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An Animal Behavior Model for Studying the Actions of LSD and Related Hallucinogens

Abstract. *Cats injected with LSD (*d*-lysergic acid diethylamide) exhibit a group of behaviors that appear to be specific to hallucinogenic drugs. Two of these behaviors, limb flick and abortive grooming, have an extremely low frequency of occurrence in normal cats, but often dominate the behavior of LSD-treated cats. The frequency of occurrence of this group of behaviors is related to the dose of LSD. The behavioral changes are long-lasting following a single injection of LSD, and exhibit tolerance following the repeated administration of LSD. They are not elicited by a variety of control drugs, but are elicited by other indole nucleus hallucinogens. Because the behavioral effects are specific, reliable, easy to score, and quantifiable, they represent an animal model that can be used in studies of the effects of LSD and related hallucinogens.*

An important step in understanding the effects of the major psychoactive drugs, and drugs of abuse, is frequently the development of an animal model or analog of the drug's action (*1*). Such models permit the undertaking of experiments which are precluded for moral and ethical reasons in humans. In addition, their use affords the opportunity for direct investigation of the physiological bases of drug actions. A case in point is the usefulness of "wet dog shakes" in rodents as a model for the abstinence syndrome that follows withdrawal from narcotics such as morphine (*2*).

In the course of examining the dose-response relationship for the behavioral effects of LSD (*d*-lysergic acid diethylamide) in the cat, we observed a group of behaviors that occur with a high probability in cats injected with LSD. The behaviors are also produced by drugs which are structurally or functionally related to LSD, but are not produced by potent psychoactive drugs from other classes.

In the experiments described here we used adult female cats weighing 2.0 to 3.3 kg. The cats were individually housed in standard stainless steel cat cages which also served as the observation chambers. To counterbalance the experiments, we subjected each cat to all treatments, allowing at least 8 days interval between consecutive treatments. The cats were given intraperitoneal injections of either saline or lysergic acid diethylamide tartrate (10.0, 25.0, 50.0, or 100.0 μg/kg, the dose being expressed as the salt). Behavioral observations, by raters who were "blind" to the treatment, were made during the hour immediately following drug administration. The frequency of occurrence of each behavior was tallied on a standard scoring sheet (Table 1).

Most of the descriptions of the cats' behaviors are self-explanatory, but a few require further comment. Abortive grooming is scored when the cat orients to the body surface as if to groom but does not emit the consummatory grooming response (bite, lick, or scratch), or emits the response in midair. Limb flicking is a behavior seen in normal cats almost exclusively in response to placing a foreign substance, such as water, on the hindpaw or forepaw. The paw is then lifted and rapidly flicked outward from the body. Investigatory or play behavior refers to pawing or sniffing at objects or in corners, chasing the tail, or batting at pieces of food or feces, for example. Hallucinatory-like behavior is scored when the cat looks around at the floor, ceiling, or walls of the cage and appears to be tracking objects visually, or when the cat either hisses at, bats at, or pounces at unseen objects (*3*).

The behavioral effects of LSD can be grouped into three distinct categories (Table 1). The frequency of the first group, which includes rubbing, treading, and vocalization, did not change significantly following the administration of LSD. The second group of behaviors, which includes staring, grooming, and head and body shakes, had a relatively high frequency of occurrence in saline-treated animals and then showed a three- to fivefold increase in frequency as a function of the dose of LSD. Many of these increases appear to be attributable simply to the arousal or activational effects of the drug.

Most important in the present context is the third group of behaviors that we describe as emergent in the LSD-treated animal. These behaviors were either nonexistent or occurred with a very low frequency in saline-injected animals, but emerged to the point of often dominating the behavior of LSD-treated cats. They include limb flicks, abortive grooming, investigatory or play behavior, and hallucinatory-like behavior. The first two of these have not been previously reported in studies of the effects of LSD on cats (*4*); and they are of particular interest because their highly stereotyped response topography makes them easy to observe and quantify.

The most impressive example of these emergent behaviors was the limb flick. After saline treatment, this response was seen only twice in the 1-hour observation period on 12 cats (mean, 0.2 per hour). However, when the same animals were given LSD (50 μg/kg), the response increased to a mean frequency of 45.8 per hour. This represents an increase of several orders of magnitude. It was not uncommon for individual animals to emit 20 to 30 flicks in a 15-minute period following the two higher doses of LSD. Two or three sequential flicks of different limbs were often seen within 2 to 5 seconds. It is worth noting that every animal tested showed at least some limb flicking in response to LSD.

Instances of abortive grooming occurred with a mean frequency of 5 to 8 per hour at doses of 25 to 100 μg/kg. While this does not represent a large ab-

Table 1. Mean frequency (± standard error of the mean) of behaviors per hour as a function of the dose of LSD (N = 12). The levels of significance for one-way analysis of variance were as follows: for grooming and hallucinatory-like behavior, $P < .05$; for staring and abortive grooming, $P < .01$; and for head and body shake and limb flick, $P < .001$. The levels of significance for t-tests (one-tail) for each drug dose compared with the saline control are given in the footnotes. LSD also produced the following behavioral changes: yawning and licking chops; standing or sitting in bizarre positions; kitten-like behavior, for example, the cats chased their tails and pawed the air while lying on their sides or backs; pawing and batting at various objects or places in the cage; sitting on the perch and staring down and back (they also frequently appeared to be responding to their own reflection in the stainless steel walls of their cages); and continual scanning of environment by moving the head about. We never observed howling, spitting, rage, or marked fear following the administration of LSD. Nor did we observe obvious salivation or lacrimation. The drug did, however, produce frequent defecation and occasional emesis.

Behavior	LSD tartrate (μg/kg)				
	0.0	10.0	25.0	50.0	100.0
Rubbing	1.9 ± 1.3	1.1 ± 1.0	2.6 ± 2.0	2.1 ± 1.6	1.7 ± 1.5
Treading or kneading	0.6 ± 0.6	0.4 ± 0.1	1.5 ± 0.4	0.8 ± 0.3	0.3 ± 0.1
Vocalization	1.1 ± 0.3	1.3 ± 0.6	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.3
Staring (other than forward) for at least 5 seconds	6.2 ± 1.9	13.1 ± 4.7*	14.6 ± 2.6†	17.9 ± 2.9‡	30.8 ± 8.0‡
Grooming	7.4 ± 2.4	18.0 ± 4.2†	22.6 ± 6.3†	16.3 ± 2.8†	11.2 ± 2.5
Head or body shake	6.3 ± 1.2	12.1 ± 2.7†	16.1 ± 4.0	28.3 ± 6.6*	17.2 ± 4.4†
Limb flick	0.2 ± 0.1	8.0 ± 2.1*	22.6 ± 3.9‡	45.8 ± 8.1‡	31.5 ± 5.9‡
Abortive grooming	0.0 ± 0.0	2.3 ± 1.2†	5.0 ± 1.5*	5.7 ± 1.2‡	7.9 ± 3.1*
Investigatory or play behavior	0.3 ± 0.1	7.0 ± 4.4†	8.8 ± 3.9†	10.8 ± 3.8*	6.8 ± 2.5*
Hallucinatory-like behavior	0.1 ± 0.1	1.5 ± 0.8†	2.7 ± 1.1*	1.3 ± 0.6†	0.7 ± 0.4†

*$P < .01$. †$P < .05$. ‡$P < .001$.

solute value, it was never observed in saline-treated animals. Every animal tested showed at least one instance of abortive grooming in response to LSD. Our impression was that the emergence of abortive grooming was reflective of the fragmentary or disjunctive nature of all behavior in these animals. They would rarely sustain any active behavior continuously for more than several seconds. It appeared as though their attention was constantly being diverted and, as a result, their ongoing behavior was frequently interrupted and changed, or even aborted prior to consummation, as in the case of grooming. They would, for example, change from play behavior to grooming and then back to play again, within the period of a few seconds.

These effects of LSD are not attributable to the inactivation of serotonin in the periphery since methysergide, a more potent blocking agent of serotonin's action in the periphery (*5*), did not elicit any of these emergent behaviors in a dose of 25 μg/kg. However, when the dose was increased to 0.1 and 0.5 mg/kg, several flicks ($\bar{X}$ = 3.2 and 4.5 per hour, respectively) and episodes of abortive grooming ($\bar{X}$ = 1.8 and 1.0 per hour, respectively) were observed. This correlates well with the facts that: (i) high doses of methysergide have been reported to be mildly hallucinogenic in humans (*6*); and (ii) methysergide (1-methyl-*d*-lysergic acid butanolamide) is an indole nucleus compound structurally very similar to LSD. Further support that the behavioral effects are not due to a peripheral action of LSD comes from the fact that its nonhallucinogenic congener, D-2-bromolysergic acid diethylamide (bromo-LSD), which has the same peripheral action as LSD, was ineffective in eliciting the emergent behaviors in doses of 25 and 100 μg/kg injected intraperitoneally (N = 5 at each dose).

The specificity of this behavioral profile is indicated by the fact that it was never seen in response to other classes of major psychoactive drugs (N = 4 or 5 for each dosage level): *d*-amphetamine sulfate (0.25, 1.0, and 5.0 mg/kg), atropine sulfate (0.5 and 2.5 mg/kg), caffeine (1.0, 5.0, and 20.0 mg/kg), and chlorpheniramine maleate (0.5, 2.5, and 5.0 mg/kg). These behaviors were, however, observed in cats whose brains were depleted of serotonin by inhibiting serotonin synthesis (*7*). We hypothesize that the similarities between the behavioral effects of LSD and serotonin depletion are based on a common functional effect. Aghajanian and co-workers have shown that LSD inactivates the brain serotonin system by directly depressing the activity of the serotonin-containing raphe neurons (*8*). Similarly, serotonin depletion inactivates the brain serotonin system by decreasing the availability of the transmitter for release into the synaptic cleft.

In an attempt to test the generality of this model, we administered psilocybin to cats. Psilocybin is a major hallucinogen that is structurally similar to LSD in that they both contain an indole nucleus. They also share the common physiological property of depressing raphe unit activity (*9*). In doses of 50 and 100 μg/kg injected intraperitoneally, psilocybin elicited 5.8 and 10.2 limb flicks per hour, respectively (N = 5 per dose). On the other hand, tryptamine, an indole nucleus compound without hallucinogenic activity, had no effect on any of the behavioral measures (50, 500, and 5000 μg/kg; N = 4 per dose). Finally, Δ^9-tetrahydrocannabinol, a hallucinogen structurally unrelated to the indole nucleus drugs, was without effect on any of these measures in doses of 500, 1000, and 5000 μg/kg (N = 4 per dose).

That these behaviors in the cat are analogous to some of the behavioral effects of LSD in humans is supported by other evidence. For example, it is well known that the affective and perceptual effects of moderate doses of LSD (1.0 to 2.0 μg/kg) last for up to 8 hours in humans (*10*). Similarly, the limb flicks and abortive grooming are still manifest 4 to 8 hours after the administration of LSD to cats. This is at a time several hours after the subsidence of most of the other behavioral effects of LSD which last for only 1 or 2 hours. Following a dose of LSD of 50 μg/kg, limb flicks still occur with a mean frequency of 25 per hour at 4 hours and 9 per hour at 8 hours after the injection. These data also argue against the interpretation of our results as being due to arousal, because 4 to 8 hours after the administration of LSD the animals are typically lying quietly in their cages, but still emitting limb flicks significantly above control levels. The behavioral effects of a 10 μg/kg dose of LSD are of a significantly shorter duration than those of the 50 μg/kg dose, which is further evidence for the dose-dependency of these behavioral changes.

Paralleling other effects of LSD in humans (*11*), LSD administered to cats also results in the development of tolerance to the drug. Such tolerance, which develops after a single dose of LSD, is also long-lasting. For example, the mean number of limb flicks emitted in response to a 50 μg/kg test dose of LSD is significantly reduced (60 percent of baseline) as long as 5 days after a single injection of the same dose. Consistent with this is the finding that an initial dose of 10 μg/kg LSD produces a shorter tolerance of ap-

proximately 3 days duration. We have also found that cats given a dose of LSD within the range employed in human studies (2.5 μg/kg) (*10, 11*) show a significant increase in limb flicks ($\bar{X}$ = 4.7 per hour; N = 5) in the absence of any other dramatic behavioral changes, a result that demonstrates the sensitivity of these measures to low doses of LSD.

In previous studies of the effects of hallucinogens in animals, investigators have utilized nonspecific behavioral measures, such as the disruption of either rope climbing or bar pressing in rats (*12*). The behaviors we describe here appear to be specific to hallucinogenic drugs and also have face validity in the sense that the constituent behaviors can be described as bizarre or inappropriate to the context in which they occur. The limb flick and abortive grooming behaviors are ideal for use as a model since they are sensitive, robust (occurring in every animal tested), reliable (stable across test sessions), quantifiable, and easy to score. They also reflect some of the major effects of LSD in humans, such as long-lasting psychological and perceptual effects, and long-lasting tolerance following a single dose.

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Plasma Membrane Vesiculation: A New Technique for Isolation of Plasma Membranes

Abstract. *Monolayer cell cultures of macrophages, monocytes, myoblasts, and density-inhibited and transformed fibroblasts form and release cell surface membrane vesicles following exposure to formaldehyde, related low-molecular-weight aldehydes, and disulfide blocking agents. Vesicles have a unique composition of proteins and lipids. They show enrichment of cholesterol and sphingomyelin content and a seven- to tenfold enrichment of 5′-nucleotidase activity. Vesicles also contain intramembranous particles and show a trilamellar unit membrane and no ultrastructural evidence of contamination with other cytoplasmic organelles. The technique is proposed as a novel method for isolating plasma membrane vesicles from cells in culture.*

A variety of techniques for the isolation of cell surface membrane fragments have been reported. These involve cell homogenization (*1*) or cell disruption by nitrogen cavitation (*2*) followed by differentiation and isopycnic centrifugation of native or "stabilized" (*1*) cells. Plasma membrane fragments have also been partially purified by affinity chromatography (*3*). We report here the development of a new procedure for the isolation of plasma membrane vesicles (PMV's) which may avoid some of the disadvantages of traditional techniques (*4*). It is based on the observation that a variety of aldehydes and disulfide blocking agents promote the formation and release of plasma membrane vesicles from cells in culture. As early as 1919 a variety of such agents were reported to produce cell surface "blebs" (*5*). We have extended these observations and have

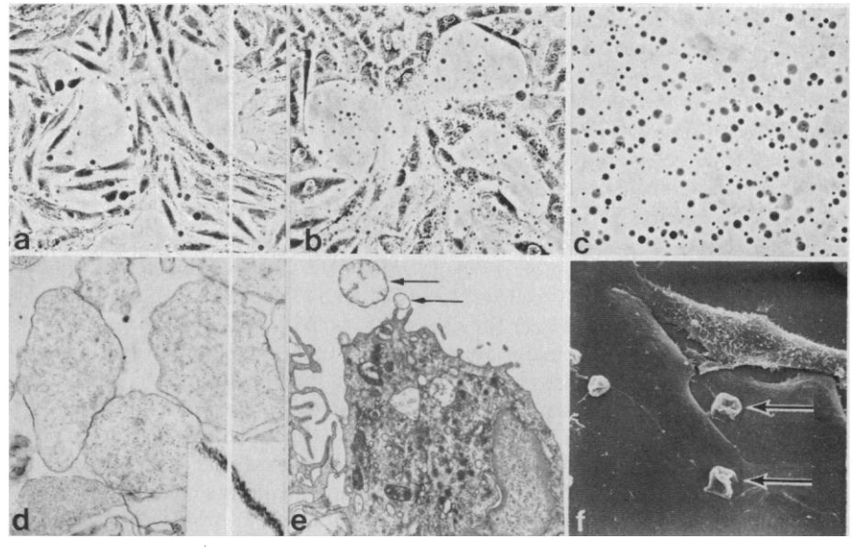

Fig. 1. (a) Phase micrograph of L_6 myoblasts exposed to 250 m*M* formaldehyde in calcium-magnesium PBS for 15 minutes, showing the formation of small cell surface membrane vesicles. (b) More extensive cell surface vesiculation with vesicles in suspension is apparent after 60 minutes incubation in 3T3 cells. (c) Vesicles decanted from 3T3 cultures represent plasma membranes. (d) Thin sections of 3T3 vesicles show no subcellular membrane contamination and (inset) a trilamellar membrane structure. (e) Analysis of macrophage vesiculation by electron microscopy also shows that vesicles are derived from the plasma membrane (arrows). (f) Scanning electron microscopy of L_6 myoblasts shows that multiple vesicles are released from individual cells (arrows). Magnifications: (a) × 140; (b) × 140; (c) × 140; (d) × 17,000; (inset) × 224,000; and (e) and (f) × 360.