

Effects of the Serotonin Releasers 3,4-Methylenedioxymethamphetamine (MDMA), 4-Chloroamphetamine (PCA) and Fenfluramine on Acoustic and Tactile Startle Reflexes in Rats

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ABSTRACT

The substituted amphetamines 4-chloroamphetamine (PCA), 3,4-methylenedioxymethamphetamine (MDMA) and fenfluramine (FEN) share the common neurochemical action of acutely releasing central serotonin (5-HT), and yet their behavioral effects are quite different. The present study evaluated the effects of these compounds on acoustic and tactile startle reflexes. PCA and MDMA were qualitatively similar in producing dose-related increases in acoustic and tactile startle reflexes that were slow in onset, but sustained throughout the 3.5-hr test session. Changes in motor activity did not account for the observed excitation of startle. In marked contrast to MDMA and PCA, FEN did not alter tactile startle and tended to depress acoustic startle. The excitatory effect of 20 mg/kg of MDMA was prevented by the 5-HT uptake blockers MDL 27,777A and fluoxetine. MDMA excitation

was not affected by a dose of the dopamine antagonist haloperidol that attenuated the startle-enhancing effect of *d*-amphetamine. MDMA excitation was greatly attenuated by a general depletion of central 5-HT produced by prior intraventricular injection of the 5-HT neurotoxin 5,7-dihydroxytryptamine. PCA and MDMA excitations of startle were attenuated in rats specifically depleted of spinal 5-HT or in rats with radio frequency lesions of the dorsal raphe nucleus. Thus, PCA and MDMA have similar prolonged excitatory effects on startle reflexes that are mediated by ascending (dorsal raphe) and descending (spinal) pathways, whereas FEN differs in its lack of excitation of startle. Differences in the neurochemical properties of these compounds or their patterns of 5-HT release may underlie their different behavioral profiles.

Ring-substituted amphetamines such as PCA release newly-synthesized 5-HT from central terminals, and additional actions, such as blockade of reuptake, inhibition of monoamine oxidase and inhibition of synthesis may contribute to the mechanism of these compounds (Fuller, 1976).

An important early observation of 5-HT releasers was that they showed fewer amphetamine-like stimulant effects in animals (Frey and Magnussen, 1968) and in man (Verster and van Praag, 1970). The hypothesized role of 5-HT in depression (Carlsson *et al.*, 1969) was supported by initial studies in which PCA or *para*-chloromethamphetamine showed potential in treating clinically depressed patients (Van Praag and Korf, 1976). However, reports of long-lasting 5-HT depletions (Sanders-Bush *et al.*, 1972) and anatomical evidence of cell body destruction (Harvey *et al.*, 1975) precluded further clinical evaluation of these compounds.

Recently, attention has focused on FEN and MDMA ("ecstasy"), two 5-HT releasers with markedly different psycho-

pharmacological profiles. Having been marketed for over 25 years as an antiobesity drug, FEN is slightly sedative, but otherwise lacks affective or psychotomimetic properties (Pinder *et al.*, 1975). FEN does not have abuse potential, as evidenced by extensive clinical experience and by lack of activity in animal models such as drug self-administration (Dahl and Gotestam, 1989; Pinder *et al.*, 1975) or self-stimulation (Hoebel *et al.*, 1988). MDMA and related compounds differ from FEN in having prominent mood altering properties. The unusual property of these "entactogens" (Nichols, 1986) to produce feelings of well-being and to promote interpersonal communication (Greer and Tolbert, 1986; Grinspoon and Bakalar, 1986; Peroutka *et al.*, 1988) lead to their informal use as psychotherapeutic adjuncts (Grinspoon and Bakalar, 1986) and as recreational drugs (Peroutka *et al.*, 1988) before being scheduled as drugs of abuse. Evidence supporting potential for abuse include the findings that MDMA decreased the thresholds for rewarding brain stimulation in primates (Lamb and Griffiths, 1987) and in rats (Hubner *et al.*, 1988), and that MDMA was self-administered by primates (Beardsley *et al.*, 1986).

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ABBREVIATIONS: 5-HT, serotonin; PCA, 4-chloroamphetamine (*para*-chloroamphetamine); MDMA, 3,4-methylenedioxymethamphetamine; FEN, fenfluramine; 5,7-DHT, 5,7-dihydroxytryptamine.

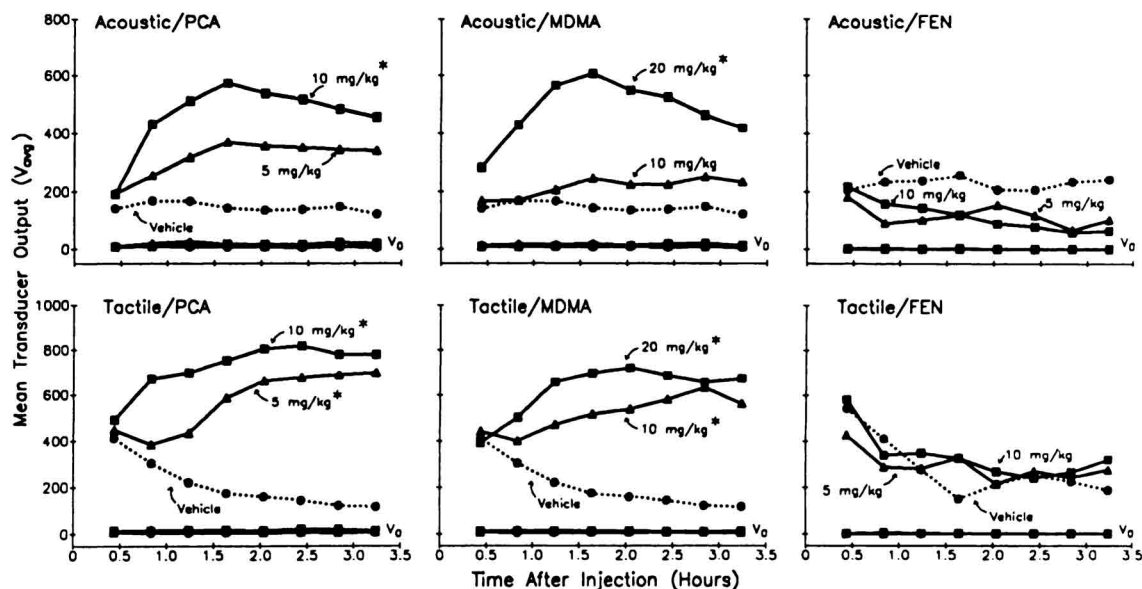


Fig. 1. Selected time courses of the effects of PCA (2.5–20 mg/kg, left panels, solid lines), MDMA (5–20 mg/kg, middle panels, solid lines) and FEN (0.625–10 mg/kg, right panels, solid lines) vs. vehicle (saline, dashed lines) on the amplitude (V_{avg}) of acoustic and tactile startle reflexes in rats. For this and all subsequent graphs, each point represents the mean of 36 stimuli (e.g., 24 min of testing). V_0 represents corresponding means sampled at the onset of the startle stimulus (e.g., background activity of the cage). Note that V_0 was essentially zero at every time point measured. *Represent significant changes in startle amplitude relative to controls, $P < .05$.

TABLE 1
Effects of PCA, MDMA or FEN on acoustic or tactile startle reflexes in rats

Drug	Dose	Mean Startle Amplitude $\pm$ S.E.M.	
		Acoustic	Tactile
PCA	Saline ^a	146 $\pm$ 25	207 $\pm$ 37
	2.5	208 $\pm$ 56	440 $\pm$ 72
	5.0	315 $\pm$ 71	573 $\pm$ 87*
	10.0	463 $\pm$ 65*	724 $\pm$ 113*
MDMA	20.0	570 $\pm$ 87*	560 $\pm$ 83*
	5.0	238 $\pm$ 44	478 $\pm$ 84*
	10.0	215 $\pm$ 32	517 $\pm$ 67*
	20.0	480 $\pm$ 76*	623 $\pm$ 62*
FEN	Saline ^b	228 $\pm$ 55	283 $\pm$ 45
	0.625	257 $\pm$ 60	436 $\pm$ 128
	1.25	185 $\pm$ 43	247 $\pm$ 56
	2.5	115 $\pm$ 44	294 $\pm$ 85
	5.0	117 $\pm$ 40	291 $\pm$ 80
	10.0	116 $\pm$ 31	336 $\pm$ 95
FEN	20.0	148 $\pm$ 59	354 $\pm$ 57

^a One-way analysis of variance for PCA, $F(4, 73) = 10.92$, $P < .0001$ (acoustic); $F(4, 73) = 9.57$, $P < .0001$ (tactile). One-way analysis of variance for MDMA, $F(3, 60) = 7.01$, $P < .0001$ (acoustic); $F(3, 60) = 7.51$, $P < .0001$ (tactile).

^b One-way analysis of variance for FEN, not significant.

* Significantly different from vehicle control, $P < .05$.

Given their apparent similar capacities to release 5-HT (Johnson *et al.*, 1986; Auerbach *et al.*, 1989; De Simoni *et al.*, 1988; Hutson and Curzon, 1989; Nichols, 1986; Schmidt, 1987; Laferrere and Wurtman, 1989), it is not clear why FEN, MDMA and PCA have differing behavioral effects in animals and in man. Possible explanations include different neurochemical actions on other mechanisms affecting 5-HT availability (e.g., uptake blockade; Fuller *et al.*, 1988), effects on other neurotransmitters (e.g., release of dopamine; Yamamoto and Spanos, 1988) or even different releasing profiles on discrete 5-HT pathways (Blier *et al.*, 1990). Further preclinical studies directly comparing the behavioral effects of the 5-HT releasers would help evaluate these and other possibilities.

The present article investigates aspects of the psychopharmacology of PCA, MDMA and FEN using a behavioral model, the startle reflex, which is known to be modulated by monoaminergic neurotransmitters, including 5-HT (see Davis, 1980, for review). Startle reflexes are short-latency, short-duration contractions of the body produced by sudden stimuli of various sensory modalities. Startle reflexes can be reliably elicited in rodents by acoustic (noise or tone burst) or tactile (air puff) stimuli and can be quantified with automated measurement systems. A common neuronal circuitry of acoustic and tactile startle involves a relatively simple polysynaptic reflex arc, coursing centrally from the brain stem to the motor neurons of the spinal cord (Davis *et al.*, 1982; J. V. Cassella and M. Davis, personal communication). Monoaminergic neurons are not intrinsic to this circuitry, but rather exert modulatory influences (*i.e.*, Kehne and Davis, 1985).

As noted for other behaviors (for review, see Gerson and Baldessarini, 1980), 5-HT neurons modulate startle reflexes in a complex fashion (see Davis, 1980; Davis *et al.*, 1986; Geyer and Tapson, 1988). Studies using acoustic startle indicate that this behavior is modulated by both descending (spinal) and ascending (supraspinal) 5-HT pathways. Different 5-HT pathways may have functionally opposite roles, and multiple 5-HT receptor subtypes appear to be involved. The overall implication of this organization is that the behavioral effect of a general 5-HT-releasing drug could be a net effect of 5-HT release from multiple, functionally opposing pathways. Thus, drug variations in the pattern of release could result in quantitatively or qualitatively different behavioral effects.

Several studies have assessed the effects of substituted amphetamines on startle reflexes. PCA produced a biphasic effect on acoustic startle, which was 5-HT mediated (Davis and Sheard, 1976). PCA produced an early inhibition (15 min postinjection) followed by a later excitation (2.5 hr postinjection), and both of these effects were prevented by 5-HT synthesis inhibition. More recently, MDMA was reported to have

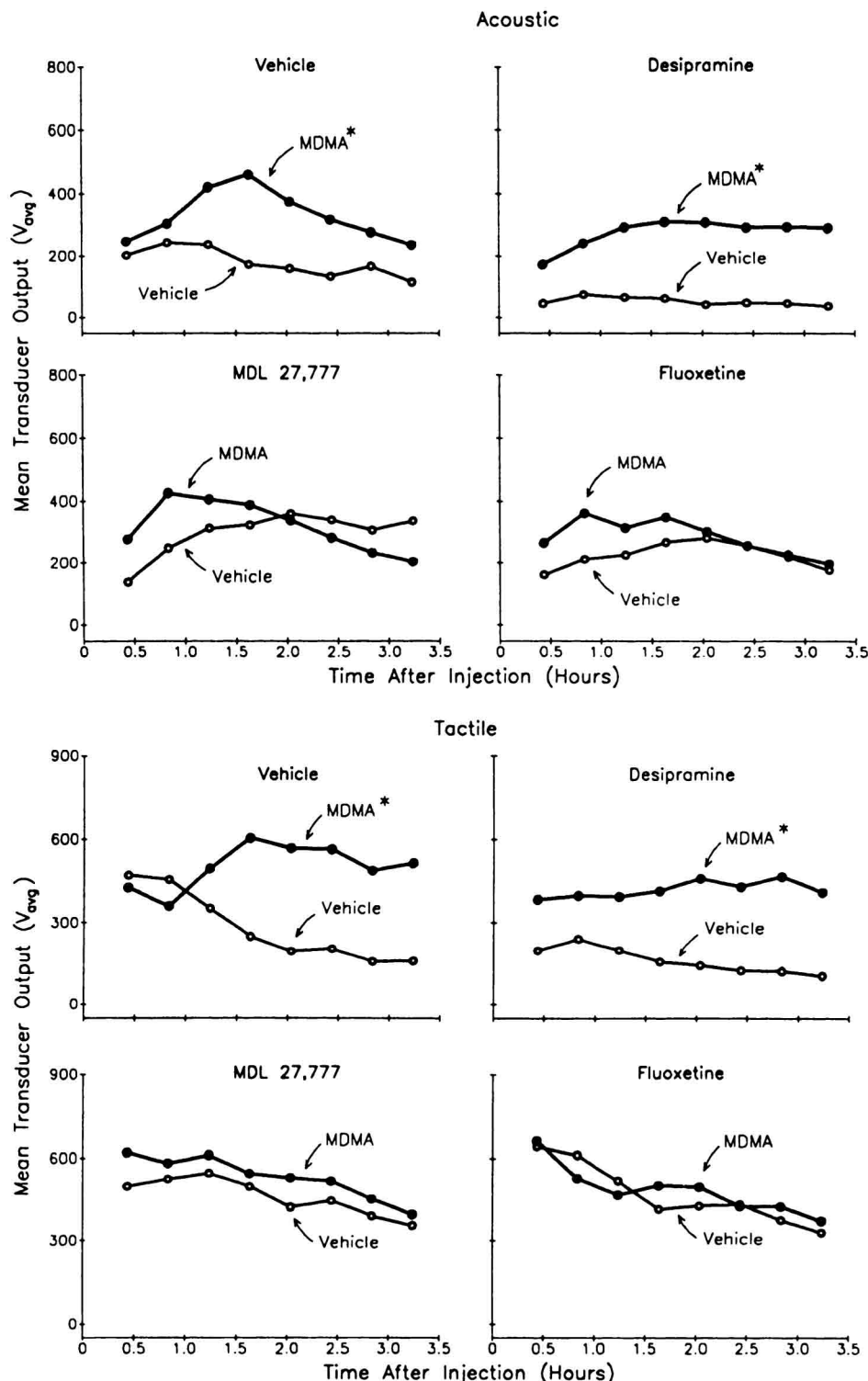


Fig. 2. Time courses of the effects of MDMA (●) or vehicle (○) on acoustic (top panels) or tactile (bottom panels) startle reflexes in rats that were pretreated with the 5-HT uptake blockers MDL 27,777A or fluoxetine, or the norepinephrine uptake blocker desipramine. Uptake blockers were administered 30 min before injection of MDMA (●) or vehicle (saline, ○). *Represent significant changes in startle amplitude relative to controls, $P < .05$.

a tendency to elevate acoustic startle amplitude and decrease prepulse inhibition (Mansbach *et al.*, 1989). No inhibition was reported, although this may be attributable to the doses and measurement times used, as well as other factors such as level of background noise (Davis and Sheard, 1976). In contrast, a recent report indicated that FEN depressed acoustic startle (Kutscher, 1987), although the effect on tactile startle was not assessed.

One goal of the present study was to systematically compare

the effects of PCA, MDMA and FEN on startle reflexes in rats. To assess for potential biphasic effects, a 3.5-hr test session was used. Startle reflexes were elicited by alternating acoustic and tactile stimuli within each test session, to assess for possible differences in drug effects on sensory modality. A second goal of this study was to use several pharmacological approaches (uptake blockers, neurotoxin/radio frequency lesions) to evaluate general and specific serotonergic contributions to the observed effects.

TABLE 2

Effects of the 5-HT uptake blockers (MDL 27,777A or fluoxetine) or the norepinephrine uptake blocker (desipramine) on the excitation of acoustic or tactile startle reflexes produced by 20.0 mg/kg MDMA

Treatment		Mean Startle Amplitude $\pm$ S.E.M.	
A	B	Acoustic	Tactile
Vehicle	Vehicle ^a	149 $\pm$ 34	191 $\pm$ 36
Vehicle	MDMA	332 $\pm$ 63 ^a	548 $\pm$ 82 ^a
MDL 27,777A	Vehicle	332 $\pm$ 101	422 $\pm$ 100
MDL 27,777A	MDMA	288 $\pm$ 79 ^b	488 $\pm$ 98 ^b
Fluoxetine	Vehicle	239 $\pm$ 74	396 $\pm$ 65
Fluoxetine	MDMA	265 $\pm$ 52 ^c	445 $\pm$ 64 ^b
Desipramine	Vehicle	47 $\pm$ 20	127 $\pm$ 36
Desipramine	MDMA	297 $\pm$ 39 ^a	437 $\pm$ 69 ^a

^a One-way analysis of variance, $F(7, 72) = 3.03$, $P < .01$ (acoustic); $F(7, 72) = 4.55$, $P < .0001$ (tactile). Data were analyzed over the second half of the test session.

^b (MDMA minus vehicle) difference scores significantly different from vehicle-pretreated controls difference scores, $P < .05$.

^c For difference scores comparison, $P = .06$.

^a Significantly different from vehicle control, $P < .05$.

Methods

General Methods

Animals. Subjects were experimentally naive, male albino Sprague-Dawley rats (Charles River, Portage facility). The rats 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.

Startle Response Testing

Acoustic and tactile startle. 5-HT may differentially modulate acoustic and tactile startle reflexes (e.g., Geyer *et al.*, 1978; Davis, 1980). As indicated above, acoustic and tactile startle reflex pathways share a common motor output component, but have different sensory input pathways which could provide differential sites of 5-HT modulation. Thus, the use of stimuli of different sensory modalities can potentially provide clues concerning the site of a drug's modulatory action. The apparatus and procedure described below were designed such that both acoustic and tactile startle reflexes could be elicited and measured within the same test session.

Apparatus. An apparatus consisting of eight separate stabilimeters measured the amplitude of startle reflexes elicited by acoustic (white noise burst) or tactile (air-puff) stimulation. Each stabilimeter consisted of a $19 \times 8.5 \times 10.5$ cm Plexiglas and wire cage that was detachable from a floating platform. The platform was wedged between compression springs at each of the four corners. Linear bearings installed at either end of the platform minimized horizontal movement of the floating platform. The transducer was a pressure-sensitive piezoelectric disc compressed between a small spring (#526; Century Spring Corp., Los Angeles, CA) and a rubber stopper, which in turn rested against the base of the platform. Movement of the platform against the transducer produced a voltage proportional to displacement. This voltage was amplified, rectified, filtered at 10 Hz and fed into a Metrabyte DAS16 I/O board, where it was digitized and sampled over a 250-msec window (measured from the onset of the eliciting stimulus). Startle amplitude was defined as the average rectified intensity that occurred over the 250-msec window, expressed in arbitrary units from 0 to 4095. Each stabilimeter was housed in a ventilated, sound-attenuating chamber illuminated by dim, red-filtered light. The acoustic startle-eliciting stimulus was a 50-msec, 120-dB burst of white noise. Background white noise of 55 dB was generated by a Grason-Stadler white noise generator. Sound level measurements were made with a General Radio Model 1988 sound level meter (peak amplitude, impulse setting). The tactile startle stimulus was a 4 lb/sq. in. burst of pressurized air, 50-msec duration, delivered to the subject's back region

through a polypropylene tube positioned over a hole in the center of the top of the test cage. Software for data acquisition and experimental control and electronics for stimulus generation were purchased from San Diego Instruments (San Diego, CA).

Matching. Because of considerable individual differences in startle amplitude, a matching procedure was used to divide rats into control and experimental groups. Rats were placed in the startle cages, and 5 min later were presented with 12 acoustic stimuli and 12 tactile stimuli delivered every 20 sec. The rats were assigned to groups with equivalent mean startle amplitudes.

Intraventricular infusion of neurotoxin. Previously described procedures (Kehne and Davis, 1985) were used to infuse 5-HT neurotoxins into the lateral ventricle (i.c.v. infusion). For i.c.v. infusions, rats were pretreated with nomifensine (20 mg/kg, 30 min) and desipramine (20 mg/kg, 30 min) before infusion of 200 μ g of 5,7-DHT (calculated as the base; dissolved in 0.9% saline + 1 mg/ml ascorbic acid) or vehicle. Surgery was carried out under halothane anesthesia. Because rats often exhibited convulsions after recovering from the anesthesia, a supplemental injection of pentobarbital (20 mg/kg) was given after the completion of surgery. This treatment greatly enhanced the survival rate. After recovering from the pentobarbital, rats were returned to their group cages. Subsequent drug testing was carried out 13 to 15 days after 5,7-DHT, a time sufficient to allow maximal degeneration of 5-HT neurons.

Intrathecal (i.t.) infusion of neurotoxin. Previously described procedures (Kehne and Davis, 1985) were used to infuse the 5-HT neurotoxin 5,6-DHT into the subarachnoid space of the lumbar spinal cord. Surgery was carried out under halothane anesthesia. Rats were anesthetized and an i.t. catheter was inserted to the lumbar level. 5,6-DHT (20 μ g) (calculated as the base) or vehicle (0.9% saline + 1 mg/ml ascorbic acid) was infused in a total volume of 20 μ l. No pretreatment was given because others have reported that this dose of 5,6-DHT has a highly selective depleting effect on lumbar 5-HT without affecting norepinephrine or dopamine (Berge and Ogren, 1984).

Raphe lesions. Sham or radio frequency lesions of the dorsal ($n = 18$) or median ($n = 19$) nuclei were made with a Radionics RFG-4A Lesion Generator with a probe tip of 0.25 mm. Coordinates and lesion parameters (determined from Paxinos and Watson, 1986, and from pilot studies) were: median raphe (from ear bar zero): 7.3 and 8.0 anterior, 2.0 above. The electrode was angled 60° from vertical. Equilibrium tissue temperature achieved was 65°C. Dorsal raphe (from bregma): 7.4, 8.2 and 9.0 posterior; depth: 6.6, 6.8 and 7.6 mm respectively, from skull; temperature: 70°C.

Drug testing. For experiments assessing the effects of a pretreatment, rats were injected with test compound A (i.e., uptake blockers or haloperidol) or vehicle, placed in the startle cages and exposed to acoustic and tactile stimuli (120 dB and 4 psi, respectively; 20 sec interstimulus interval) for 30 min. After the initial 30-min test, the rats were removed from the cages, injected with the test compound B (i.e., MDMA, PCA or *d*-amphetamine) or vehicle and returned to the cage from an additional 3.5 hr of testing. In studies that did not involve a drug combination, the above procedure was modified to have only an injection after the 30-min base-line period. The initial 30-min startle test period was not included in the data analysis.

Neurochemistry. Regional dissections of hippocampus and striatum and subsequent assay of 5-HT was carried out using high performance liquid chromatography with electrochemical detection, as described previously (Schmidt, 1987).

Drugs and injection parameters. 5-HT uptake blockers used were fluoxetine (5 mg/kg; Lilly, Indianapolis, IN) and MDL 27,777A (5 mg/kg; 2,3-dihydro-N-methyl-1-[4-(trifluoromethyl)phenoxy]-1H-indene-2-methanamine hydrochloride; Marion Merrell Dow, Cincinnati OH; see Freedman *et al.*, 1989); norepinephrine uptake blocker: desipramine hydrochloride (15 mg/kg; Marion Merrell Dow); dopamine uptake blocker: nomifensine (20 mg/kg, Hoechst, Somerville, NJ); pentobarbital (20 mg/kg, Sigma Chemical Co., St. Louis, MO); 5-HT neurotoxins: 5,6-DT and 5,7-DT (2 mg/ml and 20 mg/ml, respectively; Sigma); 5-HT releasing agents: PCA; 2.5–20 mg/kg; Sigma; MDMA;

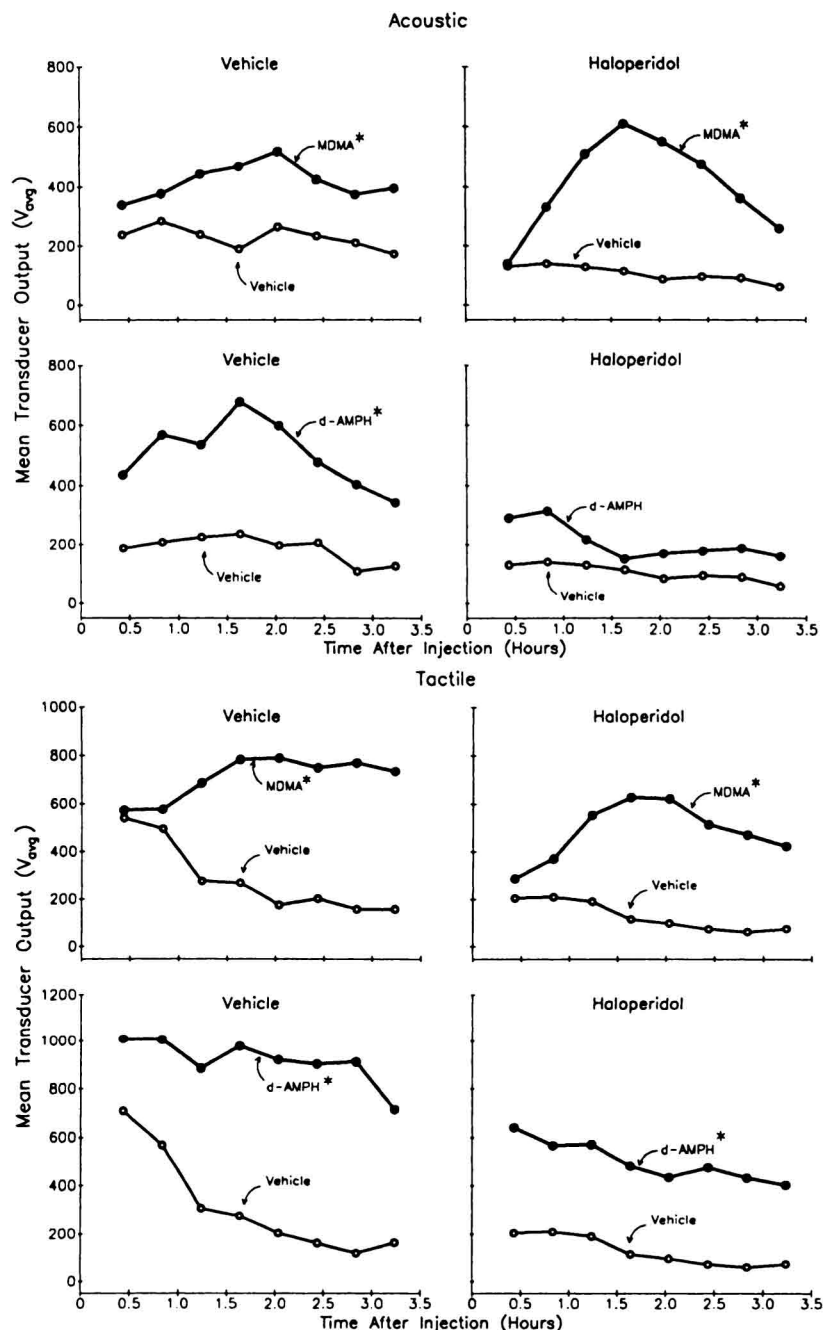


Fig. 3. Time courses of the effects of haloperidol or vehicle (dimethyl sulfoxide) on *d*-amphetamine- and MDMA-enhanced acoustic (top panels) and tactile (bottom panels) startle reflexes in rats. Haloperidol (1 mg/kg, i.p.) was given 30 min before saline (○), *d*-amphetamine (4 mg/kg, ●) or MDMA (20 mg/kg, ●). *Represent significant changes in startle amplitude relative to controls, $P < .05$.

5–20 mg/kg; National Institute of Drug Abuse, Washington, DC FEN (0.625–10 mg/kg; A. H. Robins, Richmond, VA). All drugs were dissolved in either saline or dimethylsulfoxide and were administered in a volume of 1 ml/kg body weight. PCA injections were made i.p., whereas MDMA injections were made s.c. in the back. Pilot studies in our laboratory indicated that MDMA produced an excitatory effect on startle reflexes, though it appeared to be less potent than either *d*-amphetamine or PCA, and it appeared to have a time course that more closely resembled PCA.

Statistics. Matching procedures (described above) and repeated measures designs (where rats are tested under two or more treatment conditions) are typically used to minimize the impact of inherent variability in startle amplitudes demonstrated across individual rats (see Kehne and Davis, 1985). However, the testing of compounds with neurotoxic properties precluded repeated testing of the subjects (unless specifically called for in the experimental design) and, therefore, be-

tween-subjects comparisons were typically made. Means for test sessions were calculated and (with more than two groups) appropriate analysis of variance was run, with subsequent individual comparisons (Student Neuman-Keuls test, Dunnett's test, when comparing to a control group, or Student's *t* test). Calculations of magnitudes of substituted amphetamine effects on startle were made (drug score minus control mean). Difference scores for a given pretreatment group were compared to appropriate vehicle scores (*t* test) to determine whether or not a specific pretreatment blocked the effect of a substituted amphetamine on startle. Probability levels of $P < .05$ were considered statistically significant, and marginal effects were explicitly indicated.

Experimental Protocols

Experiment 1. This experiment assessed the effects of PCA (2.5, 5, 10 and 20 mg/kg), MDMA (5, 10 and 20 mg/kg) and FEN (0.625,

TABLE 3

Effects of the dopamine antagonist haloperidol on the excitation of acoustic or tactile startle reflexes produced by 20.0 mg/kg MDMA or 4.0 mg/kg *d*-amphetamine

Treatment		Mean Startle Amplitude $\pm$ S.E.M.	
A	B	Acoustic	Tactile
Vehicle	Vehicle*	228 $\pm$ 67	284 $\pm$ 54
Vehicle	MDMA	418 $\pm$ 70*	707 $\pm$ 92*
Haloperidol	Vehicle	104 $\pm$ 53	128 $\pm$ 42
Haloperidol	MDMA	405 $\pm$ 127*	483 $\pm$ 134*
Vehicle	Vehicle ^b	186 $\pm$ 40	314 $\pm$ 65
Vehicle	<i>d</i> -Amphetamine	504 $\pm$ 75*	915 $\pm$ 120*
Haloperidol	Vehicle	104 $\pm$ 53	128 $\pm$ 42
Haloperidol	<i>d</i> -Amphetamine	206 $\pm$ 72†	502 $\pm$ 143*

* One-way analysis of variance for MDMA, $F(3, 28) = 3.20$, $P < .05$ (acoustic); $F(3, 28) = 8.09$, $P < .0001$ (tactile).

^b One-way analysis of variance for *d*-amphetamine, $F(3, 28) = 12.22$, $P < .0001$ (acoustic); $F(3, 28) = 11.09$, $P < .0001$ (tactile).

* Significantly different from vehicle control, $P < .05$.

† (MDMA minus vehicle) or (*d*-amphetamine minus vehicle) difference scores significantly different from vehicle-pretreated controls difference scores, $P < .05$.

1.25, 2.5, 5 and 10 mg/kg) on acoustic and tactile startle reflexes. After obtaining a 30-min base-line measurement of startle, rats were injected with the test compound and were then exposed to a total of 630 stimuli (315 of each modality), which comprised approximately 3.5 hr of testing.

Experiment 2. This experiment evaluated the role of 5-HT in MDMA (20 mg/kg)-enhanced acoustic and tactile startle by attempting to reverse this excitation by administration of 5-HT uptake blockers (e.g., MDL 27,777A, fluoxetine) or by general central 5-HT depletion with i.c.v. administration of 5,7-DHT. Davis and Sheard (1976) convincingly implicated 5-HT involvement in the effects of PCA on acoustic startle, and, therefore, this experiment focused on characterizing general 5-HT involvement in the effects of MDMA on startle. Controls were carried out for possible contributions of norepinephrine (using the norepinephrine uptake blocker, desipramine) and dopamine (using the dopaminergic receptor antagonist, haloperidol). In addition, the role of 5-HT in FEN (1.25 mg/kg) inhibition of startle was evaluated

by attempting to reverse this inhibition with 5.0 mg/kg of MDL 27,777A. For the uptake blocker/haloperidol studies, matched groups of rats were injected with the appropriate pretreatment and then a 30-min base-line measurement of startle was taken. The rats were then injected with either MDMA (20 mg/kg) or saline and then tested for 3.5 hr. For the neurotoxin study, matched groups of rats that had received i.c.v. infusions of 5,7-DHT or vehicle approximately 2 weeks previously were given a 30-min base-line test, after which they were injected with MDMA or saline and tested for 3.5 hr.

Experiment 3. Both ascending (brain) and descending (spinal) 5-HT pathways have been implicated in the modulation of startle (Davis, 1980; Davis *et al.*, 1986). This experiment evaluated the involvement of these pathways in MDMA (20 mg/kg)- and PCA (10 mg/kg)-enhanced acoustic and tactile startle reflexes. The first study investigated the involvement of descending 5-HT pathways in the excitatory effects of MDMA and PCA. 5-HT terminals in the caudal (lumbar/sacral) region of the spinal cord were lesioned by infusing 5,6-DHT i.t. into the subarachnoid space over the lumbar spinal cord. Two weeks later, when maximal destruction of spinal 5-HT neurons should have developed (Berge and Ogren, 1984), the rats were treated with MDMA (20 mg/kg) or PCA (10 mg/kg) and tested for their startle reflexes. In the second study, radio frequency lesion studies evaluated the individual contributions of the ascending dorsal or median raphe 5-HT fiber systems to the observed excitatory effects of PCA and MDMA. Approximately 1 to 2 weeks after matched groups of rats received sham, dorsal raphe or median raphe lesions, a 30-min base-line measure of startle was taken. Rats were then injected with PCA, MDMA or saline and then tested for 3.5 hr. Brains from the saline-treated rats were subsequently removed for neurochemical measurement of 5-HT and dopamine.

Results

Experiment 1. Figure 1 shows mean acoustic or tactile startle amplitude after injection of selected doses of MDMA (10, 20 mg/kg), PCA (5, 10 mg/kg) and FEN (5, 10 mg/kg) or after injection of saline. Each point represents the mean of 36 consecutive stimuli of one modality (or a total of 24 min of

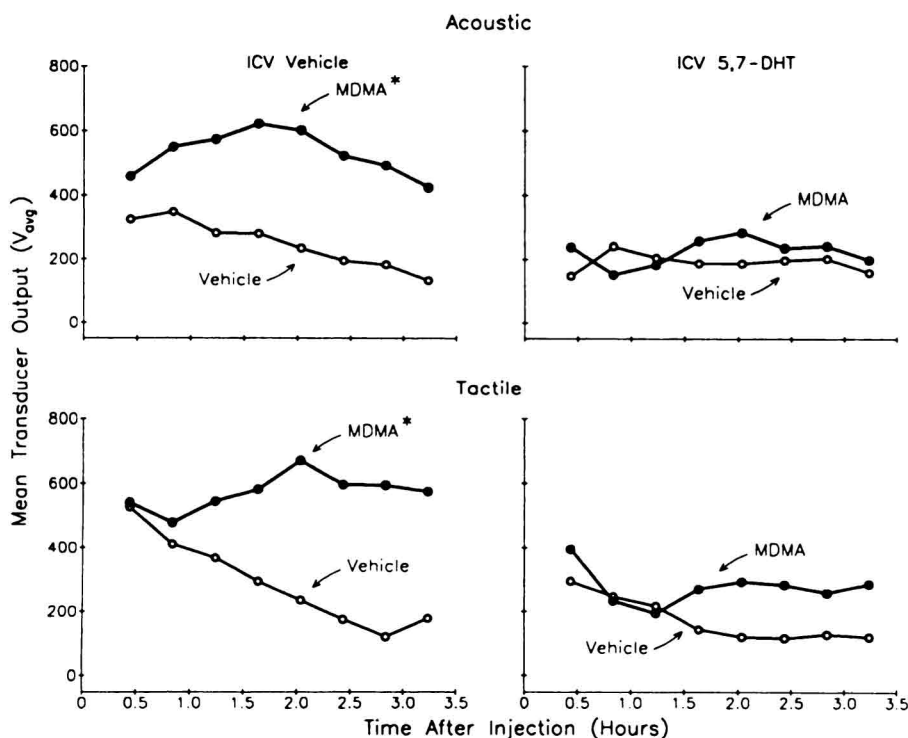


Fig. 4. Time courses of the effects of MDMA (20 mg/kg; ●) or vehicle (saline; ○) in rats pretreated with i.c.v. administration of 5,7-DHT (right panels) or vehicle (left panels) on acoustic and tactile startle reflexes in rats. *Represent significant changes in startle amplitude relative to controls, $P < .05$.

TABLE 4

Effects of i.c.v. administration of 5,7-DHT or vehicle on the excitation of acoustic or tactile startle reflexes produced by 20.0 mg/kg MDMA

Treatment		Mean Startle Amplitude $\pm$ S.E.M.	
A	B	Acoustic	Tactile
i.c.v. Vehicle	Vehicle*	246 $\pm$ 38	287 $\pm$ 48
i.c.v. Vehicle	MDMA	527 $\pm$ 85*	572 $\pm$ 67*
i.c.v. 5,7-DHT	Vehicle	186 $\pm$ 36	172 $\pm$ 46
i.c.v. 5,7-DHT	MDMA	216 $\pm$ 38†	276 $\pm$ 43†

* One-way analysis of variance, $F(3, 60) = 5.90$, $P < .001$ (acoustic); $F(3, 60) = 10.87$, $P < .0001$ (tactile).

* Significantly different from vehicle control, $P < .05$.

† (MDMA minus vehicle) difference scores significantly different from i.c.v. vehicle-pretreated controls difference scores, $P < .05$.

testing). The middle and left panels show that MDMA and PCA had excitatory effects on both acoustic and tactile startle. However, the peak effect of these excitations was delayed in that it generally occurred between approximately 1 and 3 hr after injection of the drugs. The right panels show that FEN, in contrast to PCA or MDMA, slightly inhibited acoustic

startle, although this effect was not statistically significant. FEN had no effect on tactile startle. The means for all doses are summarized in table 1. The curves at the bottom of the graphs (labeled V_0) represent the mean background activity of the cages, that is, the output from the transducer sampled at the onset of each stimulus presentation. These curves demonstrate that spontaneous movements produced by the rats, or increased general activity possibly produced by the drugs, did not contribute to the overall output of the transducer. V_{AVG} , thus, appears to reflect an accurate representation of the rat's startle reflex amplitude.

Experiment 2. Figure 2 and table 2 show that pretreatment with the 5-HT uptake blockers MDL 27,777A (5.0 mg/kg; 30-min pretreatment) or fluoxetine (5.0 mg/kg; 30-min pretreatment) prevented the excitatory effect of MDMA (20 mg/kg) on acoustic startle or tactile startle. In contrast, pretreatment with the noradrenergic uptake blocker desipramine (20 mg/kg; 30-min pretreatment) did not block this effect. Desipramine alone tended to depress base-line startle, whereas fluoxetine alone tended to elevate startle amplitude. In addition, 5.0 mg/kg MDL 27,777A was tested against the inhibition of startle

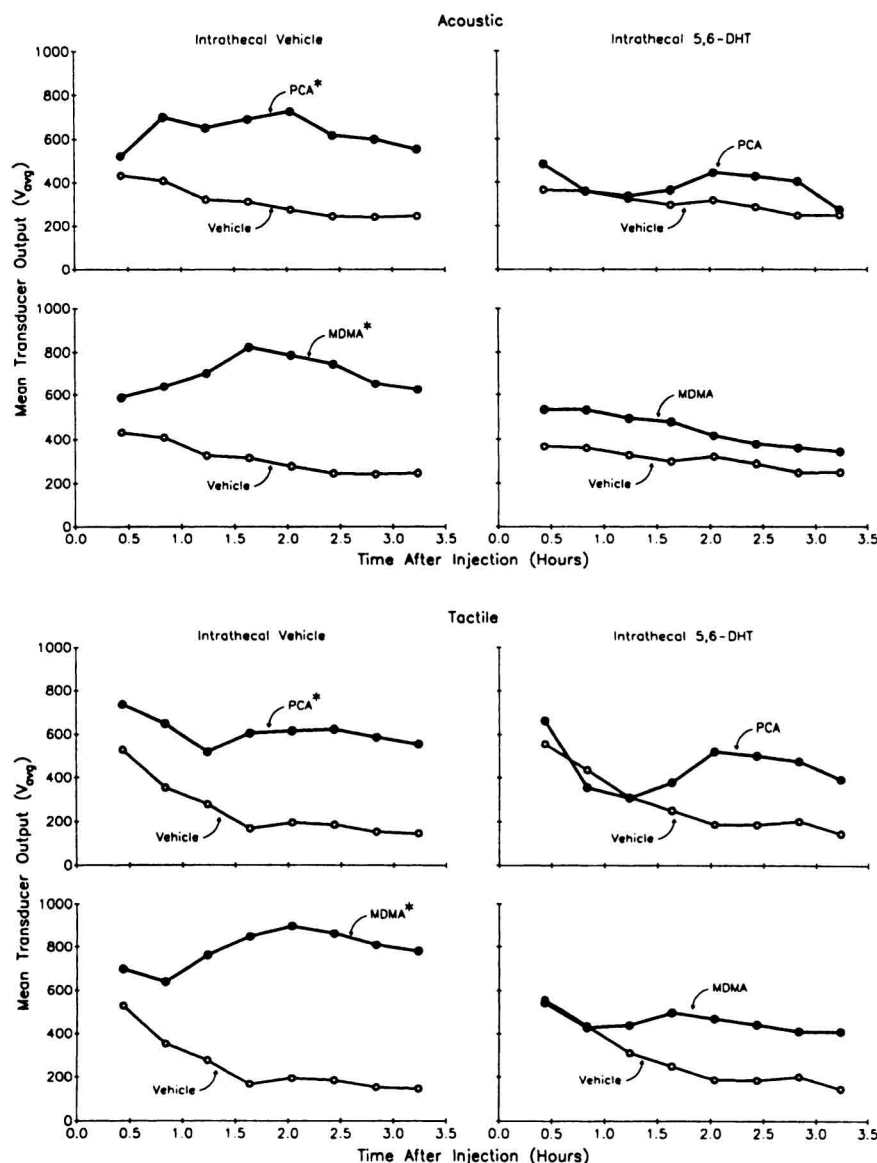


Fig. 5. Time courses of the effects of i.t. 5,6-DHT on PCA and MDMA enhancement of acoustic and tactile startle reflexes. 5,6-DHT (20 μ g; right panels) or vehicle (left panels) was administered 2 weeks before injection with MDMA (20 mg/kg, ●), PCA (10 mg/kg, ●), or vehicle (saline, ○). *Represent significant changes in startle amplitude relative to controls, $P < .05$.

TABLE 5

Effects of i.t. administration of 5,6-DHT or vehicle on the excitation of acoustic or tactile startle reflexes produced by 20.0 mg/kg MDMA or 10.0 mg/kg PCA

Treatment		Mean Startle Amplitude $\pm$ S.E.M.	
A	B	Acoustic	Tactile
i.t. Vehicle	Vehicle*	309 $\pm$ 66	249 $\pm$ 51
i.t. Vehicle	PCA	631 $\pm$ 165*	609 $\pm$ 203*
i.t. Vehicle	MDMA	696 $\pm$ 84*	789 $\pm$ 102*
i.t. 5,6-DHT	Vehicle	305 $\pm$ 40	283 $\pm$ 39
i.t. 5,6-DHT	PCA	386 $\pm$ 55 ^o	449 $\pm$ 120
i.t. 5,6-DHT	MDMA	441 $\pm$ 70†	454 $\pm$ 57†

* One-way analysis of variance for PCA, $F(3, 40) = 2.54$, $P = .07$ (acoustic); $F(3, 40) = 1.54$, not significant (tactile). One-way analysis of variance for MDMA, $F(3, 59) = 7.47$, $P < .0001$ (acoustic); $F(3, 59) = 10.46$, $P < .0001$ (tactile).

^o For difference scores comparison, $P = .084$.

* Significantly different from vehicle control, $P < .05$.

† (MDMA minus vehicle) or (PCA minus vehicle) difference scores significantly different from i.t. vehicle-pretreated controls difference scores, $P < .05$.

TABLE 6

Effects of i.t. 5,6-DHT on dorsal spinal cord 5-HT levels or striatal 5-HT or dopamine (DA) levels in rats

Condition	Dorsal Cord	Striatum	
	(5-HT)	5-HT	DA
	$\mu\text{g/g} \pm \text{SEM}$		
i.t. Vehicle	0.592 $\pm$ 0.058	0.445 $\pm$ 0.021	11.07 $\pm$ 0.509
i.t. 5,6-DHT	0.058* $\pm$ 0.012	0.425 $\pm$ 0.025	10.34 $\pm$ 0.382
Percent of control	10	96	93

* Significantly different from control group, $P < .05$.

produced by 1.25 mg/kg FEN. The FEN inhibition of startle in controls was not significantly altered by MDL 27,777A, as determined by an analysis of the (FEN minus saline) change scores for acoustic startle (FEN = -338; FEN + MDL 27,777A = -355; 105% of controls) or for tactile startle (FEN = -239; FEN + MDL 27,777A = -321; 134% of controls).

Figure 3 and table 3 show that the dopaminergic antagonist haloperidol (1.0 mg/kg) did not block the stimulatory effect of 20 mg/kg MDMA on acoustic startle. In contrast, this dose of haloperidol did attenuate the stimulatory effects of *d*-amphetamine (4.0 mg/kg) on acoustic and, to a lesser extent, tactile startle.

Figure 4 and table 4 show that MDMA stimulation of acoustic and tactile startle was blocked or greatly attenuated in rats that had been infused intraventricularly 2 weeks previously with 200 μg of the 5-HT neurotoxin 5,7-DHT. The results of neurochemical assays (reported in J. H. Kehne, T. C. McCloskey, V. L. Taylor and C. J. Schmidt in preparation) revealed that 5,7-DHT produced marked depletions of 5-HT in spinal cord (7% of control) and cortex (10% of control). Although catecholamine levels were not measured, these animals had been pretreated at the time of the 5,7-DHT infusion with the norepinephrine uptake blocker desipramine and the dopamine uptake blocker nomifensine to protect against norepinephrine or dopamine depletion.

Experiment 3. Figure 5 and table 5 show that i.t. 5,6-DHT pretreatment attenuated the excitatory effect of MDMA (20 mg/kg) on acoustic or tactile startle. 5,6-DHT alone did not affect base-line startle. Neurochemical measurements of 5-HT, summarized in table 6, showed that 5,6-DHT given i.t. produced a 90% depletion of 5-HT in the lumbar dorsal spinal cord. Striatal 5-HT was not altered, confirming the anatomical selectivity of the spinal lesion.

Figure 6 and table 7 show that MDMA stimulation of startle

was attenuated in rats that had previously received radio frequency lesions in the dorsal raphe nuclei (right panels), but not in rats that had lesions in the median raphe nuclei (middle panels).

Table 8 summarizes the 5-HT depletions produced by the radio frequency lesion experiments. The general pattern of depletions achieved by the lesions (*i.e.*, dorsal raphe: striatum > hippocampus; median raphe: hippocampus > striatum) reflected literature reports (Khanna *et al.*, 1987; Nassif-Caudarella *et al.*, 1986; Lorens and Guldberg, 1974). The magnitude of the 5-HT depletions produced by dorsal raphe lesions was consistent with literature reports, whereas the MR depletions, although statistically significant, were not as extensive as those reported in the literature or by us in a different set of experiments (J. H. Kehne, T. C. McCloskey, V. L. Taylor and C. J. Schmidt in preparation). It is possible that denervation supersensitivity in a partially lesioned 5-HT pathway might mask a deficit that would otherwise be seen with a complete lesion. Thus, definitive conclusions about the role of the median raphe are premature given the current data.

Discussion

PCA and MDMA produced qualitatively similar excitatory effects on acoustic and tactile startle (*i.e.*, a delayed peak effect and long duration of action). In contrast, FEN failed to increase tactile startle and tended to depress acoustic startle. Furthermore, the observed excitatory effects of PCA and MDMA were mediated by release of central 5-HT, with apparent contributions from 5-HT pathways that ascend from the dorsal raphe nuclei or from those that descend into the spinal cord. The remaining discussion will address possible mechanisms underlying these behavioral effects and potential relevance to understanding the actions of PCA, MDMA and FEN in humans.

Excitatory effects of PCA and MDMA on startle. The MDMA doses required to elevate startle amplitude were generally higher than those required to stimulate locomotor activity (2.5–10 mg/kg; Gold *et al.*, 1989). This is reminiscent of the pattern seen with amphetamine (see Davis, 1980). The pharmacological explanation for these dose differences is not known.

Further studies investigated serotonergic mediation of the excitatory effects of MDMA on acoustic and tactile startle. Pretreatment with 5-HT uptake blockers prevented MDMA excitation, presumably by interfering with MDMA-induced 5-HT release that is normally coupled to a carrier-mediated uptake mechanism (Fuller, 1980; Schmidt, 1987). Furthermore, depletion of central 5-HT achieved with intraventricular 5,7-DHT similarly attenuated MDMA excitation of startle. The results obtained with 5,7-DHT suggest that possible direct actions of MDMA on postsynaptic 5-HT receptors (Battaglia *et al.*, 1988; Gehlert *et al.*, 1985) were not involved. However, interactions with receptors that are located presynaptically on 5-HT terminals (which would be destroyed by 5,7-DHT) cannot be ruled out. In general, these data, combined with previous findings with PCA (Davis and Sheard, 1976), are consistent with the conclusion that the startle-enhancing effects of MDMA and PCA involve central 5-HT neurons.

The 5-HT uptake blockers alone tended to elevate startle amplitude, consistent with reports that such compounds reduced habituation of tactile startle (Geyer and Tapson, 1988) and weakly stimulated spinal flexor reflexes (Palider and Raw-

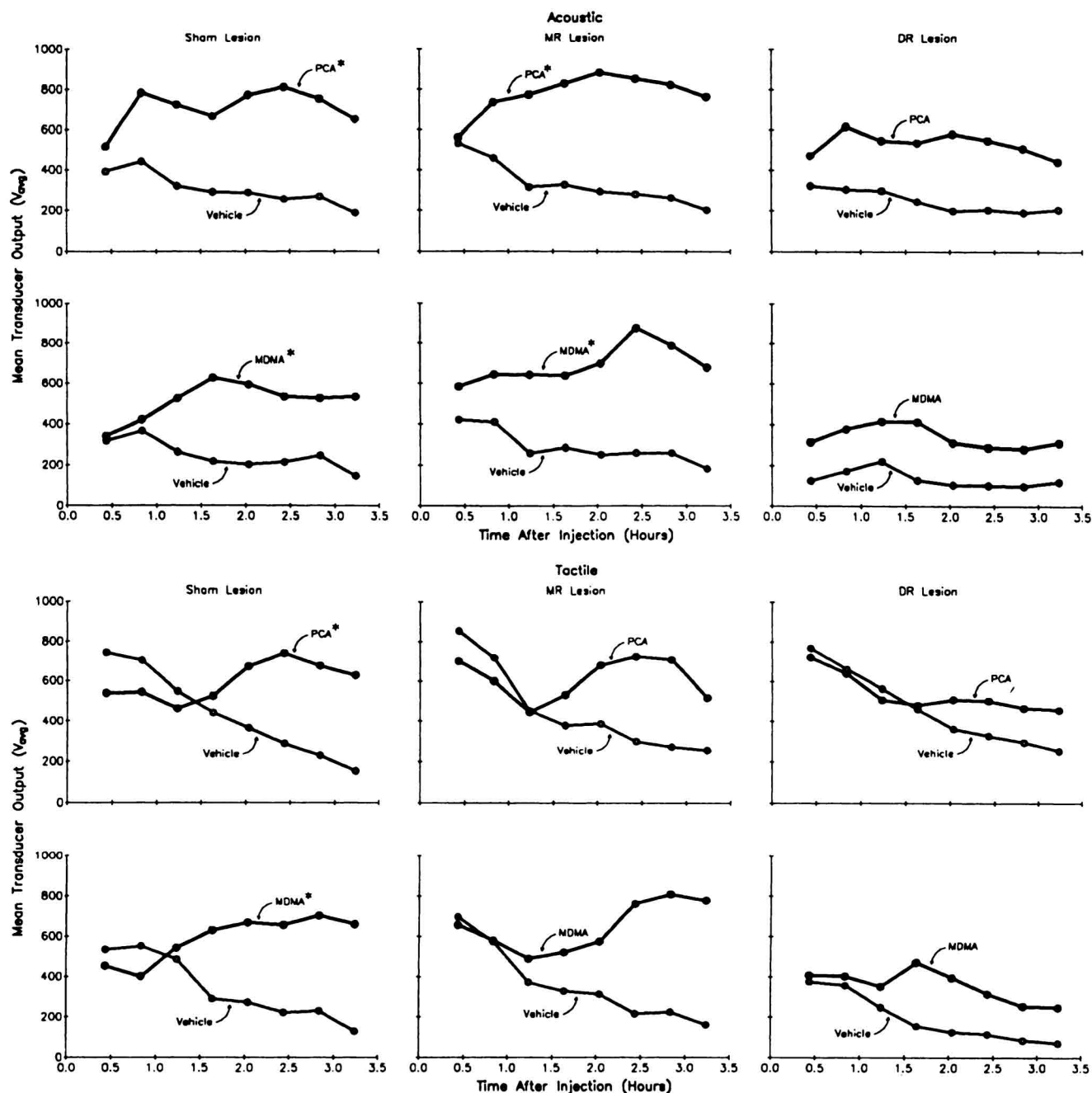


Fig. 6. Time courses of the effects of dorsal raphe (DR; right panels) or median raphe (MR; middle panels), or sham (left panels) radio frequency lesions on MDMA- or PCA-enhanced acoustic and tactile startle reflexes. Lesions were made 2 weeks before injection of MDMA (20 mg/kg, ●), PCA (10 mg/kg, ●), or vehicle (saline, ○). Asterisks represent significant changes in startle amplitude relative to controls, $P < .05$.

low, 1977). Such effects might result from enhanced synaptic availability of endogenous 5-HT, as demonstrated using *in vivo* dialysis techniques (Auerbach *et al.*, 1989). In addition, the intraventricular (i.c.v.) 5,7-DHT-treated animals tended to have lower base lines than vehicle-injected controls, suggesting that 5-HT neurons exert a tonic facilitatory influence on startle.

The simplest explanation for the MDMA/PCA effects, that these compounds release 5-HT onto receptors that are functionally excitatory to startle, does not completely fit with reported *in vivo* dialysis or voltammetry studies, which have measured 5-HT release after substituted amphetamine administration. These studies have generally found that 5-HT is

released rapidly, peaking within an hour or less after administration (PCA; Hutson and Curzon, 1989). A second explanation is that increased startle could result from disinhibition (*i.e.*, decreased activity of tonically inhibitory 5-HT neurons). Sprouse *et al.* (1989) reported that MDMA inhibits the firing of dorsal raphe neurons. Disinhibition due to depletion of 5-HT might more closely parallel the time course of the delayed increases in startle amplitude. Previous work with PCA (Davis and Sheard, 1976) noted that PCA excitation was preceded by an early inhibition, and both of these effects were prevented by 5-HT synthesis inhibition with *para*-chlorophenylalanine. In the present study, signs of early inhibition were sometimes seen (see fig. 6) and the peak effects of PCA and MDMA were indeed

TABLE 7

Effects of dorsal raphe, median raphe or sham radio frequency lesions on the excitation of acoustic or tactile startle reflexes produced by 20.0 mg/kg MDMA or 10.0 mg/kg PCA

Treatment		Mean Startle Amplitude $\pm$ S.E.M.	
A	B	Acoustic	Tactile
Sham	Vehicle*	248 $\pm$ 36	258 $\pm$ 55
Sham	PCA	849 $\pm$ 137*	776 $\pm$ 158*
Sham	MDMA	543 $\pm$ 109*	669 $\pm$ 142*
Dorsal raphe	Vehicle	192 $\pm$ 61	239 $\pm$ 92
Dorsal raphe	PCA	519 $\pm$ 168	484 $\pm$ 140
Dorsal raphe	MDMA	297 $\pm$ 128	302 $\pm$ 65†
Median raphe	Vehicle	265 $\pm$ 41	374 $\pm$ 100
Median raphe	PCA	932 $\pm$ 165*	727 $\pm$ 148
Median raphe	MDMA	761 $\pm$ 135*	730 $\pm$ 106

* One-way analysis of variance for PCA, $F(5, 76) = 14.34$, $P < .0001$ (acoustic); $F(5, 76) = 5.12$, $P < .0001$ (tactile). One-way analysis of variance for MDMA, $F(5, 64) = 15.24$, $P < .0001$ (acoustic); $F(5, 64) = 4.97$, $P < .0001$ (tactile).

* Significantly different from vehicle control, $p < .05$ or less.

† (PCA minus vehicle) or (MDMA minus vehicle) difference scores significantly different from sham controls difference scores, $P < .05$.

usually delayed for 1 to 3 hr after injection. Davis and Sheard (1976) found that, for PCA, the expression of the early inhibition (but not the later excitation) was dependent upon the level of background noise. This factor might contribute to the present findings, in which the excitatory, but not inhibitory, effects are predominantly expressed. Furthermore, Hiramatsu and Cho (1990) found that MDMA produced a decline in 5-hydroxyindoleacetic acid (5-HIAA) that peaked at 2 to 3 hr after injection, consistent with reduced 5-HT function that might result from 5-HT depletion. Evidence against the disinhibition hypothesis is the finding that 5,7-DHT-pretreated rats did not show greatly exaggerated startles as might be expected from the removal of an inhibitory 5-HT pathway, although it is possible that long-term compensatory changes occur in the chronically lesioned animal. Furthermore, FEN produces a similar 5-HT depletion (Harvey and McMaster, 1975), but clearly does not increase startle amplitude.

A third explanation for the data is that the substituted amphetamines may be releasing 5-HT from competing excitatory and inhibitory systems. Other behavioral evidence supporting the existence of this type of relationship has been previously presented (see introduction; Gerson and Baldessarini, 1980; Davis *et al.*, 1986).

MDMA and PCA also release dopamine, with the peak occurring at about an hour (PCA, Johnson *et al.*, 1990; MDMA, Hiramatsu and Cho, 1990) or later (PCA, Yamamoto and Spanos, 1988) after injection, a time course which may more generally reflect that seen on startle. Schechter (1988) reported that the discriminative stimulus properties of MDMA had two components: an early 5-HT component, and a later, mixed 5-HT and (to a lesser extent) dopamine component. 5-HT and

dopamine might interact in producing the observed excitation, although this hypothesis is not supported by the failure of haloperidol to block MDMA excitation. α -Ethyl analogs of MDMA and PCA, which have greatly diminished dopamine-releasing properties relative to their parent compounds (Johnson *et al.*, 1990), should be tested on startle.

Additionally, the lack of effect of desipramine in blocking MDMA indicates a minimal noradrenergic contribution (Nichols, 1986).

Effects of FEN on startle. FEN's failure to produce any sign of excitation of startle was surprising, given the demonstrated effects of PCA and MDMA and the qualitatively similar effects of all three compounds on 5-HT release (see introduction). One general explanation that invokes only 5-HT is that FEN's pattern of release from one or more 5-HT pathways may differ from that of MDMA or PCA. For example, FEN may fail to adequately release 5-HT from the relevant excitatory pathway. In support of this hypothesis, FEN selectively increased 5-HT metabolite levels in some 5-HT terminal areas but not in others (De Simoni *et al.*, 1988). FEN might also release 5-HT from an additional pathway that functionally inhibits the startle reflex, thereby counteracting an opposing excitatory action. In support of this possibility, drug discrimination studies have indicated that FEN generalizes to 5-HT agonists which are inhibitory to startle (*e.g.*, quipazine and the 5-HT_{1B} agonist *m*-chlorophenylpiperazine), but it does not generalize to the excitatory 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamine) tetralin (8-OH-DPAT) (see Glennon, 1988 for review). A similar explanation may explain seemingly contradictory reports that MDMA hyperactivity is attenuated by fluoxetine (Rempel and Geyer, 1990) and, yet, it is potentiated by the 5-HT antagonist methysergide (Gold and Koob, 1988). Direct comparisons of the regional 5-HT-releasing effects of FEN, MDMA and PCA using *in vivo* techniques would clarify differences in the releasing characteristics of these compounds.

A second general explanation for the observed differences is the presence of an additional neurochemical action possessed by PCA and MDMA, but not by FEN (or *vice versa*) (*i.e.*, release of a colocalized peptide or a different transmitter). PCA and MDMA release dopamine; however, a role of dopamine in the observed excitatory effect of MDMA has not been demonstrated. A "neuroleptic-like" action of racemic FEN (Invernizzi *et al.*, 1989) is probably not relevant, given the haloperidol data. Furthermore, this action is inconsistent with clinical reports that FEN is detrimental in schizophrenia (Marshall *et al.*, 1989). FEN can additionally block 5-HT uptake, although this activity is apparently weak, because FEN does not antagonize PCA-induced depletion of 5-HT (Fuller *et al.*, 1988). An intrinsic action of FEN that masks an underlying excitatory effect

TABLE 8

Regional brain levels* of 5-HT and dopamine (DA) in rats with sham or radio frequency lesions of the dorsal or median raphe nuclei

Condition	Hippocampus	Striatum		Cortex
	(5-HT)	5-HT	DA	5-HT
Sham lesion	0.415 $\pm$ 0.031	0.431 $\pm$ 0.037	10.702 $\pm$ 0.780	0.403 $\pm$ 0.021
Dorsal raphe lesion	0.270* $\pm$ 0.029 (65%)	0.146* $\pm$ 0.072 (34%)	12.365 $\pm$ 0.486 (116%)	0.201* $\pm$ 0.050 (50%)
Median raphe lesion	0.271* $\pm$ 0.045 (65%)	0.402 $\pm$ 0.012 (93%)	10.860 $\pm$ 0.403 (101%)	0.308 $\pm$ 0.023 (77%)

* Absolute values expressed as $\mu\text{g/g} \pm \text{S.E.M.}$, $n = 5$ for each region, figure in parentheses represents percent of control.

* $P < .05$ relative to sham-lesioned control.

could be tested by determining if FEN might antagonize MDMA-enhanced startle.

The slight depressant effect of FEN on acoustic startle is consistent with a previous report (Kutscher, 1987). An explanation for the different effects on acoustic and tactile startle is that FEN is suppressing neuronal transmission on the sensory side of the acoustic, but not the tactile, reflex arc. However, 5-HT uptake blockade did not reverse FEN inhibition of acoustic startle, indicating that 5-HT was not involved. *d*-FEN is a more selective releaser of 5-HT and is less sedative than is *l*-FEN (Hoebel *et al.*, 1988) and, therefore, should be tested on acoustic and tactile startle.

Central 5-HT pathways mediating PCA or MDMA excitation. Previous work suggested that there may be at least two sites in the central nervous system mediating excitatory 5-HT effects on acoustic startle: the spinal cord, mediating 5-HT₁ modulation, and a second site (possibly supraspinal), mediating 5-HT₂ modulation (see Davis *et al.*, 1986, for review). The present studies suggested that the ascending 5-HT projection from the dorsal raphe nuclei and a descending projection to the spinal cord can both contribute to the excitatory effects of MDMA and PCA on acoustic and tactile startle reflexes. 5-HT neurons exert a facilitatory influence on brain stem (McCall and Aghajanian, 1979) and spinal (Neuman and White, 1982) motor neurons, and 5-HT projections to the striatum modulate the extrapyramidal system (Giambalvo *et al.*, 1978). Thus, multiple pathways could mediate tonic and/or phasic 5-HT influences on reflex excitability. In an analogous fashion, spinal and supraspinal 5-HT pathways can contribute to the analgesia that accompanies acute PCA administration (Bjorkum and Berge, 1988).

Interpretation of substituted amphetamine effects on startle. In addition to characterizing their pharmacological basis, it is also of interest to determine the neurological actions of 5-HT releasers (*e.g.*, are these effects sensory, motor and/or motivational in nature?). MDMA and PCA could increase startle reflex amplitude by facilitating motor pathways in the brain (*e.g.*, basal ganglia, dorsal raphe projection) or in the spinal cord. Hyperreflexia and hypertonus have been reported in certain clinical cases of MDMA intoxication (Simpson and Rumack, 1981). Excessive tension in the jaw muscles, a commonly reported side effect of MDMA ingestion (Peroutka *et al.*, 1988; Greer and Tolbert, 1986) could be mediated by release from 5-HT neurons that project to the motor trigeminal nucleus (Fritschy *et al.*, 1988). Alternatively, 5-HT releasers could increase transmission in sensory pathways activated by the eliciting stimuli, a mechanism which might account for reports of enhanced tactile sensation reported by some MDMA users (Climko *et al.*, 1986–1987). Finally, startle amplitude could also be enhanced by motivational changes, such as those produced by activation of limbic system fear or anxiety pathways that ultimately impinge upon the startle reflex circuit (Davis, 1990). Currently, there is not sufficient data to distinguish between these possibilities.

In summary, three agents that share the common action of releasing 5-HT have differing effects on startle reflexes in rats. The excitatory effects of PCA and MDMA result from 5-HT released from ascending (dorsal raphe) and descending (spinal) pathways. The reason for the striking lack of effect of FEN on startle requires further experimental investigation.

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References

- AUERBACH, S. B., MINZENBERG, M. J. AND WILKINSON, L. O.: Extracellular serotonin and 5-hydroxyindoleacetic acid in hypothalamus of the unanesthetized rat measured by in vivo dialysis coupled to high-performance liquid chromatography with electrochemical detection: dialysate serotonin reflects neuronal release. *Brain Res.* **16**: 281–290, 1989.
- BATTAGLIA, G., BROOKS, B. P., KULSAKINUN, C. AND DE SOUZA, E. B.: Pharmacologic profile of MDMA (3,4-methylenedioxymethamphetamine) at various brain recognition sites. *Eur. J. Pharmacol.* **149**(1–2): 159–163, 1988.
- BEARDSLEY, P. M., BALSTER, R. L. AND HARRIS, L. S.: Self-administration of methylenedioxymethamphetamine (MDMA) by rhesus monkeys. *Drug Alcohol Depend.* **18**: 149–157, 1986.
- BERGE, O. G. AND OGREN, S. O.: Selective lesions of the bulbospinal serotonergic pathways reduce the analgesia induced by *p*-chloroamphetamine in the hot-plate test. *Neurosci. Lett.* **44**: 25–29, 1984.
- BLIER, P., SERRANO S. AND SCATTON, B.: Differential responsiveness of the rat dorsal and median raphe 5-HT systems to 5-HT₁ receptor agonists and *p*-chloroamphetamine. *Synapse* **5**: 120–133, 1990.
- BJORKUM, A. A. AND BERGE, O. G.: The relative contribution of ascending and descending serotonergic pathways in *p*-chloroamphetamine-induced antinociception. *Pharmacol. Biochem. Behav.* **31**: 135–140, 1988.
- CARLSSON, A., CORRODI, H., FUXE, K. AND HOKFELT, T.: Effect of antidepressant drugs in the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-B-ethyl-metamphetamine. *Eur. J. Pharmacol.* **5**: 357, 1969.
- CLIMKO, R. P., ROEHRICH, H., SWEENEY, D. R. AND AL-RAZI, J.: Ecstasy: a review of MDMA and MDA. *Int. J. Psychiatry Med* **16**: 359–372, 1986–1987.
- DAHL, C. B. AND GOTESTAM, K. G.: Lack of self-administration of different fenfluramine isomers in rats. *Addict. Behav.* **14**: 239–247, 1989.
- DAVIS, M.: Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci. Biobehav. Rev.* **4**: 241–263, 1980.
- DAVIS, M.: Animal models of anxiety based on classical conditioning: the conditioned emotional response (CER) and the fear-potentiated startle effect. *Pharmacol. Ther.* **47**: 147–165, 1990.
- DAVIS, M., CASSELLA, J. V., WREAN, W. H. AND KEHNE, J. H.: Serotonin receptor subtype agonists: differential effects on sensorimotor reactivity measured with acoustic startle. *Psychopharmacol. Bull.* **22**: 837–843, 1986.
- DAVIS, M., GENDELMAN, D. S., TISCHLER, M. D. AND GENDELMAN, P. M.: A primary acoustic startle circuit: lesion and stimulation studies. *J. Neurosci.* **2**: 791–805, 1982.
- DAVIS, M. AND SHEARD, M. H.: *p*-Chloroamphetamine (PCA): acute and chronic effects on habituation and sensitization of the acoustic startle response in rats. *Eur. J. Pharmacol.* **35**: 261–273, 1976.
- DE SIMONI, M. G., JURASZCZYK, Z., FODRITTO, F., DE LUIGI, A. AND GARATTINI, S.: Different effects of fenfluramine isomers and metabolites on extracellular 5-HIAA in nucleus accumbens and hippocampus of freely moving rats. *Eur. J. Pharmacol.* **153**: 295–299, 1988.
- FREY, H. H. AND MAGNUSSEN, M. P.: Different central mediation of the stimulant effects of amphetamine and its *p*-chloroanalogue. *Biochem. Pharmacol.* **17**: 1299–1307, 1968.
- FREEDMAN, J., DUDLEY, M., BARON, B., OGDEN, A. AND HOLMAN, T.: MDL 27,777A: A selective inhibitor of serotonin uptake. *Soc. Neurosci. Abstr.* **15**: 223, 1989.
- FRITSCHY, J. M., LYONS, W. E., MOLLIVER, M. E. AND GRZANNA, R.: Neurotoxic effects of *p*-chloroamphetamine on the serotonergic innervation of the trigeminal motor nucleus: a retrograde transport study. *Brain Res.* **473**: 261–270, 1988.
- FULLER, R. W.: Mechanism by which uptake inhibitors antagonize *p*-chloroamphetamine-induced depletion of brain serotonin. *Neurochem. Res.* **5**: 241–245, 1980.
- FULLER, R. W.: Pharmacology of *p*-chloroamphetamine and analogs. *Psychopharmacol. Bull.* **12**: 55–57, 1976.
- FULLER, R. W., SNODDY, H. D. AND ROBERTSON, D. W.: Mechanisms of effects of *d*-fenfluramine on brain serotonin metabolism in rats: uptake inhibition versus release. *Pharmacol. Biochem. Behav.* **30**: 715–721, 1988.
- GEHLERT, D., SCHMIDT, C. J., WU, L., LOVENBERG, W.: Evidence for specific methylenedioxymethamphetamine (ecstasy) binding sites in the rat brain. *Eur. J. Pharmacol.* **119**: 135–136, 1985.
- GERSON, S. C. AND BALDESSARINI, R. J.: Motor effects of serotonin in the central nervous system. *Life Sci.* **27**: 1435–1451, 1980.
- GEYER, M. A., PETERSEN, L. R., ROSE, G. J., HORWITT, D. D., LIGHT, R. K., ADAMS, L. M., ZOOK, J. A., HAWKINS, R. L. AND MANDELL, A. J.: The effects of lysergic acid diethylamide and mescaline-derived hallucinogens on sensory-integrative functions: tactile startle. *J. Pharmacol. Exp. Ther.* **207**: 837–847, 1978.
- GEYER, M. A. AND TAPSON, G. S.: Habituation of tactile startle is altered by drugs acting on serotonin-2 receptors. *Neuropsychopharmacology* **1**: 135–147, 1988.
- GIAMBALVO, C. T., SNODGRASS, S. R. AND URETSKY, N. J.: Effects of serotonin

- neurotoxins on rotational behavior in the rat. *Ann. N.Y. Acad. Sci.* **305**: 524-531, 1978.
- GLENNON, R. A.: Site-selective serotonin agonists as discriminative stimuli. *Psychopharmacol. Ser.* **4**: 15-31, 1988.
- GOLD, L. H., HUBNER, B. C. AND KOOB, G. F.: A role for the mesolimbic dopamine system in the psychostimulant actions of MDMA. *Psychopharmacology (Berl.)* **99**: 40-47, 1989.
- GOLD, L. H. AND KOOB, G. F.: Methysergide potentiates the hyperactivity produced by MDMA in rats. *Pharmacol. Biochem. Behav.* **29**: 645-648, 1988.
- GREER, G. AND TOLBERT, R.: Subjective reports of the effects of MDMA in a clinical setting. *J. Psychoactive Drugs* **18**: 319-327, 1986.
- GRINSPOON, L. AND BAKALAR, J. B.: Can drugs be used to enhance the psychotherapeutic process? *Am. J. Psychother.* **40**: 393-404, 1986.
- HARVEY, J. A. AND MCMASTER, S. E.: Fenfluramine: evidence for a neurotoxic action on midbrain and a long-term depletion of serotonin. *Psychopharmacol. Commun.* **1**: 217-228, 1975.
- HARVEY, J. A., MCMASTER, S. E. AND YUNGER, L. M.: *p*-Chloroamphetamine: selective neurotoxic action in brain. *Science* **187**: 841-843, 1975.
- HIRAMATSU, M. AND CHO, A. K.: Enantiomeric differences in the effects of 3,4-methylenedioxymethamphetamine on extracellular monoamines and metabolites in the striatum of freely moving rats: an in vivo microdialysis study. *Neuropharmacology* **29**: 269-275, 1990.
- HOEBEL, B. G., HERNANDEZ, L., MCCLELLAND, R. C. AND SCHWARTZ, D.: Dexfenfluramine and feeding reward. *Clin. Neuropharmacol.* **1**: S72-S85, 1988.
- HUBNER, C. B., BIRD, M., RASSNICK, S. AND KORNETSKY, C.: The threshold lowering effects of MDMA (ecstasy) on brain-stimulation reward. *Psychopharmacology (Berl.)* **95**: 49-51, 1988.
- HUTSON, P. H. AND CURZON, G.: Concurrent determination of effects of *p*-chloroamphetamine on central extracellular 5-hydroxytryptamine concentration and behaviour. *Br. J. Pharmacol.* **96**: 801-806, 1989.
- INVERNIZZI, R., BERTORELLI, R., CONSOLO, S., GARATTINI, S. AND SAMANIN, R.: Effects of the 1-isomer of fenfluramine on dopamine mechanisms in rat brain: further studies. *Eur. J. Pharmacol.* **164**: 241-248, 1989.
- JOHNSON, M. P., HOFFMAN, A. J. AND NICHOLS, D. E.: Effects of the enantiomers of MDA, MDMA and related analogues on [³H]serotonin and [³H]dopamine release from superfused rat brain slices. *Eur. J. Pharmacol.* **132**: 269-276, 1986.
- JOHNSON, M. P., HUANG, X., OBERLENDER, R., NASH, J. F. AND NICHOLS, D. E.: Behavioral, biochemical, and neurotoxicological actions of the alpha-ethyl homologue of *p*-chloroamphetamine. *Eur. J. Pharmacol.* **191**: 1-10, 1990.
- KEHNE, J. H. AND DAVIS, M.: Central noradrenergic involvement in yohimbine excitation of acoustic startle: effects of DSP4 and 6-OHDA. *Brain Res.* **330**: 31-41, 1985.
- KHANNA, J. M., CAMPANELLI, C., LIE, A. D. AND KALANT, H.: Effect of raphe lesions on the development of chronic tolerance to pentobarbital and cross-tolerance to ethanol. *Psychopharmacology (Berl.)* **91**: 473-478, 1987.
- KUTSCHER, C. L.: Action of fenfluramine, phenylpropanolamine, phentermine and diethylpropion on acoustic startle in rats. *Pharmacol. Biochem. Behav.* **27**: 749-752, 1987.
- LAFERRERE, B. AND WURTMAN, R. J.: Effect of D-fenfluramine on serotonin release in brains of anesthetized rats. *Brain Res.* **504**: 258-263, 1989.
- LAMB, R. J. AND GRIFFITHS, R. R.: Self-injection of d,l-3,4-methylenedioxymethamphetamine (MDMA) in the baboon. *Psychopharmacology (Berl.)* **91**: 268-272, 1987.
- LORENS, S. A. AND GULDBERG, H. C.: Regional 5-hydroxytryptamine following selective midbrain raphe lesions in the rat. *Brain Res.* **78**: 45-56, 1974.
- MANSBACH, R. S., BRAFF, D. L. AND GEYER, M. A.: Prepulse inhibition of the acoustic startle response is disrupted by N-ethyl-3,4-methylenedioxymethamphetamine (MDEA) in the rat. *Eur. J. Pharmacol.* **167**: 49-55, 1989.
- MARSHALL, B. D., JR., GLYNN, S. M., MIDHA, K. K., HUBBARD, J. W., BOWEN, L. L., BANZETT, L., MINTZ, J. AND LIBERMAN, R. P.: Adverse effects of fenfluramine in treatment refractory schizophrenia. *J. Clin. Psychopharmacol.* **9**: 110-115, 1989.
- MCCALL, R. B. AND AGHAJANIAN, G. K.: Serotonergic facilitation of facial motor excitation. *Brain Res.* **169**: 11-27, 1979.
- NASSIF-CAUDARELLA, S., KEMPF, E. AND VELLE, L.: Clonidine-induced sedation is not modified by single or combined neurochemical lesions of the locus coeruleus, the median and dorsal raphe nuclei. *Pharmacol. Biochem. Behav.* **25**: 1211-1216, 1986.
- NEUMAN, R. S. AND WHITE, S. R.: Serotonin-like actions of quipazine and CPP on spinal motoneurons. *Eur. J. Pharmacol.* **81**: 49-56, 1982.
- NICHOLS, D. E.: Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens. *J. Psychoactive Drugs* **18**: 305-313, 1986.
- PALIDER, W. AND RAWLOW, A.: Effect of serotonin uptake blocking agents on the flexor reflex of hind limb of the spinal rat. *Pol. J. Pharmacol. Pharm.* **29**: 367-376, 1977.
- PAXINOS, G. AND WATSON, C.: *The Rat Brain in Stereotaxic Coordinates*, 2nd ed., Academic Press, London, 1986.
- PEROUTKA, S. J., NEWMAN, H. AND HARRIS, H.: Subjective effects of 3,4-methylene-dioxymethamphetamine in recreational users. *Neuropsychopharmacology* **1**: 273-277, 1988.
- PINDER, R. M., BROGDEN, R. N., SAWYER, P. R., SPEIGHT, T. M. AND AVERY, G. S.: Fenfluramine: a review of its pharmacological properties and therapeutic efficacy in obesity. *Drugs* **10**: 241-323, 1975.
- REMPEL, N. L. AND GEYER, M. A.: Comparisons of behavioral effects of 5-HT_{1B} agonists versus MDMA in rats. *Soc. Neurosci. Abstr.* **16**: 752, 1990.
- SANDERS-BUSH, E., BUSHING, J. A. AND SULSER, F.: Long-term effects of *p*-chloroamphetamine on tryptophan hydroxylase activity and on the levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in brain. *Eur. J. Pharmacol.* **20**: 385-388, 1972.
- SCHECHTER, M. D.: Serotonergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol. Biochem. Behav.* **31**: 817-24, 1988.
- SCHMIDT, C. J.: Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. *J. Pharmacol. Exp. Ther.* **240**: 1-7, 1987.
- SIMPSON, D. L. AND RUMACK, B. H.: Methylenedioxymethamphetamine. Clinical description of overdose, death, and review of pharmacology. *Arch. Intern. Med.* **141**: 1507-1509, 1981.
- SPOUSE, J. S., BRADBERRY, C. W., ROTH, R. H. AND AGHAJANIAN, G. K.: MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin. *Eur. J. Pharmacol.* **167**: 375-383, 1989.
- VAN PRAAG, H. M. AND KORF, J.: 4-Chloroamphetamines. Chance and trend in the development of new antidepressants. *Psychopharm. Bull.* **12**: 64-74, 1976.
- VERSTER, J. AND VAN PRAAG, H. M.: A comparative investigation of methylamphetamine and 4-chloro-N-methylamphetamine in healthy test subjects. *Pharmacopsychiatria* **3**: 239-248, 1970.
- YAMAMOTO, B. K. AND SPANOS, L. J.: The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. *Eur. J. Pharmacol.* **148**: 195-203, 1988.

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