INSTRUMENTATION & TECHNIQUES

A method for determining the aversive threshold in the rat using repeated measures: Tests with morphine sulfate*

VINCENT P. HOUSER and WILLIAM P. PARÉ
Veterans Administration Hospital, Perry Point, Maryland 21902

METHOD Subjects

Six male Dublin DR (Sprague-Dawley derivatives) rats, obtained from Flow Laboratories, Dublin, Virginia, were used as Ss. They were 60 days old at the beginning of the study.

Apparatus

The test chamber consisted of a rectangular Plexiglas shuttlebox inside a sound-attenuated enclosure. The box was 18 cm deep x 41 cm long x 26 cm high. The top was plastic and hinged, and served as the entry door for the chamber. Stainless steel rods, spaced 1.25 cm apart, comprised the floor. As Fig. 1 illustrates, the entire box was pivoted in the middle, allowing the box to tilt from side to side. From a horizontal position, the box could tilt a distance of 0.20 cm before activating a light-action Acro lever switch located at the end of the cage. The switch closure would either activate the grid or turn off the shock, depending upon the position of a selector switch on the researcher's control panel. A running time meter measured the total time that shock was on during a test session, and a digital counter recorded crossings (i.e., the number of times the animal crossed from one end of the box to the other end). False walls were inserted within the box and positioned 1.50 cm from the front and back walls, 0.30 cm above the grid floor. These false walls prevented the animal from urinating on the outside walls (which held the floor rods), thus eliminating the possibility of current leakage. The shock stimulus was provided by a dc generator which produced a 60-Hz square-wave output. This unit was designed specifically to maintain a constant current across the S, even when resistance was altered radically due to the animal's movements (Reus, Houser, & Paré, 1971). The voltage across the animal was constantly monitored with an oscilloscope. A large drop in resistance, as detected by the oscilloscope, reflected an electronic short in the apparatus. Current leaks were frequently attributable to local humidity conditions. This problem was eliminated by the operation of a

The procedure described here provides an objective automated technique for the psychophysical assessment of the aversive threshold and allows the animal Ss to serve as their own controls. Sensitivity to drug effects, as well as comparisons between drugs, using the same animals are possible also. Using a rectangular tilt cage, the aversive threshold to grid shock was defined as that intensity avoided 75% of the time. Each shock intensity (30, 60, 90, 120, and 150 microA) was presented for 5 min on one side of the cage and then switched to the other side for 5 min, thereby forcing the animal to sample each shock intensity. Aversive thresholds were determined daily for each rat. The analgesic effects of morphine sulfate were compared to saline. Animals were tested for 3 days at each morphine dose level (2, 4, 8, and 16 mg/kg). Each 3-day morphine series alternated with 3 days of testing under saline. Significant differences were detected between saline and morphine at 4, 8, and 16 mg/kg.

Both the pharmacologist and behavioral scientist have been interested in developing methods which measure the analgesic potency of various pharmaceutical agents. Analgesia, of course, has been assessed by a variety of techniques. Procedures that measure simple reflexive responses include the tail-flick test, the hot plate test, the phenylbenzoquinone-induced writhing and stretching test, and the flinch-jump test. But these techniques have been reviewed elsewhere (Evans, 1965). Since these procedures involve subjective judgment on the part of the E, the behavioral scientist has attempted to incorporate more objective methods into the psychophysical assessment of aversive thresholds by using automatic recording equipment. These attempts have included the application of the conditioned emotional response (CER) procedure (Hill, Pescor, Belleville, & Wikler, 1957) and the sophisticated titration technique of Weiss and Laties (1970). While these automated techniques have eliminated the subjective element so distasteful to the behaviorist, the methods have been criticized (Evans, 1965) as being too complex, both in terms of the number of controls required and in the level of E and S training and sophistication needed.

*The authors wish to thank James Reus for his technical assistance. Gratitude is extended to J. Reeves and F. Houser for collecting the data. The photographic work was performed by Luther R. Gilliam of the Medical Illustration Service, VA Hospital, Perry Point, Maryland.

A procedure which obviates the complexity of the CER and titration techniques (Campbell, 1956, 1957) defines the aversive threshold to grid shock as the intensity of shock avoided 75% of the time by the S in a spatial preference test utilizing a rectangular tilt cage. With this method, various shock intensities are presented to the animal S and escape from shock is possible by crossing to the opposite side of the cage. Subthreshold intensities do not elicit crossing responses, whereas suprathreshold shocks of increasing intensities result in a greater percentage of time spent on the nonshock side of the cage. Responses are automatically recorded and the technique does not require extensive training of the animals. Its only drawback for investigating drug effects is that animals have typically been subjected to only one trial in order to control for the cumulative effects of drug dosage, as well as any learning factor associated with the procedure (Boldovici & Cicala, 1968; Paré, 1969b). The effects of varying drug dosages, as well as different drugs, have not been compared within the same animal.

The procedure and data reported in this paper indicate that the basic tilt cage technique can be used as an effective device for obtaining reliable threshold estimates for repeated trials on the same Ss once the technique has been modified. As such, it represents a useful tool for investigating the analgesic properties of pharmaceutical agents.

Fig. 1. Photograph of the tilt cage apparatus. The apparatus was situated in a sound-attenuated enclosure during testing.

dehumidifier in the experimental room and by subjecting the inside of the cage to a hot air blast between trials from a hair dryer mounted on the apparatus enclosure. Before each trial the shock grid was inspected for current leaks with a microammeter contained in the shock generator circuit. At the end of a daily session, the apparatus was thoroughly cleaned with special attention paid to the grid floor.

Procedure

The animal was placed in the apparatus and allowed to explore the cage for 0.5 min. After this period, the circuit which controlled delivery of the shock stimulus to the apparatus and turned on the timing devices was activated. If the animal was on the "hot" side of the apparatus, movement to the opposite side would turn off the shock. After 5 min the shock was switched to the opposite side of the box. After 10 min the

shock was turned off and the time spent on the hot side was recorded from the running time meters, along with the number of crossings. The proper shock settings were then made for testing at the next shock intensity. The intertrial interval between the various shock intensities was approximately 20 sec. This procedure was repeated for five separate shock intensities (i.e., 30, 60, 90, 120, and 150 microA) presented in an ascending series. Each animal was tested at all five shock intensities each day. In order to control for possible position preference, the initial shock presentation on a particular day was alternated from one side to another in a random fashion. The switching of each shock intensity from one side of the cage to the other every 5 min insured that each intensity was sampled by every animal. The aversive threshold was calculated daily for each S by determining the intensity of shock which an animal avoided 75% of

the time. At subthreshold intensities the animal, by chance, would spend 50% of the time on the shock side and 50% of the time on the safe side. Since time spent on the shock side diminished as shock intensity increased, the 75% threshold criteria required a simple interpolation process. Under some drug conditions, some rats spent more than 25% of the time on the shock side at the highest (150 microA) intensity. The absence of a higher test shock intensity precluded the interpolation of the aversive threshold in these cases. Therefore, a threshold value of 150 microA was arbitrarily assigned in these instances. Consequently, our threshold estimates, especially for the higher drug dosages, were very conservative.

After 5 days all animals recorded stable threshold values and the drug series was initiated. The drug investigation used 0.9% physiological saline and morphine sulfate (Eli Lilly & Co.). All injections were intraperitoneally given 0.5 h before threshold testing, and all injections were given in the same volume (1.0 cc/kg). The analgesic effect of morphine was evaluated at concentrations of 2, 4, 8, and 16 mg/kg. The various dosages were presented in an ascending series, beginning with 2 mg/kg and ending with the 16 mg/kg dose. Each dosage was given for 3 consecutive days to determine if drug habituation would occur. Each dosage series was preceded and followed by 3 consecutive saline days to insure that the animal had returned to baseline performance before the next dosage was administered. The entire study consisted of 27 consecutive daily sessions. Aversive thresholds were calculated for each S every day.

RESULTS

The mean aversive thresholds for the alternating saline and morphine series are illustrated in Fig. 2. The mean aversive threshold for all five saline series was 50.3 microA, which agrees closely with other reports using the tilt cage technique (Boldovici & Cicala, 1968; Paré, 1969a, b). Mean thresholds within each 3-day series were not significantly different. There were no statistical differences between the five individual saline series, thereby indicating that under saline Ss returned to baseline threshold levels and maintained these levels between all the morphine series. The main effect of drug treatment in the analysis of variance was highly significant ($F = 26.33$, $df = 8/40$, $p < .01$). Multiple statistical comparisons revealed that 2 mg/kg of morphine did not significantly raise the aversive

Fig. 2. Aversive thresholds (means $\pm$ SEM) for the alternating saline and morphine series for 27 consecutive testing days.

threshold ($F = 1.21$, $df = 1/40$, $p > .05$). However, the thresholds derived for the 4 mg/kg morphine series were significantly higher ($F = 23.58$, $df = 1/40$, $p < .001$). As Fig. 2 illustrates, morphine at 8 and 16 mg/kg had a greater analgesic effect and produced correspondingly higher thresholds (8 mg/kg: $F = 39.20$, $df = 1/40$, $p < .001$; 16 mg/kg: $F = 104.72$, $df = 1/40$, $p < .001$).

Activity within the tilt cage, as measured by crossings, increased for the morphine dosages of 2 and 8 mg/kg but was significantly greater only for the 4 mg/kg dose ($F = 12.01$, $df = 1/35$, $p < .005$). Morphine at 16 mg/kg resulted in a nonsignificant decrease in crossings. These data suggest that the morphine-induced changes in the aversive threshold were not attributable to locomotor impairment.

DISCUSSION

The value of this technique for the psychophysical assessment of aversive thresholds and as a tool for studying drug effects, can be evaluated in terms of the technique's: (1) sensitivity to low drug dosages, (2) repeatability or reliability of obtained data, (3) simplicity of design and procedure, and (4) lack of subjective interpretation of the behavior under study.

Morphine at 2.0 mg/kg elevated the aversive threshold and significant differences were detected at 4.0 mg/kg. Our analysis of the changes in the aversive threshold in response to morphine administration was extremely conservative. If the present data using the conventional median effective dose (ED-50) were reported, changes in the aversive threshold could be detected at 2.0 mg/kg. Thus, the present technique meets the criterion advanced by Evans (1965), which suggests that sensitive measures should be able to detect morphine-induced analgesia at 2.0 mg/kg. Other sources (Barnes & Eltherington, 1966; Farris & Griffith, 1949), however, have placed the average analgesic dose of morphine at a somewhat higher value (5.0 to 9.1 mg/kg).

The repeatability of the method was supported by the fact that analysis of thresholds within series failed to reveal any significant differences. In addition, whenever animals were administered saline, thresholds always returned to baseline levels. The reliability of the method will necessarily rely on the replication of this technique by other investigators, but our observations were relatively consistent from day to day. The possibility of obtaining repeated thresholds from the same animals provides the investigator with a tool

whereby he can use the animal as its own control and compare the effects of different drugs on the same animal.

Since extensive training of animal Ss is precluded by the tilt cage procedure, considerable time is saved. This reflects on the relative simplicity of the technique, both in the amount of sophistication required from the animal S and in the technical components of the apparatus. The control and recording equipment is minimal and the tilt cage is of simple design. The major precaution required is the critical concern to prevent current leakage across the grid. This is obviated by the false walls in the cage and the procedure of maintaining a dry environment.

REFERENCES

- BARNES, C. D., & ELTHERINGTON, L. G. *Drug dosage in laboratory animals*. Berkeley: University of California Press, 1966. P. 154.
- BOLDOVICI, J. A., & CICALA, G. A. Increased aversion thresholds in rats as a function of aspirin and meprobamate administration. *Psychological Record*, 1968, 18, 389-394.
- CAMPBELL, B. A. The reinforcement difference limen (RDL) function for shock reduction. *Journal of Experimental Psychology*, 1956, 52, 268-272.
- CAMPBELL, B. A. Auditory and aversion thresholds of rats for bands of noise. *Science*, 1957, 125, 596-597.
- EVANS, W. O. A critical review of some new methods in animal analgesimetry. *Journal of New Drugs*, 1965, 6, 179-186.
- FARRIS, E. J., & GRIFFITH, J. Q. *The rat in laboratory investigation*. Philadelphia: Lippincott, 1949. P. 358.
- HILL, H. E., PESCOR, F. T., BELLEVILLE, R. E., & WIKLER, A. Use of differential bar pressing rates for screening analgesic drugs. I. Techniques and effects of morphine. *Journal of Pharmacology & Experimental Therapeutics*, 1957, 120, 388-397.
- PARÉ, W. P. The effect of adrenalectomy, adrenal demedullation and adrenalin on the aversive threshold in the rat. *Annals of the New York Academy of Sciences*, 1969a, 159, 869-879.
- PARÉ, W. P. Age, sex, and strain differences in the aversive threshold to grid shock in the rat. *Journal of Comparative & Physiological Psychology*, 1969b, 69, 214-218.
- REUS, J. F., HOUSER, V. P., & PARÉ, W. P. An electronic constant current shock generator. *Physiology & Behavior*, 1971, 7, 635-637.
- WEISS, B., & LATIES, V. G. The psychophysics of pain and analgesia in animals. In W. C. Stebbins (Ed.), *Animal psychophysics*. New York: Academic Press, 1970. Pp. 185-210.