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Titration Procedure with Rats Using a Nose Poke Response and Tail Shock

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BURNE, T. A. AND H. A. TILSON. *Titration procedure with rats using a nose poke response and tail shock.* PHARMAC. BIOCHEM. BEHAV. 13(5) 653–656, 1980.—An operant titration procedure that provides relatively stable within-session shock thresholds (0.15–0.25 mA) within a week of training is described. Rats are placed into Plexiglas restraint tubes having a hole at one end through which the animal can poke its nose to break a photobeam; when the photobeam is broken, a response is counted. The tail of the animal is firmly held by a Plexiglas plug and an electrode connected to a programmable titration shocker is attached to the tail. Conditioned nose poke responses are made after about 15 min during the first 30 min session. The titration procedure was found to be sensitive to the analgesic effect of morphine. Significant increases in the median shock level tolerated was observed after 3 mg/kg, while response rate was not altered. Dose-related increases in threshold after 6 and 9 mg/kg of morphine were associated with decreases in the rate of nose poking. The technique offers several advantages in the study of chemical induced alterations in pain or reactivity, including rapidity of initial training, relatively short time to establish median shock thresholds, minimum involvement of motor components in the response, and sensitivity to a psychopharmacological tool.

Operant titration technique Nose poke response Tail shock

NUMEROUS techniques have been developed in recent years by behavioral pharmacologists and toxicologists to assess the responsiveness of laboratory animals to painful or aversive stimuli. Many of the more sensitive tests allow for the estimation of a shock threshold and are based on the titration principle derived from psychophysics [13]. In the titration paradigm, electric shock is applied continuously to the animal by electrodes. The intensity of the shock gradually increases with time unless the animal makes the appropriate response; each response decreases the shock intensity by a constant amount.

Most titration procedures involving rats utilize electric shock applied to the feet by grids on the floor of the test chamber. Electric foot shock has been used in a variety of operant procedures including a free operant lever press [14] and wheel turn [10], discrete trial go/no-go lever press [9], and a conditioned suppression lever press [6]. Other titration techniques not involving schedule-controlled responses include spatial preference [2,8], conditioned pole climb [12] and shuttle box [4] paradigms.

The use of electric foot shock in titration studies has received considerable criticism because it is difficult to control the current actually delivered to the animal [1,3]. To control for this problem, Azrin *et al.* [1] confined the animal to a small triangular space, fastened electrodes to the tail and required the animals to press a lever to decrease the shock. Dallemagne and Richelle [3] placed their rats in a Plexiglas cylinder, attached electrodes to the tail, and arranged for the animal to press a panel positioned in front of the animal's nose. Our laboratory has recently developed a shock titration procedure similar to that of Dallemagne and Richelle [3] except that the animal is required to poke its nose through a small aperture in order to break a photobeam. The purpose of this paper is to describe our nose-poke titration technique and validate its use by assessing the effects of a pharmacological agent known to affect reactivity of rats to electric shock. Since the intershock interval (ISI) can be an important determinant in the type of pharmacological effect observed in titration experiments [14], two different ISIs were used in the present investigation.

METHOD

Subjects

Twenty-eight male, albino rats of the Fischer 344 strain (Charles River Breeding Co., Wilmington, MA) weighing approximately 300 g served as subjects. The rats were housed in plastic cages in groups of four and permanently identified by an ear punch code. Food (NIH no. 31) and water were constantly available in the home cages. The rats were housed in a room having a constant 12/12 light-dark cycle with the lights on from 7 a.m. to 7 p.m. Temperature was maintained at 21±2°C and relative humidity at 50±10%.

Apparatus

The subjects were tested in eight identical Plexiglas tubes, each individually enclosed in a sound attenuating shell equipped with a ventilation fan. Electric shock was supplied by programmable shockers (Coulbourn Instruments, Model E-13-33) and presettable up/down counter assemblies (Model 541-28). Schedule control and data collections were accom-

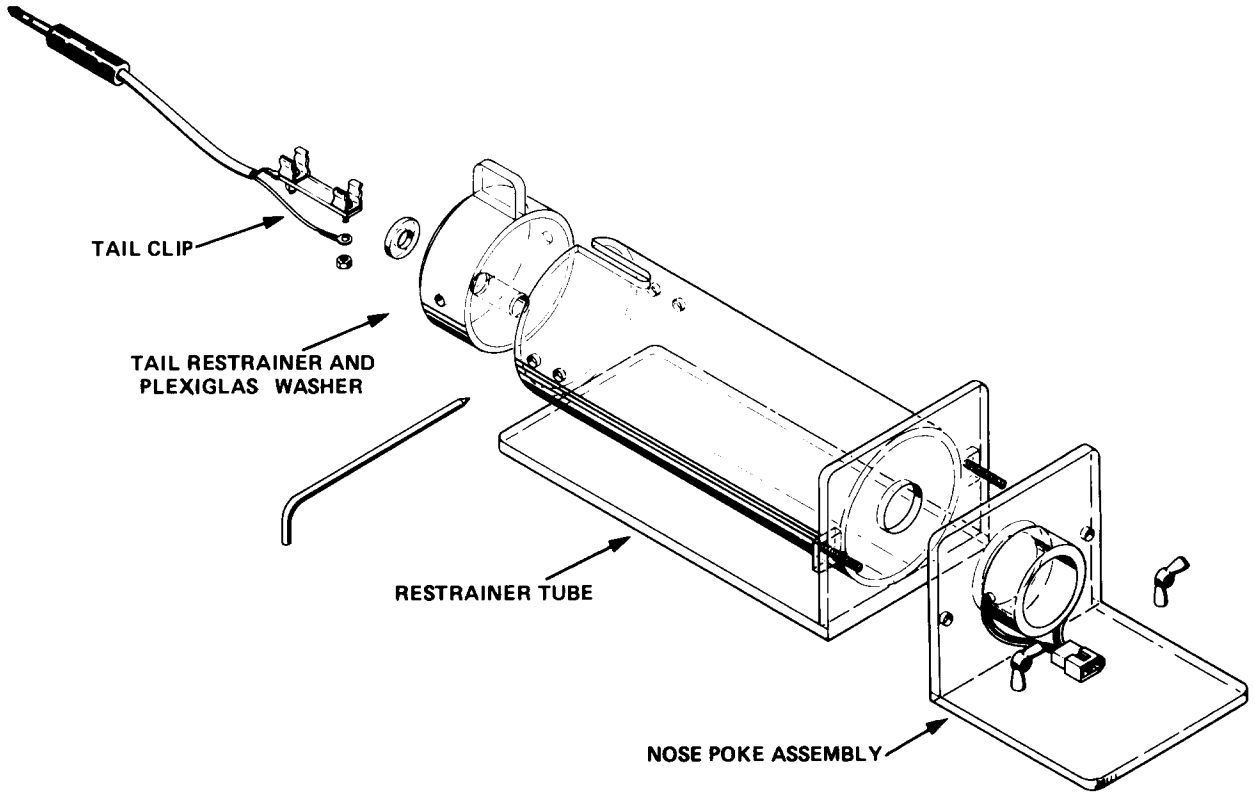

FIG. 1. Drawing of the Plexiglas restrainer tubes showing its four main parts: (1) restrainer tube, (2) nose-poke assembly, (3) tail restrainer, and (4) tail electrodes.

plished by a PDP-8/A-620 minicomputer using SuperSked software.

The Plexiglas tubes (Fig. 1) were composed of four major parts: (1) restrainer tube, (2) nose-poke assembly, (3) tail restrainer, and (4) tail electrodes (Fig. 1). The *restrainer tube* was an 8 in. cylinder of 3/16 in. clear Plexiglas with an outer diameter of 3 inches. The rear end of the tube was open to permit the subject to enter; the front end was closed by a Plexiglas panel having a 7/8 in. diameter hole in the center. The *nose-poke assembly* was a 1 in. long, 1 3/4 in. o.d., round Plexiglas tube affixed to the front plate of the restrainer tube and centered on 7/8 in. hole. A photodetector circuit was mounted across the small tube 3/8 in. from the front of the restrainer tube. The photobeam pair consisted of a MLED930 Infrared light emitting diode and a MRD300 phototransistor. The *tail restrainer* was a cyclindrical block of Plexiglas turned down to fit into the restrainer tube and drilled with two holes; one hole permitted a pin to pass from one side of the restraining tube, through the tail plug and out the other side of the restraining tube. The other hole was 3/8 in. through which the rat's tail is placed. A washer-like Plexiglas disk and a length of 1/2 in. wide adhesive tape held the tail in the tail restrainer. The tail electrode consisted of two fuse clips mounted on a 1 in.$\times$1/4 in. piece of Plexiglas and was connected to the programmable shocker by means of a phone jack.

Procedure

All rats were tested 30 min per day, 5 days a week for at least 30 sessions. The rats were placed in the restraining tube assembly and allowed to acclimate for at least 10 min prior to testing. Just before being placed into the environmental test cubicle, the tails were wiped clean with a wet towel and electrode gel (Bovie Liquid Conductor, Ritter Co., Rochester, NY) was applied to the tail at the points of electrode placement. Setup and starting procedures assured all rats were in tubes for equal times. The electrodes were attached 1.5 in. and 2.5 in. caudal to the tail restraint point and were adjusted to lie on the dorsal and ventral sides of the tail. The Plexiglas washer and adhesive tape were applied to hold the rat's tail in position and the electrode was plugged into the jack connected to the programmable shocker. The shockers were calibrated and adjusted to account for individual skin resistance.

The session was initiated with shock applied to the electrodes at zero intensity. In the absence of a nose-poke response, shock intensity was incremented 0.01 mA every 2.5 or 7.5 sec. (intershock interval, ISI) until a response was made. Each nose poke response decreased the level of shock applied to the electrodes by 0.01 mA. The average number of nose-pokes occurring when the shock was above zero (responses in shock) and, from this, the median shock level were calculated. Only data from the last 15 min of the 30 min session are discussed because of the inherent variability of the warm up period characteristic of the first 10–15 min of the session [3].

Dosing

At the start of the study, the 28 rats were randomly as-

TABLE 1

BASELINE VALUES FOR ALL ANIMALS AFTER 15 DAYS OF TRAINING ON THE NOSE-POKE TITRATION SCHEDULE MEAN ± SE*

	N	Shock level (mA)	Responses/min
2.5 sec ISI	21	0.28 ± 0.04	17.0 ± 0.2
7.5 sec ISI	21	0.19 ± 0.03	5.5 ± 0.1

*Data are means based on the response values obtained for each animal on the fifteenth day of training.

signed to the two ISI conditions. All rats were given 14 days of training and baseline performance on the titration schedule using either a 2.5 or a 7.5 sec ISI. The remaining 7 rats from each ISI group served as controls and received IP injections of the distilled water vehicle.

Seven rats from each ISI group were used to measure increases in pain or reaction thresholds. These animals were dosed with either 0, 3, 6 or 9 mg/kg of morphine sulfate dissolved in distilled water. The animals were given morphine on run days 15, 20, 25 and 30. The rats received each dose only once; doses were given in a Latin-Square design. Morphine was injected IP 30 min prior to each test session.

RESULTS

Training and Baseline

As reported by Dallemagne and Richelle [3], animals responded (nose-poked) during the first training session. Many of these responses appeared to be unconditioned reactions to the electric shock. Increases in shock to the tail electrodes induced forward lunging, which broke the photobeam and subsequently reduced the tail shock. With increased experience, responding became regular, involved very little movement and was associated with a shock threshold for each animal. After 15 days of training, the shock level was effectively controlled by all of the animals. The average shock thresholds and response rates, obtained on day 15 of training, are summarized in Table 1. As might be expected [14], animals responding on the 2.5 sec. ISI schedule had higher shock thresholds and nose-poked at a higher frequency than rats trained on the 7.5 sec ISI schedule.

Morphine

The IP administration of morphine produced marked alterations in both the average shock intensity and the rate of nose-poking (Fig. 2). Data from each drug session were expressed as a percent of the values obtained on 4 noninjection control days immediately preceding each dose of morphine (baseline) and subjected to repeated measures ANOVA [15].

ANOVA indicated that morphine produced a statistically significant increase in shock intensity, $F(2,34)=13.20$, $p<0.0024$. The shock intensity depended on the ISI, $F(1,34)=13.20$, $p<0.0009$; the ISI by dose interaction was not statistically significant, $F(2,34)=1.75$, $p<0.1897$. Pairwise comparisons (Fisher's Least Significant Differences Test) [14] between the two groups indicated that the animals responding on the 7.5 sec ISI had significantly greater changes from baseline following 6 and 9 mg/kg of morphine than the animals responding on the 2.5 sec ISI schedule.

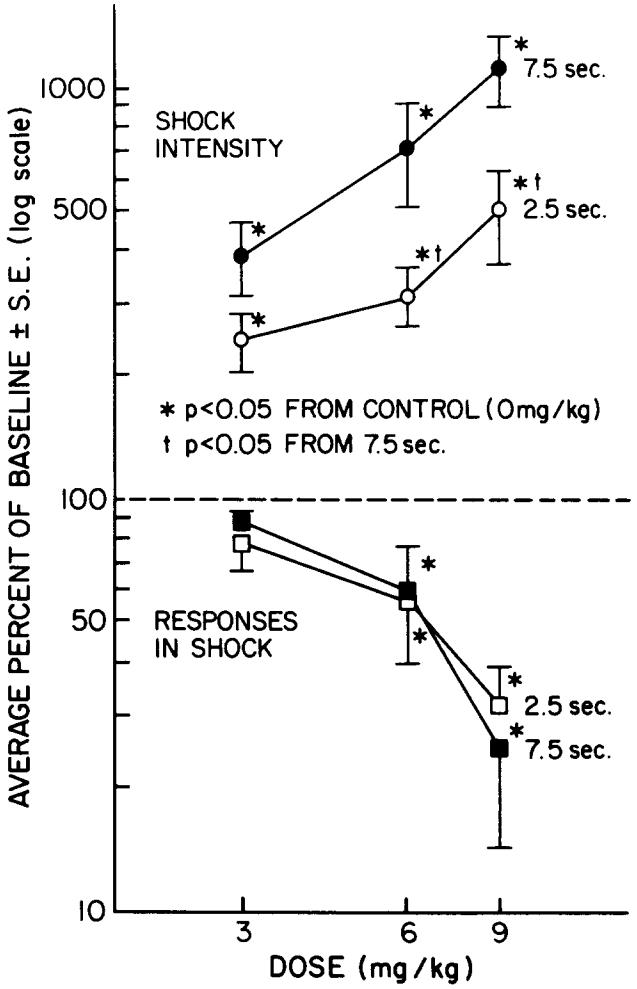

FIG. 2. The effects of morphine on median shock levels and responses in shock. Rats were trained in the titration schedule (2.5 or 7.5 sec. ISI) for 15 days. Data are average percentages±SE of shock and response rates obtained on the four (4) noninjection days preceding each dose of morphine. There were 7 animals studied at each ISI interval. Overall effects of dose and ISI interval were assessed for statistical significance using repeated measures ANOVA. Crosses indicate a significant difference between ISI intervals (Fisher's Least Significant Difference Test, $p<0.05$). The asterisks indicate a significant difference from vehicle control (0 mg/kg of morphine) for each ISI group (matched paired t-test, $p<0.05$).

Significant differences between groups in terms of responses in shock were not observed.

Matched paired t-tests [7] were used to determine morphine-induced changes in shock intensity and responses in shock relative to each animal's own baseline measure. All three doses of morphine produced significant increases in shock intensity and the effect was observed in both ISI groups.

ANOVA of the response rate measure also indicated a significant dose effect, $F(2,33)=11.29$, $p<0.0002$. However, the ISI had no significant effect ($F=0.00$) nor was the ISI by Dose interaction statistically significant ($F=0.55$). Matched pair t-tests indicated significant decreases in the response rates of both ISI groups at 6 and 9 mg/kg groups.

These data show that 3 mg/kg of morphine produced significant increases in the shock threshold of rats maintained on either a 2.5 or a 7.5 sec ISI; changes in shock intensity following 3 mg/kg of morphine were not associated with significant decreases in response rate. Increases in shock thresholds following 6 or 9 mg/kg were correlated with decreases in response rate.

DISCUSSION

The titration procedure described in this paper provides several advantages in the investigation of psychopharmacological or neurotoxicological agents. Acquisition of the nose-poke response can be done automatically without prior shaping; rapid and relatively stable within session responding can be obtained within a week of training. The nose-poke response requires a minimum of motor involvement and this could be important in the study of psychopharmacological or neurotoxicological agents that can affect both sensory and motor processes.

In general, the data obtained following the administration of morphine are in agreement with other reports that this agent increases shock levels maintained by a titration schedule [5,14]. Low doses (3 mg/kg) of morphine produced a significant increase in the median shock intensities with no significant accompanying decrease in response rate. Higher doses (6 and 9 mg/kg) of morphine produced larger increases in median shock tolerated which were accompanied by decreases in response rate. These findings were consistent for both ISIs studied, although absolute shock threshold values remained different for the two ISIs. The fact that the low dose of morphine produced increases in median shock intensities without changing response rates demonstrates the relative sensitivity of the titration procedure to detect analgesia-induced changes.

The titration technique described in the present paper offers several advantages in the assessment of the effects of chemicals on the perception of pain. The nature of the required response (nose-poke) requires fewer motor components than do responses used in other procedures (spatial preference, lever press). Since many psychopharmacological and neurotoxicological agents have effects on motor, as well as sensory systems, the nose-poke titration procedure might be more appropriate in experiments designed to assess effects on sensory processes.

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