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Measuring Morphine's Capacity to Establish a Place Preference

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REID, L. D., S. H. MARGLIN, M. E. MATTIE AND C. L. HUBBELL. *Measuring morphine's capacity to establish a place preference.* PHARMACOL BIOCHEM BEHAV **33**(4) 765–775, 1989.—A series of experiments are described providing an assessment of the procedures of conditioned place preference (CPP) testing involving an automated system having 12 separate chambers. Experiment 1 provides data to demonstrate (a) that in these chambers no initial preferences for one side over the other exists among rats, (b) that this neutrality of sides is not affected by session lengths between 15 and 60 min, and (c) that the optimal session length for tests in these chambers is on the order of 30 min. Experiment 2 demonstrates the stability of control groups' scores across a number of conditioning and testing sessions. Experiments 3 and 4 provide data to demonstrate (a) that a positive CPP can be established in our chambers using injections of morphine, (b) that a regimen of dosing with unequal numbers of days of putative and alternate conditioning is a reliable and conservative test of the opioid's ability to establish a CPP, and (c) that although the activity of rats decreases across a session, the general activity of rats before and after conditioning procedures is the same. Experiment 5 replicates the procedures employed by Scoles and Siegel (25) and demonstrates that the tendency for rats to explore novel environments is strong, and care must be taken to provide an opportunity for rats to pair different experiences with each side of the chamber in order for a CPP to emerge.

Morphine Conditioned place preference Positive affect Drug reinforcement Addictions

THERE are good reasons for developing technologies and allowing meaningful answers to the question "Does a given manipulation produce a state that increases the likelihood of a subject (perhaps a rat) experiencing positive or negative affect?" Among the germane issues is whether or not a particular drug can induce positive affect, and, if so, how. This is a salient issue, since modern theories of addiction (8,26) emphasize the potential of a drug to enhance positive affect as a critical (although not exclusive) determinant of its likelihood of becoming the focus of an addiction. Also, there are other problematic conditions, e.g., depression, that are characterized as deviations in affect.

The development of technologies for measuring affect and then using them to study affective processes, an enterprise that might be called *hedonomics,* has a long history. The issues have been approached from many perspectives ranging from introspection through systematized verbal reports to recordings of single cells thought to be part of the relevant processes.

A problem, throughout this history, is related to the possibilities that affective states might be hidden, or they might be uniquely private and subjective, or they might be uniquely human. The resolution to these kinds of problems often takes the form of (a) on the one hand, recognizing that no measure, or set of measures, is apt to capture the subtle nuances of each individual's experience, and (b) on the other hand, assuming that affective processes do manifest themselves in a sufficiently common way that, at least, some gross features of the affective states might be measureable. Considerable progress has been accomplished, for example, by making the assumption that events rats will work to achieve have some positive valence and the events rats will work to avoid have some negative valence, even though there are instances (as when rats apparently work for electric shock) in which the results do not conform to an easily understood classification of events.

It was with this general background that we faced a problem related to interpreting the observation that doses of morphine (M) could enhance rats' pressing for direct electrical stimulation of the medial forebrain bundle as it coursed through the lateral hypothalamus [a finding first clearly demonstrated by Adams, Lorens and Mitchell (1) and immediately replicated and extended (9, 12, 16)]. This observation was significant because it (a) seemed to indicate that many recreational drugs shared the ability to enhance responsiveness for positive brain stimulation, since M was one of the few that apparently did not do so from earlier observations with large doses, and (b) might indicate that the medial forebrain bundle and its fields of innervation are critical sites of action for recreational drugs (5). The general idea was that recreational drugs produced a positive affective state by enhancing activity in the medial forebrain bundle. What was needed to assess such a proposition was an independent measure of positive affect.

We (24) made the simple assumption, all other things being equal, that rats would prefer to be in places in which they had experienced positive affect to alternative places in which they had experienced no particular affective event. We then placed rats into one side of an alley under the dosing regimen of M that incremented pressing for brain stimulation. At testing, for the effects of pairing M's actions with a place, it was found that rats who had M's effects paired with one place under the circumstances in which pressing for brain stimulation was incremented (in other

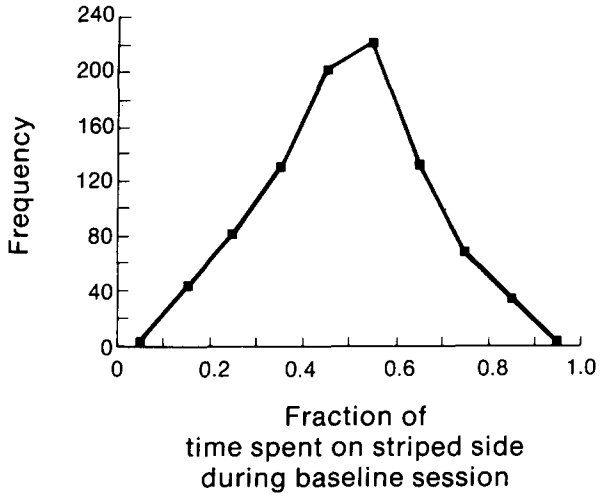

FIG. 1. Depicted are the baseline data of 924 rats in terms of the percentage of time they spent on the striped side of the experimental chamber. The mean of the distribution is 49.2%, with a standard deviation of 16.8.

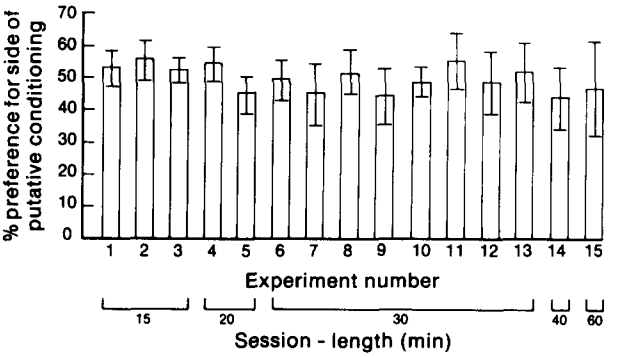

FIG. 2. These data represent the mean baseline scores and standard deviations across 15 experiments of the same 924 rats whose data are presented in Fig. 1. The data are depicted in terms of the percent preference for side of putative conditioning, an arbitrary designation baseline. The experiments are listed in terms of increasing session length, ranging from 15 to 60 min. Notice that as length of session increases, so does the variance associated with the preference scores.

procedures) did, indeed, spend more time in that place than their counterparts (24). This simple study did two things. It seemed to provide independent verification that incremented pressing for brain stimulation reflected an increment in potential for positive affect and it suggested that testing for conditioned place preference (CPP) might be a useful tool in its own right.

CPP testing was used to assess the affective properties of a synthetic analogue of enkephalin (30) and was used to confirm that the ventral tegmental area of the brain was a site of M's ability to establish a positive affective state (5). A considerable number of investigations have subsequently used CPP testing and CPP testing itself has been the subject of investigation [e.g., (2–4, 6, 15, 17, 18, 20–23, 25, 27–29, 32–37)]. The reports of this paper describe the results from a system for measuring place preferences with rats using opioids as agents to induce conditionable states. The reports also address some of the problematic issues associated with CPP testing that have appeared in the literature.

GENERAL METHOD

Subjects

All subjects of these experiments were male, Sprague-Dawley rats acquired from Taconic Farms (Germantown, NY) when they weighed more than 150 g. Upon arrival at the laboratory, they were housed individually in standard hanging cages with food and water always available. The colony room was maintained at 24°C with 12 hr of artificial light per day beginning at 0800 hr. All experimental procedures took place during the light phase. All subjects were experimentally naive when they began the procedures.

Apparatus

The apparatus are 12 nearly identical alleys having inner dimensions of 65 by 17 by 33 cm. Three sides of each alley are wood; the front walls and the tops are clear Plexiglas. The walls on the left half of each alley are painted with horizontal black and white stripes, whereas the walls on the right half are painted solid gray. The floors of the striped side of each alley are stainless steel rods running perpendicular to the length of the alley, while on the gray side, the rods run parallel to the length. The rods have 1 cm of space between them. A removable wooden barrier, painted to match respective sides of the alley, is inserted during conditioning procedures. Over each side of the alley there is a 40-W light bulb whose brightness was adjusted (prior to these procedures) so that, in general, rats showed no preference for one side over the other.

The alleys are suspended in sound-attenuating boxes, equipped with ventilation fans by way of a metal axle that passes through the top of the box between the striped and gray sides. When a subject is on a side, the box tilts to that side, closing an electrical circuit that relays signals to a single remote IBM PC. Software for the PC tabulates the amount of time spent on each side of the alley and the number of transitions made from one side to the other during a session for each subject.

Drugs

All drugs were administered subcutaneously. Placebo injections were physiological saline, the carrier of drugs. Fentanyl citrate (FEN) was administered in doses of 0.1 mg/kg (as a salt). M-sulfate was administered in doses of 2, 4, 8 or 15 mg/kg (as a salt). All injection volumes were 1 ml/kg.

Procedure

The formal experimental procedures were carried out in a darkened room (lit with a single red bulb) which held the 12 experimental spaces and was adjacent to the colony room. Subjects were transported from the colony room to the conditioning room in a rolling cart having 12 standard hanging wire cages. Each subject was randomly assigned to a particular experimental chamber and all procedures for that rat took place in the same chamber. The apparatus were washed with a mild, lemon-scented detergent prior to the start of each day's procedures and between each group of subjects to mask any odors that may have been left from previous rats.

Experiments had their own particular procedures, but followed the same pattern of a day during which habituation occurred that was followed by a day during which a baseline measure was taken. Across the next days, conditioning occurred. Subsequent to potential conditioning, there was a test.

During habituation, each subject was placed into its alley, with free access to both sides, for a specified length of time. Baseline procedures were nearly the same as habituation, with the addi-

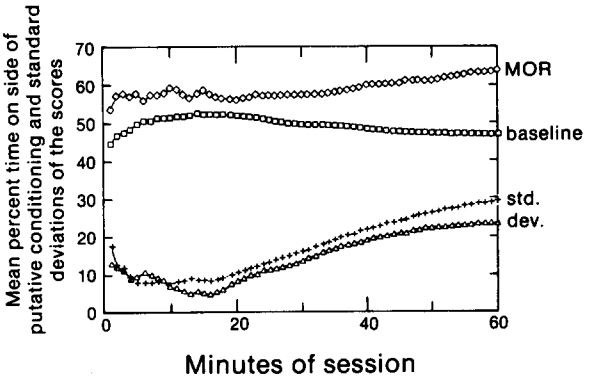

FIG. 3. Presented are mean cumulative percent preferences for the side of putative conditioning across time for some representative data (see Experiment 4). The top line shows mean test session scores for a group of 12 rats across a 60-min session after conditioning with 8 mg/kg doses of morphine (MOR). The next line shows the baseline data for all 60 rats of the experiment. The bottom two lines show the standard deviations, + and △, for the MOR and baseline data, respectively. Note that as test session length increases, both mean preference (as indicated by the difference in the top two lines) and the variances of the scores increase. Thus, the data lead to the suggestion that an optimal test session length would be 30 min.

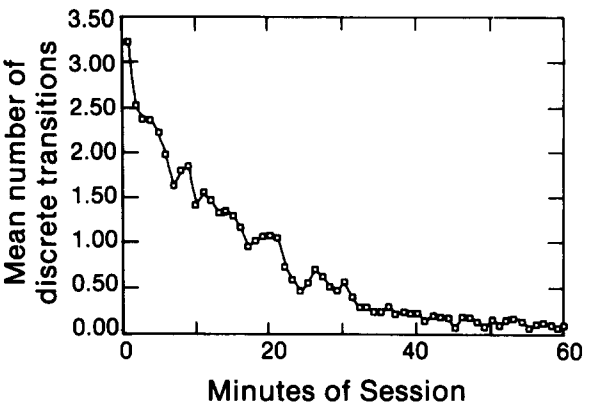

FIG. 4. These data represent the mean number of discrete transitions made in a 60-min baseline session by the 60 subjects of Experiment 4. Notice that as the length of the session increases, the number of transitions decreases.

tional aspect of measuring time spent on a preassigned side of putative conditioning, and the number of transitions. Conditioning sessions, in which subjects were confined to only one side of the alley, occurred once a day across at least 4 and no more than 12 days; subjects were administered drug prior to the start of the session on days in which they were put into the side of putative conditioning and placebo on days when they were put into the alternative side. [It is called side of *putative* conditioning rather side of conditioning because before the facts, i.e., results of a test, there is no knowledge of whether or not conditioning has succeeded. It is called conditioning as a general term and not to infer any special kind of learning process, although there are similarities to classical conditioning and secondary reinforcement procedures (10,13).] Test procedures were identical to those of baseline. Note that neither baseline nor testing procedures are conducted under the influence of injections.

EXPERIMENT 1

The data summarized here are not the results of a single formal experiment, but rather are results derived from a number of experiments, and are summaries of the way rats behave in the apparatus before putative conditioning with drugs. These baseline data are useful for interpreting results obtained from formal experiments assessing effects of drugs.

METHOD

Subjects

There were 924 subjects. Although different groups of subjects began the procedures at different times after their arrival at the laboratory, all began their procedures within one month after their arrival. These subjects' procedures involved subsequent conditioning across a number of experiments, but had only slightly different handling prior to the measurement of baseline.

Procedure

After arriving in the laboratory, subjects were handled, often daily, to ensure that they were accustomed to being handled. After a few days, subjects underwent habituation to the apparatus, i.e., they were merely placed into the apparatus for a period of time, which varied from 15 to 60 min, depending on the particular experiment. On the day after the habituation period, rats were tested for baseline preferences. In most cases, the length of the baseline session was identical to that of the habituation session. Preferences (sec on a side of the alley or percentage of total time on a side) and transitional data (number of crosses from one side to another side) from the baseline sessions were recorded.

RESULTS AND DISCUSSION

The results of the accumulated data are shown in Figs. 1, 2, 3 and 4. Across 15 experiments, the mean percentage of time spent on the striped side of the alley rats was 49.2% (see Fig. 1). This value is very close to 50%, the expected value if no preference were to be shown by the subjects for one side over the prior to any experimental manipulations. Note that the scores are depicted in terms of time on a particular side, rather than a side in which putative conditioning may have subsequently taken place.

Figure 2 displays the breakdown of the data of Fig. 1 across the individual experiments, but in a different form, i.e., mean percentage of time spent on the side of putative conditioning, the measure of relevance. Analysis of the data in terms of time spent on the side of putative conditioning indicates that there is no particular preference for a side regardless of the length of the session. Of the 15 experiments, three had baseline session lengths of 15 min, two were 20 min, eight lasted for 30 min, one was 40 min and one was 60 min in duration. The means of each individual experiment varied about 50%, regardless of the length of the session. Standard deviations are also displayed. Notice that with increasing session length, the variance in the scores increased. Figures 3 and 4 provide germane information related to the increase in variances.

The data in Figs. 3 and 4 are from Experiment 4, which had 60-min baseline and test sessions. Figure 3 shows the mean baseline scores (and standard deviations) of the 60 rats of Experiment 4 and mean test scores (and standard deviations) for a group of 12 of those rats which received an 8 mg/kg dose of M during putative conditioning. The data are depicted to reflect the

mean cumulative percentage of time spent on the side of putative conditioning up to the time noted on the abscissa.

From the figure, it is evident that as the length of the test session increases, degree of preference (difference between test and baseline scores) increases. Note, however, that the variances of scores of both baseline and test also increase with increasing session length. We have found, through informal observations, that after about 30 min in our chambers, rats with no prior experience in the boxes will "go to sleep" on either side of the chamber (on an apparently random basis). As seen in Fig. 4, the number of transitions from one side to another made per unit time decreases with time, stabilizing after 30–40 min. Figure 4 shows that during the 1-min period between minutes 39 and 40 of the baseline session, the mean number of transitions made by the 60 rats was less than 0.25. Transitional data from test sessions show a similar pattern across the length of the session. Since, at baseline, there is no apparent preference for side, the chances of a subject "going to sleep" on either side are equal, thereby increasing the overall variance.

The four figures show that in these chambers (a) there is no initial preference for either side, (b) that this neutrality is indifferent to session lengths between 15 and 60 min, and (c) that variances of the scores will increase with increasing session length. It seems that, in these apparatus, the optimal session length for baseline and test is on the order of 30 min.

EXPERIMENT 2

The results from Experiment 1 reveal no initial preference for a side in our chambers. A relevant question, however, is whether or not a preference, in terms of side of putative conditioning, will develop after repeated exposures to the chamber. The following experiment provides germane information.

METHOD

Procedure

Seventy-two subjects were randomly assigned to either experimental or control groups. Experimental subjects (n=36) were administered FEN as the drug of putative conditioning. Control animals (n=36) were further assigned to one of two groups. Subjects in one control group (n=18) were administered saline prior to all conditioning sessions, while subjects in the other group received injections of FEN prior to all conditioning sessions (n=18). Subjects received injections 15 min prior to the start of the 30-min conditioning sessions.

After the initial procedures, including habituation and baseline, 4 conditioning days ensued. Half of the subjects of each group had putative conditioning on the 1st and 3rd conditioning days, with alternate conditioning on the intervening days; the other half had putative conditioning on the 2nd and 4th days. On the day after the completion of the conditioning sequence, a test for preferences was conducted. This 5-day pattern of conditioning and testing was repeated 3 more times, resulting in a total of 16 conditioning days (8 days with FEN for experimental subjects) and 4 tests.

Statistics and Data Reduction

Initial analyses of the data revealed that some "control" factors included in design, namely Side of putative conditioning (striped or gray) and Order (whether or not days of putative conditioning were the 1st and 3rd or the 2nd and 4th days) were not reliable sources of variance. Inspection of the min by min data for the experiment revealed that the factor of Time across the test period was a reliable source of variance, and that differences among groups emerged in the later minutes of the test sessions.

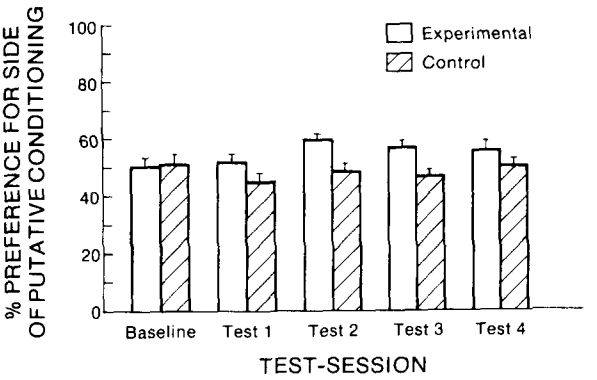

FIG. 5. This figure depicts the scores, in terms of mean percent preference for the side of putative conditioning, of the experimental and control groups across the 5 30-min test sessions of Experiment 2. Error bars represent standard errors of the means. Note the stability of the control groups' scores across all tests.

An ANOVA of the data for the two control groups (saline on both sides or FEN on both sides) showed no reliable sources of variance associated with the main effects of Group or Test (Fs<1), or with the Group by Test interaction, $F(4,136)=1.04$, $p=0.39$. Thus, the control groups' scores were combined in further analyses.

Given that the main purpose of presenting these data here is illustrative of another point, rather than an assessment of FEN itself, and given that a number of potential factors (i.e., control factors) are not reliable sources of variance, we summarized the data by collapsing it into a 2 by 5 ANOVA having repeated measures with factors associated with Groups (experimental and the combined controls) and Tests (Baseline and Tests 1–4).

RESULTS AND DISCUSSION

Figure 5 summarizes the results. There are no reliable differences between experimental and control groups' scores at baseline. The ANOVA of scores, i.e., the percentage of time spent on the putative side of conditioning, at testing indicated, however, that the experimental subjects' scores were reliably greater than those of the controls, $F(1,70)=7.70$, $p=0.007$. Student's *t*-tests, for independent measures, indicated that experimental group's scores were reliably greater than those of controls at Tests 2 and 3, $t(70)=2.97$, $p<0.01$, and, $t(70)=2.53$, $p<0.01$, respectively. These data confirm that FEN is capable of establishing a CPP (20).

Notice that the control group's mean scores do not deviate much from the expected score (i.e., 50%, if there is no systematic influence of conditioning). The mean percentage of time spent on the putative side of conditioning by the control group across all tests (including baseline) was 48.49%. At Baseline, it was 51.00%. At Tests 1, 2, 3 and 4, it was 45.22, 48.67, 46.95 and 50.62%, respectively, none of which were reliably different from 50% or from each other.

Apparently, as long as subjects are placed during conditioning on each side of the chamber an equal number of times and they experience no marked affective change in one side of the chamber, rats will show no particular preference for the putative side of conditioning across multiple tests with these apparatus. Consequently, any reliable deviation from the 50% score is apt to be due to the rats experiencing some affective change, most likely due to an experimental manipulation.

Across the last few days of the procedure, subjects receiving FEN were difficult to handle as we removed them from the

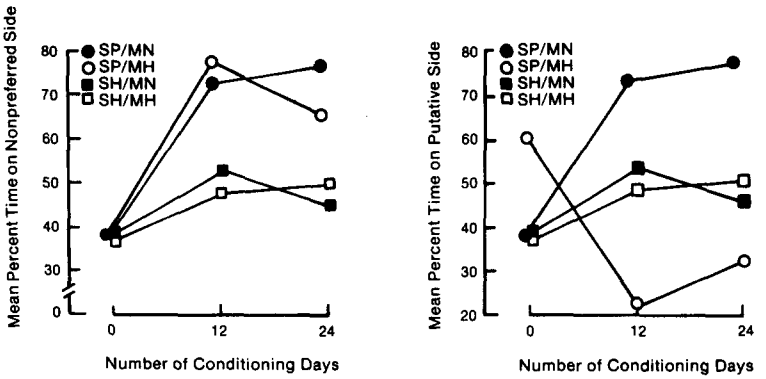

FIG. 6. The left panel is a copy of a figure from Scoles and Siegel [p. 1171, (25), reprinted with permission of both co-authors and Pergamon Press Inc.]. Four groups of subjects received differential conditioning designated by different sets of initials. S refers to getting saline prior to being confined to one side of an alley, and M refers to getting morphine. P stands for preferred side of the alley as determined by each rat's baseline performance, whereas N stands for nonpreferred. H refers to the rats being placed in a holding cage after an injection. The means of the baseline data are plotted at 0 days, and the means of the two tests are plotted at 12 and 24 days. The right panel depicts the same data, but in terms of the mean percentage of time spent on the side of putative conditioning. Compare the data of the open and closed circles of the two panels. See text for a germane discussion.

chambers. It was surmised that they were undergoing withdrawal from the quickly metabolized FEN. The possibility that the rats might have experienced negative affect toward the end of conditioning, but generally an increment in positive affect when under FEN, may account for the low magnitude of the FEN effect and contributed to us seeing greater differences between controls and experimentals at Tests 2 and 3. Given our informal observations indicative of withdrawal from FEN with its continued use, we decided that FEN was not an optimal choice of drug to use as a standard to compare the effects of other drugs.

In summary, these data confirm that FEN can establish a CPP (20). Also, and more importantly, these data show that the baseline scores, in terms of time on side of putative conditioning, are apt to remain stable provided the rats experience each side of the chamber an equal number of times and provided that no particular affective state is experienced in only one side during conditioning, i.e., control subjects' preferences are reasonably stable.

EXPERIMENT 3

The data presented in Experiments 1 and 2 indicate that chambers and procedures can be devised in which rats, during baseline, show no particular preference for one side of the chamber. Furthermore, there is no apparent development of preference with only repeated exposure to the apparatus. Data from a wide variety of experiments using a general CPP procedure, with a number of slight variations in particular procedures, have shown that M can be used to establish a CPP among rats having only limited exposure to M (3, 4, 20–22, 24, 34, 36, 37). Consequently, it was surprising to read Scoles and Siegel's (25) conclusion that the CPP test had problems that might obviate a conclusion that the effects of M would establish a preference for a place.

Scoles and Siegel's (25) design called for rats to receive morphine (M) in one side of an alley (a CPP apparatus), saline (S) in one side, and receive M in a holding cage (H). Given that there are two sides to the alley, a holding cage, and two drug conditions (M or S), a complete factorial design calls for many groups, but Scoles and Siegel (25) had only four groups, and, therein, lie the seeds of a problem. A baseline measure was taken (a measure of preference for a place before putative conditioning) and sides of the apparatus designated either preferred or nonpreferred based on the individual behavior of the rats (another potential factor of a complete design). On this basis, they then gave M or S on a schedule of putative conditioning, the results of which they tested after 12 and 24 daily trials (i.e., after a place had been paired with the effects of either M or S injections either 12 or 24 times). They had four groups whose labels characterize each group's procedure: M indicating an injection of morphine (15 mg/kg just before placing a rat in a place), S indicating an injection of saline, P indicating that rats were placed in the preferred side of the alley, N indicating placement in nonpreferred side, and H indicating placement in a holding cage away from the chamber. The initials SP/MN, thereby, indicate that on one day rats received S injections and were placed in preferred side of alley, and on another day M injections and placed in the nonpreferred side. This alternating daily schedule of putative conditioning spanned 12 days and was then followed by a test of rats' preferences without injections (in the same manner as baseline). The entire sequence of conditioning and testing was repeated so that there was a 2nd test at the end of 24 trials. Scoles and Siegel (25) had four groups: SP/MN, SP/MH, SH/MN, and SH/MH (see Fig. 6).

The left panel of Fig. 6 is a copy of the relevant figure from Scoles and Siegel [p. 1171, (25)]. As can be seen, the rats getting S on the preferred side and M on the nonpreferred side spent increased time, at testing and following putative conditioning trials, on the nonpreferred side; i.e., M seemed to establish a CPP. These results with Group SP/MN are as expected, as well as the data from Groups SH/MN and SH/MH. What is potentially problematic are the data associated with rats getting only saline in the alley (Group SP/MH).

The problem is that all data are tabulated (and analyzed) as time on nonpreferred side regardless of where rats received putative conditioning. If M is given before placing the rat in Side X during putative conditioning, the question is how much time the rat will spend on that side at testing. If S is given before placing the rat in

Side X during putative conditioning, the question is exactly the same, i.e., how much time the rat will spend on the side of putative conditioning at testing. Given these fundamentals about how to tabulate data from a CPP test, another and very different perspective is presented by Scoles and Siegel's data (25).

Recasting the data of the left panel of Fig. 6 in terms of time on side of putative conditioning, as is done in the right panel of Fig. 6, presents a very different picture. Rats of the group that received S in the preferred side of the alley at conditioning had putative conditioning on that side, i.e., the side where they experienced the effects of injections. Yet, Scoles and Siegel (25) tabulated their results *not* in terms of side of putative conditioning, but in terms of time on nonpreferred side (the side of no putative conditioning for the SP/MH group). The SP/MN group received M on nonpreferred side and that is their side of putative conditioning. At testing, they spent more time, than at baseline, on the side of putative conditioning. The SP/MH group (basically a control group), spent less time on the side in which they received S (see right panel of Fig. 6).

When the results are tabulated in terms of where rats received relevant injections, as seems the only logical way to ask the question of how the rats reacted to the effects of those injections (right panel, Fig. 6), it can be seen that M produced a substantial CPP (in comparison to controls and their own baseline). The same can hardly be said for rats getting S. The group not having an opportunity to experience effects of injections with any particular side generally showed no particular preference for a side at testing, a not unexpected result and probably related to extensive handling.

In brief, the data of Scoles and Siegel (25) provide one more confirmation that rats prefer the place of M's effects. When the data are tabulated differently, however, for the main control group and for the main experimental group in terms of where they received injections, but the same way in terms of some arbitrary designation of "nonpreferred" determined by the rats' initial experiences in the alley, the results do not provide confirmation that rats show a CPP following conditioning with M. *The determination of putative side of conditioning is,* however, *not arbitrary;* it is determined by where rats experience the effects of injections in question.

In addition to the general fact that results of no difference are subject to a number of logical limitations, the data of Scoles and Siegel (25), in themselves, when treated as the logic of the test demands, are not of the kind that should lead to the conclusion that there are potential problems with the conclusion that M can establish a CPP compared to placebos. Scoles and Siegel's paper (25) includes two additional experiments demonstrating that rats tend to move to places in which they have spent the least time and that this tendency is observed regardless of whether or not injections of saline are given. Scoles and Siegel (25) suggested, as sort of a final summary, that "nonassociative processes operating during saline trials are involved in place-preference conditioning." They could have made the statement even stronger by saying "nonassociative processes are involved in place-preference conditioning" This, however, should surprise no one. Nonassociative factors are involved in every test of animal behavior, even those in which every effort has been made to minimize the intrusion of those variables, such as tests in which learning itself is the focus. The issue is really not whether nonassociative processes are involved, but whether or not the nonassociative processes intrude to mask the processes of interest or lead to spurious results. In a poorly designed study, they can surely intrude. The interesting question, however, is do these factors intrude in apparently well-designed studies.

Since Scoles and Siegel's (25) data show that rats tend to spend more time in the place where they have been the least (as seen with the SP/MH group), and since they conclude that such tendencies obviate any CPP due to M's effects, it follows from their position that an M-induced CPP would be difficult to demonstrate if M were given in the putative side more often than placebo in the alternate side. This follows because, according to them, the rats getting M would tend to spend more time in the alternate side during testing and less time in the side of putative conditioning. The same prediction would be made for rats receiving placebos before being placed in the sides, i.e., they would tend to spend the least time at testing where they had spent the most time during conditioning. To test these inferences from their conclusions, the following experiment was done. Also, we asked whether or not differential handling (taming or habituation) among rats already accustomed to some handling would modify their responsiveness in these apparatus.

METHOD

Procedure

On the day after 72 rats arrived at the laboratory, they were assigned to one of two groups, one to receive daily special handling and the other to receive no special handling prior to the other procedures. The subjects in the group that was specially handled were, daily, removed from their home cages, transported to the conditioning room, handled there, and then returned to their home cages.

After 5 days of the special treatment for one group, a 20-min habituation period took place for all subjects. Subjects were factorially assigned to one of three dose groups: 0 (i.e., a control group), 2 or 4 mg/kg of M. Half of the subjects in each dose group were assigned the gray side as the side of putative conditioning and the other half were assigned the striped side.

Baseline scores were tabulated in a 20-min session on the next day. Across the next 6 days, subjects were given injections immediately prior to the start of 60-min conditioning sessions. The pattern of conditioning for this experiment consisted of 1 alternative (A) day followed by 2 putative (Pu) days, and this sequence was repeated (APuPuAPuPu).

A 20-min test for shifts in preferences was conducted the day after the last conditioning session (without experiencing effects of injections). Although the 20-min test session is shorter than we ultimately determined to be optimal, it is an effective period for indexing CPPs with opioids.

Statistics and Data Reduction

The initial data (time on side of putative conditioning across 20 min) were analyzed, taking into account all of the factors of the experimental design: Dose (0, 2 or 4 mg/kg), Handling (special or not special before the habituation day), Side of putative conditioning (striped of gray), and Day (baseline and test). However, the only reliable sources of variance were those associated with the main effect of Dose and the Day by Dose interaction. Consequently, we ignored the factors of Handling and Side of putative conditioning (gray or striped) in further analyses. No differences were found between the groups at baseline, so the initial design collapses into a one-way ANOVA of time spent on the side of putative conditioning during the test session for groups getting different doses of M, including 0 mg/kg, during conditioning.

RESULTS AND DISCUSSION

Figure 7 depicts the results. The ANOVA of test scores of the figure indicated that differences existed among the 3 groups, $F(2,69) = 8.18$, $p = 0.0004$. Student's t-tests, using the error term

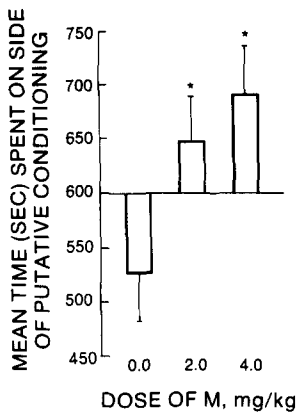

FIG. 7. Summarized are the 20-min test session scores, in terms of the mean time spent on the putative side of conditioning, for the 3 groups of Experiment 3. Error bars represent standard errors of the means. The results demonstrate morphine's ability to produce a CPP at doses of 2 and 4 mg/kg (statistical significance denoted by asterisks) relative to 0 mg/kg (controls, left bar).

from the ANOVA in the denominator, showed that both the 2 and 4 mg/kg doses of M established a CPP compared to controls, $t(69)=2.96$, $p<0.005$, and, $t(69)=4.06$, $p=0.0001$, respectively.

Although no significant differences were found in the data between the two handling conditions, the tamer and more habituated rats become, the less apt factors related to anxiety and fear are apt to intrude in an assessment. We decided, therefore, to incorporate the handling period into the usual procedure for our subsequent experiments.

As can be seen in Fig. 7, the scores of the control group are somewhat less than 600 sec, or 50% of the total time of the test. Although this difference from 50% is not statistically reliable, the data do provide some confirmation that rats tend to spend more time, at testing, in the place where they have spent the least time during conditioning, provided they only get placebos. Since, during conditioning, the rats had two experiences with the alternate side and four with the putative side, the alternate side of the alley was relatively more "novel." So, the expectation for rats experiencing no affective state (the control group) during conditioning would be to spend more time in the more novel environment. If these exploratory tendencies account for supposed M-induced CPPs, the two groups getting M should show the same tendencies. They, however, did not.

M (2 and 4 mg/kg) induced CPP indicative of positive affect in experimental spaces in which there is no reason to suppose that rats have an initial preference for a side. Furthermore, when tendencies to explore were opposite to those in which an M-induced CPP is apt to be manifest, an M-induced CPP was manifest. The conclusion is drawn that M can induce a CPP indicative of positivity, confirming a number of similar conclusions [e.g., (3, 4, 20–22, 24, 34, 36, 37)].

EXPERIMENT 4

Experiment 3 showed that a CPP could in fact be established in our chambers with M. However, there are still questions regarding some procedural variables. What influence, if any, would the order of the differential conditioning days have on the results? In Experiment 2, the order of conditioning (FEN on 1st day or saline on 1st day) made no difference. This, however, may not be the case when drugs and placebos are administered a different number of times. This experiment was designed to try to elicit an answer.

METHOD

Procedure

Sixty rats, starting 3 days after their arrival in the laboratory, were handled every day for 3 days. Over the next 2 days, each subject was placed in an alley for 1 hr each day, constituting the habituation and baseline sessions.

Subjects were randomly assigned to 1 of 5 groups. One group received saline injections prior to every conditioning period, i.e., a control group. Two groups received 4 mg/kg doses of M, the difference between the two groups being that while one group was administered M prior to every conditioning session (i.e., a "drug control"), the other group was administered M on days of putative conditioning and placebo on days of alternate conditioning. The last two groups received the same treatment, respectively, as the two groups receiving 4 mg/kg M, but were administered doses of 8 mg/kg of M when appropriate. Thus, there were 2 experimental groups which received either 4 or 8 mg/kg of M on the side of putative conditioning, and 3 control groups which received the same treatment on both sides of the alley.

Half of the subjects in each group were assigned the gray side as the side of putative conditioning, the other half were assigned the striped side. The 12 conditioning sessions, lasting 1 hr each, were conducted in 3 4-day blocks, with 3 days of no treatment intervening between each 4-day block. During each 4-day block, half of the rats in each group had 3 days of putative conditioning followed by a day of alternate conditioning (PuPuPuA), and the other half had a day of alternate conditioning followed by 3 days of putative conditioning (APuPuPu). For 3 days after the last conditioning session, subjects received no treatment. Then, all subjects were tested for shifts in preference for the side of putative conditioning in a 1-hr session allowing free access to both sides of the alley.

Statistics and Data Reduction

The data, when taking into account the "control" factors of the experiment, conform to a 2 by 2 by 5 by 6 by 2 ANOVA having repeated measures with factors associated with Side of putative conditioning (gray or striped), Order of conditioning (alternate conditioning day first or last in the 4-day block), Groups, Time (6 10-minute time segments of the sessions), and Days (Baseline and Test), respectively. The overall ANOVA performed on the data revealed a reliable 5-way interaction, $F(50,480)=2.00$, $p=0.0001$. As noted in Experiment 1 (Figs. 3 and 4), the variances associated with test session lengths above 30 min increase inordinately compared to the increase in preferences. Additionally, the factor of Time was not a reliable source of variance ($F<1$), so in subsequent analyses, we looked only at the first 30 min of the data.

The main effect of Side of putative conditioning (gray or striped) was not a reliable source of variance, nor did it reliably interact with any other factors of the design, except in the 5-way interaction. Therefore, this factor was dropped from further analyses. Although the main effect of Order of conditioning was a reliable source of variance, $F(1,50)=7.15$, $p=0.01$, it did not interact with any of the other factors, except in the 5-way interaction, and was also dropped from further analyses. None of the groups differed at baseline. Therefore, it follows that the effects seen at testing were due to differential treatments during conditioning.

Finally, since none of the 3 control groups differed from one another at test, the data from these groups were combined for the

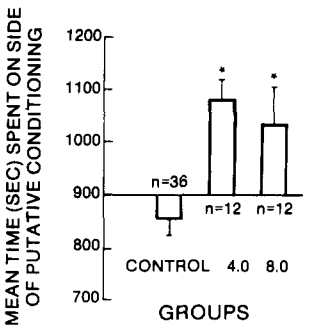

FIG. 8. Presented is a summary of the results of Experiment 4. The data is depicted in terms of the mean time (sec) spent on the putative side of conditioning during the 30-min test session. Error bars represent the standard errors of the means. The left bar shows the combined scores of the 3 control groups. The results demonstrate morphine's ability to produce CPPs (statistical significance denoted by asterisks) in doses of 4 and 8 mg/kg (middle and right bars, respectively) relative to controls.

final analysis. Thus, the analyses of the data associated with the amount of time spent on the putative side of conditioning during the test session, collapses into a one-way ANOVA for the factor associated with Groups (combined controls and experimentals getting 4 or 8 mg/kg of M).

RESULTS AND DISCUSSION

The results are depicted in Fig. 8. The ANOVA of the data of the figure revealed a reliable difference among groups, $F(2,57) = 8.37$, $p = 0.0006$. Student's t-tests, using the error term from the ANOVA, resulted in differences between the controls and both the 4 and 8 mg/kg M groups, $t(57) = 2.83$, $p < 0.007$, and $t(57) = 3.57$, $p = 0.0007$, respectively.

As mentioned above, the main effect of Order of conditioning was reliable, but this factor did not interact with other factors, except in the 5-way interaction. Subjects whose scheduled order of conditioning was PuPuPuA (where Pu stands for a day of putative conditioning and A stands for a day of alternative conditioning) had higher preference scores than did subjects whose order of conditioning was APuPuPu. However, since this factor did not reliably interact, specifically, with other potentially important factors, such as Groups or Days, it cannot be said to influence the ability of M to establish a CPP. Perhaps the two orders produce differing results because the subjects in the conditioning order PuPuPuA had been in the alternate side of the boxes on the day closest to testing, whereas the subjects in the conditioning order APuPuPu had last been in the alternate side some days prior to testing. The difference between the two means may be reflective of the relative novelty of the alternate side with APuPuPu. A choice that can be made to deal with this situation is to only use one order of conditioning.

A CPP can be established with M in our chambers. Once again, note that the control group's score at testing shows a preference less than 50%, due, in part, to the effective novelty of the alternate side. Note that the ratio of days of putative conditioning to days of alternate conditioning in this experiment is 3:1, whereas in Experiment 2, the ratio was 1:1. This difference in number of exposures to the alternate side of the chamber, is, we believe, the reason that the control group's mean preference for the side of putative conditioning decreases at testing in this experiment. This phenomenon is, theoretically, not exclusive to the control groups. The experimental groups must also overcome this tendency to stay in the place where they have been the least, i.e., the place that is most novel. So, the results of a test such as this one (and that of Experiment 3), where conditioning days were unequally divided into putative and alternate days, are actually quite conservative, since the preference for the putative side must be stronger than the tendency to explore novel environments in order to see test scores above those of the control group's.

We generally tabulate number of crossings from one side of the alley to another during baseline and test, but find that data of little interest when testing for drug effects. The typical result of these tabulations for scores at baseline is given in Fig. 4, which depicts the mean min by min data of the 60 rats of this experiment at baseline. We tabulated and analyzed the data of number of crossings in this experiment to see if differential conditioning associated with control groups and those associated with putative conditioning by M modified rats' crossing data. These analyses might be salient with respect to the argument by Scoles and Siegel (25) that when M does show a CPP that such a CPP may be due to some peculiarity in exploration. Number of crossings does provide a crude index of general exploratory behavior. Such data are probably also relevant to an argument by Swerdlow and Koob (32) who suggested that CPPs might be merely due to conditioned propensity to move.

Initial analysis determined that the 3 control groups did not differ from each other, with respect to transitions, at baseline or at test. Therefore, these groups' data were treated as if they were from a single control group in subsequent analyses. Thus, the data associated with transitions from one side of the alley to the other obtained at baseline and at test each conform to a 3 by 3 ANOVA having repeated measures with factors associated with Groups (controls, and those of 4 and 8 mg/kg M given on one side) and Time (the 3 10-min segments of the test), respectively. The ANOVA of the baseline data revealed that across time all 3 groups crossed back and forth between sides of the alley less and less, $F(2,114) = 130.4$, $p < 0.0001$, i.e., transitions decreased as a function of time. The ANOVA, however, did not reveal reliable sources of variance associated with Groups or with the Time by Groups interaction. Thus, at baseline, all groups behaved similarly, with respect to their exploration of the alleys. The min by min means of all 60 subjects are presented in Fig. 4.

The ANOVA of data at testing revealed, as expected, an overall effect associated with Time, $F(2,66) = 151.9$, $p < 0.0001$, with rats crossing the most times early in the session and steadily decreasing their rate of crossing across the length of the session. The ANOVA failed to reveal reliable sources of variance associated with the main effect of Groups or with the Time by Groups interaction. So, the 3 groups behaved similarly at testing, as well as at baseline, with respect to their exploration of the alleys. If the M-CPP we observed at testing was merely due to a conditioned propensity to move, as Swerdlow and Koob (32) would argue, then the groups should have explored the alley to different extents at testing. The data provide no evidence in support of this explanation.

The rats showing an M-CPP explore the chamber similarly to those of controls as indexed by their number of crossings from one side to another. Yet, the rats showing a M-CPP spend, by definition, more time on the side of putative conditioning. The conclusion is that rats showing a M-CPP surely explore, but prefer to spend the most time in the place of the M experience. It is doubtful whether this preference can be attributed to differential conditioned propensity to explore since the index of exploration (number of crossings) was not reliably different across groups, while time in side of putative conditioning was reliably different across groups.

In this experiment, unlike others, the sessions were not conducted across consecutive days. Although it will take a direct test to verify, it seems to make little difference to the overall outcome whether sessions are across consecutive days or not. It is

concluded that conditioning can be spaced without inordinate effects, a conclusion that is concordant with our general knowledge of conditioning.

In this experiment, and in Experiments 2 and 3, it was found that scores from groups getting FEN or MOR on both sides of the alley did not produce scores different than scores from groups getting saline on both sides. It is concluded, therefore, that the control procedure of administering the drug of putative conditioning on both sides can safely be eliminated from certain experimental designs.

EXPERIMENT 5

Scoles and Siegel (25) hypothesized that apparent CPPs following injections of M were due to the possibility that M blunted, attenuated, or blocked the rats' memory of the place of the M experience. It follows, according to their hypothesis, that a rat's preference for a place of M experience should be nearly the same as a rat's preference for a place in which they have never been. To test this hypothesis, the place preference of rats receiving M in a place was compared to the place preference of rats that had never been in that place (and, therefore, have no possibility of memory for that place).

METHOD

Procedure

Three weeks after their arrival in the laboratory, 39 subjects began the procedures. Subjects were handled for 5 days prior to a 30-min habituation session. The next day, subjects were assessed for baseline preferences in another 30-min session. Subjects were then factorially assigned to one of groups: 6 subjects to a SPu/SA condition, and 11 subjects each to MPu/SA, MPu/SH and MH/SA conditions. Group designations denote both the drug administered prior to the conditioning session (M or S for morphine or saline) and the placement of the subject during the 60-min conditioning sessions (Pu for side of putative conditioning, A for side of alternate conditioning, H for holding cage). Thus, the designation MPu/SH indicates that on days of putative conditioning, subjects of that group received 15 mg/kg of M immediately prior to being placed in the side of putative conditioning, and, on days of alternate conditioning, subjects of that group were administered saline immediately prior to the start of the conditioning session, but were left in the cages of the cart used to transport subjects to the conditioning room. Nearly half of the subjects in each group were assigned to the gray side as the side of putative conditioning and the remainder to the other.

Conditioning sessions (1 hr in length) took place in three 4-day blocks. The 1st day of each block was a day of alternate conditioning and the last 3 days of each block were days of putative conditioning. Three days of no treatment intervened between each block of conditioning. Three days after the last conditioning session, subjects were tested for shifts in preferences in a 30-min test session. In brief, this is nearly a direct replication of Scoles and Siegel's (25) procedure, but using apparatus for which rats show no initial preference (Experiment 1).

Statistics and Data Reduction

The side of putative conditioning is clear for three of the groups, i.e., the place where they received injections on 3 days of a 4-day block. Such a designation allows a direct comparison of groups SPu/SA and MPu/SA to observe whether or not the basic observation of M, in comparison to placebo, establishes a place preference. To test the hypothesis that M produces forgetting and a state similar to having never been placed in a side, it is necessary to designate the side opposite to the side of saline injections of group MH/SA as the side of putative conditioning even though the rats received no conditioning in that side. The designation of the sides in the MH/SA group is, therefore, somewhat arbitrary.

The overall design of the experiment conforms to a 4 by 2 by 2 ANOVA having repeated measures with factors associated with Groups (MPu/SA, MPu/SH, MH/SA and SPu/SA), Side of putative conditioning (gray or striped) and Days (Baseline and Test), respectively. The ANOVA revealed a reliable main effect of Groups, $F(3,31)=3.31$, $p=0.03$, and a reliable Days by Groups interaction, $F(3,31)=3.85$, $p<0.02$. Since the factor of Side of putative conditioning was not a reliable source of variance, and since it did not reliably interact with any of the other factors, it was dropped from further analyses. There were no differences among the groups at baseline ($F<1$), so the analyses of the data (time on side of putative conditioning) collapsed into a one-way ANOVA with the factor associated with Groups.

RESULTS AND DISCUSSION

The results of the experiment are presented in Fig. 9. The ANOVA of that data revealed a difference among the groups, $F(3,35)=6.40$, $p<0.002$. Hotteling's T^2 statistic revealed that there were reliable differences between the scores of the MPu/SA group and the scores of all three other groups: $F(1,20)=23.2$, $p=0.0001$, versus the MPu/SH group; $F(1,20)=5.72$, $p<0.03$, versus the MH/SA group; and, $F(1,15)=25.8$, $p=0.0001$ versus the SPu/SA group. These results indicate that M established a CPP under the usual conditions of our procedure as compared to (a) subjects never having S paired with a side of the chamber, (b) subjects never having M paired with a side of the chamber, and (c) controls (S on both sides). No differences were found between any other pairs of groups. This indicates that care must be taken to provide an opportunity for rats to pair different experiences with each side of the chamber for a strong CPP to emerge.

The tendency for rats to explore novel places is strong, as evidenced by the MPu/SH group. These animals, who had received M on the side of putative conditioning, showed no apparent CPP, even though they experienced 9 pairings of M with the putative side. The lack of a CPP with group MPu/SH may be peculiar to experimental spaces in which rats have no initial

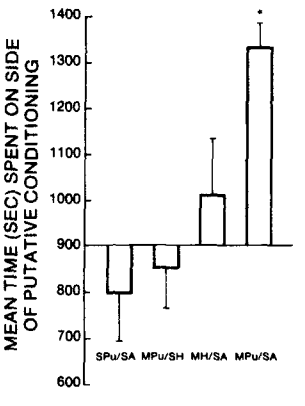

FIG. 9. Results from a test of morphine's ability to establish a CPP. See the Method section of Experiment 5 for explanation of group designations. Notice that the side of putative conditioning is designated by the initials Pu for three of the groups. The side of putative conditioning for group MH/SA was arbitrarily designated as the side opposite of saline injections, a place where in fact that group received no injections.

preference, and should not be construed to mean that procedures similar to that group's procedures would not yield meaningful data in other contexts, or with larger groups than used here.

Subjects in the MH/SA group, according to Scoles and Siegel, should have shown a strong preference for the place opposite to saline injections at testing, since the novelty of that place should have enhanced rats' preferences for that side. Although these rats did, on the average, spend more time on the side of novelty, that score is not reliably different that the control group's score or reliably different than 900 sec (the score predicted by chance alone). If one designates the side of injections of group MH/SA as the side of putative conditioning, the difference between the scores is even greater.

GENERAL DISCUSSION

These data verify the general conclusion of Rossi and Reid (24): rats having no previous experience with M will spend more time in the place that they experienced M's effects than rats that have received a placebo. In brief, the effects of M, compared to placebo, change rats' preferences for a place. The change is rather subtle. As a rat explores the alley, it gradually accumulates more time in the place of M's effects. Furthermore, M's effects will be manifest in a situation in which rats have no apparent preference for a side prior to experiencing M and when the side of putative conditioning is determined randomly before putative conditioning. Since this effect of M has been observed many times since it was first observed, across a number of laboratories and with considerable variation in the exact features of the apparatus and procedure (but all controlling for factors that might lead to spurious results), it seems reasonable to conclude M does change the average rat so that it has a bias for the place where it has experienced M's effects.

The question of whether or not M's effects as manifest in a CPP test with rats are relevant to opioid addictions as manifest by certain people is, of course, a question that is impossible to answer with confidence using only these kinds of results. Similarly, the question of whether or not M's effects as manifest by laboratory animals' self-administration of M, by way of lever-pressing, are relevant to opioid addiction as manifest by certain people is, of course, a question that is impossible to answer with confidence by way of a test with animals. The accumulation of results verifying the basic observation (i.e., M will establish a CPP or M will sustain lever pressing) merely verify the basic observation and do not by themselves address the issue of relevance. Likewise, the demonstrations that other agents that can become a focus of an addiction among people can also establish a CPP or sustain self-administration *by themselves* do not verify relevance of either measure. Indeed, the test of relevance is a much more protracted process.

The ultimate test is whether or not the theory of addictions derived from the results of these kinds of tests [and tests of the internal logic of these theories, e.g., see Mook (19)] is utilitarian. The ultimate test is, therefore, whether or not the information derived from our experiments forms a cohesive, logically sound theory leading to the remediation of the problems of addiction. Furthermore, only theories can be applied, not results of single experiments.

That rats explore the places in which they are put should surprise no one who has observed rats. It is, perhaps, somewhat more surprising that rats' tendencies to spend time in one side of an alley compared to another side can be manipulated so regularly by ratios of previous experience in one side compared to another. It is clear from the data of Scoles and Siegel (25), the experiments reported here, and from our previous experience with CPP testing, that careful controls for rats' extent of exploration is essential if a meaningful statement is to be drawn concerning a drug effect. At a minimum, there should be a control group having similar placements in the chambers to make between-group comparisons at testing. Merely using within-group comparisons between scores of baseline and test is probably not sufficient.

There has been considerable interest shown toward CPP testing. CPP testing has some advantages over the other "preclinical" tests for the potential for a drug to be positively reinforcing, hence potentially addicting (7, 14, 33). M, heroin, cocaine, amphetamine, and phencyclidine (PCP) have each been shown to sustain a CPP (6, 17, 22, 27–29). More interesting, however, than the potential for the CPP test to be an index of addiction liability, perhaps, is what the CPP test reveals about the nature of addictive drugs themselves. The effects of pairing at least some addictive drug's effects with a particular environment is to coerce subsequent behavior so that the subject is moved to spend more time in the place of the drug experience. Such demonstrations are particularly compatible with theories stressing the incentive features of addictive drugs [e.g., (11, 31, 36, 37)]. In fact, such theories seem to provide a more complete characterization of the addictive process than previous theories stressing the potential negative reinforcing features of drugs of abuse in terms of either their potential to relieve withdrawal symptoms or their potential to relieve anxiety or stress. Drugs of addiction seem to be more than antidotes for withdrawal and medicants for stress, they seem to act as incentives moving the individual to experience the place of and the experience of the drug again.

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