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Environmental Modulation of Analgesic Tolerance Induced by Morphine Pellets

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ADVOKAT, C. *Environmental modulation of analgesic tolerance induced by morphine pellets.* PHARMAC. BIOCHEM. BEHAV. **14**(2) 139–142, 1981.—The development of analgesic tolerance to the nociceptive tail flick test was examined in morphine implanted rats. Animals repeatedly exposed to a nonfunctional tail flick apparatus after implantation, were significantly more tolerant than nonexposed animals, on subsequent tests with the functional apparatus. In contrast, prior exposure to an alternate nociceptive, hot plate test, did not significantly modify tolerance on the tail flick. Facilitation of tolerance, produced by prior tail flick assessment, was maintained for at last one week following the last test, but only if the morphine pellet was not removed. If the pellet was removed the influence of prior analgesic assessment was not retained. The substantial plasticity exhibited by the spinal tail flick reflex suggests the utility of this response for investigations of neural correlates of behavior.

Tolerance Morphine Tail flick

THE importance of environmental variables in the mediation of opiate and nonopiate analgesia and tolerance has recently been demonstrated in a number of laboratories (see [4, 5, 7] for discussion and references). In response to a variety of stimuli the relatively simple nociceptive reactions used in these studies have shown considerable modulation. That these elementary behavioral systems are capable of significant adaptation in response to environmental contingencies, suggests that they may provide useful preparations for examining neural correlates of behavioral plasticity. This consideration prompted a series of investigations, using the spinally mediated tail flick reflex as an index of behavioral modulation. Previous reports have already demonstrated that the analgesic response of this nociceptive reflex can be significantly modified by environmental context ([1, 2, 3], see also [8]). The present studies extend these findings and further characterize the profound influence of environmental variables on the behavioral tolerance of the tail flick response.

METHOD

Subjects

A total of 81 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, in the University vivarium (Biologic Resources Laboratory). All animals were kept in a single room, on a 14:10 LD cycle.

Drugs

For acute administration morphine sulphate was dissolved in 0.9% saline and injected subcutaneously (SC) in a volume of 0.1 ml per 100 g of body weight.

The method of Way *et al.* [9] 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 Tests

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 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.

Hot plate tests were conducted with a 5 in. dia. copper plate maintained at 55 ± 0.5°C by a thermoregulated water circulating pump (Precision Scientific Chicago, IL). Animals were placed on the plate and confined by a 6¾ in. high Plexiglas cylinder. Latency to escape was determined manually with a stopwatch. Escape latency was defined as the time from placement until all four limbs were off the plate,

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i.e., until the animal jumped onto the rim of the cylinder. A cut off time of 30 sec was imposed on each hot plate trial.

PROCEDURE

Experiment 1

A total of 17 rats were implanted with morphine pellets (8:30–9:30 a.m.) and randomly divided into one of two groups, Pseudo-Tested (PT; N=8) and Non-Tested (NT; N=9). At intervals of 3, 6, 12 and 24 hours after implantation, Pseudo-Tested animals were removed from their cages in the vivarium and given a pseudo tail flick test. For these trials the nociceptive thermal stimulus was not turned on and none of the animals made a withdrawal response. During these tests the Non-Tested animals remained undisturbed in their cages. At 48 hours post implant all animals were tested on the functional tail flick apparatus. Immediately after this first (PRE) test all animals received a 7.5 mg/kg SC morphine injection followed one half hour later by a second (POST) tail flick test.

Experiment 2

A total of 17 animals were implanted with morphine pellets (8:30–9:30 a.m.) and randomly divided into one of two groups, Alternate-Tested (AT; N=9) and Non-Tested (NT; N=8). At intervals of 3, 6, 12 and 24 hours after implantation, Alternate-Tested animals were removed from their cages and placed on the hot plate. The latency of escape from the plate was manually recorded with a stopwatch. During the hot plate trials the Non-Tested animals remained in their cages.

At 48 hours post implant all animals received their first (PRE) test on the functional tail flick apparatus. Following this test all animals received a 7.5 mg/kg SC morphine injection followed one half hour later by a second (POST) tail flick test. At the end of this experiment an additional group of naive rats (N=10) was placed on the nonfunctional hot plate which was at room temperature (20 ± 2°C). The response of these rats to the hot plate was observed once before and once, 3 hours after, they were implanted with morphine pellets.

Experiment 3

A total of 21 animals were implanted with morphine pellets (8:30–9:30 a.m.) and randomly divided into one of two groups, Tested (T; N=10) and Non-Tested (NT; N=11). Group T received a tail flick test at 3, 6, 12, 24 and 48 hours post implant whereas group NT remained in their cages during this time. Following the 48 hr test all animals were left in their home cages for one week, with their morphine pellets intact. At the end of one week, nine days post implant all animals received a tail flick (PRE) test. Immediately after this test each animal received a 7.5 mg/kg SC morphine injection followed one half hour later by a second (POST) tail flick test.

Experiment 4

A total of 26 animals were implanted with morphine pellets (8:30–10:00 a.m.) and randomly divided into one of two groups, Tested (T; N=12) and Non-Tested (NT; N=14). The procedure followed in this experiment was the same as that of Experiment 3 with one exception: Following the 48 hr tail flick test given to group T, all pellets were removed from both groups. Therefore, all animals were withdrawn from morphine one week before their two final PRE and POST tests. All data were analyzed by non-parametric statistical tests [6], and were collected by the author, who was, of course, aware of the experimental treatments.

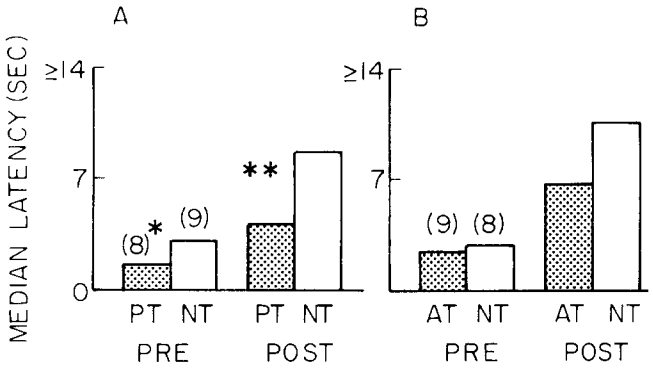

FIG. 1 (A) Median tail flick latency of two groups of rats, Pseudo-Tested (PT) and Non-Tested (NT), after implantation of a pellet containing 75 mg of morphine. The first (PRE) test occurred 48 hr after implantation and was followed by a subcutaneous injection of 7.5 mg/kg of morphine and a second (POST) test 30 min later. Group PT had received prior exposure to the nonfunctional tail flick apparatus at 3, 6, 12 and 24 hr after implantation; Group NT had not previously been exposed to the tail flick procedure or apparatus. *$p<0.01$; **$p<0.05$. (B) Median tail flick latency of two groups of rats, Alternate-Tested (AT) and Non-Tested (NT), after implantation of a pellet containing 75 mg of morphine. The first (PRE) test occurred 48 hr after implantation and was followed by a subcutaneous injection of 7.5 mg/kg of morphine and a second (POST) test 30 min later. Group AT had received prior exposure to a nociceptive hot plate apparatus at 3, 6, 12 and 24 hr after implantation; Group NT received no prior treatment before the tail flick tests.

RESULTS

The results of the first two experiments are summarized in Fig. 1. Part A shows the median tail flick latency at 48 hr post implant of the two groups, PT and NT of Experiment 1. At this time, indicated by PRE in the figure, there was a significant difference between the two groups ($U=10$; $p<0.01$). Group PT was significantly less analgesic than group NT. This difference was maintained after an acute morphine injection ($U=17.5$; $p<0.05$). In response to the morphine challenge the Pseudo-Tested animals were more tolerant than the Non-Tested animals who had not previously been exposed to the tail flick apparatus or procedure.

Part B of Fig. 1 shows the median tail flick latency of the two groups, AT and NT in Experiment 2. The implanted animals of group AT in this study showed minimal analgesia in response to the nociceptive hot plate stimulus. Median latencies of the four hot plate tests at 3, 6, 12, and 24 hours post implant were 5.5., 3.0, 3.0, and 3.5 sec, respectively. However, all responses to the ambient temperature plate were greater than 30 sec, both before and after morphine pellet implantation. Therefore, the hot plate test latencies reflect an escape response from the thermal stimulus rather than nonspecific exploratory behavior.

In contrast to the results of the first experiment the two groups in this study did not differ in tail flick latency, either

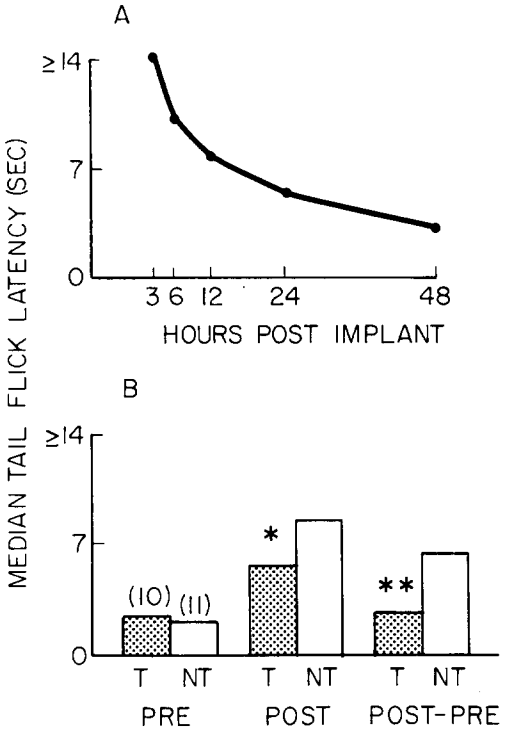

FIG. 2. (A) Median tail flick latency of rats tested at 3, 6, 12, 24, and 48 hr after implantation of a pellet containing 75 mg of morphine. (B) Median tail flick latency of two groups of rats, Tested (T) and Non-Tested (NT), before (PRE) and after (POST) a subcutaneous injection of 7.5 mg/kg of morphine. All animals were implanted nine days previously with a morphine pellet. Group T had received several prior tail flick tests, shown in Part A, after pellet implantation; Group NT was not previously tested. The change in latency of each group from the PRE test to the POST test is shown in the last two bars of the figure (POST-PRE). $*p<0.05$; $**p=0.025$.

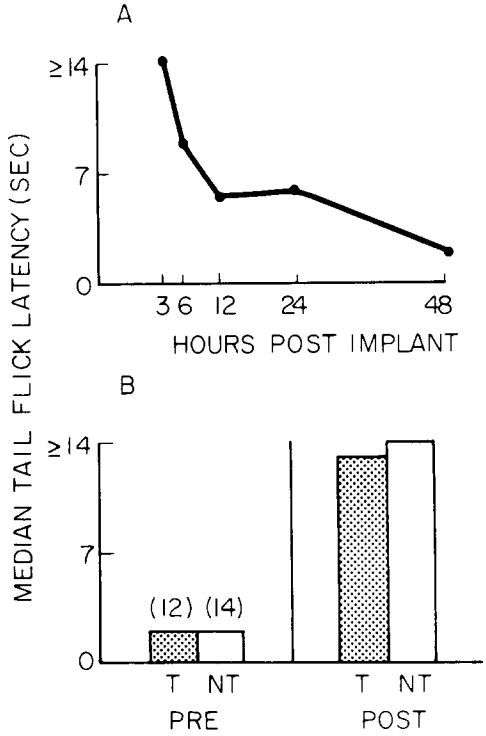

FIG. 3. (A) Median tail flick latency of rats tested at 3, 6, 12, 24 and 48 hr after implantation of a pellet containing 75 mg of morphine. (B) Median tail flick latency of two groups of rats, Tested (T) and Non-Tested (NT) before (PRE) and after (POST) a subcutaneous injection of 7.5 mg/kg of morphine. All animals were implanted nine days previously with a morphine pellet, which was removed after 48 hr, immediately after the tests administered to Group T, shown in Part A.

on the PRE or POST test. Prior nociceptive stimulation on the hot plate did not promote analgesic tolerance on the tail flick.

The results of Experiment 3 are summarized in Fig. 2. Part A of the figure shows the median tail flick latency of the Tested animals at 3, 6, 12, 24 and 48 hours post implant. It is clear that the substantial analgesia, obtained three hours after pellet implantation declined dramatically during the tests.

Part B shows the results of the tests administered nine days post implant. The morphine pellets had not been removed during this interval. On the first, PRE test, there was no difference in latency between the two groups. However, after the morphine challenge (POST) the Tested animals were significantly more tolerant than the Non-Tested animals ($U=30.5$; $p<0.05$). The difference between the two conditions is even more dramatic when the relative change in latency is taken into account, as shown by the last pair of bars in this figure (POST-PRE). These data were obtained by subtracting the PRE score of each animal from its respective POST injection score. As stated, the previously tested animals exhibited a significantly smaller increase in analgesia following the morphine injection than the nontested animals ($U=26$; $p=0.025$).

Although the baseline nociceptive responses of Tested and Non-Tested animals were comparable, Non-Tested animals were significantly less tolerant in response to a morphine challenge. These results show that the influence of prior analgesic assessment could be detected as much as seven days after the last analgesic test.

However, the results of Experiment 4 demonstrate that such retention depends upon the presence of the morphine pellet. From the results of this experiment, shown in Fig. 3, it can be seen that pellet removal produced comparable analgesic reactions in the two groups. That is, when the pellet was removed seven days before the tolerance test, there was no difference between the Tested and Non-Tested groups, either before or after the morphine challenge.

Unfortunately, it was not possible to obtain measurements of tissue levels of opiate in these experiments. Correlation of such pharmacological data with the behavioral effects observed might provide important clues concerning the mechanism of tolerance facilitation. Such studies are planned for the future.

Finally, it should be noted that, no withdrawal signs were observed in these experiments, although such symptoms were not explicitly studied. Therefore, no comment can be made concerning the effect of prior analgesic tests on opiate withdrawal.

DISCUSSION

It has recently been shown [1,2] that behavioral tolerance produced by morphine pellets can be modified by the context in which the pharmacological action is assessed. In that report, analgesic tolerance was facilitated if animals received prior experience with the analgesic test. Morphine implanted animals who had practiced the analgesic tail flick response were significantly more tolerant than their unpracticed counterparts. One possible explanation of those results was that prior testing enabled the animals to become proficient at performing the response while drugged. However the results of the present studies provide evidence against this argument. Experiment 1 clearly demonstrated that animals who are exposed to the assessment apparatus and procedure (pseudo-tested) are significantly more tolerant than naive animals. This is the case in spite of the fact that the nociceptive reflex is not elicited during the exposure period. In fact, pseudo-tested animals retained their tolerant status even after the morphine challenge. These data rule out a crucial role for motor performance in tolerance facilitation.

It might alternately be postulated that a nonspecific process underlies this phenomenon. For example, it might be the case that the test engenders an emotional response, e.g., arousal, stress, fear, which, in concert with the narcotic stimulus, attenuates analgesia. The second experiment represented one attempt to examine this possibility by providing experience with an alternate nociceptive stimulus, the hot plate. Although it is not possible to equate the aversive properties of the hot plate and the tail flick, it was clear from the response to the "nonfunctional" plate, that the hot plate did elicit an escape response. Nevertheless, prior exposure to the hot plate did not significantly modify tolerance of the tail flick reflex. The fact that there was a trend in that direction suggests that more intense nociceptive stimulation might have had a significant effect. However, it is difficult to accept this possibility when it is recalled that, in Experiment 1 tolerance was facilitated even without specific nociceptive input.

Moreover, the results of Experiments 3 and 4 also do not support a mechanism based on motor performance or nonspecific motivational processes. In each of these two experiments, animals tested nine days after morphine pellet implantation were equally responsive on their first, PRE, test regardless of whether or not they had received prior training of this response. Therefore, neither practice nor stress associated with the previously administered tail flick test, differentiated the tested animals from the nontested animals.

However, previously tested animals were still more tolerant, in response to a morphine challenge, than nontested animals (Experiment 3) as long as they retained the morphine pellet (Experiment 4). Apparently the morphine pellet must remain intact if the effect of prior testing is to be expressed. Once this opiate stimulus is removed, there is no residual influence of the behavioral or pharmacological treatment. In this respect, tolerance induced by pellets differs from that produced by intermittent, chronic injection. Behavioral tolerance of the tail flick reflex, produced by the latter technique, is retained even if the injections are spaced as much as seven days apart [3]. Therefore tolerance induced by pellets and tolerance induced by chronic injection, is not retained to the same extent. Contextual influences on pellet induced tolerance appear to be more labile than corresponding manipulations associated with chronic drug administration. In this sense, the two pharmacological procedures produce either short- or long-term retention of tolerance, respectively. As applied to the present studies, this analogy to short- and long-term memory is merely descriptive. Nevertheless, the analogy suggests that analyses of these behavioral preparations may provide insight not only into mechanisms of opiate analgesia and tolerance, but also into processes which mediate other forms of behavioral plasticity.

REFERENCES

1. Advokat C. Analgesic tolerance is facilitated by analgesic testing. *Abstr. Soc. Neurosci.* **6:** 433, 1980.
2. Advokat, C. Analgesic tolerance produced by morphine pellets is facilitated by analgesic testing. *Pharmac. Biochem. Behav.* **14:** 133–137, 1981.
3. Advokat, C. Evidence for conditioned tolerance of the tail flick reflex. *Behav. Neural Biol.* **29:** 385–390, 1980.
4. Bodner, R. J., D. D. Kelly, M. Brutus and M. Glusman. Stress-induced analgesia: neural and hormonal determinants. *Neurosci. Biobehav. Rev.* **4:** 87–100, 1980.
5. Chance, W. T. Autoanalgesia: opiate and non-opiate mechanisms. *Neurosci. Biobehav. Rev.* **4:** 55–67, 1980.
6. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences.* New York: McGraw-Hill, 1956.
7. Siegel, S. The role of conditioning in drug tolerance and addiction. In: *Psychopathology in Animals,* edited by J. D. Keehn. New York: Academic Press, 1979.
8. Urca, G., R. L. Nahin and J. C. Liebeskind. Development of tolerance to the effects of morphine: association between analgesia and electrical activity in the periaqueductal gray matter. *Brain Res.* **176:** 202–207, 1980.
9. Way, E. L., H. H. Loh and F. H. Shen. Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J. Pharmac. exp. Ther.* **167:** 1–8, 1969.