

Selective and nonselective serotonin antagonists block the aversive stimulus properties of MK212 and *m*-chlorophenylpiperazine (*m*CPP) in mice

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Received 14 June 2005; received in revised form 20 July 2005; accepted 25 July 2005

Abstract

Serotonin_{2C} (5-HT_{2C}) receptors have been implicated to treat mood disorders such as depression and anxiety. In the present study, the capacities of two 5-HT_{2C} agonists, MK212 and *m*CPP, to produce conditioned taste aversions in mice were evaluated. On two training days, Swiss-Webster male mice (19–34 g) were trained to associate the flavor of a novel solution with the injection of various doses of MK212 or *m*CPP. On two alternate training days, mice were trained to associate a different flavored solution with an injection of saline. For testing, both flavored solutions were presented simultaneously and an avoidance of the MK212 or *m*CPP-paired solution indicated conditioned taste aversion. Robust conditioned taste aversions were observed to solutions paired with 1.0 or 10 mg/kg MK212 or *m*CPP. Acquisition of conditioned taste aversions was blocked by nonselective serotonin antagonists cyproheptadine, bromo-LSD, metergoline, methysergide and mianserin. Selective 5-HT_{2B/2C} antagonist SB206,553 blocked both MK212- and *m*CPP-induced conditioned taste aversion although selective 5-HT_{2B/2C} antagonist SB200,646 only blocked *m*CPP-induced conditioned taste aversion. In a single-bottle procedure, MK212, bromo-LSD, and mianserin failed to alter acquisition rate of a LiCl-induced conditioned taste aversion. Taken together, these data indicate that the serotonin agonists MK212 and *m*CPP produce conditioned taste aversion and that these effects are mediated predominantly through 5-HT_{2C} receptors.

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Keywords: Serotonin; Conditioned taste aversion; MK212; *m*CPP; Anxiety; Mice

1. Introduction

The serotonin_{2C} (5-HT_{2C}) receptor is implicated in mood disorders such as depression, obsessive–compulsive disorder and anxiety (Baez et al., 1995; Bourin et al., 1998). For example, 5-HT_{2C} agonist *m*CPP is reported to induce mild anxiety in normal subjects and to

potentiate anxious responses in patients with generalized anxiety disorder, phobias, and panic disorder (Charney et al., 1987; Kahn et al., 1988; Sevy et al., 1994). However, in animal models, 5-HT_{2C} agonists can produce anxiolytic or anxiogenic effects depending on the test and species used. For example, in rats, *m*CPP produced anxiogenic effects in the open-field test (Guitton and Dudai, 2004), elevated T and plus-maze tests (Griebel et al., 1997; Mora et al., 1997), the Vogel drinking conflict test (Griebel et al., 1997), social interaction test (Kennett et al., 1989) and light/dark box (Bilkei-Gorzo et al., 1998). A number of antagonists

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with affinity for 5-HT_{2C} receptors such as SB200,646A, mianserin, metergoline, and cyproheptadine reversed *m*CPP-induced anxiety in the social interaction test in rats and may themselves produce anxiolytic effects in a number of these models (Griebel et al., 1997; Kennett et al., 1989, 1994a, 1994b, 1996).

In mice, *m*CPP either lacks anxiogenic effects (Dhonnchadha et al., 2003) or actually produces anxiolytic effects (Griebel, 1995). Similarly, 5-HT_{2C} non-selective antagonist mianserin and selective antagonists SB206,553 and SDZ SER082 lacked anxiolytic activity in the mouse defense test battery, four plates test, elevated plus-maze, and the light/dark choice models of anxiety (Dhonnchadha et al., 2003; Griebel et al., 1997). However, 5-HT_{2C} knock-out mice displayed decreased trait anxiety in some models, suggesting some role for 5-HT_{2C} receptors in the regulation of certain types of anxiety (Tecott, 1996). Overall, the effects of nonselective 5-HT_{2C} agonist *m*CPP and both nonselective and selective 5-HT_{2C} antagonists in the different models of anxiety are difficult to interpret for a variety of reasons such as: (1) different models of anxiety tap different ethological or conditioned fears or stresses; (2) different subtypes of 5-HT receptor and perhaps different brain regions may underlie specific anxiety responses between rats and mice, as well as strains of mice; and (3) most of the compounds studied to date do not have exclusive selectivity for 5-HT_{2C} receptors (Dhonnchadha et al., 2003).

A learning model that may be used to express the aversive properties of drugs in a variety of species is the conditioned taste aversion paradigm. Conditioned taste aversion is a classically conditioned response in which a novel flavor (conditioned stimulus) is avoided when a nausea-inducing agent (unconditioned stimulus) follows its consumption. Conditioned taste aversion is extremely robust, obtained after long delays between intake and drug administration, acquired within a few trials, and learned after drugs or doses that are innocuous or even reinforcing under other circumstances (Riley, 1998). If the organism is fluid-deprived but only offered a flavored solution that has been paired with a nausea-inducing agent, the organism can be presumed to be in a conflict between thirst, which increases drinking, and conditioned taste aversion which suppresses drinking (Ervin and Cooper, 1988). Indeed, a number of anxiolytics from a variety of drug classes will block conditioned taste aversion under certain conditions (Riley and Lovely, 1978; Ervin and Cooper, 1988).

In addition to the possible usefulness as a conflict procedure, conditioned taste aversion is a sensitive learning and memory paradigm (Welzl et al., 2001) that can be used to study the pharmacology of many agents (Riley, 1998). A number of serotonin agonists have been used as unconditioned stimuli to induce condition taste aversion in rats. For example, *l*-5-HTP (Ervin and

Cooper, 1988), *m*CPP (De Vry et al., 2000; Guitton and Dudai, 2004), fluoxetine (Prendergast et al., 1996), and fluvoxamine (Olivier et al., 1999) produce conditioned taste aversions. In the present study, we evaluated the capacity of MK212, a relatively selective 5-HT_{2C} full agonist, and *m*CPP, a nonselective 5-HT_{2C} partial agonist to induce conditioned taste aversions in mice. To speed acquisition and testing, we adopted a two-stimulus conditioned taste aversion procedure (Gommans et al., 2000) in which mice discriminated two novel flavors paired with either saline or drug (D'Mello et al., 1977; Kumar et al., 1983). We established dose–response curves for the capacity of MK212 and *m*CPP to induce conditioned taste aversions in mice. Furthermore, we evaluated a series of nonselective and selective 5-HT_{2C} antagonists as well as opioid antagonist naltrexone and 5-HT_{2A/2C} antagonist ketanserin to block the acquisition of these conditioned taste aversions. Previous investigators have suggested that a select group of 5-HT_{2A/2C} antagonists such as mianserin may impede learning while 5-HT₂ agonists may enhance learning in rabbits (Welsh et al., 1998a; Romano et al., 2000). Therefore, we examined the capacity of agonist MK212 and antagonists bromo-LSD and mianserin to alter the rate of acquisition of LiCl-induced conditioned taste aversions in mice.

2. Methods

2.1. Subjects

Male Swiss-Webster mice ($N = 280$) (Ace Animals, Inc., Philadelphia, PA) weighing between 19 and 34 g were housed individually in cages in a colony room and were maintained under a 12 h light/dark cycle. Food was freely available in the home cages and mice were water-restricted as described below. All mice were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Temple University and the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication No. 85–23, revised 1996). The highest standards of animal welfare were maintained throughout these studies and the experiments were specifically designed to reduce the number of mice required.

2.2. Apparatus

During the experiment, mice were transferred to experimental cages in a separate room. Centrifuge tubes fitted with rubber stoppers and drinking tubes were used to deliver the solutions (Allegiance Healthcare, McGraw Park, IL). The tubes were weighed on a scale set to the 1/100 g (Sartorius Laboratories, Goettingen, Germany).

2.3. Procedure

Prior to either conditioning procedure, mice were water-restricted for 23 h/day. During weekdays, mice had access to water in the centrifuge tubes for 30 min in the morning in the experimental cages (between 9:30 am and 10:30 am) and 30 min in the afternoon in the home cage (between 3:30 pm and 4:30 pm). On weekends, mice had access to water bottles in their home cages for 60 min/day. Conditioned taste aversion training began when water consumption was stable during the morning session for three consecutive days as determined by one-way analysis of variance.

2.3.1. Two-bottle, discrimination procedure

The procedure was modified from that described by Gommans et al. (2000). Mice received two injections on each of the four training days: an injection of saline or a dose of antagonist prior to drinking a flavored solution, and; an injection of MK212, *m*CPP or saline after drinking a flavored solution. Specifically, mice were injected with saline or a dose of antagonist (cyproheptadine, bromo-LSD, metergoline, methysergide, mianserin, SB200,646, SB206,533, ketanserin, or naltrexone) and placed in the experimental cages for 30 min. For two alternate training days, mice were presented with one of two novel flavored solutions (conditioned stimulus) for an additional 30 min. The flavor of the conditioned stimulus was either an almond-scented saccharin solution or a banana-scented dilute saline solution. The assignments of flavored solutions were counterbalanced within the training groups to prevent palatability preferences. In addition, tube placement (left vs. right) was also counterbalanced to avoid location preferences. Immediately after the 30 min drinking interval, the centrifuge tubes were removed and the mice were injected with MK212, *m*CPP, or saline. For each mouse, one flavored solution was paired with an injection of a dose of MK212 or *m*CPP (unconditioned stimulus) for two training days and the other flavored solution was paired with an injection of saline for two alternate training days. Each mouse completed four total training sessions [two pairings: CS⁺ (Flavor 1) + US (MK212 or *m*CPP); two pairings: CS⁻ (Flavor 2) + no US (Saline)]. After the injections of MK212, *m*CPP or saline, the mice remained in the experimental cages for 1 h before returning to their home cages. The solution bottles were weighed and recorded before and after the drinking period. Once training began, mice continued to have access to water for 30 min in their home cages and 60 min on the weekends.

Three or four days after the last training session, testing began. On the two test days, mice were injected with saline or a dose of antagonist and placed in the experimental cages. After 30 min, the mice were presented with both flavored solutions simultaneously

and allowed to drink from either tube for 30 min. No injections were administered after the test days and the mice were returned to their home cages. The positions of the two-flavored solutions were alternated on the two test days.

2.3.2. Single, bottle, conditioned taste aversion procedure

To examine the effects of an agonist, neutral antagonist, and inverse agonist on the acquisition of conditioned taste aversions, mice received injections of saline, MK212 (0.1 or 1.0 mg/kg), bromo-LSD (0.32 or 3.2 mg/kg) or mianserin (0.1 or 1.0 mg/kg). Saline, MK212, and bromo-LSD were injected 5 min prior to 60 min access to almond-saccharin solution and mianserin injected 15 min prior to 60 min access to almond-saccharin solution. After 60 min, the saccharin bottles were removed and the mice were injected with either saline or 2.4 mEq/kg LiCl, i.p. After the injections of saline or LiCl, the mice remained in the experimental cages for 1 h before returning to their home cages. Mice received LiCl pairing days for two or three days during the week alternating with 60 min access to water bottles in their home cages on the other days. The solution bottles were weighed and recorded before and after the drinking period. Once training began, mice continued to have access to water for 30 min in their home cages and for 60 min on weekends.

2.4. Drugs

The following compounds were used: D-2-bromolysergic acid diethylamine hydrogen tartrate (bromo-LSD), naltrexone HCl (supplied by the National Institute on Drug Abuse, Rockville, MD), 6-chloro-2[1-piperazinyl]pyrazine (MK212 HCl), *m*-chlorophenylpiperazine HCl (*m*CPP), lithium chloride, sodium saccharin (purchased from Sigma Aldrich, St. Louis, MO), cyproheptadine HCl, ketanserin tartrate, metergoline HCl, methysergide maleate, mianserin HCl, SB200,646 [*N*-(1-methyl-5-indolyl)-*N'*-(3-pyridyl)urea HCl] and SB206,533 [5-methyl-1-(3-pyridylcarbonyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole HCl] (Tocris Cookson Inc., Ellisville, MO). SB 206,553 was dissolved in a few drops of Tween 80 (ICI Americas, Wilmington, DE) and sterile water. SB 200,646 was dissolved in a few drops of 8.5% lactic acids and sterile water. Higher concentrations of SB 200,646 required sonication just prior to injection to maintain SB 200,646 in solution. All other drugs were dissolved in sterile water and injected intraperitoneally (i.p.) in a volume of 1.0 ml/kg. Drug doses were expressed on the basis of milligrams per kilogram of the salt.

Two-flavored solutions were used as the conditioned stimuli: almond-flavored (McCormick Flavorings, Hunt Valley, MD) 0.15% w/v saccharin solution; and

banana-flavored (McCormick Flavorings, Hunt Valley, MD) dilute 0.09% w/v sodium chloride solution. In the single-bottle experiments, 2.4 mEq/kg LiCl i.p. (0.15 M LiCl) was injected in a volume of 2.0 ml/kg, i.p., after access to almond-saccharin solution.

2.5. Data analysis

Repeated measures, one-way analyses of variance with Tukey–Kramer Multiple Comparison post hoc tests were used to compare water consumption or solution consumption across days. If water consumption was not significantly different for three consecutive days, discrimination training or conditioned taste aversion experiments could begin. In the single-bottle procedure, one-way analysis of variance was used to compare consumption between groups of mice pretreated with saline, MK212, bromo-LSD, and mianserin. In the two-bottle, discrimination experiments, dose–response data for MK212 and *m*CPP were subjected to a two-way analysis of variance with dose and solution (drug-paired vs. saline-paired) as the factors. Bonferroni post tests were used to compare differences where applicable (GraphPad Prism v. 4.0, San Diego, CA).

After training, the two test sessions were averaged together and the mean amounts of drug-paired solution were expressed as a percentage of total solution intake in the discrimination experiments. The percentages scores were analyzed with a one sample *t*-test to determine whether the % drug choice differed significantly from 50% (GraphPad Instat v. 3.01, San Diego, CA). Scores similar to 50% would indicate that conditioned taste aversion was not acquired and the mice drank both solutions equally. If % drug choice was significantly below 50%, a conditioned taste aversion was obtained.

3. Results

3.1. Dose–response curves for MK212 and *m*CPP

Agonists MK212 ($F_{(3,84)} = 3.6$; $p < 0.02$) and *m*CPP ($F_{(3,48)} = 6.7$; $p < 0.001$) dose-dependently produced conditioned taste aversions in mice (Fig. 1). The solutions associated with dose of 1.0 mg/kg MK212 ($p < 0.01$) and 10 mg/kg MK212 ($p < 0.001$) as well as 1.0 mg/kg *m*CPP ($p < 0.01$) and 10 mg/kg *m*CPP ($p < 0.001$) were consumed significantly less frequently than the solutions associated with saline when mice were presented with both solutions simultaneously on test days. Higher doses of 32 mg/kg MK212 and *m*CPP suppressed overall consumption of both the drug-paired and saline-paired solutions indicating that these doses produce an unconditioned aversion to both solutions. Based on the robust conditioned taste aversion induced

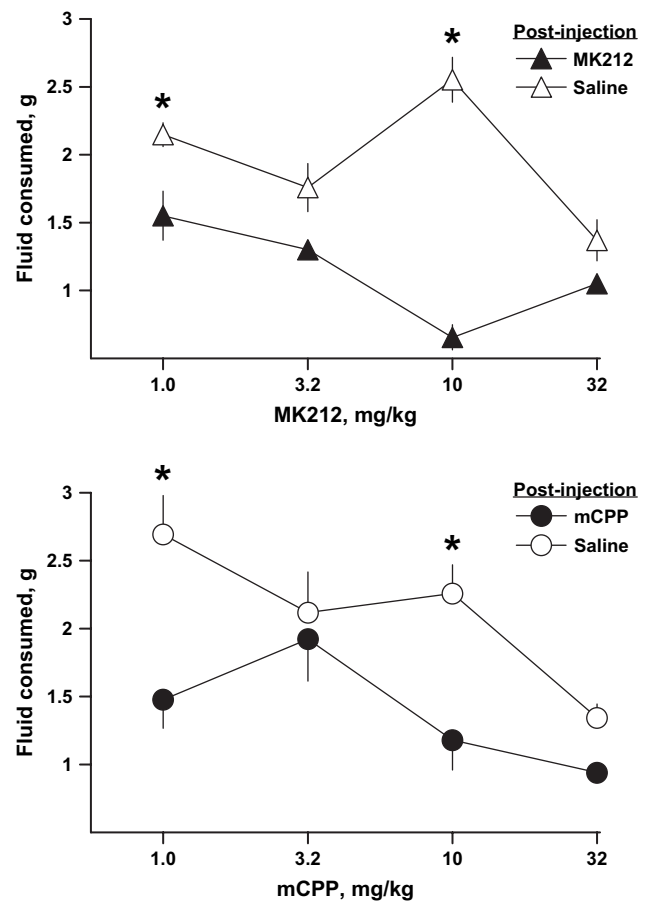


Fig. 1. Dose–response curves for MK212 (upper panels) and *m*CPP (lower panels) to induce conditioned taste aversions in mice. Ordinate: Amount of fluid consumed in grams. Both the saline-paired fluid (open symbols) and drug-paired fluid (closed symbols) were presented simultaneously. On these test days, antagonists were injected prior to fluid access but there were no injections after fluid access. Values for the two test days were averaged together for an individual mouse and then averaged into a group mean. Abscissa: Doses of MK212 and *m*CPP, in mg/kg [1.0 and 3.2 mg/kg MK212 ($N = 10$ each); 10 mg/kg MK212 ($N = 20$); 32 mg/kg MK212 ($N = 6$); 1.0 and 3.2 mg/kg *m*CPP ($N = 5$ each); 10 mg/kg *m*CPP ($N = 10$); and 32 mg/kg *m*CPP ($N = 7$)]. * Indicates a significant difference between consumption of the drug-paired solution and the saline-paired solution on test days. Vertical bars represent \pm S.E.M.

by 10 mg/kg MK212 and 10 mg/kg *m*CPP, these doses were selected for the subsequent antagonism studies.

3.2. Effects of nonselective serotonin antagonists

Doses of 10 mg/kg MK212 ($t(19) = 14$, $p < 0.0001$) and 10 mg/kg *m*CPP ($t(9) = 5.9$, $p < 0.0002$) significantly reduced the choice of the drug-paired solution to 20% and 29%, respectively, in the choice test session (Figs. 2–4). Low (0.32 mg/kg, $t(4) = 0.13$;) and high doses (10 mg/kg, $t(4) = 0.069$) of cyproheptadine blocked the acquisition of conditioned taste aversions produced by 10 mg/kg MK212, i.e., choice was not significantly different than 50% in these groups (Fig. 2,

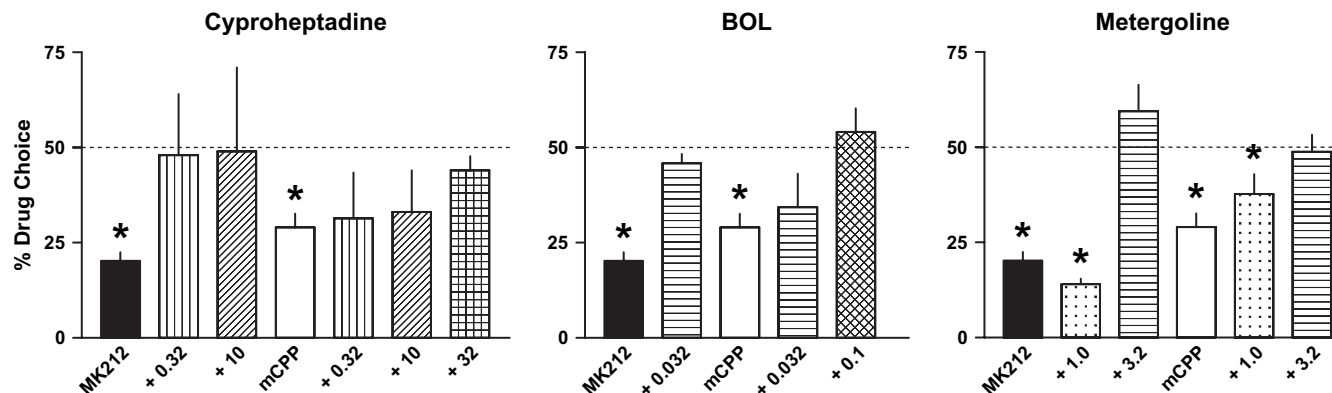


Fig. 2. Cyproheptadine (left panel), bromo-LSD (center panel), and metergoline (right panel) prevent the acquisition of MK212- and *mCPP*-induced conditioned taste aversions in mice. Ordinate: Amount of drug-paired fluid divided by the total amount of fluid consumed on the test days expressed as a percentage; 50% drug choice (dotted lines) indicates both fluids were chosen equally, i.e., no aversion to either fluid was obtained. Abscissa: pretreatment doses of antagonists in mg/kg. Data for 10 mg/kg MK212 (solid bars, $N = 20$) and 10 mg/kg *mCPP* (open bars; $N = 10$) alone are from Fig. 1. Cyproheptadine: 0.32 mg/kg ($N = 5$ each); 10 mg/kg ($N = 5$ each); 32 mg/kg ($N = 4$). Bromo-LSD: 0.032 mg/kg ($N = 7, 14$); 0.1 mg/kg ($N = 5$). Metergoline: 1.0 mg/kg ($N = 5, 10$); 3.2 mg/kg ($N = 5, 6$). * Indicates the mean consumption differs significantly from 50%, i.e., conditioned taste aversion developed to MK212 or *mCPP*. Vertical bars represent \pm S.E.M.

left panel). Similarly, low (0.32 mg/kg, $t(4) = 1.5$) and high doses (10 mg/kg, $t(4) = 1.5$; 32 mg/kg, $t(3) = 1.6$) of cyproheptadine also blocked the acquisition of conditioned taste aversions produced by 10 mg/kg *mCPP*. Bromo-LSD doses of 0.032 mg/kg ($t(6) = 1.7$; $t(13) = 1.8$) and 0.1 mg/kg ($t(9) = 0.66$) blocked acquisition of conditioned taste aversion produced by both MK212 and *mCPP*, respectively. A dose of 1.0 mg/kg metergoline ($t(4) = 24$, $p < 0.0001$; $t(9) = 2.4$, $p < 0.04$) failed to block the conditioned taste aversions produced by MK212 and *mCPP*, i.e., drug choice remained significantly less than 50%. However, higher doses of 3.2 mg/kg metergoline ($t(4) = 1.4$; $t(5) = 0.26$) did block acquisition of MK212- and *mCPP*-induced conditioned taste aversions.

Nonselective serotonin antagonists methysergide and mianserin were studied in combination with 10 mg/kg MK212 (Fig. 3). Doses of 5.6 mg/kg ($t(9) = 2.9$, $p < 0.02$) and 10 mg/kg ($t(4) = 13$, $p < 0.0002$) methysergide failed to completely block MK212 conditioned taste aversions (Fig. 3, left panel). Lower ($t(6) = 2.2$) and higher ($t(4) = 0.001$) doses of methysergide did block the acquisition of conditioned taste aversions, however. Doses of 0.032 mg/kg ($t(7) = 7.7$, $p < 0.0001$) and 1.0 mg/kg ($t(4) = 6.0$, $p < 0.004$) mianserin failed to completely block MK212-induced conditioned taste aversions (Fig. 3, right panel). Although all five mice selected the drug-paired solution between 47% and 49% in the choice test, the mean value remained significantly different than 50% due to the lack of individual

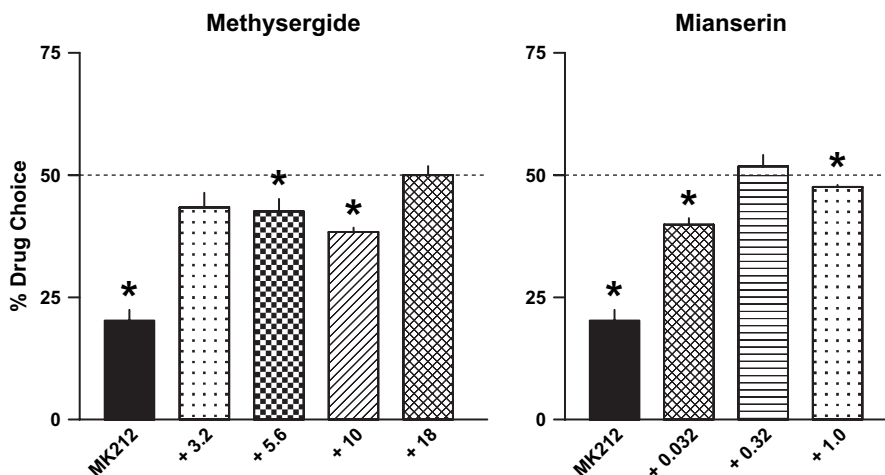


Fig. 3. Methysergide (left panel) and mianserin (right panel) prevent the acquisition of MK212-induced conditioned taste aversions in mice. Ordinate: amount of drug-paired fluid divided by the total amount of fluid consumed on the test days expressed as a percentage. Abscissa: pretreatment doses of antagonists in mg/kg. Methysergide: 3.2 mg/kg ($N = 7$); 5.6 mg/kg ($N = 10$); 10 mg/kg ($N = 5$); 18 mg/kg ($N = 5$). Mianserin: 0.032 mg/kg ($N = 8$); 0.32 mg/kg ($N = 4$); 1.0 mg/kg ($N = 5$). Other details as in Fig. 2.

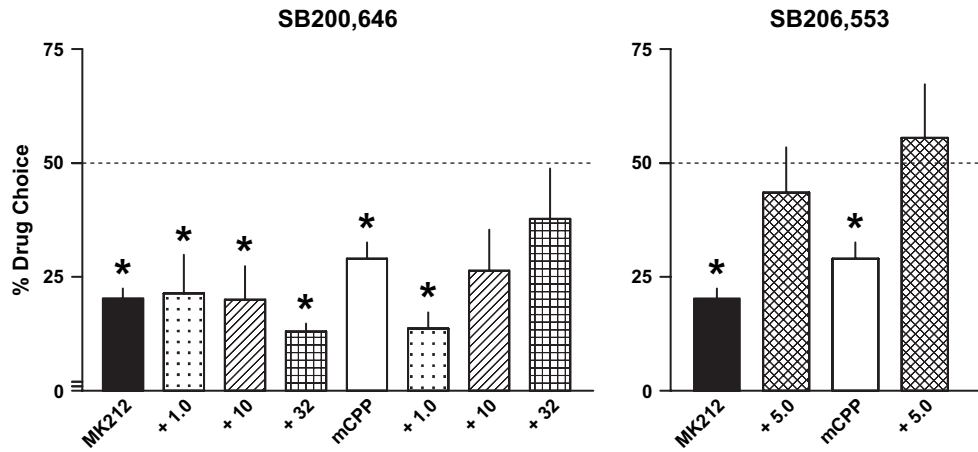


Fig. 4. Effects of selective 5HT_{2B/2C} antagonists SB200,646 (left panel) and SB206,553 (right panel) on MK212- and *m*CPP-induced conditioned taste aversions. SB200,646 blocked *m*CPP but not MK212 induced whereas SB206,553 blocked both MK212 and *m*CPP. Ordinate: amount of drug-paired fluid divided by the total amount of fluid consumed on the test days expressed as a percentage. Abscissa: pretreatment doses of antagonists in mg/kg. SB200,646: 1.0 mg/kg ($N = 5, 6$); 10 mg/kg ($N = 6, 5$); 32 mg/kg ($N = 8$ each). SB206,553: 5.0 mg/kg ($N = 6, 5$). Other details as in Fig. 2.

variability observed after the combination of 1.0 mg/kg mianserin and 10 mg/kg MK212. A dose of 0.32 mg/kg mianserin ($t(3) = 0.73$) did however, prevent the acquisition of MK212 induced conditioned taste aversions. Large doses of 3.2 mg/kg ketanserin ($t(7) = 2.8, p < 0.03$) and 1.0 mg/kg naltrexone ($t(4) = 2.7, p < 0.05$) failed to block the conditioned taste aversions produced by 10 mg/kg MK212, i.e., drug choice remained significantly less than 50% (data not shown).

3.3. Effects of selective 5-HT_{2C} antagonists

All three doses of SB200,646 (1.0 mg/kg, $t(4) = 3.4, p < 0.03$; 10 mg/kg, $t(5) = 4.1, p < 0.01$; 32 mg/kg, $t(7) = 21, p < 0.0001$) failed to prevent the acquisition of conditioned taste aversions produced by 10 mg/kg MK212, i.e., choice of the drug-paired solution was significantly less than 50% (Fig. 4, left panel). Higher doses of SB200,646 were effective, however, in preventing *m*CPP-induced conditioned taste aversions. High doses (10 mg/kg, $t(4) = 2.6$; 32 mg/kg, $t(7) = 1.1$) but not a lower dose (1.0 mg/kg, $t(5) = 10, p < 0.0002$) of SB200,646 blocked the acquisition of conditioned taste aversions produced by 10 mg/kg *m*CPP. The 5HT_{2C} antagonist SB206,553 blocked both MK212 ($t(5) = 0.65$) and *m*CPP ($t(4) = 0.48$) induced conditioned taste aversions (Fig. 4, right panel).

3.4. 5-HT_{2C} compounds and acquisition of LiCl-induced conditioned taste aversion

Neither lower (Fig. 5, upper panel) nor higher (Fig. 5, lower panel) doses of MK212, bromo-LSD, or mianserin, altered the rate of acquisition of a LiCl-induced conditioned taste aversion in mice. Injection of LiCl after 60 min access to almond-saccharin solution pro-

duced a rapid conditioned taste aversion after a single pairing ($p < 0.001$) that lasted for at least 10 trials in both experiments ($F_{(8,45)} = 8.1; p < 0.0001$; $F_{(9,45)} = 7.7; p < 0.0001$, respectively). Pretreatment doses of 0.1 and 1.0 mg/kg MK212, 0.032 and 0.32 mg/kg bromo-LSD, or 0.1 and 1.0 mg/kg mianserin failed to alter the acquisition of LiCl-induced conditioned taste aversion.

4. Discussion

Swiss-Webster mice rapidly learned to avoid novel flavored solutions paired with injections of 5-HT_{2C} agonists MK212 and *m*CPP (Fig. 1). The greatest degree of separation between consumption of the flavored solution paired with MK212 or *m*CPP and consumption of the flavored solution paired with saline was observed after doses of 10 mg/kg ($p < 0.001$). Lower doses of 1.0 mg/kg MK212 and *m*CPP also produced significant conditioned taste aversion ($p < 0.01$). The higher dose of 32 mg/kg MK212 and *m*CPP produced a generalized decrease in overall consumption on the four training days (data not shown) which was reflected in an avoidance of both flavored solutions on test days (Fig. 1). These data are the first reports to indicate dose-dependent, conditioned taste aversions produced by MK212 and *m*CPP in mice and support previous findings in other species that these 5-HT_{2C} agonists possess potential aversive stimulus effects in a variety of procedures (Alves et al., 2004; Clineschmidt et al., 1979; De Vry et al., 2000; Griebel et al., 1997; Guitton and Dudai, 2004; Sevy et al., 1994). Indeed, *m*CPP has been reported to elicit nausea as well as anxiety in humans after initial administration (Walsh et al., 1994).

There are many advantages of this two-choice training procedure (D'Mello et al., 1977; Gommans

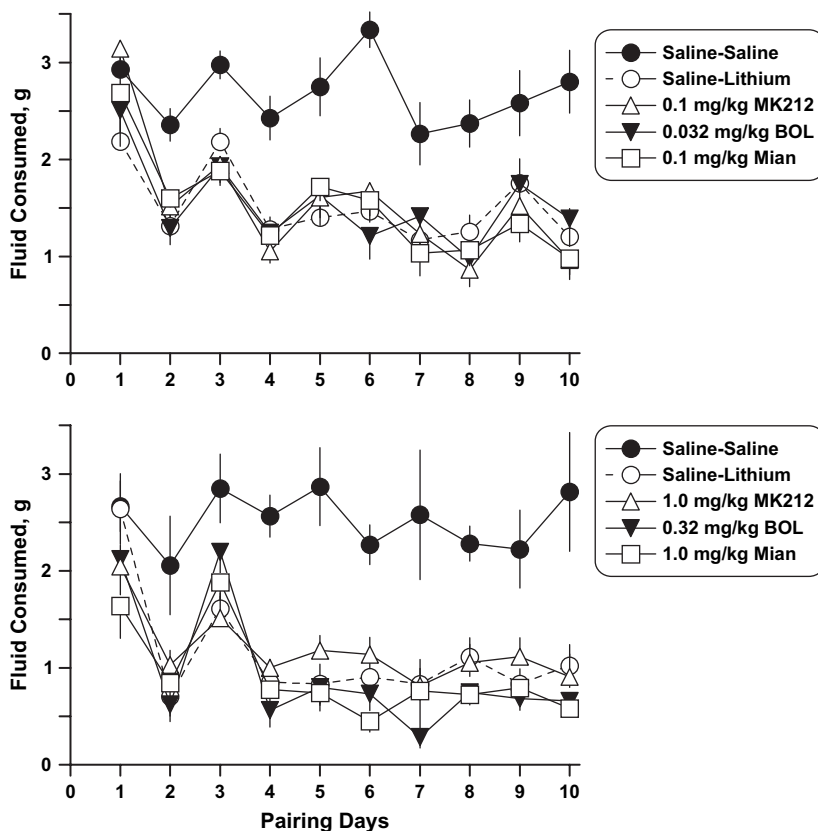


Fig. 5. Effects of saline, MK212, bromo-LSD, and mianserin on acquisition of LiCl-induced conditioned taste aversions in a single-bottle procedure. Ordinate: amount of almond-saccharin solution consumed in grams. Abscissa: almond-saccharin solution and LiCl pairing days. On alternate days, mice received 60 min access to water in the home cage (data not shown). Control mice were administered saline before and after access to almond-saccharin solution (solid circles). Top panel: Effects of saline ($N = 6$), 0.1 mg/kg MK212 ($N = 6$), 0.032 mg/kg bromo-LSD ($N = 6$), or 0.1 mg/kg mianserin ($N = 6$) pretreatments on LiCl-induced conditioned aversion. Bottom panel: effects of saline ($N = 4$), 1.0 mg/kg MK212 ($N = 6$), 0.32 mg/kg bromo-LSD ($N = 6$), or 1.0 mg/kg mianserin ($N = 6$) pretreatments on LiCl-induced conditioned aversion.

et al., 2000; Kumar et al., 1983) for studying the behavioral and pharmacological effects of serotonin agonists and antagonists in mice. Firstly, once the daily drinking schedules are stabilized, training and testing can be completed within two weeks. Secondly, graded, easily quantifiable dose–response relationships were obtained for full agonist, MK212 as well as partial agonist, *mCPP*. Thirdly, the acquisition of MK212- and *mCPP*-induced conditioned taste aversions were generally prevented in a dose-dependent manner by a series of antagonists that block 5-HT_{2C} receptors but not by antagonists such as ketanserin (Knight et al., 2004) and naltrexone (Emmerson et al., 1996) that bind to 5-HT_{2A} and opioid receptors, respectively. Therefore, this sensitive, two-bottle conditioned taste aversion procedure is well-suited to study the pharmacological relationships between 5-HT_{2C} agonists and antagonists.

All of the nonselective antagonists blocked the acquisition of MK212- and *mCPP*-induced conditioned taste aversions. In general, cyproheptadine, bromo-LSD and metergoline were equi-potent as antagonists of MK212 and *mCPP* in the present study. Methysergide and mianserin also blocked acquisition of MK212-

induced conditioned taste aversion. Taken together, these data support previous investigations indicating that cyproheptadine, bromo-LSD, metergoline, methysergide and mianserin will block 5-HT_{2C} agonists with similar potencies in other behavioral assays (Berendson et al., 1990; Gleason et al., 2001; Mansbach and Barrett, 1986; McKearney, 1990). Although, these antagonists and agonists have affinities for other receptors, their predominant actions appear to be through 5-HT_{2C} receptors (Barnes and Sharp, 1999). For example, cyproheptadine failed to block an *l*-5-HTP-induced conditioned taste aversion in rats and appeared to augment the effects of *l*-5-HTP alone (Ervin and Cooper, 1988). Indeed, in other behavioral studies, cyproheptadine, methysergide, and mianserin failed to block discriminative stimulus effects mediated by 5-HT₁ agonists such as *l*-5-HTP yet antagonized the stimulus effects mediated by 5-HT₂ agonists (Friedman et al., 1984; Walker et al., 1991; Yamamoto et al., 1991). Metergoline, with approximately equal affinity for both 5HT₁ and 5-HT₂ receptors (Hoyer et al., 1985; Kennett, 1993), antagonizes both 5HT₁ and 5-HT₂ agonists (Yamamoto et al., 1991) as well as MK212 and *mCPP*

in the present study. Although these nonselective antagonists have slightly different radioligand binding profiles (Knight et al., 2004; Nelson et al., 1999), the common receptor action that best explains the capacity to block MK212- and *m*CPP-induced conditioned taste aversions is through 5-HT_{2C} receptors.

The selective antagonist studies with SB206,533 and SB200,646 further support the role of 5-HT_{2C} receptors in the actions of MK212 and *m*CPP-induced conditioned taste aversions in mice. Selective 5-HT_{2C/2B} antagonist SB206,533 blocked both MK212 and *m*CPP-induced conditioned taste aversion. SB206,533 is a high affinity 5-HT antagonist with greater than 100-fold selectivity over 5-HT_{2A} receptors (Forbes et al., 1995; Kennett et al., 1995). Interestingly, the only antagonist that distinguished MK212 and *m*CPP in the present study was SB200,646. SB200,646 has approximately equal, modest affinity for 5-HT_{2C} and 5-HT_{2B} receptors and greater than 80-fold selectivity over 5-HT_{2A} (Forbes et al., 1993; Kennett et al., 1995). The modest affinity of SB200,646 for 5-HT_{2C} receptors may suggest that doses higher than 32 mg/kg would be required to prevent acquisition of conditioned taste aversion produced by full agonist MK212 in mice. In previous studies, SB200,646 blocked *m*CPP-induced locomotion, hypophagia, and anxiety in the rat social interaction test using similar doses to those used in the present study (Kennett et al., 1994b). The observation that SB200,646 appears to block *m*CPP but not MK212 may indicate that 5-HT_{2B} receptors play a greater role in these behavioral effects of *m*CPP relative to MK212.

5-HT_{2C} antagonists can be distinguished as neutral antagonists and inverse agonists *in vitro* (Berg et al., 1999; Schlag et al., 2004) and *in vivo* (Welsh et al., 1998b; Harvey et al., 1999). For example, some compounds such as mianserin and SB206,533 are proposed to reduce the basal activity of 5-HT_{2C} receptors and produce effects opposite to those of agonists at the receptor *in vitro* (Berg et al., 1999; Schlag et al., 2004), while 5-HT_{2C} neutral antagonist, 5-methoxygramine, has no apparent effect on basal activity (Berg et al., 1999). *In vivo* 5-HT_{2A/2C} agonists, neutral antagonists, and inverse agonists produce differential effects in the rabbit classically conditioned nictitating membrane procedure. In this assay, 5-HT_{2C} agonists such as LSD, quipazine and methylenedioxymphetamine enhanced learning, proposed neutral antagonists bromo-LSD and LY-53,857 had no effect on learning, and proposed inverse agonists mianserin and ritanserin delayed acquisition (Alhaider et al., 1993; Romano et al., 1991; Romano et al., 2000; Welsh et al., 1998b). These *in vivo* studies suggest that proposed inverse agonists SB206,533, mianserin, and cyproheptadine could impede acquisition in the present study by directly interfering with learning the association between the unconditioned stimulus (flavored solution) and conditioned stimulus (MK212 or *m*CPP).

Two observations in the present study suggest that this is probably not the case. First, both proposed neutral antagonists (bromo-LSD and methysergide) and inverse agonists (mianserin, cyproheptadine, and SB206,533) blocked agonists MK212 and *m*CPP in a similar manner. This suggests that both classes of compounds are directly competing with MK212 and *m*CPP for 5-HT_{2C} receptors as opposed to indirectly inhibiting the process of learning. Additionally, the single-bottle experiments failed to distinguish the actions of an agonist (MK212), a neutral antagonist (bromo-LSD), and an inverse agonist (mianserin) on the rate of acquisition using LiCl as the unconditioned stimulus. LiCl produces robust conditioned taste aversions in both rats and mice (Riley, 1998; Welzl et al., 2001). If mianserin, as an inverse agonist, impedes learning, the acquisition of the LiCl-induced conditioned taste aversion should be delayed. If MK212, as an agonist, facilitates learning, the acquisition of the LiCl-induced conditioned taste aversion should be more rapid. However, in the present study, the pretreatment of MK212, bromo-LSD, and mianserin failed to alter the acquisition of the LiCl-induced conditioned taste aversion under these conditions. The conditioned taste aversion paradigm may not be the optimal assay to uncover differences among agonists, inverse agonists, and neutral antagonists due to the rapid acquisition of the classically conditioned response (Riley, 1998). A better learning procedure to detect differences in learning rates may require a procedure that has a more gradual learning curve (Ma and Yu, 1993; Romano et al., 2000; Welsh et al., 1998b).

The results from the single-bottle tests also support the notion that nonselective and selective antagonists are blocking the acquisition and not the expression of MK212- and *m*CPP-induced conditioned taste aversion. In the present study, bromo-LSD and mianserin blocked MK212-induced conditioned taste aversion in the two-bottle choice procedure but failed to block LiCl-induced conditioned taste aversion in the single-bottle procedure. This pattern of antagonism is opposite to the pattern observed for traditional anxiolytics such as chlordiazepoxide, diazepam, phenobarbital, and meprobamate. Traditional anxiolytics blocked conditioned taste aversion produced by both LiCl and *l*-5HTP in a one-bottle procedure but not in a two choice procedure (Riley and Lovely, 1978; Ervin and Cooper, 1988). Conditioned taste aversions may be stronger under the two-bottle than under the one-bottle (Dragoin, 1971; Ervin and Cooper, 1988). Taken together, these studies suggest that traditional anxiolytics block the expression of conditioned taste aversion whereas the 5-HT_{2C} antagonists are blocking acquisition of MK212- and *m*CPP-induced conditioned taste aversions by competing for the same, presumably 5-HT_{2C} receptors.

Acknowledgement

This study was supported by National Institute on Drug Abuse Grant DA14673.

References

- Alhaider, A.A., Ageel, A.M., Ginawi, O.T., 1993. The quipazine- and TFMPP-increased conditioned avoidance response in rats: role of 5-HT_{1C}/5-HT₂ receptors. *Neuropharmacology* 32, 1427–1432.
- Alves, S.H., Pinheiro, G., Motta, V., Landeira-Fernandez, J., Cruz, A.P., 2004. Anxiogenic effects in the rat elevated plus-maze of 5-HT(2C) agonists into ventral but not dorsal hippocampus. *Behavioral Pharmacology* 15, 37–43.
- Baez, M., Kursar, J.D., Helton, L.A., Wainscott, D.B., Nelson, D.L., 1995. Molecular biology of serotonin receptors. *Obesity Research* 4, 441–447.
- Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. *Neuropharmacology* 38, 1083–1152.
- Berendson, H.H., Jenck, F., Broekkamp, C.L., 1990. Involvement of 5-HT-1c receptors in drug-induced penile erections in rats. *Psychopharmacology* 101, 57–61.
- Berg, K.A., Stout, B.D., Cropper, J.D., Maayani, S., Clarke, W.P., 1999. Novel actions of inverse agonists on 5-HT_{2C} receptor systems. *Molecular Pharmacology* 55, 863–872.
- Bilkei-Gorzo, A., Gyertyan, I., Levay, H.G., 1998. m-CPP induced anxiety in the light–dark box in rats – a new method for screening anxiolytic activity. *Psychopharmacology* 136, 291–298.
- Bourin, M., Baker, G.B., Bradwejn, J., 1998. Neurobiology of panic disorders. *Journal of Psychosomatic Research* 44, 163–180.
- Charney, D.S., Woods, S.W., Goodman, W.K., Heninger, G.R., 1987. Serotonin function in anxiety. II effects of the serotonin agonist mCPP in panic disorder patients and healthy subjects. *Psychopharmacology* 92, 14–24.
- Clineschmidt, B.V., Flataker, L.M., Faison, E., Holmes, R., 1979. A new in vivo model for investigating alpha1 and alpha2 receptors in the CNS: studies with mianserin. *Archives of International Pharmacodynamics* 242, 552.
- D'Mello, G.D., Stolerman, I.P., Booth, D.A., Pilcher, C.W., 1977. Factors influencing flavour aversions conditioned with amphetamine in rats. *Pharmacology, Biochemistry and Behavior* 7, 185–190.
- De Vry, J., Eckel, G., Kuhl, E., Schreiber, R., 2000. Effects of serotonin 5-HT₁ and 5-HT₂ receptor agonists in a conditioned taste aversion paradigm in the rat. *Pharmacology, Biochemistry and Behavior* 66, 797–802.
- Dhonnchadha, B.A., Bourin, M., Hascoet, M., 2003. Anxiolytic like effects of 5-HT₂ ligands on three mouse models of anxiety. *Behavioral Brain Research* 140, 203–214.
- Dragoin, W.B., 1971. Conditioning and extinction of taste aversions with variations in the intensity of the CS and UCS in two strains of rats. *Psychonomic Science* 303–305.
- Emmerson, P.J., Clark, M.J., Mansour, A., Akil, H., Woods, J.H., Medzihradsky, F., 1996. Characterization of opioid agonist efficacy in a C6 glioma cell line expressing the mu opioid receptor. *Journal of Pharmacology and Experimental Therapeutics* 278, 1121–1127.
- Ervin, G.N., Cooper, B.R., 1988. Use of conditioned taste aversion as a conflict model: effects of anxiolytic drugs. *Journal of Pharmacology and Experimental Therapeutics* 245, 137–146.
- Forbes, I.T., Ham, P., Booth, D., Martin, R., Thompson, M., Baxter, G.S., Blackburn, T.P., Glen, A., Kennett, G.A., Wood, M.D., 1995. 5-Methyl-1-(3-pyridylcarbamoyl)-2,3-dihydro-pyrrolo[2,3-*f*]indole: a selective 5-HT_{2B}/5-HT_C receptor antagonist with improved potency, selectivity and oral activity. *Journal of Medicinal Chemistry* 38, 2524–2530.
- Forbes, I.T., Kennett, G.A., Gadre, A., Ham, P., Hayward, C.J., Martin, R.T., Thompson, M., Wood, M.D., Baxter, G.S., Glen, A., 1993. *N*-(1-methyl-5-indoyl)-*N'*-(3-pyridyl)urea hydrochloride: the first selective 5-HT_{1C} receptor antagonist. *Journal of Medicinal Chemistry* 36, 1104–1107.
- Friedman, R.L., Barrett, R.J., Sanders-Bush, E., 1984. Discriminative stimulus properties of quipazine: mediation by serotonin binding sites. *Journal of Pharmacology and Experimental Therapeutics* 228, 628–635.
- Gleason, S.D., Lucaites, V.L., Shannon, H.E., Nelson, D.L., Leander, J.D., 2001. m-CPP hypolocomotion is selectively antagonized by compounds with high affinity for 5-HT(2C) receptors but not 5-HT(2A) or 5-HT(2B) receptors. *Behavioral Pharmacology* 12, 613–620.
- Gommans, J., Stolerman, I.P., Shoaib, M., 2000. Antagonism of the discriminative and aversive stimulus properties of nicotine in C57Bl/6J mice. *Neuropharmacology* 39, 2840–2847.
- Greibel, G., 1995. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacological Therapeutics* 65, 319–395.
- Griebel, G., Perrault, G., Sanger, D.J., 1997. A comparative study of the effects of selective and non-selective 5-HT₂ receptor subtype antagonists in rat and mouse models of anxiety. *Neuropharmacology* 36, 793–802.
- Guillon, M.J., Dudai, Y., 2004. Anxiety-like state associates with taste to produce conditioned taste aversion. *Biological Psychiatry* 56, 901–904.
- Harvey, J.A., Welsh, S.E., Hood, H., Romano, A.G., 1999. Effect of 5-HT₂ receptor antagonists on a cranial nerve reflex in the rabbit: evidence for inverse agonism. *Psychopharmacology* 141, 162–168.
- Hoyer, D., Engel, G., Kalkman, H.O., 1985. Molecular pharmacology of 5-HT₁ and 5-HT₂ recognition sites in rat and pig brain membranes: radioligand binding sites with [³H]5-HT, [³H]8-OH-DPAT, (-)[¹²⁵I]iodocynaocyanopindolol, [³H]mesergeline and [³H]ketanserin. *European Journal of Pharmacology* 118, 13–23.
- Kahn, R.S., Wetzler, S., Van Praag, H.M., Asnis, G.M., Strauman, T., 1988. Behavioral indications for serotonin receptor hypersensitivity in panic disorder. *Psychiatric Research* 25, 101–104.
- Kennett, G.A., 1993. 5-HT_{1C} receptors and their therapeutic relevance. *Current Opinion on Investigational Drugs* 2, 317–362.
- Kennett, G.A., Whitton, P., Shah, P., Curzon, G., 1989. Anxiogenic-like effects for mCPP and TFMPP in animal models are opposed by 5HT-1C receptor antagonists. *European Journal of Pharmacology* 164, 445–454.
- Kennett, G.A., Pittaway, K., Blackburn, T.P., 1994a. Evidence that 5-HT_{2C} receptor antagonists are anxiolytic in the rat Geiller–Seifter model of anxiety. *Psychopharmacology* 114, 90–96.
- Kennett, G.A., Wood, M.D., Geln, A., Grewal, S., Forbes, I., Gadre, A., Blackburn, T.P., 1994b. In vivo properties of SB 200646A, a 5-HT_{2C/2B} receptor antagonist. *British Journal of Pharmacology* 111, 797–802.
- Kennett, G.A., Bailey, F., Piper, D.C., Blackburn, T.P., 1995. Effect of SB 200646A a 5-HT_{2C}/5-HT_{2B} receptor antagonist in two conflict models of anxiety. *Psychopharmacology* 114, 90–96.
- Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P., Blackburn, T.P., 1996. In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. *British Journal of Pharmacology* 117, 427–434.
- Knight, A.R., Mishra, A., Quirk, K., Benwell, K., Revell, D., Kennett, G., Bickerdike, M., 2004. Pharmacological characterization of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* 370, 114–123.
- Kumar, R., Pratt, J.A., Stolerman, I.P., 1983. Characteristics of conditioned taste aversion produced by nicotine in rats. *British Journal of Pharmacology* 79, 245–253.

- Ma, T.C., Yu, Q.H., 1993. Effect of 20 (*S*)-ginsenoside-*Rg*₂ and cyproheptadine on two-way active avoidance learning and memory in rats. *Arzneimittel Forschung—Drug Research* 43, 1049–1052.
- Mansbach, R.S., Barrett, J.E., 1986. Effects of MK212 (6-chloro-2[1-piperazinyl]pyrazine) on schedule-controlled behavior and their reversal by 5-HT antagonists in the pigeon. *Neuropharmacology* 25, 13–19.
- McKearney, J.W., 1990. Effects of serotonin agonists on operant behavior in the squirrel monkey: quizapine, MK-212, trifluoromethylphenylpiperazine, and *m*-chlorophenylpiperazine. *Pharmacology, Biochemistry and Behavior* 35, 181–185.
- Mora, P.O., Netto, C.F., Graeff, F.G., 1997. Role of 5-HT-2A and 5-HT-2C receptor subtypes in the two types of fear generated by the elevated T-maze. *Pharmacology, Biochemistry and Behavior* 58, 1051–1057.
- Nelson, D.L., Lucaites, V.L., Wainscott, D.B., Glennon, R.A., 1999. Comparisons of hallucinogenic phenylisopropylamine binding affinities at cloned human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* 359, 1–6.
- Olivier, B., Gommans, J., Gugten, J., Bouwknecht, J., Herremans, A., Patty, T., Hijzen, T., 1999. Stimulus properties of the selective 5-HT reuptake inhibitor fluvoxamine in conditioned taste aversion procedures. *Pharmacology, Biochemistry and Behavior* 64, 213–220.
- Prendergast, M.A., Hendricks, S.E., Yells, D.P., Balogh, S., 1996. Conditioned taste aversion induced by fluoxetine. *Physiology and Behavior* 60, 311–315.
- Riley, A.L., Lovely, R.H., 1978. Chlorodiazepoxide induced reversal of an amphetamine-established aversion: dipsogenic effects. *Physiological Psychology* 6, 488–492.
- Riley, A.L., 1998. Conditioned flavor aversions: assessment of drug-induced suppression of food intake. In: *Current Protocols in Neuroscience*. John Wiley & Sons, Inc., pp. 8.6E.1–8.6E.10.
- Romano, A.G., Bormann, N.M., Harvey, J.A., 1991. A unique enhancement of associative learning produced by methylenediox-amphetamine. *Behavioral Pharmacology* 2, 225–231.
- Romano, A., Hood, H., Harvey, J.A., 2000. Dissociable effects of the 5-HT₂ antagonist mianserin on associative learning and performance in the rabbit. *Pharmacology, Biochemistry and Behavior* 67, 103–110.
- Sevy, S., Brown, S.L., Wetzler, S., Kotler, M., Molcho, A., Plutchik, R., Van Praag, H.M., 1994. Effects of alprazolam on increase in hormonal and anxiety levels induced by meta-chlorophenylpiperazine. *Psychiatry Research* 53, 219–229.
- Schlag, B.D., Lou, Z., Fenell, M., Dunlop, J., 2004. Ligand dependency of 5-hydroxytryptamine 2C receptor internalization. *Journal of Pharmacology and Experimental Therapeutics* 310, 865–870.
- Tecott, L., 1996. Behavioral correlates of 5-HT_{2C} receptor inactivation. *Journal of Psychopharmacology* 10, A56.
- Walker, E.A., Yamamoto, T., Hollingsworth, P.J., Smith, C.B., Woods, J.H., 1991. Discriminative-stimulus effects of quipazine and 1-5-HTP in relation to 5-HT binding sites in pigeons. *Journal of Pharmacology and Experimental Therapeutics* 259, 772–782.
- Walsh, A.E., Smith, K.A., Oldman, A.D., Williams, C., Goodall, E.M., Cowan, P.J., 1994. *m*-Chlorophenylpiperazine decreases food intake in a test meal. *Psychopharmacology* 116, 120–122.
- Welsh, S., Kachelries, W., Romano, A., Simansky, K., Harvey, J., 1998a. Effects of LSD, ritanserin, 8-OH-DPAT and lisuicide on classical conditioning in the rabbit. *Pharmacology, Biochemistry and Behavior* 59, 1–7.
- Welsh, S., Romano, A., Harvey, J., 1998b. Effects of serotonin 5HT_{2A/2C} antagonists on associative learning in the rabbit. *Psychopharmacology* 137, 157–163.
- Welzl, H., D'Adamo, P., Lipp, H.P., 2001. Conditioned taste aversion as a learning and memory paradigm. *Behavioral Brain Research* 125, 205–213.
- Yamamoto, T., Walker, E.A., Woods, J.H., 1991. Agonist and antagonist properties of serotonergic compounds in quipazine and 1-5-HTP drug discrimination in pigeons. *Journal of Pharmacology and Experimental Therapeutics* 267, 322–330.