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# DISSOCIATIONS BETWEEN THE BEHAVIORAL EFFECTS OF LSD AND TOLERANCE DEVELOPMENT DURING ONTOGENY IN CATS: A NOVEL APPROACH TO THE STUDY OF TOLERANCE MECHANISMS

Michael E. Trulson* and Gailyn A. Howell

Laboratory for Neurobiology, University of Texas at Dallas
P.O. Box 688, Richardson, Texas 75080

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## Summary

The characteristic behavioral effects of d-lysergic acid diethylamide (LSD) in cats first appeared at approximately 25 days of age and increased rapidly in magnitude over the next 10 days. However, 25 day old kittens showed no tolerance to the repeated administration of the drug. While the behavioral response to the initial dose of LSD remained relatively constant between 35 and 112 days of age, the tolerance gradually became more pronounced throughout this time period, reaching an adult level of virtually complete tolerance at 112 days. These findings provide new insight into the nature of the relationship between the primary drug action and the development of tolerance, and suggest a new strategy for investigating the neural bases of tolerance, i.e., examining the neurochemical effects of repeated LSD administration in kittens during various stages of tolerance development.

The dramatic perceptual and psychological effects of d-lysergic acid diethylamide (LSD) and theories concerning the neurochemical bases of its action have had a major impact on the fields of psychiatry, psychology, pharmacology, and cell biology over the past quarter century. One of the most intriguing aspects of LSD's action is the profound and rapid tolerance that develops following its repeated administration. LSD ingestion by humans produces a marked tolerance to the psychic effects of the drug after a single dose of 100 micrograms, and a complete tolerance develops after 3-4 consecutive daily doses. This tolerance completely disappears within 4-5 days after drug withdrawal (1-3). Despite intensive research efforts utilizing neurochemical, neurophysiological, and behavioral measures in animals, very little information is available concerning possible neural mechanisms for this tolerance effect. The only well-established fact regarding LSD tolerance is that it is not attributable to increased peripheral metabolism of the drug but, rather, clearly involves changes within the central nervous system (4,5).

During the course of examining the ontogeny of LSD-induced behaviors in cats, we observed a lack of tolerance to repeated LSD treatment in young kittens. These findings provide new insight into the nature of the relationship between the primary drug action and the development of tolerance, and suggest a new strategy for investigating the neural bases of tolerance, i.e., examining the neurochemical effects of repeated LSD administration in kittens during various stages of tolerance development.

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*Present address: Department of Pharmacology, Marshall University School of Medicine, Huntington, West Virginia 25701

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LSD administration to cats elicits a characteristic behavioral syndrome consisting of limb flicking, abortive grooming, head and body shakes, excessive grooming, staring, and investigatory and hallucinatory-like responses. Of these, the limb flick is the most sensitive and reliable behavioral index of the central action of hallucinogenic drugs and was therefore used for the quantitative behavioral analysis in this study (6). No claim is being made that cats given LSD are hallucinating, since it is impossible to know what a nonverbal organism is experiencing. The behavioral syndrome, especially the limb flick response, is a useful animal model for studying the actions of hallucinogenic drugs because (i) these behaviors are elicited only by hallucinogens, with few possible exceptions (7-10), (ii) they parallel the major characteristics of the action of these drugs in humans, (iii) they are not simply due to drug-induced somatosensory alterations, but are reflective of more complex central processes (11), and (iv) these behavorial changes are dose-dependent, sensitive to doses near the human range, robust, reliable and easy to quantify.

## Methods

Pregnant cats were individually housed in standard laboratory cages in a room kept at constant temperature (22 ± 2°C), maintained on a 12:12 hour light-dark cycle (lights on 8:00 a.m.), and were checked for births twice daily. Behavioral observations were made with the kittens placed in pairs in their home cage. Groups of kittens were administered saline followed by LSD tartrate (50 µg/kg, i.p.) at ages 4, 7, 14, 21, 25, 30, 35, 42, 49, 56, 70, 84, and 112 days post-partum, and then were re-administered the same dose of LSD 24 hours later. Five to 28 days intervened between consecutive sessions with the same kittens. Thus, each kitten received a total of 7 or 8 LSD injections. The 50 µg/kg dose of LSD was found to be approximately the peak behaviorally effective dose in our previous studies. Doses of 10 and 200 µg/kg of LSD were also tested in groups of kittens at various ages. In addition, a group of adult cats (12-26 months of age) that had never received LSD treatment was tested for their responsiveness to 50 µg/kg of LSD, as well as for tolerance development to the same dose of the drug 24 hours later. In all studies, behavioral changes were continuously monitored and recorded on a standard scoring sheet (6) for one hour post-injection, and then for the second 30-minute period of each succeeding hour until all behavorial changes had subsided.

The statistical significance of the differences in the limb flick rate following LSD administration on Day 1 versus Day 2 (tolerance) was evaluated using two tailed t-tests for each age group examined. To determine the percentage of kittens displaying a significant behavioral response to LSD on Day 1 in various age groups, the following procedure was used: A significant behavioral response occurs in an individual kitten when the number of limb flicks per hour following LSD administration is greater than two standard deviations above the mean limb flick rate per hour for all saline-treated kittens at that particular age.

## Results and Discussion

LSD (50 µg/kg) produced no significant behavioral changes in kittens between the ages of 4 and 21 days post-partum (Figure 1). At 25 days, 47% of the kittens tested (9/19) showed a significant behavioral response to LSD (Mean = 14.1 versus 1.2 limb flicks/hour for saline baseline, $P < 0.01$). When

the same dose of LSD was re-administered to these kittens 24 hours later, they showed no tolerance (Mean = 17.8 limb flicks/hour). Kittens apparently show no behavioral response to LSD prior to 25 days of age due to a lack of maturation of the neuronal substrate for the limb flick response. The limb flick is a species-specific behavior normally used for removing foreign substances, such as water, from the paws. We have determined that kittens begin to show limb flicks in response to water on their paws at age 21-25 days post-partum.

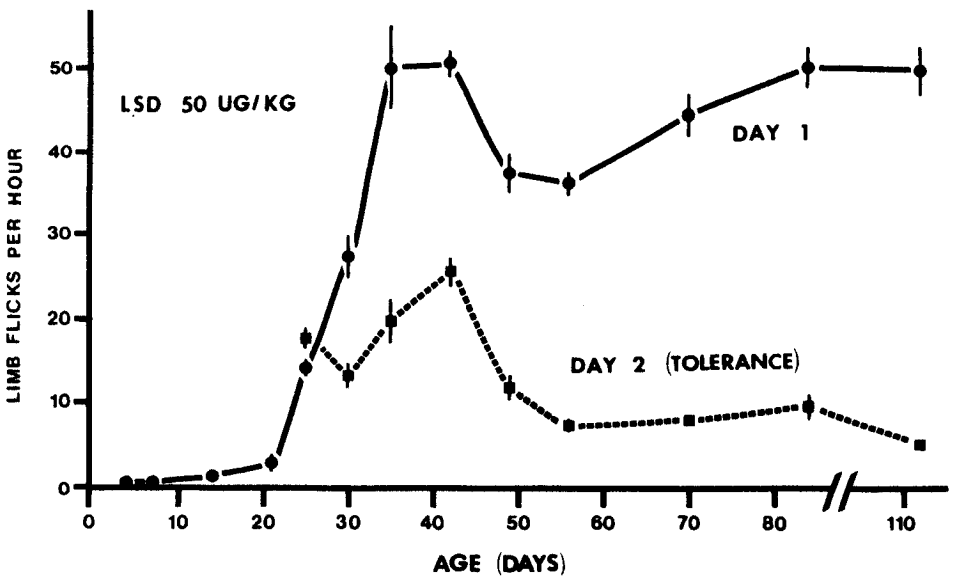


Figure 1. Mean frequency (± S.E.M.) of limb flicks per hour following administration of 50 µg/kg of LSD as a function of age post-partum. Solid line (●——●) displays the behavioral response to an initial dose of LSD (Day 1), while the dashed line (□-----□) displays the behavioral response to a second dose of LSD administered 24 hours later (Day 2-tolerance).

After 30 days, 76% (13/17) showed a significant behavioral response to LSD (Mean = 27.2 versus 2.4 limb flicks/hour for saline baseline, $P < 0.01$), and these kittens showed a significant tolerance when tested 24 hours later (Mean = 12.1 limb flicks/hour, $P < 0.05$). By 35 days of age, all kittens tested (17/17) showed a significant behavioral response (Mean = 49.9 versus 0.9 limb flicks/hour, $P < 0.05$). While the behavioral response to the initial 50 µg/kg dose of LSD remained constant between 35 and 112 days of age (except for significant decreases of approximately 20% on days 49 and 56), the tolerance gradually became more pronounced throughout this time period reaching an adult level of virtually complete tolerance at 112 days of age (Figure 1). A group of naive adult cats (12-26 months of age, N=12) displayed 41.2 ± 4.6 limb flicks/hour following their initial injection of 50 µg/kg of LSD, and displayed the typical adult tolerance effect when given a second dose of LSD

(50 µg/kg) 24 hours later (1.1 ± 0.7 limb flicks/hour, $P<0.01$). Previous studies with adult cats have shown that LSD-induced behavioral changes following a 50 µg/kg dose persist for 8-12 hours post-injection (12). The present data revealed that LSD-induced behavioral changes in kittens persist for the same duration of time (i.e., 8-12 hours) from their earliest appearance during ontogeny (25 days of age). Doses of 10 and 200 µg/kg of LSD were also tested in groups of kittens at various ages and the behavioral responses to these doses paralleled the development of the behavioral responses to the 50 µg/kg dose. Kittens displayed dose-dependent increases in the limb flick rate at 10, 50 and 200 µg/kg of LSD, which was similar to the adult responses by 35 days of age (13).

To further evaluate the resistance to tolerance development observed in young cats, we injected groups of naive kittens with 50 µg/kg of LSD every 24 hours for 5 consecutive days, beginning at age 25, 30 or 42 days. This study revealed that no significant tolerance developed during this pretreatment regimen when the injections were initiated at 25 days of age (Figure 2). When kittens received their first injection of LSD at age 30 days, no significant tolerance developed until the 5th consecutive daily injection (Figure 2). This is in sharp contrast to the significant tolerance that develops to LSD following a single injection at 30 days, when the animals had previously been exposed to the drug (Figure 1). Similarly, when naive kittens received the first injection of LSD at age 42 days, 5 consecutive daily injections were required to demonstrate a significant tolerance (Figure 2), in contrast to the significant tolerance which occurs following a single injection of the drug in animals that had previous exposure to LSD. Finally, an additional group of naive kittens was administered LSD (50 µg/kg) every 12 hours for 5 consecutive days, beginning at age 35 days. This study revealed that a significant tolerance developed after two days (4 injections) of LSD treatment (Figure 2).

The 25 day-old kittens whose data are presented in Figure 2 exhibited a significantly greater response to LSD (27.8 limb flicks/hour) than the group of kittens in Figure 1 (14.1 limb flicks/hour at 25 days). This variability among groups may be attributable to the fact that responsiveness to LSD increases very rapidly between 21 and 35 days post-partum. That is, gestational age (and, hence, stage of neural development) apparently determines the degree of responsiveness to LSD, and the gestational period varies among litters. Importantly, the lack of tolerance to LSD in naive kittens persists from day 25 to at least day 42 post-partum (Figure 2). Therefore, the neural bases of tolerance development can be investigated even though the magnitude of response to LSD at a given age varies somewhat among litters of animals.

The present data suggest a new approach for investigating the neural bases of tolerance to LSD, i.e., examining the neurochemical effects of repeated drug administration in kittens during various stages of tolerance development. This method would greatly increase the probability of isolating the critical neural change for tolerance development, since incidental neurochemical changes could be eliminated from consideration if no concomitant behavioral tolerance occurred. A number of models have been proposed to account for LSD-induced hallucinogenesis (14-17), as well as for tolerance development (18-22). 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.

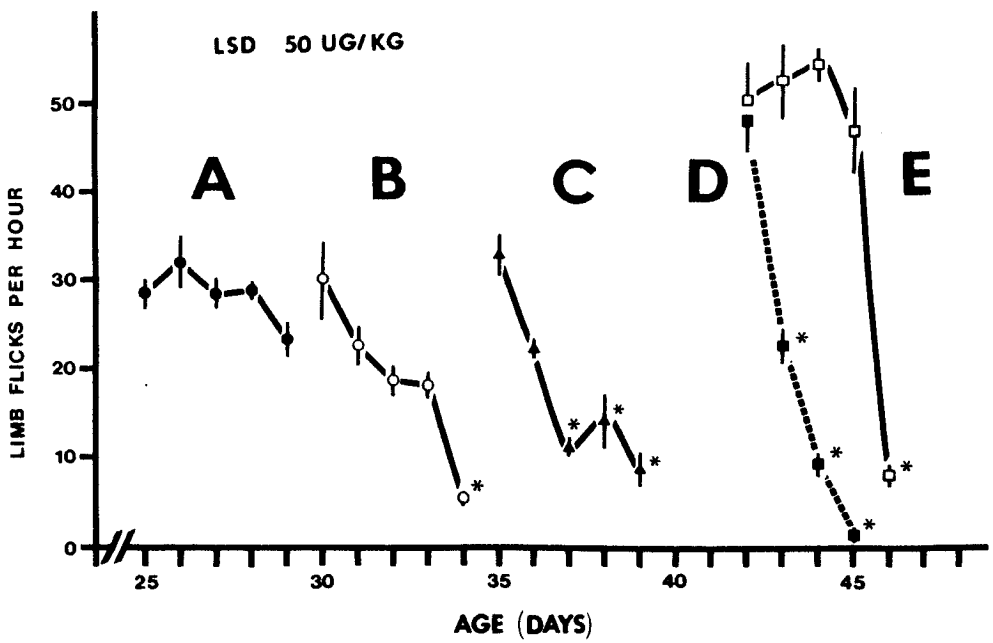


Figure 2. Mean frequency (± S.E.M.) of limb flicks per hour following repeated administration of LSD as a function of age post-partum. Naive kittens were administered LSD (50 µg/kg) every 24 hours for 5 consecutive days beginning on Day 25 (A), 30 (B) or 42 (E). A group of kittens that had had previous exposure to LSD (last injection 7 days prior to initiation of present experiment) also received daily injections of LSD (50 µg/kg) beginning on Day 42, for 4 consecutive days (D). An additional group of naive kittens received injections of LSD (50 µg/kg) every 12 hours for 5 consecutive days, beginning on Day 35 (C). N = 8 for group A, 6 for groups B, C and E, and 5 for group D. Significance of differences between the behavioral response to the first injection versus each subsequent injection for each group was evaluated by Newman-Keuls tests; *P <0.05.

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The significance of the present results is greatly enhanced by the finding that, while adult humans exhibit tolerance to LSD following a single dose, daily LSD treatment in children (ages 5-12 years) with various psychiatric and neurological disorders over periods of several weeks produced little, if any, tolerance (23). Therefore, a direct parallel exists for the dissociations between the behavioral effects of LSD and tolerance development during ontogeny in cats and humans.

The present data are of more general interest because, to our knowledge, this is the first study to report a dissociation between the primary action of a drug and the development of tolerance to that drug during ontogeny in an animal behavioral model. Furthermore, our data clearly demonstrate that prior exposure to the drug greatly accelerates the maturation of the tolerance

mechanism. These data strongly suggest that the neural mechanism for mediating the behavioral response is separate from that which mediates the tolerance effect. This finding has profound implications for the field of neuropharmacology: If the site of action for certain psychoactive drugs is separate from the site mediating tolerance, then it may be possible to develop drugs which produce the primary therapeutic effect, without producing tolerance. Therefore, it would be possible to conduct long-term therapy without the onset of tolerance, and the concomitant need to increase drug dosages, thus avoiding untoward side effects and reducing addiction liability.

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