state, but animals in the imipramine 20-mg/kg group failed to do the same until 10 weeks after cessation of treatment. This suggests that the return of hyperactivity in the OBX animals after cessation of antidepressant treatment is drug and dose (in the imipramine groups) dependant. The greater efficacy of imipramine might be due to its effect on both the norepinephrine and serotonin transporters. Further support for this theory comes from a study by Santarelli *et al.* (2003), which showed that in serotonin $(5\text{-HT})_{1A}$ receptor knockout mice, treatment with imipramine induced neurogenesis, whereas fluoxetine had no effect on neurogenesis. This might indicate that although the 5-HT_{1A} receptor is necessary for SSRIs to affect neurogenesis, imipramine does not rely on this receptor to be effective.

It is also interesting to note that escitalopram, a relatively new SSRI, has similar effects on behavior in the OBX model and that these effects after cessation of treatment are long-lasting, similar to the effects seen with imipramine. It would be interesting to study whether a higher dose of escitalopram would result in longer-lasting effects after cessation of treatment in a dose dependant manner as seen in imipramine. The fact that a tricyclic antidepressant (TCA) and an SSRI both have similar effects and that other non-antidepressant central nervous system drugs, such as haloperidol and tranylcypromine, do not have an effect on OBX-induced behaviors (Noreika *et al.* 1981) further strengthens the argument that the rat OBX model is one of the most valid animal models for depression. This strongly suggests that OBX induces changes that respond exclusively to antidepressant-like drugs.

Drawing on the notion that neurogenesis might play a role in the mechanism of action of antidepressant treatments and the fact that levels of neurotrophic factors like brain-derived neurotrophic factor (BDNF) have been shown to be decreased in depressed patients (Jiang *et al.* 2005), one would expect OBX also to have an effect on the expression of these peptides. It is interesting in the respect that previous studies have shown that chronically stressed rats also have decreased BDNF levels in the hippocampus (Murakami *et al.* 2005) and that treatment with antidepressant drugs might increase these BDNF levels (Coppell *et al.* 2003; Hashimoto *et al.* 2004), effects similar to those of antidepressant drugs in patients (Aydemir et al 2005). In a study observing the connection between exercise and chronic imipramine treatment on BDNF messenger RNA levels in the OBX rat brain, van Hoomissen *et al.* (2003) found that chronic imipramine treatment, either combined with exercise or not, increased hippocampal BDNF levels. Other studies found not increased but decreased BDNF levels after a single acute dose of an antidepressant (Coppell *et al.* 2003). Therefore, future studies might examine BDNF messenger RNA levels in the hippocampus after cessation of chronic antidepressant treatment in OBX rats.

In conclusion, chronic treatment with either an SSRI or a TCA leads to "normalized" activity levels in OBX-induced hyperactivity in rats, and these alterations might continue to manifest themselves for up to 10 weeks after cessation of treatment. It must also be mentioned that after chronic (14 days) treatment, there was full antagonism of the behavioral effects of the lesion in all the experiments, but this effect was not apparent in the weeks after cessation of treatment. A possible explanation for this might be that after cessation of treatment, all bulbectomized animals might have begun to slightly increase their activity but not enough to make them significantly more active compared with sham-treated animals. However, these changes seem to be drug and dose dependant, because the longest duration of normalization, 10 weeks, was achieved only with the highest dose of imipramine. The mechanism of action behind these changes remains unknown, although it might be that neurogenesis and BDNF levels in the brain play key roles.

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