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The Alteration of Aversive Thresholds with Cholinergic and Adrenergic Agents

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HOUSER, V. P. AND F. L. HOUSER. *The alteration of aversive thresholds with cholinergic and adrenergic agents.* PHARMAC. BIOCHEM. BEHAV. 1(4) 433–444, 1973.–Several cholinergic and adrenergic agents were administered to five squirrel monkeys in a titration schedule to ascertain their effects upon aversive thresholds. A narcotic analgesic, morphine sulfate, in several doses was able to reliably increase the aversive threshold. Scopolamine hydrobromide and *d*-amphetamine sulfate elevated the aversive threshold upon initial administration, but this effect was lost after the animals had experienced several drug sessions. Thus, animals demonstrated pronounced drug tolerance in response to these two agents. Amphetamine also produced increased general motor activity and stereotyped behaviors. Furthermore, amphetamine produced a response profile which strongly suggested that animals were responding without regard to the shock intensity presented. Pilocarpine nitrate and scopolamine methylbromide had no reliable effects upon behavior given alone or in conjuction with each other. α-Methyl-p-tyrosine in several doses reliably increased the aversive threshold and reduced response rates in all animals tested. These results suggested that adrenergic mechanisms may be involved in mediating the aversive qualities of electric shock.

Aversive thresholds Titration schedule Squirrel monkey Scopolamine Pilocarpine α-Methyl-p-tyrosine Amphetamine Morphine sulfate

THE ability of certain pharmacological agents to attenuate the motivational properties of electric shock can be studied by a technique known as a titration schedule. This term refers to an experimental situation in which increments of a stimulus are automatically programmed, and decrements in intensity are controlled by the number of specific responses emitted by an animal. The response rate thus determines the stimulus intensity presented to the animal. By recording the stimulus intensity over a period of time an aversive threshold can be computed. This threshold can be operationally defined as the intensity of shock that an individual animal encounters during any given experimental session. This technique has been reported with reference to electric shock by Weiss and Laties [7].

The present study was an attempt to delineate some of the motivational properties of several adrenergic and cholinergic agents using a titration schedule. More specifically, an attempt was made to discover whether manipulating levels of adrenergic and cholinergic activity via drugs altered the aversive threshold in a lawful manner. Experiment 1 was an exploratory study which attempted to determine whether this general approach would be fruitful and to delineate some of the problems that might be encountered. Since the data from Experiment 1 looked promising, a second experiment was conducted to more carefully ascertain the effects of the various drugs upon behavior controlled by a titration schedule.

EXPERIMENT 1

METHOD

Animals

Two male squirrel monkeys (ABBY, JERRY) with no previous training and weighing 700–730 g were used in this study. Both animals were kept on a regimen of free access to Purina monkey chow and water in their home cages throughout the experimental period.

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Apparatus

The testing apparatus consisted of a Plexiglas restraining chair, fabricated locally, which measured 13-7/8 in. high, 12 in. long and 8-1/2 in. wide. The animal sat on two metal rods which were suspended from one of the walls of the chair 2-1/2 in. above the floor. A waist lock 2 in. above the sitting rods prevented the animal from escaping a sitting posture. Furthermore, a plastic leg lock prevented the animal from turning around and thus forced it to face the front panel. The front panel was 4-5/8 in. from the waist lock. A Lehigh Valley Electronics retractable lever (Model 1405R) was mounted on the center of the front panel 3-3/4 in. above the waist lock. The front panel was painted black, unlike all the other panels which were clear Plexiglas. The tail electrodes were two 8 in. long brass square rods, 3/4 in. x 1/2 in. with semicircles carved in them to fit snugly over the tail. A tail lock was situated above the electrodes directly behind the sitting bars and thus prevented any tail movement. The electrodes were separated from each other by 5/16 in. Tail resistance was brought down to 1000 Ω every session by applying Sanborn Redux Electrode Paste between the tail and the two electrodes.

The entire chair was encased in a sound attenuating chamber which was equipped with: a speaker which supplied 85 db of white noise from a Grason Stadler generator (No. 901B), a houselight (TS 304) powered by 30 V a.c. from a Lehigh Valley isolation panel (No. 1429), and a fan to insure a supply of fresh air during the session.

The electric shock to the tail was provided by a stimulator, built locally, which essentially consisted of an a.c. transformer which provided 680 V, a 25 position stepping switch which was operated by 28 V d.c., and various resistors placed in series with the animal's tail which reduced current flow to the proper level. This stimulator was of the constant current variety which placed a fixed resistance (50 k) in series with the animal's tail. Thus, any fluctuations in the tail resistance during the session would not significantly affect current flow. Various other resistors were placed in series with the 50 k resistor to provide 25 discrete current levels. The current levels in mA (assuming a tail resistance of 1000 Ω) were as follows: 0, 0.7, 1.2, 1.7, 2.2, 2.7,...etc., with a final value of 12.2.

A Varian recorder was connected in series to the stimulator through a separate arm of the stepping switch so that each increment in current was followed by a deflection of the recording pen in one direction while a decrease in current led to a deflection in the opposite direction. Thus, a permanent ink record of current intensity throughout the session was obtained.

Finally, an assortment of electromechanical equipment was programmed to increase the current intensity one step every two sec. Every bar press response drove the stimulator and Varian recorder down one step. Finally, the total number of bar press responses were recorded for each animal during every experimental session.

Procedure

Animals' tails were shaved and electrode paste was placed between the tail and electrodes before each session. The resistance between the two electrodes was monitored to insure that the resistance was between 1.0 and 1.5 k at the beginning of each session. Measurements made after the 2-hr sessions revealed an increase in resistance to between 3.0 and 5.0 k due to the electrode paste drying out. This change in resistance, however, would not affect current flow to any great extent. Furthermore, very little drifting of the threshold occurred across the control sessions, indicating that resistance changes were probably not detected by the animal.

Initial training. Both animals were initially trained on a Sidman nondiscriminated avoidance schedule (response–shock and shock–shock interval of 20 sec) with a shock intensity of 2.2 mA. This shock was presented for a maximum duration of 3 sec. The animal could escape or avoid the shock by making a lever-press response. This schedule was continued for two successive days in 4-hr sessions. On the third day the titration schedule was introduced with a 4 sec incremental period. Thus, every four seconds the current level increased one step from 0 to 12.2 mA, and every bar-press response reduced the level by one step. Under the titration schedule shock was presented continuously. Only the intensity was altered in response to the animal's behavior. On the following day the incremental period was reduced to 2 sec where it remained for the rest of the experimental period. The first 7 days of the titration schedule consisted of 4-hr daily sessions. Both animals showed signs of fatigue during the latter part of these sessions, and so they were reduced to two hours for the remainder of the experimental period.

Both animals were then run 10 days (20 hr) on the titration schedule until a stable baseline was established. On the last two of these sessions saline (0.5 cc/kg) IM was injected immediately before the session.

Drug administration. Five drug series were then run on each of the two animals. These series consisted of several doses of each drug given in an ascending order on consecutive days. After the various doses had been given at least two saline days were run before the next drug series to insure that baseline performance had been regained.

Five drugs were tested in this procedure. They were administered in the following order to each animal: morphine sulfate (0.5, 1.0, 3.0, 5.0); scopolamine hydrobromide (0.5, 1.0, 3.0, 6.0 mg/kg); *d*-amphetamine sulfate (1.0, 3.0, 5.0 mg/kg); and the soluble methylester hydrochloride of *dl* α-methyl-p-tyrosine (α-MT). All drugs were dissolved in 0.9% saline and given intramuscularly (IM) in a volume of 0.5 cc/kg. The first three drugs listed above were given immediately before each session so that the onset of drug activity could be established. α-MT was given in multiple doses of 50 mg/kg. On the first day of drug administration both animals received 100 mg/kg of the drug in two separate 50 mg/kg injections 6 and 3 hr before the session. On the next day each animal received 150 mg/kg in three separate 50 mg/kg injections 22, 6 and 3 hr before the session.

Animals were run in the morning throughout the experimental period (seven days a week) with the exception of the last two (α-MT) days when both animals were run in the afternoon. This change was necessitated by the delay between administration and peak physiological effects of this particular drug. Weissman and Koe [9] have noted that α-MT depletes norepinephrine and dopa from whole rat brains with a peak action occurring about 5 to 7 hr after injection. Each session began with onset of the houselight and extension of the retractable lever into the chamber. Shock level at the beginning of the session always started at the first step (0 mA). At the end of 2 hr the bar retracted and the houselight was turned off along with the shock, thus terminating the session. Data from this study consisted

of the records made on the Varian recorder showing the various levels of current each animal was subjected to during each drug or saline session, and the total number of bar-press responses during each 2-hr experimental period.

RESULTS AND DISCUSSION

Figure 1 presents the mean number of responses and mean maximum current intensity step recorded under saline and the various drug dosages for the two animals. This figure presents a general summary of the data collected in Experiment 1. Morphine did not affect response rate to any degree but was able to raise the maximum current intensity step in dosages of 0.5, 3.0, and 5.0 mg/kg. Scopolamine hydrobromide reduced response rate and augmented the maximum current step recorded, but these effects were inversely related to the dose administered. Thus, the lower doses were more potent than the higher ones. *D*-amphetamine sulfate reduced response rate in all doses tested, while the maximum current intensity was augmented only after 3.0 mg/kg. Finally, α-MT in both doses reduced response rate and augmented the maximum current intensity step recorded.

Table 1 presents a more detailed summary of the response and threshold data. This table contains the mean number of responses per session and the mean maximum current intensity step encountered for both animals under all experimental drug and saline conditions in the order in which they were administered. In an attempt to indicate the variability encountered in these two measures, the standard error of the mean is also included in Table 1 for each of the entries.

A brief scan of the data summarized in Table 1 indicates that the drugs employed had a substantial effect on both response rate and the maximum current intensity step recorded. As is indicated in Table 1, morphine sulfate slightly reduced the total number of bar-press responses emitted per session while substantially augmenting the maximum current intensity step encountered by the two squirrel monkeys. The drug did seem to affect maximum current step in a dose dependent manner in that higher mean intensities were recorded under 3.0 and 5.0 mg/kg than under 0.5 mg/kg. No effect was recorded in reference to current intensity under the 1.0 mg/kg dosage. Since only one session was given under this dosage, it is possible that the injection for this day was administered in a relatively fatty portion of the animal's leg thus retarding the absorption of the drug into the general circulation. In any case, further replication, as outlined in Experiment 2, indicates that 1.0 mg/kg is able to raise the maximum current encountered under a titration schedule. No dose response relationship was noted with reference to response rate.

Figure 2 is a photograph of the Varian records taken for one animal (ABBY) during the first saline session of the experiment and while it was under the influence of morphine sulfate (5.0 mg/kg).

The first thing to note about this data is that the current level was normally maintained between Step 1 and 8 (i.e., 0–3.7 mA). Usually, the current would increase by three or four steps before the animal responded thus resetting the

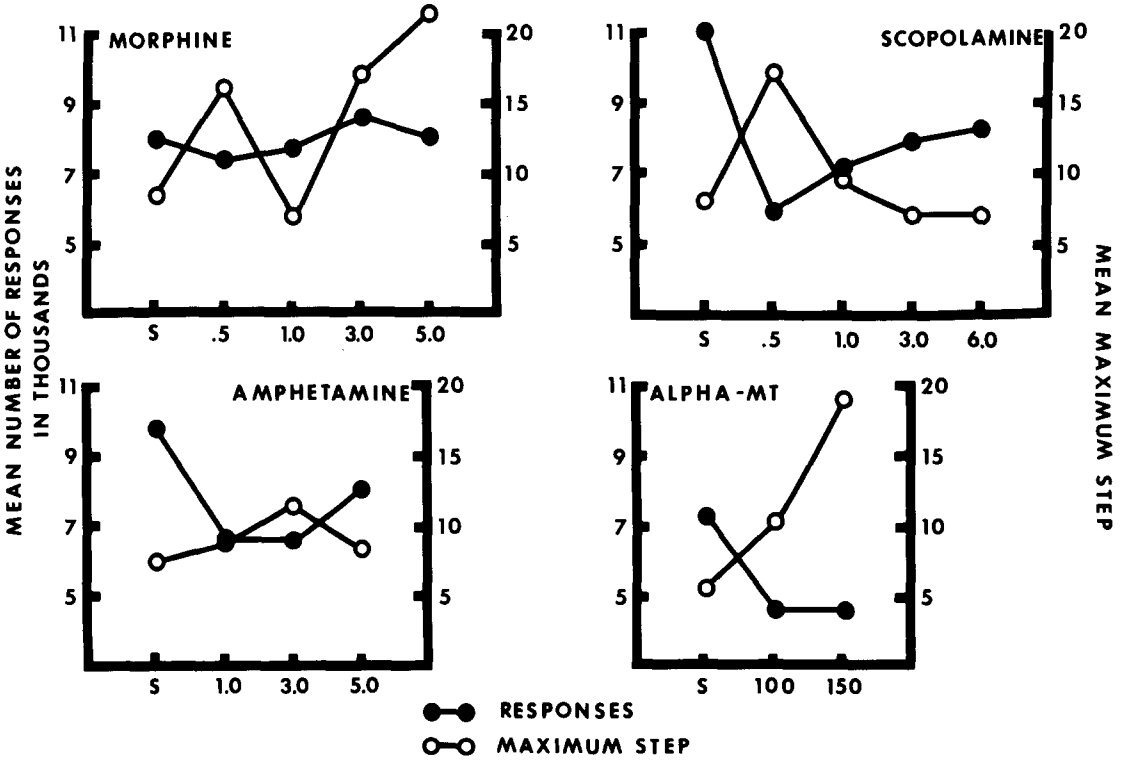

FIG. 1. Mean number of responses in thousands and mean maximum current intensity step measured under saline (S) and various dosages of morphine sulfate, scopolamine hydrobromide, *d*-amphetamine sulfate, and α-MT. All dosages are given in mg/kg and each point represents the mean of two animals. The current intensity steps represent increments of 0.5 mA and range from 0–12.2 mA (i.e., Steps 1–25).

TABLE 1

MEAN NUMBER OF RESPONSES AND MAXIMUM CURRENT INTENSITY STEP TOLERATED PER SESSION ALONG WITH THE CORRESPONDING STANDARD ERROR OF THE MEANS FOR EXPERIMENT 1

Condition	Number of Sessions	Mean Responses Per Session	S.E.	Mean Maximum Step	S.E.
Saline	2	8,010	±517	8.50	±1.55
Morphine 0.5 mg/kg	3	7,386	±1388	16.16	±3.69
Saline	2	11,147	±408	8.75	±1.10
Morphine 1.0 mg/kg	1	7,653	±1126	7.00	±0.00
Morphine 3.0 mg/kg	1	8,647	±4313	17.00	±8.00
Morphine 5.0 mg/kg	1	8,111	±3419	22.50	±2.50
Saline	1	11,081	±2355	8.00	±3.00
Scopolamine 0.5 mg/kg	1	5,961	±158	17.00	±2.00
Scopolamine 1.0 mg/kg	1	7,179	±383	9.50	±0.50
Saline	2	10,832	±537	7.00	±1.29
Scopolamine 3.0 mg/kg	1	7,816	±332	7.00	±1.00
Scopolamine 6.0 mg/kg	1	8,117	±599	7.00	±1.00
Saline	1	9,846	±777	7.50	±1.50
Amphetamine 1.0 mg/kg	1	6,746	±2247	9.00	±6.00
Amphetamine 3.0 mg/kg	1	6,768	±1578	11.50	±6.50
Amphetamine 5.0 mg/kg	1	8,059	±514	8.50	±1.50
Saline	3	7,387	±909	5.83	±0.40
α-MT 100 mg/kg	1	4,711	±109	10.50	±3.50
α-MT 150 mg/kg	1	4,810	±592	19.00	±6.00

current to 0. Under morphine sulfate (5.0 mg/kg), however, the current rose steadily until during the last half-hour it reached the highest possible level (12.2 mA) or Step 25, as seen in Fig. 2. It should be noted, however, that the animal was still making bar-press responses even when the current intensity was relatively high. Figure 2 indicates this by the fact that the animal did not allow the intensity to remain at Step 25 for any length of time before it responded to drive the intensity down. Table 1 also indicates that both animals showed only a slight reduction in the number of bar-press responses emitted under 5.0 mg/kg of morphine sulfate. Thus, it would appear that a reduction in the number of bar-press responses was not the primary cause of the increase in the aversive threshold noted under morphine. In order to keep the current in any given level the animal need make only one response every two seconds (i.e., 3600 responses per session). Thus, under morphine the animal was not sedated to the point where too few bar-press responses were being made. Furthermore, visual observation of the animals indicated that they were alert and relatively active under all doses of morphine sulfate. This suggests that the titration procedure may have been able to reflect the analgesic properties of this drug through mechanisms other than general sedation of the animal.

The scopolamine data showed what appeared to be clear dose response relationships with reference to both measures. As Table 1 indicates, the maximum current level step encountered was elevated above control values under the two lower doses (0.5, 1.0 mg/kg), but returned to control values under the higher dosages (3.0, 6.0 mg/kg). Response rate was similarly affected with the two lower doses severely reducing the number of responses emitted, while the higher doses reduced response rate to a lesser degree. Although one might be tempted to conclude from these data that scopolamine reduces the motivational properties of electric shock at low doses, while having equivocal results at doses above 3.0 mg/kg, it must be noted that these doses were given in an ascending order on consecutive days. Thus, the effects noted could be attributed to drug tolerance rather than to the effects of increasing dosage. The second experiment, to follow, was an attempt to determine whether drug tolerance or dose level was responsible for the above results.

The amphetamine data, summarized in Table 1, indicated that the two lower doses (1.0, 3.0 mg/kg) raised the maximum current intensity step while the highest dose (5.0 mg/kg) did not. The two lower doses also reduced the response rate to a greater degree than did the higher dosage. The standard error of the means, however, indicate that there was a considerable amount of variance noted in the two measures for this particular drug series. A careful analysis of the data for both animals indicated a differential pattern of results for each animal. One animal (ABBY) showed a gradual increase in the aversive threshold (i.e., maximum current step encountered) under both 1.0 and 3.0 mg/kg of *d*-amphetamine, as the 2-hr experimental session progressed. Figure 3 is a photograph of the Varian record showing onset of the effects of 1.0 mg/kg of *d*-

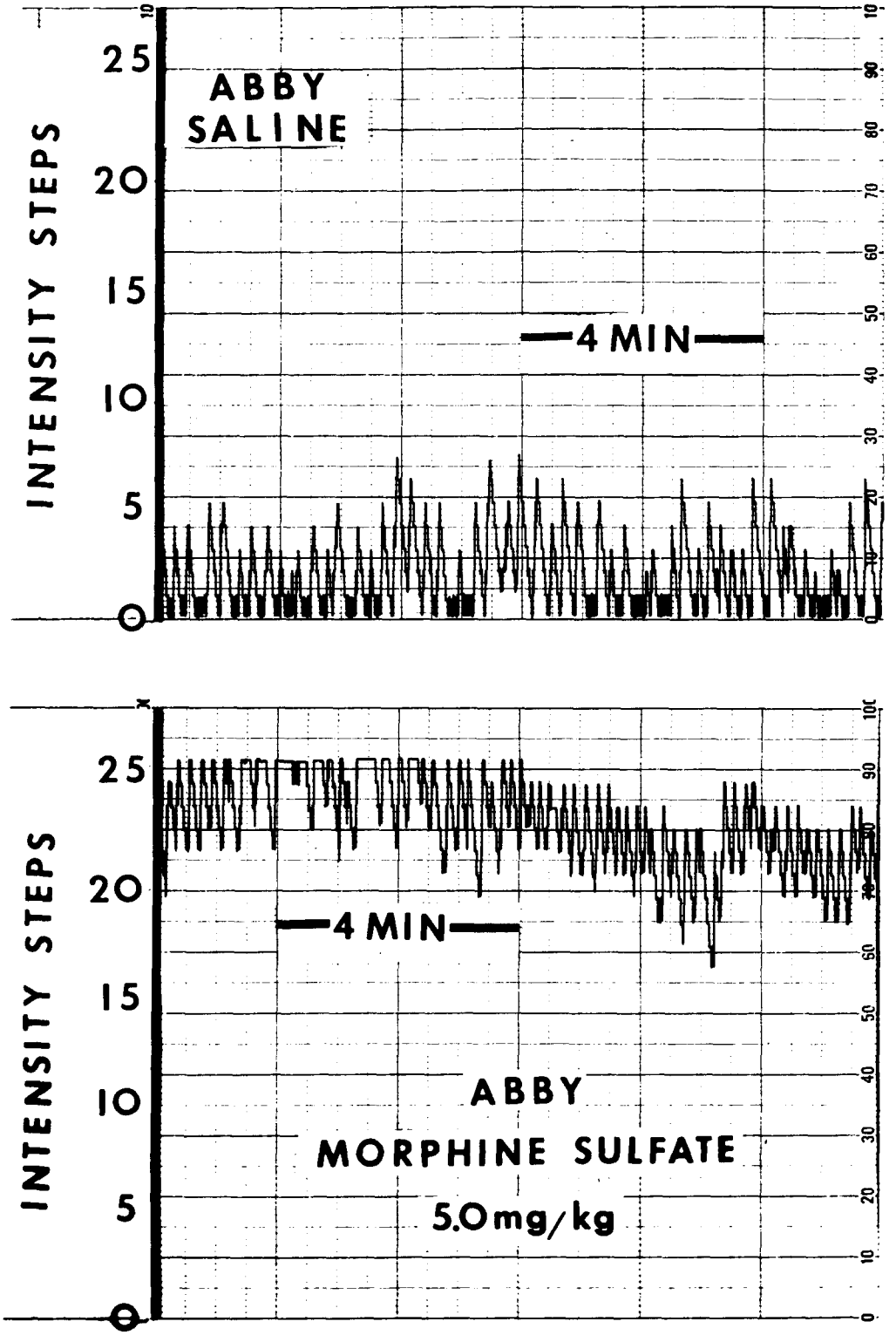

FIG. 2. Varian records comparing the effects of saline and 0.5 mg/kg of morphine sulfate on the aversive threshold 1-1/2 hr after drug administration for one animal, ABBY. The records should be read from right (earliest time) to left. Both records represent data collected during the last half-hour of these particular 2-hr sessions.

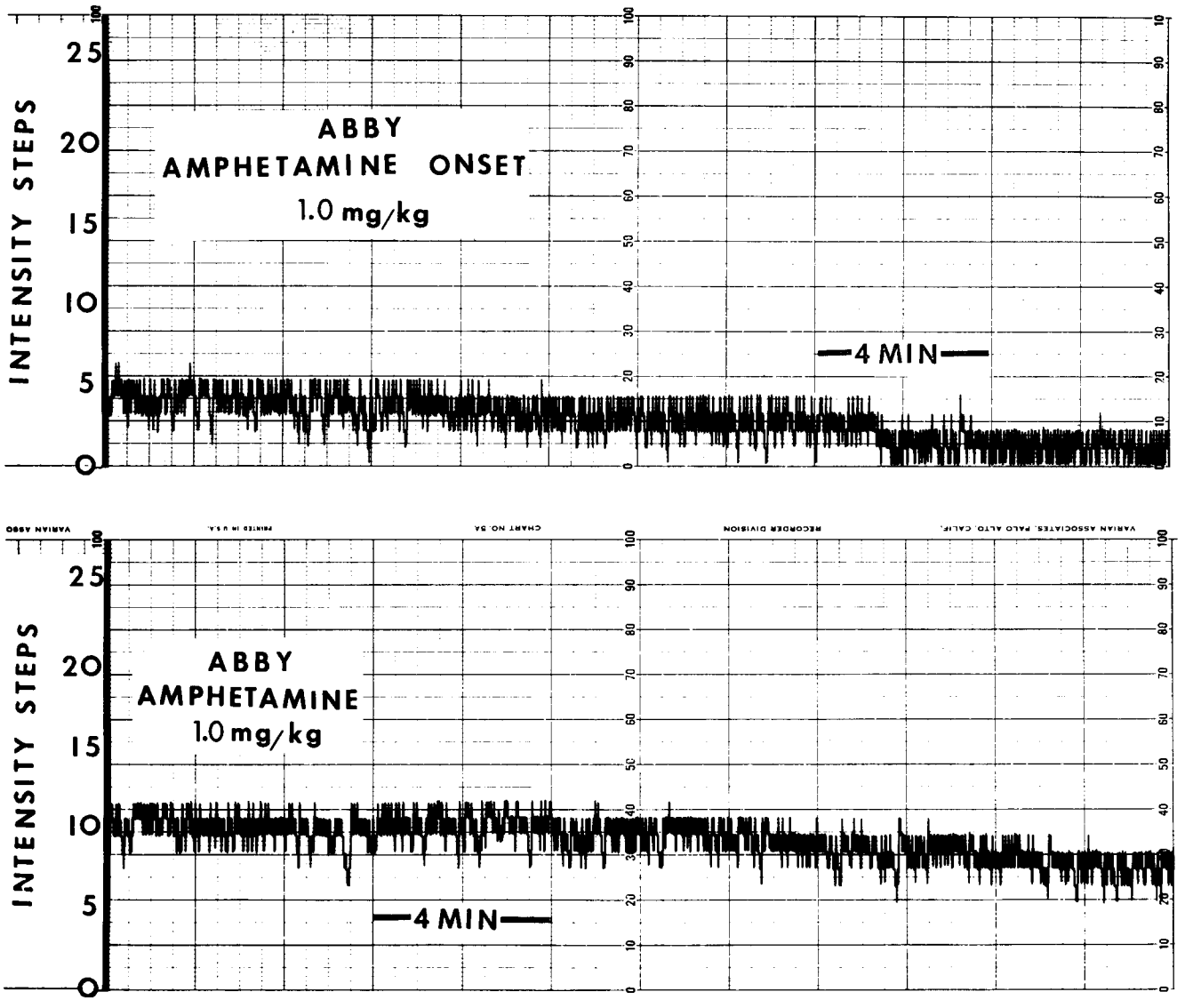

FIG. 3. Varian records comparing the effects of amphetamine onset and peak drug activity on the aversive threshold for one animal, ABBY. The top portion of this figure represents performance immediately after injection of 1.0 mg/kg of *d*-amphetamine sulfate, while the lower portion of the figure represents performance one hour after injection of the drug. Records should be read from right (earliest time) to left.

amphetamine sulfate, and performance one hour after administration of the drug for ABBY.

This figure indicates that amphetamine raised the aversive threshold in this animal one hour after administration. A comparison between the saline record in Fig. 2 and the data in the lower portion of Fig. 3 confirms this effect. Furthermore, amphetamine reduced the variability in the aversive threshold normally seen under saline control conditions. The second animal, however, did not show an increase in the maximum current intensity step encountered under any of the dosages of amphetamine. In contrast to the first animal, JERRY maintained a rapid response rate that kept the current intensity at very low levels for the entire 2-hr drug sessions. Visual observation of this animal indicated that all the dosages of amphetamine employed produced stereotyped behavior patterns that led to rapid depression of the response lever. Normally this animal would depress the response lever only with its hands. Under amphetamine, however, this animal demonstrated stereotyped rocking motions which led it to depress the lever with its head or other portions of its body. On several occasions while this animal was under the influence of amphetamine the shock was turned off. Under these no-shock conditions JERRY continued to respond by pressing the lever at a high rate. When the shock was turned off under saline conditions, however, this animal would quickly cease its lever-pressing response. Thus, amphetamine caused this animal to respond irrespective of the shock stimulus. The first animal, ABBY, also showed stereotyped behavior patterns along with a reduction in the aversive threshold to control levels under the highest dose (5.0 mg/kg) of *d*-amphetamine.

The α-MT data indicated that this drug in both dosages (100, 150 mg/kg) severely reduced response rate while substantially augmenting the maximum current encountered by both animals. Visual observation of both experimental animals indicated that although they experienced high current intensities (i.e., 9–12.2 mA) they seemed relatively calm.

The above data strongly suggest that alterations in adrenergic and cholinergic tone did alter behavior under the control of a titration schedule. Several problems arise, however, when one attempts to interpret these results. As was pointed out above, since a particular dose was given on only one occasion no measure of reliability is available. Furthermore, all the drug treatments were given in an ascending series on consecutive days, thus confounding the effects of drug tolerance and drug dosage. Finally, one other methodological point became apparent from this first exploratory study. The graded changes in current intensity were fairly large (0.5 mA) and the possible number of intensities too few (i.e., 25) to allow this procedure to reflect small changes in the aversive threshold produced by the various agents. The second experiment attempted to address these shortcomings so that a more definitive statement could be made about the effects of changes in adrenergic and cholinergic tone upon behavior under the control of a titration schedule.

EXPERIMENT 2

METHOD

Animals

Three naive male squirrel monkeys (*Saimiri sciureus*) weighing between 736–790 g served in this experiment. All animals had free access to Purina monkey chow and water throughout the experimental period while they were housed in their home cages.

Apparatus

The testing apparatus consisted of a Plexiglas restraining chair, fabricated locally, which was similar to the unit described in the first experiment. The only differences noted in the two chairs was that the brass electrodes in the unit used in Experiment 2 were 1-1/2 in. apart rather than only 5/16 in. apart as in Experiment 1. This increased separation of the two brass rods insured that the electrode paste applied between the rods and the shaven portion of the animal's tail would not accidentally cause a short between the two electrodes. Furthermore, the chair in Experiment 2 had a fixed nonretractable response lever manufactured by Lehigh Valley Electronics (Model 121-05). This lever required a downward pressure of 10 g to close the microswitch and it was mounted directly in front of the animal as described in Experiment 1. The chair was mounted in a sound attenuated wooden chamber. White noise (85 db) was presented throughout the experimental period to mask any extraneous laboratory sounds via a speaker mounted inside the chamber. Shock was supplied by a constant current stimulator which placed a minimum of 50 k in series with the animal's tail. The animal's tail resistance was monitored before each session to insure that the resistance between the two electrodes was approximately 3200 Ω (±200 Ω) after the electrode paste had been applied. As in the first experiment, tail resistance was altered only slightly throughout the session due to the paste drying out. A recording attenuator (Grason Stadler Model E7110A) was linked in series with the stimulator to provide the animal with 52 discrete electric stimuli. The range in intensity was 0–15.3 mA in 0.3 mA steps. The recording attenuator was equipped with an up-down recording pen which allowed one to make permanent ink records of the current intensity encountered during all portions of each 2-hr session. Finally, standard electromechanical scheduling and recording equipment was located in an adjacent room.

Procedure

Animals were initially trained to press the response lever to escape shock using the same procedure as outlined above in Experiment 1. The titration schedule was similar to that noted above in that shock intensity was automatically programmed to increase every 2 sec while each bar-press response decreased the current intensity by one discrete step. The only difference in Experiment 2 was that the steps were more numerous (i.e., 52 vs. 25) and each step represented an increase of only 0.3 mA instead of 0.5 mA. Furthermore, the maximum possible current available to the animals was higher in Experiment 2 (15.3 mA) than in Experiment 1 (12.2 mA). The experimental sessions were two hr in duration and were begun by closure of the experimental chamber door and onset of the 28 V houselight. Termination of the 2-hr period was signaled by offset of the houselight and tail shock and removal of the animal to its home cage.

Drug series. Unlike the procedure in Experiment 1, all dosages of each drug were given in three consecutive daily sessions followed by at least one saline session. This procedure was adopted in order to reveal the reliability of behavior under any given dose of a particular agent. Secondly, drug dosages were either given in a random order or given in ascending series with the dosages replicated to see if drug tolerance could account for some of the effects noted in the first experiment. In all cases, return to baseline performance was insured before a new dose of drug was administered. Thus, some attempt was made to insure that the effects of a previous dosage would not carry over to the next dosage series. All drugs were dissolved in physiological saline (0.9%) and given IM in a volume of 0.5 cc/kg. Identical drugs to those administered in Experiment 1 were given in Experiment 2. An attempt, however, was made to give a wider range of dosages so that more information concerning the dose–response relationships involved could be ascertained. Furthermore, a cholinomimetic, pilocarpine nitrate, was included to give some indication as to the role of cholinergic systems in modulating behavior under the control of a titration schedule. Data for this study consisted of the records made on the recording attenuator showing the various levels of current each animal was subjected to during each drug or saline session. The total number of bar-press responses during each session were also recorded.

RESULTS AND DISCUSSION

Figure 4 presents the mean number of responses and mean maximum current intensity step measured under saline and the various drug dosages for all three animals. This figure presents a general summary of the data collected in Experiment 2. Scopolamine hydrobromide reduced mean response rate and augmented the mean maximum current step recorded upon initial administration of the drug. After repeated exposure to scopolamine, however, animals

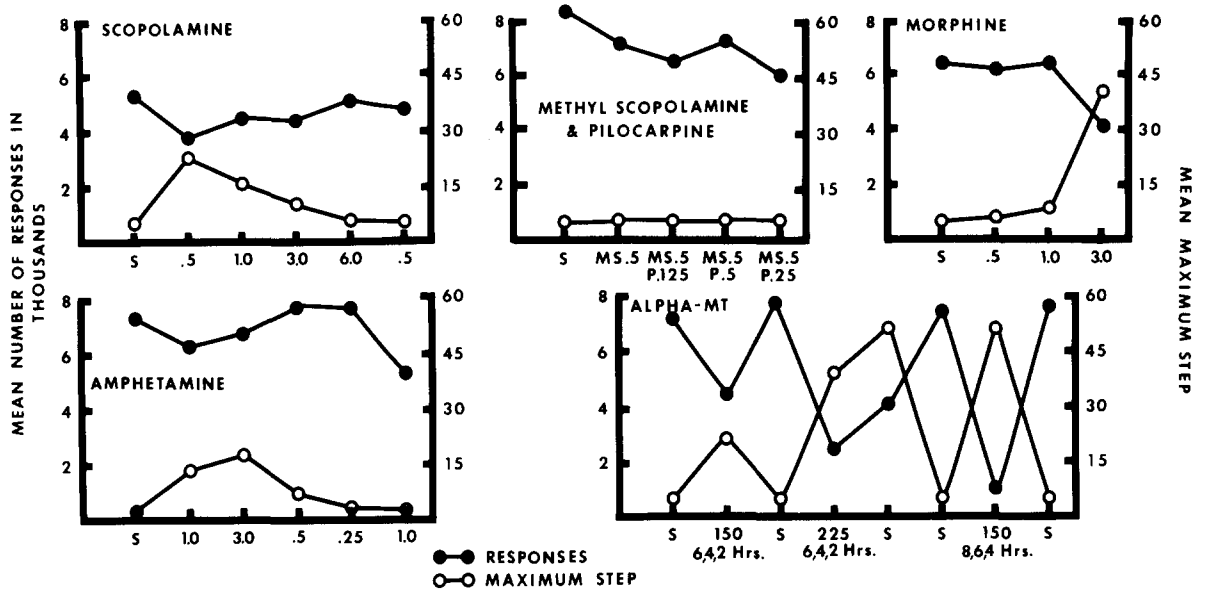

FIG. 4. Mean number of responses in thousands and mean maximum current intensity step measured under saline (S) and various dosages of scopolamine hydrobromide, scopolamine methylbromide given alone or in conjunction with pilocarpine nitrate, morphine sulfate, *d*-amphetamine sulfate or α-MT. All dosages are given in mg/kg. Each current intensity step represents an increment of 0.3 mA. There were 52 current steps available with a range of 0–15.3 mA. Each data point represents the mean of three animals.

demonstrated considerable drug tolerance. Thus, the second exposure to 0.5 mg/kg of scopolamine produced no increase in the mean maximum current step recorded, even though previous exposure had raised the maximum step to a level of 23.22 (approximately 6.7 mA). Scopolamine methylbromide, a peripheral acting anticholinergic, had no effect on the maximum current step recorded when it was administered alone (0.5 mg/kg) or in conjunction with pilocarpine nitrate in several doses. Lever-pressing rate, however, was reduced somewhat in response to these two agents. Morphine sulfate reliably increased the maximum current step recorded in a dose-dependent manner. Under the highest dose (3.0 mg/kg) tested animals received a maximum current intensity of 12.0 mA (i.e., Step 41). Response rate was reduced under morphine sulfate, especially under the 3.0 mg/kg dosage. *d*-Amphetamine sulfate was similar to scopolamine hydrobromide in that it produced increments in the mean maximum current intensity upon initial exposure to the drug. After repeated administration of amphetamine, however, animals demonstrated drug tolerance, and thus the current intensity measure showed no effect when 1.0 mg/kg was tested for a second time. α-Methyl-p-tyrosine (α-MT) showed consistent dose and time related effects when tested in the titration procedure. Both dosages reliably reduced response rate and augmented the mean maximum current step. Injecting animals 8, 6 and 4 hr before testing produced greater effects than when the injections were given 2 hr earlier (i.e., 6, 4, 2 hr). Finally, substantial carry-over effects were noted during the first saline day following the 225 mg/kg dosage. Although response rate was augmented somewhat during this session, the maximum current intensity continued to rise to the highest possible step (i.e., 52), thus subjecting all three animals to 15.3 mA of electric shock.

Table 2 presents a more detailed summary of the response and threshold data. This table contains the mean number of responses per session and the mean maximum current intensity step encountered for both animals under all experimental drug and saline conditions. In order to indicate the variability encountered in these two measures, the standard error of the mean is also included in Table 2 for each of the measures.

Since there were at least three replications of each dose for each of the three animals, much more data were available on the reliability of the various measures. With several replications the data were amenable to statistical analysis. The results of comparisons between saline control and the various drug manipulations using the nonparametric sign test (two-tailed) are presented in Table 2. Since control values tended to change somewhat during the course of the experiment (i.e., response rates increased with a consequent decrease in the maximum current step encountered), two different series of saline sessions are used as controls for the various statistical comparisons. The first three days of the experiment (saline) are used as controls for all the statistical comparisons made with reference to scopolamine hydrobromide. The three saline days preceding and following the scopolamine methylbromide drug series serve as the controls for all statistical comparisons made concerning the remaining drugs. In all cases the control sessions are clearly marked in Table 2.

As the data in Table 2 indicate, scopolamine hydrobromide raised the aversive threshold while substantially reducing mean response rate under the three lower doses (0.5, 1.0, 3.0 mg/kg). It is interesting to note, however, that these effects were attenuated as the dose of scopolamine was increased until no significant effects were noted at 6.0 mg/kg. As was mentioned earlier, this could have been due to drug tolerance as well as to the increments

TABLE 2

MEAN NUMBER OF RESPONSES AND MAXIMUM CURRENT INTENSITY STEP TOLERATED PER SESSION ALONG WITH THE CORRESPONDING STANDARD ERROR OF THE MEANS FOR EXPERIMENT 2

Condition	Number of Sessions	Mean Responses Per Session	S.E.	Mean Maximum Step	S.E.
Saline (Control)	3	5,353	±158	5.33	± 0.52
Scopolamine 0.5 mg/kg	3	3,826*	±561	23.22†	± 6.00
Saline	1	5,121	±1059	5.66	± 1.20
Scopolamine 1.0 mg/kg	3	4,503*	±326	16.00*	± 5.08
Saline	3	6,469*	±498	5.55	± 0.37
Scopolamine 3.0 mg/kg	3	4,416*	±301	11.44*	± 1.80
Saline	1	5,881	±406	5.00	± 0.57
Scopolamine 6.0 mg/kg	3	5,175	±386	6.77	± 0.61
Saline	1	5,599	±377	5.33	± 1.33
Scopolamine 0.5 mg/kg	3	4,855	±115	6.55	± 0.52
Saline (Control)	1	8,346	±875	3.00	± 0.00
Methyl Scopolamine 0.5 mg/kg	3	7,185	±204	3.22	± 0.22
Saline (Control)	2	7,764	±374	3.00	± 0.00
Methyl Scop. 0.5 mg/kg & Pilocarpine 0.125 mg/kg	3	6,448†	±464	3.22	± 0.14
Saline	1	7,239	±1101	3.00	± 0.00
Methyl Scop. 0.5 mg/kg & Pilocarpine 0.5 mg/kg	3	7,126	±631	3.22	± 0.14
Saline	2	6,819	±707	3.33	± 0.21
Methyl Scop. 0.5 mg/kg & Pilocarpine 0.25 mg/kg	1	5,889	±651	3.00	± 0.00
Saline	3	6,322*	±377	3.11	± 0.20
Morphine 0.5 mg/kg	3	6,180†	±217	6.33†	± 0.44
Saline	1	7,255	±330	3.33	± 0.33
Morphine 1.0 mg/kg	3	6,370*	±343	8.77†	± 0.46
Saline	1	7,618	±530	3.33	± 0.33
Morphine 3.0 mg/kg	3	4,174†	±334	40.88†	± 5.76
Saline	1	7,365	±673	2.66	± 0.33
Amphetamine 1.0 mg/kg	3	6,364	±719	13.44	± 5.72
Saline	1	5,974	±110	3.00	± 0.00
Amphetamine 3.0 mg/kg	3	6,764	±1058	17.33*	± 6.31
Saline	2	6,799	±847	3.83	± 0.74
Amphetamine 0.5 mg/kg	3	7,778	±830	7.33	± 2.95
Saline	4	7,749	±701	2.91	± 0.33
Amphetamine 0.25 mg/kg	3	7,751	±325	3.88	± 0.51
Saline	2	7,391	±890	3.50	± 0.67
α-MT 150 mg/kg 6, 4, 2 hr	1	4,474	±1637	21.00	±15.50
Saline	1	7,720	±1857	3.00	± 0.57
α-MT 225 mg/kg 6, 4, 2 hr	2	2,415*	±1093	38.50*	± 8.58
Saline	1	4,090	±1230	52.00	± 0.00
Saline	2	7,466	±1001	3.50	± 0.56
α-MT 150 mg/kg 8, 6, 4 hr	2	1,005*	±528	52.00*	± 0.00
Saline	2	7,622	±870	3.00	± 0.44
Pilocarpine 0.25 mg/kg	1	5,757	±430	3.00	± 0.00
Saline	1	8,922	±1511	2.66	± 0.49
Amphetamine 1.0 mg/kg	1	5,349	±1197	3.00	± 1.00

*Significantly different from the saline control sessions at $p<0.05$. All comparisons made using a two-tailed sign test. †$p<0.01$

in dosage. In order to address this problem, the 0.5 mg/kg dosage series was replicated a second time and no significant changes were recorded in either the response rate or the aversive threshold. Furthermore, a careful analysis of the threshold data revealed that a drug tolerance effect was apparent within the initial drug sessions. All three animals showed a progressive decline in the maximum current tolerated and a steady increase in response rate within each 3-day dosage series. As Table 2 indicates, each experience with a subsequent dosage led to less of an effect than the previous dosage had produced. These data indicate that scopolamine hydrobromide exerts an effect on behavior under the control of a titration schedule only during the initial period of drug administration. After sufficient experience with the drug (i.e., in the present data nine sessions) the animal will show no change in behavior under a titration schedule as far as response rate and maximum current step are concerned. Thus, drug tolerance would seem to account for the reduction in the aversive threshold noted in the first experiment under the higher dosages of scopolamine hydrobromide.

The 3-day series in which scopolamine methylbromide was administered indicated that this anticholinergic, which exerts its primary influence on the peripheral nervous system [3], had no significant effect on the two measures employed in this study. Since methyl scopolamine (0.5 mg/kg) did not exert any effects on behavior, it was administered as a pretreatment in the pilocarpine nitrate drug series. This was done to protect the animals from the toxic side effects of this cholinomimetic agent. Previous experience [4] has indicated that pilocarpine nitrate can lead to vomiting, diarrhea, and excessive salivation which can be abolished by pretreatment with a peripheral acting anticholinergic. As Table 2 indicates, pilocarpine nitrate has no significant effect on either response rate or the aversive threshold. The only exception to this was during the initial 3-day drug period when pilocarpine reduced response rate significantly without affecting the threshold. In order to insure that the lack of effects were not due to an interaction between pilocarpine and methyl scopolamine, pilocarpine (0.125 mg/kg) was administered alone for one session at the end of the experimental period. Again no effect was noted with respect to the aversive threshold, while response rate was somewhat reduced below control values. It would appear that cholinergic stimulation via pilocarpine administration in the range of doses employed has no effect on the aversive threshold measured by means of a titration schedule.

Morphine sulfate in the doses administered (0.5, 1.0, 3.0 mg/kg) demonstrated a dose–response relationship with reference to the two measures employed in the present study. Response rate showed significant decrements, while the maximum current step recorded was augmented as the dosage of morphine was increased. These data agree with the results of Experiment 1 and indicate that these drug effects did not diminish over time. A careful analysis of each 3-day series indicated no evidence of drug tolerance. Thus, it would appear that a drug with known analgesic properties, such as morphine sulfate, augments the aversive threshold as defined by an increase in the maximum current tolerated, and substantially reduces the response rate in a dose-dependent manner.

The results summarized in Table 2 with reference to the various dosages of amphetamine indicate a similar pattern to that noted for scopolamine hydrobromide. Initial experience with the drug led to a reduction in response rate and a concomitant increase in the aversive threshold. After repeated drug sessions, however, these effects were reduced. Furthermore, as can be seen in Table 2, although the initial drug series (1.0 mg/kg) substantially decreased the mean response rate while augmenting the maximum current step encountered, none of these differences were statistically significant. This was simply because the data both between and within the animals were very erratic. In order to determine whether drug tolerance could account for the reduction in the behavioral effects of amphetamine over time, the initial dosage (1.0 mg/kg) was replicated at the end of the experimental period. As Table 2 indicates, this replication produced a substantial reduction in the mean response rate without any increase in the aversive threshold. This failure to replicate an increase in the aversive threshold with the same dosage (i.e., 1.0 mg/kg) that had previously been effective indicated that drug tolerance rather than a change in dosage could account for the behavioral effects of amphetamine.

Visual observation of all three animals confirmed that, as in the first experiment, amphetamine produced stereotyped rocking movements that led the animals to depress the response lever with various portions of their bodies, rather than with their hands, as was the usual custom under control conditions. This stereotyped behavior along with increased motor activity was apparent at all dosages. Furthermore, in the cases where the aversive threshold was not elevated, the animals appeared to be responding without reference to the shock intensity presented. This was evidenced by the fact that turning the shock off while the animals were performing under the influence of amphetamine (3.0 mg/kg) did not influence their behavior. Under control conditions turning the shock off led to a cessation of responding, thus allowing the ink recorder to be elevated to the highest step. Under amphetamine (3.0 mg/kg), however, animals would continue to respond at a rapid rate under no-shock conditions, keeping the recording attenuator in the first two steps. Thus, amphetamine was able to remove behavior from the control of the shock stimulus. To summarize it would appear that amphetamine had several effects upon behavior under the control of a titration schedule. First, amphetamine was able to increase the aversive threshold during the first few sessions that the drug was administered. This initial increment in the aversive threshold was not reliable between or within the animals, however, and all animals rapidly developed drug tolerance. Any reliable increments in threshold appeared to be masked by a substantial increase in motor activity and the appearance of stereotyped rocking behavior which led the animals to emit high response rates even when no shock was presented. Any attempt in the future to monitor changes in the aversive threshold in response to amphetamine administration must require a behavioral response that will not be substantially affected by increments in motor activity or the existence of stereotyped behavior. This might be accomplished by placing the response lever in the restraint chair some distance from the animal so that it could be reached only by the outstretched arms of the animal. This would reduce the possibility that random stereotyped movements would lead to a lever-press response.

The administration of α-MT in both dosages (150, 225 mg/kg) produced effects as seen in Table 2, which included reductions in response rate with significant increments in the aversive threshold. The drug was given in three injec-

tions spaced at 2-hr intervals to avoid the toxic effects of single large doses [6]. α-MT appears to affect the aversive threshold in a dramatic manner, especially when given 8, 6, and 4 hr before testing. Figure 5 presents ink records made by the recording attenuator for one animal, AL, under saline and 150 mg/kg of α-MT administered in three 50 mg/kg injections 8, 6, and 4 hr before testing. As these records indicate, the aversive threshold was quickly elevated to the highest level within minutes after the initiation of a drug session. The animal reacted as if the shock were turned off even though 15.3 mA was being delivered to its tail. Furthermore, no signs of drug tolerance were noted across the five drug sessions in any of the animals tested. As a matter of fact, the behavioral effects noted appeared to intensify during the latter drug sessions.

It should be noted that these drug effects demonstrated dramatic carry-over effects during the saline session that followed the highest dosage of α-MT. As Table 2 and Fig. 4 indicate, the first saline day after the 225 mg/kg drug series, produced behavioral results that were similar to those noted during drug sessions. The aversive threshold was at its maximum while the response rate was substantially reduced. It would appear that this agent can produce behavioral effects even 24 hr after injection.

GENERAL DISCUSSION

Throughout the present report the term aversive threshold has been employed rather than pain threshold. A change in the pain threshold following the administration of a drug would imply that the chemical agent interfered with a sensory system in some manner. Pain, however, consists not only of a sensory component but also an affective one. For example, with therapeutic doses of morphine the perception of a painful stimulus itself is not always decreased, even in patients who obtain satisfactory pain relief (i.e., analgesia). There is instead an altered motivational reaction to the painful stimulus; patients frequently report that the pain is still present, but that they feel more comfortable [3]. In the present context the aversive threshold can conceivably be altered by modulation of sensory or motivational processes by the various chemical agents. Using the titration technique one is not able to discern which of these processes is being affected by a particular analgesic agent. An animal that allows the current intensity to rise may be doing so because he cannot feel pain or because pain no longer has its usual motivational properties that would lead him to respond to the noxious stimulus. Finally, other effects (e.g., increase in general activity,

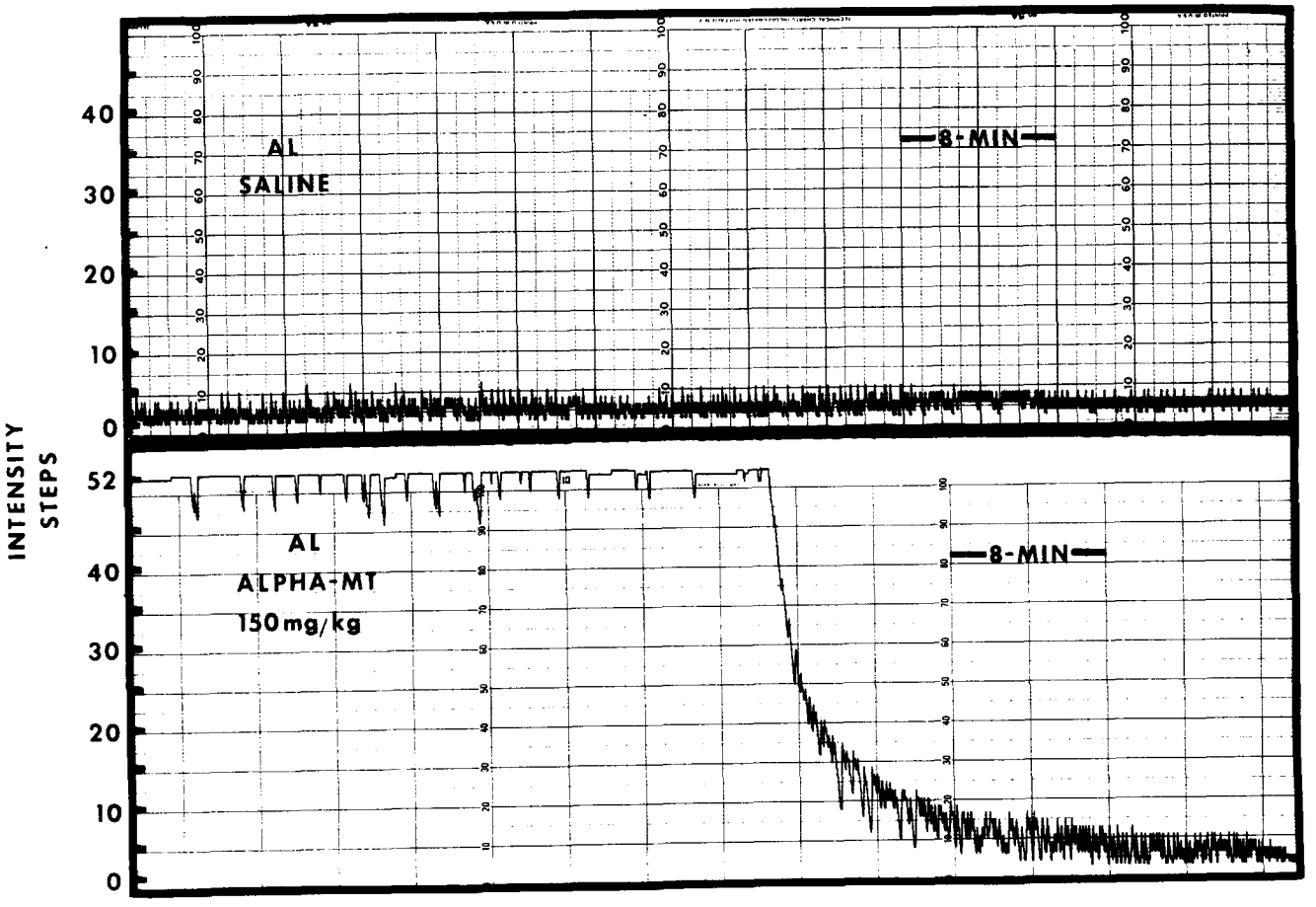

FIG. 5. Ink records made on the recording attenuator representing performance of one animal, AL, under saline and 150 mg/kg of α-MT administered in three separate 50 mg/kg injections 8, 6, and 4 hr before testing. Each record represents performance during the first hour of the 2-hr experimental sessions. Records should be read from right (earliest time) to left.

nausea, diarrhea, sedation, ataxia, etc.) produced by the various drugs could possibly affect an animal's ability to respond under the titration schedule, and thus alter the aversive threshold.

Morphine sulfate has a long history of use as an analgesic agent [3] and thus it is not surprising that it significantly raised the aversive threshold in a dose-dependent manner. This finding was in agreement with other reports [10,11] that used the titration technique in monkeys with surgical implants in the gasserian ganglion. Thus, it would appear that a true analgesic agent such as morphine sulfate produces a response profile which includes a reduction in response rate with a consequent increase in the aversive threshold. The only agent tested in the present set of experiments that consistently produced similar results was α-MT. This may suggest that the depletion of the catecholamines produced by α-MT [6] may be involved in mediating analgesia. Corrodi and Hanson [2] have shown that the soluble methylester hydrochloride of *dl*-α-MT decreases the catecholamine levels of whole brain in rats to their lowest levels between 16 and 20 hr after administration. Complete recovery from one dose of 250 mg/kg is noted in 36–48 hr [2]. Rech, Borys and Moore [6] have noted that multiple doses of α-MT (50 mg/kg) made every 4 hr for three doses decreased levels of norepinephrine and dopamine in the telencephalon and brain stem to their lowest levels 12 hr after the first injection and did not return to normal until 24–48 hr after the first injection. The fact that considerable time is required before norepinephrine and dopamine levels recover from high doses of α-MT may suggest why in Experiment 2 the aversive threshold remained elevated 24 hr after administration of the drug. It may be that adrenergic systems must completely recover from the depleting effects of α-MT before the organism can fully appreciate the aversive qualities of electric shock.

The above conclusion, however, rests on the assumption that the titration procedure reflects primarily the analgesic properties of drugs. Some investigators [1], however, have suggested that the titration procedure is complicated by discriminated avoidance behavior with lower current levels acting as warning stimuli for the higher currents. This may or may not be the case, but the fact remains that the titration procedure used in the present study has been able to detect changes in the aversive threshold for several different pharmacological agents (i.e., morphine sulfate [11] and the salicylates [8]) that are known to be analgesic in man. Boren and Malis [1] have suggested that a variation of the titration technique (where responding on a fixed ratio schedule terminated the stimuli and reset it to zero) was more likely to be controlled by the immediate consequence of escaping the stimulus rather than avoiding future stimulation [1]. Malis [5] has compared this variation (FR reset to zero) and the continuous reinforcement (CRF) procedure used in the present study to investigate the effects of analgesic agents. He concluded that the CRF technique is more sensitive because in addition to measuring peak current tolerated, it also measured the minimum current, relief level, that the animal escaped to. This author [5] noted that analgesic agents could produce changes in the relief level at doses which did not affect peak current tolerated.

In conclusion, although the titration technique is undoubtedly influenced by the various properties of chemical agents, it does seem to reflect changes in the aversive threshold that can be described under the general term analgesia. Since other effects may also influence behavior under a titration schedule, validation of the analgesic effects of a drug should be obtained by other tests (i.e., tail–flick, hot–plate, clinical data, etc.) before any firm conclusions are drawn. With reference to the present data, however, it seems reasonable to suggest that depletion of the catecholamines by the administration of α-MT may reduce the ability of an organism to fully appreciate the aversive qualities of electric shock. Thus, adrenergic mechanisms may be involved in mediating the sensory or motivational components of pain.

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