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Analgesic Tolerance Produced by Morphine Pellets is Facilitated by Analgesic Testing

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ADVOKAT, C. *Analgesic tolerance produced by morphine pellets is facilitated by analgesic testing.* PHARMAC. BIOCHEM. BEHAV. **14**(2) 133–137, 1981.—Analgesic tolerance induced by morphine pellets was examined in rats using the nociceptive tail flick reflex. Analgesic responses of animals who received preliminary tail flick tests after morphine implantation were significantly lower than responses of naive, nontested animals. Previously tested animals were also significantly more tolerant to a morphine challenge than nontested animals. A dose response curve to morphine was not obtained, at the doses used here, from previously tested animals, whereas naive animals responded to morphine in a dose dependent manner. Environmental modulation of the tail flick reflex represents an elementary form of behavioral plasticity which may prove useful for neural analyses of simple reflex systems.

Tolerance Opiate analgesia Tail flick

ALTHOUGH the tail flick response has been used extensively in analgesia research, the plastic capacities of this spinally mediated reflex have not received much attention. Yet, the fact that the tail flick undergoes behavioral tolerance to narcotics suggests that it may prove useful in analyses of brain behavior relationships. This possibility is especially appealing in view of increasing interest in spinal mechanisms of opiate action [13, 14, 15]. Knowledge of the concomitant behavioral capacities of this elementary reflex may permit correlation with emerging neurophysiological and neurochemical concepts, not only concerning opiate analgesia and tolerance but other forms of behavioral plasticity as well.

These studies are part of a systematic investigation of environmental influences on opiate analgesia and tolerance utilizing the tail flick withdrawal reflex as a behavioral nociceptive index.

METHOD

Subjects

A total of 109 naive, male albino Sprague-Dawley derived rats (King Laboratories, Oregon, WI) served as experimental animals. The rats weighed 225–250 g at the beginning of each experiment and were housed four to six to a cage with ad lib access to food and water. The animals were housed in the University vivarium (Biologic Resources Laboratory) in a single room, which was on a 14:10 LD cycle (5:00 a.m.–7:00 p.m. light).

The laboratory was located five floors above the vivarium in a different building. When necessary, subjects were transported to the laboratory, where the tail flick (TF) apparatus was located, or, the TF apparatus was brought to the vivarium. There were usually several neurophysiological experiments being conducted in the laboratory, which, in general provided a more stimulating environment relative to the rather quiet vivarium conditions.

Drugs

For acute administration morphine sulfate was dissolved in 0.9% saline and injected subcutaneously in a volume of 0.1 ml per 100 gram of body weight.

The method of Way *et al.* [12] was used for the preparation of morphine and placebo pellets. Tolerance was induced by the subcutaneous implantation, under ether anesthesia, of a single morphine pellet, containing 75 mg of morphine base, under the dorsal skin surface.

Analgesia Test

The tail flick technique was used to assess nociceptive thresholds and morphine analgesia. Tail flick latency was automatically recorded and was defined as the elapsed time between onset of a high intensity light beam focused on the

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tail and the reflex withdrawal (flick) response. Each test consisted of the mean score of three successive trials. For each trial the tail was replaced on the apparatus so that a different patch of skin was stimulated. To avoid excessive injury, a cut-off value of 14 sec was automatically imposed on the response.

PROCEDURE

Experiment 1

A total of 30 rats were divided into two groups. One group (N=18) received a morphine implant (Morphine) while a second group (N=12) received a placebo implant (Placebo). All pellets were implanted between 9:00–10:00 a.m. in the vivarium where the animals were housed. One half of the animals in each of the two groups were brought to the laboratory where they received a tail flick test at 3, 24 and 48 hours after their implant (Tested condition). The other half of the animals in each of the two groups remained in their cages, undisturbed for 48 hours (Nontested condition). At this time they received their first exposure to the laboratory and their first analgesic test. Immediately after their 48 hr test all animals received a 7.5 mg/kg subcutaneous (SC) injection of morphine, followed by a final analgesic test one half hour later.

Experiment 2

Three groups of rats (N=9 in each case) were implanted with morphine pellets in the vivarium between 9:00–10:00 a.m. One group was tested in the vivarium at 3, 6, 12, 24 and 48 hrs after the implant (Tested group). A second group received its first tail flick test at 48 hours post implant; this test was also conducted in the familiar environment of the vivarium (Nontested group). The third group also received its first test 48 hours post implant; however, for this group the test was conducted in the novel laboratory environment (Nontested-Novel group). After the 48 hr analgesia test each animal received a 7.5 mg/kg SC morphine injection followed by a final test one half hour later.

Experiment 3

A total of 52 rats were implanted with morphine pellets in the vivarium between 9:00–10:00 a.m. One half of these animals were brought to the laboratory where they were tested at 4, 8, 24 and 48 hours post implant (Tested group; N=24). At 48 hours post implant the second half of the animals were brought to the laboratory where they received their first test (Not Tested group; N=28). Following the 48 hr test each of the two main groups were divided into 3 subgroups. Each subgroup received a SC morphine injection of either 2.5, 5.0 or 7.5 mg/kg prior to a final test one half hour later. This experiment was conducted twice with approximately half of the data collected each time.

During the course of the three experiments the pellets were not removed. However, after Experiment 2 was completed the animals were sacrificed and all pellets were recovered. All data were collected by the author, who was, therefore, aware of the respective group treatments.

All data were analyzed by non-parametric statistical tests [8]. Comparisons among groups were made with the Kruskal-Wallis one-way analysis of variance; differences between groups were evaluated with a Mann-Whitney U Test; within group comparisons by the Wilcoxin test.

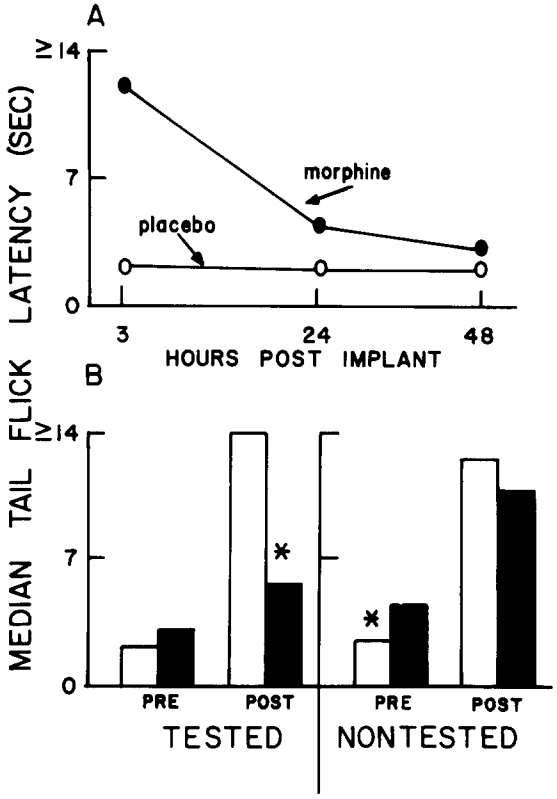

FIG. 1. (A) Median latency of tail flick at several intervals after implantation of a morphine pellet containing 75 mg of morphine (filled circles; N=9) or a placebo pellet (open circles; N=6). (B) Median latency of tail flick before (PRE) and after (POST) a subcutaneous injection of 7.5 mg/kg of morphine. All animals were implanted 48 hours previously with either a morphine pellet (filled bar) or a placebo pellet (open bar). One half of each group (TESTED), shown in Part A, had been tested on the tail flick prior to the morphine injection, the other half (NONTESTED) had not. *$p<0.025$, one-tailed.

RESULTS

The results of the first experiment are summarized in Fig. 1. In part A of the figure are the median tail flick latencies of the Placebo-Tested and Morphine-Tested groups during the post implant analgesic tests. On the first test, 3 hours after the implant there was no overlap between the two groups ($U=0$; $p<0.001$), indicating substantial analgesia of the morphine implanted animals. However, the groups no longer differed on the third test 48 hours post implant ($U=14$; NS). Furthermore, as shown in Part B, the Placebo-Tested group was significantly more analgesic than the Morphine-Tested group after a morphine challenge given 48 hrs post implant ($U=9$; $p<0.025$). These results demonstrate tolerance in the Morphine-Tested group.

In contrast, there was still a slight, but significant difference in latency between the Placebo-Nontested and Morphine-Nontested groups on their first test at 48 hours ($U=9.5$, $p<0.025$). Moreover, these two groups did not differ in their analgesic response to the acute morphine injection. Therefore, the Morphine-Nontested group was still not tolerant 48 hours after implantation.

However, the Morphine-Nontested group did show a relatively decreased response to the morphine challenge

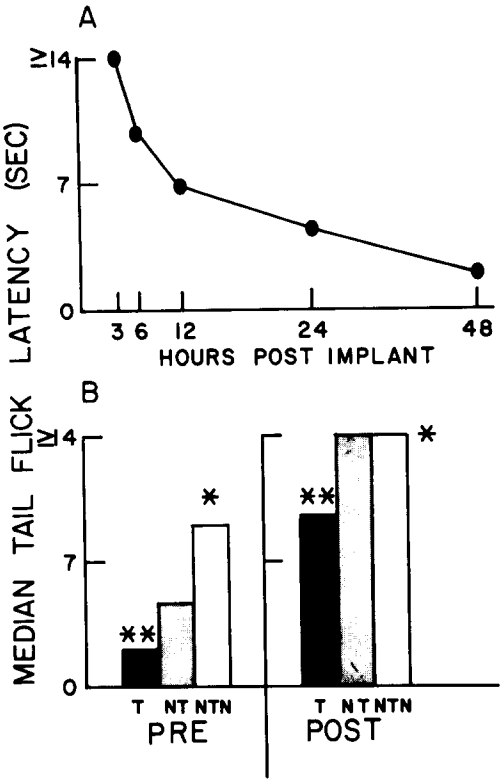

FIG. 2. (A) Median latency of tail flick at several intervals after implantation of a morphine pellet containing 75 mg of morphine (N=9). (B) Median latency of tail flick of three groups of rats before (PRE) and after (POST) a subcutaneous injection of 7.5 mg/kg of morphine, administered 48 hours after implantation of a morphine pellet. Group T (filled bar) had received several prior tail flick tests, shown in Part A; Group NT (stippled bar) was not previously tested on the tail flick; Group NTN (open bar) was also not previously tested on the tail flick, but unlike group NT, was examined in a novel environment, which was different from the environment in which the animals were housed and implanted. *significant difference among the three groups; **significant difference between Group T and each of the other two groups.

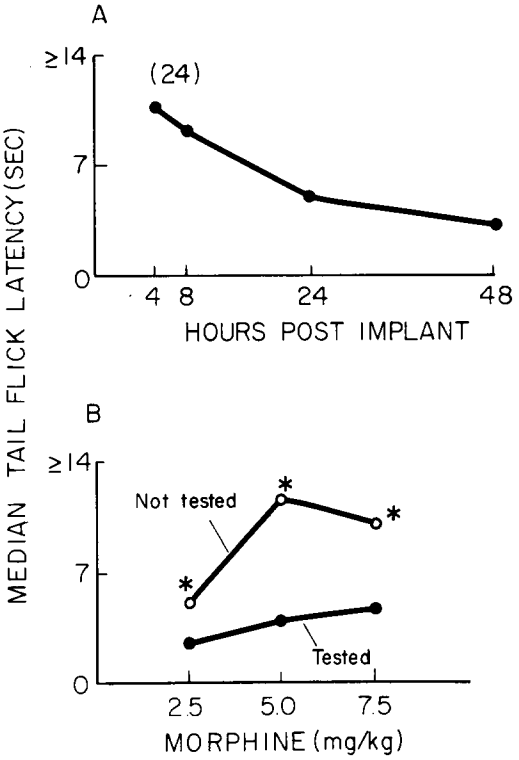

FIG. 3. (A) Median latency of tail flick at several intervals after implantation of a morphine pellet containing 75 mg of morphine (N=24). (B) Median tail flick latency in response to each of three doses of morphine administered 48 hours after implantation of a morphine pellet. Animals in the Tested group received several tail flick tests shown in A, prior to the injection of either 2.5 (N=8), 5.0 (N=8) or 7.5 (N=8) mg/kg SC; animals in the Not-Tested group were tested only once, at 48 hours post implant, prior to the SC injection of 2.5 (N=9), 5.0 (N=9) or 7.5 mg/kg (N=10) of morphine. *$p<0.01$ Tested vs Not-Tested.

compared with its Placebo control. This observation was quantified by subtracting the latency of each animal prior to the injection from the latency obtained after the injection. Statistical analysis confirmed the fact that the difference scores of the Placebo Non-tested group were significantly greater than those of the Morphine-Nontested group ($U=5.5$; $p<0.02$, two tailed). Therefore, in spite of the fact that the Nontested Placebo and Morphine groups did not differ in their absolute analgesic response to a morphine challenge, it was possible to demonstrate some degree of tolerance in the Morphine-Nontested group.

In order to specifically examine the influence of the assessment procedure, the next two experiments compared the effect of analgesic testing only in morphine implanted animals.

The results of the second experiment are shown in Fig. 2. In this study, the tested group received a total of five analgesic tests, shown in part A of the figure. Part B of the figure summarizes the results of the analgesic tests of all three groups at 48 hours post implant, both before and after the acute morphine injection. As indicated in the figure, there was a significant difference among the three groups before the morphine challenge ($p<0.01$). The Tested group was significantly less analgesic than both, the Nontested ($U=17$, $p<0.025$) and Nontested-Novel ($U=3$; $p<0.001$) groups. These latter two groups did not differ from each other.

The overall difference among the three groups was maintained after the morphine injection ($p<0.001$). At this time the Tested group again differed significantly from each of the other two groups ($U=14$; $p<0.01$ in each case). It was not possible to determine whether the Nontested and Nontested-Novel groups differed from each other because so many of the scores within these groups reached the 14 sec cut off point.

At the end of this experiment the pellets were removed and all were found intact. Therefore, the possibility that the analgesic scores of the tested group declined because the pellets had been crushed during the tests, was ruled out.

The third experiment, summarized in Fig. 3, shows the effect of the challenge dose of morphine on behavioral tolerance in Tested and Nontested animals. As seen in Part A of the figure, analgesia had declined in the tested groups by 48 hrs post implant. At this time in agreement with the results of the first two experiments there was a slight but significant difference between the Tested and Nontested animals (3.2 sec vs 5.0 sec; $p<0.0013$).

In part B of the figure it can be seen that the two main groups differed in their response to the morphine challenge. The Tested group did not show a dose response relationship to the morphine challenge ($0.05<p<0.10$) whereas the Not-tested animals did ($p<0.01$). (These results, of course, do not rule out the possibility that higher doses would have produced a dose response curve in Tested animals.) Furthermore, at each of the three doses the previously untested animals were significantly more analgesic than the previously tested rats ($p<0.01$ in each case). Finally, as might have been expected, no withdrawal signs were noted during the course of these experiments. This is not surprising in view of the relatively brief implantation time, and the fact that only one pellet was implanted.

DISCUSSION

Morphine pellet implantation is a common means of rapidly inducing both tolerance and dependence [6,12]. Most studies using this method have been concerned with the neuropharmacological substrates underlying these phenomena. The present experiments provide evidence for the importance of environmental variables in the development of behavioral tolerance due to morphine pellets. Specifically, these results demonstrate that tolerance of the tail flick reflex is facilitated by prior analgesic assessment.

The importance of environmental contingencies in the development of narcotic tolerance has been well recognized; the results of several recent studies have been incorporated into a Pavlovian conditioning model [9,10]. Evidence supporting this model has been obtained from many systems, including the tail flick reflex [1]. In such investigations each drug administration represents a conditioning trial; the opiate is either specifically paired or unpaired with the environmental context in which the pharmacological effect is assessed. Under these conditions, tolerance develops in response to successive drug treatment only in the context with which the drug has been paired.

However, in contrast to acute, intermittent injections, pellet implantation chronically elevates tissue drug levels, at least for the duration used in these experiments [7,11]. As a result, the pharmacological opiate stimulus is not only paired with the context of drug assessment, it is present outside of that context as well. Morphine pellet implantation disrupts the specific temporal association of the drug, with the drug assessment environment. Therefore, facilitation of tolerance by this method appears to be due to the motor and/or sensory components induced by prior testing, rather than a conditioning process. Furthermore, the results of the second experiment indicate that the test procedure itself is the crucial variable. In that study tolerance was primarily determined by the administration of prior tail flick tests rather than the familiarity of the test environment. Although environmental novelty potentiated analgesia in that study, it did not do so to a significant degree (group NT was not statistically different from group NTN).

The simplest explanation of these results is that practice of the withdrawal reflex while under the influence of the drug can improve motor performance relative to unpracticed animals. However there are some weaknesses to this argument. For one thing, the last two tail flick tests were always 24 hours apart, which is a substantial period of time in which to retain such motor improvement.

In addition, the first experiment showed that tolerance developed more rapidly after only two prior tests. On the first of these tests, many animals were so analgesic they did not even make a withdrawal response and so, did not actually practice the reflex. Therefore, the tested animals actually received a minimal amount of practice with the tail flick reflex relative to non-tested animals.

More recent results also argue against an explanation based solely on motor performance [2]. These studies have found that prior exposure to the test procedure and apparatus alone, without the nociceptive heat stimulus (and consequently, the withdrawal reaction) was sufficient to promote behavioral tolerance in this system. Therefore tolerance can be facilitated without prior elicitation of the tolerant response.

The mechanism by which prior practice of and/or exposure to the analgesic test procedure facilitates behavioral tolerance, remains to be seen. One obvious approach is to determine whether analgesic testing modifies tissue levels of the opiate. It is possible, for example, that an emotional response, engendered by the assessment procedure, may trigger a physiological reaction that counteracts opiate analgesia. There is, in fact, ample evidence that environmental stress can modify nociceptive responsivity (see [3, 4, 5] for references). Such modulation could be mediated by endogenous opiate systems. On the other hand, there is no reason to assume that the mechanism responsible for facilitating tolerance is expressed through an opiate system. At present, there is no reason to rule out non-opiate interactions as the bases for this phenomenon. Both possibilities warrant further investigation.

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