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Selective Neurotoxin Lesions of the Lateral Septum: Changes in Social and Aggressive Behaviours

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CLARKE, A. AND S. E. FILE. *Selective neurotoxin lesions of the lateral septum: Changes in social and aggressive behaviours*. PHARMAC. BIOCHEM. BEHAV. 17(4) 623–628, 1982.—Bilateral microinjection of 5,7-dihydroxytryptamine into the lateral septum resulted in a behavioural profile in the Social Interaction test of anxiety similar to that seen after chronically-administered benzodiazepines. In contrast, bilateral microinjection of 6-hydroxydopamine into this locus did not alter the profile of rats tested in this model. In a colony-intruder model of aggression, the 5,7-dihydroxytryptamine lesioned rats showed decreased dominance, while the 6-hydroxydopamine lesioned rats showed increased dominance and reduced subordination. The results suggest that lateral septal 5-hydroxytryptamine, but not catecholamines, is important in the control of anxiety, whereas both lateral septal 5-hydroxytryptamine and catecholamines are involved in the control of aggression.

Aggression Anxiety Neurotoxins Septum Social interaction

DESPITE the volume of work investigating the pharmacological effects of anxiolytic drugs such as the benzodiazepines rather fewer studies have been aimed at determining the neurochemical basis of anxiety itself.

Early studies showed that benzodiazepines could reduce the turnover of both noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in several brain regions [12,14]. The extent to which the effects of benzodiazepines on NA and 5-HT systems contribute to the anxiolytic and sedative effects of these drugs has been investigated by Stein, Wise and Berger [29]. These workers suggested that the sedative effects are largely a result of the reduction in the turnover of NA, whereas the reduced turnover of 5-HT appears to underlie the anxiolytic effects. Several other studies support this conclusion. The sedative effect of oxazepam is reduced after chronic administration: the anxiolytic activity is not [24]. The tolerance to the sedative effects correlates well with tolerance to the effects of oxazepam on the turnover of NA, whereas the reduction in the turnover of 5-HT persists during chronic administration [32].

Consistent with the view that a reduction in the turnover of 5-HT might underlie anxiolysis, Tye, Everitt and Iversen [30] showed that selective lesions of 5-HT-containing neurones in the median forebrain bundle could elicit an anxiolytic profile in a conflict test. More specifically, selective neurotoxin-induced lesions restricted to the dorsal, but not median, raphe area produces an anxiolytic profile in the Social Interaction test of anxiety [20], whereas large depletions of central NA produced by injection of 6-hydroxydopamine (6-OHDA) into the locus coeruleus do not [15,19].

The limbic system has long been implicated in the control of anxiety. Since the ascending monoamine projections from the locus coeruleus and the dorsal raphe nucleus innervate the lateral septum [4, 5, 8], it was decided to investigate the effects of selective lesions of monoamine-containing neurones in this area on performance in an experimental model of anxiety.

As the septum has been implicated in the control of aggression (e.g., [2]), the lesioned animals were also tested in a colony intruder aggression model.

METHOD

Drugs

5,7-Dihydroxytryptamine creatine sulphate (5,7-DHT), 6-hydroxydopamine hydrochloride (6-OHDA) and standards of 5-hydroxytryptamine creatine sulphate (5-HT), noradrenaline hydrochloride (NA) and dopamine hydrochloride (DA) were all obtained from Sigma Ltd., London, Des-

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methylimipramine was supplied by Ciba-Geigy Ltd., Horsham.

Apparatus

The test arena for social interaction was 60×60×36 cm, with wooden walls and floor. Rats in the box were observed via a closed-circuit television system, and a measure of locomotor activity derived from infra-red beams that crossed the box. All animals were tested between 0700 and 1130 hr. Two levels of illuminance were used: 338 and 23 scotopic lux, for the high and low levels respectively. The aggression colony box was made of clear Perspex and had three storeys: each was 90×60 cm, the middle and lower storeys were 20 cm high while the upper floor was 15 cm high. Water was available on the upper and lower floors; food was available on the upper floor only. Rats in the box had free access to each floor. During testing, the light levels (in scotopic lux) were as follows: upper 115, middle 41.5 and lower 3.32. The animals were tested between 1300 and 1730 hr. The colony of rats consisted of 10 animals, was established six months before the beginning of these experiments, and had previous experience of intruder rats.

Animals and Surgery

Male hooded rats (Rattus norvegicus) from Olac Ltd., Bicester, weighing 100–120 g were anaesthetised with halothane (1% v/v in oxygen) and lesions of both lateral septa (A 7.4; L ±1, [23]) were induced by three injections of 5,7-DHT (0.4 μg in 0.05 μl) or 6-OHDA (0.4 μg in 0.05 μl) at levels V 4.6, 4.2 and 3.8. Three injections of such small volume into each locus were made in order to localize the neurotoxins to as discrete an area as possible. Injections were delivered from a 1 μl microsyringe (Hamilton, Nevada #7001): each injection was given gradually over 30 sec and the injection needle was left in place for 30 sec after the final injection. The animals in the 5,7-DHT group were pretreated with desmethylimipramine (25 mg/kg,IP) 60 minutes before surgery in order to prevent damage to NA-containing fibres [6]. The neurotoxins were dissolved in 0.9% (w/v) saline containing 1 mg/ml of ascorbic acid; control animals received equal volume injections of vehicle only (as the 5,7-DHT and 6-OHDA experiments were performed at different times, there were separate controls for the two lesion groups). All animals were allowed two weeks postoperative recovery time. During the second week after surgery, the water consumption of all animals was measured, as the increase in aggression seen after septal ablations has been reported to be secondary to hyperdipsia [21].

The animals were housed in groups of six in a room maintained at 25°C, in a 11 hr light:13 hr dark cycle (lights on at 0700 hr). Food and water were available ad lib.

Procedure

Social Interaction test. The animals were singly housed for six days prior to testing and were allocated to a partner with whom they were to be tested (rats in a pair did not differ in weight by more than 10 g). The pairs of animals were randomly assigned to be tested in either high or low light; there were initially 7 pairs of animals in each test group. On the first day of testing, pairs of rats were placed together in the test arena (which at this time was unfamiliar to them) in the assigned light level, and the time spent in active social interaction was scored by two observers who had no knowledge of either the light level or the lesion-state of the animals. The behaviours scored included: sniffing, grooming, wrestling, following and crawling on or under the partner. The same afternoon, all rats were placed singly into the test box for 7.5 minutes in order to familiarise them, and on the second day, pairs of rats were re-tested in the now familiar arena. Each test lasted 7.5 minutes. At the end of each test, boluses were removed, and the test box was wiped with detergent then dried. For full details of this method see File [17].

Colony intruder test. On the day after completion of the Social Interaction test, all animals (28 lesioned and 28 control) were placed singly into the aggression colony in a random order, and the frequency of aggressive encounters with the residents scored. This test lasted 5 minutes.

Behaviours scored included sniffing, grooming, wrestling, approaching, and attacking the resident (the total frequency of these behaviours was calculated to give an index of dominance), being sniffed, being groomed, submitting, squeaking, boxing (the total frequency of these behaviours was calculated to give an index of subordination).

After all behavioural testing was complete, six animals from each lesion group and each of the control groups were randomly chosen, were killed by cervical dislocation, and their brains rapidly removed and fixed in 10% Formalin in 0.9% saline. Serial lateral sections of fixed brain 30 μm thick were prepared and mounted, and the placements of injection sites were inspected.

The remaining animals were killed by decapitation and their brains were quickly removed and frozen. The entire septal areas were dissected out, and assayed for either 5-HT [16] or NA and DA [28]. The cingulate cortex was chosen as a control area to determine whether the neurotoxins had leaked into non-septal loci, and thus 5-HT or catecholamine content of the overlying cingulate cortex was also measured. Whole septal samples were used in order to provide sufficient tissue for assay. It is therefore not possible to determine the extent of amine depletion within the lateral septum, however, as an approximate guide, the lateral septum contains about 20% of total septal 5-HT and NA [11,27]; thus the data from rats having greater than 90% of control levels of 5-HT or NA were discarded. Septal depletions to 90% of control would represent a lateral septal depletion of approximately 50%.

RESULTS

Lesion Placements

Inspection of the histological sections revealed that vestigial traces of the injection needle tract were located, without exception, in the lateral septum (i.e., within the lateral boundaries described by the anterior horns of the lateral ventricles and the medial septum, and the rostro-caudal boundaries located at A 7.2 to A 7.8 (König and Klippel, [23]). It was assumed therefore that the microinjections into rats chosen for biochemical verification of the lesions were located likewise.

Analysis of Results

The data for the time spent in social interaction and the motor activity scores were analysed with three-way split-plot analyses of variance with lesion-state and light level as the independent factors and familiarity as the repeated measure. The water consumption data were analysed with

TABLE 1

MEAN (± S.E.M.) AMINE LEVELS (μg/g WET WEIGHT) IN THE SEPTUM AND CINGULATE CORTEX AFTER LATERAL SEPTAL INJECTIONS OF NEUROTOXIN OR VEHICLE

	5-HT	NA	DA
Septum			
Control	0.85 ± 0.14 (n=22)	0.83 ± 0.07 (n=22)	0.99 ± 0.11
Lesion	0.45 ± 0.007 (n=19)	0.61 ± 0.09 (n=22)	0.83 ± 0.10
Cingulate Cortex			
Control	0.17 ± 0.02 (n=22)	0.13 ± 0.08 (n=22)	0.26 ± 0.09
Lesion	0.18 ± 0.02 (n=19)	0.14 ± 0.08 (n=22)	0.26 ± 0.10

5-HT was measured after injection of 5,7-DHT and the catecholamines were measured after injection of 6-OHDA.

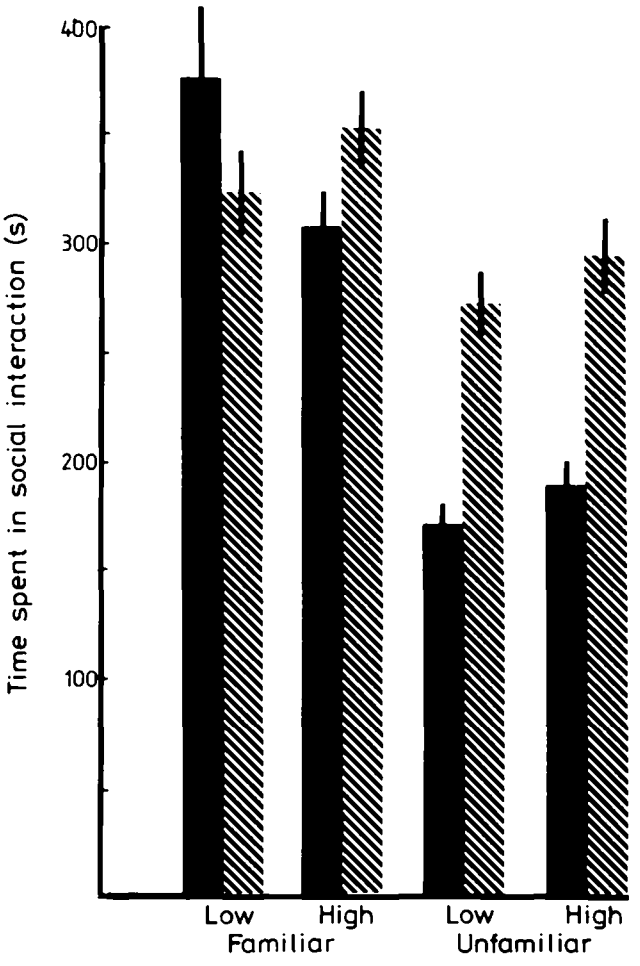

FIG. 1. The effects of 5,7-DHT-induced lesions of the lateral septum on the mean time spent in social interaction by pairs of male rats. The control responses are shown as solid bars, and the responses of lesioned rats as hatched bars. There were 7 pairs of rats in each test condition, except the lesion group low and high light rats where there were 6 and 5 respectively. 5,7-DHT caused an overall increase in the levels of social interaction, $F(1,21)=7.7$, $p<0.011$.

t-tests, and the aggression data were analysed with Mann-Whitney U-tests.

5,7-DHT lesions. In the lesion group, 1 rat from the low light and 2 rats from the high light groups failed to show reductions in septal 5-HT below the 90% of control level criterion, and hence the data from these animals was excluded from further analysis. The mean (±SEM) 5-HT levels in the septum and cingulate cortex are shown in Table 1.

The water consumption of lesioned animals did not differ significantly from that of control animals, $t(30)=0.79$. Overall, the time spent in active social interaction was increased in the lesion group, $F(1,21)=7.7$, $p<0.011$. Manipulation of the light level did not have a significant effect on social interaction. Overall, increasing familiarity resulted in significantly higher levels of social interaction, $F(1,21)=37.7$, $p<0.001$, but since in the lesion group the levels of social interaction were high regardless of the test condition, a significant lesion × familiarity interaction was obtained, $F(1,21)=13.2$, $p<0.02$ (see Fig. 1). The lesion did not affect locomotor activity, $F(1,21)=1.65$.

Although the total aggression score of lesioned rats was not significantly different from that of control rats, $U=46$, the lesion significantly reduced the frequency of dominance behaviours, $U=33$, $p<0.05$ (see Table 2).

6-OHDA lesions. All rats in the lesion groups showed reductions in septal catecholamines below the 90% of control levels criteria, thus it was not necessary to exclude any data. The mean (±SEM) levels of NA and DA in the septum and cingulate cortex are shown in Table 1.

The lesion did not affect water consumption, $t(30)=0.92$. Overall, the time spent in active social interaction was not altered by the lesion, $F(1,24)=0.37$. Increasing the light level or decreasing familiarity resulted in overall decreases in the levels of social interaction, $F(1,24)=16.3$, $p<0.001$ and $F(1,24)=144.45$, $p<0.001$, respectively, but since the manipulation of the test conditions produced equal changes in the levels of social interaction in both lesioned and control animals, the lesion × light and lesion × familiarity interactions did not reach significance, $F(1,24)=2.70$ and $F(1,24)=0.42$, respectively (see Fig. 2). Locomotor activity was unaffected by the lesion, $F(1,24)=2.76$.

In the aggression colony, the total frequency of behaviours was not altered by the lesion, $U=34.5$. However, the total frequency of dominance behaviours was increased, $U=18$, $p<0.02$, and the total frequency of subordination behaviours decreased, $U=22.5$, $p<0.05$. The latter effect was due to significant reductions in the number of submissions, $U=10$, $p<0.002$ and the number of squeaks, $U=23$, $p<0.05$ (see Table 3).

DISCUSSION

The results presented here demonstrate that depletion of lateral septal 5-HT results in a behavioural profile in the Social Interaction test similar to that seen after chronic administration of benzodiazepines. It is thought, therefore, that this reflects an anxiolytic action of this lesion. Although an

TABLE 2

EFFECTS OF 5,7-DHT LESIONS ON THE MEAN FREQUENCY OF BEHAVIOURS IN THE AGGRESSION COLONY

Behaviour	Control	Lesion
Sniff	1.00	0.30
Anogenital sniff	0.00	0.00
Approach	1.83	1.10*
Groom	0.08	0.08
Wrestle	1.50	1.30
Jump onto	0.00	0.00
Bite	1.67	1.10
Move between floors	0.92	0.90
Be sniffed	1.50	1.00
Be anogenitally sniffed	0.08	0.08
Be groomed	0.17	0.40
Submit	1.25	1.00
Squeak	3.92	2.60
Total Dominance	5.42	3.70*
Total Subordinate	7.92	5.50
Total Behaviours	13.33	9.20

*$p<0.05$, Mann-Whitney U-tests.

TABLE 3

EFFECTS OF 6-OHDA LESIONS ON THE MEAN FREQUENCY OF BEHAVIOURS IN THE AGGRESSION COLONY

Behaviour	Control	Lesion
Sniff	0.60	1.20
Anogenital sniff	0.00	0.10
Approach	0.70	1.50
Groom	0.00	0.30
Wrestle	2.80	3.50
Jump onto	0.00	0.70
Move between floors	1.20	0.90
Box	1.10	1.90
Be sniffed	2.60	3.10
Be anogenitally sniffed	0.40	0.10
Be groomed	3.00	2.30
Submit	3.20	0.60†
Squeak	7.10	3.30*
Total Dominance	6.30	9.20†
Total Subordinate	17.40	11.20*
Total Behaviours	23.70	20.30

*$p<0.05$; †$p<0.002$, Mann-Whitney U-tests.

effect of the lesion on social interaction per se cannot be excluded, it is thought unlikely that such an explanation is valid, since the changes in the levels of social interaction seen in the lesioned animals were not the same in all test conditions. On the other hand, depletion of septal NA and DA to an extent which was sufficient to affect aggression, did not alter social interaction significantly.

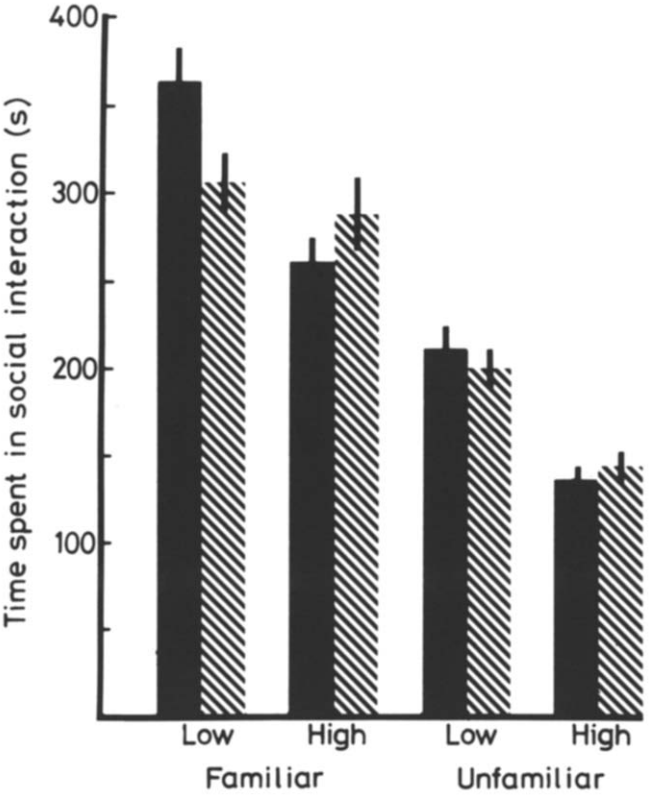

FIG. 2. The effects of 6-OHDA-induced lesions of the lateral septum on the mean time spent in social interaction by pairs of male rats. The control responses are shown as solid bars, and the responses of lesioned rats as hatched bars. There were 7 pairs of rats in each test group. There was no significant difference between the control and lesioned rats.

The results are in agreement with previous social interaction studies involving neurotoxin-induced lesions. Lesions in the locus coeruleus induced with 6-OHDA did not alter the behavioural profile of rats tested in the Social Interaction test, and as the septum is a projection site of the ascending monoamine pathway originating in the locus coeruleus, the present results could be largely predicted. Lesions of the dorsal raphe nucleus, induced with 5,7-DHT, result in an anxiolytic profile in the Social Interaction test, whereas gross lesions of both dorsal and median raphe nuclei result in sedation [18]. The septum receives its 5-HT innervation largely from the median raphe, but the lateral septum receives its 5-HT innervation from the dorsal raphe [5]. As such, the results of the present study are in agreement with those of the previous study.

A major problem of interpretation of behavioural data after neurotoxin-induced lesions is often encountered, since the degree of spread of neurotoxin to sites adjacent to the locus of injection is difficult to estimate. However, it is felt that in this study the spread of neurotoxin was extremely small for three reasons: (1) The monoamine assays of parietal cortex showed no evidence of leakage of toxins from the lateral septum to adjacent extra-septal loci, (2) the volume of microinjection was extremely small, and (3) the neurotoxins would be much more likely to diffuse into the

cleft where the injection needle had rested, and hence maintain a localised depot of neurotoxin, rather than diffuse through local tissue to reach more distal sites.

It was the primary objective of this study to investigate the effects of selective monoamine depletions in the lateral septum on the behavioural profile of rats in models of anxiety and aggression. Thus neurotoxins were used in preference to electrolytic lesioning procedures. Furthermore, the specificity of the two neurotoxins for neurones containing 5-HT or catecholamines is well documented: 5,7-DHT at the concentration injected does not affect catecholamine levels if the animals are pretreated with desmethylimipramine at the dose used in this study [6], and 6-OHDA at the concentration injected does not affect 5-HT levels [31].

Overall, therefore, the results of this study considered in concert with the results of the previous studies involving neurotoxin lesions and social interaction tests, suggest that the lateral septum may be involved in the control of anxiety. More specifically, septal 5-HT rather than catecholamines is an important transmitter involved.

Since the early reports of Brady and Nauta [9,10] that surgical lesions of the septum result in hyper-reactivity or "septal rage," much work has been performed in order to further investigate the role of the septum in emotional behaviour.

Septal rage following ablation of the septum is characterised by hyper-reactiveness to the experimenter, including resistance to capture, and by biting attacks [9]. Large septal ablations result in this syndrome, whereas medial septal ablations do not [13,26]. On the other hand, lateral septal ablations, particularly of the anterolateral region, do increase responsiveness to handling [7]; this effect can also be achieved by injection of local anaesthetic into this region [3]. It would seem, therefore, that the changes in aggression seen after whole septal ablation are probably mediated mainly via antero-lateral septal ablation.

In this study, depletion of lateral septal 5-HT resulted in a reduction in dominance, whereas the catecholamine depleted rats showed increased dominance and decreased subordination. These results would suggest that these two monoamine transmitters, in the lateral septum, are physiologically antagonistic in the control of aggression. Neither of the two groups of lesioned animals showed overt overreaction to handling compared with controls: although this contrasts with most studies, it has been reported that preoperative [22,25] or postoperative [1] handling of septally-ablated rats can reduce overreaction to handling. It is possible, therefore, that the failure to see gross changes in aggression and in ease of handling may have been because the rats used in this study were extensively handled both before and after surgery.

Since neither of the groups of lesioned animals showed alterations in water consumption compared with control animals, but did show altered aggression, the results do not support the suggestion [21] that increased aggression in animals with septal lesions is secondary to increased water intake.

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