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Using Antagonists to Assess Neurochemical Coding of a Drug's Ability to Establish a Conditioned Place Preference

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BILSKY, E. J., S. H. MARGLIN AND L. D. REID. *Using antagonists to assess neurochemical coding of a drug's ability to establish a conditioned place preference.* PHARMACOL BIOCHEM BEHAV **37**(3) 425–431, 1990.—Rats were given morphine as an agent of putative conditioning to establish a place preference. Doses of 4 and 8 mg/kg of morphine did establish reliable conditioned place preferences (CPP's). Other rats were given one of the doses of morphine and one of a number of antagonists in procedures designed to assess which antagonists would specifically block morphine's ability to establish a CPP indicative of positivity. Doses of naloxone and larger doses of naltrexone but not smaller ones did antagonize morphine's effects. A dose of the benzodiazepine antagonist Ro 15-1788 did not attenuate morphine's effects. It was concluded that morphine's positivity is dependent upon actions by way of receptors sensitive to naloxone and naltrexone, but that morphine's positivity is less sensitive to naltrexone's effects than morphine's analgesia.

Morphine Drugs of abuse Opioids Naloxone Naltrexone Affect Conditioned place preference Positive reinforcement Reward

A number of technologies are available for assessing the potential positivity of drugs. Some use laboratory rodents, others use people, and some can be used with both. These include the systems associated with (a) drug self-administration (30), (b) how drugs modify responsiveness to positive brain stimulation (8,19), (c) how drugs modify responsiveness to novel tastes, i.e., conditioned taste aversions or preferences (14,17), (d) drug discrimination procedures (18), (e) when possible, systematized verbal reports (9), and (f) procedures of conditioned place preference (CPP) testing (4, 15, 26, 29). Each technology has advantages and limitations which are explored in a recently published text (5).

Given that there are ways to measure positivity of a drug, salient questions revolve about the issues of neurochemical coding of a drug's positivity. Given that a wide array of relatively specific receptor antagonists for different neuroreceptors are available, and that these are associated with specific neurochemical systems, one might presume that determining the neurochemical coding of a particular drug's capacity to elicit positive affect (or positive reinforcement or positive incentive values depending on theoretical orientation) is rather simple. One can choose a system for measuring a drug's positivity and then make assessments with and without available antagonists until the critical systems have been isolated. Unfortunately, it is not that simple.

The major problem is associated with the fact that the antagonists themselves may (or, even, are likely to) produce an affective change. The case is exemplified by naloxone (NX), the classic antagonist at opioid receptors (opioceptors). NX produces an aversive state in rodents as indexed by CPP testing and by conditioned taste aversion procedures (17). So, NX's ability to antagonize a morphine-induced CPP or taste preference may be due to NX's effects by itself; since the expected response, a decrement in responsiveness, is the same with either antagonism or aversion. NX will, of course, precipitate withdrawal signs in subjects dependent on opioids, so giving NX to animals working for an opioid, say morphine (M), and observing a decrease in work for that opioid does not provide unequivocal results. The decrease in work may be due to NX's ability to antagonize the opioceptors associated with M's positivity (or discriminability) or due to withdrawal malaise. NX decreases pressing for brain stimulation among rats never having received exogenous opioids (3, 23, 27), so decreases in M-induced acceleration in pressing for brain stimulation may be due to NX antagonizing M's positivity, or due to NX's effects themselves. With less well-studied antagonists, the potential problems are at least as great.

Tests of CPPs have some advantages over other procedures for measuring positivity, including the ability to measure the effect of

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a drug that produces motor disturbances (10). Here, we assess procedures that might be useful in determining whether or not an antagonist blocks a drug's positivity. We use M and NX as our prototypic drugs.

Tests for CPPs typically use rats and an experimental space with, at least, two discriminable places. After measuring the time a rat has spent in each place during an initial session (a baseline), conditioning trials are begun. Conditioning involves (a) during some sessions and under the influence of drug, putting a rat in one place; and (b) during other sessions and under the influence of placebo (usually the drug's vehicle), putting the rat in another place. After a number of these conditioning sessions, a rat's preference for a place is again measured by tabulating the time spent in the place of previous drug experience (place of putative conditioning). Rats, whose procedure serves as a control, get placebos throughout conditioning. Testing usually occurs in a drug-free state, as does the measurement of baseline. Our apparatus and procedures have been described extensively (21).

One approach for assessing NX's effects on M-induced CPPs would be, during conditioning, to give NX and M before rats were put into their putative place of conditioning and vehicles of agents before they were put into an alternative place. The antagonist's effects, then, supposedly would be indexed by a lack of a CPP indicative of positivity at testing, i.e., M's effects supposedly would be antagonized. As mentioned, however, the lack of a positive CPP may be due to one or more of NX's other effects, e.g., its own aversiveness. What is needed is a procedure similar to that employed by Mucha *et al.* (16) in which NX's effects are paired with both places. We reasoned that if, during conditioning, (a) NX and M were given before rats were put in one place, and (b) NX and M's vehicles were given before rats were put into an alternate place, then NX's aversive effects would be paired with each place. Any effects seen with respect to M's ability to sustain a CPP, therefore, would then be due to NX's antagonizing effects with respect to M and not NX's nonspecific effects. A variant of that procedure was used in these experiments.

GENERAL METHOD

Subjects

The procedures used 264 experimentally naive, male, Sprague-Dawley rats purchased from Taconic Farms (Germantown, NY). Rats weighed between 175 and 200 g when they were shipped from the supplier. Upon arrival, rats were housed individually in standard hanging wire cages. All cages were kept in a colony room maintained at 24°C with artificial light for 12 hr a day beginning at 0800 hr. Rats had food and water always available in their home cages.

Apparatus

The apparatus is a system having 12 separate alleys. Each alley has two distinctive sides (places), one painted with black and white stripes, and the other painted solid grey. The floor of the striped side has steel rods running parallel to the length of the alley; the rods of the grey side are perpendicular to the length. During conditioning, a removable barrier, painted as the walls, divides the two sides. When the rat is on one side of the alley, a switch is closed allowing information about the rat's position to be recorded by a computer using data-acquisition software. The alleys are enclosed by an outer shell which, in turn, is ventilated and lit so that each side of the alley has roughly equal reflected light. The arrangement has two discriminable places for which rats typically show no mean preference prior to conditioning (21).

Drugs

Throughout the experiments, a number of doses of drugs were used. Morphine sulfate (M), naloxone HCl (NX), naltrexone HCl (NTX), and scopolamine methyl bromide (SCO) were all dissolved in physiological saline and administered subcutaneously (SC). The benzodiazepine receptor antagonist RO15-1788 (RO), dissolved in a glycol solution, was administered interperitoneally (IP). All injections were of equal volume (1.0 ml/kg). The times of injections, specified elsewhere, were assigned based on earlier work showing that drug effects, as manifest by behavioral changes, would likely be extant during the periods of conditioning (7, 12, 13).

Procedure

These procedures followed a standard cycle across consecutive days involving: (a) habituation, (b) a measurement of baseline, (c) several conditioning sessions, one a day, and (d) finally a test. With some procedures, further conditioning and testing were performed.

The 1st day after rats' arrival, they began a schedule of handling which included daily weighing. On Days 1–5, the rats were placed in a mobile cart (12 rats to a cart, each rat in its own holding cage) and wheeled into the room containing the apparatus. Rats were then handled, one at a time, and returned to their cages.

Days 6 and 7 were formal habituation and baseline days. Prior to these days, rats were assigned randomly to groups being tested during that cycle. Rats were again brought into the room, and each placed into an alley for 30 min with access to both sides. Scores (time spent on putative side of conditioning) for each rat were recorded on Day 7 and served as a baseline measure. All boxes were cleaned after a rat was taken from it.

The conditioning phase of the experiment began on the 8th day. The exact schedule of conditioning was dependent on the experiment. In general, rats received the drug(s) of putative conditioning on four occasions and the agent(s) of alternative conditioning on two occasions, so that 2 days of putative conditioning preceded each day of alternative conditioning. There was a lapse of 10 min between each rat's last injection and it being placed in the alley. All testing and conditioning sessions were 30 min. The procedure of having more pairings with side of putative conditioning than with alternative side is a control for exploratory effects (21). This control procedure, however, does introduce a complication that is addressed subsequently (Experiment 2).

The 14th day of the procedure was a test for place preference. Rats were placed in their respective alleys with no injections and having access to both sides. With some procedures, further conditioning and testing were done.

EXPERIMENT 1

One hundred and forty-eight rats were tested. Since with even 12 alleys it is difficult to assess this number at the same time, groups were tested at different times. With each cycle of testing, there was a group that only received placebos (saline or S-control). Also, with each cycle, there was a group that received M before being placed in the side of putative conditioning (M-continuance). There were no reliable differences across cycles among the S-control groups. Furthermore, there were no differences between the M-continuance groups across cycles. Consequently, we combined the data of each cycle and treated the results as if they were

from a single experiment. One consequence of this procedure was that the number of subjects in these two groups were larger than the other groups.

The procedures involved two conditioning periods and two tests (Baseline, conditioning, Test 1, conditioning, Test 2). Prior to baseline, rats were randomly assigned to two groups, a M-group (n=95) and a saline group (n=53). The M-group received 8 mg/kg M on days of putative conditioning and saline (S) on alternative days (the procedure is designated M/S). The S-group received saline on all conditioning days (S/S).

Following the first test, the two groups were subdivided: rats that had previously received M were subdivided into 6 groups based on their Test 1 scores, so that each subgroup was nearly equal. Rats that had previously received saline were divided similarly into 2 subgroups. Each new group was characterized by the agents administered prior to subsequent putative conditioning.

Subjects which had previously received M were assigned to one of 6 groups: (a) A group of 29 that continued the conditioning just as they had before Test 1, i.e., they were given M (plus saline) before being placed in the putative side and saline (two injections) before being placed in the alternate side. This group (S+M/S+S) is designated as the group with continuance of M-conditioning and is labelled M-Continuance in Fig. 1. (b) A group of 12 received saline (two injections each day) before being placed into a side. This group's procedures (S+S/S+S) are analogous to an extinction procedure and, therefore, is labelled Extinction in Fig. 1. (c) A group (n=12) which received NX (10 mg/kg) plus an injection of M before being put into the putative side and two injections of saline before alternative side placement (NX+M/S+S). This group is the one whose results may confound NX's specific effect of blocking M with NX's other, more nonspecific effects. (d) A group (n=21) which received NX plus an injection of M before being placed into the putative side and NX plus saline before alternate side placement (NX+M/NX+S). If an antagonist neutralizes the effects of M, this group should perform at Test 2 as do subjects in the Extinction group, which first had conditioning with M (putative side only) and then received no further M but continued being placed into the chamber. (e) A group (n=10) which received RO (3 mg/kg) on both sides while M was given only before placement into putative side (RO+M/RO+S), and (f) a group (n=11) which received SCO (1 mg/kg) as a potential antagonist, or (SCO+M/SCO+S). Note that this drug (SCO) only acts peripherally, and, therefore, is another control procedure.

The rats which had previously received only saline (up to Test 1) were assigned to one of two groups. One group (n=29) continued to receive only saline (S+S/S+S), i.e., it served as the control group for the effects of the general procedures without active drugs. It is designated S-Control in Fig. 1. The other group (n=24) received NX as a drug of putative conditioning, i.e., NX+S/S+S. The results with this group should reflect the effects of NX injections by themselves. After 6 more conditioning days (2 cycles of 2 days putative conditioning followed by 1 day of alternative conditioning), all groups were tested for their preferences.

Data Reduction and Statistics

The data conform to a 2 by 8 by 3 analysis of variance (ANOVA) having repeated measures with factors associated with Side of putative conditioning, Groups (as specified by 2nd set of injections), and Tests (Baseline, Test 1, Test 2), respectively. This ANOVA revealed that the factor associated with side of putative conditioning (grey or striped) was not a reliable source of variance

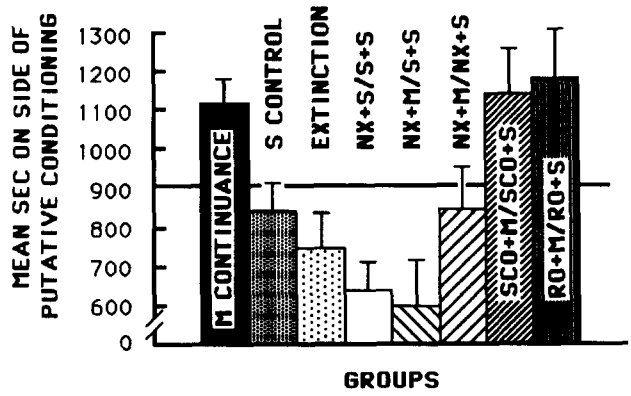

FIG. 1. The results, expressed in terms of mean sec on side of putative conditioning, for Test 2 are depicted. Prior to Test 2, there was putative conditioning, Test 1, and further putative conditioning. The S-control group received no drugs other than saline across all conditioning. All groups with the exception of those designated S-Control and NX+S/S+S received M on side of putative conditioning prior to a second phase of the procedure. Between Test 1 and Test 2, the eight groups received different types of drugs which are designated by the abbreviations. The abbreviations before a slash denote type of injection received prior to rats being placed in the putative side and abbreviations after the slash denote type of injections received prior to being placed in the alternative side. M stands for morphine; NX stands for naloxone; RO for RO15-1788; SCO for scopolamine methylbromide; and S for placebo (saline, the vehicle of most drugs).

nor did it interact with either of the other two factors separately or in combination (i.e., there was no reliable three-way interaction). Thus, this factor was ignored in subsequent analyses of the data. Consequently, the data conform to an 8 by 3 ANOVA having repeated measures, with factors associated with Groups and Tests, respectively.

Results

The ANOVA, an 8×3 factorial for the scores of the eight groups across the three tests (Baseline, Test 1, Test 2), yields an $F(14,280)=3.19$, $p=0.0001$ for the critical Drug group by Test interaction. Such a result calls for more specific analysis. A one-way ANOVA across groups at Baseline yields an $F(1,146)=0.22$, $p=0.64$, indicating that the groups' scores were not unlike one another, in terms of preference, prior to putative conditioning. At Test 1, the morphine group had a mean score of 1106 sec spent on side of putative conditioning compared to the saline group's score of 868 sec. A one-way ANOVA indicated that the subjects who received M compared to those who received placebo exhibited a CPP, $F(1,146)=17.33$, $p<0.0001$. The critical tests concern the comparisons at Test 2 (Fig. 1). The one-way ANOVA of scores at Test 2 yielded an $F(7,140)=5.49$, $p<0.0001$, indicating that there were reliable differences among groups at Test 2.

For there to be a test of a drug's ability to antagonize M's effects at Test 2, the scores of the group having continued experience with M on side of putative conditioning (M-continuance) must differ from those of the group getting only saline between Test 1 and Test 2 (M-extinction) as well as the saline control group. Student *t*-tests indicated that these basic conditions were met. The comparison of M-continuance to M-extinction yields a $t(39)=2.25$, $p=0.01$. The comparison of M-continuance to saline controls yields a $t(56)=2.72$, $p=0.001$. With these basic

conditions being met, the question of whether or not a drug treatment antagonized M's effects can be addressed by comparing the scores of the M-extinction group to the scores of the other groups.

Scores of M-extinction did not reliably differ from the scores of any group receiving NX ($ps>0.20$). The scores of M-extinction are reliably less than the scores of the groups for which RO or SCO were given, $t(20)=2.83$, $p=0.01$ and $t(19)=2.16$, $p=0.04$, respectively. Furthermore, the scores of RO and SCO did not differ reliably from scores of M-continuance. Consequently, it is reasonable to conclude that neither the benzodiazepine receptor antagonist nor the peripheral cholinergic antagonist, at the doses tested, modified M's ability to sustain a CPP and, by inference, that M's ability to sustain a CPP is not dependent on neurocircuitry involving either neurotransmitter.

The question of whether or not M's effects are mediated by way of systems sensitive to NX is complicated by the fact that NX itself is reactive: compare the mean score of S+NX/S+S to that of S-control (Fig. 1), $t(51)=1.98$, $p=0.053$. Although the control of having NX given before rats were placed in both putative and alternate side seems to provide the appropriate conditions to come to the conclusions that M's ability to establish a positive CPP is sensitive to NX, there are some other perspectives to take into account.

Discussion

These data confirm that M is capable of establishing a place preference in an apparatus for which rats have no preference for a place prior to conditioning. M establishes a CPP among rats for which side of putative conditioning is determined randomly prior to any measurements. In brief, the data of Test 1 confirm the notion that M establishes a CPP (21,22). Apparently, M's positivity does not wane with repeated administrations among rats, a conclusion that is concordant with the assessment of M in studies involving measures of responsiveness to rewarding brain stimulation (6, 8, 11). As might be expected, M's effects show extinction when M is no longer given.

NX was effective at producing an aversion even after considerable experience with the two sides of the boxes under saline. The basic finding that NX's effects by themselves are aversive replicates previous findings (16,29). The findings also support the idea that the extent of familiarity with the alleys, after some habituation has occurred, is of little consequence provided that a powerful drug effect is introduced (21).

The schedule of twice the times on side of putative conditioning as on alternative side during "conditioning" with saline produces a mean score at testing that is somewhat less than 900 sec (half the testing time) and this replicates previous findings (21,24). With no other coercion, rats spend more time in the place they have previously spent the least time (21,24). The variance of Saline-control is large (SD = 836 sec; range = 1221 sec) as might be expected if there is little to determine which side of the alley a rat is to be. The large variance of a control group, when there is no strong motivation to stay in a side, probably increases the probability of committing a Type II statistical error (saying no difference when there is a difference). If one uses an apparatus for which rats do show a marked preference, such as when one side is dark compared to another, then other problems emerge (such as a drug affecting timidity rather than producing another affective state). This limitation is inherent to CPP testing and (a) necessitates the use of large numbers of subjects to guard against Type II errors, (b) should make one doubly cautious in drawing inferences based on no apparent effect, and (c) relatedly, forces one to be cautious about some of the conclusions to be drawn subsequently.

These results confirm what might be expected from these kinds of procedures. The place preference established with M is sustained (and often strengthened) with repeated trials. Trials without M (saline instead) leads to extinction. These expected results, from a perspective of general knowledge of conditioning, strengthen the idea that the test itself is indexing conditioned drug effects rather than an artifact.

The results of this experiment are very encouraging from a number of perspectives. They seem to indicate that (a) the test is sensitive, (b) the results from the test indicate that M's positivity is sensitive to NX but not to RO or to the peripheral effects associated with SCO, and (c) on the surface, using the procedure of pairing the effects of a putative antagonist with both sides of the alley provides a control for an antagonists' nonspecific effects. Nevertheless, there is a potential problem with the procedure.

EXPERIMENT 2

Results of Experiment 1 indicate that NX tends to be aversive. The t-value comparing the groups NX+S/S+S and S-Control yields a $t(51)=1.98$, $p=0.053$. It remains a possibility that rats of a procedure involving M+NX/S+NX could be driven to the NX+S side to avoid a conditioned aversion rather than showing no preference. Consequently, we began searching for another way to control for an antagonist's own effects.

When injections of a drug are given just before putting a rat in a side of the chamber, the drug's effects stand a good chance of being associated with that particular side of the chamber. If, however, a drug's effects are continuous (or nearly so), there is no opportunity to associate any given effect in the rat's life with the drug effect, i.e., there is no specificity of drug effect for a particular side. Given this reasoning and given that naltrexone (NTX) is a longer acting antagonist at opioceptors (13), we potentially have another way of controlling for antagonists' effects themselves. If NTX is given 4 hr before a rat was placed into either side, then the general effects of the antagonist would be equally associable (therefore, not particularly associable) with all aspects of the procedure.

To test if NTX would block M's positivity, we ran the following pilot study. After rats had received extensive experience with M, we split them into two groups. One group continued to receive M on side of putative conditioning (designated M/S). This group also received an injection of placebo 4 hr prior to time of conditioning. Another group also received the procedure M/S, but got 10 mg/kg of NTX 4 hr before the conditioning sessions. There were 6 days of conditioning during this phase, four with M on putative side and two with S on alternate side and with NTX given to one group on every one of the 6 days. The results indicated that M+NTX produced a stronger CPP than M+S (or M-continuance), a surprising result.

The data with NTX lead to two plausible conclusions: (a) M's positivity is not sensitive to NTX's antagonism, or (b) something is amiss with respect to the rationale underlying the testing. The conclusion of something amiss with the testing is apt to be the proper conclusion, because of the considerable data demonstrating NTX's ability to act as an antagonist. On the other hand, one cannot discount the possibility that either M's positivity is not sensitive to NTX or that larger doses of NTX are necessary to block M's positivity than one might suppose from knowing about NTX's ability to antagonize other opioid's effects such as analgesia. Given these multiple considerations, we assessed NTX's ability to antagonize M's positivity using a number of doses of NTX.

Method

Ninety-six rats were randomly assigned to 10 groups, each

having a different regimen of drugs to be administered. After the standard handling and baseline measurements, the rats began conditioning. Eight of the groups (n = 10/group) received an injection of NTX 4 hr before conditioning on both putative and alternative days. Four doses of NTX were used: 0 (saline), 10, 30, and 56 mg/kg with two groups receiving each dose. Each of these two groups then received either an injection of 4 or 8 mg/kg M, 10 min before conditioning, on putative days; or, saline, on alternative days. The two remaining groups (n = 8) served as controls. One group received only saline for each of its two injections per day while the other group received 56 mg/kg NTX each day along with an injection of saline. The conditioning was similar to that of Experiment 1 and rats were given M on side of putative conditioning twice and saline on side of alternative conditioning once across a 3-day period and this cycle was repeated twice. Animals were then tested for their preferences for a side.

NTX was also tested for its ability to block M's analgesia with a regimen of dosing similar to that used in testing for CPPs. Two weeks after the 60 rats that received NTX and M were tested for the CPPs, they were randomly assigned to one of 8 groups. Two groups served as controls, one receiving only saline (n = 8) and one (n = 4) receiving NTX (10 mg/kg) but no M just before testing. The remaining 6 groups' procedures conform to a factorial design with three levels of dosing with NTX (0, 3, and 10 mg/kg, 4.5 hr before testing) and two levels of M (4 and 8 mg/kg, 20 min before testing). The test was a standard tail-flick test and involved placing the rat on a board so that its tail was over a hole. Once the tail was in place, a light, under the hole, producing heat was turned on. The light was turned off after the rat moved (flicked) its tail or after 10 sec lapsed. The time to flick was recorded and the procedure was repeated two more times. The mean of the three measures with a rat was taken as the score reflecting antinociception for that subject.

An initial inspection of the tail-flick data indicated that a simple one-way ANOVA across the 10 groups with *t*-tests comparing individual groups was an adequate assessment, since the scores of the control group and of the groups getting either 3 or 10 mg/kg doses of NTX were very similar to one another.

Results

The results of the CPP testing are depicted in Fig. 2, but in terms that need explanation. The mean scores of the 10 groups at baseline were not reliably different from one another, $F(9,86) = 0.7$, $p < 0.70$. The overall mean baseline score was 877.8 sec, and no group's mean was reliably different from 900 sec (the score expected if rats had no preference for a side). Since groups' did not differ reliably at baseline, any differences seen at testing are probably due to differential drug effects.

The two control groups' scores at testing were 659.2 and 787.0 sec for the group of saline only and the group getting NTX but no M, respectively. These scores do not represent reliable differences between these two control groups, at testing, $t(14) = 1.34$, $p > 0.20$, thereby allowing them to be considered as a single group. The reduction from the mean score of baseline is expected since rats tend to spend more time in the place where they have been the least (21,24). The mean of these two groups' scores on their side of putative conditioning (designated randomly before any testing), therefore, represents the best estimate of what the other groups' scores would be if they had received no M. Consequently, the control groups' mean score (723.1 sec) was used to transform each other rat's score so that we would have a complete factorial design (test score/723.1 × 100 or percent of controls). The transformed scores of the 8 groups conform to a factorial design for a 4 by 2 ANOVA having factors of dose of NTX (0, 10, 30, or 56 mg/kg) and of dose of M (4 or 8 mg/kg).

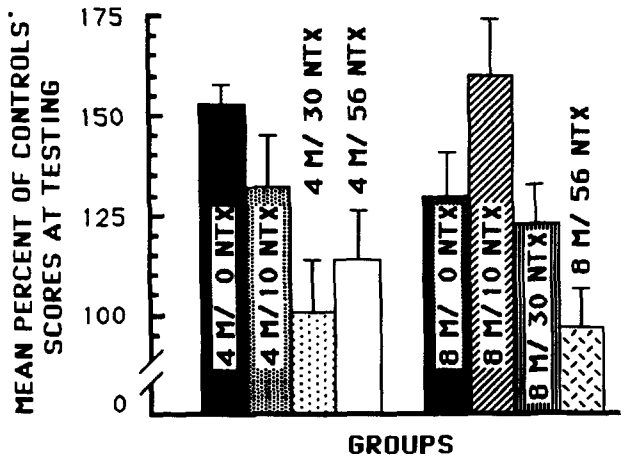

FIG. 2. The results of a CPP test are displayed in terms of how the groups performed compared to a control group. The key denotes the doses of naltrexone (NTX) and morphine (M) given to each group on putative conditioning days. On alternate days, saline was given rather than a dose of M.

The means of transformed scores (% of controls) are depicted in Fig. 2. The ANOVA of the scores of Fig. 2 yields for the factor of dose of M an $F(1,72) = 0.1$, $p < 0.8$, for the factor of dose of NTX an $F(3,72) = 6.6$, $p = 0.0005$, and for the factor of the interaction, M by NTX, an $F(3,72) = 2.8$, $p = 0.048$. Such results call for more specific comparisons.

From inspection of the scores of Fig. 2, it seems that both doses of M established a CPP indicative of positivity, as would be expected from previous results using similar procedures (21). This was confirmed by results of *t*-tests comparing the raw scores of the controls to that of the raw scores of the group getting 4 or 8 mg/kg dose of M (with no NTX). The respective tests yield $t(24) = 5.65$ and 2.48, respectively, $ps < 0.03$.

Inspection of the scores of Fig. 2 will lead to the conclusion that the two groups getting 56 mg/kg of NTX (plus a dose of M) do not differ remarkably from 100% (i.e., from controls' scores), but do differ from groups getting M with 0 mg/kg of NTX. The *t*-tests comparing the score of group of 8 mg/kg M plus 56 mg/kg NTX to the scores of the controls is $t(24) = 0.28$, $p = 0.8$. The *t*-test comparing scores of group getting 4 mg/kg M plus 56 mg/kg NTX to controls' scores yields $t(24) = 1.08$, $p = 0.3$. The group getting 56 mg/kg NTX and 4 mg/kg M scored reliably differently than the group getting 0 mg/kg NTX and 4 mg/kg of M, $t(18) = 3.02$, $p = 0.007$. The group getting 56 mg/kg of NTX and 8 mg/kg M scored reliably less than the group getting 0 mg/kg NTX and 8 mg/kg of M, $t(18) = 2.30$, $p = 0.03$. These results indicate that 56 mg/kg of NTX antagonizes M's ability, at doses of 4 and 8 mg/kg, to establish a CPP indicative of positivity. The dose of 30 mg/kg of NTX antagonized the effects of 4 mg/kg of M, but 30 mg/kg of NTX only attenuated the effects of 8 mg/kg of M (Fig. 2). The 10 mg/kg dose of NTX did not completely antagonize the effects of either dose of M.

The enhanced CPP established by 10 mg/kg of NTX plus 8 mg/kg of M is of considerable interest. The mean score of the group receiving 10 mg/kg NTX plus 8 mg/kg of M is the largest of any group. Furthermore, that groups' mean score approaches being reliably different than the mean score of the group getting 8 mg/kg of M and 0 NTX, $t(18) = 1.73$, $p = 0.10$. That groups' (10 mg/kg NTX and 8 mg/kg M) score is reliably greater than

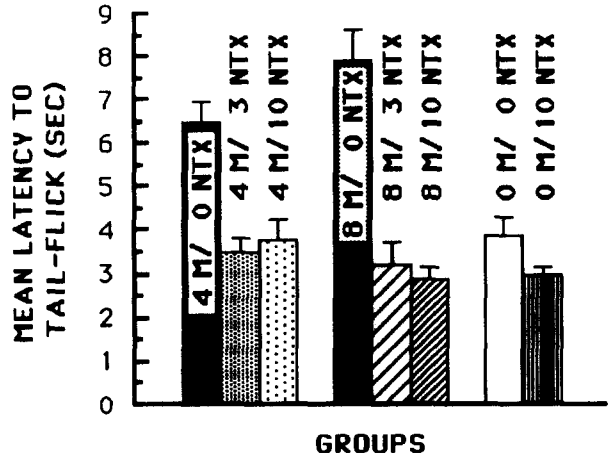

FIG. 3. The effects of naltrexone (NTX) on morphine's (M's) antinociception are depicted as mean number of sec for a rat to flick its tail. Rats received one of three doses of NTX 4 hr before testing. One of three doses of M was administered 20 min before testing.

the control groups' score, $t(24)=4.33$, $p=0.0002$. Perhaps the 10 mg/kg dose of NTX was sufficiently large to antagonize some aversive properties of a larger dose of M, but not sufficiently large to antagonize M's positive effects. This conclusion is concordant with other research (1,2).

In summary, M's positivity is sensitive to NTX's antagonistic effects, but it takes a dose of 56 mg/kg NTX (given 4 hr before) to block the effects of 8 mg/kg of M and a 30 mg/kg dose of NTX to antagonize a 4 mg/kg dose of M. The effects of 10 mg/kg NTX on an 8 mg/kg dose of M's ability to establish a positive CPP is surely not one of complete antagonism. That dose of NTX may, in fact, potentiate M's typical effect (pilot study and the more formal study).

Figure 3 depicts the scores of the groups tested for antinociception by way of the tail-flick test. The ANOVA of scores of Fig. 3 yields an $F(7,52)=14.96$, $p<0.0001$. Only the means of groups receiving 4 or 8 mg/kg M plus saline (0 mg/kg NTX) are reliably greater than those receiving 0 mg/kg of M plus 0 mg/kg NTX, $ts(14)=4.18$ and 4.79, respectively, $ps<0.002$.

Discussion

As mentioned in the introduction to this experiment, data from a pilot study indicated that the effects of a 10 mg/kg dose of NTX plus a dose of 4 or 8 mg/kg M produced a substantial CPP indicative of positivity. It was suggested that such a result leads to two plausible conclusions, one being that M's positive effects were not sensitive to NTX and one being that the CPP test was not adequate to test for NTX's antagonism. With the dose-response data, a clearer picture emerges. M's positivity is sensitive to NTX, but it is necessary to use considerably larger doses of NTX to antagonize M's positivity than to antagonize M's antinociception. There seems to be nothing amiss with respect to the rationale underlying the CPP test. The situation is merely that M's positivity is not sensitive to low doses of NTX, as defined by results from tests such as the tail-flick test.

GENERAL DISCUSSION

The CPP test may have some advantages over alternative methods in assessing the neurochemistry of drug-elicited positive affect. The procedures such as those used in Experiment 2 or similar ones (25), apparently control for an antagonists' nonspecific effects. Other procedures do not provide for such control.

All of the data lead to the conclusion that M's ability to elicit positive affect, as indexed by CPP testing, is sensitive to the effects of naloxone and naltrexone. In as much as naloxone sensitivity is indicative of involvement of opioceptors, it follows that M's positivity is elicited by way of opioceptors. This report provides no evidence to support the idea that M's positivity involves systems having major circuits with GABAergic receptors.

Although it is clear that M's positivity is sensitive to NTX's effects, it is also clear that large doses of NTX are necessary to antagonize M's positivity. The doses are large in comparison to those necessary to antagonize M's antinociception, as indexed by the tail-flick test. When using naltrexone to treat addiction to opioids, large doses of naltrexone are probably necessary.

The differences in dose response for NTX's antagonism with respect to the tail-flick test and the CPP test provide additional evidence for the conclusion that M's positivity is disassociable from M's analgesia. There are a number of lines of converging evidence to support such a conclusion (6,19). Among them are: (a) M's positivity, as indexed by testing involving brain stimulation and CPP testing show little tolerance, whereas M's analgesia shows rapid tolerance. (b) Opioids which block other opioids' analgesia can also induce signs of positivity that are NX-sensitive, e.g., diprenorphine. (c) Not all opioids which produce antinociception also produce signs of inducing positive affect, e.g., the negative enantiomer of ethylketocyclazocine (EKC). (d) The dose-response curve for NTX antagonizing M's positivity is to the "right" of that curve for NTX antagonizing M's analgesia (19).

Given that M's positivity is disassociable from M's analgesia, it is likely that two different kinds of opioceptors are involved. Since, however, all schemes of classifying kinds of opioceptors use assays that cannot be sensitive to M's positivity, it is impossible to determine if M's positivity is a product of one of the postulated kinds of opioceptors. Since there are apt to be more kinds of opioceptors than those presently characterized (28), it remains a possibility that one type of opioceptor may be linked selectively to M's positivity (20).

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