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Stimulus-Response Relationships in a Quickly Learned Escape From Shock: Effects of Morphine

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BABBINI, M., M. GAIARDI AND M. BARTOLETTI. *Stimulus-response relationships in a quickly learned escape from shock: Effects of morphine.* PHARMAC. BIOCHEM. BEHAV. 11(2) 155–158, 1979.—The relationship between stimulus intensity and analgesic effectiveness of morphine was investigated by means of an operant technique. Various doses of morphine were tested in rats trained to press a bar to escape from shocks of varying intensity. Under control conditions a good linear relationship between the log of the stimulus intensity and the log of the speed to press the lever was found. Morphine showed inhibitory effects upon this escape behavior, which were greater at any given dose with greater intensity of the shock. These effects were dose related, that is, the slopes of the shock-response lines decreased by increasing the dose. The data obtained do not appear to be a consequence of a general depressant effect of the drug upon behavior and are in line with several experimental observations showing that in animals, as well as in humans, the magnitude of the analgesic effect of morphine tends to increase as pain severity increases.

Escape from shocks of different intensity Morphine Analgesia

IN SPITE of the many pharmacological tests currently used to study the analgesic properties of narcotic drugs in animal experimentation, few works have investigated the influence of stimulus intensity upon the analgesic effects of morphine and morphine-like compounds [7, 11, 12]. This is especially true for the procedures which make use of an operant response to measure analgesia. In the most popular of these procedures, the shock "titration" method, an electric shock is continuously delivered to rats or monkeys. The intensity of the shock is adjusted by an add-subtract device so that animals have to press a lever to reduce the intensity by a fixed amount. Failure to press the lever will cause the shock to be periodically increased by the same amount. In this manner the animals are able to modulate the level of shock received and will maintain it at a tolerable intensity which is taken as a titration threshold. This threshold has shown to be significantly raised by narcotic [9,19] and non-narcotic [17] analgesics.

Apart from methodological difficulties, the tests which employ an operant response to measure an analgesic effect seem to be nearer than others to clinical situations since the response to a painful stimulus in humans is a complex and a learned one. The titration procedure however does not investigate the relationship between stimulus intensity and analgesic effectiveness, which can be very important as pointed out by various authors [6,16] for a more complete understanding of analgesic action. Because of this in the present work a bar pressing escape from shocks of varying intensities was used to examine the analgesic effects of morphine. The method is similar to the shock titration procedure since an operant response is required to get rid of a noxious stimulus but, unlike the titration method, it measures the latency of the response to each intensity of shock given to the animal.

METHOD

Animals

The animals were male albino rats of the Sprague-Dawley strain (Nos Farm) weighing about 400 g. They were housed four to a cage in a room maintained on a 12 hr day-night cycle with constant temperature (21 ± 1°C). Food and water were always available in the home cages.

Apparatus

The apparatus consisted of four conventional operant chambers equipped with a grid floor, two levers located on opposite walls and a light placed directly above each lever. The chambers were housed in sound-attenuating containers supplied with a ventilating fan and a buzzer to provide a low level background noise. The shocks were delivered to the experimental chamber by a constant-current stimulator through a scrambler. Standard electromechanical scheduling and recording equipment was located in an adjoining room. It was used to automatically present the various shock intensities and to record the response latencies to each shock (to the nearest 0.2 sec) as well as the number of lever presses made in each experimental session.

Procedure

Experiment 1. In this experiment, performed to investigate the stimulus-response relationship of a bar pressing escape under control conditions, 20 rats were used. The experiment included three daily sessions. In the first one the rats were placed in the operant chambers and were given a series of 60 shocks (500 μA, constant current) at a variable interval schedule (mean 90 sec). If the rat did not respond the duration of the shock was 20 sec. A response to either one of the levers during this 20 sec period terminated the shock; a lever press outside these periods had no consequence. The pilot light above each lever remained on for the whole session.

The next day the rats were again run on an escape program similar to the preceding one but the maximum length of the shock was 10 sec and the intensity of the shocks was varied.

Nine separate intensities (40, 70, 130, 200, 300, 450, 600, 800 and 1000 μA) were presented in a randomized order and then the whole series was repeated three times. Thus each rat was submitted to 36 shocks of nine different intensities. Twenty-four hours later the rats were again run on the same program but they received an IP saline injection 15 min before starting the session.

Only the animals which had learned the escape response and had shown a good stimulus-response relationship on the second day (i.e., decreasing latencies as the shock levels increased) were used in the saline session.

Experiment 2. This experiment followed the same general methodology described for the previous one except that a series of thirteen separate intensities (35, 55, 80, 110, 160, 220, 290, 380, 490, 610, 740, 870 and 1000 μA) was used. Sixty rats were submitted to the escape program. On the third day the animals showing a good stimulus-response relationship were randomly subdivided into five groups. They received IP saline (13 rats) or 2.5, 5, 10, 20 mg/kg of morphine hydrochloride (5–6 rats for group). Injections were always made 15 min before starting the session.

Data Analysis

Two measures of performance were taken for each animal: (1) average speed (reciprocal latency) to press the lever at each shock intensity; (2) total number of lever presses during the no-shock periods.

The average speed data of both experiments were submitted to the analysis of variance applied to regression (after a log transformation of both the independent and the dependent variables) taking into account that the first experiment followed a repeated measure one factor design while the second one was a 5×13 two-factor design with repeated measures on one factor [18].

The number of lever presses during the no-shock periods of Experiment 2 were log transformed and analyzed by Dunnett's test.

RESULTS

Experiment 1

For thirteen out of 20 rats the speed of lever pressing in response to the electric shock was found to increase with the intensity of the stimulus in a good linear relationship to the logarithm of the μA used.

Individual stimulus-response regression lines differed from rat to rat only in slope and in scattering around the line. Regressions of representative animals are depicted in Fig. 1 together with the average regression line. The slope of this regression was statistically significant ($p<0.01$) while the deviation from linearity was very small and insignificant.

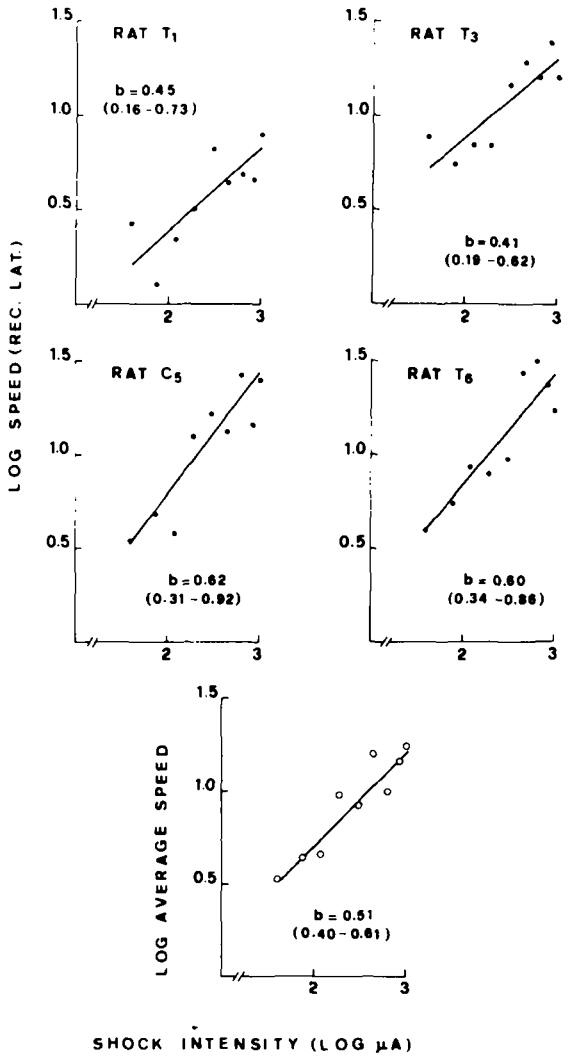

FIG. 1. Stimulus-response regression lines of some representative animals (●) and the average (n=13) regression line (○). For each line the regression coefficient and its 95% confidence limits are shown.

Experiment 2

Thirty-five out of sixty rats showed a good stimulus-response relationship on the second day session. The effects of the treatment of these animals with saline or with various doses of morphine are depicted in Fig. 2. The analysis of variance of these data gave a significant "dose" effect, $F(4,360)=8.92$, $p<0.01$, and "stimulus" effect, $F(12,360)=31.95$, $p<0.01$. The overall dose by stimulus interaction was not significant but the dose by linear component of this interaction was significant, $F(4,360)=3.15$, $p<0.05$, indicating that the slopes of the regression lines at

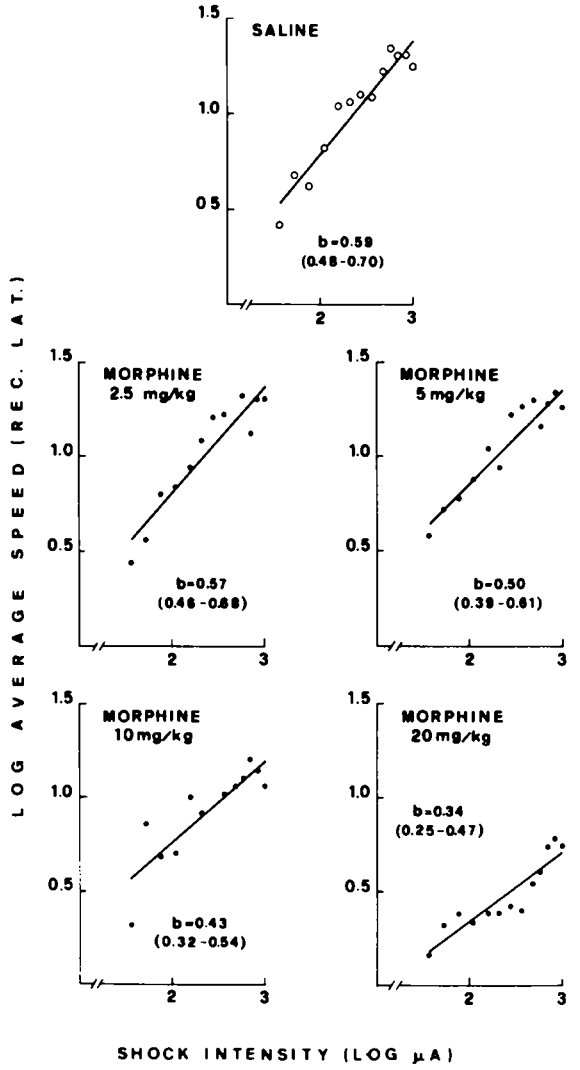

FIG. 2. Stimulus-response regression lines of animals treated with various doses of morphine. Each point represents the mean of 13 (○) or 5–6 (●) scores. For each line the regression coefficient and its 95% confidence limits are shown.

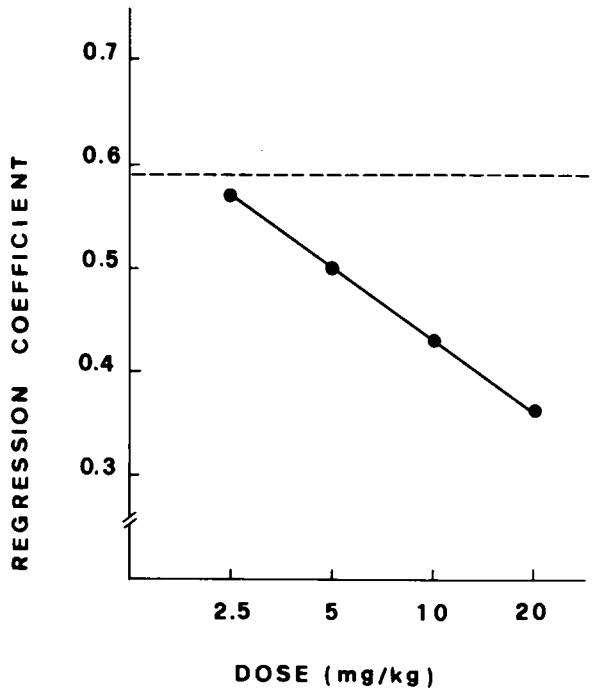

FIG. 3. Relationship between doses of morphine and the regression coefficients of various stimulus-response regression lines. Dotted line refers to animals treated with saline.

TABLE 1

EFFECT OF MORPHINE ON THE NUMBER OF LEVER PRESSES EMITTED OUTSIDE THE SHOCK PERIODS

Drug	Dose mg/kg	n	Number of lever presses (log transformed) mean ±S.E.
Saline	—	13	2.182 ± 0.041
Morphine	2.5	6	2.112 ± 0.089
	5	5	2.186 ± 0.115
	10	6	2.139 ± 0.088
	20	5	1.775 ± 0.188*

*Statistically different from saline by Dunnett's test ($p<0.01$).

various dose levels are different from each other. Further analyses of these slopes showed that, in any case, the linear relationship between the log of stimulus intensity and the log of average speed of bar responses was maintained (see the confidence limits of regression coefficients). Morphine however decreased these slopes in a dose-related way. In Fig. 3, where the regression coefficients are plotted against doses, it can be seen that a very good and highly significant, $F(1,360)=8.08$, $p<0.01$, relationship exists between dosages of morphine and its increasing capacity of retarding the reaction speed of rats as the shock intensity increases.

The number of lever presses emitted outside the shock periods is reported in Table 1. Only at a 20 mg/kg dose did morphine significantly decrease this number, while for the other dosages the scores were very similar to those obtained under saline.

DISCUSSION

The results of Experiment 1 demonstrate that a rat can reliably learn a bar pressing escape from shock in only two days and that the speed of its response is a function of shock intensity. The stimulus-response relationship obtained in this experiment can be adequately described (transforming back the linear regression equation) by a power function of the following form $R=a\ \mu A^b$ where R is the speed to press the lever and a and b are constants. This function has been generally used to explain the relationship between stimulus and response in neurophysiology [4] and a direct relationship between the intensity of the stimulus and the magnitude of the response has been described several times in conditioning experiments [8,10].

The main purpose of the present work, however, was to see if this relationship could be used to study the analgesic effects of morphine. From the results of Experiment 2 it appears that morphine decreased the reaction speed and that, at any given dose, this decrease is proportional to the intensity of the shock used (the slopes of the shock-response line under morphine are always lower than that under saline). Moreover, the magnitude of this decrease is a function of the dose used (see Fig. 3).

When a behavioral technique is used to measure the analgesic action of a drug, any obtained effect could be due to a number of factors (i.e., changes in general stimulus set, recent memory, or motor output) not directly related to the perception of the noxious stimulus. Even though these factors cannot be completely excluded in the present study, some data do suggest that the obtained effects of morphine are not due to a general depressive action of the drug upon behavior. In fact from the results of Fig. 2 it appears that, at any given dose, the starting points of the stimulus-response lines are very similar (except at the 20 mg/kg dose). This indicates that morphine does not appreciably decrease the reaction speed when shocks are very weak but it does so when more intense stimuli are presented. With the highest dose used however the starting point of the stimulus-response line is lower than the other ones suggesting a general depressant effect of the alkaloid in addition to its stimulus-related analgesic action. This is confirmed by the results of the analysis of the number of bar presses during the no-shock periods: only with the 20 mg/kg dose is this number lower than that obtained under saline.

There is some controversy in the literature about the relationship between stimulus intensity and potency of the analgesic effect of opiates. Using a tail-flick procedure Gray and coworkers [6] found that the potency of morphine and meperidine remained constant irrespective of stimulus strength. Since a ratio of treatment to control reaction times was used to measure the analgesic effect their results mean that the effects of the two drugs were greater with greater intensity of the stimulus.

Granat and Saelens [5] however, using the same procedure and the same index of analgesic effect, reported that the potency of meperidine varied inversely with the intensity of the heat source and similar results were obtained by O'Callaghan *et al.* [11] for morphine using the hot plate method.

The results obtained in the present work are well in keeping with those of Gray *et al.* [6] and also agree with several experimental studies in humans [13,14] which pointed out that morphine effects tend to increase as pain severity increases.

A final point is worth noting. As stated in the introduction, among the many algesimetry methods, those which make use of an operant response to measure effects of narcotics that are useful to predict clinical analgesia can present certain theoretical advantages. The shock titration method however has been criticized on various grounds [1, 3, 15]. Furthermore it gives only a measure of pain threshold and it requires a long animal training period. The method employed in the present work possesses the important features of being quick and of exploring the stimulus response relationship over a large range of shock intensities.

If the present results can be confirmed and extended to other compounds, the method might constitute an useful tool in the experimental evaluation of narcotic analgesics.

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