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The Effects of Fluoxetine and Zimeldine on the Behavior of Olfactory Bulbectomized Rats

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JOLY, D AND D J SANGER *The effects of fluoxetine and zimeldine on the behavior of olfactory bulbectomized rats* PHARMACOL BIOCHEM BEHAV **24**(2) 199–204, 1986 —Previous work has shown that subchronic administration of antidepressant drugs can reverse the behavioral and physiological changes produced by removal of the olfactory bulbs of rats It has also been reported that acute administration of drugs believed to enhance serotonergic transmission can improve passive avoidance performance in bulbectomized rats In order to follow up this observation the effects of the serotonin reuptake inhibitor, fluoxetine, were studied in bulbectomized and control rats Fluoxetine produced a dose-related improvement in the passive avoidance behavior of bulbectomized rats in a step-down task and in a Y-maze The effect of fluoxetine on step-down avoidance was blocked by metergoline and was also shown by zimeldine, another inhibitor of serotonin reuptake However, in tests of active avoidance responding in a shuttle box and exploratory locomotion, fluoxetine produced similar disruptions of behavior in both bulbectomized and control animals Thus, the effects of fluoxetine on the behavior of bulbectomized rats are dependent upon the behavioral test

Fluoxetine Zimeldine Bulbectomy Avoidance Rats

IT has been known for many years that surgical removal or destruction of the olfactory bulbs of rats and other rodents gives rise to a variety of behavioral and physiological changes [11,20] These may include increased exploration and locomotor activity [17], aggressiveness [12], hyperirritability [5], impaired acquisition in appetitive or aversive learning tasks requiring response inhibition [15, 16, 18] and facilitated acquisition of an active avoidance response [15]

Several studies have demonstrated that some or all of these effects of olfactory bulbectomy can be reversed by subchronic administration of antidepressant drugs, including tricylic agents such as imipramine and atypical agents such as mianserin [3, 4, 14, 19] Broekkamp and his colleagues [2] have also reported that acute administration of single doses of several drugs believed to facilitate serotonergic activity (fluoxetine, fenfluramine and quipazine) reversed the passive avoidance deficit shown by bulbectomized rats In the same study it was found that metergoline, a serotonin antagonist [6], blocked the effect of fenfluramine and quipazine and also the similar effect produced by subchronic administration of imipramine and mianserin Furthermore, this same research group found that injections of imipramine, amitriptyline, fluoxetine and serotonin into the medial amygdala improved the passive avoidance learning of bulbectomized rats and this effect also was blocked by metergoline [7]

In contrast to these results Jancsàr and Leonard [9] have recently presented evidence that the effects of mianserin to reverse the hyperactivity of bulbectomized rats in an open field were mediated through noradrenergic mechanisms, although these workers did not rule out completely the involvement of serotonin The present study, therefore, was carried out to further investigate the role of serotonin in the behavioral changes produced by olfactory bulbectomy in rats The effects of the serotonin reuptake inhibitor fluoxetine [21] were studied at several dose levels in three behavioral procedures In addition, the effects of a second drug also having a relatively specific action to inhibit serotonin reuptake, zimeldine [13], were investigated using the acquisition of a passive avoidance response

GENERAL METHOD

Subjects

Male Wistar rats weighing 250–350 g were used in all experiments After arrival from the supplier the animals were kept in group housing for several days before the surgical procedure was carried out After surgery the rats were individually housed and allowed two weeks recovery before the behavioral test They were kept under standard laboratory conditions with food and water constantly available and lights on from 7 00–19 00 hr Each rat was used on a single occasion

Surgical Procedure

Surgery was carried out as previously described [2] Rats were anaesthetized with pentobarbital, the skull exposed and two 2 mm holes trepanized through the skull 7 mm anterior to bregma The olfactory peduncles were cut and the bulbs

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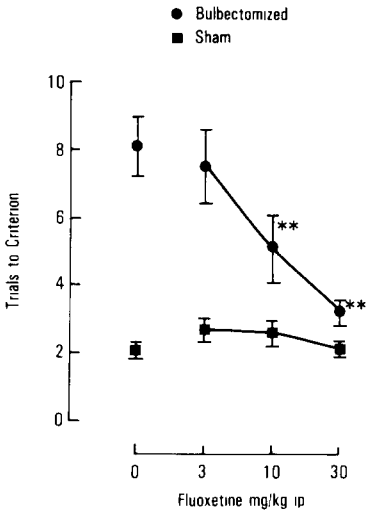

FIG 1 The effects of fluoxetine on the acquisition of step-down passive avoidance responding in bulbectomized and control rats Each animal was tested until it reached a criterion of remaining on the platform for 3 min or had received 10 trials n=10 rats/group **$p<0$ 01 difference from undrugged rats

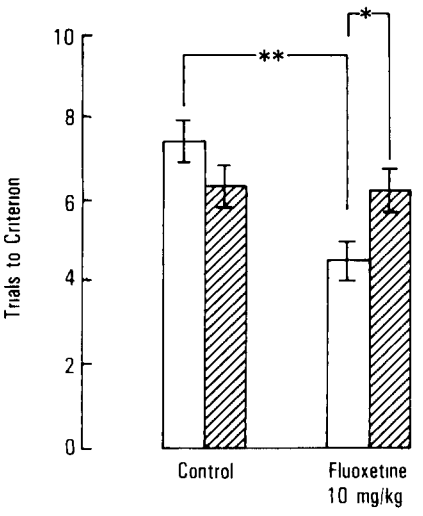

FIG 2 The effects of fluoxetine and metergoline on the passive avoidance acquisition of bulbectomized rats Fluoxetine reduced the number of trials to criterion and this effect was blocked by metergoline *$p<0$ 05, **$p<0$ 01

removed by aspiration Sham lesioned rats were treated in an identical manner except that the peduncles were not cut nor were the bulbs removed

After experimentation all animals were killed with ether and the lesions verified by visual inspection Bulbectomized rats were removed from the study if less than approximately 75% of the bulbs had been removed or if damage extended into the frontal cortex Sham operated rats were removed from the study if there was evidence of damage to the olfactory bulbs Approximately 10% of animals were removed for these reasons and were replaced with other rats No attempt was made to correlate the extent of the damage to the bulbs with the degree of behavioral change

EXPERIMENT 1

The purpose of the first experiment was to attempt to replicate and extend the finding that acute administration of a dose of fluoxetine would improve the passive avoidance learning of bulbectomized rats [2] Three doses of fluoxetine were studied and the drug was administered to both bulbectomized and sham operated controls

Method

A total of 80 rats (40 bulbectomized and 40 sham operated) was used in this experiment The rats were injected with either fluoxetine hydrochloride (3, 10, 30 mg/kg, IP) or the injection vehicle (distilled water, 5 ml/kg) and 30 min later were tested in a step-down passive avoidance task Each rat was placed on a platform (7 5×30 cm) mounted 4 5 cm above the grid floor of a cage (30×30×28 cm) When the animal stepped off the platform with all four paws it received an electric shock through the grid floor (0 6 mA for 1 sec, Grason-Stadler shocker 700) The rat was then returned to its home cage for 1 min after which it was given a second trial This procedure was continued until the animal remained on the platform for 3 min without stepping off or until 10 trials had been given

Results

The effects of fluoxetine on the acquisition of the step-down passive avoidance response are shown in Fig 1 The data are expressed as the mean number of trials to the criterion of 3 min on the platform without stepping down Rats which did not reach this criterion in 10 trials were given the value 10 The figure shows that while control animals learned very rapidly to remain on the platform, bulbectomized rats required more trials The number of trials taken by bulbectomized rats showed a dose-related reduction after fluoxetine, but the drug did not affect the passive avoidance behavior of sham operated controls It was observed informally that the highest dose of fluoxetine (30 mg/kg) reduced the activity of sham operated animals in the home cages and appeared to increase the latency of these animals to step off the platform on the first trial However, these rats were not grossly debilitated by the drug as indicated by the fact that all did step off the platform on the first trial, but learned to stay on the platform in two or three trials Statistical analysis using a two-way ANOVA showed significant effects of the operation, $F(1,72)=62$, $p<0$ 001, of drug dose $F(3,72)=6$ 5, $p<0$ 001, and a significant interaction, $F(3,72)=6$ 2, $p<0$ 001 Individual comparisons using Duncan's test showed that fluoxetine produced significant reductions in the number of trials to criterion in the bulbectomized rats at 10 and 30 mg/kg

EXPERIMENT 2

In this experiment an attempt was made to antagonise the effects of a dose of fluoxetine (10 mg/kg, IP) by metergoline on bulbectomized rats

Method

Forty bulbectomized rats were injected with fluoxetine or distilled water 30 min after receiving an oral administration of metergoline (10 mg/kg) or its vehicle which consisted of

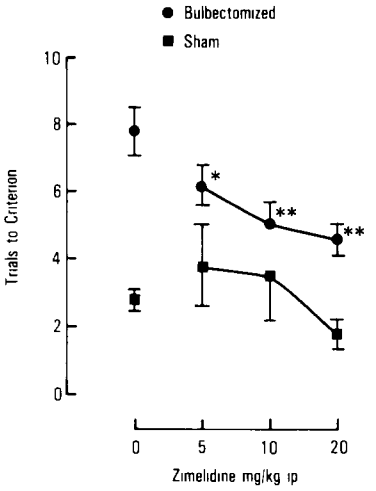

FIG 3 The effects of zimeldine on the acquisition of a step-down passive avoidance response in bulbectomized and control rats Data are expressed as in Fig 1

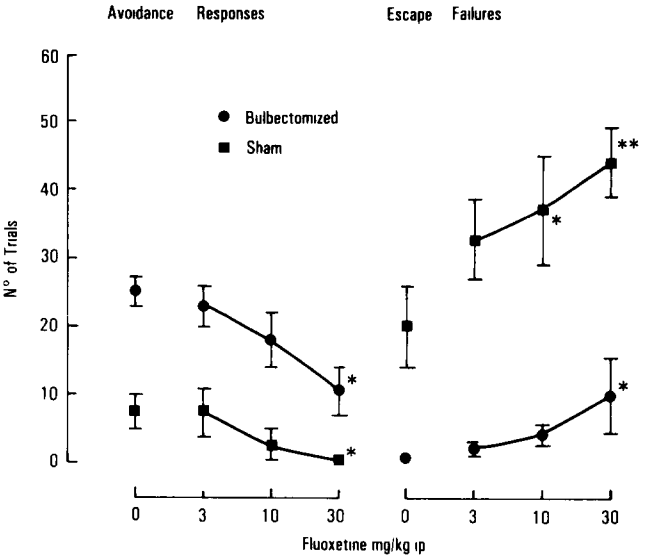

FIG 4 The effects of fluoxetine on two-way shuttle-box escape/avoidance responding in bulbectomized and control rats Each rat was given a session of 60 trials The results are shown as the mean number of trials on which animals avoided the shock and the mean number of trials on which they failed to escape the shock *$p<0$ 05, **$p<0$ 01 difference from undrugged rats

distilled water containing two drops of Tween 80 Thirty minutes after the second injection each rat was tested in the step-down passive avoidance procedure as described above Sham lesioned animals were not used in this experiment

Results

Figure 2 shows that fluoxetine at 10 mg/kg reduced the number of trials to learn the passive avoidance response in bulbectomized rats and this effect was blocked by metergoline at 10 mg/kg This dose of metergoline had no effect when given alone These differences were confirmed statistically using Duncan's test, the results of which are indicated in Fig 2

EXPERIMENT 3

The purpose of this experiment was to investigate the effects of another compound known to inhibit the reuptake of serotonin Zimeldine [13] was chosen for this purpose and its effects studied on the acquisition of the step-down passive avoidance response in bulbectomized and sham lesioned rats

Method

A total of 125 rats was used The procedure for this experiment was identical to that of Experiment 1 except that zimeldine was used in place of fluoxetine Bulbectomized or sham operated rats were injected with a dose of zimeldine dihydrochloride (5, 10, 20 mg/kg, IP) or the vehicle and 30 min later were tested in the acquisition of the step-down passive avoidance response

Results

The effects of zimeldine on the acquisition of the step-down passive avoidance response in bulbectomized and control rats are shown in Fig 3 A more limited range of doses of zimeldine than of fluoxetine was used but it is clear from the figure that zimeldine had a similar effect Zimeldine produced a dose-related reduction in the number of trials to criterion in the bulbectomized animals Statistical analysis showed a significant effect of the lesion, $F(1,117)=28\ 9$, $p<0\ 001$, and of drug dose, $F(3,117)=3\ 0$, $p<0\ 05$, although the interaction was not significant Figure 3 shows the results of individual statistical comparisons using Duncan's test which demonstrated that all doses of zimeldine reduced trials to criterion in bulbectomized rats without producing statistically significant effects in sham operated controls

EXPERIMENT 4

In addition to showing slower acquisition of a passive avoidance response, bulbectomized rats may acquire active avoidance responding faster than controls This experiment was therefore carried out to investigate the effects of fluoxetine on avoidance behavior in a shuttle-box in bulbectomized and sham lesioned rats

Method

A total of 80 rats was used Thirty minutes after administration of fluoxetine (3, 10, 30 mg/kg, IP) or the vehicle, rats were given 60 conditioned escape/avoidance trials in a shuttle-box (Ugo Basile) The shuttle-box was of dimensions 50×28×28 cm and was divided into two equally sized compartments by a partition with a hole 7 cm wide × 8 cm high Movement of an animal between the two compartments was detected by a microswitch operated by the pivoted grid floor of the cage Each trial consisted of a conditioned stimulus (CS—tone + light) of 4 sec followed by a period of stimulus presentation with footshock (65 V, scrambled) which lasted for 3 sec The trials were separated by 7 sec intertrial intervals A passage across the box during the intertrial interval had no effect, whereas shock could be avoided during the CS or escaped during the CS + shock by passing from one compartment to the other Each animal was tested only once

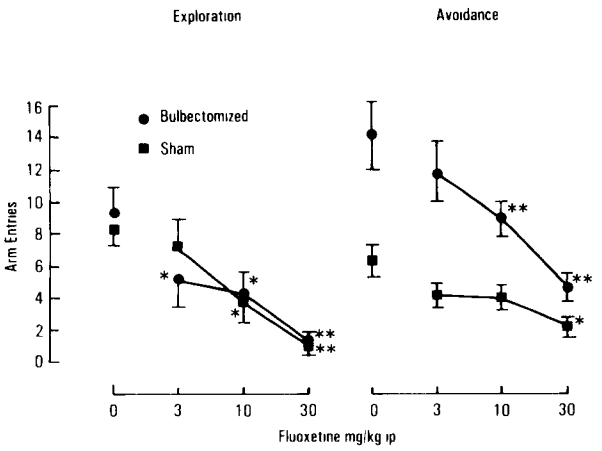

FIG 5 The effects of fluoxetine on behavior in the Y-maze of bulbectomized and control rats The rats were given a 3 min period of exploration followed by a 3 min period of passive avoidance training during which they were required to remain on a central platform to avoid continuous shock *$p<0.05$, **$p<0.01$ difference from undrugged rats

Results

The effects of fluoxetine on two-way active avoidance responding in the shuttle-box in bulbectomized and sham operated rats are shown in Fig 4 The data are presented as the mean number of trials on which rats avoided the shock presentation by crossing the box during the CS, and the mean number of trials on which they failed to escape the shock by crossing the box neither during the CS nor during the shock It can be seen from Fig 4 that the performance of the control rats was poor as these animals showed relatively few avoidance responses and failed to escape a substantial number of shocks This performance is typical of that shown by control rats during the first 60 trials of shuttle-box conditioning using these parameters in our laboratory The performance of the undrugged bulbectomized rats was considerably better, however These animals avoided more shocks than the control rats and showed very few escape failures

Statistical analysis showed significant effects of the lesion (avoidance responses $F(1,72)=63$, $p<0.001$, escape failures $F(1,72)=73$, $p<0.001$) and of drug dose (avoidance responses $F(3,72)=7.0$, $p<0.001$, escape failures $F(3,72)=4.1$, $p<0.01$), but the interactions were not statistically significant As shown in Fig 4, individual statistical comparisons using Duncan's test confirmed that fluoxetine produced similar disruptions of two-way escape/avoidance behavior in both sham operated and bulbectomized rats

EXPERIMENT 5

In this experiment the effects of fluoxetine on the exploration and avoidance behavior of bulbectomized and control rats were studied in a Y-shaped maze

Method

The Y-maze consisted of three arms each of dimensions 40×12×25 cm high and having grid floors At the intersection of the three arms was a small perspex platform raised 2 cm above the grid floor A total of 80 rats was used Each rat was injected with fluoxetine (3, 10, 30 mg/kg, IP) or the injection vehicle and 30 min later was placed at the intersection of the arms of the Y-maze For the first 3 min of the trial the exploratory activity of the animal was recorded as the number of times the rat entered the arms of the maze, an arm entry being defined as all four of the animal's paws being within the arm After 3 min of exploration the rat was left for a further 3 min in the maze during which time it received continuous electric footshock (0 6 mA, scrambled, Grason-Stadler shocker 700) whenever all four paws left the central platform This can be considered a passive avoidance procedure as, in order to avoid shock, an animal was required to stay on the central platform The number of entries into the arms of the maze during this second 3 min period was also counted

Results

The effects of fluoxetine on the behavior of bulbectomized and control rats in the Y-maze are shown in Fig 5 This figure presents the data for the 3 min period of exploration and also for the second 3 min period during which the rats received shocks when they left the central platform of the maze There was no difference in levels of exploration between the undrugged bulbectomized and control rats and fluoxetine produced similar, dose-related reductions of exploration in both The ANOVA showed a statistically significant effect of drug dose, $F(3,72)=13.3$, $p<0.001$, but neither the effect of the lesion nor the interaction was significant The results of individual comparisons using Duncan's test are shown in Fig 5 Doses of 10 and 30 mg/kg of fluoxetine reduced activity in both bulbectomized and sham animals At the 3 mg/kg dose there was a statistically significant effect in bulbectomized but not in sham operated rats As the ANOVA did not show significant effects of the lesion nor a significant interaction, however, the results show generally similar effects of the drug in both lesioned and non-lesioned rats

Figure 5 also shows that during the second 3 min period in the maze, bulbectomized rats showed more arm entries than controls It is interesting to note that the number of arm entries of undrugged bulbectomized rats was greater during this second 3 min period than during the 3 min of unshocked exploration While control rats generally learned to remain on the central platform and thus avoid shock, the shock appeared to have an activating effect in the lesioned animals Fluoxetine produced a dose-related reduction in shocked arm entries and the highest dose also reduced arm entries in the controls Statistical analysis showed statistically significant effects of the lesion, $F(1,72)=45$, $p<0.001$, and of drug dose, $F(3,72)=11.4$, $p<0.001$, but the interaction did not reach an acceptable level of significance The results of the individual comparisons using Duncan s test are shown in Fig 5 and suggest that fluoxetine was more effective at reducing arm entries in bulbectomized than in sham lesioned rats

DISCUSSION

Surgical removal of the olfactory bulbs of rats was found to produce a deficit in the acquisition of passive avoidance responding using both a step-down task and a Y-maze In contrast, bulbectomized rats showed greatly improved performance of a two-way shuttle avoidance response These results are thus consistent with many previous studies [11, 15, 16, 17] In the present experiment bulbectomy did not alter the level of exploration of rats during a three minute

trial in the Y-maze Previous studies have shown that under some experimental conditions bulbectomized rats can show greatly elevated levels of exploration or locomotor activity (e g , [9]) Other studies however, have reported only small or marginal differences between activity levels of control and lesioned animals [5,15] Bulbectomized rats may show impaired habituation in novel environments [11] and thus the short trial duration used in the present study may have ensured that little habituation occurred in either control or lesioned animals and thus minimized the possibility of a difference in levels of locomotion Although the present experiments were not aimed at investigating the nature of the behavioral deficit produced by removal of the olfactory bulbs, these results are consistent with the proposal of Leonard and Tuite [11] that bulbectomized rats have a tendency to show an "all-purpose' avoidance response of active locomotion in novel environments This would lead to improved performance of an active avoidance response, deficient passive avoidance learning and a tendency for shock punishment of exploration in the Y-maze to have an activating rather than a suppressant effect on behavior

Fluoxetine was found to produce a dose-related improvement in the passive avoidance behavior of bulbectomized rats in the step-down task and in the Y-maze The drug had little effect on these measures in the control rats except to produce a small effect in the Y-maze at the highest dose These results are thus consistent with those of Broekkamp and his colleagues [2] but also extend this previous work by providing data for several doses of this drug in two passive avoidance tests in both bulbectomized and control rats The conclusion drawn from previous work, that serotonin is involved in the passive avoidance deficit of bulbectomized rats [2,7], is also supported by the present results Thus, the effect of a dose of fluoxetine on step-down passive avoidance in bulbectomized rats was antagonized by metergoline and the improvement in passive avoidance performance was also produced by zimeldine, which, like fluoxetine, has been shown to be a relatively specific inhibitor of the reuptake of serotonin into presynaptic neurons [13]

In contrast to the apparently specific effects of fluoxetine and zimeldine in blocking the deficit produced by olfactory bulbectomy on passive avoidance learning, fluoxetine did not have specific effects on the behavior of bulbectomized rats when measures of active avoidance and exploration were taken Bulbectomized rats showed greatly improved performance in the shuttle-box but fluoxetine had similar effects in both lesioned and control animals These results can be compared with those of Archer and coworkers [1] who recently found that acute administration of zimeldine and fluoxetine disrupted the acquisition of two-way but not of one-way avoidance in normal rats In the present study it was also found that fluoxetine produced similar reductions in exploratory behavior in both bulbectomized and control rats Previous studies have shown that after repeated administration of other antidepressant drugs the hyperactivity of bulbectomized rats is reduced [9] It must be noted, however, that the measure of exploratory activity used in the present study was not sensitive to the effects of the operation

Most studies of the effects of repeated administration of antidepressant drugs in bulbectomized rats [3, 4, 19] suggest that these drugs reverse many or all of the behavioral and hormonal changes produced by the lesion The present results, in contrast, show that acute administration of fluoxetine can affect passive avoidance but not active avoidance in bulbectomized rats One interpretation of this result is that the passive avoidance deficit may involve different neurochemical mechanisms Other researchers [9] have proposed that different behavioral changes produced by bulbectomy may involve different mechanisms, and it is known that the lesion gives rise to changes in a variety of neurochemical systems [8,10]

Finally, although the present results suggest that inhibitors of serotonin reuptake produce specific effects on passive avoidance behavior of bulbectomized rats, the possibility needs to be considered that this effect is produced by a tendency for these drugs to give rise to a general depressant effect on behavior Thus, it might be argued that the apparently improved passive avoidance learning might have resulted from a tendency for fluoxetine and zimeldine to reduce activity levels, an effect not observed on the passive avoidance behavior of control rats because of the rapid rate of learning of these animals Consistent with this possibility are the findings that fluoxetine at 10 and 30 mg/kg reduced exploratory behavior in both bulbectomized and control rats Certainly this interpretation cannot be ruled out, particularly with the highest dose of fluoxetine, which was observed to have a general depressant effect However, other observations suggest that the actions of fluoxetine and zimeldine on the passive avoidance behavior of bulbectomized rats may be specific In particular, a number of previous studies have shown that administration of depressant drugs at relatively high doses, including chlordiazepoxide, chlorpromazine and clonidine, does not affect passive avoidance acquisition in bulbectomized rats [2, 3, 4, 19] Also, other, unpublished, work from the present authors' laboratory and the work of Archer and his colleagues [1] suggests that the doses of zimeldine active in this study do not have grossly debilitating effects on behavior

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