Pharmacology Biochemistry & Behavior, Vol. 22, pp. 377–382, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Manipulations of 5-HT Activity and Memory in the Rat[1]

ROBERT LALONDE AND VAIRA VIKIS-FREIBERGS[2]

Department of Psychology, Université de Montréal, C. P. 6128, Succursale "A", Montréal, P. Q., H3C 3J7

Received 21 June 1982

LALONDE, R. AND V. VIKIS-FREIBERGS. *Manipulations of 5-HT activity and memory in the rat.* PHARMACOL BIOCHEM BEHAV 22(3) 377–382, 1985.—The activity of 5-HT was manipulated by means of the peripheral injection of 5-HT reuptake inhibitors, fenfluramine and fluoxetine. These drug treatments, at doses higher than 1 mg/kg, produced retrograde amnesia in a one-trial appetitive learning task in rats. A non-specific inhibitor of 5-HT reuptake, imipramine, did not produce this amnesic effect, nor did the combination of fenfluramine with the MAOI tranylcypromine, although it produced, as expected, the "serotonergic syndrome." Results for the metabolic precursor of 5-HT, 5-HTP, also administered peripherally, were inconsistent, with amnesic effects seen at 5 and 20 mg/kg but none at 10 mg/kg.

Memory 5-HT activity Amnesia

5-HYDROXYTRYPTAMINE (5-HT) manipulations have been linked to learning and memory processes in animals [6]. In avoidance tests, it has been found that 5-HT depletion facilitates learning [22,24], though this result depends on such factors as the method (lesions, drugs) by which 5-HT depletion is produced, the extent of 5-HT depletion and the nature (1-way or 2-way) of the avoidance task itself [18]. On the contrary, an increase in 5-HT activity, either by peripheral administration of the 5-HT metabolic precursor, 5-hydroxytryptophan (5-HTP), or the 5-HT releasing agent, p-chloroamphetamine, impairs avoidance learning [15,19]. These results are based on studies where the drugs were administered before the learning session. The facilitation of avoidance learning by 5-HT may be due to increased pain sensitivity caused by this type of manipulation [2,22] and not to cognitive processes as such. Additional information may be obtained relating 5-HT specifically to memory processes by means of administration of drugs after the learning session.

It has been shown that post-trial intracerebral administration of 5-HT impairs retention of avoidance learning [6,25]. These results suggest that activation of 5-HT neurons produces retrograde amnesia in animals.

Intracerebral 5-HT, however, may cause a non-specific effect by activating neurons not containing 5-HT [2]. It is the purpose of this study to increase 5-HT activity by means of peripheral administration of drugs known to affect 5-HT reuptake or release. The hypothesis being tested is that these drugs cause retrograde amnesia in rats.

Fenfluramine (FF) and fluoxetine (FLX) are inhibitors of 5-HT reuptake [8, 16, 27]. FF is also a 5-HT releaser [4] and there is evidence that FF decreases appetite in animals because of its 5-HT stimulating properties [1, 10, 13.]. These two drugs will be compared to imipramine (IMI), a drug which inhibits the reuptake of 5-HT and noradrenaline [17,21]. 5-HTP will also be given in order to find out whether the 5-HT precursor which increases brain 5-HT levels [20] has the same effect as the 5-HT reuptake inhibitors.

The one-trial passive avoidance test has been the task most often used in the evaluation of drug effects on memory. In order to provide a wider approach to such an evaluation, a new one-trial appetitive task will be used here. After four days of exploration in a box, rats either find a food-filled hole or an empty hole. On the sixth day, the day of the retention test, rats reinforced on the previous day visit the now empty hole more often than nonreinforced rats. Amnesic agents, administered immediately after the one-trial learning session, should decrease the number of hole visits of reinforced but not of non-reinforced rats.

METHOD

Series of male Sprague-Dawley rats (Charles River, St-Constant, Quebec) served as subjects. On arriving at the lab the rats, weighing 240 g (±40 g), were placed in groups of 5 to 7 in metal cages (Hoeltge, Cincinnati, OH).

The learning apparatus consisted of two wooden cells of identical size (30×25 cm), separated by a partition of cardboard boxes (30×22 cm). At the end of each cell, there was a round hole (diameter: 4 cm, depth: 2 cm). On the first two exploration days, the floor of the box was covered with an opaque layer of black Plexiglas. A few days after their arrival at the lab, the rats, deprived of food (Purina Rat Chow) for 23 hr (water available at all times), explored the learning apparatus for 15 minutes a day in groups of 4 to 6. At that time the hole of each cell was covered with the layer of Plexiglas. On days 3 and 4, the rats, still food deprived for 23 hr, explored the cells one by one for 4 minutes (since there were two cells, the experimenter sent in two rats at a time).

[1]Supported by Grant No. A0700 of the Natural Sciences and Engineering Research Council of Canada.
[2]Requests for reprints should be addressed to V. Vikis-Freibergs.

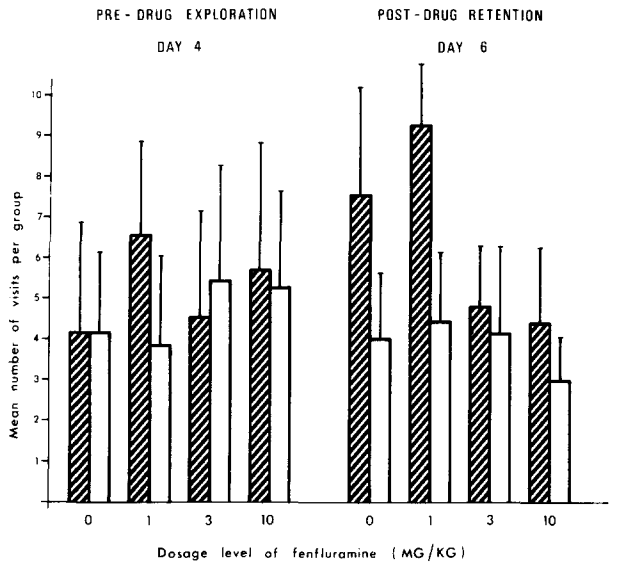

FIG. 1. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=7) on day 6 (retention test) as a function of a fenfluramine injection on day 5 and pre-drug responding on day 4.

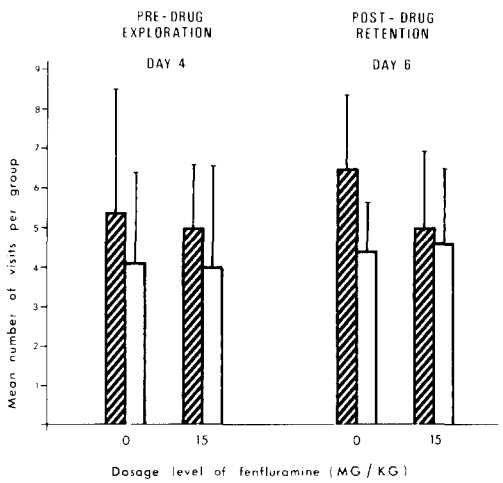

FIG. 2. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=10) on day 6 (retention test) as a function of a fenfluramine injection on day 5 and pre-drug responding on day 4.

During these two days, the Plexiglas layer being removed, the experimenter counted the number of times a particular rat would approach the empty hole and sniff about by putting its nose around or into the hole; this behavior is said to be a "visit" to the hole. The hole is visited only when the nose of the rat touches the border of the hole or enters into the hole. For a second visit to occur, a rat must take a few steps about the box before returning to the hole. In other words, there is no accumulation of visits when a rat remains fixed in one spot while its nose enters and leaves the hole continuously. A subject is accepted for the learning test only if it makes at least one visit either on day 3 or day 4. The fifth day is the day of the learning session. A few hours before the session, the rats were separated and placed in individual cages (Hoeltge). The rats were then divided according to a factorial design into different groups according to dose level. Half of the subjects found 45 mg Noyes food pellets in the hole of the cell (Reinforced rats), while the other half found, as on the two previous days, an empty hole (non-Reinforced rats). Immediately after the four minute session, the rats received an IP injection of one of the drugs. The rats were lifted from the learning box, injected, then put back in their home cages.

Drug treatments, given in separate experiments, included fenfluramine, FF (A. H. Robins, Montreal), fluoxetine, FLX (Lilly, Indianapolis) or imipramine, IMI (Sigma, St-Louis) at 1, 3 and 10 mg/kg. 5-HTP methyl ester (Sigma) was administered at 5, 10 and 20 mg/kg. Different groups of rats (n=7 or 10) were used for each dose level. Distilled water or saline were used as the drug vehicle and placebo, with an injection volume of 1 ml/kg. FF was also administered in combination with tranylcypromine (TCP 20 mg/kg, dissolved in saline, source: Sigma), an inhibitor of monoamine oxydase (MAOI), to induce the "serotonergic syndrome" [12]. In a separate experiment, as a test for the possibility that taste aversion may affect the results of this study, lithium chloride (LiCl, Matheson, Coleman and Bell), a drug known to induce taste aversion [14], was injected at 3 mEq/kg.

All injections were done on day 5 through the intraperitoneal route, immediately after the learning session, except in the case of the combination of TCP and FF where the latter drug was given 30 min after the former drug. After the injection, the rats were placed in individual cages (as described above) but, unlike the previous days, they received no food. This treatment is necessary to ensure that drugged and non-drugged rats do not consume different amounts of food, thus creating differential motivation a day later. Day 6, the day of the retention test, is the same as days 3 and 4. The rats, deprived of food for 47 hours, were placed one by one in the box for 4 min (the experimenter being blind as to the drug treatment any rat received the day before), and the number of visits to the hole were counted for each rat. The floor of the box was cleaned after each retention test with warm water (to reduce and diffuse odor cues). For learning to be demonstrated, the number of visits by the Reinforced (R) group must be higher than the number of visits by the non-Reinforced (non-R) group.

RESULTS AND DISCUSSION

The number of hole visits were recorded on day 4 (pre-drug exploration) and on day 6 (post-drug retention). The number of visits on day 4 is regarded as a baseline measure so that group differences on day 6 may be ascribed to drug treatments on day 5 and not to sampling error. No group differences (1-Way ANOVA, $p<0.05$) were found on day 4 in any of the experiments. The variances on the day 6 data were always homogeneous according to the Hartley test ([26] pp. 92–94), so that t-tests (one-tail) were done for all paired group comparisons. In all experiments, learning was demonstrated in non-drugged rats by comparing the number of visits on day 6 of the R group to the non-R group. The R group always had more visits to the hole than the non-R group among these non-drugged rats ($p<0.05$).

Fenfluramine (FF)

It was found that the post-trial administration of FF at higher doses decreased the number of visits during the re-

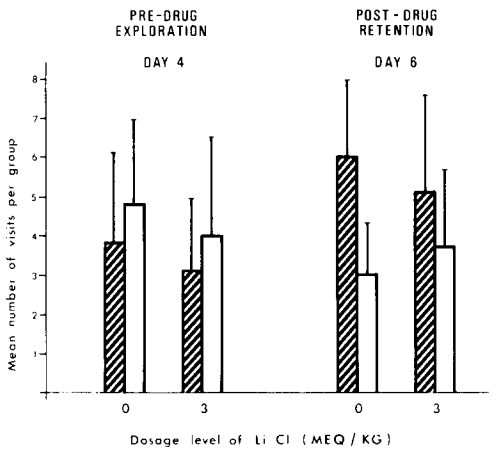

FIG. 3. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=10) on day 6 (retention test) as a function of a LiCl injection on day 5 and pre-drug responding on day 4.

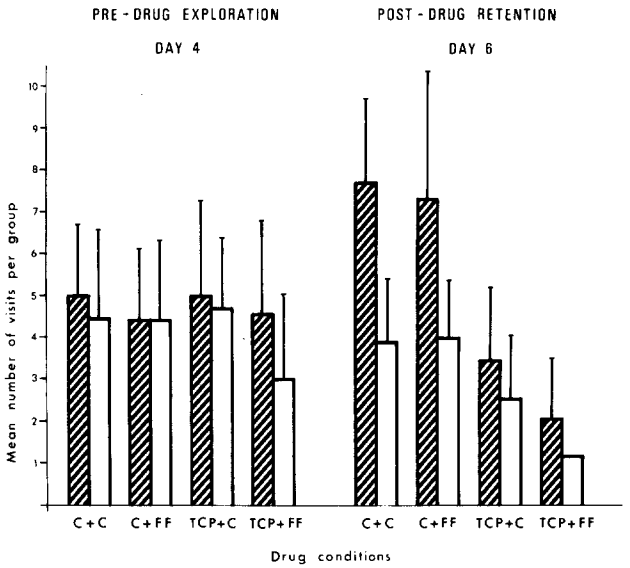

FIG. 4. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=7) on day 6 (retention test) as a function of drug injections on day 5 and pre-drug responding on day 4 (either saline or trancypromine, followed by either saline or fenfluramine 30 min later).

tention session for R rats but not for non-R rats. A 2×4 analysis of variance (ANOVA) was performed on the results of day 6 with 2 reinforcement conditions (reinforcement or no reinforcement) and 4 dose levels of FF (0, 1, 3 and 10 mg/kg). There were significant effects for the reinforcement factor, $F(1,48)=33.3$, $p<0.01$, the dose factor, $F(3,48)=8.7$, $p<0.01$, and the interaction term, $F(3,48)=4.3$, $p<0.01$. As shown in Fig. 1, FF at 3 mg/kg ($t(12)=2.44$, $p<0.025$) and 10 mg/kg ($t(12)=2.66$, $p<0.01$) decreased the number of visits of R rats compared to placebo.

Among non-R rats, there is no difference between any of the groups ($p>0.05$). The same result was found in a separate experiment with a higher dose (15 mg/kg) of FF, as shown in Fig. 2, where the FF group again had fewer visits than the placebo group among R rats ($t(18)=1.85$, $p<0.05$), but not among non-R rats. Only the low-dose 1 mg/kg FF group showed results not significantly different from the placebo group. Within the 3 to 15 mg/kg dosage range, the number of hole visits by R rats was reduced to the same level as that for non-R animals, the drug apparently eliminating the learning produced by reinforcement which can be seen in the placebo and 1 mg/kg groups.

Lithium (LiCl)

In order to test whether the decrease in visits of R rats found among drugged animals might be due to taste aversion to the Noyes pellets given as reinforcement on day 5, LiCl was injected instead of FF in a different group of rats. A 2×2 ANOVA was performed with 2 reinforcement conditions and 2 dose levels of LiCl (0 and 3 mEq/kg). There was a significant effect for the reinforcement factor, $F(1,24)=6.2$, $p<0.05$, but not for the dose factor, $F(1,24)=0$, $p>0.05$, or the interaction term, $F(1,24)=1.0$, $p>0.05$. As shown in Fig. 3, LiCl at 3 mEq/kg did not decrease the number of visits of R rats ($t(18)=0.92$, $p>0.1$) or of non-R rats. The failure of LiCl to decrease the number of visits of R rats suggests that the decrease found with FF is not due to taste aversion learning.

Tranylcypromine Plus Fenfluramine (TCP + FF)

FF at the highest dose (15 mg/kg), given 30 min after 20 mg/kg of the MAOI tranylcypromine (TCP) produced the "serotonergic syndrome" (hind limb rigidity, forepaw treading and Straub tail). These signs, known to be due to increased brain 5-HT activity [12], would have been expected to be linked to retrograde amnesia. No such amnesic effect, however, could be demonstrated in the present experiment. Rats receiving TCP + FF had a significantly smaller number of hole visits than the C + C control group receiving two saline injections at 30 min intervals, but this occurred for both R ($t(12)=3.73$, $p<0.005$) and non-R ($t(12)=6.61$, $p<0.0005$) rats. Similar results were found with the TCP + C groups, which received saline 30 min after TCP. This group also showed a significant reduction in hole visits in comparison to the C + C control group, but again, this occurred equally for the R animals ($t(12)=3.73$, $p<0.005$) and the non-R animals ($t(12)=2.37$, $p<0.025$). All rats receiving TCP, whether jointly with FF or with saline, appeared behaviorally depressed during the retention test. They tended to huddle in the same area of the box and barely moved at all. There were no lethal effects, however. The general behavioral toxicity of TCP is thus too large to permit detecting any potential cognitive differences between R and non-R animals.

A third group shown in Fig. 4, C + FF, received saline immediately after training and 15 mg/kg FF 30 min later. Contrary to the results with the same dosage injected immediately after training (Fig. 2), where an amnesic effect was found, the delayed FF group was not significantly different from the control group (C + C) either for the R or the non-R animals. The reinforced animals made significantly more hole visits than the non-reinforced ones, both in the drug group and in the control group ($p<0.005$). This suggests that there is a time gradient for the learning impairment due to FF, which is much like the time-gradient effect found for electroconvulsive shock, a well-known amnesic treatment [5]. This result also helps to reinforce the hypothesis that the

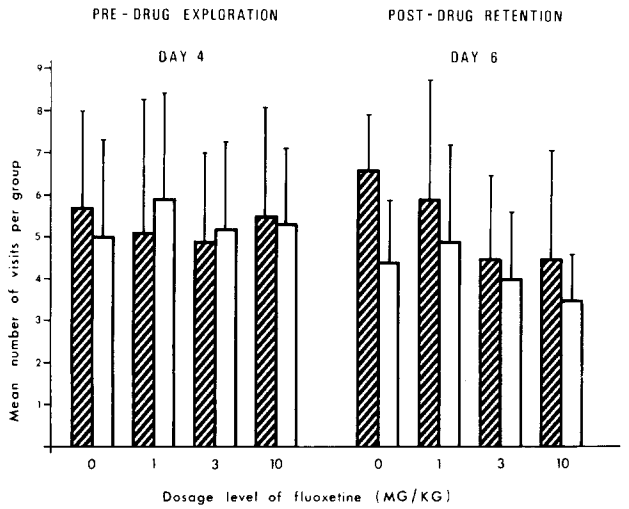

FIG. 5. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=10) on day 6 (retention test) as a function of a fluoxetine injection on day 5 and pre-drug responding on day 4.

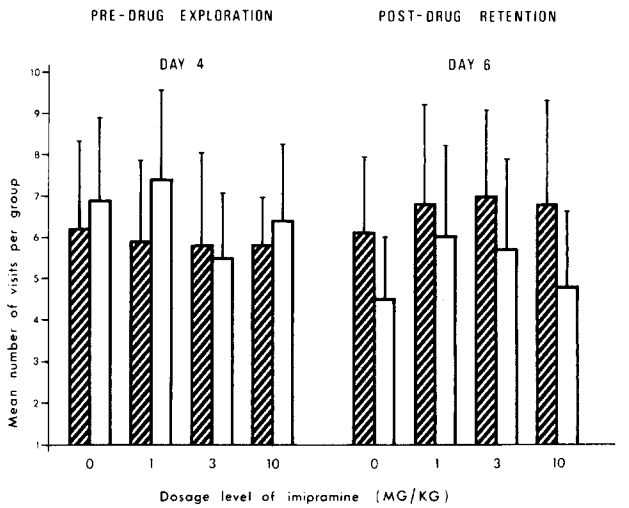

FIG. 6. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=10) on day 6 (retention test) as a function of an imipramine injection on day 5 and pre-drug responding on day 4.

effect of FF in our paradigm is retroactive rather than proactive. Since FF can cause long-term depletion of 5-HT [3,9], the effect of the drug in our test might have been proactive. If so, then the 30 min delay of injection after training should not have made any difference on the testing session one day later. The fact that the amnesic effect is eliminated by a 30 minute delay after training suggests, on the contrary, a time-dependent retrograde effect.

Fluoxetine (FLX)

In order to test whether the apparent amnesic effect of FF can be generalized to other drugs presumed to increase 5-HT activity, fluoxetine (FLX) was given in the same paradigm as before. FLX has been reported to cause inhibition of the pre-synaptic reuptake of 5-HT but not that of dopamine or noradrenaline [8,27].

A 2×4 ANOVA was performed with 2 reinforcement conditions and 4 dose levels of FLX (0, 1, 3 and 10 mg/kg). There was a significant effect for the reinforcement factor, $F(1,72)=6.8$, $p<0.01$, but not for the dose factor, $F(3,72)=2.9$, $p<0.05$, or the interaction term, $F(3,72)=0.8$, $p<0.05$.

As shown in Fig. 5, the effect of FLX at the two higher dose levels resembles that of FF. At these two dose levels, there is no significant difference ($p<0.1$) in the number of visits between R rats and non-R rats, suggesting an effect of retrograde amnesia, while the control non-drugged animals continue to show an effect of past training by a significant difference ($p<0.05$) between reinforced and non-reinforced animals. Rats receiving 1 mg/kg of FLX, on the other hand, were closer to the results of the placebo groups, suggesting that this dosage was too low to produce significant behavioral effects.

Imipramine (IMI)

As a further test on the generality of these results, imipramine (IMI) was given in the same paradigm. *In vitro* studies on rat cerebral tissues have shown that IMI inhibits

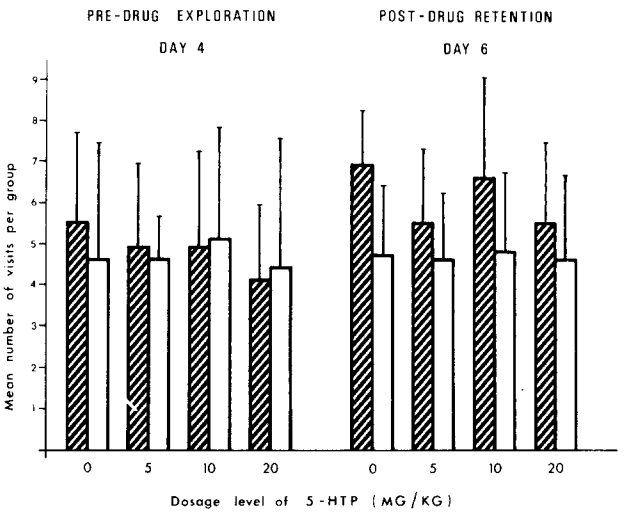

FIG. 7. Mean number of visits (±SD) by Reinforced (striped bar) and non-Reinforced (open bar) rats (n=10) on day 6 (retention test) as a function of a 5-HTP injection on day 5 and pre-drug responding on day 4.

the neuronal reuptake of 5-HT [17,21]. A 2×4 ANOVA was performed with 2 reinforcement conditions and 4 dose levels of IMI (0, 1, 3 and 10 mg/kg). There was a significant effect for the reinforcement factor, $F(1,72)=9.3$, $p<0.01$, but not for the dose factor, $F(3,72)=1.0$, $p>0.05$, or the interaction term, $F(3,72)=0.3$, $p>0.05$. No effects of retrograde amnesia similar to those observed for FF and FLX can be found (Fig. 6). The R rats make significantly more visits than the non-R rats at 10 mg/kg IMI ($t(18)=2.04$, $p<0.05$) as well as in the no-drug condition ($t(18)=2.10$, $p<0.025$), showing that the learned response has not been affected by the drug. While the difference between R and non-R subjects indeed disappears under 1 mg/kg and 3 mg/kg of IMI, this lack of differ-

ence is due to a slight increase in the number of responses by non-R animals as compared to the placebo level, rather than to a decrease in responding by reinforced animals, as would be expected for an amnesic effect. Although IMI has the same neuropharmacological effect (reuptake inhibition) regarding 5-HT activity as FF and FLX, it differs from these other two drugs in that it also blocks the reuptake of another neurotransmitter, norepinephrine [17,21]. The lack of an amnesic effect for IMI may thus possibly be linked to its lack of specificity. A similar lack of specificity may also account for the lack of an amnesic effect in the TCP + FF group which otherwise exhibited signs of the "serotonergic syndrome." How the activation of norepinephrine activity by IMI interfered with the amnesic effect expected from its increase in 5-HT activity is unclear at this moment.It may be that, while drugs stimulating the 5-HT system produce amnesia, this effect is blocked by increased activity of other neuropharmacological systems.

5-HTP

In a final experiment, 5-HTP, the metabolic precursor of 5-HT, was administered. Logically, one would expect this substance to produce behavioral effects analogous to those of the 5-HT reuptake inhibitors, FF and FLX. The results, shown in Fig. 7, were inconsistent. A 2×4 ANOVA for 4 dose levels of 5-HTP (0,5,10 and 20 mg/kg) revealed a significant effect for the reinforcement factor, $F(1,72)=12.0$, $p<0.01$, but not for the dose factor, $F(3,72)=1.0$, $p>0.05$, or the interaction term, $F(3,72)=0.6$, $p>0.05$. It was found that expected amnesic effects were indeed obtained for two of the drug groups, in that there was a significant decrease of hole visits for R rats at 5 mg/kg ($t(18)=2.03$, $p<0.05$) and at 20 mg/kg of 5-HTP ($t(18)=1.92$, $p<0.05$) as compared to placebo. But no such effect was obtained at 10 mg/kg of 5-HTP. The reason for this non-linear relation is not known. Sampling error is always a possibility, but seems unlikely in this case because on day 4 of pre-drug exploration all experimental groups were closely similar in performance (see Fig. 7). It is possible that the decrease in visits found at 5 and 20 mg/kg is due to different neuropharmacological effects, only one (or none) of which being due to some 5-HT related function. But until further experiments are performed with this drug, all theoretical statements are highly speculative.

In summary, the results of this study show that the specific 5-HT reuptake inhibitors, FF and FLX, and the 5-HT precursor 5-HTP are potential amnesic agents. Whether these drugs produce amnesic effects in other behavioral tasks remains to be determined. These results, however, offer support for the hypothesis that the amnesic effect of intracranial 5-HT is due to the specific activation of 5-HT neurons [6,25]. This is not inconsistent with the finding that retrograde amnesia can be caused by electrical stimulation of dorsal raphe neurons, an effect which is blocked by prior administration of the tryptophan hydroxylase inhibitor p-chlorophenylalanine [7].

A parallel may be drawn between the results reported here and the amnesic effect of electroconvulsive shock [5]. Since it has been shown that shock treatment increases brain levels of 5-HT [6], one may argue that an increase in 5-HT activity is responsible for the amnesia. While it is certain that electroconvulsive shock does not stimulate specifically the 5-HT system alone, it is possible to consider that, in spite of the general activation of the brain caused by this treatment, the amnesic effect is at least partially due to an activation of 5-HT neurons. If amnesia as such is due largely to an activation of 5-HT neurons, then one would predict that drugs decreasing this activity may prevent the amnesia caused by electro-shock as well as that caused by drug manipulation.

ACKNOWLEDGEMENTS

We are grateful to A. H. Robins, Montreal and Lilly Labs, Indianapolis for the supply of fenfluramine and fluoxetine.

REFERENCES

1. Blundell, J. E., C. J. Latham and M. B. Leshem. Biphasic action of 5-hydroxytryptamine inhibitor on fenfluramine-induced anorexia. *J Pharm Pharmacol* **25:** 492–494, 1973.
2. Chase, T. N. and D. L. Murphy. Serotonin and central nervous system function. *Annu Rev Pharmacol Toxicol* **13:** 181–197, 1973.
3. Clineschmidt, B. V., J. A. Totaro, J. C. McGuffin and A. B. Pflueger. Fenfluramine: long term reduction in brain serotonin (5-hydroxytryptamine). *Eur J Pharmacol* **35:** 211–214, 1976.
4. Costa, E., A. Gropetti and A. Revulta. Action of fenfluramine on monoamine stores of rat tissues. *Br J Pharmacol* **41:** 57–64, 1971.
5. Duncan, C. P. The retroactive effect of electroshock on learning. *J Comp Physiol Psychol* **42:** 32–44, 1949.
6. Essman, W. B. Serotonin in learning and memory. In: *Serotonin in Health and Disease, Vol. 3, The Central Nervous System*, edited by W. B. Essman. New York: Spectrum Publications, 1978, pp. 69–143.
7. Fibiger, H. C., F. G. Lepiane and A. G. Phillips. Disruption of memory produced by stimulation of the dorsal raphe nucleus: mediation by serotonin. *Brain Res* **155:** 380–386, 1978.
8. Fuller, R. W. and D. T. Wong. Inhibition of serotonin reuptake. *Fed Proc* **36:** 2154–2158, 1977.
9. Harvey, J. A. and S. E. McMaster. Fenfluramine: evidence for a neurotoxic action on midbrain and long-term depletion of serotonin. *Psychopharmacol Commun* **1:** 217–228, 1975.
10. Hollister, A. S., G. N. Ervin, B. R. Cooper and G. R. Breese. The role of monoamine neural systems in the anorexia induced by (+)-amphetamine and related compounds. *Neuropharmacology* **14:** 715–723, 1975.
11. Holman, R. B., E. Seagraves, G. R. Elliott and J. D. Barchas. Stereotyped hyperactivity in rats treated with tranylcypromine and specific inhibitors of 5-HT reuptake. *Behav Biol* **16:** 507–514, 1976.
12. Jacobs, B. L. An animal behavior model for studying central serotonergic synapses. *Life Sci* **19:** 777–786, 1976.
13. Jespersen, S. and J. Scheel-Krüger. Evidence for a difference in mechanism of action between fenfluramine and amphetamine-induced anorexia. *J Pharm Pharmacol* **25:** 49–54, 1973.
14. Jolicoeur, F. B., M. J. Wayner, A. D. Merkel, D. B. Rondeau and R. B. Mintz. The effects of various barbiturates on LiCl induced taste aversion. *Pharmacol Biochem Behav* **12:** 613–617, 1980.
15. Joyce, D. and H. M. B. Hurwitz. Avoidance behaviour in the rat after 5-hydroxytryptophan (5-HTP) administration. *Psychopharmacologia* **5:** 424–430, 1964.
16. Kannengiesser, M. H., P. F. Hunt and J. P. Raynaud. Comparative action of fenfluramine on the uptake and release of serotonin and dopamine. *Eur J Pharmacol* **35:** 35–43, 1976.

17. Lidbrink, P., G. Jonsson and K. Fuxe. The effect of imipramine-like drugs and antihistamine drugs on uptake mechanisms in the central noradrenaline and 5-hydroxytryptamine neurons. *Neuropharmacology* **10:** 521–536, 1971.
18. Lorens, S. A. Some behavioural effects of serotonin depletion depend on method: a comparison of 5,7-dihydroxytryptamine, p-chlorophenylalanine, p-chloroamphetamine and electrolytic raphe lesions. *Ann NY Acad Sci* **305:**532–555, 1978.
19. Ögren, S. O. Forebrain serotonin and avoidance learning: behavioural and biochemical studies on the acute effect of p-chloroamphetamine on one-way active avoidance learning in the male rat. *Pharmacol Biochem Behav* **16:** 881–895, 1982.
20. Penn, P. E., W. J. McBride, J. N. Hingtgen and M. H. Aprison. Differential uptake, metabolism and behavioral effects of the D and L isomers of 5-hydroxytryptophan. *Pharmacol Biochem Behav* **7:** 515–518, 1977.
21. Shaskan, E. G. and S. H. Snyder. Kinetics of serotonin accumulation into slices from rat brain: relationship to catecholamine uptake. *J Pharmacol Exp Ther* **175:** 404–418, 1970.
22. Tenen, S. S. The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behaviour in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
23. Trulson, M. E. and B. L. Jacobs. Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. *Eur J Pharmacol* **36:** 149–154, 1976.
24. Vorhees, C. V., G. J. Schaefer and R. J. Barrett. p-Chloroamphetamine: Behavioral effects of reduced cerebral serotonin in rats. *Pharmacol Biochem Behav* **3:** 279–284, 1975.
25. Wetzel, W., V. M. Getsova, R. Jork and H. Mathies. Effect of serotonin on Y-maze retention and hippocampal photein syntheses. *Pharmacol Biochem Behav* **12:** 319–322, 1980.
26. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Book Company, 1962.
27. Wong, D. T., F.P. Bymaster, J. S. Horng and B. B. Molloy. A new selective inhibitor for uptake of serotonin into synaptosomes or rat brain: 3 (p-trifluoromethylphenoxy)-n-methyl-3-phenylpropylamine. *J Pharmacol Exp Ther* **193:** 804–811, 1975. 811, 1975.