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The Up-and-Down Method for the Determination of Nociceptive Thresholds in Rats

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CROCKER, A. D. AND R. W. RUSSELL. *The up-and-down method for the determination of nociceptive thresholds in rats.* PHARMACOL BIOCHEM BEHAV 21(1) 133–136, 1984.—The up-and-down method applied to the jump-flinch technique provided estimates of shock thresholds for jump and flinch with high statistical efficiency and a much reduced amount of experimentation compared with traditional methods. Reproducible results were obtained after repeated testing and flinch and jump thresholds were significantly increased by treatment with morphine or oxotremorine.

Nociception Analgesia "Up-and-down" method Threshold measurement

METHODS for measuring the magnitude of a stimulus necessary to produce an observable response have received attention in pharmacology, physiology, psychology and toxicology for well over a hundred years [4] and have been applied to the measurement of thresholds under various environmental and experimental conditions. The "Method of Limits" has received particular attention for determining stimulus thresholds. This method requires the presentation to a subject of a range of stimulus magnitudes within which the threshold is presumed to lie in several alternating ascending (from subthreshold) and descending (from suprathreshold) series. The multiple repetition of such series is not only time consuming, but also raises problems of interpretation when ever there is a possibility that the repetition may lead to habituation or tolerance. A much briefer procedure for achieving the same results has been proposed by Fechner [4], i.e., the so-called "up and down" method. Although devised originally for use in determining median lethal thresholds, the method has significant advantages for other applications. The present paper reports a series of experiments designed to illustrate the use of the method for determining nociceptive thresholds in rats.

A sensitive assay for nociception in rats involves the determination of thresholds for the jump and flinch responses elicited by foot shock [13]. The jump-flinch assay is based upon observations of innate (unconditioned, unlearned) responses of rats to inescapable (non-contingent) foot shock [8]. Low shock intensities produce a flinching response (crouch, startle, twitch or front paw elevation) and increased shock intensities give rise to such skeletal activities as rear paw elevation, jumping and running. Standardized procedures have generally involved the recording of three major features of this response sequence: (a) no response; (b) flinch; and (c) jump and studies have shown that these categories can be distinguished with reliability coefficients of 0.95–0.99 between independent observers. The responses have been shown to be sensitive to the effects of moderate doses of narcotic analgesics, narcotic antagonists and antipyretic analgesics [1, 3, 10, 11].

In the present study the shock thresholds to produce jump and flinch responses have been determined using an adaptation of the up and down method for small samples [2] in which the shock intensities presented are determined sequentially. The up and down method has the advantage that it requires a reduced amount of experimentation to obtain the accuracy of more traditional methods [2]. The results of repeated testing of the same animals and the effects of morphine and oxotremorine injections are presented as evidence of the reliability and validity of this method for the determination of nociceptive thresholds.

METHOD

Animals

Male Sprague-Dawley rats (250–300 g; Simonsen, Gilroy, CA) were housed in individual cages for 3–10 days before being used in any experimental procedures. Water and food were available ad lib.

Drugs

Morphine (Mallincrodt) was dissolved in water which was

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TABLE 1

THRESHOLD SHOCK INTENSITIES (EI_{50}) FOR FLINCH AND JUMP RESPONSES OBTAINED IN THREE EXPERIMENTS CARRIED OUT IN DUPLICATE ON DIFFERENT DAYS IN THE SAME ANIMALS

		1		2		3	
Day		1	2	1	2	1	2
Series	N	(mAmp)		(mAmp)		(mAmp)	
Flinch	6	0.20 ±0.02	0.29 ±0.03	0.20 ±0.02	0.21 ±0.04	0.24 ±0.05	0.24 ±0.05
Jump	6	0.50 ±0.06	0.41 ±0.05	0.42 ±0.05	0.47 ±0.07	0.47 ±0.11	0.50 ±0.08

Results expressed as means ± SEM.

acidified by the addition of hydrochloric acid (1 M) to give a final concentration of 26 μmol/ml.

Oxotremorine (Sigma) was dissolved in water to give a final concentration of 1 μmol/ml.

The volume of both drug solutions injected was 1 ml/kg.

Apparatus

The assay apparatus consisted of a test chamber 30.5×30.5×30.5 cm in its three dimensions. The top and two sides of the chamber were made of transparent plastic. It was illuminated from above by a single 15 W bulb, there being no other light in the room. The floor consisted of stainless steel rods (28 mm dia, 1.2 cm centers). Shock was delivered by a Grason-Stadler Shock Generator, Model E1064GS. The shock pattern on the electrified grid was continuously scrambled. By mounting the test chamber at the observer's eye level an unobstructed view could be obtained of the animal's paws. Observation was further facilitated by the placement of a mirror on the opposite side of the chamber from the experimenter.

Procedure

All rats were given a 2 min habituation period prior to the start of an experiment. On an experimental day each was placed in the chamber for 2 min before a shock series began and after the grid floor had been cleaned with steel wool and water. Each shock pulse had a duration of 0.5 sec and shocks were delivered at 10 sec intervals. Shock intensities were available from 0.05 to 4.0 mA in 20 steps arranged logarithmically. The full range was not used in determining thresholds. The ranges of intensities within which thresholds were to be found were estimated from preliminary observations. The midpoints of these ranges served as the beginning intensities in the experiments proper.

A "flinch" was defined as elevation of one paw and "jump" as rapid movement of three or more paws, both responses involving withdrawal from the floor.

An adaptation of the "up-and-down" method for small samples [2] was used for determining the order of presentation of shock intensities during each series. The steps in the procedure were as follows: (1) The first series began with a shock intensity as close as possible to the flinch or jump threshold for the treatment being observed. (2) A series of trials was carried out in accordance with the rules that: 2.1—the responses, flinch or jump, were followed by a decrease (of 0.1 $\log_{10}$ unit) in shock intensity; 2.2—nonresponses (no flinch or jump) were followed by a similar increase in shock intensity. (3) Trials were continued in each series until a change in behaviour occurred (nonresponse to response or response to nonresponse) and were terminated four trials thereafter.

Int (mA)	Example of a Test Series Response*							
0.16	X				X		X	
0.13		X		0		0		
0.10			0					

*"X" = flinch; "0" = no flinch

The estimate of the median effective "intensity" (EI_{50}) was calculated by the formula $EI_{50} = X_f + kd$, where X_f = last intensity administered, k is the value in Table 1 provided by Dixon [2] and d is the log interval between shock intensities. The k values in the table are maximum likelihood estimates for each possible configuration of responses assuming a normal cumulative distribution. In the above example the sequence of responses and nonresponses is: XXOOXOX. The value of k for this sequence is −0.935; the final shock intensity, 0.16 mA; and the log interval between shocks, 0.1. Inserting these values into the above equation, EI_{50}=0.13 mA.

Preliminary experiments were conducted to verify the inter-observer reliability of the assay as reported by other investigators. Agreement between two independent observers on the sequence of behaviours within test series was very high, differences appearing only occasionally and then when shock intensities were near to threshold values. Such differences did not affect EI_{50}'s calculated by the Dixon method. This inter-observer reliability in observing the flinch and jump responses is consistent with that reported for similar observations [1].

RESULTS

High reliability was shown in the consistency by which the assay measured flinch and jump thresholds. Table 1 summarizes results from three experiments using the same subjects in each of which EI_{50}s were determined in duplicate. These values are very similar both between series within

TABLE 2

THRESHOLD SHOCK INTENSITIES (EI_{50}) DETERMINED IN DUPLICATE AFTER SALINE 1 ml/kg AND MORPHINE (26 μmol/kg) INJECTION SUBCUTANEOUSLY

	Saline		Morphine	
Experiment Series	1 (mAmp)	2	1 (mAmp)	2
Flinch	0.16 ± 0.02	0.15 ± 0.02	0.83 ± 0.02 (519%)*	0.89 ± 0.12 (593%)*
Jump	0.45 ± 0.05	0.39 ± 0.04	1.40 ± 0.12 (311%)*	1.30 ± 0.19 (333%)*

*Values in parentheses are EI_{50} expressed as % of saline baseline.
Results expressed as means ± SEM.

each experiment and between experiments carried out on different days.

Validity of the assay was tested using a drug, morphine, known to produce analgesia. Baseline EI_{50}s were obtained after injection (subcutaneously between the shoulders) of 1.0 ml/kg of saline (0.154 M). Three hours later the same animals were injected SC with 26 μmol/kg morphine and IE_{50}s determined after 30 min. The results are presented in Table 2. EI_{50}s for both flinch and jump following saline injections were similar to those reported in Table 1. Comparison of the two treatment conditions shows highly elevated thresholds, i.e., analgesia resulting from treatment with morphine. Differences in flinch thresholds between saline and morphine treatments for the two series of tests were highly significant: $t(10)=23.69$, $p<0.001$ and $t(10)=6.08$, $p<0.001$, and percent increases 519 and 593 respectively. For jump thresholds the differences were also highly significant: $t(10)=7.31$, $p<0.001$ and $t(10)=4.69$, $p<0.001$, percent increases being 311 and 333.

The results of 1 μmol/kg oxotremorine, a potent cholinergic agonist with analgesic properties, or 1 ml/kg saline on thresholds for flinch and jump are shown in Table 3. Anova for repeated measures established that time effect relationships were significant (Flinch, $F(4,24)=5.4$, $p<0.005$; Jump, $F(4,24)=11.5$, $p<0.005$).

Oxotremorine significantly increased flinch thresholds at 30 minutes (172%: $p<0.05$) and 60 minutes (178%: $p<0.05$) after injection (Newman-Keuls test). Jump thresholds were also significantly increased at 30 minutes (185%: $p<0.01$) and at 60 minutes (169%: $p<0.01$). Thereafter flinch and jump thresholds returned to pretreatment levels. There were no significant changes in thresholds in the saline injected group again confirming the reproducibility of the assay.

TABLE 3

THRESHOLD SHOCK INTENSITIES (EI_{50}) OBTAINED IN RATS UP TO 240 MIN AFTER INJECTIONS OF SALINE 1 ml/kg OR OXOTREMORINE (1 μmol/kg)

	Time after injection (min)				
Experiment	0	30	60	120	240
Flinch EI_{50} (mAmp)					
Saline	0.19 ±0.02	0.21 ±0.03 (111%)	0.17 ±0.02 (89%)	0.19 ±0.02 (100%)	0.22 ±0.03 (116%)
Oxotremorine	0.18 ±0.01	0.31 ±0.05 (172%)	0.32 ±0.03 (178%)	0.21 ±0.01 (117%)	0.20 ±0.02 (111%)
Jump EI_{50} (mAmp)					
Saline	0.45 ±0.07	0.50 ±0.07 (111%)	0.41 ±0.05 (91%)	0.37 ±0.06 (82%)	0.40 ±0.05 (89%)
Oxotremorine	0.48 ±0.05	0.89 ±0.11 (185%)	0.81 ±0.11 (169%)	0.44 ±0.05 (92%)	0.42 ±0.04 (88%)

Results expressed as means ± SEM.
Values in parentheses are EI_{50} expressed as % of pretreatment baseline.

DISCUSSION

The jump-flinch technique has been established as a satisfactory assay of nociception with good sensitivity to narcotic and non-narcotic analgesics [1]. The determination of jump and flinch thresholds by the application of the up-and-down method [2] has provided a means of reducing the amount of experimentation involved in such assays and gives estimates of high statistical efficiency. It is particularly suited to procedures which involve a clear end point (e.g., alive or dead) such as the estimation of the LD_{50}. In our laboratories estimates of LD_{50} have been carried out on as few as six animals which resulted in considerable savings in the number of animals used and in experimenters' time compared with traditional methods, with no loss of reliability (Crocker, Russell and Booth unpublished observations). Because the jump-flinch technique involved clear behavioural end-points it seemed reasonable that the up-and-down design could be applied.

Values for jump and flinch thresholds obtained with the up-and-down method are similar to those reported by other workers using more traditional experimental designs [1,7] and did not vary when the same animals were tested several times within a four hour period or daily for three days. However, in our study as few as six shocks were administered to an animal for the estimation of the jump or flinch threshold.

This can be compared with more traditional designs where, for example, five successive shock presentations were delivered at each level until thresholds for each behavioural category were achieved [1].

The validity of the method was confirmed by the demonstration that the narcotic analgesic, morphine, produced a significant increase in jump and flinch thresholds of the same magnitude as reported previously [1]. The cholinergic agonist, oxotremorine, also increased jump and flinch thresholds which is consistent with other reports that cholinergic receptors are involved in nociceptive responses [5, 6, 9].

There are many other stimulus-response relations to which this up-and-down method may be applied. It should prove to be especially useful when it is desirable to minimize the number of stimulus presentations and when the determination of threshold values is but one element among several in a battery of different assays.

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