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The Effects of Lysergic Acid Diethylamide and Mescaline-Derived Hallucinogens on Sensory-Integrative Function: Tactile Startle[1]

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ABSTRACT

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Tactile startle responding by male Sprague-Dawley rats given 60 presentations of air-puff stimuli (37.5 psi) was measured after the intraperitoneal administration of graded doses of hallucinogens and other psychoactive drugs. Among the drugs tested were the indoleamine-derived compounds, lysergic acid diethylamide (LSD), N,N-dimethyltryptamine and psilocin, and the phenylethylamine-derived compounds, mescaline, 2,5-dimethoxy-4-methylamphetamine and a series of active and inactive congeners of 2,5-dimethoxy-4-methylamphetamine. All of the active phenylethylamines increased startle response magnitudes throughout the test session. This pattern of augmented startle suggests that these drugs increase reactivity. However, none of the indoleamine hallucinogens increased startle responding. Of the nonhallucinogenic drugs tested, only apomorphine increased startle responding, while clonidine significantly decreased it, and amphetamine, chlorimipramine, scopolamine and methysergide had no effect. In additional studies with LSD, it was found that LSD increased the response to only the first stimulus when more intense air-puffs were used (50 psi). Furthermore, when the number of stimuli was increased from 60 to 240 (1 hr) so that appreciable habituation was evident in controls, LSD impaired this habituation. Whereas the response magnitudes of the control group decreased by 70% across the session, the responses of LSD-treated rats decreased by only 32%. These results suggest that LSD and phenylethylamine-derived hallucinogens may differ in their effects on tactile startle responding.

A number of behavioral and neurophysiological paradigms have been used to explore the role of the serotonin systems of the brain in the modulation of sensory and sensory-integrative functions in which drugs thought to act through their effects on serotonergic transmission are combined with tasks that are at least partially dependent on the degree of perceptual sensitivity. Such studies have encompassed a variety of sensory functions, including auditory evoked potentials (Key and Bradley, 1960), auditory startle responses (Davis and Sheard, 1974a; Carlton and Advokat, 1973; Connor *et al.*, 1970), visual evoked potentials (Winters, 1975), lower cord reflexes (Meek and Fuxe, 1971), modulation of hunger (Myers, 1975; Hoebel, 1977), tempera-

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ture regulation (Myers, 1975) and tactile startle responses (Geyer *et al.*, 1975, 1976b). Characteristically the studies have focused on the effects of serotonin system modulators on stimulus-evoked initial responses and the responses after repeated presentations of the stimuli, and the paradigms have been used to explicate such psychological constructs as arousal (Key and Bradley, 1960), the orienting reflex (Sokolov, 1960; Izquierdo, 1975), the defensive reflex (Sokolov, 1960), reactivity (Miliaressis and St. Laurent, 1974; Geyer *et al.*, 1976b), sensitization (Groves and Thompson, 1970; Davis and Sheard, 1974a) and habituation (Connor *et al.*, 1970; Kandel, 1976). Species studied in this way have ranged from aplysia (Tremblay *et al.*, 1976; Kandel, 1976) to man (Winters, 1975).

Focusing on tactile startle responses to air-puffs delivered to the backs of rats, we have found that startle magnitudes after one or repeated stimuli are inversely related to the level of brain serotonin, particularly in such limbic forebrain structures as the hippocampus. Infusions of serotonin into the lateral ventricle (Geyer *et al.*, 1975) or directly into the hippocampi (Geyer, 1978) reduce startle. Conversely, tactile startle responding is increased by various manipulations that reduce limbic serotonin: electrolytic lesions of the median raphe (B8) nucleus, which projects to the hippocampus and septal nuclei (Geyer *et al.*, 1976a,b); injections of 5,7-dihydroxytryptamine (either into the ventricles or directly into the median raphe (L. R. Petersen and M. A. Geyer, unpublished observations); or tryptophan-free diets (A. J. Mandell, P. Markham and M. A. Geyer, unpublished observations). In general, functional levels of serotonin appear to influence the responses after the initial as well as the repeated presentations of stimuli. The role of serotonin in the modulation of startle is also supported by studies using auditory stimuli. Acoustic startle is increased by midbrain raphe lesions (Davis and Sheard, 1974a) or by depletions of serotonin by the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Carlton and Advokat, 1973; Connor *et al.*, 1970) or *p*-chloroamphetamine (Davis and Sheard, 1976). Although the findings suggest that startle response provides a useful measure of the functional activity of ascending serotonergic systems, that conclusion does not belie the fact that other neuronal systems are also important in the modulation of startle responding. For example, lesions of the noradrenergic locus coeruleus markedly reduce startle amplitudes (Geyer, 1978).

The argument has been developed that some aspects of the complex human experience induced by hallucinogenic drugs involve the inhibition of serotonin turnover (Brawley and Duffield, 1972), perhaps resulting from the sudden arrest of the spontaneous discharges of serotonin cells in the raphe nuclei (Aghajanian *et al.*, 1970), with resulting "release" of sensory and sensory-integrative processes from serotonin system inhibition (Purpura, 1956; Key and Bradley, 1960; Mandell and Geyer, 1976; Aghajanian and Haigler, 1976). Such sensory disinhibition has been inferred in part from studies showing facilitation of startle responding and retardation of habituation to repeated sensory stimuli in rats given hallucinogenic drugs (Miliaressis and St. Laurent, 1974; Izquierdo, 1975). Davis and his co-workers have reported that lysergic acid diethylamide (LSD), N,N-dimethyltryptamine or psilocybin increases acoustic startle in the presence of background noise (Davis and Sheard, 1974b, c; Davis and Walters, 1977), an effect they attribute to the inhibition of firing of serotonergic neurons. Similarly, we have found that mescaline and 2,5-dimethoxy-4-methylamphetamine also increase tactile startle responding (Geyer, 1978).

Taken together, these findings suggest that startle response measures may be useful in assessing the effects of hallucinogens in rats. However, direct comparisons among hallucinogens are difficult to make because of the variety of paradigms and stimulus modalities used. The present study was designed to determine whether a variety of hallucinogens produce similar increases in tactile startle responding when tested under standardized conditions. To assess the specificity of a change in startle responding produced by hallucinogens, we also tested several other psychoactive drugs in the same paradigm.

Methods

Animals. The animals were 750 experimentally naive male Sprague-Dawley rats (200–250 g). Upon receipt from the supplier (Hilltop Laboratories, Scottsdale, Pa.), the rats were housed in group cages of five rats each, located in a temperature-regulated (25 ± 2°C) animal room on a 12/12 hr light/dark cycle (lights on from 0600 to 1800 hr). Purina Rat Chow and water were available *ad libitum*. After 5 to 9 days in the animal room, the rats were transferred to the laboratory so they could adjust to that environment for between 1 and 2 hr before behavioral testing. Behavior measures were made between 0700 and 1700 hr.

Apparatus. Two separate stabilimeter devices were used to measure startle amplitudes. Each stabilimeter consisted of a cylindrical Plexiglas cage 8.2 cm in diameter and adjustable in length, surrounded by a 12.5 × 25.5 cm Plexiglas frame. Within this frame the cage was sandwiched between four rubber cylinders, two above and two below the cage. A microphonic transducer, sandwiched between the bottom of the cage and the frame, detected cage movement. Each stabilimeter was housed in a 29.5 × 39.5 × 55.0 cm sound-attenuated, illuminated and ventilated box. Visual observations of animals in the startle response devices were made through wide-angle lenses mounted in the enclosing chambers.

Air-puff stimuli, 20 msec in duration, were administered through a 1/4-inch pipe located 10 mm above the center of the cage. The cylindrical shape of the cage along with its adjustable length ensured that the stimulus would strike the animal's back. During the 250 msec interval after stimulus onset, the maximum voltage produced by the microphonic transducer was recorded by a sample-and-hold circuit. This signal was then amplified, digitized (0 to 1999) *via* an analog-to-digital converter, and simultaneously recorded on magnetic tape and printed on paper tape.

While the air-puff stimulus does produce an audible noise, the responses to this noise when the air-puff is directed away from the animal range from 0 to 10% of the mean amplitude of the responses to the air-puffs.

Behavioral procedure. For a typical experiment a total of 40 or 50 naive rats were tested on 2 consecutive days. On the 1st day each animal was placed in a stabilimeter for a 5-min warm-up period and then received 30 air-puff stimuli (37.5 psi) on a 15-sec fixed interval schedule. This test provided a baseline startle response level for each animal. Each animal was assigned to one of four to five groups ($N = 10$) matched on the basis of the baseline data because variability among subjects was high relative to the variability of each subject from day to day. On the next day one group received an i.p. injection of isotonic saline (1 ml/kg) while the other groups each received an i.p. injection of one dose of the test drug. To counterbalance variability due to circadian rhythms (Horlington, 1970) the rats were tested in approximately the same order as on the baseline day, and the average time of testing was similar for each drug group. The drugs were all tested in a predetermined sequence within a 3-month period.

After the injection, each animal was placed in single cage for the pretest interval and then placed in the stabilimeter. After 5 min, 50-air-puff stimuli (37.5 psi) were delivered on a 15-sec fixed interval schedule. The stabilimeters were cleaned with soap and water between tests. As noted in "Results," some additional experiments were performed in which the intensity or number of stimuli differed from this standard paradigm (table 4).

Drugs. The following drugs were tested: LSD tartrate; N,N-dimethyltryptamine fumarate (DMT); psilocin; mescaline SO_4; 2,5-dimethoxy-4-methylamphetamine HCl (DOM); 2,5-dimethoxy-4-ethylamphetamine HCl (DOET) (all supplied by the Research Technology Branch, National Institute of Drug Abuse); 2,5-dimethoxy-4-propylamphetamine HCl (DOPR); 2,5-dimethoxy-4-*n*-butylamphetamine HCl (DOBU); 2,5-dimethoxyamphetamine bioxalate (DMA); 2,5-dimethoxy-4-amylamphetamine HCl (DOAM); 2,5-dimethoxy-4-t-butylamphetamine HCl (DOTB) (donated by Dr. A. T. Shulgin); *d*-amphetemine SO_4 (Sigma Chemical Company, St. Louis, Mo.); apomorphine HCl (Merck & Company, Rahway, N.J.); clonidine HCl (provided by Dr. P. Skolnick); chlorimipramine HCl (donated by Geigy Pharmaceuticals, Ardsley, N.Y.); methysergide maleate (donated by Sandoz Pharmaceuticals, Hanover, N.J.); scopolamine HBr (Sigma); and methylscopolamine HBr (Sigma). Doses (tables 1–3) refer to the salt form. Drugs were generally dissolved in isotonic saline immediately before use and were injected i.p. at a volume of 1 ml/kg.

Among the drugs tested was a homologous series of DOM derivatives for which relative human potencies have been reported (Shulgin and Dyer, 1975). This series varies only in the number of carbons (0–5) in the alkyl chain of position 4 (fig. 1). Their psychoactive properties in humans (Shulgin and Dyer, 1975) and apparently in rats (Morin *et al.*, (1975) are maximal with from 1 to 3 carbons and virtually absent with 0 to 5 carbons. Only high doses (4 mg/kg) of the less potent phenylethylamines (DMA, DOAM and DOTB) were tested because these drugs are relatively inactive in humans (Shulgin and Dyer, 1975) and rats (Morin *et al.*, 1975).

Statistical analysis. All of the data were stored initially in digital form on a Kennedy incremental tape unit. The tapes were read into a Burroughs 6700 computer for reduction and analyses of the data. The results were analysed in several ways. One-way analyses of variance (ANOVA) were done on the mean startle response amplitude over all 60 trials, the differences between the means of the 30 baseline trials and the means of the 60 test trials and the ratios between the baseline and test means. Only the first of these ANOVAs is presented because all three ANOVAs yielded comparable results. A separate one-way ANOVA was done on the first response when appropriate.

	R =
DMA	H
DOM	CH_3
DOET	C_2H_5
DOPR	n-C_3H_7
DOBU	n-C_4H_9
DOAM	n-C_5H_{11}
DOTB	t-C_4H_9

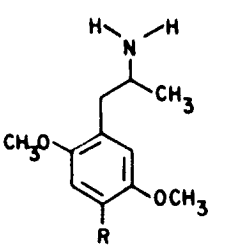

Fig. 1. The general structure of the series of the phenylethylamine hallucinogens is indicated on the right, with the specific substituents on the left. Only DOM, DOET and DOPR significantly increased startle responses.

For an index of sensitization, we calculated the difference between the first response and the mean of responses 2 through 10 (*cf.* Geyer *et al.*, 1976b). This measure was generally insensitive to drug treatments and will be mentioned only where significant results were found. Habituation was assessed in a repeated-measures ANOVA with drug and blocks of 20 trials as factors. The trials factor was routinely significant and only significant drug-by-trials interactions, indicative of a drug effect upon habituation, will be mentioned. In general, specific comparisons were made between each drug dose and the corresponding saline group mean according to Dunnett's method (Winer, 1971). Linear regression analyses and tests of the significance of differences between slopes used *t* tests as described by Winer (1971). Two-tailed sampling distributions were used for determining significance.

Results

Drug Screening with the Standard Paradigm

Hallucinogens. The effects of the hallucinogens are summarized in table 1. The first five drugs are the active hallucinogens that share the basic phenylethylamine structure: mescaline, DOM, DOET, DOPR and DOBU. Each (with the exception of DOBU, the weakest in man of the active compounds in the series) reliably increased startle magnitudes. On the basis of percent change from saline controls, mescaline, DOM and DOPR produced the largest effects (37–47%). These results confirm our previous findings with the first two of these drugs (Geyer,

TABLE 1

Effects of phenylethylamine hallucinogens on startle responding

Startle response amplitudes are expressed in arbitrary units as group means. The *F* values reported refer to a one-way ANOVA on the mean response amplitudes over 60 trials.

Dose per kg	*N*	Overall Mean ± S.E.	Dunnett's		ANOVA	Percent Change
			t	P		
Expt. 1: Mescaline (pretest interval = 30 min)						
0	9	186 ± 10.1				
5 mg	9	232 ± 16.0	N.S.		$F = 3.57$	+25
10 mg	9	255 ± 23.9	2.79	<.025	df = 3,32	+37
20 mg	9	257 ± 16.5	2.86	<.025	P < .025	+38
Expt. 2: DOM (35 min)						
0	9	163 ± 15.9				
0.125 mg	9	158 ± 12.4	N.S.		$F = 5.83$	−3
0.25 mg	9	190 ± 13.5	N.S.		df = 4,40	+16
0.5 mg	9	239 ± 19.1	3.77	< .005	P < .01	+47
1.0 mg	9	214 ± 13.4	2.55	< .025		+31
Expt. 3: DOET (35 min)						
0	9	158 ± 11.3				
0.125 mg	10	153 ± 19.2	N.S.		$F = 2.66$	−3
0.25 mg	9	184 ± 8.9	N.S.		df = 4,42	+16
0.5 mg	10	211 ± 16.5	2.41	< .05	P < .05	+33
1.0 mg	9	192 ± 19.2	N.S.			+22
Expt. 4: DOPR (35 min)						
0	10	149 ± 16.1				
0.125 mg	9	156 ± 23.4	N.S.		$F = 3.08$	+5
0.25 mg	9	184 ± 8.9	N.S.		df = 4,42	+23
0.5 mg	10	205 ± 12.6	2.68	< .025	P < .05	+37
1.0 mg	9	198 ± 11.4	2.34	< .05		+33
Expt. 5: DOBU (35 min)						
0	9	193 ± 15.3				
0.25 mg	9	216 ± 13.7	N.S.		N.S.	+12
0.5 mg	9	218 ± 14.7	N.S.			+13
1.0 mg	9	217 ± 15.2	N.S.			+13
Expt. 6: (35 min)						
Saline	10	236 ± 14.1				
DMA						
4.0 mg	10	228 ± 19.1	N.S.		N.S.	−3
DOAM						
4.0 mg	10	226 ± 13.0	N.S.			−4
DOTB						
4.0 mg	10	267 ± 26.5	N.S.			+13

1978). With DOM, DOET and DOPR maximal responses were obtained at doses of 0.5 mg/kg. The lower doses had no significant effects, while the higher dose (1.0 mg/kg) was less effective than the 0.5 mg/kg dose for all three drugs. These dose-response relationships suggest an inverted U-shaped curve. The first responses after these four phenylethylamine drugs were increased proportionately (maximal effects ranged from 24–38% above control) although not significantly since such single-response measures were highly variable. In no case was our index of sensitization—the difference between the first response and the mean of responses 2 through 10—significantly affected by these drugs. Similarly, there were no significant drug-by-trials interactions after the phenylethylamine hallucinogens. While DOBU produced mean increases in startle responding at all three doses tested, the increases were not statistically significant. The high doses (4.0 mg/kg) of the three congeners of DOM reported to be inactive in man (Shulgin and Dyer, 1975)—DMA, DOTB and DOAM—had no detectable effects.

As shown in table 2, none of the indoleamine-derived hallucinogens increased tactile startle responding significantly, even though the doses used were comparable to those reported to increase acoustic startle responding in the presence of background noise (Davis and Sheard, 1974b,c; Davis and Walters, 1977). Neither LSD nor DMT had a significant effect at any dose or trial block. The 10 mg/kg dose of psilocin significantly reduced startle amplitudes according to Dunnett's test, although the overall ANOVA was nonsignificant. This dose of psilocin also produced some hindlimb ataxia similar to that produced by both mescaline and LSD at doses higher than those used in the present study (M. A. Geyer and D. S. Segal, unpublished observations). Further studies examining this disparity between the effects of indoleamines and phenylethylamines are described below.

Nonhallucinogens. Of the nonhallucinogenic drugs shown in table 3, only clonidine and apomorphine had significant effects on startle response amplitudes. Low doses of clonidine, an *alpha* adrenergic agonist, reduced startle amplitudes in a dose-dependent fashion ($F = 14.8$; df = 3,32; $P < .001$); specific comparisons indicated that the effect was most significant at doses of 25 μg/kg ($t = 2.73$; df = 32,4; $P < .025$) and 50 μg/kg ($t = 5.5$; df = 32,4; $P < .001$). Clonidine reduced startle throughout the test session; there was no interaction with trial blocks, and the effect was significant in the first block of five trials ($F = 7.36$; df = 3,32; $P < .001$). Apomorphine increased startle amplitudes over the first 20 trials ($F = 3.1$; df = 3,32; $P < .05$), although the effect was not significant over all 60 trials (table 3). In the repeated measures ANOVA, apomorphine produced a significant drug-by-trials (blocks of 20) interaction ($F = 3.26$; df = 6,72; $P < .01$). This early increase in startle was dose-dependent and was not followed by a later decrease, as has been reported for apomorphine (Davis and Aghajanian, 1976), and is therefore visible in the overall means shown in table 3.

TABLE 2

Effects of indoleamine hallucinogens on startle responding

Startle response amplitudes are expressed in arbitrary units as group means. One-way ANOVA was performed on the mean response amplitudes over 60 trials.

Dose per kg	N	Overall Mean ± SE	Dunnett's		ANOVA	Percent Change
			t	P		
Expt. 1: LSD (pretest interval = 35 min)						
0	7	152 ± 7.7			N.S.	
20 μg	7	144 ± 9.6				−5
40 μg	7	135 ± 8.2				−12
80 μg	7	149 ± 17.3				−2
Expt. 2: DMT (5 min)						
0	9	157 ± 10.6			N.S.	
0.25 mg	10	170 ± 15.0				+8
0.5 mg	9	161 ± 10.3				+3
1.0 mg	10	167 ± 10.6				+7
Expt. 3: Psilocin (35 min)						
0	10	223 ± 17.8			N.S.	
2.5 mg	10	199 ± 19.1				−11
5.0 mg	10	184 ± 15.5				−18
10.0 mg	10	173 ± 10.9	2.39	<.05		−22

TABLE 3

Effects of nonhallucinogenic psychoactive drugs on startle responding

Startle response amplitudes are expressed in arbitrary units as group means. The *F* value reported refers to a one-way ANOVA on the mean response amplitudes over 60 trials.

Dose per kg	*N*	Overall Mean ± S.E.	Dunnett's *t*	Dunnett's P	ANOVA	Percent Change
Expt. 1: Amphetamine (pretest interval = 30 min)						
0	10	215 ± 9.4			N.S.	
0.5 mg	10	245 ± 19.3				+14
1.0 mg	10	240 ± 18.5				+12
2.0 mg	10	245 ± 11.6				+14
Expt. 2: Apomorphine (30 min)						
0	10	188 ± 10.6			N.S. except	
0.25 mg	10	186 ± 12.2			trials 1–20	−1
0.5 mg	10	218 ± 20.3			(see "Results")	+16
1.0 mg	10	233 ± 22.7				+24
Expt. 3: Clonidine (30 min)						
0	10	228 ± 21.9			$F = 14.8$	
12.5 μg	10	232 ± 16.5	N.S.		df = 3,32	+2
25.0 μg	10	186 ± 13.1	2.73	< .025	P = .01	−18
50.0 μg	10	145 ± 10.8	5.5	< .001		−36
Expt. 4: Chlorimipramine (40 min)						
0	10	211 ± 16.6				
5.0 mg	10	207 ± 12.3				−2
10.0 mg	10	200 ± 10.9				−5
20.0 mg	10	236 ± 20.3				+11
Expt. 5: Methysergide (40 min)						
0	9	224 ± 22.9			N.S.	
1.5 mg	9	226 ± 17.5				+1
3.0 mg	9	263 ± 18.8				+17
6.0 mg	9	215 ± 13.1				−4
Expt. 5: (40 min)						
Scopolamine						
0	10	218 ± 22.6			N.S.	−5
2.0	10	207 ± 16.6				
Methylscopolamine						
2.0 mg	10	246 ± 26.2				+13

The short duration of the effect of apomorphine may be related to the rapid metabolism of this dopaminergic agonist. Methysergide, amphetamine, chlorimipramine, scopolamine and methylscopolamine had no significant effect on the overall startle response at any of the doses tested.

Further Experiments with LSD

Time-response and stimulus intensity studies. The results are summarized in table 4. As indicated in table 2 we had previously begun the startle test 35 min after 20, 40 or 80 μg/kg of LSD. The first experiment listed in table 4 shows that doses of 40, 80 or 160 μg/kg LSD had no effect 10 min after injection. The second experiment summarized in table 4 compared saline and 80 μg/kg of LSD at two different stimulus intensities: our standard 37.5 psi and 50 psi. While no differences in the overall startle magnitudes were found at either stimulus intensity (table 4, expt. 2), the first response to the higher intensity stimulus was nearly doubled in the LSD group (280 ± 40.7 *vs.* 454 ± 62.3; $P < .05$, *t* test). At the standard intensity no such difference was obtained (278 ± 76.4 *vs.* 290 ± 56.1; N.S.). To determine whether this striking contrast between the first and all subsequent responses at the higher stimulus intensity was a reliable effect of LSD, we repeated the experiment with 100 μg/kg and larger group sizes (to compensate for the inherent variability of single-response measures). In this instance, we waited 90 to 120 sec after each of the first two stimuli before beginning a series of 30 stimuli with a fixed interstimulus interval of 15 sec. Once again, the first 50 psi stimulus produced a significant difference between the saline and LSD groups (226 ± 15.3 *vs.* 366 ± 55.0; $P < .02$, *t* test). The next two responses were similar for both groups (285 ± 34.4 *vs.* 248 ± 29.3; N.S.); as was the mean of all 30 subsequent responses (table 4, expt. 3).

Habituation studies with LSD. As is characteristic of "defensive reactions" to noxious

stimuli (Sokolov, 1960) the rate of habituation to the air-puffs in control animals is rather slow, typically amounting to 15% reduction in response over three blocks of 20 trials each. Even if LSD were completely preventing habituation it would have been difficult to detect this effect without more appreciable habituation being evident in control animals. Therefore, we tested animals injected with either 100 μg/kg of LSD or saline with 240 presentations (1 hr) of the 50 psi air-puff. The results are presented in figure 2 in blocks of 30 trials each. As expected from

TABLE 4

Effects of LSD on startle responding

Startle response amplitudes are expressed in arbitrary units as group means.

Expt. (psi)	Dose	Pretest Interval	N	First Response	Trial Blocks			Overall Mean ± S.E.	ANOVA
					1	2	3		
	μg/kg	min							
					blocks of 20				
1 (37.5)	0	10	9	178	139	123	121	128 ± 11.4	N.S.
	40		9	174	133	144	134	137 ± 7.8	
	80		9	164	157	123	126	135 ± 13.5	
	160		9	148	142	140	135	139 ± 11.9	
2a (37.5)	0	35	10	278	231	185	197	205 ± 12.8	N.S.
	80		10	290	203	186	195	195 ± 13.8	
2b (50)	0	35	10	280	230	204	222	218 ± 5.8	N.S.
	80		10	454*	231	233	242	235 ± 13.7	
					blocks of 10				
3 (50)	0	35	20	226	205	204	196	202 ± 13.4	N.S.
	100		20	366**	205	229	220	218 ± 28.2	

* $P < .05$; significantly different from control by Student's *t* test.
** $P < .02$.

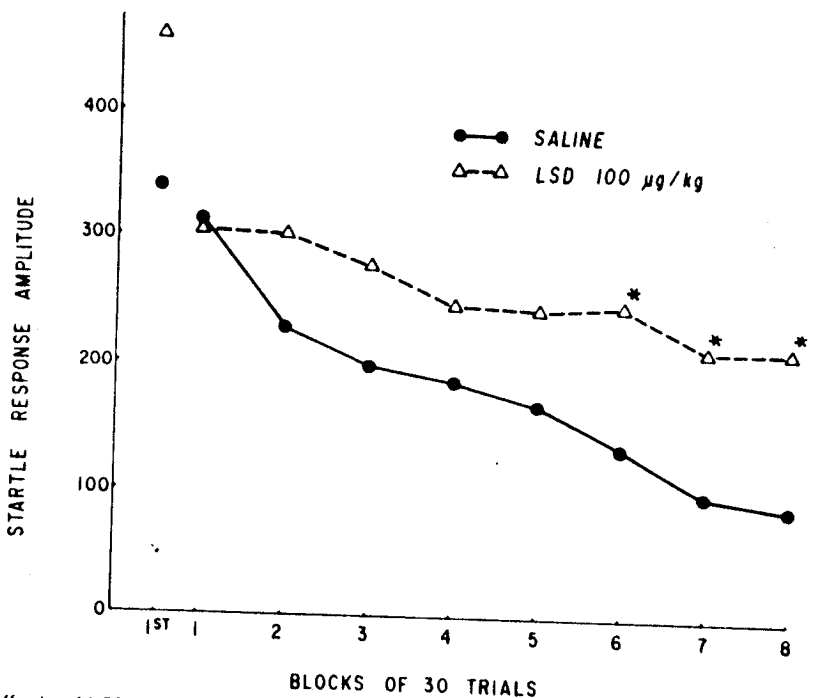

Fig. 2. The effects of LSD on the responses to 240 air-puff stimuli (15 sec interstimulus interval) are shown for the first trial and 8 blocks of 30 trials each. Each point represents the mean startle amplitude for groups of 13 rats each. Testing began 10 min after i.p. injections of either saline or 100 μg/kg of *d*-LSD. LSD-treated rats habituated significantly less than did controls (see "Results"). Trial blocks 5 and 6 correspond to the time after injection used in the 60 trial experiments in which 100 μg/kg of LSD had no significant effect (expt. 2 and 3, table 4), indicating that this result is not due simply to the time course of action of LSD. * Significantly greater than corresponding control mean (ANOVA), $P < .05$.

experiments 2 and 3, table 4, the initial response was somewhat higher in LSD-treated animals, although the difference was not significant due to the smaller sample size. As in the previous experiments, no significant differences were found during the first 60 trials. However, in the sixth and each subsequent block of 30 trials, LSD significantly increased startle magnitudes (F's = 5.0–10.2; df = 1,24; $P < .05$). Furthermore, the drug-by-trials interaction was significant ($F = 2.24$; df = 7,168; $P < .05$). Habituation was also assessed as the percent reduction in response amplitude for each subject from the first to the last trial block. As shown in figure 2, control animals exhibited 70 ± 5.1% habituation while the response in LSD-treated animals decreased by only 32 ± 6.9% ($t = 4.67$; df = 24; $P < .001$). A linear regression analysis of the means displayed in figure 2 demonstrated that both groups' curves had slopes that were significantly less than 0 (−28.8 ± 3.1; $t = 9.31$; df = 6; $P < .001$ and −14.2 ± 1.85; $t = 7.71$; df = 6; $P < .001$) and that these slopes differed from each other ($t = 21.7$; df = 6 $P < .001$). Virtually identical results have recently been found with 240 presentations of standard 37.5 psi stimulus and with doses of 50 or 200 μg/kg of LSD in the 50 psi, 240 trial paradigm (M. A. Geyer, R. L. Hawkins and D. L. Braff, unpublished observations).

Discussion

Drug screening with the standard paradigm. In general, the phenylethylamine hallucinogens (mescaline, DOM, DOET and DOPR) did reliably increase startle response amplitudes in the standard 60 trial test, and the relative potencies of the phenylethylamines on tactile startle paralleled their hallucinogenic potencies in man rather well. Within the homologous series of DOM derivatives, those with 1 (DOM), 2 (DOET) or 3 (DOPR) carbons in the alkyl side chain augmented startle amplitudes. These three also exhibit the greatest potencies as hallucinogens in man (Shulgin and Dyer, 1975). According to Shulgin and Dyer, DOM, DOET and DOPR are approximately equipotent, while DOBU is approximately one-third as potent as the previous three, and DMA, DOAM and DOTB are essentially inactive. The effects of DOBU, while increasing startle response amplitude, were not statistically significant.

Of the nonhallucinogenic drugs tested, only clonidine and apomorphine had significant effects on startle response amplitudes. The fact that clonidine depresses startle responses to air-puff stimuli is consistent with the results found with acoustic stimuli (Davis *et al.*, 1977a). It has been argued that clonidine facilitates the habituation of acoustic startle (Davis *et al.*, 1977a). However, in our study with tactile stimuli, clonidine reduced virtually all responses, and no interaction between clonidine and successive trial blocks was found. Our data are more consistent with a general decrease in reactivity after clonidine. Very low doses of clonidine given either systemically (10 μg/kg) or directly by iontophoresis, inhibit the firing rate of norepinephrine-containing neurons in the locus coeruleus (Svennson *et al.*, 1975). The depressant effect of clonidine on tactile startle is consistent with depression of startle amplitudes by electrolytic locus coeruleus lesions (Geyer, 1978). Our data with apomorphine parallel previous findings with acoustic stimuli. Davis and Aghajanian (1976) found that apomorphine increased startle amplitudes until 40 min after i.p. injection; at longer periods apomorphine had a depressant effect on startle amplitudes. Our finding that apomorphine increased tactile startle only during the first 20 trials is consistent with these results since our test began 30 min postinjection, but the absence of any later decrease suggests that the time course of action of apomorphine may be quite short.

Methysergide, *d*-amphetamine, scopolamine and chlorimipramine had no significant effects on tactile startle at any dose tested. It has been reported recently that chlorimipramine also has no significant effect on acoustic startle (Davis *et al.*, 1977b). Our results with amphetamine are consistent with previous reports that amphetamine has no effect on startle response amplitudes except at doses above 2.5 mg/kg (Kirkby *et al.*, 1972; Davis *et al.*, 1975; Horlington, 1970). The doses of amphetamine used in the present study produce striking increases in most measures of behavioral arousal, such as locomotor activity (Segal and Mandell, 1974). If the sympathetic system arousal common to hallucinogens were responsible for their effects on startle, these doses of amphetamine should have also increased startle responding. As Horlington (1970) demonstrated, activity measures and reactivity measures such as startle reflect different and dissociable aspects of behavior.

The above results indicate that our standard paradigm measuring tactile startle over 60 trials provides a reasonably sensitive measure for phenylethylamine-derived hallucinogens that correlates well with their psychotropic activity in man. That apomorphine was the only non-

hallucinogen that increased startle responding may not be surprising. In the study by Silva and Calil (1975) of three potential screening tests for hallucinogens, this dopaminergic agonist was the most similar to the hallucinogens of all the drugs tested. Certainly the involvement of dopaminergic systems in the actions of hallucinogens is a current hypothesis with some support (Nichols, 1976; Fog, 1969; Pieri *et al.*, 1974; Kelly and Iversen, 1975; Trulson *et al.*, 1977).

Most attempts to develop a screening measure for hallucinogens have been based on the premise that the class of hallucinogens is a distinct category of drugs sharing some common effect (Silva and Calil, 1975). However, membership in this class has largely been defined by the subjective reports of humans, often with little or no rigorous observation (Mandell and Geyer, 1976). There is some evidence for qualitative differences in the psychological effects of indoleamine or phenylethylamine-based hallucinogens in man (Hoch *et al.*, 1952; Naranjo, 1975; Shulgin, 1973). Similarly, in animals, behavioral, electrophysiological and biochemical differences between these two structurally distinct drug groups have been reported (Schechter and Winter, 1971; Winters, 1975; Aghajanian *et al.*, 1970; Freedman *et al.*, 1970). Our studies suggest that tactile startle is useful in predicting hallucinogenic drug activity within the phenylethylamine series and may also discriminate between the actions of this family and the indolethylamines. The absence of effects with indoleamine hallucinogens was unexpected in light of the work of Davis's group who have reported that LSD, DMT and psilocin, relatively inactive in our screening study, augmented the amplitude of the startle response to acoustic stimuli at the doses used in our study (Davis and Sheard, 1974b,c; Davis and Walters, 1977). To clarify the effects of indoleamine hallucinogens on tactile startle, we undertook several additional studies with LSD.

Habituation studies with LSD. The results of these experiments further indicate that there may be qualitative differences between the effects of phenylethylamine drugs and LSD on the tactile startle response. Drugs such as mescaline or DOM increased startle magnitudes in response to virtually every air-puff stimulus, a response pattern indicative of a general increase in reactivity. In contrast, LSD had no significant effect over 60 trials with the standard paradigm. When more intense tactile stimuli were used, LSD significantly augmented only the first one of 60 responses. Further study is needed to explain the uniqueness of the first response in its sensitivity to LSD.

Perhaps the most intriguing difference between the effects of LSD and DOM or mescaline is that LSD appears to retard the habituation of startle without producing an increase in general reactivity. Habituation is defined as a decrement in response magnitude that accrues as the stimulus is presented repeatedly. In the case of DOM, for instance, startle is increased in the first block of 20 trials, long before habituation is evident in controls. Since it is difficult to interpret any change in the rate of response reduction when the initial values are different, we have not yet attempted to assess the rates of habituation after DOM or mescaline in the 240-trial paradigm. However, LSD has no detectable effect on startle during the first block of 30 trials (fig. 2) and no significant effect over 60 trials (table 4). Yet by the sixth block of 30 trials, LSD-treated rats are significantly more responsive than controls (fig. 2). While the mean response magnitude in controls decreased 70% from the first to the eighth trial block, the response of LSD-treated rats decreased only 32%. This impairment of habituation was not complete; the responses of the LSD animals during the eighth trial block were significantly lower than those during the first block (dependent t test, $t[12] = 4.45$; $P < .001$). That this accruing effect is not simply due to the time course of the distribution or action of LSD is indicated by its significant effect on the initial response and by the lack of any effect of LSD over the first 60 trials when the interval between injection and testing was 35 min (table 2; expt. 2 and 3, table 4), a time corresponding to trial blocks 5 and 6 of figure 2. This result is reminiscent of the impairment of habituation of the electroencephalographic arousal response demonstrated by Key and Bradley (1960) and Key (1961). More recently, Miliaressis and St. Laurent (1974) suggested that LSD impaired habituation of acoustic startle and Izquierdo (1975) presented data on orienting responses indicative of similar phenomena. However, in their reports it is not clear that the early responses were not also increased by LSD, as discussed by Davis and Sheard (1975).

As described by Pavlov (1941) and Groves and Thompson (1970), unconditioned ressponses to repeated presentations of startling stimuli appear to be affected by two distinct processes: habituation and sensitization. Although it is generally believed that sensitization is most evident during the initial trials (Groves and Thompson,

1970), when LSD had no effect on our measure, it is conceivable that the accruing effect of LSD seen in figure 2 was due to an increase in sensitization rather than (or in addition to) an impairment of the process of habituation. In support of this possibility is the observation by Davis and Sheard (1974c) that the augmentation of acoustic startle after LSD is attributable to an increase in sensitization to the background noise. Our operational measure of sensitization has been the difference between the first response and the mean of responses 2 through 10 since the initial response is the only measure that is not contaminated by either sensitization or habituation (*cf.* Geyer *et al.*, 1976b; Geyer, 1978). However, because LSD has a selective effect on the first response (table 4), such a measure of sensitization is not applicable. Thus, we cannot be certain whether the LSD-induced impairment of habituation, as defined operationally, is due to an alteration in the process of habituation or the process of sensitization, or both.

Our results suggest that while LSD and the phenylethylamine hallucinogens share the common effect of increasing startle responses, the specific natures of these effects may be quite different. Work is now in progress to determine whether other indoleamine-derived hallucinogens such as DMT or psilocin impair the habituation of tactile startle responding in a manner similar to LSD. If so, such a dissociation between the effects of phenylethylamine and indoleamine drugs would suggest that these two classes of hallucinogens differ in their mechanisms of action.

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