

ORIGINAL INVESTIGATION

John H. Kehne · Robert A. Padich
Timothy C. McCloskey · Vicki L. Taylor
Christopher J. Schmidt

5-HT modulation of auditory and visual sensorimotor gating: I. Effects of 5-HT releasers on sound and light prepulse inhibition in Wistar rats

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Abstract Increasing evidence suggests an important role for serotonin (5-HT) neurons in the etiology and treatment of schizophrenia. The prepulse inhibition paradigm is used as a model for sensorimotor gating processes that are disrupted in schizophrenia. The present study assessed the general role of 5-HT in modulating auditory and visual prepulse inhibition in Wistar rats. A general overactivation of central serotonergic pathways was produced pharmacologically by four different agents which all shared the common property of releasing 5-HT, i.e., *p*-chloroamphetamine, 3,4-methylenedioxymethamphetamine, *N*-ethyl-3,4-methylenedioxymethamphetamine, or fenfluramine. Within each test session, both sound and light prepulses were used to obtain a cross-modal assessment of auditory and visual sensory gating processes. All four 5-HT releasing agents produced dose-related disruptions of auditory and visual prepulse inhibition, with *p*-chloroamphetamine being the most potent. The releasers depressed baseline to varying degrees. The α_2 -adrenergic agonist clonidine decreased baseline startle without substantially disrupting prepulse inhibition, demonstrating that the two effects were dissociable. Using fenfluramine as the most selective 5-HT releaser, two approaches were used to demonstrate 5-HT mediation of its disruptive effect on prepulse inhibition. In the first approach, the selective 5-HT uptake blocker MDL 28,618A was used to prevent fenfluramine-induced 5-HT release. In the second approach, prior exposure to a neurotoxic dose of *p*-chloroamphetamine (10 mg/kg) was used to produce a substantial, sustained depletion of cortical 5-HT, presumably reflecting the loss of 5-HT terminals. Both approaches reduced the disruptive effect of fenfluramine on auditory and visual prepulse inhibition, thereby demonstrating 5-HT mediation of these effects. Neither manipulation significantly affected the depressant effect of fenfluramine on startle baseline, demonstrating that the baseline-reducing and prepulse inhibition-reducing

effects of fenfluramine could be dissociated. MDL 28,618A alone did not affect prepulse inhibition or basal startle levels, demonstrating an important functional difference between pharmacologically induced 5-HT uptake blockade and 5-HT release. In summary, these data indicate that serotonergic overactivation can disrupt auditory and visual sensorimotor gating as measured using sound and light prepulse inhibition in rats. These data support a potential role of excessive 5-HT activity as a contributing factor to disrupted sensory gating processes seen in schizophrenia and possibly other neuropsychiatric disorders.

Key words Prepulse inhibition · Sensorimotor gating · Schizophrenia · Wistar rats · Serotonin · *p*-Chloroamphetamine, PCA · 3,4-Methylenedioxymethamphetamine, MDMA · *N*-Ethyl-3,4-methylenedioxymethamphetamine, MDEA · Fenfluramine · Clonidine

Introduction

The "dopamine (DA) hypothesis" formulated by Carlsson traditionally emphasized the critical role of dopaminergic neurons in the etiology and treatment of schizophrenia. This hypothesis is based primarily on the two findings that agents that stimulate central dopaminergic neurons can produce or exacerbate psychotic symptoms in man, and that most agents currently used as antipsychotics block D_2 dopamine receptors. Increasing preclinical and clinical evidence supports a potential contribution of serotonin (5-HT) neurons (see Meltzer et al. 1989; Meltzer 1991; Schmidt et al. 1993, 1995; Breier 1995). For example, selected 5-HT agonists can produce psychotic-like symptoms in man, and some antipsychotic agents, including the atypical antipsychotic clozapine and the new antipsychotic risperidone, affect 5-HT receptors.

An important aim of preclinical studies is to supplement existing therapeutic models with improved tests

J.H. Kehne (✉) · R.A. Padich · T.C. McCloskey
V.L. Taylor · C.J. Schmidt
Hoechst Marion Roussel Inc., 2110 E. Galbraith Road,
Cincinnati, OH 45215, USA

which more closely reflect the etiology and/or symptomatology of the clinical condition, and which therefore might improve our understanding of the disease as well as providing better predictive value for identifying and characterizing novel therapeutic agents. The prepulse inhibition model was used in the present study with this aim in mind.

A hallmark of schizophrenia is an inability properly to process ("gate") sensory information (e.g., Braff and Geyer 1989). Prepulse inhibition is a behavioral test used objectively to quantify sensory gating in the brain, and has the advantage of being measurable in both animals and in humans. In this model, a weak stimulus such as an auditory stimulus (sound) or a visual stimulus (light) presented at a short interval (e.g. ≤ 100 ms) before a strong, startle-reponse-eliciting stimulus, reduces the normal response to the strong stimulus. This reduction in response amplitude by the weak prestimulus is operationally defined as prepulse inhibition. Prepulse inhibition using an eyeblink component of the startle response is disrupted in schizophrenics (Braff and Geyer 1989; Geyer et al. 1990; Grillon et al. 1992; Judd et al. 1992; Karper et al. 1993). Furthermore, drugs which produce psychosis in humans can disrupt prepulse inhibition in humans and in animals (Mansbach et al. 1988; Swerdlow et al. 1990, 1991; Sipes and Geyer 1994).

Though the role of DA neurons has been emphasized, several previous studies suggested that excessive serotonergic activation can also disrupt prepulse inhibition. Agents which produce excess serotonergic activity such as the DA/5-HT releasing agents 3,4-methylenedioxymethamphetamine (MDMA) or *N*-ethyl-3,4-methylenedioxymethamphetamine (MDEA) tend to disrupt auditory prepulse inhibition in rats (Mansbach et al. 1989). Although the contribution of DA to these effects was emphasized, *in vitro* studies have shown that MDEA is a more potent releaser of 5-HT than DA (Schmidt 1987). Further work has implicated several 5-HT receptor subtypes in modulating prepulse inhibition. Thus, disruption of auditory prepulse inhibition is produced by agonists for the 5-HT_{1A} (Rigdon and Weatherspoon 1992) and 5-HT_{1B} (Sipes and Geyer 1994) receptors. Recently, it was reported that the hallucinogen ($\pm$)1,4-iodo-2,5-dimethoxyphenyl-2-aminopropane (DOI), which directly stimulates 5-HT_{2A/2C} receptors, disrupted auditory prepulse inhibition in rats and this effect was reversed by the 5-HT_{2A}/ α_1 -adrenergic antagonist ketanserin (Sipes and Geyer 1994).

The overall goal of this and the accompanying study (Padich et al. 1996) was to provide further insight into a possible role of serotonergic neurons in general (present study) and 5-HT_{2A} receptors in particular (Padich et al. 1996) using the prepulse inhibition paradigm in rats. The present study investigated the hypothesis that excess serotonergic activation would disrupt prepulse inhibition. The aim of the first experiment was to use pharmacological agents which release 5-HT to produce excessive stimulation of central 5-HT receptors, and to evaluate the effects on prepulse inhibition. Testing was carried out using four agents which shared the common property of being 5-HT

releasers, i.e. *p*-chloroamphetamine, PCA; MDMA; MDEA; or fenfluramine (Schmidt and Kehne 1990; Schmidt and Taylor 1990; Berger et al. 1992). It is important to emphasize that three of these agents can release DA as well, with the rank order in decreasing potency being PCA >MDMA >MDEA (Schmidt and Kehne 1990). Fenfluramine is unique amongst these four compounds in being essentially devoid of DA-releasing properties *in vitro* (Schmidt and Kehne 1990). Fenfluramine has been shown to stimulate DA release *in vivo* (Schwartz et al. 1989; Shimizu and Bray 1989), though this effect may occur indirectly as a result of increased 5-HT release (Invernizzi et al. 1989; Benloucif and Galloway 1991).

Previous work has shown that some of these 5-HT releasers (i.e. fenfluramine) tend to depress baseline startle (Kehne et al. 1992), an effect which could potentially confound the interpretation of observed reductions in prepulse inhibition. Thus, in addition to evaluating 5-HT releasers, the present study assessed the effects of a compound with a different mechanism of action but which is known to depress startle baseline. It might be possible to show that a compound could decrease startle amplitude without necessarily producing a substantial disruption of prepulse inhibition. Given previous demonstrations that the α_2 -adrenergic agonist clonidine reduces baseline acoustic startle (i.e., Davis et al. 1989), the present study evaluated the dose-related effects of clonidine on prepulse inhibition.

The aim of the second experiment was to evaluate 5-HT mediation of the disruptive effects of fenfluramine on prepulse inhibition. Fenfluramine was chosen as being the most selective 5-HT releaser of the four agents tested in the first experiment. Two approaches were taken, the first of which was to attempt to block the effects of fenfluramine by preventing uptake carrier-mediated release of 5-HT (Schmidt and Kehne 1990; Schmidt and Taylor 1990; Berger et al. 1992). The present study used MDL 28,618A, a selective blocker of the 5-HT uptake site (Cregge et al. 1990). MDL 28,618A is the dextrorotatory and active isomer of the selective 5-HT uptake blocker MDL 27,777A (Freedman et al. 1989; Schmidt and Taylor 1990). *In vitro*, MDL 28,618A inhibited [³H]5-HT uptake into rat cortical synaptosomes with an IC₅₀ of 0.23 μ M. In an *in vivo* measurement of 5-HT uptake inhibition (reversal of cortical 5-HT depletion induced by 10 mg/kg PCA), MDL 28,618A and fluoxetine had ED₅₀ values of 1.2 and 2.5 mg/kg, IP respectively. MDL 28,618A in doses of up to 40 mg/kg, had no effect on *in vivo* norepinephrine uptake inhibition as measured by reversal of xylamine-induced depletion of rat cortical norepinephrine levels. Furthermore, MDL 28,618A did not show high affinity binding to a variety of receptors *in vitro*, including α_1 - or α_2 -adrenergic, D₂ dopaminergic, 5-HT_{1A} or 5-HT_{2A} serotonergic, H₁ histaminergic or muscarinic cholinergic receptors (IC₅₀ >1 μ M). On the basis of these results, a 5 mg/kg dose of MDL 28,618A was chosen to induce selective 5-HT uptake blockade.

The second approach taken was to attempt to block the effects of a 5-HT releaser by producing a profound

and sustained depletion of central 5-HT using a dose of PCA known to be neurotoxic to 5-HT pathways (Schmidt and Kehne 1990; Schmidt and Taylor 1990). Sustained (i.e. >1 week) 5-HT depletion should theoretically decrease MDMA- and fenfluramine-induced 5-HT release, either by damaging 5-HT terminals or by greatly reducing the availability of releasable 5-HT. This diminished 5-HT release would be reflected by diminished functional effects, though it is possible that compensatory processes (i.e. development of denervation supersensitivity) could overcome this effect.

Evaluations of prepulse inhibition are typically made using an auditory stimulus such as a weak tone or noise-burst as a prepulse. However, inhibition can be produced using prepulses of other sensory modalities, including the visual modality (Hoffman and Ison 1980). Evaluating drug effects on cross-modal prepulse inhibition (i.e. prepulse inhibition produced by prepulses of different sensory modalities) might provide valuable information relevant to better understanding the neurobiology of sensory gating processes in the brain. In the present study, cross-modal prepulse inhibition was assessed using an auditory stimulus (weak noise-burst) or a visual stimulus (light flash). The test conditions were such that a rat would be exposed to both light and sound prepulses within a given session.

Finally, Wistar rats were chosen for use in all studies, given their robust levels of startle amplitude relative to other strains (unpublished observations; Rigdon and Viik 1991). A non-zero baseline is important when measuring a phenomenon such as prepulse inhibition in which the desired response is a reduction of startle amplitude from a control baseline.

Materials and methods

Animals

Subjects were experimentally naive, male albino Wistar rats which were housed in groups of four in a colony room maintained on a 14:10 light/dark cycle (lights on at 6.00 a.m.). Food and water were available *ad libitum*. Rats were acclimated for at least 1 week from the date of receipt before beginning the experiments.

Prepulse inhibition testing

Apparatus

An apparatus consisting of eight separate stabilimeters measured the amplitude of startle reflexes elicited by acoustic stimulation (see Kehne et al. 1992, for a detailed description). Movement of the platform against a transducer produced a voltage proportional to displacement and was reported in units from 0 to 4095. Each stabilimeter was housed in a ventilated, sound-attenuating chamber illuminated by dim, red-filtered light.

Stimulus parameters

The startle-eliciting stimulus was a 40-ms burst of white noise at a sound pressure level of 120 dBA. The auditory prepulse stimulus was a 20-ms, 78-dB burst of white noise presented 100 ms prior to the eliciting stimulus against a constant 64-dB background of

white noise. These parameters were selected to be similar to those used in the majority of the studies reviewed by Geyer et al. (1990).

As mentioned in the Introduction, cross-modal prepulse inhibition by a light stimulus was included to determine if the reported prepulse effects were limited to a single (auditory) modality or more generalized across two modalities. The visual prepulse was created by turning on three incandescent bulbs in the animal chamber (one mounted on the ceiling and one mounted at each end of the test chamber) 75 ms prior to the onset of the startle stimulus. The visual stimulus was turned off 75 ms later. This visual stimulus produced no perceptible or electronically measurable sound, even when the 65-dB white masking noise was turned off. The estimated rise time of the light prepulse to a peak of about 175 lux was approximately 25 ms. The resultant interval between peak intensity and startle stimulus corresponds to the 50-ms inter-stimulus interval reported in the literature to be optimal for visual prepulses (Hoffman and Ison 1980). The dim, red background illumination in the chambers averaged <2 lux.

Standard test procedure

After an appropriate pretreatment time, rats were placed in the startle chamber for a 5-min acclimation period. The rats were then presented with three pre-test trials: the first was the startle stimulus alone (no prepulse), followed 20 s later by a sound prepulse alone (no startle stimulus), and then 20 s later by a light prepulse alone (no startle stimulus). The purpose of these pre-test trials was first, to reduce the variability typically seen on the presentation of the first startle stimulus, and second, to demonstrate that the sound or light prepulses do not by themselves produce a startle response. This was followed by 10 min of prepulse inhibition testing: ten trials with a startle stimulus alone (no prepulse), ten trials with a startle stimulus preceded by a sound prepulse, and ten trials with a startle stimulus preceded by a light prepulse. These trials were presented in a pseudorandom order. The intertrial interval of 20 s resulted in a session length of about 16 min, including the 5-min acclimation period. Prepulse inhibition was operationally defined as a rat's decrease in startle amplitude for the *startle stimulus+prepulse* trial, relative to the *startle stimulus alone* trial. Prepulse inhibition was quantified as an absolute change score [$(\text{startle stimulus alone}) - (\text{startle stimulus+prepulse})$] or as a % change score (change score as a percentage of baseline startle).

Experimental design

In all experiments, independent groups of rats were used to test different pharmacological agents, or different doses of a given agent. Thus, "Treatment" was a between-subjects variable. All rats were tested only once. Within a given test session, individual scores were generated for each rat for each of three trial types: (1) acoustic startle stimulus preceded by no prepulse ("No Prepulse" condition; NP), which gives a measure of the baseline reactivity of the rat; (2) acoustic startle stimulus preceded by a sound prepulse ("Sound Prepulse" condition; SP) to produce auditory prepulse inhibition; (3) acoustic startle stimulus preceded by a light prepulse ("Light Prepulse" condition; LP) to produce visual prepulse inhibition. Thus, "Trial Type" was a within-subjects variable. Because there were eight individual startle test cages, two independent samples of eight rats each were used for each treatment condition to give a total $n=16$ per group (unless otherwise noted). For simplicity, within a given test session, all eight rats received the same treatment (i.e. vehicle or specific dose of drug). The second group of eight rats was tested at a different time of day. All testing was carried out during a 6-h window of the light cycle. Our experience has indicated that this test design results in stable, reproducible prepulse inhibition in control and drug-treated rats.

Statistical analysis

For each rat, several scores were derived from the data. "Raw scores", defined as the session means of the ten trials of each trial

type (NP, SP, and LP, as described above) were calculated. "Change Scores" were calculated as the difference between baseline (NP) session mean for a rat and either of the two prepulse condition means (i.e. NP minus SP; NP minus LP). "Percent (%) Change Scores" were calculated as the difference between baseline (NP) session mean for a rat and either of the two prepulse condition means, expressed as percent of the NP alone score (i.e. NP minus SP/NP $\times$ 100%; NP minus LP/NP $\times$ 100%). The Raw Scores and % Change Scores were each analyzed by two-factor analysis of variance (ANOVA) with "Trial Type" as a within-subjects (repeated measures) factor and "Treatment" as a between-subjects factor. Subsequent individual comparisons were made using Dunnett's *t*-test for direct comparisons to vehicle controls. When necessary, LS Means test was used to evaluate additional desired comparisons. ED₅₀ determinations were calculated using linear regression methods.

Neurochemistry

Cortex was dissected out and a subsequent assay of 5-HT was carried out using high performance liquid chromatography with electrochemical detection, as described previously (Schmidt et al. 1992).

Drugs and injection parameters

All drugs were given intraperitoneally (IP). 5-HT releasing agents used included *p*-chloroamphetamine (PCA; Sigma), 3,4-methylenedioxymethamphetamine (MDMA), *N*-ethyl-3,4-methylenedioxy-methamphetamine (MDEA), and fenfluramine. MDMA, MDEA, and fenfluramine were obtained from the National Institute of Drug Abuse (NIDA). Doses were chosen on the basis of literature references (Mansbach et al. 1989; Schmidt and Kehne 1990; Kehne et al. 1992; Kehne et al. 1996b) and on the basis of pilot studies in our lab. All releasers were racemic ($\pm$). Clonidine (Boehringer-Ingelheim) was dissolved in distilled water. MDL 28,618A ((1*R*,2*S*)-(+)-2,3-dihydro-*N*-methyl-1-[4-(trifluoromethyl)phenoxy]-1*H*-indene-2-methanamine, hydrochloride) was synthesized at Hoechst Marion Roussel, Cincinnati, Ohio. Dose (5 mg/kg, IP) and pretreat time (1 h) were chosen on the basis of internal studies indicating that these parameters produced selective 5-HT uptake blockade (see Introduction). The dose (10 mg/kg) and pretreat time (2 weeks) of PCA was chosen from studies indicating that these parameters produced substantial (>80%) and long-lasting depletion of 5-HT in forebrain projection areas (see Schmidt and Kehne 1990).

Experimental protocols

In all experiments, rats were placed singly in a portable transport device modified from a standard lab rodent housing rack, and then moved from the animal rooms into the test rooms. They remained in these cages (including following pretreatment injections) until being placed in the startle apparatus for prepulse inhibition testing as described below.

Experiment 1

This experiment assessed the effects of PCA (0.16–2.5 mg/kg), MDMA (1.25–20 mg/kg), MDEA (2.5–10 mg/kg), and fenfluramine (1.25–10 mg/kg) on auditory and visual prepulse inhibition. The rats were injected with vehicle or a single dose of the test compound, and 15 min later were placed in the startle cages, and tested for prepulse inhibition for 11 min following a 5-min acclimation period. In addition, a dose-response study was run using clonidine (0.01–0.04 mg/kg), a compound known to have depressant effects on startle baseline (i.e., Davis et al. 1989) but which has a neurochemical mechanism (α -adrenergic agonism) different from the 5-HT releasers used in this study. The purpose of this

study, as discussed in the Introduction, was to evaluate whether baseline depressant effects necessarily produce disruptions of prepulse inhibition. Doses were chosen on the basis of previous studies (i.e., Davis et al. 1989).

Experiment 2

This experiment evaluated the role of 5-HT in mediating the disruptive effects of 5 mg/kg fenfluramine on prepulse inhibition. In the first study, rats were injected with vehicle or 5 mg/kg of the 5-HT uptake blocker MDL 28,618A, and, 50 min later, were injected with vehicle or 5 mg/kg fenfluramine. 15 min later were placed in the startle cages, and tested for prepulse inhibition for 11 min following a 5-min acclimation period. In the second study, rats were injected with vehicle or 10 mg/kg PCA and returned to their home cages in the animal room. Two weeks later, the rats were injected with vehicle or 5 mg/kg fenfluramine. 15 min later were placed in the startle cages, and tested for prepulse inhibition for 11 min following a 5-min acclimation period. One week later, the rats were killed and their brains were removed, dissected regionally, and assayed for cortical 5-HT levels. The 1-week lag between testing and sacrifice was chosen to allow for recovery of any acute effects of fenfluramine on 5-HT parameters (Schmidt and Kehne 1990). Previous work indicates that 5-HT reductions produced by PCA are stable over this period of time after PCA administration (Fuller et al. 1975; Sanders-Bush and Steranka 1978). DA levels were not measured in this experiment, though previous work indicates little effect of PCA (Sanders-Bush and Steranka 1978).

Results

Experiment 1

Figures 1–4 and Table 1 summarize the dose-related effects of PCA, MDMA, MDEA, and fenfluramine on auditory and visual prepulse inhibition in Wistar rats. The left panel of each graph shows the Raw Scores, whereas the right panel shows the Change Scores.

Figure 1 shows that PCA decreased baseline acoustic startle and reduced auditory and visual prepulse inhibition.

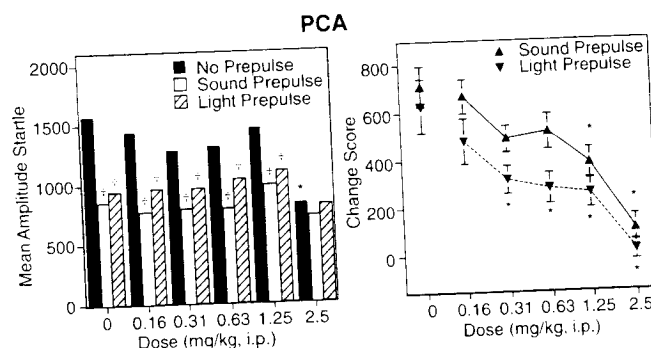


Fig. 1 Effects of IP administration of vehicle or PCA (0.16–2.5 mg/kg) on auditory or visual prepulse inhibition in Wistar rats. The left panel represents mean acoustic startle amplitude elicited in the absence of a prepulse (filled bar) or in the presence of an auditory prepulse (open bar) or visual prepulse (diagonally hatched bar). The right panel represents the Change Scores (no prepulse minus prepulse) for the sound prepulse condition (triangles with solid line) or for the light prepulse condition (inverted triangles with dotted line). Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition.

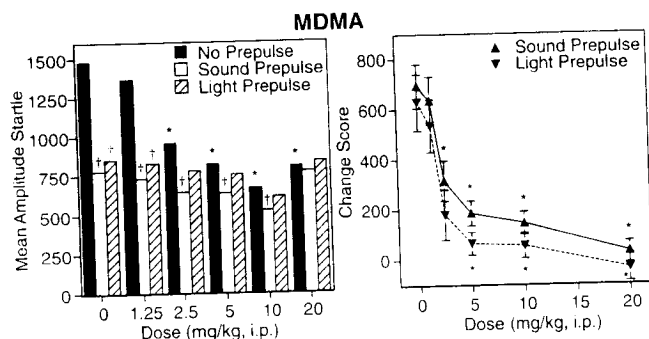


Fig. 2 Effects of IP administration of vehicle or MDMA (1.25–20 mg/kg) on auditory or visual prepulse inhibition in Wistar rats. The left panel represents mean acoustic startle amplitude elicited in the absence of a prepulse (filled bar) or in the presence of an auditory prepulse (open bar) or visual prepulse (diagonally hashed bar). The right panel represents the Change Scores (no prepulse minus prepulse) for the sound prepulse condition (triangles with solid line) or for the light prepulse condition (inverted triangles with dotted line). Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition

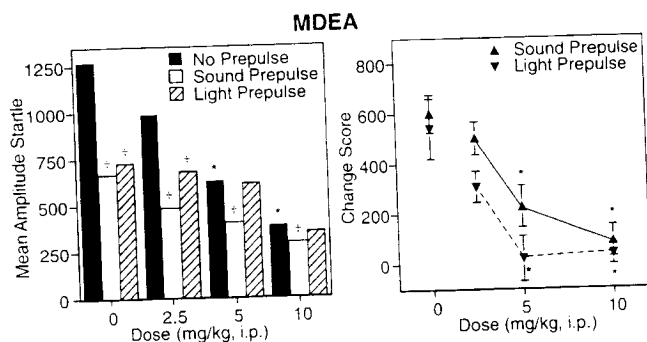


Fig. 3 Effects of IP administration of vehicle or MDEA (2.5–10 mg/kg) on auditory or visual prepulse inhibition in Wistar rats. The left panel represents mean acoustic startle amplitude elicited in the absence of a prepulse (filled bar) or in the presence of an auditory prepulse (open bar) or visual prepulse (diagonally hashed bar). The right panel represents the Change Scores (no prepulse minus prepulse) for the sound prepulse condition (triangles with solid line) or for the light prepulse condition (inverted triangles with dotted line). Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition

tion. A two-factor ANOVA of the Raw Scores revealed a significant main effect of Trial Type, $F(2,180)=136.05$, $P<0.0001$, and a Trial Type $\times$ Treatment interaction, $F(10,180)=6.01$, $P<0.001$. Individual comparisons to a rat's own NP condition (Dunnett's test) revealed statistically significant auditory and visual prepulse inhibition in all dose groups with the exception of the 2.5 mg/kg dose. The 2.5 mg/kg dose also significantly depressed baseline relative to vehicle controls. A two-factor ANOVA of the % Change Scores revealed significant main effects of Trial Type, $F(1,90)=17.62$, $P<0.0001$ and Treatment $F(5,90)=8.89$, $P<0.0001$. Dunnett's test on the Change Scores or % Change Scores revealed significant differences relative to vehicle-injected controls for

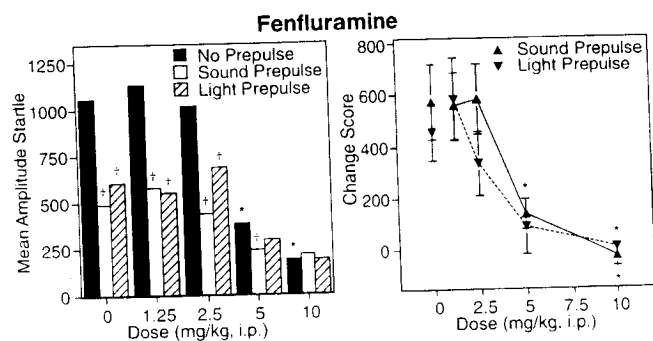


Fig. 4 Effects of IP administration of vehicle or fenfluramine (1.25–10 mg/kg) on auditory or visual prepulse inhibition in Wistar rats. The left panel represents mean acoustic startle amplitude elicited in the absence of a prepulse (filled bar) or in the presence of an auditory prepulse (open bar) or visual prepulse (diagonally hashed bar). The right panel represents the Change Scores (no prepulse minus prepulse) for the sound prepulse condition (triangles with solid line) or for the light prepulse condition (inverted triangles with dotted line). Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition

auditory prepulse inhibition for the 1.25 (Change only) and 2.5 mg/kg doses, and for the 0.31 (Change only), 0.63 (Change only), 1.25, and 2.5 mg/kg doses for visual prepulse inhibition. Thus, these analyses support the conclusion that PCA significantly disrupted auditory and visual prepulse inhibition.

Figure 2 shows that MDMA decreased baseline startle and reduced auditory and visual prepulse inhibition. A two-factor ANOVA of the Raw Scores revealed significant main effects of Trial Type, $F(2,180)=57.69$, $P<0.0001$, and a Trial Type $\times$ Treatment interaction, $F(10,180)=8.16$, $P<0.0001$. Dunnett's test revealed significant auditory prepulse inhibition at all doses except 20 mg/kg, and significant visual prepulse inhibition at the 1.25 mg/kg dose. All doses except 1.25 mg/kg significantly decreased baseline startle. A two-factor ANOVA of the % Change Scores revealed significant main effects of Trial Type, $F(1,90)=11.60$, $P<0.001$, and of Treatment, $F(5,90)=4.07$, $P<0.0022$. Dunnett's Test on the Change Scores or % Change Scores revealed significant differences relative to vehicle-injected controls for auditory prepulse inhibition for the 2.5 (Change only), 5.0 (Change only), 10, and 20 mg/kg doses, and for the 2.5, 5.0 (Change only), and 20 mg/kg doses for visual prepulse inhibition. These results support the conclusion that MDMA reduced auditory and visual prepulse inhibition.

Figure 3 shows that MDEA decreased baseline startle and reduced auditory and visual prepulse inhibition. A two-factor ANOVA of the Raw Scores revealed significant main effects of Trial Type, $F(2,88)=44.29$, $P<0.0001$, and Treatment, $F(3,44)=4.78$, $P<0.0057$, and a significant interaction, $F(6,88)=7.22$, $P<0.0001$. Dunnett's test revealed significant auditory prepulse inhibition at all doses, and significant visual prepulse inhibition at the 2.5 mg/kg dose. The 5 and 10 mg/kg doses significantly decreased baseline startle. A two-factor

Table 1 Effects of PCA, MDMA, MDEA, fenfluramine, or clonidine on auditory and visual prepulse inhibition in Wistar rats

5-HT agonist	Dose (mg/kg)	n	No prepulse±SEM (baseline)	Auditory			Visual		
				Pre-pulse	Change ±SEM	% Change ±SEM	Pre-pulse	Change ±SEM	% Change ±SEM
PCA	Vehicle	16	1573±81	865	708±85	46±5	946	626±113	39±7
PCA	0.16	16	1443±100	779	664±69	46±4	964	479±95	34±6
PCA	0.31	16	1282±89	797	486±56	42±6	969	314±58*	27±6
PCA	0.63	16	1316±81	800	516±73	42±6	1038	278±61*	23±5
PCA	1.25	16	1362±120	982	381±65*	29±5	1106	256±62*	18±5*
PCA	2.5	16	831±90*	725	106±55*	5±7*	816	15±43*	3±6*
MDMA	Vehicle	16	1481±90	788	693±87	49±6	853	628±111	44±7
MDMA	1.25	16	1369±128	733	636±96	46±6	835	534±104	28±12
MDMA	2.5	16	964±152*	648	316±79*	32±5	783	181±99*	-19±30*
MDMA	5.0	16	826±135*	640	185±50*	26±7	759	66±44*	4±6
MDMA	10.0	16	670±99*	525	145±46*	21±12*	615	56±50*	9±11
MDMA	20.0	16	805±134*	776	29±42*	1±7*	839	-34±55*	-10±9*
MDEA	Vehicle	12	1267±152	666	601±76	55±7	727	540±118	44±8
MDEA	2.5	12	987±127	485	501±65	53±4	680	307±64	30±7
MDEA	5.0	12	630±123*	403	227±82*	31±9	612	18±90*	5±13*
MDEA	10.0	12	383±82*	297	86±63*	-3±17*	348	35±41*	-8±15*
Fenfluramine	Vehicle	8	1063±236	493	571±146	53±7	609	454±107	47±8
Fenfluramine	1.25	8	1135±183	580	556±129	50±8	553	582±161	49±10
Fenfluramine	2.5	8	1022±203	441	582±137	47±12	690	333±125	30±18
Fenfluramine	5.0	8	382±173*	246	136±57*	32±13	296	86±106	-7±22
Fenfluramine	10.0	8	190±78*	217	-27±42*	-12±17*	187	3±20*	-15±18*
Clonidine	Vehicle	16	1317±136	747	569±78	48±6	668	648±99	55±7
Clonidine	0.01	8	1177±134	587	590±154	53±12	673	504±111	47±10
Clonidine	0.02	8	889±161	387	502±107	62±10	414	475±82	59±7
Clonidine	0.04	16	603±101*	227	376±60	66±5	313	290±50*	54±7

* $P < 0.05$ vs. Vehicle controls

ANOVA of the % Change Scores revealed significant effects of Trial Type, $F(1,44)=10.80$, $P < 0.002$ and Treatment, $F(3,44)=6.49$, $P < 0.001$. Dunnett's test on the Change Scores or % Change Scores revealed significant differences relative to vehicle-injected controls for the 5.0 (Change only) and 10 mg/kg doses for auditory prepulse inhibition, and for the 5.0 and 10.0 mg/kg doses for visual prepulse inhibition. These results indicate that MDEA reduced auditory and visual prepulse inhibition.

Figure 4 shows that fenfluramine decreased baseline startle and reduced auditory and visual prepulse inhibition. A two-factor ANOVA of the Raw Scores revealed significant main effects of Trial Type, $F(2,70)=28.56$, $P < 0.0001$, and Treatment, $F(4,35)=3.55$, $P < 0.016$. Dunnett's test revealed significant auditory prepulse inhibition at all doses 10 mg/kg, and significant visual prepulse inhibition the 1.25 and 2.5 mg/kg doses. All doses except 1.25 and 2.5 mg/kg significantly decreased baseline startle. A two-factor ANOVA of the % Change Scores revealed a significant main effect of Treatment, $F(4,35)=6.31$, $P < 0.0006$. Dunnett's test on the Change Scores or % Change Scores revealed significant differences relative to vehicle-injected controls for auditory prepulse inhibition for the 5.0 (Change only) and 10 mg/kg doses, and for the 10 mg/kg dose for visual prepulse inhibition. These data support the conclusion that

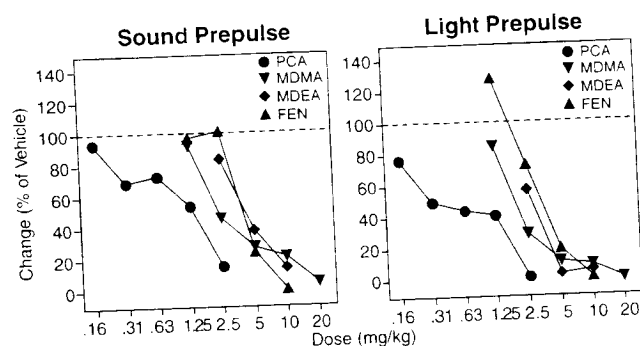


Fig. 5 Summary of the effects of PCA, MDMA, MDEA, and fenfluramine on auditory and visual prepulse inhibition in Wistar rats. Each point represents the mean Change Score for each group, calculated as % of the Vehicle Change Score mean

fenfluramine disrupted auditory and visual prepulse inhibition.

Figure 5 summarizes the disrupting effects of the four 5-HT releasers on auditory prepulse inhibition (Sound Prepulse; left panel) and on visual prepulse inhibition (Light Prepulse; right panel) using Change Scores expressed as % of vehicle control. Two points are worth noting from this figure. First, all four agents were able to completely or nearly completely disrupt auditory or visual prepulse inhibition. Second, PCA appeared to be the

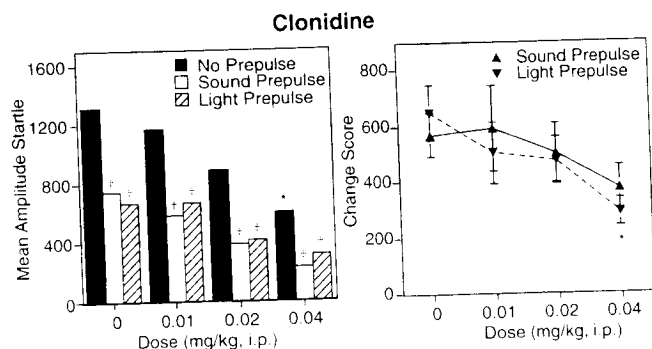


Fig. 6 Effects of IP administration of vehicle or clonidine (0.01–0.04 mg/kg) on auditory or visual prepulse inhibition in Wistar rats. The left panel represents mean acoustic startle amplitude elicited in the absence of a prepulse (filled bar) or in the presence of an auditory prepulse (open bar) or visual prepulse (diagonally hashed bar). The right panel represents the Change Scores (no prepulse minus prepulse) for the sound prepulse condition (triangles with solid line) or for the light prepulse condition (inverted triangles with dotted line). Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition

most potent of the four agents in disrupting auditory or visual prepulse inhibition. ED_{50} calculations (mg/kg, 95% confidence limits) were as follows: PCA: Sound, 0.97 (0.69–1.50); Light, 0.46 (0.26–0.69); MDMA: Sound, 3.28 (2.22–4.43); Light, 2.15 (1.07–3.25); MDEA: Sound, 4.53 (3.19–6.07), Light, 2.32 (0.21–3.73); fenfluramine: Sound, 3.84 (2.70–5.68); Light, 3.85 (2.47–6.37). Thus, PCA was 3.3–4.7 times more potent than the other agents in disrupting auditory prepulse inhibition, and 4.7–8.4 times more potent in disrupting visual prepulse inhibition.

Figure 6 and Table 1 show the effects of a α_2 -adrenergic agonist clonidine on auditory and visual prepulse inhibition. A two-factor ANOVA of the Raw Scores revealed significant main effects of Trial Type, $F(2,88)=93.79$, $P<0.0001$, and Treatment, $F(3,44)=4.14$, $P<0.012$, and a significant Trial Type $\times$ Treatment interaction, $F(6,88)=2.32$, $P<0.04$. Dunnett's test revealed significant auditory and visual prepulse inhibition at all doses. The 0.04 mg/kg dose significantly decreased baseline startle. A two-factor ANOVA of the % Change Scores revealed no significant terms. Dunnett's test on the Change Scores or % Change Scores revealed a significant difference relative to vehicle-injected controls for visual prepulse inhibition for the 0.04 mg/kg dose (Change only).

It should be noted that the disruption of visual prepulse inhibition only occurred at one dose of clonidine (0.04 mg/kg), and statistical significance was attained when Change Scores (but not % Change Scores) were analyzed. Furthermore, the reduction by 0.04 mg/kg was not complete. Thus, 0.04 mg/kg clonidine reduced baseline to 45% of controls, and visual prepulse inhibition was reduced to 45%. These numbers can be compared to doses of selected 5-HT releasers that produced about a 50% reduction in baseline: 10 mg/kg MDMA reduced

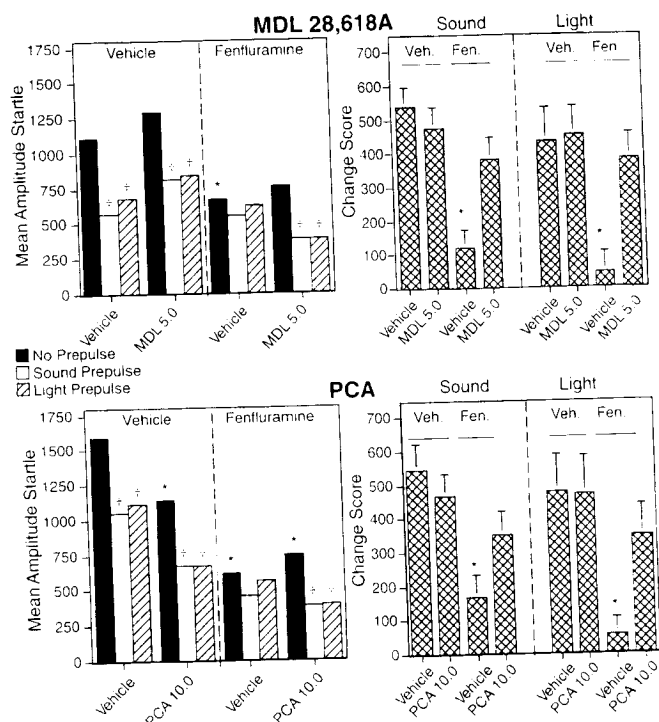


Fig. 7 Top two panels, effects of IP administration of the 5-HT uptake blocker MDL 28,618A (5 mg/kg) or vehicle 50 min prior to treatment with 5 mg/kg fenfluramine or vehicle on auditory or visual prepulse inhibition in Wistar rats. The left side of each panel represents mean acoustic startle amplitude elicited in the absence of a prepulse (filled bar) or in the presence of an auditory prepulse (open bar) or visual prepulse (diagonally hashed bar). The right side of each panel summarizes the mean Change Scores (no prepulse condition minus prepulse condition) for each group of auditory prepulse inhibition or visual prepulse inhibition. Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition. Bottom two panels, effects of IP administration of the 5-HT neurotoxin *p*-chloroamphetamine (PCA; 10 mg/kg) or vehicle 2 weeks prior to treatment with 5 mg/kg fenfluramine or vehicle on auditory or visual prepulse inhibition in Wistar rats. Bars represent scores as described above. Stars represent significant differences from vehicle-injected controls. Crosses indicate significant differences from No Prepulse Condition

baseline to 45% of controls, but reduced visual prepulse inhibition to 9%; 5.0 mg/kg MDEA, 50%, 3%; and 2.5 mg/kg PCA, 57%, 2%. A two-way ANOVA on the Change Scores of these doses revealed significant main effects of Trial Type, $F(1,56)=17.28$, $P<0.0001$, and Treatment, $F(3,56)=5.98$, $P<0.0013$. A two-way ANOVA comparing the % Change Scores of these groups revealed significant main effects of Trial Type, $F(1,56)=8.19$, $P<0.0001$, and Treatment, $F(3,56)=9.97$, $P<0.0001$. Dunnett's test on the Change Scores or the % Change Scores revealed that the clonidine reduction of visual prepulse inhibition was significantly less relative to the reductions produced by MDMA, MDEA, or PCA. Taken together, these analyses support the conclusion that a substantial depressant effect on startle baseline by a drug is not necessarily accompanied by a substantial disruption of auditory or visual prepulse inhibition.

Table 2 Effects of MDL 28,618A (5 mg/kg), or PCA (10 mg/kg; 2 weeks before) on fenfluramine (5.0 mg/kg)-disrupted auditory and visual prepulse inhibition in Wistar rats

Treatment A dose (mg/kg)	Treatment B dose (mg/kg)	n	No prepulse ± SEM (baseline)	Auditory			Visual		
				Pre-pulse	Change ± SEM	% Change ± SEM	Pre-pulse	Change ± SEM	% Change ± SEM
Vehicle	Vehicle	16	1117±77	576	539±75	51±5	681	435±96	40±8
MDL 28.618 5.0	Vehicle	16	1293±116	819	467±65	38±4	842	452±83	38±6
Vehicle	Fenfluramine 5.0	16	678±120*	559	169±64*	10±8	633	45±60*	-11±11*
MDL 28.618 5.0	Fenfluramine 5.0	16	767±114	385	353±66	29±24	386	382±77	50±7
Vehicle	Vehicle	16	1593±120	1054	539±75	38±6	1113	480±107	33±7
PCA 10.0	Vehicle	15	1146±135*	679	467±65	47±6	675	471±13	44±9
Vehicle	Fenfluramine 5.0	16	625±111*	455	169±64*	27±9	567	58±48*	4±10
PCA 10.0	Fenfluramine 5.0	16	743±104*	390	353±66	46±6	395	348±91	21±20

* $P < 0.05$ vs. Vehicle/Vehicle controls

Table 3 Mean cortical tissue levels (ng/g±SEM) of 5-HT and 5-HIAA in Wistar rats treated with 10 mg/kg PCA or vehicle 3 weeks prior to death. One week prior to death, rats were tested for prepulse inhibition following administration of either vehicle or 5.0 mg/kg fenfluramine (FEN)

Treatment	n	5-HT ng/g	SEM	% of Control	SEM	5-HIAA ng/g	SEM	% of Control	SEM
Vehicle	16	328.04	12.56	100	4	272.86	23.22	100	9
+Vehicle	16	287.39	8.53	88	3	229.97	7.00	84	3
Vehicle +Fen	15	54.10*	3.75	16	1	21.97*	2.35	8	1
PCA +Vehicle	15	50.60*	4.00	15	1	24.20*	4.24	9	2
PCA +Fen									

* $P < 0.05$ vs. Vehicle controls

Experiment 2

Figure 7 and Table 2 shows the effects of pretreatment with either the 5-HT uptake blocker MDL 28,618A (5 mg/kg, 1 h before testing; top panels) or with a neurotoxic dose of PCA (10 mg/kg, 2 weeks before testing; bottom panels) on the disruption of prepulse inhibition produced by 5 mg/kg fenfluramine. The left panels show the Raw Scores, and the right panels summarize the Change Scores for this experiment.

For the MDL 28,618A experiment, consistent with experiment 1, 5.0 mg/kg fenfluramine depressed startle amplitude and decreased auditory and visual prepulse inhibition relative to vehicle injected controls. Pretreatment with MDL 28,618A alone did not either depress startle baseline, nor did it disrupt prepulse inhibition. MDL 28,618A reversed the decrease in prepulse inhibition produced by fenfluramine without antagonizing the fenfluramine-induced decrease in baseline. A two-factor ANOVA of the Raw Scores revealed significant main effects of Trial Type, $F(2,120)=70.17$, $P < 0.0001$, and Treatment, $F(3,60)=4.88$, $P < 0.0042$, and a significant interaction, $F(6,120)=5.05$, $P < 0.0002$. Dunnett's test revealed statistically significant auditory and visual prepulse inhibition in all groups with the exception of the vehicle/fenfluramine group. Baseline startle was also significantly depressed in the vehicle/fenfluramine treated group. A two-factor ANOVA of the % Change scores revealed a significant main effect of Treatment, $F(3,60)=6.14$, $P < 0.001$. Dunnett's test on the Change

Scores or % Change Scores revealed significant differences relative to vehicle/vehicle controls for only the vehicle/fenfluramine group for auditory prepulse inhibition (Change only) and visual prepulse inhibition.

For the PCA experiment (bottom panels of Fig. 7 and Table 2), it is seen that again fenfluramine depressed startle baseline and disrupted auditory and visual prepulse inhibition. Rats which had been exposed to 10 mg/kg PCA 2 weeks before showed reduced startle amplitudes relative to vehicle-exposed controls. (This is reminiscent of reduced acoustic startle amplitude seen in rats which were depleted of central 5-HT by prior-ICV administration of the neurotoxin 5,7-dihydroxytryptamine, as reported by Kehne et al. 1992). Furthermore, the magnitude of auditory or visual prepulse inhibition as reflected by the Change Scores was not diminished in these rats. The fenfluramine-induced disruption of either auditory or visual prepulse inhibition was reversed in the PCA-exposed rats. Again, similar to the MDL 28,618A experiment, the depressant effect of fenfluramine on startle baseline did not appear to be altered in the PCA-exposed rats. Thus, for both MDL 28,618A-treated and for PCA-exposed rats, fenfluramine-disrupted prepulse inhibition, but not fenfluramine-reduced baseline, was attenuated.

A two-factor ANOVA of the Raw Scores revealed significant main effects of Trial Type, $F(2,118)=51.88$, $P < 0.0001$, and Treatment, $F(3,59)=9.94$, $P < 0.0001$, and a significant interaction, $F(6,118)=3.39$, $P < 0.004$. Dunnett's test revealed significant visual prepulse inhibition

in all groups except the vehicle/fenfluramine group. Baseline startle was significantly decreased in the three other Treatment groups relative to vehicle/vehicle controls. A two-factor ANOVA of the % Change Scores revealed a significant main effect of Trial Type, $F(1,59)=4.68$, $P<0.035$, and a Treatment effect that approached significance, $F(3,59)=2.42$, $P<0.075$. Dunnett's test on the Change Scores or % Change Scores revealed significant differences in auditory and visual prepulse inhibition for the vehicle/fenfluramine group, as compared to the vehicle/vehicle controls (Change Scores only).

Table 3 shows the results of the neurochemical assays of vehicle and PCA-treated rats. PCA pretreatment 3 weeks before death resulted in approximately an 80% reduction in cortical 5-HT or 5-HIAA levels, indicating that the desired level of depletions had been achieved. In summary, blockade of the uptake carrier by MDL 28,618A or central depletion of 5-HT with PCA prevented the disruptive effects of 5-HT releasers on auditory and visual prepulse inhibition, supporting the conclusion that 5-HT mediated these effects.

Discussion

The first experiment found that PCA, MDMA, MDEA, and fenfluramine consistently disrupted auditory and visual prepulse inhibition in Wistar rats. The second experiment found that the fenfluramine disruptions of auditory and visual prepulse inhibition were prevented by either neurotoxin-induced 5-HT depletion or by 5-HT uptake blockade. Thus, agents which share the property of releasing 5-HT disrupt cross-modal prepulse inhibition, and at least for fenfluramine, the data suggest that this disruption involves 5-HT neurons.

These data support the findings of previous studies in which MDMA and MDEA were reported to disrupt auditory prepulse inhibition (Mansbach et al. 1989). The present study increases the generality of these findings to include the Wistar strain and visual prepulse inhibition. The reported findings with PCA and fenfluramine on cross-modal prepulse inhibition are novel.

The present study focused on identifying a possible contribution of 5-HT to the fenfluramine-induced disruption of prepulse inhibition. MDMA and MDEA, agents which are psychoactive agents in humans (Greer and Tolbert 1986; Peroutka et al. 1988; Climko et al. 1989), can release DA, as can PCA (Schmidt and Kehne 1990). In the absence of mechanistic studies, no conclusions can be made regarding 5-HT mediation of the effects of MDMA, MDEA, and PCA.

Furthermore, though fenfluramine does not have notable DA releasing effects in vitro (Schmidt and Kehne 1990), in vivo studies have demonstrated stimulation of DA release after acute administration (Schwartz et al. 1989; Shimizu and Bray 1989). Thus, 5-HT release by fenfluramine could be indirectly stimulating DA release (Invernizzi et al. 1989; Benloucif and Galloway 1991). The manipulations used in the present experiment (PCA

destruction of 5-HT terminals; 5-HT uptake blockade) could achieve their effects by indirectly reducing fenfluramine-induced DA release. However, Padich et al. (1996) report that the DA antagonist haloperidol does not attenuate the disruption of prepulse inhibition produced by fenfluramine, suggesting that, under these experimental conditions, DA release onto D_1 receptors does not contribute to the observed disruption of prepulse inhibition. Padich et al. (1996) reported similar findings for MDMA disrupted prepulse inhibition.

Dose and time factors also need to be considered before ruling out a role of DA. For example, in vivo microdialysis studies measuring DA and 5-HT release (Yamamoto and Spanos 1988; Hutson and Curzon 1989) or behavioral studies (Schechter 1988) have reported different time courses of 5-HT and DA mediated effects. The present studies focused on early time points (i.e. <30 min post-injection), times at which 5-HT release might be expected to be maximal.

Interpretation of the prepulse inhibition data is complicated by the finding that the 5-HT releasing agents had varying degrees of depressant effects on baseline acoustic startle amplitude. Thus, it could be argued that the reduction in prepulse inhibition results from a measurement problem associated with a low baseline level of startle. Two lines of evidence are relevant to this issue. First, other experiments (Padich 1993) found that 10 mg/kg of either PCA or MDMA tested in a different rat strain (Charles River CD) blocked prepulse inhibition without significantly depressing baseline startle. Furthermore, experiment 2 is an example in which specific manipulations (5-HT uptake blockade; lesion of 5-HT terminals) reversed fenfluramine-induced disruptions of prepulse inhibition without concomitantly reducing its depressant effect on baseline. Thus, the baseline- and prepulse inhibition-lowering effects of fenfluramine could be dissociated, a finding which is consistent with literature reports for effects of 5-HT releasers on baseline startle (Kehne et al. 1992) or on locomotor activity (Callaway et al. 1993).

A second line of evidence indicates that drugs that work through different neurochemical mechanisms can depress baseline without fully disrupting prepulse inhibition. In the present study, the α_2 -adrenergic agonist clonidine was shown to decrease startle baseline without significantly disrupting auditory prepulse inhibition (Fig. 6), demonstrating that the two effects can be dissociated. The high dose of clonidine did appear marginally to disrupt visual prepulse inhibition, though the magnitude of this effect was significantly less than that produced by doses of the 5-HT releasers which produced a comparable decrease in startle baseline. It should also be noted that clonidine can, depending upon the dose, increase or decrease 5-HT transmission (Mongeau et al. 1994), presumably by an indirect mechanism involving presynaptic α_2 -adrenergic heteroreceptors on 5-HT neurons (Trendelenburg et al. 1994). This action might account for the slight effect of clonidine seen on visual prepulse inhibition.

Another example of compounds which depress baseline startle without fully disrupting prepulse inhibition are certain competitive NMDA antagonists (Mansbach 1991; Kehne et al. 1994). Taken together, these data indicate that the effects of the releasers seen in the present studies probably reflect disrupted prepulse inhibition, though a possible contribution of baseline depressant effects cannot be completely ruled out.

A 5-HT uptake blocker might be expected to have functional effects similar to those of a 5-HT releaser, since both agents increase the availability of 5-HT in the synapse. However, as seen in Fig. 7, administration of the 5-HT uptake blocker MDL 28,618A alone did not disrupt prepulse inhibition, nor did it depress startle baseline, effects in contrast to those of the 5-HT releasers. Thus, these data demonstrate a clear difference in the functional consequences of these two classes of agents. One explanation for these differences is that the doses of fenfluramine (or other 5-HT releasers) used achieved a much greater elevation of synaptic 5-HT and, by inference, a greater degree of postsynaptic versus presynaptic receptor stimulation. *In vivo* microdialysis studies in Wistar rats could help address this question.

In addition to supporting previously reported effects of 5-HT releasers on auditory prepulse inhibition, the present studies reported disruptive effects on visual prepulse inhibition as well. Thus, the 5-HT releasers produced a "cross-modal" disruption of auditory and visual prepulse inhibition. Given the current lack of data on the pharmacology of visual prepulse inhibition, it will be important for future work to enhance our understanding of the pharmacology and anatomy of cross-modal prepulse inhibition. It will be of particular interest to determine the location(s) of the 5-HT receptors which modulate auditory and visual prepulse inhibition. For example, they might have a common location as part of a general filter at the level of the thalamus. Alternatively, they may be anatomically distinct, acting independently at sites along the different sensory pathways. The present studies cannot discriminate between these possibilities.

An implicit assumption in the present studies is that the relative contributions of a neurotransmitter system on cross-modal prepulse inhibition can be quantitatively assessed using sound and light prepulses. However, slight differences in drug effects on the two modalities might be attributable to differences in salience of the two types of stimuli (Davis et al. 1990; Keith et al. 1991). In this regard, direct quantitative comparisons between sound and light prepulse inhibition need to be interpreted carefully. The preliminary nature of these results notwithstanding, the results generally highlight the potential utility of incorporating multiple prepulse sensory modalities within the same experiment.

5-HT releasers in humans

The results of the present preclinical studies would predict that fenfluramine would disrupt prepulse inhibition in humans. To our knowledge, this study has not been

carried out. However, fenfluramine has been used clinically for the treatment of obesity for many years without any apparent "psychoactivity" in the general population (see Pinder et al. 1975 for review). The doses used in the present study to disrupt prepulse inhibition may be high relative to those used clinically, thereby producing excessive 5-HT stimulation. Fenfluramine has, however, been reported to worsen schizophrenic symptomatology in schizophrenic populations (Marshall et al. 1989), suggesting the possibility that patients in certain clinical populations might be more susceptible to the effects of fenfluramine.

Although a theoretical link has been made (see Introduction for references), the precise relationship between disrupted prepulse inhibition and the symptoms of schizophrenia is not totally clear. Prepulse inhibition in rats can be disrupted by agents which produce psychosis in man, and schizophrenics show disrupted prepulse inhibition. However, deficits in non-psychotic, "schizotypal" humans have been reported (Cadenhead et al. 1993), suggesting the possibility that disrupted prepulse inhibition may be a measure of a susceptibility towards psychosis. Deficits in other neurological conditions such as Huntington's disease (Swerdlow et al. 1995) and obsessive compulsive disorder (Swerdlow et al. 1993) suggest that prepulse inhibition reflects a fundamental process of sensory gating that may be disrupted in a variety of CNS disorders. Evaluations of 5-HT releasers such as fenfluramine on prepulse inhibition in humans should help improve our understanding of the relationship between disrupted prepulse inhibition, sensory gating processes, and specific psychopathologies in humans.

MDMA neurotoxicity in humans

Fenfluramine disruption of prepulse inhibition was reduced in rats that were depleted of cortical 5-HT by prior exposure to a neurotoxic dose of PCA. In addition to demonstrating the importance of 5-HT for the fenfluramine effect, these data also indicate that PCA-induced 5-HT neurotoxicity is expressed functionally as a reduction in fenfluramine's disruptive effect on prepulse inhibition. That is, a functional deficit specifically attributable to a reduction in 5-HT levels was demonstrated. 5-HT deficits following exposure to ring-substituted amphetamines have been shown (Schmidt and Kehne 1990). The present data support the use of a "challenge" approach, in which a deficit is measured by a decrease in the ability of a 5-HT releaser to produce a given behavioral effect, in this case, a reduction of prepulse inhibition. This approach could be used further to assess for possible functional neurotoxic effects of other 5-HT releasers, i.e. MDMA or PCA.

The findings with PCA have potential clinical relevance in that they suggest that fenfluramine may possibly be used as a pharmacological tool in humans for assessing functional deficits attributable to substituted amphetamine-induced 5-HT neurotoxicity. It has been suggested that humans who have repeatedly ingested

MDMA may develop 5-HT neurotoxicity as measured, for example, by a reduction in cerebrospinal fluid 5-HIAA levels (Krystal et al. 1992). However, these measurements are indirect, and it would be useful to have a method to measure for possible functional changes in 5-HT function. The PCA study suggests one possible approach to this problem. Prepulse inhibition can be readily measured in humans (e.g. Grillon et al. 1992), and fenfluramine can be administered to humans. If reliable deficits in prepulse inhibition following fenfluramine administration can be measured in humans, then it is possible that this "challenge" approach can be used to provide functional evidence for 5-HT neurotoxicity. Such a possibility remains to be tested.

In summary, the present studies using 5-HT releasing agents to disrupt auditory and visual prepulse inhibition implicate the role of 5-HT in the modulation of prepulse inhibition, though further work is needed to identify the 5-HT receptor subtypes mediating these effects. Previous work suggests that 5-HT_{1A} (Rigdon and Weatherspoon 1992), 5-HT_{1B} (Sipes and Geyer 1994) and 5-HT_{2A} (Sipes and Geyer 1994) receptors can modulate auditory prepulse inhibition. The accompanying report (Padich et al. 1996) uses MDL 100,907, a selective 5-HT_{2A} antagonist and potential atypical antipsychotic (Palfreyman et al. 1993; Schmidt et al. 1993, 1995; Sorensen et al. 1993; Kehne et al. 1996; Moser et al. 1996) to evaluate the contribution of 5-HT_{2A} receptors to 5-HT agonist disrupted cross-modal prepulse inhibition.

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Note added in proof Two recent studies are relevant to the present studies. First, Martinez et al. (Abstracts, Soc. Neuroscience, Vol. 21, Abstr. #663.18, p. 1693, 1995) reported that α -ethyltryptamine, a monoamine releaser which does not depress acoustic startle baseline, disrupted auditory prepulse inhibition in a fluoxetine-reversible manner, indicating that excess serotonergic activation disrupts auditory prepulse inhibition. Second, Campeau and Davis (Psychopharmacology 177:267–274, 1995) reported that the dopamine agonist apomorphine disrupted both auditory and visual prepulse inhibition in rats, providing pharmacological evidence for dopaminergic modulation of cross-modal prepulse inhibition.