J. Neural Transmission 50, 1—12 (1981)

Journal of
Neural Transmission

Depletion in Amygdaloid 5-Hydroxytryptamine Concentration and Changes in Social and Aggressive Behaviour

Sandra E. File, T. A. James, and **N. K. MacLeod**

Department of Pharmacology, The School of Pharmacy, University of London, London, U.K.

With 1 Figure

Received February 18, 1980

Summary

5, 7-Dihydroxytryptamine (10 and 20 μg) was microinjected bilaterally into the amygdaloid complex of rats and resulted in 55 % and 80 % depletion in 5-hydroxytryptamine concentration, respectively. The lesioned animals exhibited fewer dominance behaviours and submitted more often to an intruder into their home-cages than did the vehicle-injected controls. The lesioned rats were also more submissive than were the controls when they were intruding into another rat's territory. Only the higher dose of toxin altered social investigatory behaviour when this was measured in an arena in which neither rat had established territory. The lesioned rats displayed less social interaction and had reduced levels of motor activity. The results are compared with those of other studies in which there has been regional or general depletion of brain 5-hydroxytryptamine concentration.

Introductions

Several sources of evidence support the suggestion of *Stein et al.* (1973) that a reduction in serotonergic neurotransmission results in a reduction in anxiety. Parachlorophenylalanine, an inhibitor of 5-hydroxytryptamine (5-HT) synthesis, produces an anxiolytic profile in several animal tests (*Robichaud* and *Sledge*, 1969; *Geller* and *Blum*, 1970; *Cook* and *Sepinwall*, 1975; *File* and *Hyde*, 1977; *File* and

0300-9564/81/0050/0001/$ 02.40

on a video monitor in an adjacent room. The following behaviours were scored as active social interaction: sniffing, following, grooming, kicking, boxing or wrestling with, mounting and crawling under or over the partner. At the end of the trial, the rats were removed from the arena and the floor wiped clean.

The rats were tested in a randomized order, between 7.30 a.m. and 11.30 a.m.

The pairs of rats allocated to the LF test condition were given two further tests, on the following two days. On the first day half of the lesioned rats and half of the controls were tested 5 min after an i.p. injection of $ACTH_{1-24}$ (50 μg/kg); the others were tested after a saline injection. On the second day, those previously tested with ACTH now received saline, and vice versa.

Half of the lesioned and control rats from the LU, HF and HU test conditions were singly housed for a further 5 days after the social interaction test. An unoperated intruder rat was then introduced into the home-cage of each rat and the interactions that occurred between the two rats were scored for 5 min. A different intruder rat was used for each experimental rat.

The remaining lesioned and control rats were housed in groups of six for 10 days after the social interaction test. They were then introduced as intruders into the home-cages of singly-housed unoperated rats, and the interactions were scored for 5 min. The unoperated rats had been singly housed for 10 days before this test and a different resident rat was used for each experimental rat.

At the end of behavioural testing, the rats were stunned and decapitated and their brains rapidly dissected out and frozen. 5-HT concentrations in the amygdala were estimated by the method of *Curzon* and *Green* (1970). Since 5, 7-DHT is also taken up into noradrenaline-containing neurones, noradrenaline concentrations were also estimated, by the method of *Jacobs et al.* (1977).

Results

Biochemical Verification of Lesions

The mean ($\pm$ S.E.M.) 5-HT concentration in the amygdala of the vehicle-injected control rats was 2.36 ± 0.06 μg/g. The data from any lesioned rat not showing 50 % depletion were excluded. Table 1 shows the number of pairs of rats for which scores were included in the social interaction test. These lesioned rats had a mean ($\pm$ S.E.M.) 5-HT concentration of 1.07 ± 0.06 μg/g, a 55 % depletion. The neurotoxin injections also resulted in a small depletion (20 %) in noradrenaline concentration in the amygdala. The mean ($\pm$ S.E.M.) concentration in control rats was 1.53 ± 0.08 μg/g and in lesioned rats 1.23 ± 0.11 μg/g.

Social Interaction

Table 1. *Active social interaction scores (in secs) (mean ± S.E.M.) for pairs of lesioned and control rats tested for 10 min in four different test conditions. Low and high refer to the light levels in the test arena and familiar/unfamiliar to the rat's familiarity with the test arena*

	Low		High	
	Familiar	Unfamiliar	Familiar	Unfamiliar
Lesioned rats				
Active social interaction	245 ± 34.5	210 ± 23.5	133 ± 24.2	91 ± 19.1
Number of pairs tested	6	4	6	5
Vehicle-injected controls				
Active social interaction	224 ± 34.2	215 ± 28.8	122 ± 29.5	83 ± 19.0
Number of pairs tested	6	5	6	4

Table 1 shows the mean time spent in active social interaction by the pairs of lesioned and vehicle-injected control rats tested in the 4 test conditions. It can be seen from this table that the lesion did not alter the amount of social interaction in any of the test conditions. The level of motor activity was also unaffected by the lesion, with the control animals having a mean (± S.E.M.) score of 458.1 ± 34.2 and the lesioned rats a score of 490.9 ± 34.6.

The control rats that were given two further tests in the low light, familiar condition had a mean score of 331 sec after a saline injection and this was significantly reduced to a mean of 226 sec after 50 μg/kg of ACTH ($t(5) = 2.97$, $p < 0.025$). The lesoned rats showed a similar reduction in their social interaction scores when given ACTH, from a mean of 294 to 208 sec ($t(5) = 2.7$, $p < 0.025$).

Home-cage Aggression

Table 2 a shows the mean incidence of each type of interaction that took place between lesioned rats and unoperated intruders placed in the home-cages; and the interactions between vehicle-injected control rats and unoperated intruder rats. The lesioned and control rats did not differ in the total number of interactions that took place with the intruder. The intruders sniffed the lesioned rats significantly more (6.7 times more, see Table 2) than they sniffed the control rats. The

Table 2. *The mean incidence of various social behaviours in the home-cage intruder test*

A. *Unoperated intruders introduced into home-cage of 5,7-DHT lesioned and vehicle-injected control rats*

	I submits	Submits	Box	Wrestle	Kick, jump, stand on	Groom intruder	Self-groom	I self-groom	Sniff intruder	I sniff resident	Total
Lesioned n = 10	4.4	2.6*	3.1	4.4	4.5*	1.7*	0.8	1.2	5.2	2.0**	29.9
Controls n = 11	5.0	1.4	2.3	4.5	9.0	2.9	1.2	1.2	3.5	0.3	31.3

Lesioned and control scores were compared with Mann-Whitney U-tests; those that differed significantly are asterisked.
* $p < 0.05$, ** $p < 0.025$.

B. *5,7-DHT lesioned and vehicle-injected rats introduced as intruders into home-cages of unoperated resident rats*

	I submits	Submits	Box	Wrestle	Kick, jump, stand on	Groom intruder	Self-groom	I self-groom	Sniff intruder	I sniff resident	Total
Lesioned n = 10	7.1*	2.7	5.5*	10.3	11.0	1.6	1.4	1.6	4.3	0.6	40.1
Controls n = 10	4.7	1.9	1.7	8.6	9.6	1.3	1.3	0.9	3.9	1.9	35.8

Lesioned and control scores compared with Mann-Whitney U-tests. * $p < 0.05$.
In all cases the scores are those for the resident rats, unless preceded by the prefix I, in which case they are for the intruder rat.

lesioned rats in turn groomed, jumped, kicked and stood on the intruder less (half as much, see Table 2) than did the controls and submitted more often to the intruder than did the control rats. The lesioned rats, therefore, showed a reduction of dominance behaviours when faced with a home-cage intruder.

Table 2 b shows the mean incidence of each type of interaction that took place when lesioned and vehicle-injected control rats were introduced as intruders into the home-cages of unoperated resident rats. The lesioned rats showed significantly more defensive behaviours —boxing and submitting.

Experiment 2

Methods

Animals and Surgery

These were similar to those for Experiment 1 but a higher dose of toxin was injected, 20 μg bilaterally in a volume of 5 μl. This high dose was chosen to ensure a major degree of 5-HT depletion, since the lower dose had only produced a 55 % depletion of amygdaloid 5-HT concentration. Control rats received equal-volume injections of vehicle.

In order to assess the extent of any damage resulting from the neurotoxin injections, rats were injected either with 5, 7-DHT (20 μg bilaterally) or with vehicle. Two weeks after injection, the animals were anaesthetized and perfused with formol saline. The brains were removed and serial sections taken of the amygdaloid nuclei were stained with cresyl violer (1 %).

Apparatus

As for Experiment 1.

Procedure

Within each group (lesioned rats or vehicle-injected controls), rats were randomly allocated to two light levels: low and high. They were allocated to test partners on the basis of weight.

On the first test day all rats were unfamiliar with the test arena. Each pair was observed and scored for 10 min, as in Experiment 1. The next day each rat was placed singly in the test arena for a 10-min familiarization period. The following day every pair of rats was given a second social interaction test.

At the end of behavioural testing, the rats were killed and their brains dissected out and assayed, as in Experiment 1.

Results

Biochemical Verification of Lesions

The 5-HT concentration (mean ± S.E.M.) in the amygdala of the lesioned rats was 0.53 ± 0.06 μg/g, representing an 80 % depletion. The noradrenaline concentration was depleted by 29 %. Five pairs of lesioned rats were tested in the low light condition, and 6 pairs in the high light. Six pairs of vehicle-injected controls were tested in each light condition.

Histological Evidence

Careful examination of the amygdala from control and lesioned rats showed little non-specific damage deriving from the toxin injection.

Social Interaction

Fig. 1 shows mean social interaction scores for lesioned and control rats, tested under low or high light. Lesioned rats showed significantly less social interaction than controls ($F\ [1, 19] = 4.94$, $p < 0.05$). All animals manifested significantly more interaction when they were familiar with the test arena ($F\ [1, 19] = 24.5$, $p < 0.001$) and under low rather than high light ($F\ [1, 19] = 11.5$, $p < 0.005$). The lesion × light and lesion × drug interactions were not significant, *i.e.* lesioned rats were as responsive as controls to changes in test conditions.

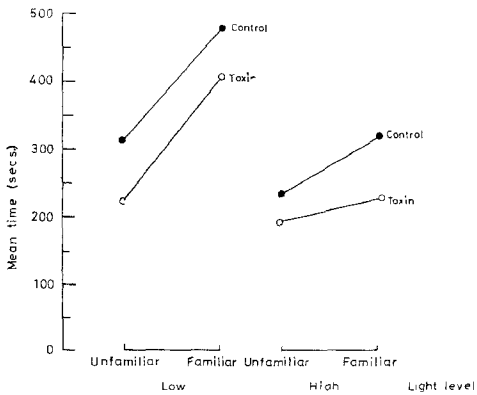

Fig. 1. Mean time (secs) spent in active social interaction by pairs of rats tested under low and high light level. Each pair of rats was tested when it was unfamiliar with the test arena and then retested after a further 10-min familiarization period. 5, 7-DHT lesioned rats (○——○), vehicle-injected controls (●——●)

As well as having reduced social interaction scores, the lesioned rats also showed significantly reduced motor activity (mean ± S.E.M.) compared with controls (459.5 ± 44.9 *vs.* 586.5 ± 26.7, t [22] = 2.44, $p < 0.05$).

Discussion

Compared with controls, rats that received the high dose of toxin showed significantly less social interaction and had reduced motor activity in the social interaction test. This finding is similar to the pattern of results previously observed after acute administration of benzodiazepines (*File*, 1978) or after joint lesions of the dorsal and median raphé nuclei resulting in 90 % depletion of forebrain 5-HT (*File* and *Deakin*, 1980). However, the data contrast with the "anxiolytic" profile seen after treatment with parachlorophenylalanine (*File* and *Hyde*, 1977) and after chronic administration of benzodiazepines (*File* and *Hyde*, 1978), where there is no hypoactivity and social interaction scores remain high throughout all the test conditions. We think it unlikely that depletion in amygdaloid noradrenaline concentration is responsible for the behavioural changes found in the present study because the change was only small and there was no difference between low and high toxin-treated animals, whereas the behaviour of these two groups was different. Also, there was no evidence, from light microscopic examination of the lesion site, of non-specific damage. However, any relationship between 5-HT and anxiety reduction is clearly a complex one and depends on extent and regional distribution of depletion, as well as on possible changes in receptor sensitivity.

ACTH reduced social interaction in both lesioned and control animals. Thus, either there were sufficient 5-HT receptors still available in the lesioned animals to mediate the behavioural effects of ACTH, and/or the amygdala is only one of several sites of action of ACTH.

Although the animals that received the low dose of toxin did not differ from controls in the social interaction test, where the behaviour is mainly investigatory, they did show differences in the home-cage intruder tests where most of the behaviours exhibited are aggressive. When the lesioned rats were on their home territory, they exhibited fewer dominance behaviours to the intruder and indeed submitted to him more often than did the controls. The lesioned rats were also more submissive than were the controls when they were intruders into another rat's home-cage. This loss of dominance is similar to that reported for rhesus monkeys following amygdalectomy (*Rosvold*

et al., 1954) and suggests that a reduction in amygdaloid 5-HT may underlie such a change in aggressive behaviour. The roles of specific amygdaloid nuclei were not studied in this experiment since it was not possible to localize the toxin injections to this extent. The investigation of whether 5-HT depletion in particular nuclei will produce distinctive changes in aggression behaviour will have to await the refinement of existing techniques. But within the raphé nuclei, there is evidence that the effects of 5-HT depletion on aggressive behaviour depend on the particular regions depleted. Rats with 5, 7-DHT lesions of the median raphé nucleus showed increases in dominance behaviours, those with dorsal raphé lesions showed a decrease in both dominance and submissive behaviours when faced with an intruder (*File et al.*, 1979) and those with joint lesions of both raphé nuclei showed no significant changes in home-cage aggression (*File* and *Deakin*, 1980).

Acknowledgements

This work was supported by a Medical Research Council grant to S.E.F. The video equipment was purchased with a grant from the Central Research Fund of the University of London. We are grateful to Lynn Rooney for technical assistance.

References

Azmitia, E. C., Segal, M.: An autoradiographic analysis of the differential ascending projections of the dorsal and median raphé nuclei in the rat. J. comp. Neurol. *179*, 641—668 (1978).

Bosmann, H. B., Case, K. R., DiStefano, P.: Diazepam receptor characterization: Specific binding of a benzodiazepine to macromolecules in various areas of rat brain. FEBS Letters *82*, 368—372 (1977).

Cook, L., Sepinwall, J.: Behavioral analysis of the effects and mechanisms of action of benzodiazepines. In: Mechanism of Action of Benzodiazepines, pp. 1—28. New York: Raven Press. 1975.

Curzon, G., Green, A. R.: Rapid method for determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. Br. J. Pharmac. *30*, 653—655 (1970).

Endroczi, E., Lissak, K., Bohus, B., Kovacs, S.: The inhibitory influence of archicortical structures on pituitary-adrenal function. Acta Physiol. Acad. Sci. Hung. *16*, 17—22 (1959).

Fernandez de Molina, A., Hunsperger, R. W.: Central representation of affective reactions in forebrain and brain stem: Electrical stimulation of amygdala, stria terminalis, and adjacent structures. J. Physiol. *145*, 251—265 (1959).

Fernandez de Molina, A., Hunsperger, R. W.: Organization of the subcortical system governing defence and flight reactions in the cat. J. Physiol. *160*, 200—213 (1962).

File, S. E.: Anxiety, ACTH and 5-HT. Trends in Neuroscience *1*, 9—11 (1978).

File, S. E., Deakin, J. F. W.: Chemical lesions of both dorsal and median raphé nuclei and changes in social and aggressive behaviour in rats. Pharmac. Biochem. Behav. *12*, 855—859 (1980).

File, S. E., Hyde, J. R. G.: The effects of p-chlorophenylalanine and ethanolamine-O-sulphate in an animal test of anxiety. J. Pharm. Pharmac. *29*, 735—738 (1977).

File, S. E., Hyde, J. R. G.: Can social interaction be used to measure anxiety? Br. J. Pharmac. *62*, 19—24 (1978).

File, S. E., Hyde, J. R. G., Mac Leod, N. K.: 5, 7-Dihydroxytryptamine lesions of dorsal and median raphé nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. J. Affect Dis. *1*, 115—122 (1979).

File, S. E., Rodgers, R. J.: Partial anxiolytic action of morphine sulphate following microinjection into the central nucleus of the amygdala in rats. Pharmac. Biochem. Behav. *11*, 313—318 (1979).

File, S. E., Vellucci, S. V.: Studies on the role of ACTH and of 5-HT in anxiety, using an animal model. J. Pharm. Pharmac. *30*, 105—110 (1978).

Gallagher, M., Kapp, B. S.: Manipulation of opiate activity in the amygdala alters memory processes. Life Sci. *23*, 1973—1978 (1978).

Gastaut, H., Vigouroux, R., Corriol, J., Badier, M.: Effets de la stimulation électrique (par électrodes à demeure) du complexe amygdalien chez le chat non-narcosé. J. Physiol. *43*, 740—746 (1951).

Geller, I., Blum, K.: The effects of 5-HT on p-chlorophenylalanine (pCPA) attenuation of "conflict" behaviour. Eur. J. Pharmac. *9*, 319—324 (1970).

Haefely, W. E.: Behavioral and neuropharmacological aspects of drugs used in anxiety and related studies. In: Psychopharmacology: A Generation of Progress (*Lipton, M. A., DiMascio, A., Killam, K. F.*, eds.), pp. 1359 to 1374. New York: Raven Press. 1978.

Jacobs, B. L., Simon, S. M., Ruimy, D. D., Trulson, M. E.: A quantitative rotational model for studying serotonergic function in the rat. Brain Res. *124*, 271—281 (1977).

James, T. A., Mac Leod, N. K., Mayer, M. L.: A functional antagonism between benzodiazepines and ACTH? Br. J. Pharmac. *66*, 115—116P (1979).

Kapp, B. S., Frysinger, R. C., Gallagher, M., Haselton, J. R.: Amygdala central nucleus lesions: effect on heart rate conditioning in the rabbit. Physiol. Behav. *23*, 1109—1117 (1979).

Knigge, K. H.: Adrenocortical response to stress in rats with lesions in hippocampus and amygdala. Proc. Soc. Exp. Biol. Med. *108*, 18—21 (1961).

König, J. F. R., Klippel, R. A.: The Rat Brain. Baltimore: Williams and Wilkins. 1963.

Lidbrink, P., Corrodi, H., Fuxe, K., Olson, L.: The effects of benzodiazepines, meprobamate, and barbiturates on central monoamine neurons. In: The Benzodiazepines (*Garattini, S., Mussini, E., Randall, L. O.*, eds.), pp. 203—223. New York: Raven Press. 1973.

Mohler, H., Okada, T.: The benzodiazepine receptor in normal and pathological human brain. Br. J. Psychiat. *133*, 261—268 (1978).

Nagy, J., Zambo, K., Decsi, L.: Anti-anxiety action of diazepam after intra-amygdaloid application in the rat. Neuropharmacology *18*, 573 to 576 (1979).

Pratt, J., Jenner, P., Reynolds, E. H., Marsden, C. D.: Clonazepam induces decreased serotoninergic activity in the mouse brain. Neuropharmacology *18*, 791—799 (1979).

Robichaud, R. C., Sledge, K. L.: The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. Life Sci. *8*, 965—969 (1969).

Rosvold, H. E., Mirsky, A. F., Pribram, K. H.: Influence of amygdalectomy on social behaviour in monkeys. J. comp. physiol. Psychol. *47*, 173—178 (1954).

Stein, L., Wise, C. D., Berger, B. D.: Antianxiety action of benzodiazepines: Decrease in activity of serotonin neurons in the punishment system. In: The Benzodiazepines (*Garattini, S., Mussini, E., Randall, L. O.*, eds.), pp. 299—326. New York: Raven Press. 1973.

Tye, N. C., Everitt, B. J., Iversen, S. D.: 5-Hydroxytryptamine and punishment. Nature *268*, 741—743 (1977).

Authors' address: Dr. *Sandra E. File*, Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, U.K.