Pharmacology Biochemistry and Behavior, Vol. 3, pp. 47–55. ANKHO International Inc., 1975. Printed in the U.S.A.

A Modification of the Jump-Flinch Technique for Measuring Pain Sensitivity in Rats[1]

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(Received 28 March 1974)

BONNET, K. A. AND K. E. PETERSON. *A modification of the jump-flinch technique for measuring pain sensitivity in rodents.* PHARMAC. BIOCHEM. BEHAV. 3(1) 47–55, 1975. – The jump-flinch procedure provides a sensitive alternative to the hot-plate and tail-flick procedures. Analysis of the components of motor responses to increasing intensity of foot shock presentation has allowed the observational discrimination of five reliably elicited categories of unlearned responses to inescapable foot shock. Morphine sulfate differentially altered response category thresholds in rats. Response category thresholds also differed between Wistar and Fisher strain rats in analgesic effects of morphine sulfate.

Footshock sensitivity Analgesia Strain difference Morphine

A SENSITIVE method for quantitating foot shock sensitivity in animals has been provided by the jump-flinch technique [6]. As an alternative to the tail-flick procedure, it has allowed discrimination between drug states and elicitation of responses more analogous to human responses to pain. The pharmacological usefulness of this technique has been demonstrated by its sensitivity to analgesic effects of moderate doses of narcotic analgesics, narcotic antagonists, and antipyretic analgesics, as well as to the pseudoanalgesic effects of chlorpromazine but not of amphetamine [6, 14, 15]. Hypersensitivity to foot shock also has been detected in animals treated with parachlorophenylalanine (pCPhe) or chronic lithium chloride [10, 14, 15].

Various investigators reporting the use of the jump-flinch technique have used differing response categories as criteria for dichotomizing flinch and jump. Procedures employing sufficiently low levels in a shock series report differential effects of treatment conditions in altering both flinch and jump responses, as well as elicited vocalization when recorded [4,14]. Flinch is usually defined as crouch, startle, or twitch, but it has also been defined to include front paw elevation in direct response to foot shock. Jump most often has included rear paw elevation as a criterion but has also variously included whole body withdrawal and running. Vocalization is usually accepted as an audible cry.

In standardizing the jump-flinch technique for use in our laboratory, we observed a sequence of unlearned behavioral responses to inescapable foot shock with ascending shock levels. The response sequence include all responses specified in the various operational definitions for flinch and jump found in the literature. The respone sequences resembled those described for animals given ascending levels of shocks to the tail root that involved ascending levels of brain stem and limbic forebrain integration for response execution [2]. The present studies were directed toward determining the reliability of the response heirarchy with ascending foot shock intensity and the effects of a standard analgesic compound, morphine sulfate. The response heirarchy and analgesic effects of morphine sulfate were tested in two strains of rats.

METHOD

Animals

Except where otherwise noted, male Wistar and Fisher strain rats 35–45 days old were received from Simonsen Laboratories, Gilroy, California. Animals were maintained in individual wire mesh hanging cages for 3 to 10 days before being used in any experimental procedures. Purina Lab Chow and water were available ad lib. Twelve hr light cycles were automatically regulated, light on from 7 a.m. to 7 p.m. At the time of testing, Wistar weights ranged from 190 to 240 grams and Fisher weights from 165 to 210 g.

Apparatus

The test apparatus consisted of a clear methacrylate cylinder (24 × 38 cm) supported by a stainless steel rod

[1] This work was supported in part by grant number 1R01-DA-00356-01 awarded to the senior author by NIMH.
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grid floor (0.8 cm dia, 1.5 cm centers). Shock was delivered by a constant voltage variable shock source and pulsed at 12.5 pulse/sec. Shock was automatically delivered for a 200 msec duration with a 20 sec interhsock interval. A 2 sec duration remote warning light (not visible to test animals) cued the observer beginning 1.8 sec before shock onset. The apparatus was indirectly illuminated by a 25 W bulb suspended over the semiopaque cylinder top (the sole light source in the testing room).

Procedure

Testing was conducted uniformly between 9 a.m. and 4 p.m. Observers had no knowledge of animal treatments before testing. The animal was introduced into the cylinder and allowed to adapt to the apparatus for 2 min during which time the total vertical behavior (rearing and grooming) was timed. A single starting shock of 0.4 mA was delivered to reduce subsequent spontaneous motor activity and provide a more uniform response set across animals for subsequent low-level shock presentation. A series of 5 successive shock presentations was delivered at each level in an ascending order of shock intensity until a target threshold for all behavioral response categories was achieved. A 50% shock threshold was interpolated from the shock level at which a response category, or its more intense successive category, was observed for three (60%) or more of the 5 presentations at that level. Thus, independent thresholds were determined for flinch, vocalization, and 3 other response categories (described below), and a measure of vertical behavior was taken for each animal in a single session lasting approximately 20 min. The full scale of shock levels was 30, 50, 70, 90, 120, 150, 180, 210, 250, 290, 320, 370, 420, 470, 520, 570, 630, 690, 750, 810, 880, 950, 1050, 1150 μA. Shock levels above 520 μA were seldom encountered except when specific drugs caused extreme analgesic effects.

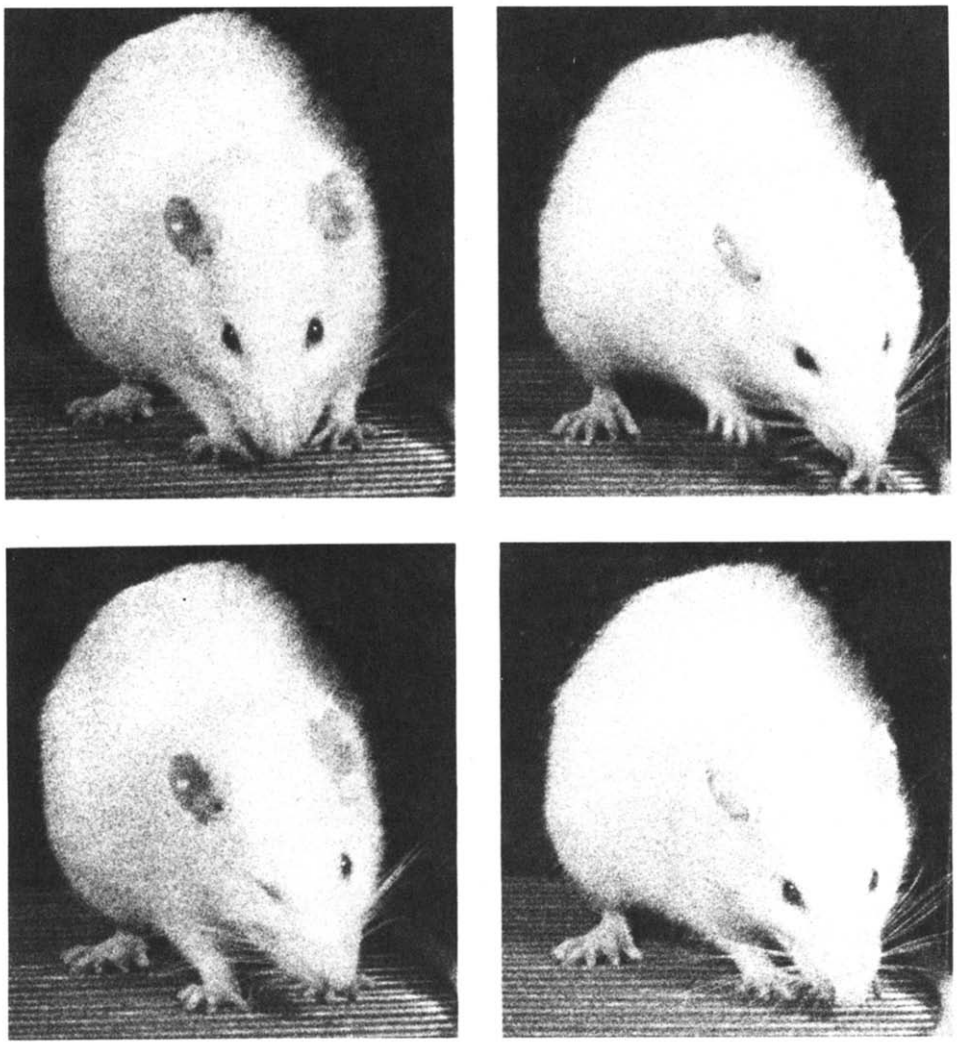

FIG. 1. Sequential execution of Response Category 2. Top left: Onset of 0.2 sec duration shock. Bottom left: Early response to shock perception. Top right: Right front paw flexion. Bottom right: Return to resting posture.

RESULTS

Behavioral responses to inescapable foot shock were recorded on film (24 frame/sec) and by videotape recordings. Still-frame analysis of motor responses to a 200 msec duration foot shock verified that all shock-induced responses of interest lasted less than 200 msec following shock onset. Secondary responses such as running, bar biting, and rearing or jumping occurred rarely, except at high shock levels, and always followed initial rapid responses of the following types.

Flinch at lower shock levels was verified to consist of crouch, startle, or any rapid body response distinguishable from ongoing physiological activity and not attended by elevation of any paws (Response Category 1). Ambiguity was encountered only in instances of observable flexion of the scrotal area in males, which often occurred in response to extraneous noises. At somewhat higher shock levels, the flinch response was replaced by the rapid elevation of a single paw (usually one forepaw). Elevation of one or two paws was treated as a single response category (Category 2). This response, rarely accompanied by vocalization or followed by running, is illustrated in Fig. 1. Occasionally, rear paw elevation was seen instead of front paw movement in one or two trials for a given animal. Subsequent shock increments frequently elicited successive or simultaneous elevation of two paws as a rapid response.

One or two paw elevation was succeeded in intensity of response by a response consisting of the elevation of three or more paws in a variety of configurations, usually composed of two distinct movement components, or by the direct vertical elevation of all four paws simultaneously (Response Category 3). Running was not a part of this response but infrequently followed the completion of the rapid sequence. The response is depicted in Fig. 2 as it is most commonly encountered. Although a variety of two-component movement configurations is seen, none appears to be a more intense response or to occur at higher shock levels than any other. The majority of response configurations that are observed closely resemble those in Fig. 2.

The most intense motor sequence occurring in rapid response to shock consisted of a three-component tetany or rapid oscillation of paws (Response Category 4). Because of the rapidity of this response, detection of it by visual observation alone is sometimes difficult. However, auditory cues from concomitant vocalization or limb-bar noises make this response type easily distinguishable (Fig. 3). Placement of the grid floor at observer's eye level permitted unobstructed view of the animal's paws, and mirrors behind the grid frame permitted maximal observation of limb movement and aided in the visual resolution of Response Category 4. An experienced observer can reliably detect Response Category 4 occurrence even in the absence of vocalization cues. This three-component movement was related to the number of shock pulses detected by the animal during the 200 msec duration shock delivery at high levels.

Analysis of responses at higher shock intensities revealed the execution of 2 or 3 of the above responses in very rapid succession; this complex of responses may be related to the frequently reported running or agitation. The order of the responses observed appeared to be related more to the number of ascending shocks experienced than to the absolute value of the shock.

A description of response categories that can be reliably observed and their mean 50% shock thresholds are presented in Table 1. Also given are the interrater reliabilities for two trained observers independently rating 20 animals. Generally, a naive observer is capable of reliably scoring the full complement of behavioral categories after training in only 10 or 12 animal sessions. Figure 4 presents the occurrence of each response category or its higher intensity successor as a function of shock level.

Repeated testing of 15 Wistar animals at 1-hr intervals for 4 hrs was assessed by an analysis of variance for repeated measurems. No significant effect of repeated testing was evident for any of the response categories, although a slight trend toward a lower threshold with successive test sessions was evident for all response categories except Category 1 (Flinch) and Vocalization.

Animals tested on 2 successive days showed good correspondence between days for Categories 2, 3, and 4, with a trend for slightly lower thresholds on the second day. For Response Category 1 and Vocalization, animals showing low initial thresholds evidenced a subsequent increase in threshold; those showing high initial thresholds showed a subsequent decrease. Test–retest correlations for 15 Wistar animals tested on two successive days were 0.652, 0.415, 0.630, 0.689, and 0.586 for Response Categories 1 through 4 and Vocalization, respectively, ($p<0.05$ for all but Category 2).

Paré [12] has reported that the threshold for aversive shock in rats is directly related to body weight. Correlations between body weight and shock threshold for 32 animals were 0.514, 0.269, −0.022, 0.438, and 0.172 for Response Categories 1 through 4 and Vocalization. Whereas Paré used animals ranging from 34 to 500 days of age, the narrow range of male Wistar rats used in the present study revealed good correlations with body weight only for Response Categories 1 and 4 ($p<0.01$ and $p<0.02$, respectively).

The effect of morphine sulfate on the response categories was assessed. Wistar animals were tested 30 min after receiving a subcutaneous injection of sterile isotonic saline (1 ml/kg). The following day, each animal was tested at the same time as on the previous day and 30 min following subcutaneous injection of saline or 2.5 or 10.0 mg/kg morphine sulfate in sterile saline. All animals were injected and tested under a blind procedure. Statistical comparison was made between change in threshold for saline controls and that for the two morphine groups. Results are presented in Table 2.

Response Categories 2, 3 and Vocalization were the most sensitive to the analgesic effects of morphine sulfate. At 2.5 mg/kg, thresholds were significantly elevated for all response categories except Response Category 1. Category 2 was most sensitive, being elevated in threshold by 146%. Categories 3, 4 and Vocalization showed mean percent threshold increases of 117, 47, and 97%, respectively. At 10 mg/kg, the order of sensitivity to morphine sulfate was maintained for the 5 response categories. Thus thresholds for Categories 2, 3, 4 and Vocalization were elevated by 320, 224, 155, and 265%, respectively. However, Category 1 was also significantly analgesic at this dosage with a mean percent threshold elevation of 42.5%.

Albino rats of the Fisher strain were studied to assess cross-strain validation of response categories and to make interstrain comparison of morphine sulfate-induced alterations in response category thresholds. Table 3 lists response category thresholds for male 50-day-old Fisher rats. Comparison of these with control data for Wistar rats (Table 1) shows that the Fisher animals have lower thresh-

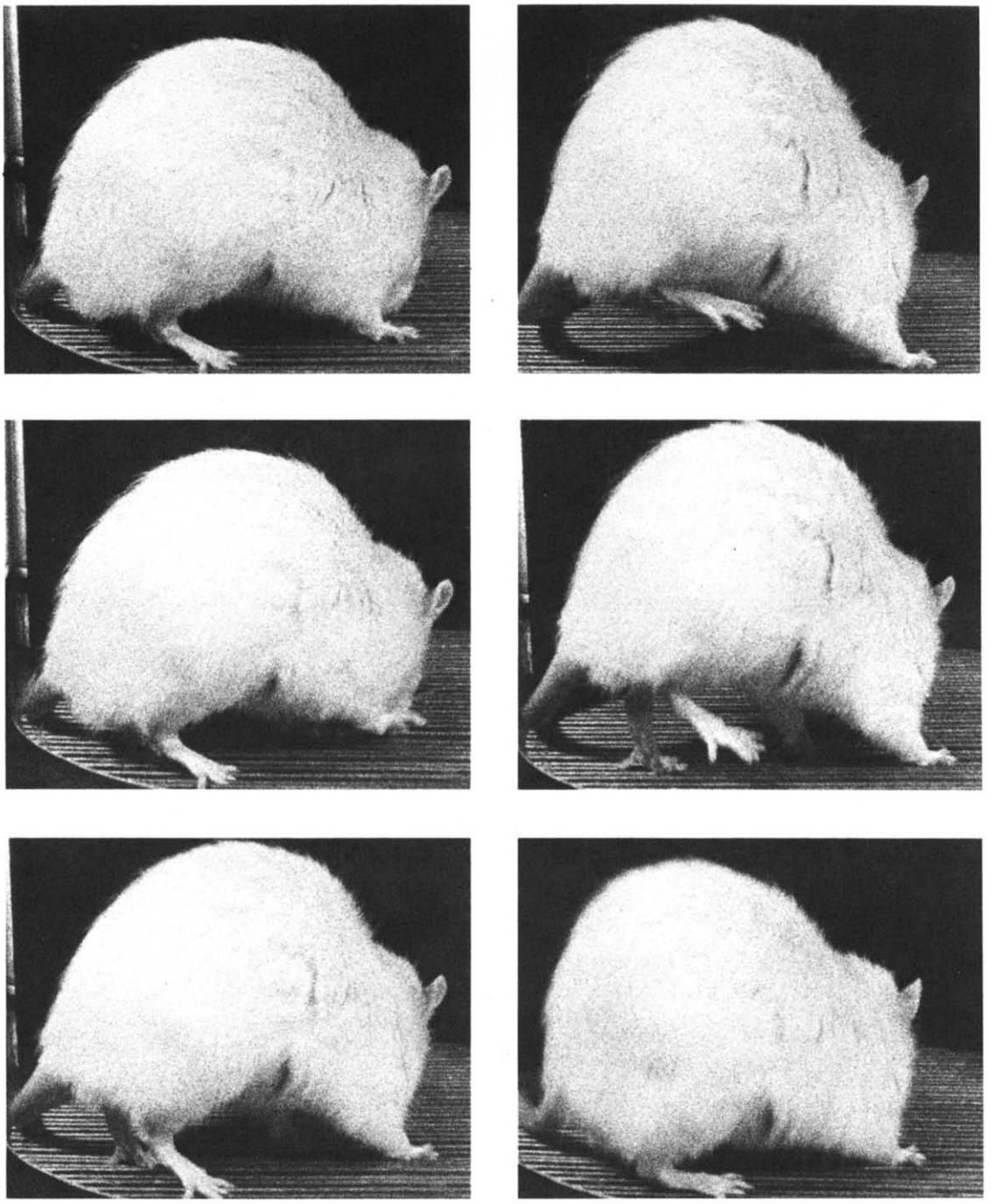

FIG. 2. Sequential execution of Response Category 3. Left column: Shock onset followed by front paw flexion and subsequent rear paw extension. Right column: Continued rear paw extensor response resulting in elevation of hindquarters, followed by return to resting posture.

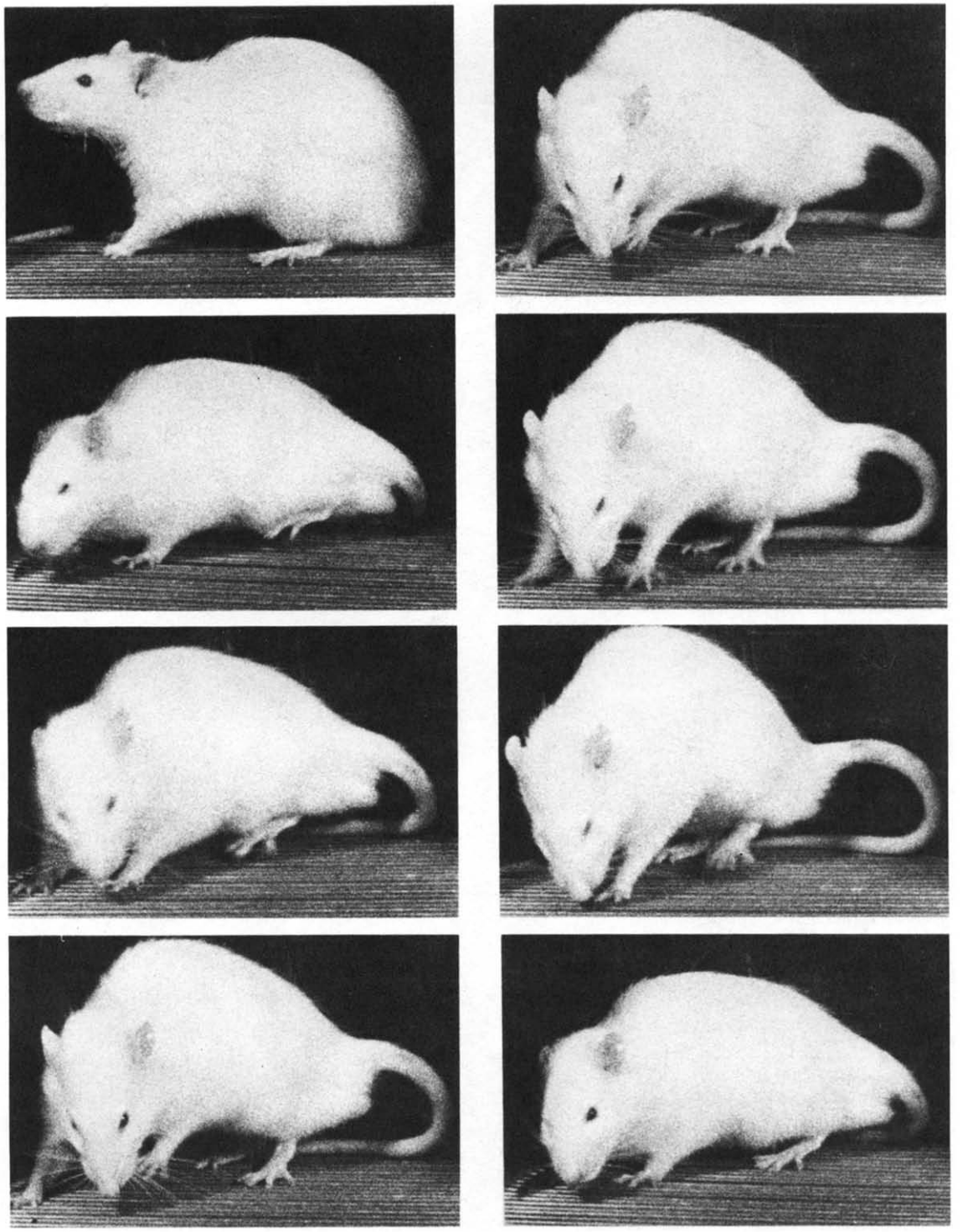

FIG. 3. Sequential execution of Response Category 4. Left column: Resting posture gives way to rear paw elevation with subsequent front paw elevation. Right column: Completion of front paw elevation, followed by a second rear paw elevation and final resumption of the resting posture.

TABLE 1

CHARACTERISTICS OF RELIABLY ELICITED RESPONSE CATEGORIES, BASED ON A REPRESENTATIVE SAMPLE OF 20 MALE 50-DAY-OLD WISTAR RATS

Category	Response	Threshold (μA ± SEM)		Interrater Reliability
1	Flinch, crouch, or startle	56.6	(3.33)	0.953
2	Elevation of one or two paws only	118.3	(17.6)	0.999
3	Rapid movement of three or more paws	198.3	(17.4)	0.998
4	Rapid oscillation or tetany of opposing paw pairs	451.6	(69.0)	0.997
Vocalization	An audible cry	156.6	(23.4)	0.999

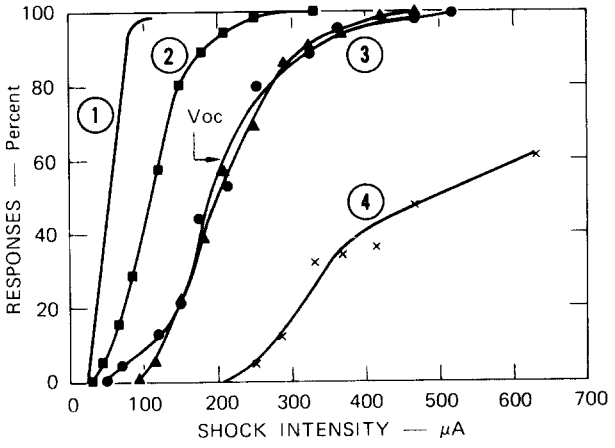

FIG. 4. Cumulative frequency of occurrence of each response category or its successively higher intensity complement as a function of foot shock intensity (———Category 1; ■ ——■Category 2; ▲ ——▲ Category 3; ● ——● Vocalization; X ——X Category 4).

TABLE 2

EFFECT OF MORHPINE SULFATE ON BEHAVIOURAL RESPONSE CATEGORY THRESHOLD FOR MALE, 50-DAY-OLD WISTAR RATS*

Response Category	Drug		
	Saline	Morphine 2.5 mg/kg	Morphine 10.0 mg/kg
N	15	14	14
1	94.3	106.8	142.5†
2	118.8	246.8‡	419.9‡
3	106.3	217.6‡	324.0‡
4	111.1	147.4†	255.5‡
Vocalization	99.3	197.3†	365.7‡

*Figures are mean percentage of control test threshold. Changes were tested by the *t*-test for matched pairs.
†$p<0.02$
‡$p<0.001$

TABLE 3

REPRESENTATIVE RESPONSE CATEGORY THRESHOLDS (μA) AND PERCENTAGE CONTROL THRESHOLD (% C) FOR MALE, 50-DAY-OLD FISHER RATS*

Response Category	Saline	Drug			
		Morphine			
		2.5 mg/kg	% C	10.0 mg/kg	% C
N	8	6		6	
1	48.0	42.2	87	59.0	123
2	85.6	84.2	98	185.8‡	217
3	121.0	220.3†	182	351.3‡	290
4	435.0	562.6†	129	712.8‡	163
Vocalization	181.5	252.0†	138	405.3‡	223

*Figures are mean 50% thresholds in μA. Differences were tested by *t*-test for matched pairs.
†$p<0.05$
‡$p<0.01$

olds to Response Categories 1, 2, and 3 but equivalent thresholds for Category 4 and Vocalization.

Since correlations between body weight and thresholds for Response Categories 1 and 4 were evident for Wistar animals, it seemed possible that lower shock thresholds in Fisher animals were attributable to their inherently lower body weights for a given age. However, linear regression lines relating Wistar body weight and thresholds did not predict Fisher thresholds for any Response Category. Moreover, regression of response category thresholds on Fisher body weights bore no relationship to the slope of the regression lines for Wistar animals. Correlations for Fisher body weights were 0.289, 0.435, 0.253, 0.242, and 0.204 to thresholds for Response Categories 1 through 4 and Vocalization. Only the correlation between Fisher body weight and Response Category 2 threshold approaches statistical significance.

Table 3 also presents thresholds for Fisher animals given 2.5 mg/kg or 10.0 mg/kg morphine sulfate 30 min before testing. At 2.5 mg/kg Fisher animals are not altered on Response Categories 1 and 2, whereas Wistar animals showed 6.8 and 146.8% elevation in threshold for these categories. In Categories 3, 4 and Vocalization, Fisher thresholds were elevated by 82, 29 and 38%, respectively, whereas the Wistar thresholds were more highly elevated. At 10 mg/kg both strains of animals show analgesic effects in all response category thresholds, although the percentage of increase was less in Fishers than in Wistar. Thus, Fisher thresholds were elevated by 23, 117, 190, 63 and 123% for Response Categories 1 through 4 and Vocalization, respectively (all s,$p<0.01$ except for Response Category 1 which was nonsignificant). In Wistar animals the effect of morphine sulfate was most pronounced in Category 2 and Vocalization. In Fishers the greatest effect was in Category 3 and Vocalization. At 10 mg/kg only Wistar animals were significantly analgesic in Response Category 1, although the Fisher animals showed a similar trend.

Data were transformed to percent of control threshold to circumvent nonadditivity effects in the analysis of variance for repeated measures. Significant dose, $F(1,20) = 29.8$, $p<0.001$, strain, $F(1,20) = 11.1$, $p<0.005$, and dose × strain interaction, $F(1,20) = 5.1$, $p<0.05$, effects were evident. In addition significant category, $F(4,80) = 16.5$, $p<0.001$, dose × category interaction, $F(4,80) = 6.5$, $p<0.001$, and strain × category interaction, $F(4,80) = 8.4$, $p<0.001$, effects were found. The three-way interaction of dose × strain × category was not significant.

DISCUSSION

The jump-flinch technique, as employed by other investigators and as reported here, provides an alternative to the tail-flick procedure [3]. It retains the advantages of good sensitivity to narcotic and nonnarcotic analgesics and the measurement of simple responses to a nociceptive stimulus. In addition, the technique allows for free movement of the animal and for the systematic recording of all behavioral responses. Multiple levels of nociceptive stimulation may be studied in a single animal session, and stimulus duration and intensity may be more discretely applied.

The response categories derived from observations of the components of motor responses to inescapable foot shock permit the determination of a behavioral response profile. It is likely that each of these response types involves different central nervous system structures mediating rapid responses to foot shock. Because of the distinct resemblance of these response categories to the behaviors elicited by shock to the rat tail root, it is tempting to equate the nervous system structures involved.

The flinch response is a locally mediated spinal reflex. This reflex is elicited by peripheral local stimulation or direct trigeminal nerve stimulation [2]. It is likely that alterations in thresholds of spinally mediated reflexive responses such as the flinch responses are effected through the influence of lesions or drugs acting on supraspinal structures which exert inhibiting influences on nociceptive spinal reflexes [2]. Lesions of the mesencephalic reticular

formation elevate the threshold of this response, and lesions of the anterior thalamus eliminate the low-level analgesic effects of morphine on this response. Lesions of rat brain ventromedial hypothalamus (in males) or medial forebrain bundle (in either sex) lowers flinch as well as jump and vocalization thresholds [4]. The results presented here indicate a significant dose-related inhibition of such reflexes by systemic morphine sulfate. The lack of sensitivity to alterations in threshold by Evans [6] and Tenen [14] may relate to their unusually stringent criteria for threshold, the duration of the test period, and the recent observation in this laboratory that the peak analgesic effect of morphine on the flinch response develops later than for any other response category described (unpublished observations). Hence, the threshold for flinch is probably changing with successive trials in an ascending-descending alternating shock series paradigm.

Responses entailing the elevation of paws from the grid during electric shock have been reported in a variety of configurations as the criterion for jump responses. Few authors have used the elevation of one or two paws only (Response Category 2) as a behavioral criterion. Doty and Forkner [5] include the elevation of one or two front paws as part of the flinch response criterion; however, this motor component was pooled with "response failure" and no data was given regarding two paw elevation responses during footshock. Tilson *et al.* [16] employed a jump criterion which included one or two paw elevation in combination with vocalization; this composite criterion showed threshold changes sensitive to morphine analgesia, tolerance development and hyperalgesia accompanying abstinence.

A criterion for jump responses frequently reported is the elevation of the rear paws from the grid, possibly corresponding to Response Category 3 in many instances. The threshold for this response has been shown to be lowered by chronic lithium chloride treatment. This effect is reversed by administration of the serotonin precursor 5HTP [8]. Lesions of the dorsomedial tegmentum, septal nucleus, medial forebrain bundle or male ventromedial hypothalamus also lower the shock threshold for this response. Lesions of the ventrolateral tegmentum may produce a transient elevation in threshold for this response [11].

Composite and less well defined criteria have been reported for dichotomizing jump and flinch responses. Frequently these criteria include elevation of the rear paws or running. Though useful, these criteria are insufficiently discrete to allow the reader to assess the range or qualitative nature of responses actually observed. These criteria may be comparable to Response Category 3 or 4, depending on the actual nature of the response components elicited. Such criteria have been reported to show elevated thresholds with analgesic compounds and selective antagonism of the narcotic analgesics by prior depletion of brain serotonin levels [14,15]. Lesions of the septal nucleus significantly increase the occurrence of this response category to foot shock at moderate intensities [5].

Vocalization has been systematically observed by only a few authors in the assessment of responses to foot shock. The vocalization response during foot shock is a vagal reflex, probably mediated at the medulla [1]. Dennis determined vocalization thresholds in male and female animals [4]. While males had higher threshold than females, lesions of the male VMH lowered the male threshold to approximately that of the female. No lesion effect was seen in the female. MFB or septal lesions lowered vocalization thresholds for both sexes. Lateral amygdaloid lesions elevate thresholds for vocalization to electroshock and eliminate the analgesic effect on motor responses following administration of 10 mg/kg morphine sulfate. The importance of this response in the measurement of responses to noxious stimulation [1] and the effects of analgesics indicates that valuable data may be obtained from simply recording vocalization as it occurs in the testing procedure.

Most response criteria employed in the above studies have been variously sensitive to the effects of brain lesions or pharmacological treatments. Standardization of elicited responses in the jump-flinch procedure would simplify replications by other investigators. The determination of thresholds for all response categories reported here permits the construction of a behavioral response profile which may be useful in differentiating between treatment levels or between effects of differing treatments. Determination of thresholds for one or more response categories may permit selection of an optimal end point in assessing a treatment effect, or in differentiating treatment effects in a specific strain of rats.

Strain differences in the response to morphine-induced analgesia have been reported recently for mice. Gebhart and Mitchell [8] selected mouse strains having different levels and turnover rates of brain 5-hydroxytryptamine (5-HT). The native pain sensitivity of CFI and CFW strains did not differ, but the CFI strain having lower brain 5-HT levels was considerably more sensitive to morphine-analgesic activity than the CFW strain. No differences in tolerance development were found between these strains. Rosecrans and Schechter [16] have reported that Fisher strain rats have significantly higher levels of telencephalon 5-HT and lower estimated 5-HT turnover rates than Sprague Dawley rats. It is not known currently whether these differences hold between Fisher and Wistar animals. If brain 5-HT levels of the Wistar animals are comparable to those of the Sprague Dawley animals, the difference in morphine analgesic response between Fishers and Wistars may reflect that observed between CFI and CFW mice and is counter to the relationship between morphine analgesia and brain 5-HT levels reported by several authors [9,15].

Since the work reported in this paper was completed, a report by Tilson and Rech [17] appeared indicating a relationship between Sprague-Dawley and Fisher rats similar to that reported here for Wistar and Fisher. The Fisher strain had lower jump-flinch shock-thresholds and were less responsive to morphine treatment. Pretreatment with the serotonin depletor parachlorophenylalanine attenuated morphine-induced analgesia in Sprague-Dawley but not in Fisher animals.

The sensitivity of the multiple response category procedure for assessing shock sensitivity to modulation by drug treatment is indicated in part by the differential elevation of thresholds by 2.5 mg/kg morphine sulfate in Wistar rats, and the elevation of all thresholds by 10 mg/kg. Repeated testing at hourly or daily intervals does not appear to alter significantly thresholds of any of the response categories recorded in this study. In another series of studies under way, a differential response profile is evident for analgesic effects of morphine and Δ^9-tetrahydrocannabinol. Finally, differential effects of morphine injected into various subcortical structures currently are being assessed as they are reflected in the response category profile.

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