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ALTERATIONS OF SEROTONIN AND LSD RECEPTOR BINDING FOLLOWING REPEATED ADMINISTRATION OF LSD

Michael E. Trulson and Barry L. Jacobs

Program in Neuroscience
Department of Psychology
Princeton University
Princeton, New Jersey 08540

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Summary

Repeated administration of LSD to rats (100 µg/kg every 6 hours for 4 days) resulted in significant decreases in both the K_D (-17 & -23%) and Bmax (-25 %& -30%) for [^{3}H]- 5HT binding in forebrain and brainstem plus spinal cord. [^{3}H] - LSD binding showed significant changes in Bmax values (-19%) in forebrain and brainstem plus spinal cord, while K_D values were not significantly changed. Neither a single injection of LSD (100 µg/kg) nor repeated administration of brom-LSD (100 µg/kg every 6 hours for 4 days) produced any significant changes in binding. In addition, repeated LSD administration produced no significant changes in [^{3}H] - spiroperidol binding.

One of the most interesting characteristics of the action of LSD is the profound and rapid tolerance which develops to its repeated administration. In humans, the psychic effects of LSD are significantly diminished in response to the second or third consecutive daily dose. They are virtually abolished by the fourth or fifth day, and are reestablished by 4 or 5 drug free days (1-3). In animal studies, which have generally employed higher dose levels, behavioral evidence of the development of tolerance can be seen within several hours after the initial injection of LSD. A complete loss of the behavioral effects in animals occurs by the second or third daily injection, and a return to full responsiveness is observed after 5-7 drug-free days (4-8).

A number of studies have shown that the magnitude of changes in brain neurochemistry produced by repeated administration of LSD are significantly smaller than those which follow a single

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administration (9-12). However, none of these studies has reported changes of sufficient magnitude to account for the almost complete tolerance to the effects of LSD that is often seen in behavioral experiments.

The most prominent current theory of the action of LSD is that it acts to preferentially suppress the activity of serotonin-containing neurons in the mammalian central nervous system. Thus, when LSD, or related indole nucleus hallucinogens, such as psilocin or 5-methoxy N, N dimethyltryptamine, are systemically administered in very low doses, they produce a rapid and dramatic decrease in the discharge rate of serotonergic neurons (13-15). Furthermore, if these compounds are iontophoretically applied directly onto the perikarya of serotonergic neurons, they also produce this depression, whereas, if they are applied directly to serotonergic target neurons, they have little effect (16,17). Based on these data, a reasonable hypothesis would be that following repeated administration, LSD loses its effectiveness in depressing the activity of serotonergic neurons. In direct tests of this, both in anesthetized rats and freely moving cats, we found no support for the hypothesis since LSD was equally effective in depressing the activity of serotonergic neurons in the tolerant and non-tolerant conditions (18, 19 and Trulson and Jacobs, submitted).

Based on these negative data, the next most reasonable hypothesis would be that tolerance to LSD is mediated by some change in target neurons postsynaptic to serotonergic neurons. Utilizing a rat behavioral model, which is specific to activity in serotonin-mediated synapses, we found that repeated administration of high doses of LSD produced a dramatic shift to the right (over three fold) in the dose-response curve for the direct postsynaptic serotonergic effects of LSD (20).

In an attempt to understand the mechanisms which might alter the sensitivity of postsynaptic neurons following the repeated administration of LSD, the present study examines the receptor specific binding of serotonin, spiroperidol and LSD.

Methods

Male Sprague Dawley rats weighing 200-250 grams were administered d-lysergic acid diethylamide tartrate (LSD, 100μg/kg, i.p.), brom-LSD (BOL, 100 μg/kg, i.p.) or saline (1 ml/kg, i.p.) every 6 hours for 4 days. Six or 24 hours after the final injection the rats were killed by decapitation and the brains and spinal cords were rapidly removed and dissected into forebrain (all tissue anterior to a coronal cut through the optic chiasm) and brainstem (all tissue posterior to a coronal cut immediately anterior to the superior colliculi minus the cerebellum) plus spinal cord (C_1 through T_6). The tissues were

assayed for [^{3}H]-5HT and [^{3}H] -LSD binding using the methods of Bennett and Snyder (21). The tissues from two animals were pooled, weighed and homogenized in 40 volumes of 0.05M tris-HCl buffer, centrifuged at 50,000 X g for 10 min., and the pellet washed either one or four times by re-homogenization and centrifugation. The washed pellet was resuspended in 0.05M tris-HCl buffer (15 mg original wet tissue weight per ml) containing 0.1% L-ascorbic acid, 10 µM pargyline and 5mM $CaCl_2$. Two-ml aliquots were incubated in duplicate for 10 min at 37°C after addition of tritiated and unlabeled ligands. [^{3}H]-5HT (27.8 Ci/mmol) was added in concentrations of 3,6,9,18,27,36 and 54 nM. [^{3}H]-LSD (18.3 Ci/mmol) was added in concentrations of 1,2,4,6,12,18 and 27 nM. For [^{3}H]- 5HT and [^{3}H]-LSD binding, 10 µM unlabelled 5HT and 1 µM unlabelled LSD were included as blanks for non-specific binding, respectively. The samples were then rapidly filtered through Whatman GF/B filters, and the incubation tubes rinsed with three 5 ml aliquots of tris buffer. The filters were then immersed in 15 ml of Hydromix, extracted overnight at 4°C, and the radioactivity quantitated in a Searle model 6868 liquid scintilation counter at 46-52% counting efficiency. K_D and Bmax values were determined by Scatchard analysis. To determine the acute effects of LSD on the binding measures, rats were given a single injection of LSD (100 µg/kg, i.p.) or saline (1 ml/kg) and were killed one hour later and assayed for [^{3}H] -5HT binding as described above.

Additional groups of rats were pretreated with LSD (100 µg/kg, i.p.) every 6 hours for 4 days, and were killed 24 hours following the final injection for assay of [^{3}H]-spiroperidol binding by the method of Fields, Reisine and Yamamura (22). The brains were dissected into striatum and "limbic forebrain" (all tissue anterior to a coronal cut through the optic chiasm minus the striatum and cerebral cortex). The tissues from two animals were pooled, weighed, homogenized and centrifuged as described above. The pellet was washed twice and then resuspended (8-10 mg original wet tissue weight per ml) in 0.05 M tris-HCl buffer containing ascorbic acid, 0.1%; NaCl, 120 mM; KCl, 5mM; $CaCl_2$, 2mM; and $MgCl_2$, 1mM. One-ml aliquots were incubated in duplicate for 10 min at 37°C after addition of [^{3}H]-spiroperidol (0.10, 0.20, 0.30, 0.50, 0.75, 1.0 and 1.5 nM, 23.6 Ci/mmol), and (+)-Butaclamol (0.1 µM) in blanks for non-specific binding. The samples were then filtered, rinsed (five times) extracted and quantitated as described above.

For all binding studies, control and experimental assays were paired at each concentration of ligand in each replication of the experiment.

Results

Chronic LSD administration resulted in significant decreases in both the K_D (-17%) and Bmax (-25%) for [^{3}H]-5HT binding in forebrain. Similarly, [^{3}H]-5HT binding showed significant decreases in K_D (-23%) and Bmax (-30%) in brainstem plus spinal cord (Figure 1 and Table 1). [^{3}H]-LSD binding showed significant changes in Bmax values (-19%) in forebrain and brainstem plus spinal cord, while K_D values were not significantly changed (Figure 2 and Table 2).

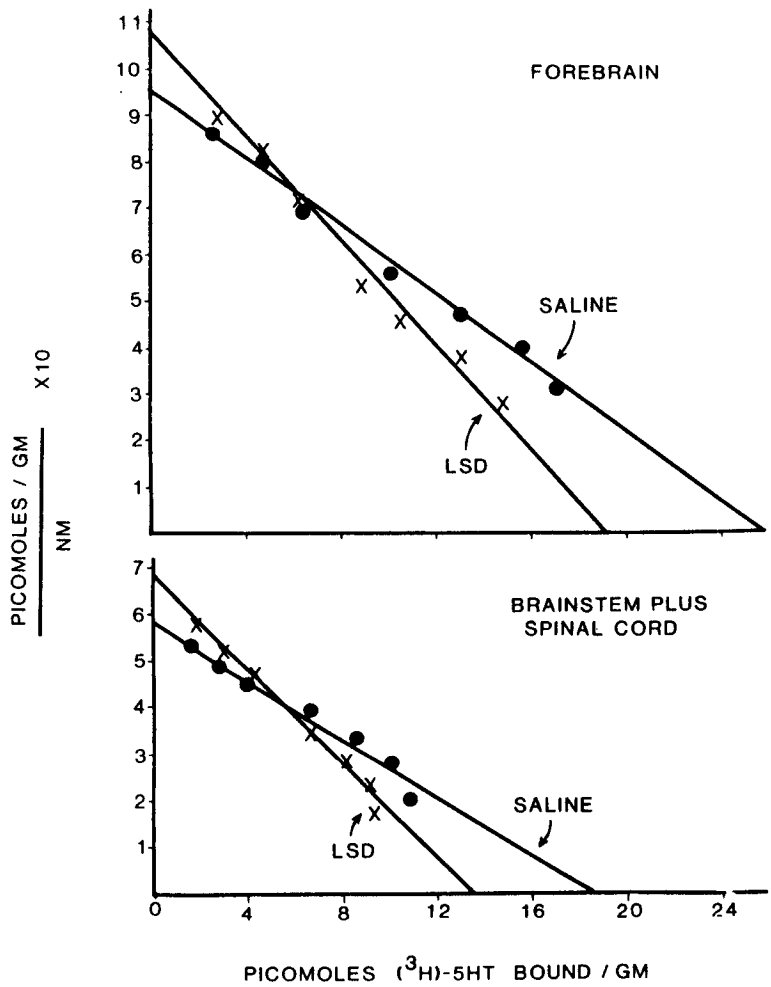

FIGURE 1

Effects of LSD (100 μg/kg, i.p.) or saline (1 ml/kg, i.p.) administered every 6 hours for 4 days on [^{3}H] -5HT binding in forebrain (upper panel) and brainstem plus spinal cord (lower panel). Scatchard analysis revealed significant decreases in both K_D and Bmax following chronic LSD administration. The experiment was replicated 8 times. Correlation coefficients for individual Scatchard plots ranged from 0.91 to 0.99.

TABLE I

[^{3}H]-5HT Binding Following Chronic LSD or BOL Administration

		Forebrain		Brainstem plus Spinal Cord	
Treatment	n	K_D(nM)	Bmax(pmol/g)	K_D(nM)	Bmax(pmol/g)
Saline	8	30.3±1.1	24.8±1.0	29.4±1.0	17.2±0.9
LSD (% Change)	8	25.1±1.0** (-17%)	18.7±0.9** (-25%)	22.6±1.2** (-23%)	12.1±0.7** (-30%)
Saline	8	31.8±1.9	25.3±1.1	28.5±2.0	13.3±0.6
BOL (% Change)	8	30.4±2.1 (-4%)	25.1±1.4 (-1%)	28.5±3.2 (0%)	13.4±0.8 (+1%)

Rats were administered either LSD or BOL (100 µg/kg, i.p.) or saline (1ml/kg, i.p.) every 6 hours for 4 days and were killed for binding assays 24 hours after the last injection. Data are presented as means ± S.E.M. Statistical significance of differences between saline and LSD or BOL groups evaluated using 2-tailed-t-tests; ** $p < .01$.

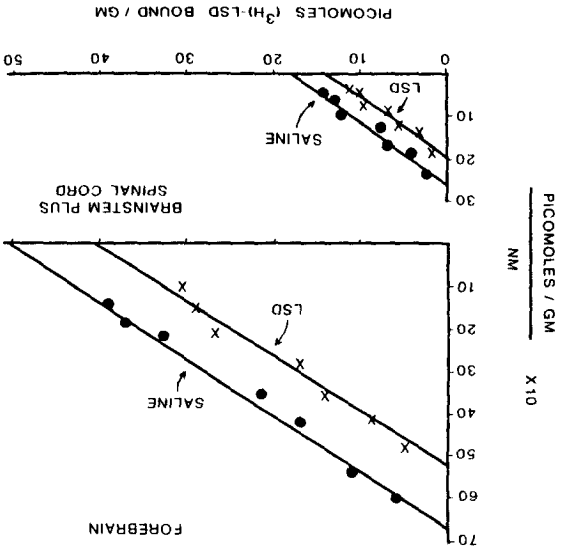

FIGURE 2

Effects of LSD (100 µg/kg, i.p.) or saline (1 ml/kg, i.p.) administered every 6 hours for 4 days on [^{3}H]-LSD binding in forebrain (upper panel) and brainstem plus spinal cord (lower panel). Scatchard analysis revealed a significant decrease in Bmax following chronic LSD administration. The experiment was replicated 8 times. Correlation coefficients for individual Scatchard plots ranged from 0.93 to 0.99.

TABLE II

[^{3}H]-LSD Binding Following Chronic LSD or BOL Administration

		Forebrain		Brainstem plus Spinal Cord	
Treatment	n	K_D (nM)	Bmax (pmol/g)	K_D (nM)	Bmax (pmol/g)
Saline	8	8.2±0.6	49.3±2.2	10.2±0.6	17.8±0.6
LSD (% Change)	8	8.8±0.8 (+7%)	40.0±1.6** (-19%)	9.3±3.9 (-9%)	14.5±1.3* (-19%)
Saline	4	6.8±0.4	51.2±1.4	10.4±0.5	16.8±0.9
BOL (% Change)	4	6.7±0.2 (-1%)	50.7±3.0 (-1%)	11.0±0.8 (+6%)	17.4±1.0 (+4%)

Rats were administered either LSD or BOL (100 µg/kg, i.p.) or saline (1ml/kg, i.p.) every 6 hours for 4 days and were killed for binding assays 24 hours after the last injection. Data are presented as means ± S.E.M. Statistical significance of differences between saline and LSD or BOL groups evaluated using 2-tailed-t-tests ; * $p < .05$; ** $p < .01$.

When assays were performed 6 hours following the final injection of LSD, the Bmax values for [^{3}H]-5HT binding were decreased approximately 27%, while the K_D values were decreased 31 and 28% in forebrain and brainstem plus spinal cord, respectively. The latter values reflect an even greater change than that seen when assays were performed 24 hours following the final LSD injection. The changes observed 24 hours after the final LSD injection were virtually identical when the tissue was washed one vs. four times prior to the binding assay. A single injection of LSD resulted in no significant changes in [^{3}H]-5HT binding. Chronic administration of BOL produced no significant changes in either [^{3}H]-5HT or [^{3}H] -LSD binding (Tables 1 & 2). Chronic administration of LSD produced no significant changes in [^{3}H] -spiroperidol binding (Table 3).

Discussion

The present data demonstrate that repeated administration of LSD, on a regimen known to produce tolerance using behavioral measures (4,6,7), results in decreased maximal [^{3}H] -5HT and [^{3}H]-LSD specific receptor binding, while acute LSD administration resulted in no changes in binding. The effects of chronic LSD administration shows some specificity in that [^{3}H] -spiroperidol binding was not altered by this treatment. Furthermore, chronic administration of BOL, a non-hallucinogenic congener of LSD, produced no significant changes in either [^{3}H] -5HT or [^{3}H] -LSD binding. While several previous studies have reported that repeated administration of LSD produces an attenuation in certain neurochemical changes hich occur following a single LSD administration (9-12), the pre-

TABLE III

[^{3}H]-Spiroperidol Binding Following Chronic LSD Administration

		Striatum		Limbic Forebrain	
Treatment	n	K_D (nM)	Bmax (pmol/g)	K_D (nM)	Bmax (pmol/g)
Saline	4	0.21±0.02	20.9±1.5	0.27±0.01	11.4±1.4
LSD (% Change)	4	0.23±0.03 (+10%)	20.7±1.2 (-1%)	0.23±0.03 (-15%)	11.2±1.7 (-2%)

Rats were administered LSD (100 µg/kg, i.p.) or Saline (1ml/kg, i.p.) every 6 hours for 4 days and were killed for binding assays 24 hours after the last injection. Data are presented as means ± S.E.M. No significant differences were found between saline and LSD groups.

sent study is the first to report an actual directional change in any neurochemical measure in LSD tolerant animals, as compared to the effects of acute LSD administration.

The magnitude of the observed changes in maximal binding of [^{3}H]-5HT and [^{3}H]-LSD (i.e., 19-30%) is similar to that produced by chronic neurotransmitter depletion or receptor blockade (e.g., in the dopamine system (23,24). In view of these latter findings, it has been hypothesized that small changes in neurotransmitter binding may be sufficient to mediate large behavioral or pharmacological effects. Similarly, the decreased receptor binding found in the present study may account for the profound tolerance that develops to LSD following its repeated administration.

In addition to the decreased maximal binding of [^{3}H]-5HT, the affinity of [^{3}H]-5HT for its receptor was increased following repeated LSD administration. The relative importance of changes in affinity versus changes in maximal binding capacity is not known. It is possible that the increased affinity of [^{3}H]-5HT for its receptor following repeated administration of LSD offsets the effects of decreased maximal [^{3}H]-5HT binding. [^{3}H]-LSD binding, on the other hand, displayed decreased maximal binding with no change in affinity, suggesting a net decrease in the ability of LSD to affect its receptor during tolerance. Since serotonergic neurons are equally sensitive to the depressant effects of LSD in the tolerant and non-tolerant conditions (18,19, and Trulson & Jacobs, submitted), the present data suggest that tolerance may be mediated by a subsensitivity to LSD on the part of their postsynaptic target neurons.

Considerable evidence suggests that LSD binds to 5HT receptors in the CNS. For example, [^{3}H]-LSD binding shows a regional distribution very similar to [^{3}H]-5HT binding (21,25). Furthermore, of all neurotransmitters examined, 5HT was found to be the most effective in displacing [^{3}H]-LSD from its binding sites (26-28). It is unlikely, however, that LSD and 5HT bind to identical macromolecular structures. Displacement studies have revealed that 5-hydroxy-

substituted tryptamines are more potent on [^{3}H]-5HT than [^{3}H]-LSD binding sites, while 5HT antagonists are more potent on [^{3}H]-LSD compared to [^{3}H]-5HT binding sites (21). The present finding that chronic LSD administration decreases the maximal binding of both [^{3}H]-5HT and [^{3}H]-LSD, but changes the affinity only of the [^{3}H]-5HT binding site, is further evidence that 5HT and LSD bind to similar, but not identical, macromolecular structures.

LSD has also been shown to bind to brain dopamine receptors (29). While several recent electrophysiological, behavioral and neurochemical studies have revealed that LSD has potent dopamine receptor agonist effects (29-32), tolerance to LSD is apparently not mediated by a change in this system. We found no change in the binding of [^{3}H]-spiroperidol, which labels dopamine receptors, in the present study. This conclusion is supported by the finding that LSD-induced contralateral turning in rats with unilateral destruction of the nigral-striatal dopamine system shows no change with repeated LSD administration (33).

The present data are of more general interest in that they demonstrate a change in [^{3}H]-5HT receptor binding that occurs independent of brain 5HT depletion. The only manipulations that have previously been shown to change [^{3}H]-5HT binding are those which dramatically deplete brain 5HT, such as reserpine, p-chlorophenylalanine, or 5,7-dihydroxytryptamine (21). Administration of 5HT reuptake blockers, agonists, or antagonists produce no change in [^{3}H]-5HT binding (34). Therefore, the LSD molecule apparently has a somewhat unique interaction with the 5HT system.

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