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Long-Lasting Alterations in Behavior and Brain Neurochemistry Following Continuous Low-Level LSD Administration

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KING, W., JR. AND G. ELLISON. *Long-lasting alterations in behavior and brain neurochemistry following continuous low-level LSD administration.* PHARMACOL BIOCHEM BEHAV **33**(1) 69–73, 1989.—Groups of rats were administered either 80 μg LSD-25 continuously over seven days using subcutaneous minipumps, or were given the same total amount of drug in seven daily injections, or were administered vehicle. When tested long after cessation of drug administration, persisting alterations in behavior and brain were found in the continuous LSD groups. In social open-field tests, this consisted of decreased social distance between animals; this effect increased upon repeated testing. In uptake of labeled ligands, this was reflected predominantly by decreased ^{3}H-LSD binding in several limbic regions. LSD appears to have especially persisting neurotoxic effects when administered in a continuous, low-level fashion.

LSD-25 Neurotoxicity Behavior Autoradiography Limbic brain

SOME psychoactive drugs have become especially noted for inducing persisting effects on behavior or biochemistry. While the most notable examples are of clear neurotoxins such as MPTP (25) or long-lasting amphetamines (6,18), clinical reports of lasting effects of hallucinogen ingestion have occurred periodically in the literature, and a primary involvement of LSD is often presumed (1,2).

This effect may also be of theoretical importance, for many of the after-effects of LSD resemble psychotic reactions of a schizophrenic type (19,21), and there is a close resemblance between the psychotic after-reactions to hallucinogens and the symptoms of psychiatric patients with "good premorbid" or "schizophreniform" psychotic reactions that are apparently unrelated to drug use (2). Varden and Kay (26) reported that "LSD psychotics" were quite similar to nondrug schizophrenics in most aspects of symptomatology.

It has been extensively documented that amphetamine administered continuously via a subcutaneous delivery system has marked and persisting neurotoxic effects, whereas the same amount of drug administered via daily injections does not (6), and by analogy it seems possible that LSD is especially able to induce long-lasting effects in part because of its unusually long-lasting action compared to other hallucinogens. Most animal studies of chronic hallucinogens have relied upon daily injections of drugs, but these produce both highly fluctuating drug level within the animal from day to day and marked tolerance to the drug.

The present study was designed to determine whether persisting after-effects of continuous, low-level administration of LSD in rats would be greater than those after daily injections of the same amount of drug. Rats received chronic administration of 80 μg of d-LSD-25 tartrate over a period of seven days either by daily injections or by a subcutaneous delivery system. The animals were tested 30 days later for behavioral and neurochemical changes.

METHOD

Behavioral Observations

For the behavioral tests, 48 male albino rats (Simonsen) were divided into two replications of 24 rats each. Within each replication 8 rats were assigned to each of 3 groups, such that all groups had the same mean body weight (307 and 352 g respectively) in the two replications. The rats were housed individually in standard stainless steel cages in a quiet room under a 12-hr reversed light-dark cycle. Food and water were available ad lib in the home cage.

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Drug Administration

To control for differential handling during the drug administration period, all animals received both subcutaneous implants and daily injections. For pellet implantation, the animal was restrained, and after a local injection of lidocaine an incision was made and the pellet was inserted into the cavity. Three groups were prepared: a "minipump" group was implanted with Alzet osmotic minipumps (Alza Corp.) containing 80 μg of d-LSD-25 tartrate (Sandoz) dissolved in distilled water to yield a total volume of 170 μl (1.0 μl/hr for 170 hr or 7 days). The other two groups were implanted with control pellets (25-mm lengths of 7.9-mm silastic tubing) filled with physiologic saline. The day following pellet implantation, and continuing for seven days, all groups were given subcutaneous injections at the back of the neck. A second group (the "injection group") was injected with a total of 80 μg of LSD in distilled water in seven equal daily injections (approximately 34 μg/kg per day). The other two groups were injected with equal volumes of physiologic saline.

Several hours following the last injection the pellets were removed, and the rats were left undisturbed, except for routine feeding and cleaning, in their home cages for 30 days, whereupon behavioral testing began and continued for 10 days. During all testing the observer was blind as to the subjects' previous drug treatment.

The open field was a circular enclosure 130 cm in diameter with a flat black interior and a floor divided by white lines into 22 cm squares. It was illuminated by a 15-W bulb 70 cm above the center of the floor. The observer sat quietly at the edge of the field and recorded the animals' behavior. For testing, the rats were tested following procedures described previously (5). Paired rats were treated identically except that they were placed under separated boxes at the beginning of the test, and locations of each rat were recorded every 12 sec. One rat of each pair was marked on the fur and at the base of the tail with a felt marker so that the two rats were easily distinguishable by the observer. In the "together" condition all incidence of fighting or of upright and sideways aggressive postures (11) as well as all attempts at mounting were noted. Data from the open field were analyzed by breaking time samples into 1-min periods (5/min), with locomotion calculated for each rat as the sum of the straight line distances between the centers of squares occupied in successive time samples. In the "together" condition social attraction was determined by calculating the straight line distance between the centers of squares occupied by the two rats at each time sample and taking the mean of these values for each minute.

Autoradiography

Twenty-one female albino rats (223–263 g) were similarly divided into three groups. The "minipump" group had minipumps surgically implanted in their backs which administered 11.27 μg of LSD per day for seven days; the "injection" group received daily IP injections of 11.3 μg of LSD for seven days. The control group had control, empty pellets surgically implanted in their backs for seven days. After seven days, the minipumps and control pellets were removed and the daily injections stopped. The animals were allowed to recover for 25 days. They were then tested twice in the open field using procedures like those described above so as to make sure the behavioral results obtained with the females were similar to those obtained with the males. They were then sacrificed by decapitation, the brains quickly removed, and frozen in dry ice. For each rat, three adjacent sections were taken from six different brain areas, corresponding respectively to sections from the Pellegrino and Cushman atlas of AP 11, 9.0, 7.5, 4.0, 2.0, and −1.0 mm. Sections were cut at 20 μ and stored in the cryostat at −20°C. The duplicate sections for each area were taken for each of the three ligands, ^{3}H-spiroperidol, ^{3}H-serotonin, and ^{3}H-LSD.

For the ^{3}H-spiroperidol sections, after allowing the slides to come to room temperature, they were incubated for 30 minutes (23°C) in 0.17 M Tris HCl buffer (pH 7.7) containing 0.4 nM ^{3}H-spiroperidol, 0.001% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. After 30 min, the slides were rinsed quickly in distilled water, placed into 2 consecutive 5-min washes in the buffer without the ^{3}H-spiroperidol, rinsed in distilled water and dried under a stream of cool, dry air. While the slide boxes were being filled, the box was kept under a stream of dry air, and once filled a small amount of desiccant was added to one end of the box, the box was taped shut and stored for up to two weeks in a refrigerator at 4°C.

^{3}H-LSD incubation used a 50 mM Tris-maleate buffer (23°C) containing 6 nM ^{3}H-LSD for 60 minutes. After incubation, the slides were rinsed in distilled water, and washed twice, each time for 10 min in ice-cold buffer (0°C). The slides were then rinsed in distilled water and dried and stored as described above. For ^{3}H-serotonin, the slides were incubated in a 0.17 M Tris-HCl buffer (pH 7.6) containing 1 nM ^{3}H-5HT for 60 minutes at 23°C. The buffer also contained 0.001% ascorbic acid, 10 M pargyline, and 4 mM $CaCl_2$. After incubation, the slides were then rinsed in distilled water, dried, and stored as above. All ligands were from New England Nuclear.

For assembly with the Ultrofilm (LKB-Tritium-sensitive), 11 × 14″ cassettes with the slides glued onto an exposed piece of X-ray film as close as possible to each other were used. The Ultrofilm was then placed emulsion side down over the slides, and the cassette was stored in a refrigerator at 4°C until exposure was completed. The Ultrofilm was developed using Microdol-X at 1:3 dilution for 11 minutes at 68°F. It was fixed for 10 minutes using Kodak fixer and then rinsed in distilled water for 20 minutes. The films were then scored for optic density using a Sergeant-Welch photo densitometer. A total of 32 brain regions were scored on each animal and the resulting data was analyzed using repeated measures analyses of variance. When significant interactions were found between drug treatment and brain region, Dunnett's tests were performed in order to determine which specific brain regions were responsible for the interaction.

RESULTS

Behavioral Experiments

There were no differences within groups in data obtained from the two replications and so the data were combined.

Individual open field. Analysis of variance revealed a significant difference between groups in locomotion, $F(2,33) = 4.389$, $p = 0.02$. Duncan's Multiple Range test showed that there were no significant differences between the control and injection groups, while both of these groups locomoted significantly more than the minipump group. No significant differences were found between groups in time spent near the wall of the open field or in number of fecal boli.

Social open field. Three-way analysis of variance revealed significant main effects of groups, $F(2,21) = 4.825$, $p < 0.02$, and of days of testing, $F(6,126) = 4.172$, $p < 0.001$. Two-way analysis of variance performed on each day's data revealed significant differences between groups only on day 5, $F(2,21) = 6.61$, $p = 0.006$, day 6, $F(2,21) = 9.664$, $p < 0.001$, and day 7, $F(2,21) = 13.852$, $p < 0.0001$. A similar difference on day 4 was not significant, $F(2,21) = 2.481$, $p = 0.11$. These data are summarized in Fig. 1, which shows social distance plotted over days. Duncan's Multiple Range tests revealed that on days 5 and 7 the minipump

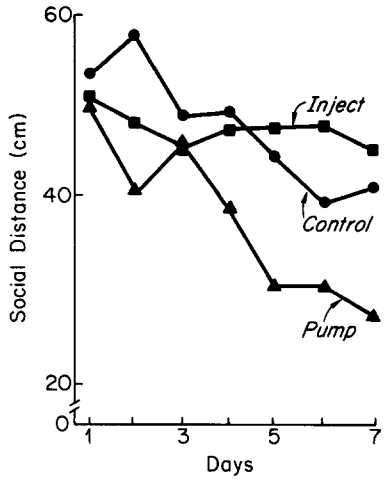

FIG. 1. Changes in average social distance (in cm) in the open field across days. The data have been collapsed across the 5 minutes of testing. Progressively the "pump" animals become more distinguishable from the other groups.

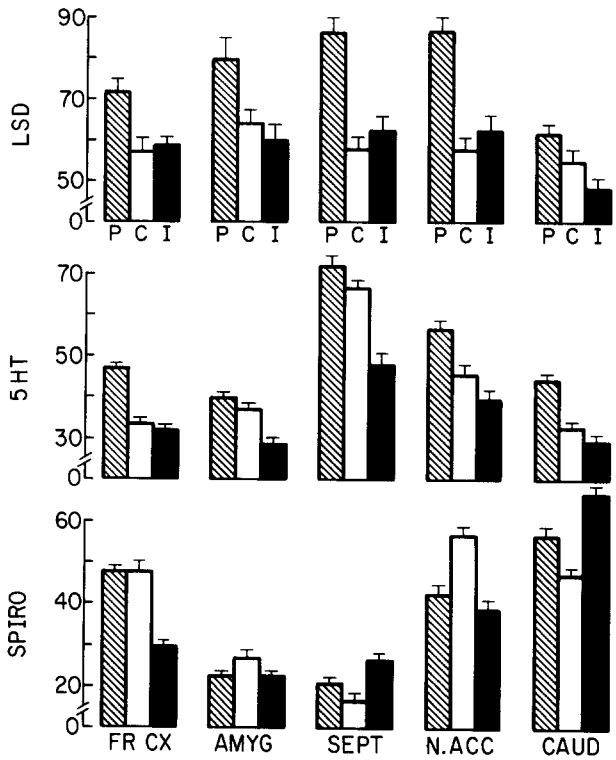

FIG. 2. Average densitometry readings (±s.e.m.) for 6 brain regions using, as a ligand, tritiated LSD (top), 5HT (middle), and spiroperidol (bottom). "P"=LSD pellet group; "C"=Controls; "I"=LSD injection group. LSD binding is especially low in both LSD groups in visual cortex.

group was significantly more social than the injection and control groups, while the differences between the latter two groups were not significant. On day 6 each group was significantly different from the other two, the minipump group again being the most

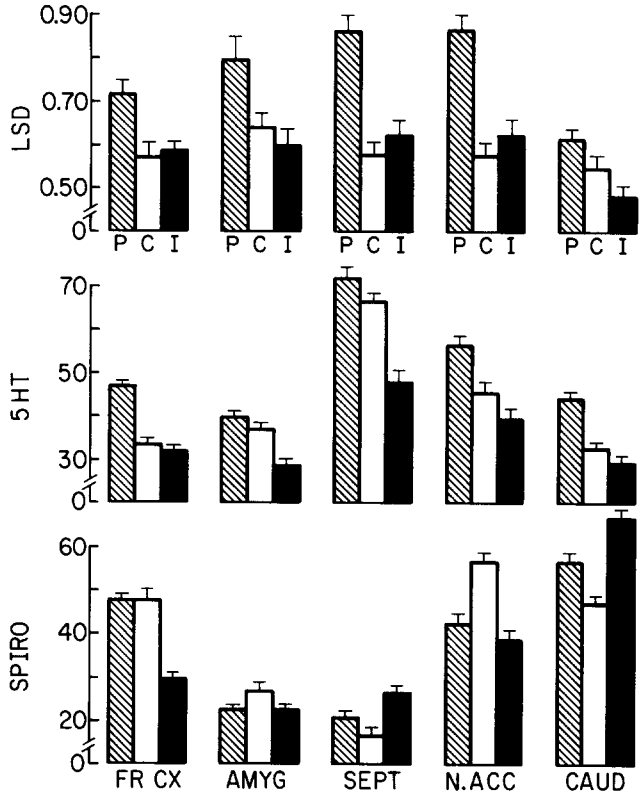

FIG. 3. Average densitometry readings ± for 6 other brain regions over the three ligands. Ligands and group labels as in Fig. 2. The increased binding of ^{3}H-LSD in temporal brain regions in the "Pellet" group is especially apparent.

social and the injection group the least.

It was noted that rats tended to spend increasing amounts of time in physical contact across days of testing. For the most part, the rats' behavior in these situations consisted of crawling over, under, or pushing past (11); huddling was not often seen and an attempted mount was seen on only one occasion. Fighting and aggressive postures were common, becoming most frequent on the later days of testing. Aggressive behavior was most common in the minipump group, nearly as common in the controls, and was rarely seen in the injection group. As in the individual open-field test, there were no significant differences between groups in time spent near the wall or in number of fecal boli.

Autoradiography

There were significant group effects using all three ligands. The differences between groups were significant for ^{3}H-LSD, ^{3}H-5HT, and ^{3}H-haloperidol [$F(2,18)=5.1$, 6.4, and 6.6 respectively, all $p<0.05$]. Similarly for all three ligands there were highly significant differences between the various brain regions [$F(19,342)=108$, 142, and 87, respectively for the three ligands, all $p<0.001$]. There were also significant interactions between brain regions and groups with all three ligands, $F(38,342)=3.4$, 3.3, and 2.9 respectively, all $p<0.05$]. Figure 2 shows that in regions (including visual system lateral geniculate and two areas of visual cortex), the only significant effects were of a slight decrease in LSD and 5HT uptake in both drug-treated animals, and that there were no significant effects found for any of the three ligands in the hypothalamus, the dentate gyrus, and the substantial nigra.

There were highly significant effects, however, for LSD binding in several limbic regions (Fig. 3). The pellet animals showed much higher uptake of ^{3}H-LSD than did the other two groups in the frontal cortex, amygdala, septum and nucleus accumbens. The differences in caudate nucleus were not significant. This difference was not clearly due to either 5HT or dopamine binding by LSD as shown by the lack of consistent effects for either 5HT or spiroperidol binding in these same three brain regions.

DISCUSSION

Chronic administration of LSD over a period of seven days was found to result in alterations in behavior of rats that were detectable more than thirty days after the drug treatment. It is unlikely that the effect can be attributed to a depot of drug remaining in the brain, for Rosecrans, Lovell and Freedman (20) have shown that the half-life of LSD in rat brain is approximately 15 min and that the drug is essentially cleared at the end of 30 min.

Nor is this effect due to the administration of extremely large doses of the drug. The rats in this experiment received the equivalent of 34 μg/kg per day of LSD, whereas in reports in the literature typical doses of LSD range from about 100 μg/kg to over 1 mg/kg in rats. Freedman, Aghajanian, Ornitz and Rosner (8) reported tolerance in the deficit in rope climbing speed produced by 130 μg/kg of LSD in rats, and in other experiments (9, 12, 27), roughly the same dose was given.

In other experiments, changes in behavior have been produced by much smaller doses. Cameron and Appel (3) reported that 20–40 μg/kg of LSD was about the threshold dose that rats could discriminate from saline in a two-lever discrimination task, whereas a dose of 80 μg/kg produced a reliable discriminative stimulus. Ellison, Ring, Ross and Axelrod (7), using the same dose and method of administration as was used in the present experiment, found changes in behavior in a colony experiment as well as an increase in head twitches and other behaviors in isolated animals. Finally, Silverman (24) reported alterations in the nature of social interactions between rats after as little as 1–4 μg/kg of LSD. Thus, the daily dose of LSD received by rats in the present experiment must be considered a low dose, though not so low as to be below the threshold for producing changes in behavior.

An important finding is that the long-lasting alterations in behavior reported here did not occur after daily injections of LSD but only after continuous delivery of the drug. In this respect, then, LSD has been shown to act in an analogous fashion to amphetamine, which has also been shown to produce differing behavioral effects after continuous versus daily administration (15,16).

In humans, it has been reported that long-lasting psychiatric sequelae of LSD ingestion have resulted both from chronic use as well as from a single dose of the drug (10,21). It has been speculated, based on this and other evidence, that schizophrenia may result when a hallucinogenic compound is produced endogenously in the brain (4, 15, 17, 22, 23). If this is true, then continuous, chronic administration of hallucinogens might more closely mimic the endogenous production of the schizophrenogenic compound in the brain of humans and might, therefore, provide a better model of the schizophrenic process.

The behavioral effect of LSD administered in this fashion to humans has not been studied for obvious reasons. Nevertheless, it might be speculated that such a regimen of drug administration would result in a syndrome that much more closely resembles the schizophrenic state as typically described, especially if the subject were unaware that he was receiving a hallucinogen [see (14) for a discussion].

The results using autoradiography suggest that the locus of these long-lasting effects produced by continuous LSD was in the temporal lobes. This finding is congruent with earlier suggestions linking LSD's hallucinogenic effects with alterations in temporal and limbic structures.

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