Pharmacology Biochemistry & Behavior, Vol. 12, pp. 855–859. Printed in the U.S.A.

Chemical Lesions of Both Dorsal and Median Raphe Nuclei and Changes in Social and Aggressive Behaviour in Rats

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Received 14 February 1980

FILE, S. E. and J. F. W. DEAKIN. *Chemical lesions of both dorsal and median raphe nuclei and changes in social and aggressive behaviour in rats.* PHARMAC. BIOCHEM. BEHAV. 12(6) 855–859, 1980.—Microinjections of 5,7-dihydroxytryptamine into both the dorsal and median raphe nuclei resulted in 90% depletions of striatal and hippocampal 5-HT concentrations. Compared with vehicle-injected controls the lesioned rats showed reduced active social interaction scores in all four of the test conditions and also reduced levels of locomotor activity. The lesioned rats did not differ from the controls in their latency to start drinking in a novel environment; or in their response to intruder rats placed in their home cages, or in their behaviour as intruders when they were placed in the home-cages of unoperated rats. The difficulties of interpreting the behavioural effects of a lesion when the lesion produces hypoactivity, and the differences between the effects of these joint lesions of both dorsal and median raphe nuclei and the effects of separate lesions of each nucleus are discussed.

Raphe nuclei Social interaction Aggression Locomotor activity

A REDUCTION in serotonergic neurotransmission has been suggested as a mechanism underlying anxiety reduction [2, 6, 11, 15, 18], and on the basis of biochemical findings it was suggested that 5-HT pathways in the midbrain might be of particular importance [10]. Further support for this suggestion came from the finding that rats with lesions of the 5-HT (5-hydroxytryptamine) pathways ascending from the dorsal raphe nucleus had a behavioural profile in the social interaction test very similar to that seen after chronic treatment with benzodiazepines [9]. The lesions were produced by microinjections of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), and they resulted in about 50% depletions of 5-HT in the caudate and hippocampus. The extent of the hippocampal depletion suggested that there might have been transport of toxin from the dorsal to the median raphe nucleus and that the anxiolytic profile was therefore produced in rats with partial lesions of both nuclei. 5,7-DHT lesions of the median raphe nucleus alone were without significant effect in the social interaction test of anxiety [9].

We now report the effects of more complete depletions of striatal and hippocampal 5-HT concentrations obtained by 5,7-DHT injections into both dorsal and median raphe nuclei in two tests of anxiety, the social interaction test [8] and the anxiosoif test [17].

Depletion of 5-HT following parachlorophenylalanine (PCPA) or 5,7-DHT administration increases muricidal behaviour in rats [1, 5, 12, 14, 19], and PCPA increases shock-elicited fighting in rats [16] and aggressiveness in mice [13]. Changes in aggression might be dependent on the extent of 5-HT depletion, and results from one of our previous studies [9] suggest that lesions of the median raphe nucleus might lead to different results from lesions of the dorsal raphe nucleus. Rats with 5,7-DHT lesions of the median raphe nucleus showed an increased frequency of dominance behaviours compared with vehicle-injected controls when an intruder rat was placed in their home cages. In contrast, rats with 5,7-DHT lesions aimed at the dorsal raphe nucleus showed a reduction in all forms of interaction with an intruder into their home-cage. In view of these changes, in the present experiment we therefore also examined the effects of joint lesions of both dorsal and median raphe nuclei in home-cage aggression tests, both when the lesioned rat was the resident and when it was the intruder rat.

METHOD

Animals and Surgery

Male hooded rats (200–250 g) were anaesthetised with

sodium pentobarbitone (60 mg/kg) and injected with 5,7-dihydroxytryptamine (9 μg in 3 μl of 2 mg/ml ascorbic acid in saline) into both the dorsal and the median raphe nuclei at the rate of 1 μl/min. Control rats received equal volume injections of vehicle (0.2% ascorbic acid in saline). The microinjections were made using a Kopf stereotaxic frame with the incisor bar 1 mm above the interaural line. The coordinates, referring to an interaural zero, were AP+0.5, D+4.0 (for dorsal) +2.3 (median), in the mid-line. One week was allowed for post-operative recovery. The rats were then housed singly for 5 days before the social interaction test. Food and water were available ad lib. The rats weighed approximately 300 g at the time of behavioural testing, and there were no significant differences in weight between the lesioned and control animals.

Apparatus

Social interaction test. The test arena was a wooden box (65×65×47 cm), with infrared photocells in the walls of the box. These provided an automated measure of motor activity. The low and high light levels were 13 and 333 scotopic lux, respectively. A camera was mounted vertically above the test arena, and the rats were observed on a video monitor in an adjacent room.

Home-cage intruder test. The home cages were 21×34×17 cm and were made of white plastic. They had wire grid bases and flat wire lids.

Anxiosoif test. The rats were tested in a clear perspex box, 29×29×30 cm, with a small opening in one wall giving access to a water spout.

Procedure

Social interaction test. The lesioned rats were randomly allocated among the four test conditions: low light, familiar (LF); high light, familiar (HF); low light, unfamiliar (LU); high light, unfamiliar (HU). The control rats were likewise randomly allocated 6 pairs to each of the four test conditions.

The rats allocated to the "familiar" test condition were placed singly in the test arena, under the appropriate light level, for a 10-min trial on the 2 days preceding the social interaction test. Those allocated to the "unfamiliar" test conditions were placed in the test room, under the appropriate light level, but remained in their home cages.

The rats were allocated to test partners on the basis of weight, so that the members of a pair did not differ by more than 10 g from each other. Each pair of rats was placed in the centre of the test arena and observed for 10 min on a video-screen 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 randomised order, between 0730 and 1130 hrs.

The lesioned and control rats allocated to the LF test condition were given two further social interaction tests. In one, they were tested 5 min after an IP injection of saline, and in the other, 5 min after IP injection of $ACTH_{1-24}$ (5 μg/100 g); half of the rats received the saline injection first. The effects of ACTH were assessed in the low light, familiar condition since it is the test condition in which we have previously found ACTH injections to have the greatest effect [11].

After behavioural testing, the 5-HT concentrations in the hippocampus and in the caudate were determined for each rat, and the data from animals with poor depletions were excluded from the analysis. This left 6 pairs of rats in each test condition for the control rats, 6 pairs of lesioned rats in LF, 2 pairs in HF, 5 in LU and 6 in HU.

Home-Cage Intruder Test

A group of lesioned rats (n=14) and control rats (n=14) were housed singly for 5 days (after a one-week period of post-operative recovery). Another 12 lesioned and 12 control rats were housed in large cages with 5 per cage. These rats had not been used in any previous behavioural tests.

A large, unoperated intruder rat (mean weight 500 g) was introduced into the home-cage of each singly housed rat (a different intruder was used for each rat) and the interactions that occurred between the resident rat and the intruder were scored for 5 min. Each of the group-housed lesioned or control rats was introduced into the home-cage of a singly-housed, unoperated rat. A different resident rat was used for each intruder (the unoperated rats had been housed singly for 5 days before this test). The interactions that occurred between the resident and intruder rats were scored for 5 min.

Anxiosoif Test

Twelve lesioned and 12 control rats were selected randomly from the rats that had been singly-housed for the home-cage intruder test. They were water-deprived for 24 hrs. Each rat was then placed singly in the test box and the time taken to first sniff the spout and the time taken to start drinking were measured. Defecation was also scored and any boluses were removed and the floor wiped clean before the next rat was tested.

A group of 80 unoperated rats were randomly allocated, ten each to the following drug-treatment groups: Flurazepam (0.5 mg/kg), flurazepam (2 mg/kg), flurazepam (0.5 mg/kg for 4 days), and water, all of these 4 groups received an injection 30 min before testing; Parachlorophenylalanine (PCPA 300 mg/kg), saline controls, these two groups were injected 72 hours before the test; ACTH (50 μg/kg), saline, these two groups were injected 3 min before the test.

Biochemistry

The day after behavioural testing ended the rats were stunned, decapitated and their brains rapidly removed; striata and hippocampi were rapidly dissected out and stored at −70°C until assayed for 5-HT according to the method of Curzon and Green [4]. Since it is not possible to obtain histological verification of neurotoxin lesions, our lesions were verified by assaying 5-HT in the main projection sites of the DRN and MRN.

RESULTS

Social Interaction Test

Both the vehicle-injected control rats and the lesioned rats showed a decrease in the time spent in active social interaction when the light level was high, $F(1,35)=6.0$ $p<0.02$, and when the test arena was unfamiliar, $F(1,35)=13.2$, $p<0.001$, as is shown in Fig. 1. The lesioned rats showed a significantly lower level of social interaction than did the controls, $F(1,35)=9.5$, $p<0.01$, but there was no significant lesion × light or lesion × familiarity interaction, $F(1,35)=<1.0$ in both cases. As well as showing reduced

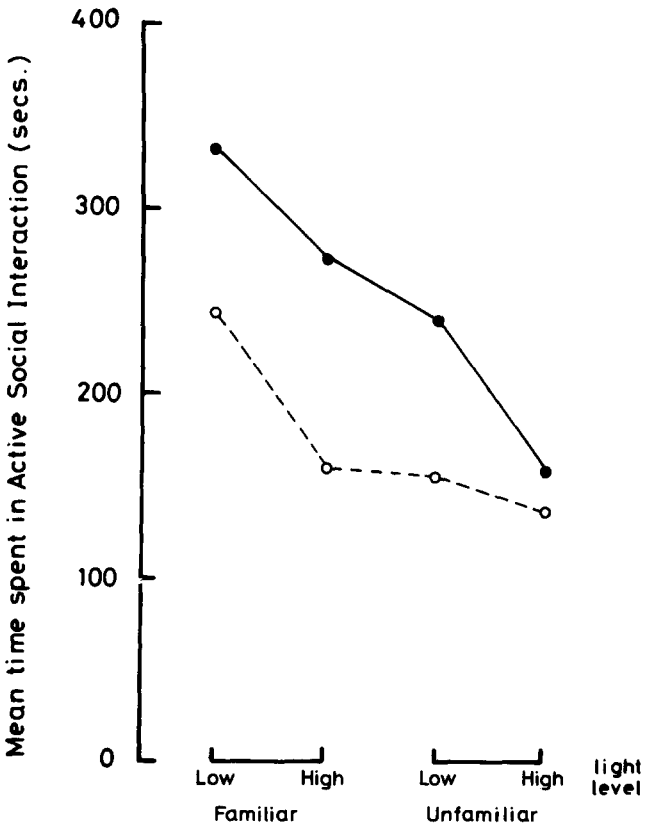

FIG. 1. Mean time spent in active social interaction by pairs of rats tested in low or high light, in a familiar or unfamiliar test arena, O---O 5,7-DHT injected rats, ●——● vehicle-injected controls.

active social interaction the lesioned rats also had lower levels of motor activity, $F(1,35)=19.5$, $p<0.001$.

The mean active social interaction score of the lesioned rats retested after saline injections was 285 secs, and the mean score when they were retested after ACTH injections was 183 secs, (paired t-test; $t(5)=4.4$, $p<0.01$. The control rats also showed a significantly lower level of social interaction after injections of ACTH than after saline injections, $t(5)=3.6$, $p<0.02$.

Home-Cage Aggression Test

When unoperated intruder rats were placed in the home-cages of the experimental rats the lesioned rats engaged in a mean of 19.8 interactions with the intruder, and the control rats in a mean of 18.4 interactions. Thus the two groups did not differ in the total number of interactions with the intruder, and, as can be seen from Table 1, none of the individual behaviours differed in incidence between the 2 groups.

When the experimental rats were placed in the home-cage of an unoperated rat, there was again no difference between the lesioned and control rats in the number of interactions that took place between them and the resident rats, or in the incidence of individual behaviours.

Anxiosoif Test

Acute flurazepam (0.5 and 2.0 mg/kg) was without effect on any measure in this test, but chronic flurazepam and

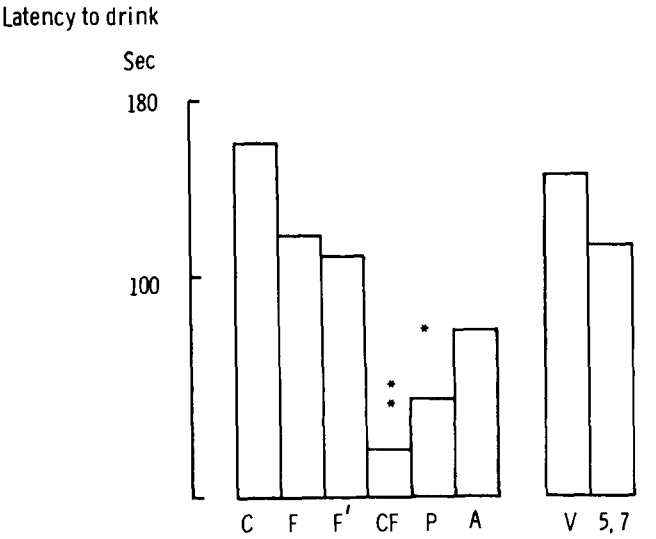

FIG. 2. Mean latency to start drinking in an unfamiliar environment. C=controls F=acute flurazepam (0.5 mg/kg); F'=acute flurazepam (2.0 mg/kg); CF=chronic flurazepam (0.5 mg/kg for 4 days); P=parachlorophenylalanine (300 mg/kg); V=vehicle microinjections into raphe nuclei; 5,7=5,7-DHT microinjections into raphe nuclei; A=ACTH (50 μg/kg). *$p<0.01$ **$p<0.005$ Mann-Whitney U-tests.

PCPA pretreatment both significantly reduced the latency to drink (see Fig. 2). The mean latency to drink of the ACTH-injected rats did not differ significantly from the mean of their saline-injected controls, but ACTH produced a bimodal distribution of scores with some extremely short latencies and some long latencies.

There was no significant difference on any measure between the lesioned and control rats in this test.

Biochemical Confirmation of Lesions

The 5-HT concentrations in the hippocampus and striatum of the vehicle-injected control animals were 0.41 ± 0.2 μg/g and 0.32 ± 0.02 μg/g, respectively. In the lesioned animals the concentrations were 0.04±0.005 μg/g and 0.035±0.004 μg/g, respectively, representing about 90% depletion. The values were not corrected for recovery, which was 70%.

DISCUSSION

In the social interaction test of anxiety the decrement in social interaction between pairs of rats that occurs with increasing light intensity and unfamiliarity is abolished by chronic treatment with benzodiazepines [8], by PCPA [7] and by partial depletion of hippocampal and striatal 5-HT concentrations produced by microinjections of 5,7-DHT into the dorsal raphe nucleus (DRN) [9]. These findings support the view that 5-HT neurones may be involved in the mediation of anxiety (see Introduction). It was therefore predicted that the 90% depletions produced by combined DRN and MRN 5,7-DHT microinjections would result in an anxiolytic behavioural profile in the social interaction model of anxiety. This was not found and, indeed, the 5,7-DHT lesioned animals showed significantly less social interaction in all the test conditions.

TABLE 1

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 sub-mits	sub-mits	box	wrestle	Kick Jump Stand on Intruder	groom intruder	self-groom	I self groom	sniff intruder	I sniff resident	Mean No. of interactions
Lesioned	1.0	0.3	3.9	1.8	3.3	1.2	0.1	0.3	6.7	1.0	18.4
Controls	1.5	0.3	2.8	2.5	3.5	1.8	0.2	0.6	5.9	1.9	19.8

B) 5,7-DHT and vehicle-injected control rats introduced into home-cage of unoperated resident rats

	I sub-mits	sub-mits	box	wrestle	Kick Jump Stand on Mount Intruder	groom intruder	self-groom	I self groom	sniff intruder	I sniff resident	Mean No. of interactions
Lesioned	0.8	0	2.2	0.7	4.8	1.1	0.4	0.6	4.8	2.8	17.8
Controls	0.4	0.3	2.8	0.9	3.8	0.5	0.6	0.8	4.4	3.2	18.8

The scores are for the resident rats, unless preceded by the prefix I, in which case they refer to the intruder rat.

In the anxiosoif test the fear-inducing effects of an unfamiliar environment were quantified by measuring the latency of water-deprived animals to drink from a water spout in the novel environment. To validate the test the effects to flurazepam were investigated and, as with the social interaction test, chronic but not acute administration was effective in that drinking latencies were shortened. Furthermore, PCPA administration reproduced the benzodiazepine effect as it does in the conflict and social interaction tests [6,11]. However, severe depletions of forebrain 5-HT following 5,7-DHT lesions were without effect in the anxiosoif test.

The reduced social interaction scores shown by the lesioned animals were accompanied by a significant reduction in motor activity. Hypoactivity was also found in a group of rats with similar lesions when they were tested singly in the open-field and in the holeboard [4]. This hypoactivity may have masked the expected anxiolytic effect; and, indeed, there was little change in the social interaction scores of the lesioned animals in the high light familiar and the two unfamiliar test conditions. A similar pattern of results has been found with acute administration of benzodiazepines, where the sedative effects mask the anxiolytic profile [6]. Any anxiolytic effect in the anxiosoif test may also have been masked by hypoactivity as this would have had the effect of increasing the latency to start drinking. The hypoactivity is unlikely to be the result of non-specific effects of 5,7-DHT injections, i.e. a reduction in catecholamine levels. Control animals that received injections of the catecholamine neurotoxin, 6-hydroxydopamine, into the MRN and DRN displayed no hypoactivity [4].

In a previous report ACTH was found to increase 5-HT turnover [10] and the ACTH effect on social interaction was abolished by partial depletions in forebrain 5-HT produced by 5,7-DHT microinjections into the DRN [9]. This suggested that ACTH exerted its putative anxiogenic effect by increasing activity in 5-HT neurones. The present study casts doubt on this interpretation since more complete 5-HT depletions produced by combined DRN and MRN 5,7-DHT microinjections failed to block the ability of ACTH to diminish social interaction. Indeed the ACTH effect on social interaction may be independent of anxiety processes in view of its lack of effect on the anxiosoif test.

What is clear from this study is that the behavioural consequences of joint lesions of the DRN and of the MRN cannot be predicted from the results seen after 5,7-DHT injections separately into each nucleus. The rats with joint lesions were hypoactive, whereas those with lesions of the dorsal raphe alone did not show reduced exploration or motor activity and those with median raphe lesions were hyperactive at some stages of the holeboard test [4]. A further difference between lesions of both nuclei and lesions of each nucleus separately can be seen in the home-cage aggression test. Rats with 5,7-DHT injections into the median raphe nucleus showed more dominance behaviours to an intruder placed into their home-cage, than did controls, whereas rats with 5,7-DHT injections into the dorsal raphe showed fewer interactions of all kinds with the intruder [9]. In the present experiment the rats with lesions of both raphe nuclei did not differ in any way from the controls in their behaviour towards an intruder, or in their behaviour as an intruder.

The pattern of results seen in our lesioned rats highlights the difficulty of interpreting the behavioural effects of a lesion when this changes the baseline levels of responding. If only one behaviour is studied it might erroneously be concluded that the lesion had a specific effect of reducing that behaviour. We were able to see reductions in more than one behaviour, but it was not possible to determine whether the hypoactivity was also masking other significant behavioural changes. It has been common practice to lesion both DRN and MRN nuclei in order to destroy all the ascending serotonergic projections. The results of the present experiments illustrate the limitations of such a strategy: the lesion may reduce spontaneous behaviours to an extent sufficient to mask other changes, and the behavioural changes resulting from joint lesions might be quite different from those seen after separate lesions of each individual nucleus.

ACKNOWLEDGEMENTS

We are grateful to Roche Products Ltd for the gift of Flurazepam. This work was supported by a Medical Research Council project grant held by S.E.F. The video recording apparatus was purchased with a grant from the Central Research Fund, University of London.

REFERENCES

1. Breese, G. R. and B. R. Cooper. Behavioural and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intra-cisternally to adult and developing rats. *Brain Res.* **98:** 517–527, 1975.
2. Cook, L. and J. Sepinwall. Behavioural analysis of the effects and mechanisms of action of Benzodiazepines. In: *Mechanism of Action of Benzodiazepines,* edited by E. Costa and P. Greengard, New York: Raven Press, 1975, pp. 1–28.
3. Curzon, G. and A. R. Green. Rapid method for determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac.* **30:** 653–655, 1970.
4. Deakin, J. F. W., S. E. File, J. R. G. Hyde and N. K. MacLeod. Ascending 5-HT pathways and behavioural habituation. *Pharmac. Biochem. Behav.* **10:** 687–694, 1979.
5. DiChiara, G., R. Camb and P. F. Spano. Evidence for inhibition by brain serotonin of mouse killing behaviour in rats. *Nature, Lond.* **233:** 272–273, 1971.
6. File, S. E. Anxiety, ACTH and 5-HT. *Trends Neurosci.* **1:** 9–11, 1978.
7. File, S. E. and J. R. G. Hyde. The effects of p-chlorophenyl-alanine and ethanolamine-O-sulphate in an animal test of anxiety. *J. Pharm. Pharmac.* **29:** 735–738, 1977.
8. File, S. E. and J. R. G. Hyde. Can social interaction be used to measure anxiety? *Br. J. Pharmac.* **62:** 19–24, 1978.
9. File, S. E., J. R. G. Hyde and N. K. MacLeod. 5,7-Dihydroxytryptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. *J. Aff. Dis.* **1:** 115–122, 1979.
10. File, S. E. and S. V. Vellucci. Behavioural and biochemical measures of stress in hooded rats from different sources. *Physiol. Behav.* **22:** 31–36, 1979.
11. Geller, L. and K. Blum. The effects of 5-HT on p-chlorophenylalanine (pCPA) attenuation of 'conflict' behaviour. *Eur. J. Pharmac.* **9:** 319–324, 1970.
12. Hole, K., G. E. Johnson and O-G. Berge. 5,7-Dihydroxytryptamine lesions of the ascending 5-hydroxytryptaminergic pathways: habituation, motor activity and agonistic behaviour. *Pharmac. Biochem. Behav.* **7:** 205–210, 1977.
13. Matte, A. C. and H. Tornow. Parachlorophenylalanine produces dissociated effects on aggression "emotionality" and motor activity. *Neuropharmacology* **17:** 555–558, 1978.
14. Miczek, K. A., J. L. Altman, J. B. Appel and W. O. Boggam. Para-chlorophenylalanine, serotonin and behaviour. *Pharmac. Biochem. Behav.* **3:** 355–361, 1975.
15. Robichaud, R. C. and K. L. Sledge. The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. *Life Sci.* **8:** 965–969, 1969.
16. Sheard, M. H. The effect of p-chlorophenylalanine on behavior in rats: Relation to 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Brain Res.* **15:** 524-528, 1969.
17. Soubrie, P., L. de Angelis, P. Simon and J. R. Boissier. Effets des anxiolytiques sur la prise de boisson en situation nouvelle et familiere. *Psychopharmacologia* **50:** 41–45, 1976.
18. Stein, L., C. D. Wise and B. D. Berger. Antianxiety action of benzodiazepines—Decrease in activity of serotonin neurons in the punishment system. In: *The Benzodiazepines,* edited by S. Garrattini, E. Mussini and L. O. Randall. New York: Raven Press, 1973, pp. 229–326.
19. Vergnes, M., C. Penot, E. Kempf and G. Mack. Lésion sélective des neurones sérotoninergiques du raphé par la 5,7-dihydroxytryptamine: effects sur le compartement d'agression interspécifique du Rat. *Brain Res.* **133:** 167–171, 1977.