

# The effect of reversible lesions in the posterior hypothalamus on conditioned suppression<sup>1</sup>

MARK E. DULLE, PAUL D'ENCARNACAO, and CHARLES J. LONG, *Memphis State University, Memphis, Tenn. 38111*

*Three rats with cannulae implanted in the posterior hypothalamic area and one nonimplant rat were tested in a conditioned suppression paradigm. During the acquisition phase of conditioned suppression, xylocaine hydrochloride was administered bilaterally via cannulae to two of the implant animals and an equal volume of normal saline was administered to the third animal. The xylocaine-injected animals required approximately twice the number of CS-UCS pairings as the saline-injected animal and five times the number of CS-UCS pairings as the normal control animal to acquire conditioned suppression. The maintenance of conditioned suppression was generally unaffected by xylocaine lesions.*

Research directed at assessing the relation between structures of the brain and behavior have continually implicated the limbic system as an important component of the neural substrate of emotion (Papez, 1937; Brady & Nauta, 1955; Ingram, Barris, & Ranson, 1936). Recently, research employing electrophysiological techniques has indicated that the posterior hypothalamus plays a major role in activating the various structures of the limbic system and thus could be a key structure mediating emotional behavior (Kawamura, Nagamura, & Tokizane, 1961). Kawamura & Oshima (1962) demonstrated that an intact posterior hypothalamus is required for activation of the limbic system following injections of epinephrine or norepinephrine.

Ingram et al (1936) reported that lesions involving the caudal part of the hypothalamus and upper part of the mesencephalic tegmentum of cats may produce a syndrome which includes initial somnolence, vacuity of expression, and strong suppression of emotional reactions. Lesions in the caudal part of the hypothalamus produced somnolence in 10 out of 11 monkeys (Ranson, 1939). The animal which failed to exhibit somnolence was the only one in which the mamillary bodies and mamillothalamic tracts were intact, suggesting that the mamillary bodies may be involved in somnolence. While these

studies report gross behavioral effects of posterior hypothalamic lesions, no systematic behavioral studies have been conducted attempting to assess more subtle effects on the emotionality of the animal resulting from these lesions.

The aims of the present study were to assess the effect of drug-induced lesions in the posterior hypothalamus on both the acquisition and maintenance of conditioned suppression. Reversible, drug-induced lesions were used because they allow within-S control, which is precluded by the use of permanent lesions. In addition, reversible lesions have the advantage of avoiding permanent debilitating effects such as reported by Haetig & Masserman (1940). In this study, 16 of 19 cats with permanent lesions in the posterior hypothalamus died of bronchopneumonia within 8 days of operation.

There are several ways of evaluating the emotionality of an animal, such as active avoidance, passive avoidance, conditioned suppression, etc. How emotionality is defined will influence the way emotionality is assessed. In this study, emotionality is defined as a disruption of ongoing behavior (Estes & Skinner, 1941; Pribram, 1967). Defined in this manner, a sensitive measure of emotionality is thought to be found in a conditioned suppression paradigm. Briefly stated, this method consists of presenting a previously neutral stimulus, e.g., light or tone (CS), paired with an aversive stimulus, e.g., shock (UCS) to animals.

## SUBJECTS

The Ss consisted of four male albino rats of the Sprague-Dawley strain, approximately 100 days of age at the beginning of the experiment. They were housed individually and maintained on ad lib food. Water was presented for approximately 15 min per day immediately following the experimental session. Of the four animals, two served as drug animals, one as a saline control, and one as the normal control (nonimplant).

## APPARATUS

The apparatus consisted of a Plexiglas operant conditioning chamber with grid floor wired to a Grason-Stadler shock generator. A 24-V houselight was mounted on the outside of the chamber. The operant chamber was enclosed in a sound-attenuated cabinet and an exhaust fan and white noise were presented in order to decrease the possibility of auditory cues. The cannulae

were composed of a 27-ga stainless steel tube, 18½ mm in length, attached to a 3-in. polyethylene tube which was secured to the skull by eyeglass screws and dental acrylic.

## PROCEDURE

Following cannulae implantation and a postoperative recovery period of 3 days, the animals were placed on a 24-h water-deprivation schedule and conditioned to lever-press for water reinforcement delivered on a VI 1 schedule. The criterion for stability was a fluctuation from baseline during the CS period not exceeding  $-.3$  for 6 consecutive days, using the formula  $(B - A)/A$ , where B equals the number of responses during the CS period and A equals the number of responses during an equal period immediately prior to the CS onset (Brady & Nauta, 1955).

The animals were run one session each day for 30 min. A light which served as the CS was presented twice each session. Suppression ratios were then determined. To be considered stable, the suppression ratios for both CS periods per session had to meet the  $-.3$ -or-above criterion for 6 consecutive days. As each individual animal met this criterion for stabilization, shock was introduced. The implant animals were injected immediately before each session and midway through the session to assure continued anesthetization. After the animal had been responding for several minutes at a rate judged by the E to be stable by visual inspection of the cumulative record, the CS was presented for a 2-min period, terminating with the UCS, which was a 0.5-sec 1.0-mA foot-shock.

To assess the effects of reversible lesions in the posterior hypothalamus on the acquisition of conditioned suppression, ½ microliter of 2% xylocaine-hydrochloride (20 mg/cc) was injected into the posterior hypothalamus of the experimental animals. It has been shown that a drug such as xylocaine when used in a similar manner, employing small doses, produces no enduring injurious effects on the animals (Epstein, 1960). Isotonic saline was injected into the posterior hypothalamus of the saline control. The normal control animal (nonimplant) was run under the same conditions as the other animals.

After completion of the experimental manipulations, the implant animals were perfused with 0.9% saline and 10% formalin. The brains were sectioned at 42 microns and stained with cresylecht violet.

## RESULTS

The mean suppression ratios for each animal over the experimental days are depicted in Fig. 1. As can be seen from this figure, a dramatic difference was found between the performance of xylocaine-injected animals and the performance of normal control and saline

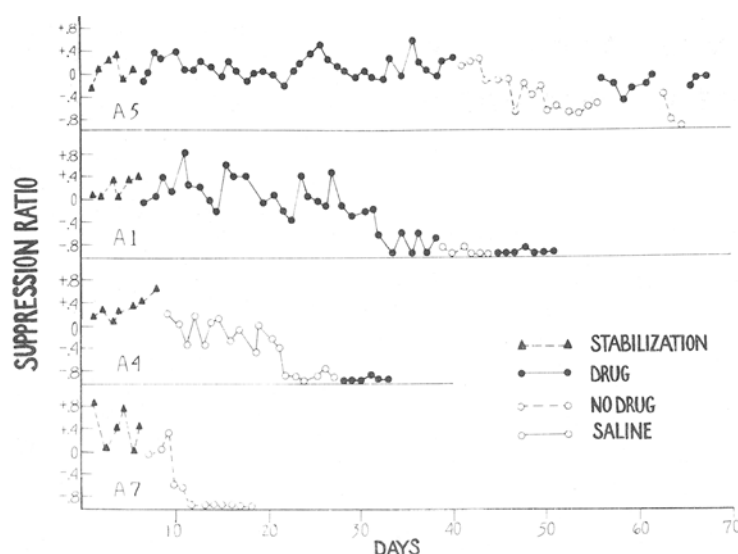


Fig. 1. Average suppression ratios for each session during stabilization criterion and experimental manipulations.

control animals. The xylocaine-injected animals did not acquire suppression (A-5), or took much longer to acquire suppression (A-1) than either the saline control (A-4) or the normal control animal (A-7).

Histologies revealed that, with the exception of A-5, all cannulae were in the correct position. A-5 was found to have one cannula tip on the midline and the other tip ½ mm lateral to the posterior hypothalamus in the right hemisphere. All implant animals were found to have permanent lesions varying from slight (A-5) to extensive (A-1) damage.

Animal 5 failed to acquire conditioned suppression after as many as 72 pairings of the CS-UCS. Thus, even after being run more than five times longer than the normal control animal and more than twice as long as the saline control, this animal evidenced no signs of suppression. Xylocaine-induced lesions were then terminated and the animal acquired suppression within 16 pairings of the CS-UCS. To assess the effects of xylocaine on the maintenance of conditioned suppression, the drug injections were resumed for 6 days. The average suppression ratio for this phase was  $-.28$ . The drug injections were then terminated for a 3-day period. The average suppression ratio for this period dropped to  $-.77$ . A subsequent 3-day period of xylocaine injections resulted in raising the suppression ratio for this period to  $-.25$ .

Seventy presentations of the CS-UCS were necessary for A-1 to acquire conditioned suppression. This was nearly twice the number of pairings required for the saline control and more than five times that required for the normal control animal. The average suppression ratio for the 6 days of suppression criterion was  $-.82$ . When the xylocaine injections were discontinued for

the 6 days following the attainment of suppression criterion, an enhancement of suppression was produced. The average suppression ratio for this period was  $-.98$ , indicating that once conditioned suppression had been established, xylocaine had no effect on lessening the suppression of this animal. Although A-1 did eventually develop suppression, it was not as consistent or at as low a level as when xylocaine injections were discontinued.

The saline control animal (A-4) required 38 pairings of the CS-UCS before the conditioned suppression criterion was reached. The average suppression ratio during criterion was  $-.95$ . Although it took A-4 approximately twice as long to acquire conditioned suppression as did the normal control, once the suppression had been established, it was more dramatic than either of the xylocaine animals. It was found that during the maintenance phase, for the 6 days during which xylocaine injections were substituted for saline injections, A-4 continued to exhibit suppression, its ratio being  $-.99$  for this period.

After only eight pairings of the CS-UCS, the normal control animal (A-7) exhibited significant suppression and continued to suppress until criterion was reached. Perfect suppression ( $-1.0$ ) was exhibited for the final eight pairings of the criterion phase. While only one normal control animal was run, the performance of this animal compares well with the majority of the literature on the acquisition of conditioned suppression, e.g., Stein et al (1958), Brady & Nauta (1955), as well as with the behavior of previous animals run in this apparatus.

#### DISCUSSION

The major finding of this study was that reversible lesions induced by injections of small amounts of xylocaine into the

posterior hypothalamic area retard the acquisition of conditioned suppression. The fact that xylocaine-induced lesions of the posterior hypothalamus selectively prevented or slowed the establishment of conditioned suppression, while not significantly modifying the animals' baseline performance, suggests that different neural structures underlie these two behaviors. This finding further suggests that since conditioned suppression has often been considered as a measure of emotionality (Estes & Skinner, 1941), interference with the normal functioning of the posterior hypothalamus has a dampening effect on the emotionality of the animal.

An interesting finding was that once conditioned suppression had been established in A-5, it was able to be lessened by xylocaine injections to the posterior hypothalamic area, whereas A-1 continued to suppress once this behavior had been established. As has been pointed out, the cannula placement of A-5 varied slightly from the other animals. Since the medially placed cannula was close to the third ventricle in this animal, there is a high probability that drug injections spread to other hypothalamic and extra-hypothalamic areas. Thus, cannula placement may account for the differential maintenance effect found between A-5 and A-1. Another factor which may have contributed to the difference between these two animals is the permanent lesions which may have been caused by the chronic presence of the cannulae in the brain (Margules & Stein, 1969).

The finding that the saline control animal took longer than the normal control to acquire conditioned suppression indicates that perhaps the saline injections created reversible lesions which, while not as severe as xylocaine-induced lesions, were severe enough to cause some dysfunctioning of that structure. Hanai & Delgado (1967) state that saline injections in various structures of the brain did not modify spontaneous electrical activity. Their implicit assumption seems to be that these saline injections in no way modified the behavior of the animals. The present study, however, demonstrates that with a sufficiently sensitive measure as conditioned suppression an effect of the saline injections can be discerned.

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maximum score of 60 for each hour for each behavior (six animals x 10 bins). To check for accuracy of observation and scoring, two Os recorded independently during one session in each of the six "parts" of the day.

## RESULTS AND DISCUSSION

The categories of behavior that were scored are listed and defined below:

- (1) Ambulation: Moving or turning on a level surface.
  - (2) Climbing: Moving up or down hardware cloth, branches, or around boxes.
  - (3) Investigation: Sniffing environment (exclusive of nosing behavior).
  - (4) Nosing: Placing nose near another animal.
  - (5) Grooming: Cleaning self with mouth, paw, face, or tongue.
  - (6) Consummatory: Ingestion of solid food, or water (which were available at all times).
  - (7) Resting: Sleep or resting (resting is distinguished from observing behavior by the general lack of body movements except for an occasional adjustment of body position which occurs even during sleep).
  - (8) Observing: Periods of wakefulness during which the animal is not doing anything that would place him in another category.
  - (9) Passive social contact: Sleeping, resting, sitting, or standing in physical contact with another animal.
  - (10) Active social contact: Nosing, pawing, or biting another animal. Also includes climbing on or around another animal as well as growling at another animal.
- Behaviors scored, but not graphically analyzed because they occurred too rarely:
- (11) Aggression: Biting or growling at another animal.
  - (12) Submission: Retreating after an aggressive display of another animal.
  - (13) Yawning: Self-descriptive.

The scores of the principal O and the second Os correlated highly ( $r = .95$ ).

Figure 1 shows graphs which plot the time of day against the total number of bins in which a particular behavior was observed for the six animals; each point represents the sum of 2 h of observation. Graphs A-E demonstrate clear diel patterns of activity. For each of these behaviors—ambulation, climbing, investigation, nosing, and active social—there seems to be an approximately 10-h activity period beginning about 10:00 p.m. (Hour 22, approximately 1 h after sunset), and lasting until approximately 8:00 a.m. (approximately 1 h after sunrise). Consummatory behavior shows an activity period that is slightly later, and also a burst of activity in the afternoon, but the frequency of this category is too small to warrant conclusions. Graphs G and H, observing and grooming, show a much

## Daily activity schedule of captive opossums

ROCCO A. BOMBARDIERI, JR., *Zoology Department*, and JOHN I. JOHNSON, JR., *Biophysics, Psychology, and Zoology Departments, Michigan State University, East Lansing, Mich. 48823*

*A stratified time-sampling technique was used to describe quantitatively, in terms of observed probabilities, the time of occurrence and variety of activities in captive opossums. Opossums are awake and grooming between 6:00 PM and 8:00 AM, with most types of other activities occurring between 10:00 PM and 8:00 AM. The opossums spend the rest of the day, 8:00 AM through 6:00 PM, resting or sleeping.*

In general references on opossums, *Didelphis marsupialis* (Hartman, 1952; Keefe, 1967; Reynolds, 1953), there are few data concerning their diel activity patterns. Therefore, the quantitative data that we have obtained may prove valuable to other investigators of opossum behavior in the laboratory. They show, in a definite and discrete way, the distribution of activities in time. This study was designed to describe the time of occurrence and the varieties of activities observed in captive opossums studied over a period of 30 days.

### METHODS

Six animals, two adult females, two adult males, and two juvenile females, were selected for this study. They were kept in a colony with six other animals, one adult and five juvenile females. The animals were originally obtained from commercial sources in North Carolina and Texas. The study was conducted in August 1968, in a

barn on the Michigan State University farms near East Lansing, Michigan. All animals were marked for identification with Nyanzol-D dye, and the entire colony was kept in a 3.9 x 2.8 m (x 2.3 m high) hardware cloth enclosure within the barn. Within the enclosure, there were three elevated boxes that were used by the animals for rest or sleep, and tree branches that the animals used to climb from the floor to the boxes. All observations were made from outside the pen. At night a small flashlight or the barn lights were used to make the animals visible.

Observations were made using a stratified time-sampling technique in which the 24-h day was divided into six 4-h "parts" (Hours 1-4, Hours 5-8, etc.). Observations were conducted on 6 days of each week, and each "part" of the day was represented by one observation in each of the 4 weeks. A random-number table was used to select the time of observation for each day during the study, always maintaining the criteria that each of the six "parts" of the day was represented once in every week, and each hour of the day was represented once in the study. The order of animals observed was also randomized.

Beginning at the designated time, each animal was observed for 5 min, divided into 10 consecutive 30-sec time bins. Everything the animal did in that period was recorded in a notebook, or on tape and later transcribed to a notebook. At the end of all the observation periods, various categories of behavior were defined, and the notebook was scored by noting the presence or absence of each behavior, in each time bin, for each animal. There was, therefore, a