Pharmacology Biochemistry and Behavior, Vol. 2, pp. 675–683. ANKHO International Inc., 1974. Printed in the U.S.A.

Effects of Lysergic Acid Diethylamide (LSD) on Habituation and Sensitization of the Startle Response in the Rat[1]

MICHAEL DAVIS[2] AND MICHAEL H. SHEARD

Yale University School of Medicine and Connecticut Mental Health Center
New Haven, Connecticut 06508

(Received 1 February 1974)

DAVIS, M. AND M. H. SHEARD. *Effects of lysergic acid diethylamide (LSD) on habituation and sensitization of the startle response in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(5) 675–683, 1974. – In 4 experiments the effect of d-lysergic acid diethylamide (LSD) on the acoustic startle response in rats was measured. A low dose (20 μg/kg) facilitated startle but a high dose (160 μg/kg) at first facilitated but then depressed startle somewhat relative to an intermediate dose (40 μg/kg). 2-brom LSD (199 μg/kg) had no detectable effect and 40 μg/kg LSD did not change startle in raphe-lesioned rats. LSD appeared to augment sensitization rather than acting on the startle circuit directly since it did not increase startle unless given in conjunction with either background noise or repetitive tones. LSD did not prevent between session habituation. Relationships between habituation, sensitization, and the midbrain raphe nuclei were discussed.

Habituation LSD Lysergic acid diethylamide Raphe Sensitization Startle

IT HAS long been recognized that reflex behavior can be diminished by repetitive presentation of the eliciting stimulus. This phenomenon has been called habituation and has been reported in many experiments [13,18]. There is now considerable evidence, however, which indicates that reflex behavior may also increase with repetitive stimulus exposure. This phenomenon has been termed sensitization and has also been seen in a variety of experimental situations [11]. If these two divergent consequences of repetitive stimulus exposure represent fundamentally different processes, then it should be possible to find separate physiological mechanisms underlying each.

Recently it was reported that lesions of the midbrain raphe nuclei result in a substantial increase in the amplitude of the acoustic startle reflex in the rat [10]. More interesting, a tone by tone analysis of the data suggested that the raphe lesion accentuated sensitization but did not interfere with either within or between-session habituation. These results suggest that the raphe neuronal system may be especially important in the modulation of startle and/or the regulation of sensitization as well as suggesting that habituation and sensitization at the behavioral level may be dissociated by a physiological manipulation.

It is possible that the effects associated with lesions of the raphe nuclei were not specific to the raphe but instead a consequence of damage of fibers of passage, or surrounding areas, or even non-specific lesion effects. A method which would specifically alter raphe function but which would not involve a lesion of the raphe area would therefore be important to further study the relationship between startle and the raphe nuclei.

There is now considerable evidence that systemic administration d-lysergic acid diethylamide (LSD) specifically inhibits cells within the midbrain raphe nuclei. These cells have been shown to contain serotonin by histochemical techniques [5]. Very low doses of LSD given systemically cause a marked inhibition of the spontaneous firing rate of raphe neurons [1] and direct microiontophoretic ejection of LSD onto raphe neurons also inhibits their rate of firing [2]. More recently it has been demonstrated that raphe cells are much more sensitive to the inhibitory effects of LSD than cells which are postsynaptic to them [12]. Further, LSD administered to animals with a mesencephalic-diencephalic transection still inhibits raphe firing rates [12]. Taken together these results suggest that systemic administration of LSD causes a specific inhibition

[1] This research was supported by United States Public Health Service Grants MH-17856 and MH-07114, by National Science Foundation Grant GB-23685 and the State of Connecticut. Our thanks to Lee Schulhof and Sue Williams for their assistance in data collection and analysis and to the Food and Drug Administration, Public Health Service, Psychotomimetic Agents Advisory Committee for supplying the LSD used in this study.

[2] Reprint requests should be sent to Michael Davis, Yale University School of Medicine, 34 Park Street, New Haven, Connecticut 06508 U.S.A.

of cells in the raphe nuclei that is not mediated by a neuronal feedback loop.

If the augmentation of startle amplitude associated with lesion of the raphe nuclei was caused by specific damage to raphe neurons and if LSD can specifically inhibit these neurons, then LSD should also produce an augmentation of startle amplitude. The purpose of the present series of studies was to evaluate this possibility.

EXPERIMENT 1

The purpose of Experiment 1 was to evaluate how various doses of LSD would affect acoustic startle amplitude. Intraperitoneal (I.P.) doses of 20, 40 and 160 μg/kg were chosen since previous studies have shown that similar doses can have either facilitatory (with low doses) or inhibitory (with high doses) effects on operant performance in the rat [15,17].

Method

Animals. In this and all subsequent experiments the animals were experimentally naive male albino rats of the Sprague-Dawley strain that weighed between 300 and 350 g. Upon receipt from the supplier (Charles River Co.) the rats were housed in group cages of 4 to 5 rats each in a large colony room that was maintained on a 12:12 light–dark schedule. Food and water were continuously available.

Apparatus. Five separate stabilimeter devices were used to record the amplitude of the startle response. Each stabilimeter consisted of a 3.5 × 6 × 6 in. Plexiglas and wire mesh cage suspended within a 10 × 8 × 8 in. wooden frame. Within this frame the cage was sandwiched between 4 compression springs above, and a 2 × 2 in. rubber cylinder, below, with an accelerometer (M.B. Electronics Type 302) located between the bottom of the cage and the top of the rubber cylinder. Cage movement resulted in displacement of the accelerometer and the resultant voltage was fed through a matched accelerometer amplifier (M. B. Electronics Model N504), the output of which was proportionate to the velocity of accelerometer displacement.

The amplified signal was then fed to a specially designed sample and hold circuit. Basically this circuit consisted of 5 channels, 1 for each stabilimeter, and was used to sample the peak accelerometer voltage that occurred during a 200-msec time band immediately after the onset of the startle-eliciting stimulus. Immediately prior to this sample period, each channel was discharged so that any spontaneous activity occurring between stimulus exposures was erased. In this way the amplitude of the startle response of 5 rats was recorded simultaneously and stored in one of each of the 5 channels. Immediately after the sample period the output of each of the 5 channels was digitized through a specially designed analog to digital convertor and fed into a 14 channel Newport Printer. With 2 printing channels per cage, startle amplitude could vary from 0 to 99, allowing appreciable resolution among various startle amplitudes.

The 5 stabilimeters were located in an 8 × 8 × 7 ft, dark, ventilated sound-attenuated chamber (Industrial Acoustic Co. - IAC). They were placed 45 in. from an Altec, high-frequency loud speaker, which was used to provide a 4000 Hz, 90 msec tone which was generated by a Hewlett Packard audio generator, amplified through an Altec 100 W power amplifier and shaped through a Grason-Stadler electronic switch to have a rise decay time of 5 msec. Background white noise was provided by a Grason-Stadler white noise generator. The intensity of the tone (120 dB) and the white noise (46 dB) was measured with a General Radio Model 1551-C sound level meter (A scale) by placing the microphone in each cage and positioning the cages to have comparable readings.

Procedure. The general procedure in this and all subsequent experiments employed a crossover design in which half the rats were injected with LSD on Day 1 and then with an equivalent volume of saline (1 cc) on Day 2 and the other half with saline on Day 1 and LSD on Day 2. Different groups of rats were given I.P. LSD bitartrate doses of either 20 (n = 20), 40 (n = 40) or 160 (n = 40) μg/kg, dissolved in 0.9% saline. In this way each animal served as his own control with respect to drug type with order of drug administration and dose varied across animals. Within each dose group the order in which the drug conditions were run within a day was varied so that the average time of day in which testing occurred after injection of saline or LSD was similar for both conditions.

To evaluate the time course of LSD action, a total of 240 tones were presented at a 10-sec interstimulus interval (ISI) immediately after a group of 5 rats was injected. Using 2 experimenters, 1 to inject and 1 to put the rats in the test cages, the injection procedure took about 60 sec so that about 60–70 sec elapsed from the time the first rat was injected until the first tone was presented.

Results and Discussion

Figure 1 shows the mean amplitude startle response following injection of LSD or saline over blocks of 12 tones (i.e., over successive 2-min periods) for each of the 3 doses. The results were collapsed over the 2 test days since each day's results were fairly similar. The graphic results represent, therefore, the within-subject difference in startle amplitude following injection of LSD or saline.

Figure 1 shows that LSD resulted in a substantial increase in startle amplitude. At the 20 μg/kg dose, the effect began about 10 min after the injection and lasted for about 15 min. At the 40 μg/kg dose the effect began in about 6–8 min and lasted for most of the session. With the 160 μg/kg dose the effect occurred within about 4 min but then decayed rapidly during the middle of the session.

Using the average startle amplitude across the entire session, an analysis of variance revealed a highly significant difference between LSD and saline conditions, $F(1,97) = 47.06$, $p<0.001$, and a significant Drug × Dose interaction, $F(2,97) = 4.58$, $p<0.02$. Subsequent individual comparisons indicated that while there was no overall LSD-saline difference at the 20 μg/kg dose, there was a reliable Drug by Trials interaction, $F(19,361) = 2.03$, $p<0.01$. This reflected the augmentation of startle by 20 μg/kg LSD between Minutes 12–24 ($t = 2.93$, $df = 19$, $p<0.01$). At the 40 and 160 doses the overall LSD-saline differences were highly reliable, $F(1,39) = 13.10$, $p<0.001$ and $F = 48.02$, $p<0.001$, respectively.

To visualize both the relative size and the time course of the effects at the various doses more easily, the data were transformed to percentage scores at each of the 20 time points. These results are shown in Fig. 2 for each of the 3 doses where percent increase = [(LSD amplitude minus saline amplitude)/saline amplitude] × 100.

Figure 2 indicates that with doses of 20 and 40 μg/kg, startle was potentiated maximally in about 16 min, with a

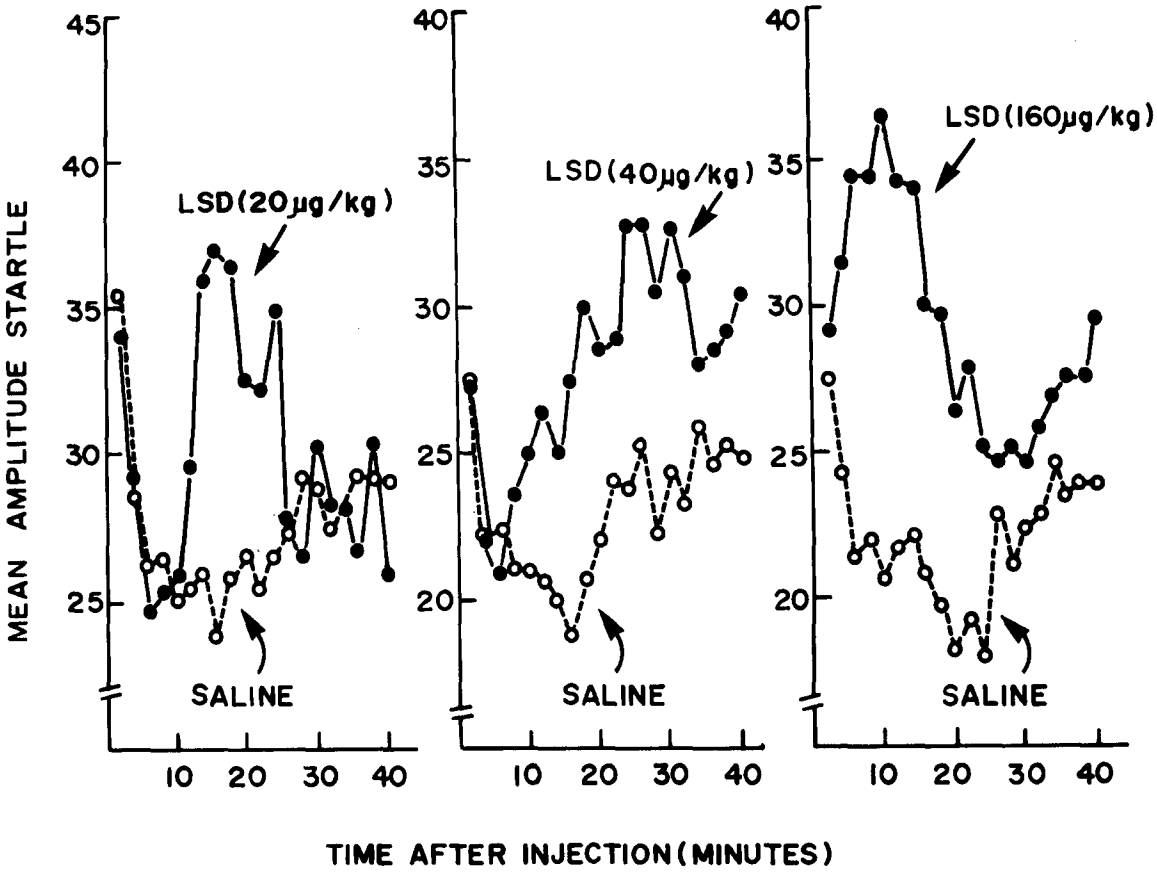

FIG. 1. Mean amplitude startle response over blocks of 12 tones (2-min periods) after injection of either 20, 40, or 160 μg/kg LSD or saline.

longer lasting effect using 40 vs 20 μg/kg. With the 160 μg/kg dose there was an even greater and more rapid onset of potentiation, so that by 4 min the 2 conditions were reliably different (t = 5.37, df = 39, p = <0.001). This high dose did not, however, continue to augment startle for an even longer period than the 40 μg/kg dose. In fact, the size of the LSD-saline differences was actually somewhat smaller toward the end of the session after the 160 compared to the 40 μg/kg dose. The effects of LSD on startle did not conform, therefore, to a simple increase in both the size and duration of potentiation with successively higher doses.

Both the time course and the duration of the effects of LSD on startle agree quite well with the time course and duration of the effects of LSD given intraperitoneally on the firing of raphe neurons (Aghajanian, personal communication). Inhibition of raphe neurons following an I.P. dose of 50 μg/kg begins in about 5–6 min and lasts for about 40 min. With higher I.P. doses (100 μg/kg) inhibition occurs in 2–3 min and may last over an hour. However, at these high doses LSD is also capable of inhibiting cells that are post-synaptic to the raphe [14], which may explain the rather rapid decline in potentiation observed with the 160 μg/kg dose.

EXPERIMENT 2

The changes in startle amplitude following injection of LSD in Experiment 1 show considerable correspondence with the effects of LSD on the firing of raphe neurons and support the expectation that inhibition of the raphe will be associated with an enhancement of startle. It is still possible, however, that some of the LSD effect could be attributed to changes in peripheral serotonin sensitive systems (e.g., smooth muscle). In an attempt to control for some of these peripheral effects, Experiment 2 compared the effect of d-LSD with that of 2-brom-LSD (BOL). BOL is a form of LSD that causes similar peripheral effects [4] but only minimally influences raphe firing rates [1].

Method

A total of 20 rats were used. The design was identical to that in Experiment 1 except in this case half the rats were injected with d-LSD on Day 1 and then with BOL on Day 2 and half with BOL on Day 1 and d-LSD on Day 2. The dose of d-LSD bitartrate was 160 μg/kg and the dose of 2-brom-LSD bitartrate was 199 μg/kg to yield equivalent doses of the bitartrate salts, given the added molecular weight of the bromine.

Results and Discussion

The left panel of Fig. 3 shows the mean amplitude startle over blocks of 12 tones (i.e., 2 min periods) following injection of LSD or BOL, collapsed over both test days. Again, the results on each day were fairly similar and were therefore combined to show the within-subject comparison

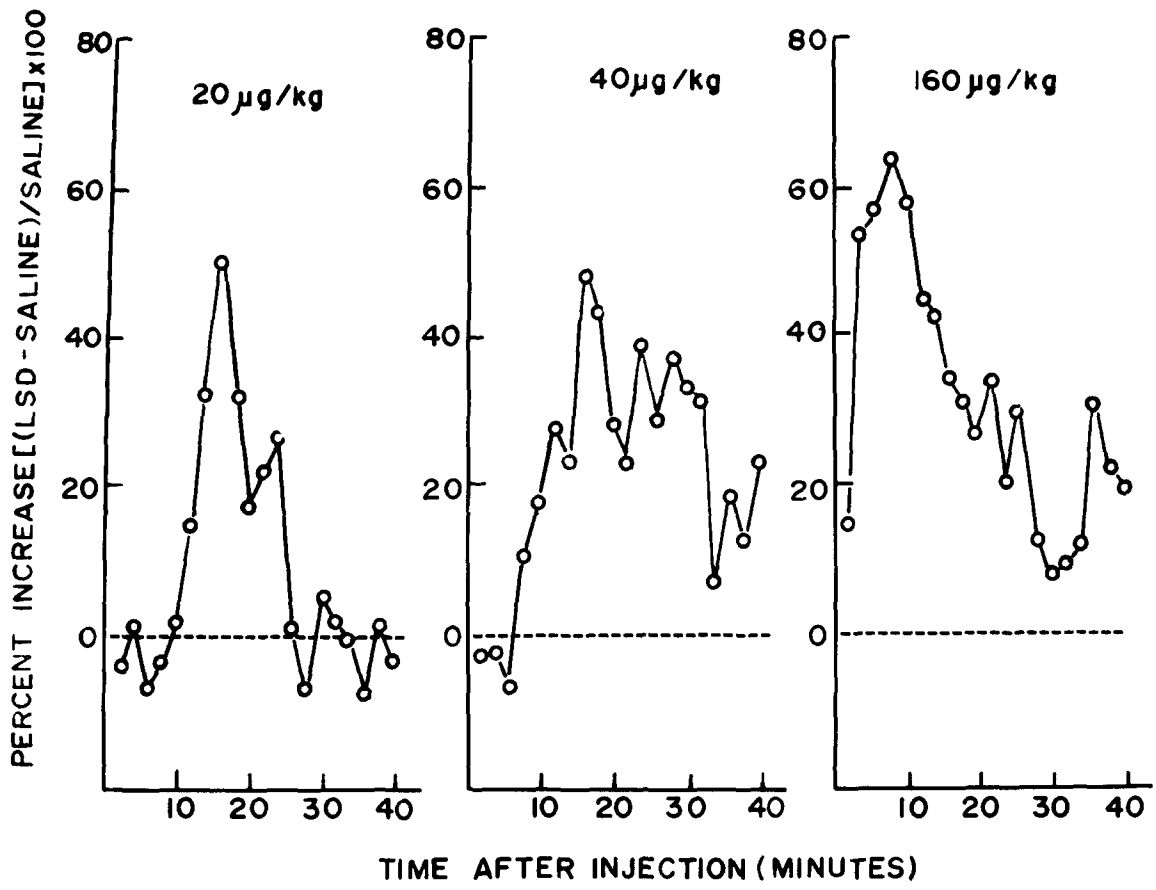

FIG. 2. Percent increase in startle, defined as [(LSD amplitude minus saline amplitude)/saline amplitude] × 100 at doses of 20, 40, and 160 µg/kg LSD.

of startle under the 2 drug conditions. Figure 3 shows that startle performance following injection of BOL was similar to that following saline in Experiment 1. LSD again caused a substantial increase in startle amplitude relative to the BOL condition, peaking in about 10 min and then declining abruptly over the next 15 min. The overall difference in startle amplitude over the session was highly significant, $F(1,19) = 12.11$, $p<0.005$. Further, the shape of the LSD-BOL facilitation curve, which is shown in the right panel of Fig. 3, was similar to the 160 µg/kg LSD-saline facilitation curve in Experiment 1 (Fig. 2). These results indicate that startle amplitude was not detectably influenced by BOL but only by LSD and indicates that the LSD effect on startle was mediated centrally.

Given the crossover design that was employed in Experiments 1 and 2, it was of interest to evaluate whether between session habituation occurred and whether this was different when exposure to tones on Day 1 was given after injection of LSD vs saline or BOL. To make this analysis, the decrease in startle amplitude from the first block of 6 tones on Day 1 to the first block of 6 tones on Day 2 was computed separately for the animals that received saline or BOL on Day 1 and LSD on Day 2 and the animals that received LSD on Day 1 and saline or BOL on Day 2, across Experiments 1 and 2 (i.e., n = 60 in each of these 2 groups). The first block of 6 tones was chosen since the control and LSD conditions did not differ on this block on Day 1 and Day 2. That is, since LSD had not yet begun to influence startle during the first min after injection, this allowed a drug free estimate of startle amplitude in the 2 groups on both Days 1 and 2. In addition, previous work has shown that the change in startle amplitude over the first several tones on successive days is the most sensitive index of habituation since it is uncontaminated by sensitization effects that may develop over the rest of the session [6]. The results showed that while both groups had statistically reliable decreases in startle amplitude from Day 1 to Day 2 (Group Saline-LSD decreased 21% from 34.23 to 26.96, $t = 4.96$, $df = 59$, $p = <0.001$ and Group LSD-saline decreased 28% from 34.53 to 24.86, $t = 5.96$, $df = 59$, $p<0.001$); the size of these decreases did not differ between the two groups ($t = 0.34$, $df = 118$, $p<0.10$). This indicates that as much habituation from Day 1 to Day 2 occurred in animals that were exposed to tones on Day 1 after injection of LSD as in animals that were exposed to tones on Day 1 after injection of saline or BOL. Unless it is assumed that the first several tones and/or the last several tones (i.e., before and after LSD had been absorbed) were sufficient to produce all of the measured between session habituation, which seems unlikely, these results indicate that these doses of LSD did not interfere with between session habituation.

EXPERIMENT 3

If the low dose effects of LSD on startle amplitude are a

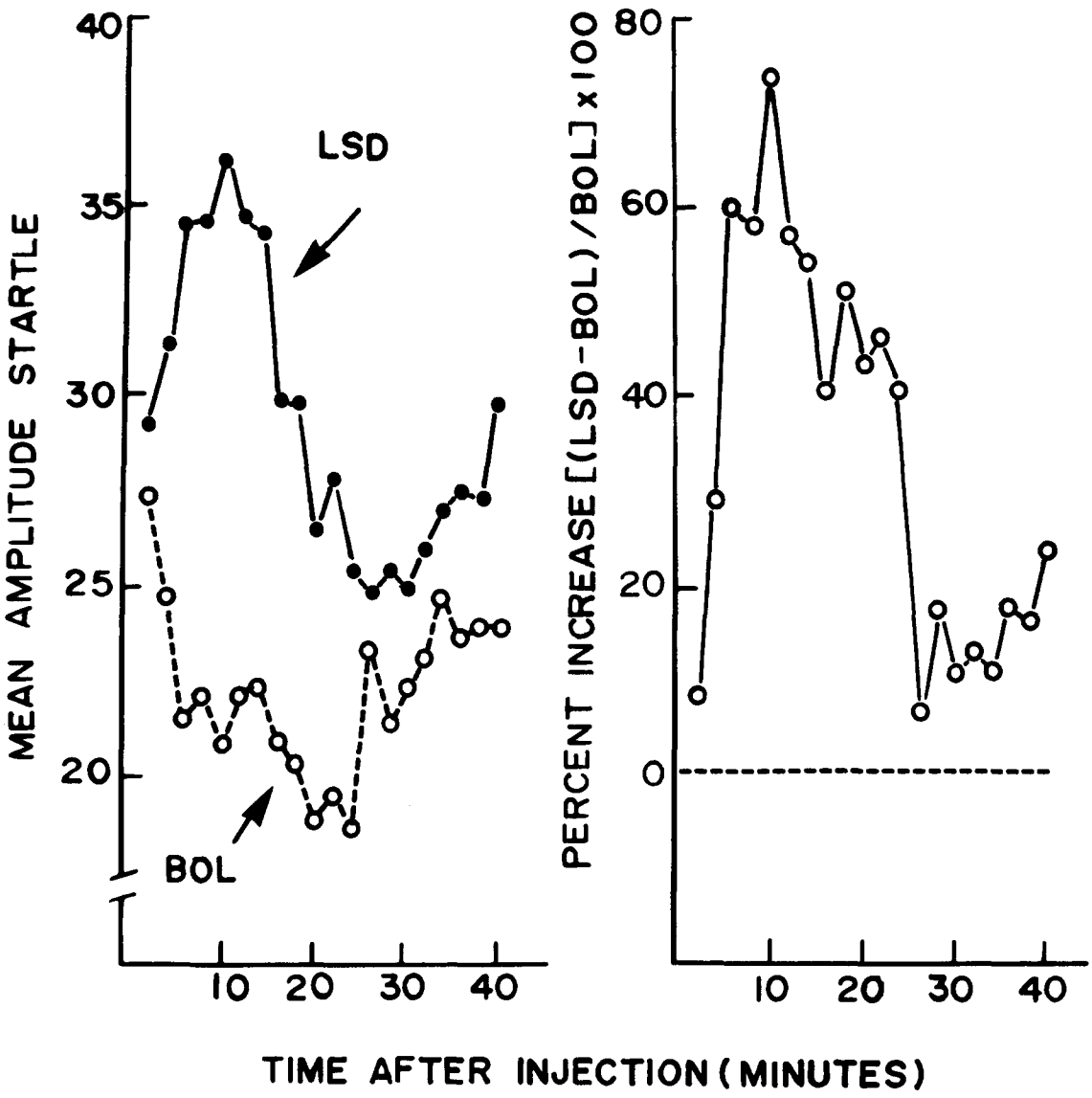

FIG. 3. Left panel shows the mean amplitude startle response over blocks of 12 tones (2-min periods) after injection of 160 μg/kg LSD or an equivalent dose of BOL. Right panel shows same data as a percent increase in startle caused by LSD relative to BOL.

consequence of inhibition of the raphe nuclei, then low doses of LSD should have no effect on rats that have no intact raphe to inhibit. The purpose of Experiment 3, therefore, was to evaluate whether a dose of LSD that was effective in enhancing startle in the normal animal (e.g., 40 μg/kg) would alter startle in rats with raphe lesions.

Method

A total of 20 rats were used. Lesioning was accomplished by placing the animal, anesthetized with chloral hydrate, in a stereotaxic instrument. An insulated 0.25 mm dia. stainless steel electrode with 0.5 mm uninsulated tip was lowered through a burr hole into the midbrain raphe region. A 1 mA constant cathodal current was passed for 10 sec at depths from the skull surface of 6.5 and 8.5 mm at A 350 μ [16]. Electrodes were removed and the skin sutured.

On the first test day, which occurred 3 weeks after lesioning, half the rats were injected with LSD on Day 1 and then with saline on Day 2 with the reverse conditions for the other 10 rats. All other parameters of tone presentation were identical to those in Experiments 1 and 2. Prior to testing, 3 rats died, leaving a total of 17 rats.

Following completion of behavioral testing the rats were perfused with 10% Formalin and the brains removed. Serial 35 μ frozen sections around the area of the lesion were cut and stained with cresyl violet to identify the site of lesion.

Results and Discussion

In 4 rats the lesions were somewhat off the midline,

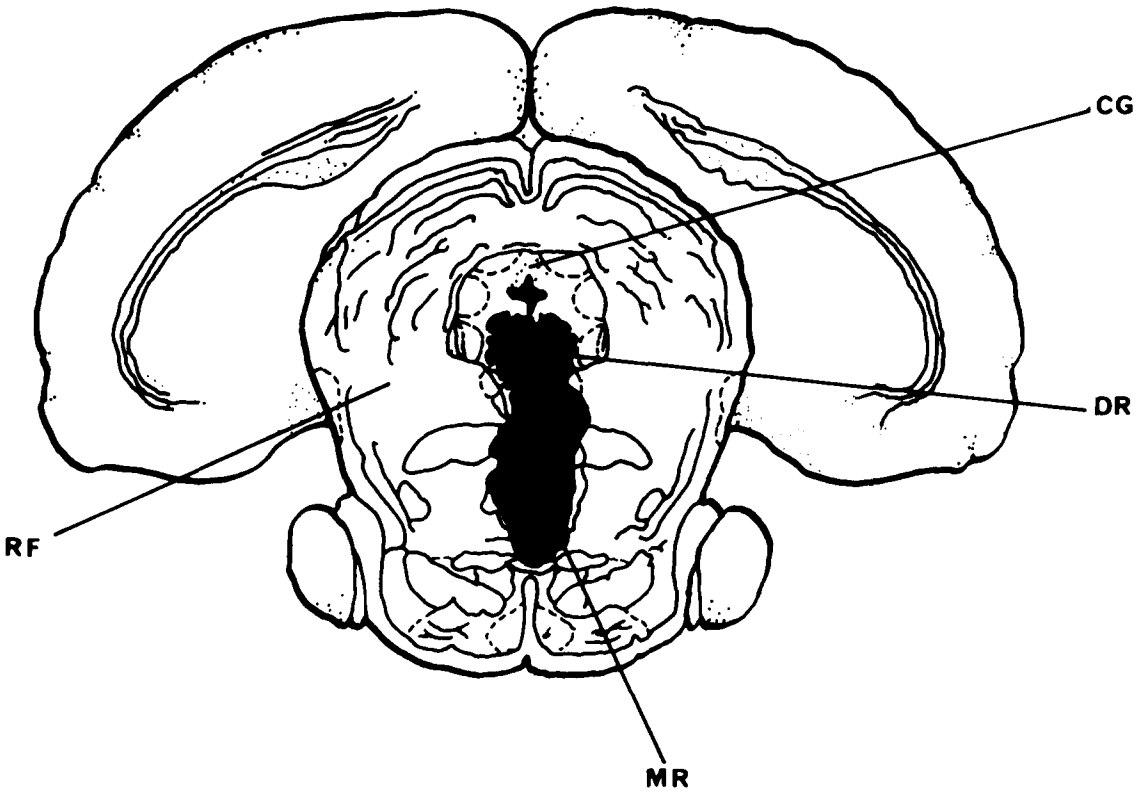

FIG. 4. Schematic diagram of rat midbrain section at A-P 350 μ [16] to show lesion involving the dorsal and median raphe nuclei. CG = Central Grey, DR = Dorsal Raphe Nucleus, MR = Median Raphe Nucleus, RF = Reticular Formation.

although partial destruction of the dorsal and median raphe nuclei did exist. In the other 13 rats, 9 had extensive midline lesions 1.5–2 mm in diameter, destroying the areas of the dorsal and median raphe nuclei encompassing A350–A60 [16]; 3 had an extensive lesion of the dorsal raphe nucleus with a moderate lesion of the median raphe nucleus and 1 had extensive lesion of the median raphe nucleus with a smaller lesion of the dorsal raphe nucleus. Figure 4 shows a schematic diagram of a typical lesion with destruction of both midbrain raphe nuclei.

The left panel of Fig. 5 shows the mean amplitude startle response over blocks of 12 tones for raphe lesioned rats following injection of saline or LSD, collapsed over the 2 test days. Two points are evident. First, the raphe animals showed a general, all be it variable, increase in startle over the session in contrast to the previous saline curves shown earlier. This is consistent with the previous finding that lesions of raphe nuclei result in exaggerated sensitization to repetitive tone exposure which is only later followed by habituation [10]. Most important, LSD was apparently ineffective in augmenting startle in these raphe-lesioned animals, since there were no systematic or statistically significant LSD-saline differences across the session as illustrated in the right panel of Fig. 5. These results should be viewed with some caution, however, since it is possible that the general increase in startle over the session displayed by the raphe animals after injection with saline could have masked an underlying effect of LSD. They are nonetheless consistent with the view that the effects of LSD on startle amplitude in the normal animal are mediated by the raphe neuronal system.

EXPERIMENT 4

Having established that low doses of LSD augment startle amplitude it was of interest to explore some ways by which LSD may have brought about this change. The simplest possibility is that the raphe exerts tonic inhibition on the startle reflex arc and that LSD, by inhibiting the raphe, reduces this tonic level of inhibition, producing a release of startle. The LSD-saline difference curves shown in Fig. 2 would then represent the time course of this release of inhibition.

Another possibility is that LSD interfered with habituation. Overall response amplitudes in the LSD condition would then be greater by virtue of a slower rate of response decrement rather than by a release of direct tonic inhibition. As mentioned earlier, LSD did not interfere with between session habituation which implies a normal development of habituation during tone exposure. Further, Figs. 1 and 3 show that the LSD curves were not simply flatter than the saline curves, as would be expected if the difference had been caused by a difference in rate of habituation. Rather they showed abrupt increases over time with startle amplitudes at later points in the sessions actually being higher than at the beginning of the sessions.

The third possibility is that LSD augments sensitization. Inspection of Figs. 1 and 3 indicates that sensitization did occur under these conditions. Thus, in each of the 4 saline curves, startle showed an initial decrease in amplitude which was followed by a gradual increase in amplitude toward the end of the session. Since the lesion data [10] suggested that the midbrain raphe neurons normally inhibit

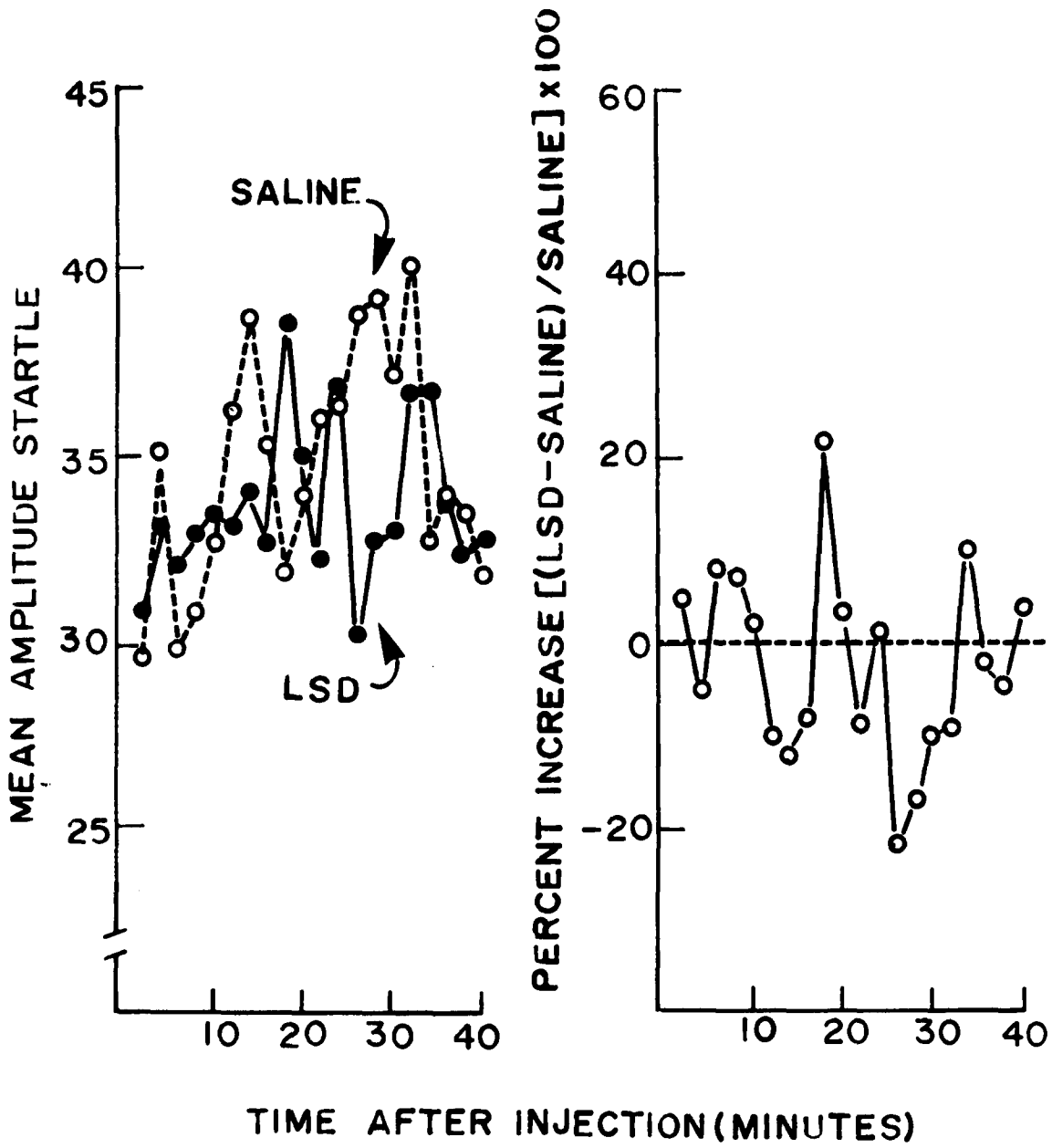

FIG. 5. Left panel shows the mean amplitude startle response over blocks of 12 tones (2-min periods) after injection of 40 μg/kg LSD or saline in rats with lesions of the midbrain raphe nuclei. Right panel shows the same data as a percent increase in startle caused by LSD relative to saline.

systems that are involved with sensitization, rather than inhibiting the startle reflex directly, perhaps LSD, by inhibiting these neurons, also influences sensitization rather than affecting the startle reflex directly.

As mentioned above, there are at least two ways by which sensitization can be produced. One is that repetitive exposure to loud tones produces sensitization. If LSD potentiates this tone-produced sensitization, then repetitive exposure to tones after injection of LSD should be necessary to produce the LSD-saline differences obtained in the previous experiments. If, therefore, a group was injected with LSD, placed in the chamber but not presented with any tones until a later time (e.g., 15 min, since this was the point at which a peak LSD-saline difference occurred in the other experiments using doses of 20 and 40 μg/kg) there should be no LSD-saline difference at that initial test point.

The other way that sensitization of the startle can be produced is by continuous exposure to background noise. If LSD potentiates this noise-produced sensitization then continuous exposure to background noise after injection of LSD should be necessary to produce the LSD-saline differences obtained in the previous experiments. This would predict that the group mentioned above would show an LSD-saline difference when first tested after 15 min in the

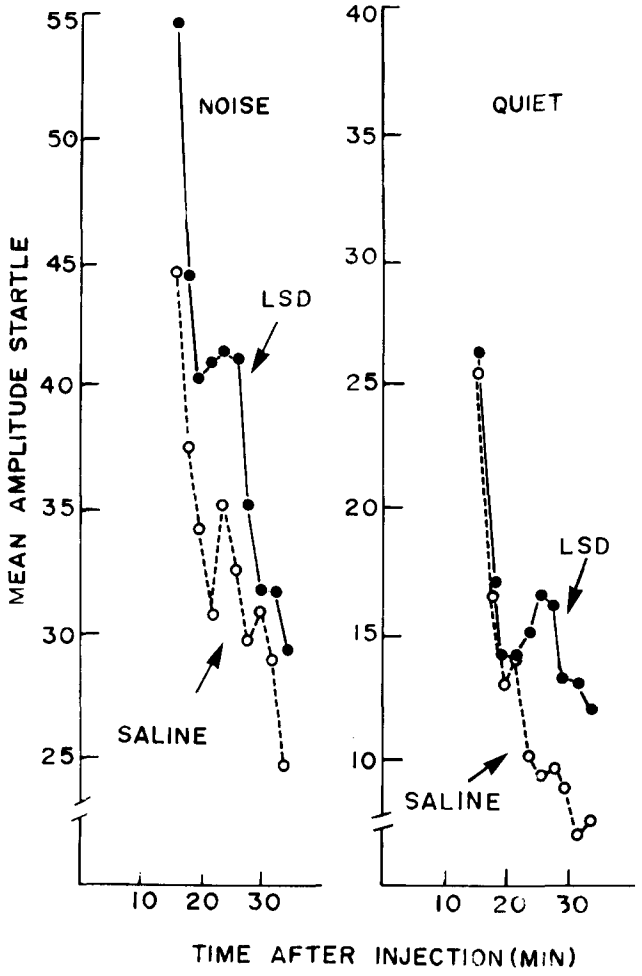

FIG. 6. Mean amplitude startle response over blocks of 12 tones (2-min periods) after 40 μg/kg LSD or saline when tone presentation was delayed for 15 min after injection, and background white noise was either present (left panel) or absent (right panel) throughout.

chamber if background noise was included but would not if no background noise was present. The purpose of Experiment 4 was to test these possibilities.

Method

Two groups of 20 rats each were used. Within each group, half the rats were injected with LSD on Day 1 and saline on Day 2 and half with saline on Day 1 and LSD on Day 2. The dose of LSD was 40 μg/kg in all cases. Following injection the animals were placed in the test chamber and 15 min later a total of 120 tones was presented. For one group the background noise was 46 dB, identical to the noise level used in the previous experiments. For the other group the noise generator was disconnected so that only the ambient noise level (28 dB) of the IAC room remained.

Results and Discussion

Figure 6 shows the mean amplitude startle response over blocks of 12 tones (i.e., over 2-min periods) for the 2 groups after injection of LSD or saline, collapsed over Days 1 and 2. Three findings are evident. First, overall startle levels were higher in the group that was tested in the presence of background noise. This is consistent with previous research that has shown that both the background level of noise that is present at the time startle is elicited as well as prior to its elicitation is a critical parameter in determining startle amplitude [7,8]. Second, the curves in Fig. 6 showed only a steep decrease across the session, in contrast to the curves shown in earlier figures where startle showed both decreases and increases across the session. This is consistent with previous work which showed that the length of the time animals spend in the chamber prior to testing is a critical variable in determining whether a habituation or a sensitization curve will result [8].

Most important for the present discussion, however, was that over the first several min of startle testing, LSD augmented startle in the noise group but not in the quiet group. This indicates that, although prior exposure to loud tones was not necessary for LSD to subsequently augment startle, exposure to noise was. It suggests, moreover, that LSD did not increase startle directly by altering tonic inhibition on the reflex arc itself, since this type of effect should be independent of background stimulation. Rather LSD appears to potentiate the sensitizing effects of noise, since the presence of noise was necessary to see a reliable LSD augmentation of startle.

Figure 6 also indicates that even in the quiet group LSD did finally augment startle toward the end of the session. Since this difference only emerged after several tones had been presented and occurred well after the normal peak LSD-saline difference seen in the previous experiments, it suggests that LSD may also augment tone-produced sensitization, which itself may take quite a long time to develop.

To evaluate the statistical reliability of these results, the data were analyzed with an analysis of variance using noise level as a between subject factor and drug condition and blocks of tones as within subject factors. This revealed significantly higher overall startle amplitudes in the group tested with noise vs quiet, $F(1,38) = 26.06$, $p<0.001$, significantly higher startle amplitudes after LSD vs saline, $F(1,38) = 11.69$, $p<0.005$, and a significant decrease in startle over blocks of tones, $F(9,342) = 25.01$, $p<0.001$. Most important was that none of the interactions was significant except the Noise Level × Drug × Blocks of Tones interaction, $F(9,342) = 2.31$, $p<0.02$. This confirms the conclusions stated above, namely, that LSD did not augment startle over the initial test tones in the quiet group but only after several tones had been presented, whereas it did augment startle both over the initial test tones and over the later test tones in the noise group. Subsequent individual comparisons indicated that over Minutes 16–22 there was a reliable LSD-saline difference in the noise group ($t = 2.56$, $df = 19$, $p<0.02$) but not a reliable difference in the quiet group ($t = 1.08$). Over Minutes 24–32, however, both the noise group and the quiet group did have reliable LSD-saline differences ($t = 2.11$, $df = 19$, $p<0.05$, and $t = 2.80$, $df = 19$, $p<0.02$, respectively).

In sum, whether or not LSD augments startle seems to be critically dependent on the level of sensory stimulation either during or prior to testing. When both background noise and tones are presented during testing, LSD causes a rapid and robust augmentation of startle. When tones are eliminated but noise is still present prior to testing, the effect still occurs. When both are eliminated prior to testing, the effect does not appear until some time after the

tones are reinstated. The time course of the LSD effect on startle, therefore, is not simply a function of the time after injection, but also a function of the length of time sensory stimulation has been present after LSD has been given.

GENERAL DISCUSSION

The present results provide further evidence that the midbrain raphe nuclei are important sites in the regulation of the startle reflex. Startle amplitude can be increased either by lesioning these nuclei or by low doses of LSD which inhibit cells within the raphe nuclei. Moreover, these two different methods of altering raphe function appear to influence the process of sensitization without substantially interfering with habituation. In the case of the raphe lesion, this was shown by a rapid increase in startle over the first several tone presentations [10]. In the case of LSD it was shown by the interaction of background noise and number of tones and LSD, so that the augmentation of startle by LSD was dependent on noise level and the number of tones presented rather than solely on the time after injection.

It should be pointed out that there are two ways in which noise influences startle amplitude. One is the noise level that is present at the time the reflex is elicited and the other is the noise that has been present prior to the time the reflex is elicited. In Experiment 4 these two effects were confounded, since the noise group was tested at a higher noise and also exposed to higher noise during the 15 min period prior to testing. Since it has been shown that the raphe lesion does not alter the function relating startle amplitude to the noise level that is present at the time the reflex is elicited [10], one would expect that the interaction between LSD and noise in Experiment 4 was caused by different noise levels during the 15 min preexposure period. These LSD results would predict, then, that raphe lesioned animals should be more sensitized by a 15 min period of noise exposure than non-lesioned rats, although this is yet to be tested.

At the highest dose of LSD used, startle was potentiated quickly but then declined abruptly over the rest of session. Given an LSD half-life of about 20 min and the fact that doses of 20 and 40 μg/kg were effective in augmenting startle, a high dose of LSD should continue to augment startle for an even longer period, if its only effect was to inhibit the raphe. What must happen, therefore, is that high doses of LSD, in addition to inhibiting the raphe, also depress startle by influencing other systems. Suppose, for example, that excitation in a group of cells that are post-synaptic to and inhibited by the raphe enhance startle. Low doses of LSD inhibit the raphe and thus increase excitation in these cells, thereby enhancing startle. High doses could therefore enhance startle (by inhibiting the raphe) but at the same time depress startle (by inhibiting the post-synaptic cells), so that the net result would be less than an intermediate dose. Direct evidence, using single unit recording techniques, has in fact shown this type of biphasic LSD dose response curve in cells which are post-synaptic to the raphe [14]. It is of particular interest in this regard that relatively high doses of N-N-Dimethyltryptamine (DMT) depress startle, [9] even though DMT reduces the rate of firing in raphe neurons [1]. The present results would predict, therefore, that low doses of DMT, by virtue of inhibiting only raphe neurons should augment startle.

Finally, it should be mentioned that the startle results agree with earlier reports that low doses of LSD enhance reactivity to sensory stimulation [3]. The present results suggest, however, that one should be cautious in attributing altered rates of response decrement during repetitive stimulus presentation seen after LSD to an interference with habituation without exploring the relationship between LSD and sensitization.

REFERENCES

1. Aghajanian, G. K., W. E. Foote and M. H. Sheard. Action of psychotogenic drugs on single midbrain raphe neurons. *J. Pharmac. exp. Ther.* **171**: 178–187, 1970.
2. Aghajanian, G. K., H. J. Haigler and F. E. Bloom. Lysergic acid diethylamide and serotonin: Direct actions on serotonin-containing neurons. *Life Sci.* **11**: 615–622, 1972.
3. Bradley, P. B. and B. J. Key. The effect of drugs on arousal responses produced by electrical stimulation of the reticular formation of the brain. *Electroenceph. clin. Neurophysiol.* **10**: 97–110, 1958.
4. Cerletti, A. and E. Rothlin. Role of 5-hydroxytryptamine in mental diseases and its antagonism to lysergic acid derivatives. *Nature* **176**: 785–786, 1955.
5. Dahlstrom, A. and K. Fuxe. Evidence for existence of monoamine-containing neurons in the central nervous system: I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.* **62**: Suppl. 232, 1–55, 1965.
6. Davis, M. Differential retention of sensitization and habituation of the startle response in the rat. *J. comp. physiol. Psychol.* **78**: 260–267, 1972.
7. Davis, M. Signal-to-noise ratio as a predictor of startle amplitude and habituation in the rat. *J. comp. physiol. Psychol.* **86**: 812–825, 1974.
8. Davis, M. Sensitization of the rat startle response by noise. *J. comp. physiol. Psychol.* (In press).
9. Davis, M. and H. D. Bear. Effects of N-N-Dimethyltryptamine on retention of startle response habituation in the rat. *Psychopharmacologia. (Berl.)* **27**: 29–44, 1972.
10. Davis, M. and M. H. Sheard. Habituation and sensitization of the rat startle response: Effects of raphe lesions. *Physiol. Behav.* **12**: 425–431, 1974.
11. Groves, P. M. and R. F. Thompson. Habituation: A dual process theory. *Phychol. Rev.* **77**: 419–450, 1970.
12. Haigler, H. J. and G. K. Aghajanian. Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving a serotonergic input. *J. Pharmac. exp. Ther.* **188**: 688–689, 1974.
13. Harris, J. D. Habituatory response decrement in the intact organism. *Psychol. Bull.* **40**: 385–422, 1943.
14. Horn, G. and J. M. McKay. Effects of lysergic acid diethylamide on the spontaneous activity and visual receptive fields of cells in the lateral geniculate nucleus of the cat. *Expl Brain Res.* **17**: 271–284, 1973.
15. Jarrard, L. E. Effects of d-lysergic acid diethylamide on operant behavior in the rat. *Psychopharmacologia* **5**: 39–46, 1963.
16. Konig, J. F. R. and R. A. Klippel. *The Rat Brain.* Baltimore, Maryland: The Williams and Wilkins Co., 1963.
17. Tilson, H. A. and S. B. Sparber. Similarities and differences between mescaline, lysergic acid diethylamide-25 (LSD) and d-amphetamine on various components of fixed interval responding in the rat. *J. Pharmac. exp. Ther.* **184**: 376–384, 1973.
18. Thompson, R. F. and W. A. Spencer. Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* **73**: 16–43, 1966.