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Stimulus Properties of the Selective 5-HT Reuptake Inhibitor Fluvoxamine in Conditioned Taste Aversion Procedures

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OLIVIER, B., J. GOMMANS, J. VAN DER GUGTEN, J. A. BOUWKNECHT, A. H. J. HERREMANS, T. PATTY AND T. H. HIJZEN. *Stimulus properties of the selective 5-HT reuptake inhibitor fluvoxamine in conditioned taste aversion procedures.* PHARMACOL BIOCHEM BEHAV **64**(2) 213–220, 1999.—Previous attempts to train pigeons and rats to discriminate between the antidepressant fluvoxamine and its vehicle as assessed in a drug discrimination paradigm have been without success. The present experiments were, therefore, designed to assess in a conditioned taste aversion procedure (CTA) whether or not fluvoxamine possesses stimulus properties. Rats were exposed to a conditioned taste aversion (CTA) procedure. In Experiment I, subjects were given 15 mg/kg fluvoxamine PO or vehicle after drinking a novel tasting saccharin solution. In Experiment II, a comparison was made between the effects of 15 mg/kg fluvoxamine IP, 30 mg/kg fluvoxamine IP, NaCl, and lithiumchloride (LiCl). In Experiment III, subjects were treated with either 10 mg/kg fluoxetine IP, 30 mg/kg fluvoxamine IP, or LiCl. CTA was observed after treatment with LiCl, but never after treatment with fluvoxamine or fluoxetine, suggesting that fluvoxamine does not have clear stimulus properties, which can serve as a discriminative stimulus in operant procedures. In a crossfamiliarization CTA procedure in mice, however, fluvoxamine elicited a reliable CTA, suggesting that under certain conditions (species, dose?) selective serotonin reuptake inhibitors (SSRIs) may lead to certain discriminable effects. It is as yet unclear why SSRIs apparently produce such weak and species or situation-dependent discriminable effects. © 1999 Elsevier Science Inc.

Fluvoxamine Serotonin Conditioned taste aversion Rat Mouse Crossfamiliarization

DRUG discrimination procedures provide a sensitive measure of the stimulus properties of drugs. Subjects are required to make one response in the presence of the training drug and another response in the absence of the drug. Psychoactive compounds from various classes have been shown to gain stimulus control over behavior, but the length of the training period seems to be dependent on the type of drug, dose, and species studied. Most drug discrimination studies employ operant procedures in the Skinnerbox. Generally spoken, training subjects to discriminate a drug from its vehicle in such procedures is very time consuming, in the sense that a large number of sessions is required before the stimulus properties of a given compound can be assessed.

Fluvoxamine is an antidepressant that acts by selectively inhibiting 5-HT reuptake. In our laboratory we have previously attempted to train different groups of rats with different doses of fluvoxamine in a two-lever operant conditioning procedure with an FR10 schedule of reinforcement. The discrimination criterion was defined as no more than three responses on the incorrect lever before the first food presentation (FRF $\leq$ 13) on at least 8 out of 10 consecutive training sessions. Five out of 12 rats achieved the discrimination between 10.0 mg/kg IP fluvoxamine and saline after an average of 90 training sessions. Eleven out of 12 rats attained criterion when the training dose was raised to 15 mg/kg (mean number of sessions: 97.1). Another group of rats was trained with 15 mg/kg IP

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fluvoxamine and their mean number of sessions to criterion averaged 61.7. Dose–response curves were obtained, but the subjects' performance deteriorated so that it became impossible to perform any further generalization testing (31). Still another group of rats was trained with fluvoxamine 15 mg/kg PO; only two rats reached criterion after the completion of 90 training sessions.

An attempt to train pigeons to discriminate between 10 mg/kg PO fluvoxamine and vehicle was also without success. In this case, an FR30 conditioning procedure was employed. The discrimination criterion was defined as no more than nine responses on the incorrect key before the first food presentation (FRF $\leq$ 39) on at least 8 out of 10 consecutive training sessions. Two birds out of a group of 12 reached criterion within 75 sessions (mean number of sessions: 61.5). Subjects were then given 10 remedial training sessions during which only the key that corresponded with the injection condition was illuminated. In the course of the following 55 sessions during which both keys were illuminated again, 10 out of 12 pigeons reached criterion (mean number of sessions: 22.2). However, the behavior never stabilized, and generalization tests were not performed.

Although these studies were rather disappointing regarding the discriminative stimulus effects of fluvoxamine, these results at least gave some indication that fluvoxamine does possess stimulus properties. The present experiments were designed to investigate whether such stimulus properties could be more readily detected by means of conditioned taste aversion (CTA) procedures. In a frequently used method, CTA refers to the reduced intake of a preferred solution due to a previous pairing of a novel taste with LiCl-induced sickness (12). Taste-aversion learning is historically viewed as a form of Pavlovian learning in which the taste stimulus serves as a conditioned stimulus (CS) for the aversive properties of LiCl injection (unconditioned stimulus effects, UCS). However, it is now well accepted that gastrointestinal distress is a sufficient but not a necessary prerequisite for the development of CTA [e.g., (17)]. A wide range of psychoactive drugs, including those which are self-administered by rats such as amphetamine, barbiturates, morphine and alcohol, are capable of inducing CTA. As such, CTA is nowadays more broadly defined as resulting from an association between a novel tasting solution and the stimulus properties of the drug.

Following this line of reasoning this would mean that drugs that induce CTA can be detected, and it may thus be expected that rats are able to discriminate these drugs in other procedures. In Experiment IA, water-deprived rats received 15 mg/kg PO fluvoxamine after ingestion of a novel tasting saccharin solution. In Experiment IB, treatment for the different groups consisted of either 15 mg/kg or 30 mg/kg IP fluvoxamine. Control subjects received LiCl or NaCl. Experiment IC compared the effects of LiCl, fluvoxamine 30 mg/kg, and fluoxetine 10 mg/kg on saccharin consumption when the test session was given either 1 or 4 days after the conditioning trial.

Recently, it was shown that the SSRIs fluoxetine (5) and fluvoxamine (16) have stimulus properties when measured in a crossfamiliarization CTA procedure in mice. In this paradigm, a drug is administered immediately after drinking a glucose solution. On a second occasion when the glucose solution is presented again, the animals drink less. It is assumed that a new internal cue is associated with the taste of glucose (9). When animals are preexposed to the drug, however, the taste aversion is prevented and animals drink the glucose solution readily. In the last experiment (II) the stimulus properties of the SSRI fluvoxamine in this crossfamiliarization CTA procedure in mice are described.

EXPERIMENT I (A, B, AND C)

Experiment IA used an injection with 15 mg/kg fluvoxamine as an UCS in a CTA paradigm to rapidly assess whether fluvoxamine indeed possesses stimulus properties and to determine how readily rats can detect the effects of this drug.

In Experiment IB, different groups of rats were treated with fluvoxamine after they were exposed to a saccharin solution. Fluvoxamine was administered intraperitoneally to find out whether the results of Experiment IA were confounded by the unwanted effects of water deprivation on food consumption. Eight rats were given 15 mg/kg IP to be able to make a comparison with the results of Experiment IA. Another group was treated with 30 mg/kg IP because it would also be possible that the lack of results of Experiment IA was due to too low a dose. Both a positive (LiCl) and a negative (NaCl) control group were included in Experiment IB.

Lorden and Nunn (28) showed that fluoxetine (10 mg/kg), another selective 5-HT reuptake inhibitor, induced CTA when the test trial was given 4 days after the conditioning trial. Although they did not discuss their motive for delaying the test trial, and although it is not clear why a drug that induces a CTA 4 days after conditioning would not do so on the day immediately after the conditioning trial, we decided to replicate their experiment (Experiment IC) to see whether fluvoxamine is able to induce a CTA if we test on the fourth day after the conditioning trial.

Materials and Methods

Subjects. Male Wistar rats were obtained from Harlan (Zeist, The Netherlands) when they were approximately 7 weeks old. Upon arrival in the laboratory, subjects were individually housed under a reversed light–dark cycle (lights on 1900–0700 h). All tests were performed during the dark portion of the light–dark cycle. Food was always available.

Drugs. Fluvoxamine (Solvay Pharmaceuticals B.V., Weesp, The Netherlands) was freshly suspended in tragacanth before oral use or dissolved in destilled water for IP use. Fluoxetine was dissolved in destilled water. Drugs were administered in a volume of 2 ml/kg. Saccharin was obtained from Sigma. Lithiumchloride (LiCl; OPG, Utrecht, The Netherlands) was dissolved in distilled water and injected IP in a volume of 8 ml/kg.

Procedure

Experiment IA. All subjects (n = 16) were adapted to a restricted drinking schedule. The water bottles were removed from the cages. Following 23.5 h of water deprivation, subjects were given access to tap water in the home cage once a day between 1330–1400 h for 3 consecutive days. Subjects were matched on water consumption following the drinking period on day 3, and were assigned to one of two groups (n = 8 per group). Subjects drank an average of 14.5 ($\pm$0.2) ml tap water during the last day of adaptation. On day 4, all subjects were given access to a 0.1% w/v saccharin solution for 30 min. For one group of subjects saccharin consumption was followed by an injection with fluvoxamine (15 mg/kg), while the remaining subjects received vehicle. Saccharin consumption on day 5 was registered to assess whether conditioned taste aversion had occurred.

Experiment IB. The procedure was identical to that employed in Experiment IA. Subjects (n = 32) drank an average

of 13.8 (±0.3) ml tap water on the third day of adaptation. They were matched on water consumption and assigned to one of four groups, consisting of eight subjects each. Following saccharin consumption on day 4, subjects were injected with 15 mg/kg fluvoxamine; 30 mg/kg fluvoxamine; LiCl 1.2 mEq (48.0 mg/kg); or NaCl. All drugs were given IP. Saccharin consumption on day 5 was taken as a measure of conditioned taste aversion.

Experiment IC. The procedure was identical to that employed in Experiment IA. Subjects ($n = 48$) drank an average of 16.7 (±0.6) ml tap water on the third day of adaptation. They were matched on water consumption and assigned to one of six groups, consisting of eight subjects each. Following saccharin consumption on day 4 (conditioning trial), two groups of subjects were injected with 10 mg/kg fluoxetine IP, two other groups of subjects were treated with 30 mg/kg fluvoxamine IP, and the remaining two groups were given LiCl 1.2 mEq (48.0 mg/kg). Within each drug condition, one group of subjects was given a test session on day 5, the day immediately after the day the conditioning trial was given. The remaining groups were tested at day 8, the fourth day after the conditioning trial. These latter groups received 30 min access to tap water on days 5, 6, and 7.

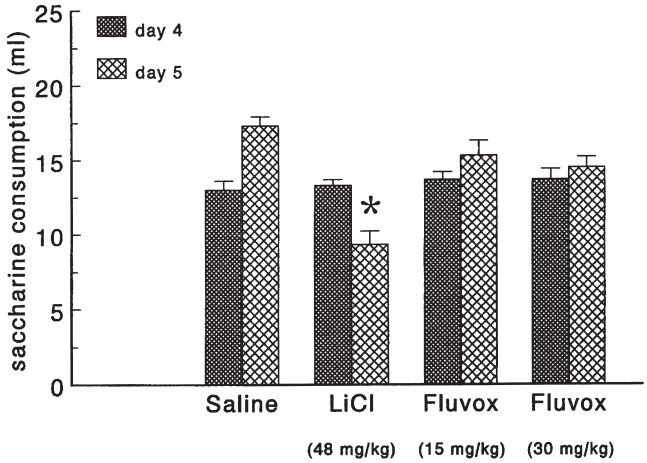

FIG. 1. Mean saccharin consumption (ml) for different groups of rats ($n = 8$ each) before (conditioning trial; day 4, dark bars) and after (test trial; day 5, hatched bars) IP treatment with vehicle (NaCl), Lithium Chloride (LiCl), 15 or 30 mg/kg fluvoxamine. Asterisks (*) indicate a significant ($p < 0.05$) effect compared to NaCl-controls on day 5.

Results

Experiment IA. Saccharin intake on the conditioning trial (day 4) averaged 14.3 (±0.62) and 13.4 (±0.59) ml for vehicle- and fluvoxamine-treated rats, respectively. Saccharin consumption on the test trial (day 5, the day following vehicle or fluvoxamine treatment) averaged 16.3 (±1.06) and 15.5 (±0.84), respectively. Differences between groups were not observed (Student's *t*-test, $t = 0.60$, $p > 0.5$).

Experiment IB. Figure 1 shows the mean (±SEM) saccharin intake for the different groups of rats before (day 4, conditioning trial) and after (day 5, test trial) treatment with NaCl, LiCl, and fluvoxamine. Saccharin consumption on the test trial was subjected to an overall analysis of variance (ANOVA) with the factor group (four levels). ANOVA revealed a highly significant effect of treatment on saccharin consumption on day 5, $F(3) = 22.27$, $p < 0.01$. Subsequent post hoc analyses showed that LiCl-injected subjects drank less than any of the other three groups (all $p < 0.01$). Differences between NaCl- and fluvoxamine-treated subjects were not observed (all ps > 0.01).

Experiment IC. Figure 2 shows mean (± SEM) saccharin intake for the different groups of rats before (conditioning trial, day 4) and after (test trial, day 5 or 8) treatment with LiCl, fluoxetine, and fluvoxamine. Saccharin consumption on the conditioning trial (day 4) did not differ significantly between groups, $F(5, 42) = 1.05$, $p > 0.4$. The difference between saccharin consumption during conditioning and test trial was subjected to an overall analysis of variance (ANOVA) with the factors drug (three levels) and test trial (two levels). ANOVA revealed a significant effect of drug treatment on saccharin consumption during the test trial, $F(2, 21) = 11.42$, $p < 0.01$. When the results of both test trials were combined, ANOVA revealed that LiCl-treated subjects consumed less (2.8 ml) and fluoxetine-treated subjects drank more (3.9 ml) of the saccharin compared with the conditioning trial (both $p < 0.01$). Fluvoxamine-treatment did not alter saccharin consumption ($p > 0.9$). It was also shown that the effects were more strongly observed when the test trial was given immediately following the conditioning trial (day 5) compared to when the test trial was delayed for 4 days, $F(1, 21) = 4.88$, $p < 0.05$. So when subsequent post hoc analyses were performed for all groups separately, it was concluded that only LiCl was able to induce a CTA when the test trial was given immediately (1 day) following the conditioning trial ($p = 0.02$). All other comparisons were statistically not significant.

Discussion

It is not clear why fluvoxamine in the present experiments failed to induce CTA, because others have reported that drugs that are known to alter serotonergic neurotransmission are indeed capable of inducing CTA. Fletcher (11) showed that rats refrain from drinking saccharin water after the taste had been paired with tryptamine. The effect was only observed after repeated injections with high (80.0 mg/kg) doses, and extinguished quite rapidly. The authors explained the relative weak action by referring to onset and duration of action on the one hand, and to low penetration of the blood–brain barrier on the other. More clearly demonstrable effects were obtained with the 5-HT reuptake blockers zimeldine (13) and fluoxetine (28). Both drugs induced strong taste aversions at moderate doses (zimeldine 20.0 mg/kg; fluoxetine 10.0 mg/kg). Similar results were reported by Berendsen and Broekkamp (4) in mice. Because zimeldine and fluoxetine in other operant learning tasks (DRL 72-s) both have a behavioral profile that is directly comparable to that of fluvoxamine (38), it is most likely that the differences as observed in the present experiments result from procedural variables, and not so much from differences in mechanisms of action between the compounds.

The most striking difference between the present Experiments IA and IB on the one hand and the experiments with zimeldine and fluoxetine on the other is the interval between conditioning and test days. In the study of Gill et al. (13) the conditioning trial was given 6 days before the test trial. In Lorden and Nunn's study (28), 4 days elapsed between the conditioning and the test trial. In Experiment IA and IB of the present study the test session was given the day immedi-

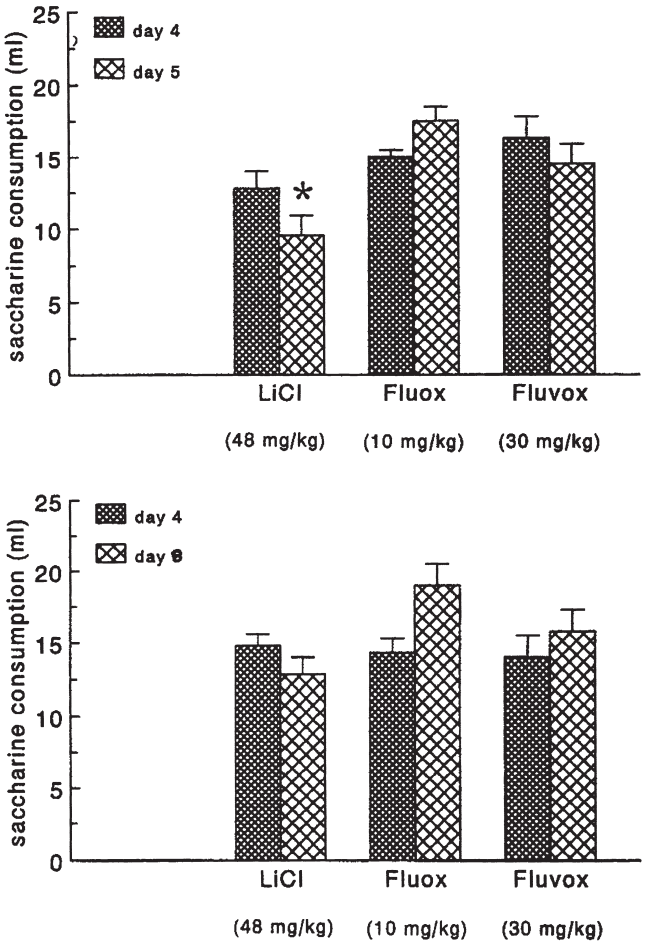

FIG. 2. Mean saccharin consumption (ml) for different groups of rats before (dark bars) and after (hatched bars) treatment with Lithium Chloride (LiCl48), 10 mg/kg fluoxetine (Flx10) or 30 mg/kg fluvoxamine (Flu30). Drugs were IP administered. The upper panels show the results for subjects that were tested the day immediately after the conditioning trial (day 5); the lower panel shows the data when the test trial was given 4 days after the conditioning trial (day 8). *Indicates a significant difference ($p < 0.05$) compared to day 4.

ately following conditioning. It could, thus, have been that fluvoxamine also had induced a CTA if we had waited longer between conditioning and testing. To test this hypothesis we performed a third experiment in which we compared the effects of fluvoxamine, fluoxetine, and LiCl on saccharin consumption either 1 or 4 days after the conditioning trial was given. Only LiCl produced a reliable CTA when tested immediately after conditioning. Fluvoxamine had no effect. Fluoxetine, if anything, increased saccharin consumption. As such, the findings reported by Lorden and Nunn (28)were not confirmed. Kreiss and Lucki (26) were able to train the SSRI sertraline (10 mg/kg) from saline in a discriminated taste procedure using a two-flavor choice test. The SSRIs fluvoxamine, fluoxetine, and paroxetine substituted completely for sertraline, but also noradrenergic uptake inhibitors (desipramine and maprotiline). The results are difficult to interpret, because sertraline and all other compounds exerted hypodipsic effects; moreover, different receptor agonists (trifluoromethylphenylpiperazine (TFMPP), 8-OH-DPAT, and serotonin (5-HT) itself) generalize (partly) to sertraline's cue. The fact that serotonin, systemically administered, substituted for the stimulus properties of sertraline, suggests that peripheral effects mediate sertraline's cue, because 5-HT does not cross the blood–brain barrier (30). This may mean that aversive peripheral effects induced by the various drugs tested may cause the generalization. The quite lengthy procedure and frequent drug injection used in the Kreiss and Lucki (26) experiments may indicate that animals have to learn these aversive effects, and that single administrations as we did in Experiment I are not sufficient to establish drug effects.

These results indicate that in the present CTA procedure orally administered fluvoxamine at 15 mg/kg has no clear discriminable effects in rats. However, it is also possible that such effects of the drug have been obscured by other unintended variables. Water-deprived rats are known to postpone their eating period until they are allowed to drink, even if food is freely available at all times. In Experiment IA, fluvoxamine was orally administered immediately after the drinking period, and thus, most probably just before the rats started to eat. It could very well be then that food consumption interfered with the effects of the drug, thereby preventing CTA to occur.

EXPERIMENT II

Many drugs may induce a CTA as a result of the aversion to a novel experience due to the first-time exposure to a particular effect of the drug (18,22). This interpretation lends weight to the discriminable properties of a drug rather than intrinsic aversive effects. Such discriminable properties may resemble those obtained in drug-discrimination learning experiments. Preexposure experiments with CTA would, therefore, allow assessment of the degree of similarity of the effects of drugs, notably the intrinsic stimulus properties. Basically, animals are familiarized with the drug stimuli during preexposure, thereby removing the novelty aspects of the drug stimuli. De Beun et al. (9) referred to this procedure as "crossfamiliarization" CTA, and showed that this procedure was suitable to study similarities in stimulus properties of different serotonergic drugs, including SSRIs like fluoxetine (5). Therefore, we investigated the stimulus properties of the SSRI fluvoxamine in this procedure and compared them to those of fluoxetine.

Materials and Methods

Animals. For all experiments naive male mice of the CD-1(IcR)BR strain (Broekman, The Netherlands), weighing 30–35 g, or the Balb/C strain (GDL Utrecht, The Netherlands) were used, weighing 20–25 g at the start of the experiments. Mice were housed in groups of five in macrolon cages, under a controlled 12 L:12 D cycle (light on at 0700 h), at a room temperature of 21–22°C. Mice had free access to standard food pellets but assess to tap water was restricted to a period of 20 min in the experimental session (between 0830 and 1220 h), and an additional period of 30 min in the home cage (from 1530 to 1600 h). Home cages were enriched with some nesting material (environ-dry, BMI, Helmond, The Netherlands), and a PVC tube to reduce fighting. Fifteen mice per experimental group were used, taken randomly from different home cages. One week before the experiments started the mice arrived in the laboratory and were weighed every other day.

Procedure

One day before the experiment started the water bottles were removed from the home cage. On the first 4 days (preex-

posure days), every morning mice were moved to the experimental chamber and placed individually in an experimental cage equipped with two calibrated pipettes filled with water. During 20 min the mice were allowed to drink and the amount of consumed water from each pipette was recorded; 15 mice were studied at the same time. On these preexposure days, between 1330 and 1430 h (at least 1 h after the mice had drunk in the test cage), mice were injected subcutaneously with a preexposure test drug or saline. In the afternoon, from 1530 to 1600 h, all mice had access to water in the home cage. On the fifth day (conditioning day) both pipettes were filled with a glucose solution (5% w/v). On this conditioning day, immediately after drinking from the glucose solution, mice were injected with the conditioned taste aversion-inducing reference drug or saline. From day 5 (1600 h) until day 7 (1100 h), mice were left undisturbed in their home cages and had free access to water and food. At 1100 h on day 7 the water bottles were removed again. On day 8 (test day), one pipette was filled with water, the other with glucose, and each mouse could choose what to drink during 20 min. On the test day no injections were made. All test sessions took place between 0830–1230 h.

The dose of drug used on the conditioning day was twice the ED_{50} obtained in separate conditioned taste aversion experiments (Table 1)

Data analysis. Mice have to drink a minimal amount of glucose solution for the conditioning on day 5, and data from mice that drunk less than 0.25 ml were discarded. For each animal the amount of glucose drunk as percentage of total fluid intake on the test day is calculated, and results are expressed as the mean (±SEM) percentage glucose solution drunk by each group. Data were submitted to a one-way analysis of variance and, if there was a significant overall effect, individual groups were compared using Fishers protected *t*-test. Familiarization (i.e., drug completely prevents the taste aversion of a reference drug) was defined in the following way: a group treated with a dose of preexposure drug differs significantly from the group treated with the conditioned reference drug, but not from the unconditioned saline control group.

Drugs. Fluvoxamine and flesinoxan (Solvay Pharmaceuticals B.V., Weesp), fluoxetine HCl (Lilly Laboratories), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) (RBI), 6-chloro-2(1-piperazinyl) pyrazine (MK-212) (Solvay Pharmaceuticals B.V.) 8-Hydroxy-di-N-dipropyl-amino-tetralin (8-OH-DPAT). All drugs were dissolved in sterile saline (0.9% NaCl) and freshly prepared. Injections were made subcutaneously using a volume of 10 ml/kg body weight.

Results

The ED_{50} of fluvoxamine induced conditioned taste aversion was 24 mg/kg, SC (Table 1) and twice the ED_{50} (50 mg/kg) was used for conditioning in subsequent experiments. Injection of this dose of fluvoxamine on day 5 (conditioning day) immediately after the mice drank the glucose solution caused a reliable aversion to the glucose on the test day. Preexposure to fluvoxamine, 8-OH-DPAT, and flesinoxan dose dependently familiarized to fluvoxamine. DOI did not familiarize to fluvoxamine and MK-212 only partially familiarized to fluvoxamine (Table 2).

In a direct comparison between the two SSRIs fluvoxamine and fluoxetine it was shown that preexposure to fluvoxamine familiarized completely to fluoxetine, whereas fluoxetine preexposure only partially familiarize to fluvoxamine (Table 2).

To test crossfamiliarization between fluvoxamine and flesinoxan, fluvoxamine was used as a preexposure drug to flesinoxan. Table 2 shows that preexposure to fluvoxamine did not familiarize to flesinoxan. Because flesinoxan preexposure familiarized completely to fluvoxamine, it was investigated whether this drug would also familiarize to fluoxetine, which occurred (Table 2).

Discussion

This study clearly shows that fluvoxamine has distinct stimulus properties that can be reliably measured in a crossfamiliarization (CTA) procedure in mice; the stimulus properties differ from fluoxetine. Preexposure to flesinoxan and 8-OH-DPAT prevented fluvoxamine-induced conditioned taste aversion. Flesinoxan binds with high affinity to 5-HT_{1A} receptors (40), and is a full agonist for this receptor (34,35). The discriminative stimulus properties of flesinoxan in rats are mediated by 5-HT_{1A} receptors (15,41,43–45). 8-OH-DPAT is a prototypical 5-HT_{1A} receptor agonist of which the discriminative stimulus properties are also mediated by 5-HT_{1A} receptors (42). It can, therefore, be argued that the 5-HT_{1A} receptor is involved in the CTA stimulus of fluvoxamine.

The highest dose of MK-212 partially prevented the fluvoxamine-induced taste aversion. MK-212 binds preferentially to 5-HT_{2C} receptors (6,21), and its discriminative stimulus properties are mediated by 5-HT_{2C} receptors (8,20). This suggests that 5-HT_{2C} receptors play a role in fluvoxamine's stimulus properties. Preexposure to DOI did not prevent this fluvoxamine-induced taste aversion. DOI binds to 5-$HT_{2A/2C}$ receptors (6) but its discriminative stimulus properties are

TABLE 1

SUMMARY OF CTA RESULTS

Compound	ED_{50} in CTA (mg/kg, SC)	Dose of Drug to Familiarize to Fluvoxamine (50 mg/kg, SC)*	Dose of Fluvoxamine (mg/kg, SC) needed to Familiarize†
Fluvoxamine	24	50	50
Fluoxetine	5	>10	50
8-OH-DPAT	0.1	0.6	n.d.
Flesinoxan	0.05	0.3	>50
DOI	0.4	—	n.d.
MK-212	3	—	n.d.

*Doses that cause a complete prevention of the fluvoxamine-induced taste aversion.
†The dose fluvoxamine needed to prevent the taste aversion induced by different drugs.
— no familiarization; n.d. not determinable.

TABLE 2

THE EFFECTS OF PREEXPOSURE (DAYS 1–4) ON THE CONDITIONED TASTE AVERSION INDUCED BY A DRUG (EXPOSURE) ON DAY 5 IS SHOWN SCHEMATICALLY

	Exposure					
Preexposure	Fluvoxamine (50 mg/kg)	Fluoxetine (10 mg/kg)	Flesinoxan (0.1 mg/kg)	8-OH-DPAT (0.22 mg/kg)	MK 212 (4.6 mg/kg)	DOI (1.0 mg/kg)
Fluvoxamine	+	+	+/−	+*	+*	+/−*
Fluoxetine	+/−	+*				
Flesinoxan	+	+				
8-OH-DPAT	+	+/−*				
MK 212	+/−	+*				
DOI	—	—*				

The dose of the drug used for exposure is given in parentheses.

+ = complete generalization; +/− = partial generalization; − = no generalization. The data with "a" are derived from (5).

mediated preferentially by 5-HT_{2A} receptors (14,36). The lack of familiarization to fluvoxamine or DOI in the CTA procedure strongly suggests that 5-HT_{2A} receptors are not involved in the stimulus properties of fluvoxamine. Previously it has been shown that MK-212 familiarizes completely to fluoxetine (5). The results show that the stimulus properties of both fluoxetine and fluvoxamine are mediated by 5-HT_{1A} and 5-HT_{2C} receptors, but, possibly, not to the same degree.

Fluoxetine has some (although rather low) affinity for the 5-HT_{2C} receptor (19,27,31,38,39), whereas fluvoxamine has not (2,7,19). However, it has been shown that fluoxetine has antagonistic effects at this receptor (27,32), whereas MK-212 behaves as an agonist for this receptor (10). Therefore, it is unlikely that a direct effect of fluoxetine at the 5-HT_{2C} receptor can explain the familiarization to MK-212. Moreover, MK-212 also familiarizes fully to paroxetine, an SSRI that does not bind to 5-HT_2 receptors (24).

For the crossfamiliarization tests of fluvoxamine and fluoxetine twice the ED_{50} dose, as determined in separate conditioned taste aversion experiments, was used as the highest dose. This way taste aversion effects appear about equally strong for both drugs. There was an asymmetrical familiarization between both SSRIs. Preexposure to fluoxetine only partially prevented the taste aversion induced by fluvoxamine, whereas preexposure to fluvoxamine completely prevented the taste aversion induced by fluoxetine, indicating that the stimulus properties of the two SSRIs are somewhat different.

We found an asymmetrical crossfamiliarization between fluvoxamine and flesinoxan, i.e., fluvoxamine preexposure did not prevent flesinoxan-induced taste aversion completely. Previously, it was found that the taste aversion induced by 8-OH-DPAT could be prevented by preexposure to fluoxetine (5). It seems plausible that preexposure to an SSRI familiarizes to most specific serotonergic receptor agonists (including flesinoxan) because it is expected that most serotonin receptors can be stimulated by an SSRI, resulting from the nonselective increase of extracellular 5-HT by blockade of the 5-HT transporter. But the effects mediated by each single receptor could be modest, and the cue evoked by 5-HT_{1A} receptor stimulation after fluvoxamine may have been too weak to familiarize to flesinoxan. At higher doses, fluvoxamine might stimulate the 5-HT_{1A} receptor enough to familiarize to flesinoxan completely. Preexposure to flesinoxan, on the other hand, could induce a strong specific stimulus that is recognized by the mice when they receive fluvoxamine on the conditioning day. Although the fluvoxamine stimulus might consist of more than solely 5-HT_{1A} receptor stimulation, the recognition of the flesinoxan 5-HT_{1A} receptor "cue" is strong enough to prevent taste aversion conditioning.

All-in-all, there is a high degree of overlap between the stimulus properties of fluvoxamine and fluoxetine, although there are some differences which emphasize the need for further studies.

There are only few reports in the literature on the discriminative properties of antidepressants. This may be due to the fact that these drugs are difficult to train as discriminative stimuli. Part of the problem with the tricyclic antidepressants is that doses required for discriminative control are often toxic after repeated administration. Jones et al. (25) reported a failure to train rats in a two-lever operant discrimination with 10 mg/kg imipramine within 60 sessions. The mortality rate with this dose was 25%. Shearman et al. (37) trained rats to discriminate 10 mg/kg desipramine. Only one-half of the subjects learned the discrimination, the other half of the animals died before generalization tests could be performed. Schechter (33) reported transfer of stimulus control in rats from imipramine (10 mg/kg) to amitriptyline and desmethylimipramine, but again, in this experiment 50% of the subjects died. These low survival rates and slow learning speed are, however, probably species dependent, because Zhang and Barrett (46) succeeded in training pigeons to discriminate 3.0 or 5.6 mg/kg imipramine from saline within an average of 26 sessions. Discrimination performance remained stable for more than 2 years, and they tested a number of compounds for generalization. The pigeons did not show any signs of toxicity over this period. This means that the pigeon offers a better model for profiling stimulus properties of tricyclic antidepressant drugs than rats.

The failure or difficulties to induce stimulus control with all kind of antidepressant drugs in rats, using two-lever drug discrimination procedures, is remarkable. We were not able to train fluvoxamine in either rats or pigeons (see introduction), whereas reports on other compounds are scarce. Jones et al. (25) did train the phenylaminoketone bupropion in rats, and obtained stimulus control within 40 sessions with high (20 mg/kg) but not with low (5.0 or 10.0 mg/kg) doses of the drug. Järbe and Archer (23) published some preliminary data on drug discrimination training with 20 mg/kg zimeldine, a 5-HT reuptake inhibitor, vs. saline. They obtained dose–response curves for 2.5–20.0 mg/kg zimeldine both under a fixed ratio (FR) 10 and a differential reinforcement of low rate (DRL) 10-s schedule of reinforcement. Generalization tests to drugs

other than the training compound were not reported. It is conceivable that the mechanism of action of antidepressants acting via inhibition of monoamine-uptake (5-HT, NA, or DA) does not lead to clear receptor activating effects in the brain. Although, for example, SSRIs enhance 5-HT release in the brain, they do so primarily at the raphé level (at least acutely), and have limited effects in the synaptic cleft (1,3). Moreover, dependent on the brain area involved, differential effects have been found (1,3). This may be one of the main factors why SSRIs (and NRIs) induce weak (or no) stimulus effects; monoaminergic receptors are not strongly enough activated to lead to discriminable effects, or have too diffuse effects over the brain leading to confusing stimuli.

Recently (29), citalopram (2.5 mg/kg, IP) was successfully trained in a two-lever, food-reinforced, drug discrimination procedure against vehicle, at a dose leading to enhanced 5-HT release in several brain regions. Two other SSRIs, sertraline and paroxetine, fully substituted for the citalopram cue. Citalopram is the most potent and selective SSRI available, and it may suggest that other SSRIs are not sufficiently potent and/or selective for the serotonergic system to induce discriminative stimuli.

Using a paradigm in mice, the crossfamiliarization CTA procedure, it appeared possible to induce discriminative effects, at least for two SSRIs, fluvoxamine and fluoxetine. Much more research is needed, using this paradigm to find out whether such effects are really mediated via the CNS or that peripheral effects are also included.

Based on the results of the present experiments in rats, and also on our previous experience with fluvoxamine as a stimulus cue in operant drug discrimination procedures, we must conclude that fluvoxamine possesses, at best, only weak, if any, stimulus properties that can acquire discriminative control over behavior in rats. However, using the crossfamiliarization CTA in mice, it appeared possible to detect stimulus properties of fluvoxamine and fluoxetine. In how far species differences play a role is unclear yet. This crossfamiliarization CTA should be tried out in rats using SSRIs. Further work is needed to find our whether different procedures or different species are important.

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