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Delayed Emergence of Antidepressant Efficacy Following Withdrawal in Olfactory Bulbectomized Rats

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NOREIKA, L., G. PASTOR AND J. LIEBMAN. *Delayed emergence of antidepressant efficacy following withdrawal in olfactory bulbectomized rats.* PHARMAC. BIOCHEM. BEHAV. 15(3)393–398, 1981.—Repeated antidepressant treatment attenuates the step-down passive avoidance deficit which is induced by olfactory bulbectomy in rats. Using a shuttlebox passive avoidance procedure, the effects of antidepressants were investigated after various drug withdrawal intervals. Imipramine, amitriptyline, doxepin, bupropion and mianserin were effective at 48 and usually 72 hours after withdrawal, but no significant attenuation of the deficit was seen 4 hours after withdrawal from any antidepressant tested. At least 4 to 7 days of imipramine treatment were required for efficacy. A high dose of *d*-amphetamine (5 mg/kg) produced similar results while tranylcypromine and haloperidol were inactive at all withdrawal intervals tested. The olfactory bulbectomy syndrome may reflect functional serotonin deficiency, which would be ameliorated through antidepressant-induced alterations in serotonin receptor sensitivity.

Antidepressants *d*-Amphetamine Olfactory bulbectomy

BILATERAL destruction of the olfactory bulbs in rats impairs acquisition of a passive avoidance response and induces hyperirritability as well as other behavioral changes [9, 18, 19]. Superficially, such a behavioral syndrome would not appear to mimic depressive disorders. Considerable interest was, therefore, aroused by the claim [2, 3, 21] that repeated treatment with amitriptyline, mianserin or viloxazine reversed the passive avoidance acquisition deficit in rats with complete, bilateral ablation of the olfactory bulbs. Subsequently, other investigators reported that imipramine was also effective in this procedure [1]. However, chlordiazepoxide or chlorpromazine failed to attenuate the passive avoidance deficit [2,21]. On the basis of these results, Cairncross *et al.* [2] characterized the olfactory bulbectomized rat as a new model for detecting antidepressant activity.

As we attempted to reproduce these results, additional characteristics of this model became apparent. In particular, we report that the effects of antidepressants in this model emerge most strongly after 24 to 48 hours of withdrawal from antidepressant treatment. The effects of various treatment durations, and the comparative effects of *d*-amphetamine and haloperidol, are also described.

METHOD

Surgical Procedures

Male Wistar (Charles River) rats (250–400 g) were used. The animals were anesthetized with 0.2 to 0.4 ml (IM) of 100 mg/ml ketamine solution, to which acepromazine (0.75 mg/ml) had been added to induce muscle relaxation. An incision was made in the skin overlying the skull and three 2-mm diameter holes were drilled to expose the olfactory bulbs. The bulbs were then bilaterally aspirated with a glass pipette attached to a vacuum apparatus. Sham-operated animals were treated identically except that the bulbs were not removed. After completion of surgery, rats were individually housed and were allowed to recover for 2 to 3 weeks before drug treatments were initiated.

Behavioral Testing

After cessation of drug treatment (see below), each rat was tested once in a passive avoidance acquisition test. The rat was placed on the electrified grid floor (0.2 mA, continuously scrambled) of the "shock" side of a shuttlebox (overall dimensions 17 1/2 in. × 8 in. × 7 in.). The partition was immediately lifted to allow the rat to escape to the other side, which was not electrified at any time. The rat was then allowed to re-enter the "shock" side, but when all four paws crossed the center line, electrical footshock was delivered by the experimenter until the rat again escaped. Each re-entry was considered as a trial to criterion and was scored by the investigator, who was "blind" to the experimental treatments. The initial escape response was not included in the trials-to-criterion measure. If acquisition (defined as 120 consecutive seconds on the "safe" side) was not completed within 20 trials, the animal was given a score of 20 and was removed from the apparatus.

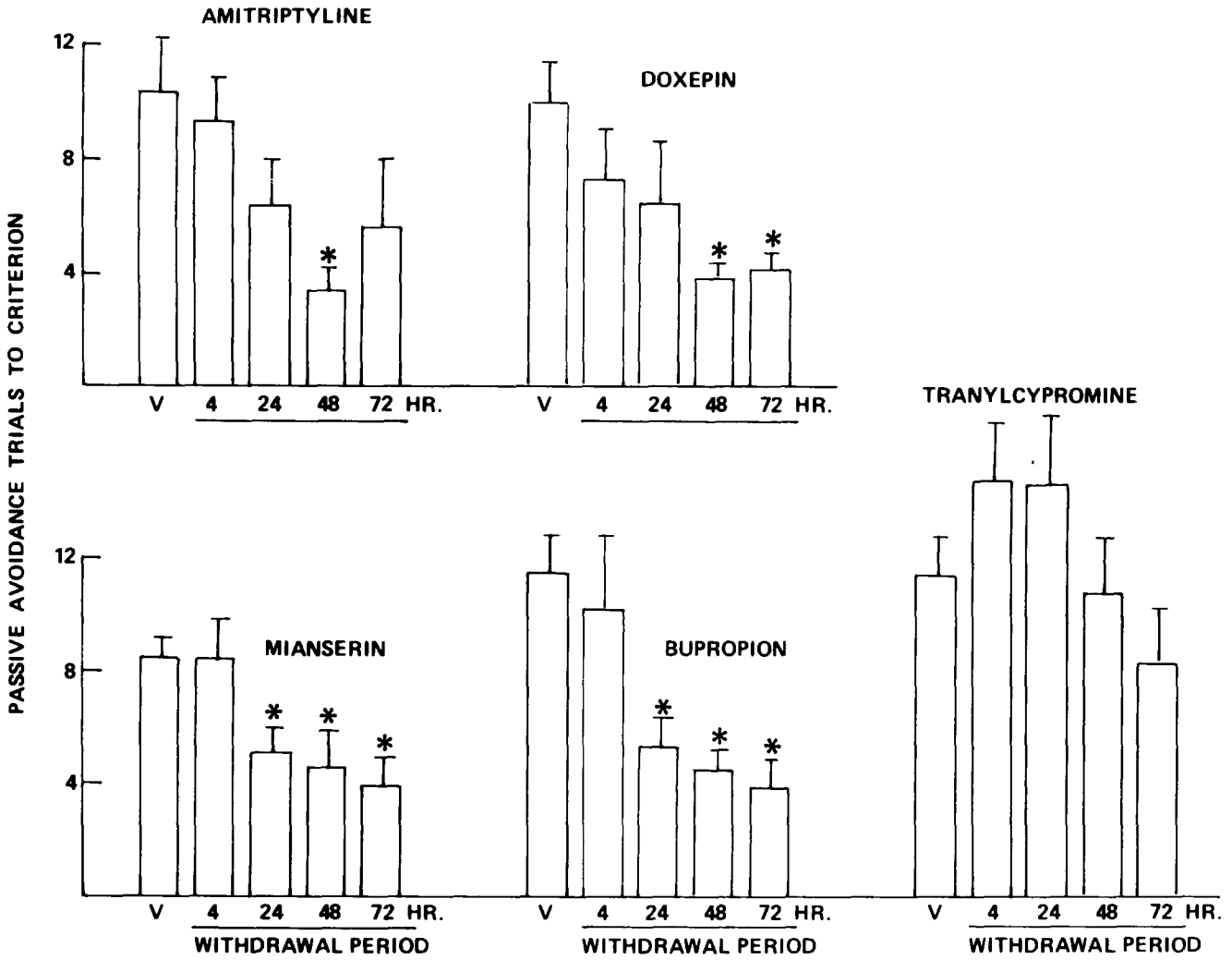

FIG. 1. Effects of repeated treatment with amitriptyline, doxepin, mianserin, bupropion and tranylcypromine on passive avoidance acquisition in olfactory bulbectomized rats. Bars indicate mean (±S.E.) trials to criterion. The number of animals per group ranged from 4 to 11. *Significantly different from vehicle control, $p<0.05$.

Drug Treatments

Drugs administered were: amitriptyline hydrochloride (Merck, West Point, PA), bupropion hydrochloride (synthesized by CIBA-GEIGY chemists), *d*-amphetamine (Smith Kline, Philadelphia, PA), doxepin hydrochloride (Pfizer, Groton, CT), haloperidol (McNeil Laboratories, Fort Washington, PA), imipramine hydrochloride (CIBA-GEIGY, Summit, NJ), mianserin hydrochloride (synthesized by CIBA-GEIGY chemists) and tranylcypromine sulfate (Smith Kline, Philadelphia, PA). All doses were expressed as the respective salt forms. Amitriptyline, *d*-amphetamine and imipramine were administered in saline solution, while bupropion, diazepam, doxepin, haloperidol, mianserin and tranylcypromine were prepared in 3% (w/v) colloidal cornstarch containing 5% (w/v) PEG-400 and 0.34% (w/v) Tween 80. All injections were administered intraperitoneally in a volume of 1.0 ml/kg body weight.

Amitriptyline, bupropion, doxepin, mianserin and tranylcypromine were each administered once daily for seven consecutive days. Separate subgroups of rats were tested at 4, 24, 48 or 72 hr after the last drug treatment. The injection procedure itself seemed to be unusually stressful for the olfactory bulbectomized rats because of their extreme irritability. Therefore, the experimental design was devised so as to equate handling prior to testing. In this treatment schedule, every rat received one injection daily for 10 days. For example, in the subgroup that was tested 72 hr after drug withdrawal, vehicle was injected daily instead of drug during the last three days before testing. Conversely, in the subgroup that was tested 4 hr after the last injection, vehicle was administered for the first three days and drug for the last seven days. A vehicle group was run concurrently with each drug treatment, and was tested 4 hr after the last injection. Amitriptyline-treated animals did not receive saline injections after drug withdrawal, but the results were nevertheless comparable to those from the other drugs tested.

Various treatment durations were also compared systematically in experiments using imipramine (5 mg/kg). Imip-

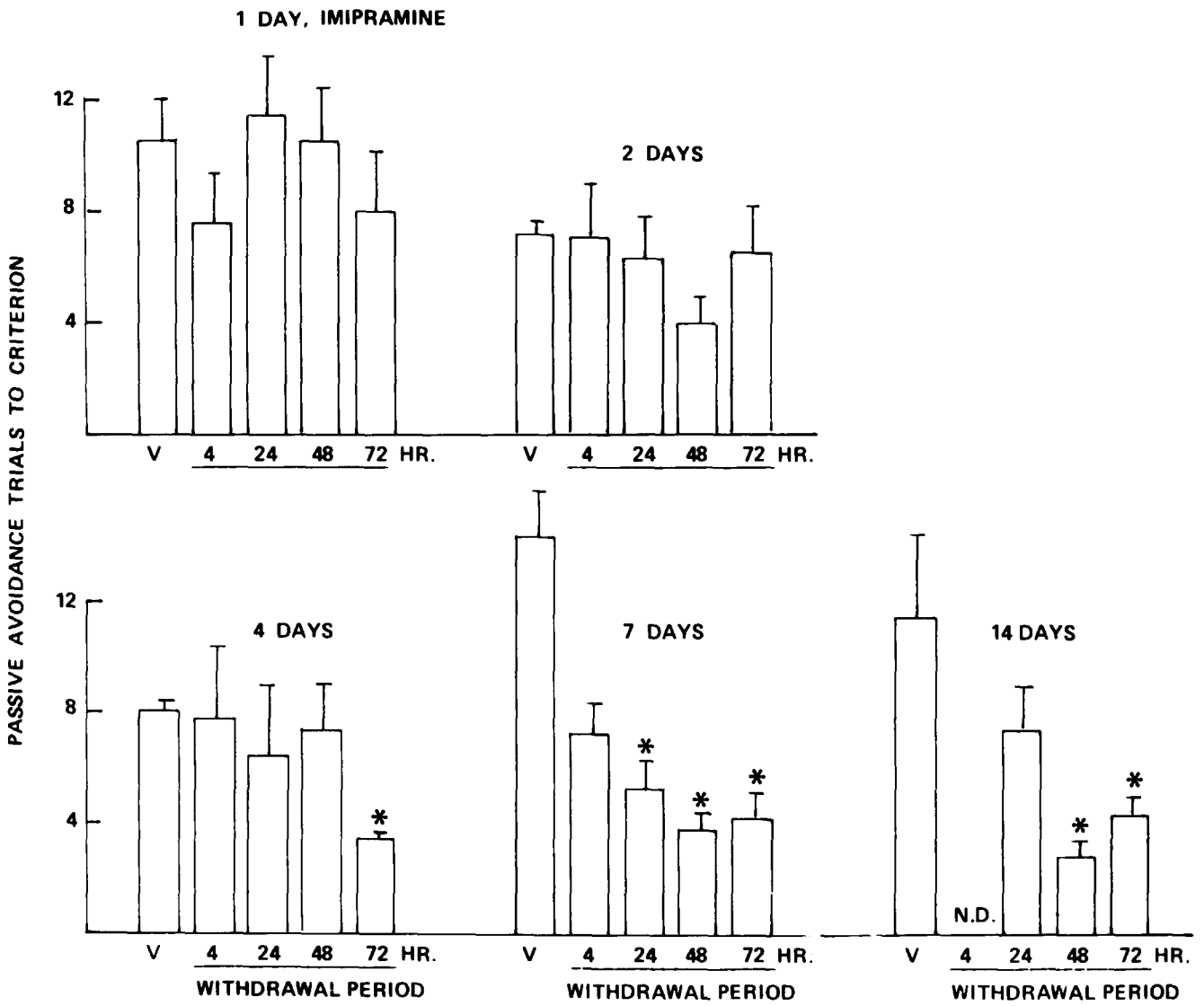

FIG. 2. Effects of repeated treatment with imipramine for 1, 2, 4, 7 and 14 days on passive avoidance acquisition in olfactory bulbectomized rats. The number of animals per group ranged from 6 to 11. See legend, Fig. 1 for further explanation.

ramine was administered for 1, 2, 4, 7 or 14 consecutive days to separate groups of olfactory bulbectomized rats. Again, separate subgroups of rats were tested at 4, 24, 48 or 72 hr after the last drug injection. Haloperidol and *d*-amphetamine were administered for 7 days to olfactory bulbectomized rats which were tested at either 4 or 48 hr after withdrawal.

Surgical Verification and Data Analysis

After completion of the passive avoidance acquisition task, each rat was sacrificed and its brain was removed for examination. Data from individual animals were included in the analysis only if both olfactory bulbs were completely ablated and there was no apparent destruction of the adjoining frontal cortex. Judgments of lesions were made without knowledge of behavioral results from an individual animal. Typically, 60 to 90% of the brains in any given experiment met these criteria.

The Kruskal-Wallis k-sample location test [12] was used for statistical analysis of differences among treatment groups, and was followed by pairwise comparisons using Dunn's multiple comparison procedure [8].

RESULTS

Bilateral olfactory bulbectomy induced a marked and highly reproducible passive avoidance acquisition deficit. For example, in one typical experiment, a group of untreated olfactory bulbectomized rats (n=27) required an average of 9.1±1.3 trials to reach criterion. A group of untreated, sham-operated rats (n=20) acquired the task rapidly with a mean of 0.8±0.1 trials. This low value reflects the fact that some sham-operated rats never re-entered the shocked side after the initial escape response.

After repeated treatment with amitriptyline (10 mg/kg) or doxepin (30 mg/kg) this deficit was significantly ameliorated

at the 48 hr withdrawal interval and, in the case of doxepin, the 72 hr interval (Fig. 1). Strikingly, no attenuation of the deficit was evident at the 4 hr withdrawal interval. Mianserin (20 mg/kg) and bupropion (30 mg/kg) yielded even more pronounced effects (Fig. 1). The deficit was significantly attenuated at the 24, 48 and 72 hr intervals after treatment with either of these non-tricyclic antidepressants. Again, no effect was apparent 4 hr after the last drug injection. Tranylcypromine (10 mg/kg) was, however, inactive at all withdrawal intervals tested.

When imipramine (5 mg/kg) was administered for 1 or 2 consecutive days, no significant amelioration of the acquisition deficit was seen at 4, 24, 48 or 72 hr after treatment (Fig. 2). After 4 days, a significant improvement in acquisition occurred at the 72 hr interval but not at the shorter withdrawal intervals. Treatment with imipramine for 7 days attenuated the deficit significantly at the 24, 48 and 72 hr withdrawal intervals. No greater improvement was noted as a result of 14 days of imipramine treatment.

At a high dose (5 mg/kg), *d*-amphetamine significantly attenuated the passive avoidance acquisition deficit 48 hr after withdrawal (Fig. 3). At the 4 hr withdrawal interval, a strongly bimodal response pattern emerged, probably due to behavioral competition by amphetamine-elicited stereotyped response patterns in some treated animals. The lower dose (0.5 mg/kg) had no effect at either withdrawal interval. Haloperidol (0.1 and 1 mg/kg) failed to reverse the acquisition deficit.

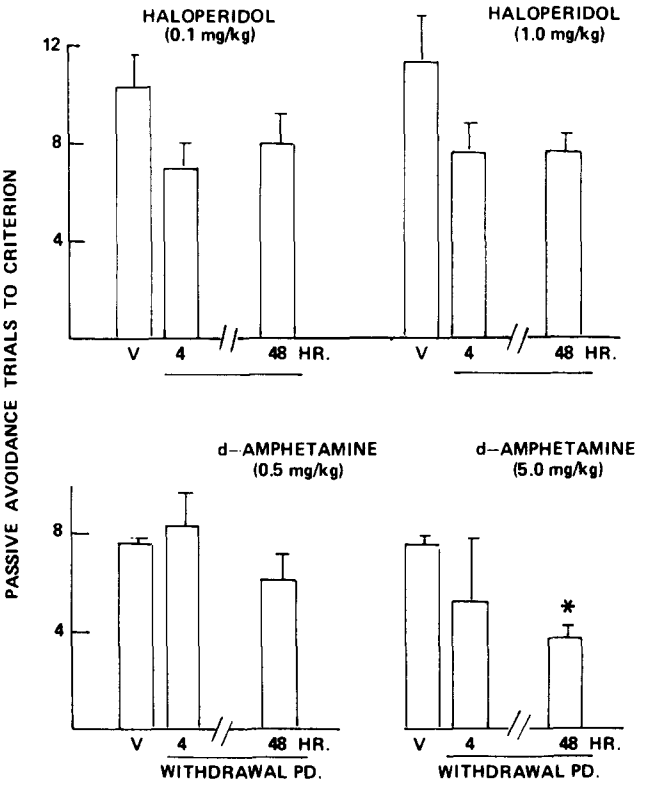

FIG. 3. Effects of repeated treatment with *d*-amphetamine and haloperidol on passive avoidance acquisition in olfactory bulbectomized rats. The number of animals per group ranged from 5 to 10. See legend, Fig. 1 for further explanation.

DISCUSSION

The present results show that the effects of antidepressants in the olfactory bulbectomized model are more readily detected after withdrawal from repeated treatment. Significant improvement of passive avoidance acquisition begins to appear only at a withdrawal interval (24 to 48 hr) when at least some of the antidepressant would be expected to have been metabolized and eliminated. These results suggest that the effects of antidepressants in the olfactory bulbectomy model do not result simply from their direct pharmacological action(s). A further indication that the actual presence of antidepressant does not ameliorate the deficit is that a minimum of four to seven days of repeated treatment was required for efficacy. Rather, it appears that an unspecified event which accompanies withdrawal from repeated antidepressant treatment may cause the deficit to be attenuated.

In agreement with previous reports [1, 2, 3, 4, 21], repeated treatment with imipramine, mianserin and amitriptyline improved acquisition of a passive avoidance task by olfactory bulbectomized rats. The present experiments extend these results to two other clinically effective antidepressants having diverse mechanisms of action, doxepin and bupropion. The doses of amitriptyline and imipramine were comparable to those used by other investigators [1, 4, 21]. A dose of 20 mg/kg mianserin was used because pilot work had suggested that 10 mg/kg had only weak efficacy. Although two reports indicated that mianserin was effective at 5 to 15 mg/kg [4,21] another report evaluated mianserin in this model at a cumulative daily dose of 20 mg/kg (10 mg/kg twice daily) [1].

Our results were obtained with a shuttlebox passive avoidance test, which differed slightly from the "step-down" passive avoidance procedure used by others [1, 2, 3, 4, 21]. In our hands, the shuttlebox passive avoidance procedure was easier to perform and resulted in smaller experimental variability. This procedure also caused a larger separation between the performance of olfactory bulbectomized and sham-operated rats. For example, it was reported [1] that the mean trials to criterion for untreated bulbectomized rats was 6.6 and that for sham-operated rats was 2.8, a mean difference of 3.8 trials. In the present procedures, the corresponding mean difference between bulbectomized and sham rat performance was 8.3 trials. In both cases, however, antidepressants appeared to reduce the number of trials to criterion by approximately 50%.

It has been shown that repeated treatment with mianserin or amitriptyline reduces locomotor activity in olfactory bulbectomized but not in sham-operated rats [21]. These results would suggest that amelioration of a passive avoidance deficit could be secondary to such an induced reduction in motor activity. This possibility would be equally true for the step-down procedure as for the shuttlebox procedure, as motor hyperactivity is potentially detrimental to performance in either task.

The failure of tranylcypromine to improve passive avoidance in olfactory bulbectomized rats might seem surprising, given its effectiveness as a monoamine oxidase inhibiting antidepressant. If anything, tranylcypromine slightly retarded acquisition. This finding is consistent with a previous report [2]. Further experimentation would be needed using other monoamine oxidase inhibitors before it could be

concluded that the olfactory bulbectomy model does not detect antidepressants of this type.

That an induced serotonin deficiency could contribute to the olfactory bulbectomized-induced behavioral deficits was first suggested by the finding [3] that the syndrome could be reproduced by microinjecting 5,6-dihydroxytryptamine, a serotonin neurotoxin, into the olfactory bulbs of rats. Moreover, acute administration of serotonin agonists or releasing agents also reversed bulbectomy-induced deficits [1]. Provided that the following recent findings are taken into account, the present results are compatible with this hypothesis.

Firstly, ^{3}H-mianserin binding in brain appears to be serotonergic, and many other known antidepressants compete for binding of this ligand [23]. Secondly, many antidepressants also compete for *in vivo* ^{3}H-spiroperidol binding to rat frontal cortex, a preparation which is said to be sensitive to serotonin antagonists [6]. Furthermore, mianserin, amitriptyline and possibly other antidepressants antagonize behavioral impairment produced by d,l-5-hydroxytryptophan when administered acutely [11,16] and have other "antiserotonin" effects [13].

However, repeated antidepressant treatment does not block, but actually causes increased sensitivity to the behavioral effects of 5-hydroxytryptophan [11,15]. Correspondingly, repeated antidepressant treatment induces hypersensitivity as measured electrophysiologically in amygdaloid, hippocampal and lateral geniculate neurons [7,22]. This apparent hypersensitivity of serotonin receptors may occur in response to repeated antidepressant blockade of these receptors, and could compensate functionally for olfactory bulbectomy-induced deficiencies in availability of brain serotonin. The acute presence of antidepressant, as at the 4-hr withdrawal interval, would block the expression of this hypersensitivity, which would emerge functionally only after longer withdrawal intervals. The present findings regarding the withdrawal interval would be consistent with this suggestion.

The effects of repeated treatment with a high dose of *d*-amphetamine (5 mg/kg) may also be consistent with this hypothesis. Repeated high doses of *d*-amphetamine and methamphetamine have been reported to deplete tryptophan hydroxylase and serotonin in brain [17,20]. Such an action could result in hypersensitivity of serotonin receptors.

Certainly, the evidence in favor of the serotonin hypersensitivity hypothesis advanced here is far from conclusive. For example, bupropion has not been reported to antagonize serotonin receptors, but appears to block the uptake of dopamine [5]. Alterations in several other neurotransmitter systems are known to result from olfactory bulbectomy [10]. Drug-induced augmentation of serotonergic neurotransmission may simply compensate indirectly for bulbectomy-induced damage to a non-serotonergic pathway. Evaluation of these questions will be aided by a better understanding of the neurochemical events involved, as well as by investigations of the relationships among dose, onset of action, and withdrawal period. Regardless of the neurochemical mechanism involved, a better knowledge of the neuronal substrates of the olfactory bulbectomy syndrome may contribute to our understanding of antidepressant actions in the clinic.

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