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The Activation and Expression of Endogenous Pain Control Mechanisms in Rats Exposed to Nociceptive Stimulation under the Influence of Morphine or Naloxone: Implications for Models of Environment-Specific Tolerance to the Analgesic Effect of Morphine.

Joseph Rochford

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ABSTRACT

The Activation and Expression of Endogenous Pain Control Mechanisms in Rats Exposed to Nociceptive Stimulation Under the Influence of Morphine or Naloxone: Implications for Models of Environment-Specific Tolerance to the Analgesic Effect of Morphine

Joseph Rochford, Ph.D.
Concordia University, 1985.

The present experiments investigated the effect of morphine and naloxone administration on the development of conditioned autoanalgesia. It was predicted that, by attenuating the intensity of nociceptive stimulation to which animals are exposed during conditioning, morphine would attenuate the development of conditioned autoanalgesia. In addition, it was predicted that naloxone administration would enhance the development of conditioned autoanalgesia by magnifying the perceived intensity of nociceptive stimulation (i.e., eliciting hyperalgesia). Experiments 1-3 were conducted to investigate the prediction that morphine would attenuate the development of conditioned autoanalgesia. The results of these experiments confirmed the prediction, although the degree of attenuation was dependent upon the intensity of the nociceptive stimulus employed.

Experiments 4-9 tested the prediction that naloxone administration would enhance the development of conditioned autoanalgesia. As predicted, naloxone did enhance conditioned autoanalgesia. However, contrary to prediction, naloxone administration did not elicit hyperalgesia, but produced a robust analgesia that developed over

repeated analgesia tests. It was found that the naloxone-induced analgesia resulted partly from the attenuation of the habituation of novelty/stress-induced analgesia, and also in part from exposure to nociceptive stimulation. In addition, it was found that the naloxone-induced analgesia was mediated by a non-opiate antinociceptive substrate.

The results were discussed in terms of the activation and expression of both opiate and non-opiate pain control mechanisms, and their implications for models of environment-specific tolerance to morphine analgesia were addressed.

For The Lary and Pops, with love and affection.

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Much of the pleasure that is derived from the completion of a production of this magnitude comes from reflecting upon the aid and good will of the people who helped in every aspect of its preparation. The roll call that follows may appear overly lengthy, but to exclude any one of its members would be to deny an expression of gratitude that is rightfully due them.

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Thanks also to my colleague and office mate, Paul Vezina. Paul's incessant desire (some might even call it obstinacy) to discover "truth" would not permit my natural inclination to overlook the nagging inconsistencies that plague all ideas and theories. His persistence forced me to evaluate more critically both self-generated ideas and those garnered from other sources. Indeed, only after I was able to respond to the objections of the "Grand Inquisitor" did I feel

comfortable with the hypotheses and interpretations reported in the thesis. And despite my frequent attempts to silence him through personal insult and slander, he maintained a dignified air of humanity and kindness that I have rarely seen in others.

A gracious acknowledgement must also be offered to Nicole Milhomme. Nicole always had the presence of mind to respond to my hurried and anxious demands with a knowing resignation and a friendly smile. How she ever accomplished this feat is beyond me, but to her credit she did. Thanks, as well, to my other office mate, John Mitchell, who was the quintessance of patience when I descended upon him with newly acquired data and my premature interpretation of them. He (and Paul) must also be commended for the benign acceptance of what undoubtedly appeared to be an endless series of bad and tasteless jokes. For some reason, they always laughed.

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Last, but by no means least, thanks to my wife, Joelle. A gentle touch, a loving kiss, a knowing glance, a soft hug, a well timed display of humour-- these were the things that made me realize there is more to life than science and experimentation. They gave me more pleasure than any words can describe.

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In a series of studies published in the late sixties and early seventies, Mitchell and his associates demonstrated that the development of tolerance to the analgesic effect of morphine is maximized if tolerance induction and analgesic testing are conducted in the same environment (Adams, Yeh, Woods & Mitchell, 1969; Ferguson, Adams & Mitchell, 1969; Gebhart & Mitchell, 1971; 1972; Kayan, Ferguson & Mitchell, 1973; Kayan, Woods & Mitchell, 1969). These studies were important because they they were the first to demonstrate that environmental manipulations play a functional role in the development of tolerance. Until that time it was assumed that tolerance reflected a change in one or more physiological processes, such as receptor occupancy (Axelrod, 1956; Seevers, 1958), receptor disuse (Collier, 1968; Sharpless & Jaffe, 1969), or the formation of new receptors (Collier, 1965), solely as the result of iterative drug administrations. These theories clearly did not anticipate, nor could they account for, the work emanating from Mitchell's laboratory.

Although Mitchell and his associates were the first to demonstrate environment-specific tolerance, they were unable to specify a mechanism for the effect. Adams et al. (1969) suggested that environment-specific tolerance is attributable to a drug-stress interaction. This hypothesis was so poorly formulated, however, that the assessment of its validity was precluded (Kesner & Baker, 1981). As such, the major impetus of tolerance research in the last fifteen years has been the delineation of the mechanism(s) responsible for environment-specific tolerance.

The Pavlovian Model of Environment-Specific Analgesic Tolerance:

In a series of seminal papers, Siegel (1975; 1976; 1977) argued that Pavlovian conditioning is the mechanism underlying environment-specific tolerance to the analgesic effect of morphine. According to Siegel, morphine serves as an unconditioned stimulus (UCS) that elicits unconditioned responses (UCRs) such as analgesia. When morphine is routinely administered in a particular environment, the environment comes to serve as a conditioned stimulus (CS) capable of eliciting conditioned responses (CRs). The hallmark of Siegel's hypothesis is that the CR elicited by a CS is not identical to the UCR elicited by the drug (as is often observed during Pavlovian conditioning with more conventional reinforcers, see Eikelboom & Stewart, 1982; Mackintosh, 1974; 1983), but rather is opposite in direction to the UCR. Thus, when a given environment is paired with morphine administration, it acquires the ability to elicit a compensatory response that counteracts the direct, unconditioned effects of the drug. It is this compensatory CR that is assumed to mediate environment-specific tolerance. In the case of morphine-induced analgesia, for instance, the CR is assumed to be hyperalgesia. With repeated environment-morphine pairings, the environment comes to elicit a conditioned hyperalgesic response that attenuates the potency of morphine-induced analgesia, thereby resulting in environment-specific tolerance to morphine analgesia.

The hypothesis that Pavlovian conditioning is involved in the development of environment-specific tolerance to morphine analgesia is consistent with demonstrations that tolerance development is similarly influenced by manipulations known to affect Pavlovian conditioning with

more conventional reinforcers. For instance, it has been shown that: (1) analgesic tolerance can be extinguished by exposing animals to the CS without drug (Siegel, 1975; 1977; Siegel, Sherman & Mitchell, 1980); (2) the development of analgesic tolerance can be attenuated by intermittent reinforcement (i.e., pairing the CS with drug administration on less than a 100% basis, Siegel, 1977); (3) analgesic tolerance is subject to "latent inhibition" (Lubow & Moore, 1959), that is, tolerance development is diminished if animals are exposed to the CS prior to CS-drug pairings (Siegel, 1977); (4) the development of analgesic tolerance to a CS can be overshadowed if that CS is presented in compound with a more salient CS (Walter & Riccio, 1983); (5) the development of analgesic tolerance to one component of a compound CS is reduced if the second component of the compound CS is paired with drug delivery prior to pairing the compound CS with drug (i.e., the Kamin (1969) "blocking" effect, Dafters, Hetherington & McCartney, 1983); and (6) a CS can elicit tolerance if it is paired, not directly with morphine administration, but with another CS that is subsequently paired with drug delivery (i.e., sensory preconditioning, Dafters et al., 1983).

Although these results are consistent with the idea that a Pavlovian mechanism is operative in the development of morphine analgesic tolerance, they do not in and of themselves provide direct support for Siegel's hypothesis. Unequivocal support for the validity of this hypothesis requires the demonstration that the CS paired with morphine administration can elicit a conditioned compensatory hyperalgesic response when animals are tested for analgesia under

saline. Siegel and his associates have reported just this effect (Krank, Hinson & Siegel, 1981; Siegel, 1975). The design of each of these studies involves two major groups. One group, the experimental group, is administered morphine in the presence of a distinctive set of cues, the CS. The second group, the control group, is administered morphine that is explicitly unpaired with the CS. Following the morphine administration, or tolerance induction, phase of the experiment, both groups are administered tests for analgesia in the presence of the CS. The typical result is that animals in the experimental group are more responsive to nociceptive stimulation than those in the control group (i.e., they are hyperalgesic).

The idea that environment-specific tolerance represents the conditioning of a compensatory response has much intuitive appeal; more importantly, it has been the directing force behind a considerable body of research. There are, however, a number of theoretical and empirical problems with the hypothesis. The first is that Siegel fails to specify a mechanism through which compensatory conditioning may occur. Why is it that morphine administration elicits a CR that is antagonistic to the UCR? The implicit assumption is that compensatory CRs are adaptive, at least insofar as they allow the animal to maintain homeostatic equilibrium. This logic, however, does not appear to be ideally suited to certain response systems, one of which is the system mediating pain responsivity. To illustrate, consider first the system controlling body temperature. The advantage inherent in the ability to either increase or decrease body temperature in response to changing environmental conditions is obvious. The ability to increase or

decrease body temperature in turn suggests that both of these responses are potentially conditionable, such that if administration of a hyperthermia-inducing drug is consistently predicted by a given set of cues, these cues can come to elicit a compensatory, hypothermic CR that would help to maintain equilibrium.[1]

The logic of homeostasis is not as readily applicable to the mechanism mediating an animal's responsivity to nociceptive stimulation, however. Under what environmental circumstances would it be advantageous for an animal to lower its pain threshold? There are environmental conditions in which an advantage would be conveyed by an increase in pain thresholds (see Bolles & Fanselow, 1980), but delineating circumstances in which increased pain-responsivity would be advantageous is more difficult. If an animal cannot lower its pain threshold, then it is unlikely that such a response is conditionable.

These speculations notwithstanding, the compensatory conditioning hypothesis can be attacked on empirical grounds (see Baker & Tiffany, 1985; Kesner & Baker, 1981; for reviews). Most damaging to the hypothesis have been studies that have failed to find evidence for conditioned hyperalgesia when tests for analgesia were conducted under saline, in spite of the fact that environment-specific tolerance was observed when animals were tested under morphine (Abbott, Melzack & Leber, 1982; LaHoste, Olson, Olson & Kastin, 1980; Morris, Jonzen, Welsh & Cahusec, Note 1; Sherman, 1979; Tiffany, Petrie, Baker & Dahl, 1983). These studies suggest that conditioned hyperalgesia is <u>not</u> the mechanism mediating environment-specific tolerance (Baker & Tiffany, 1985).

The Opponent Process Model of Tolerance: Like Siegel's model, Solomon's (1977; 1980) opponent process model of tolerance is founded on the concept of homeostasis. According to Solomon, all hedonically important stimuli or events, one example of which could be the effect of a narcotic drug, elicit primary hedonic responses, defined as a-processes. The strength of a given a-process is assumed to be determined by the intensity, quality and duration of the hedonic stimulus that elicits the process. Moreover, it is assumed that the ability of a given hedonic stimulus to elicit an a-process of a given strength does not diminish as a function of repeated presentations of the stimulus. Rather, a-processes diminish in strength because they in turn elicit antagonistic b-processes that counteract the effects of the a-processes and function to return the organism to equilibrium. In constrast to a-processes, b-processes are assumed to have longer latencies to onset, to increase in strength over iterative stimulus presentations, and to decay more slowly over time, such that b-processes can persist for longer periods of time than a-processes.

It is the incremental increase in the strength of b-processes over repeated morphine presentations that is presumed to constitute tolerance. This occurs because the overall effect of morphine is assumed to be an additive function of the strength of the a-process with the counterdirectional b-process. As the b-process grows in strength over repeated drug presentations, the apparent strength of the a-process is reduced.

The opponent process theory of tolerance accounts for situation-specific tolerance by assuming that the environment in which the

stimulus is presented becomes capable of directly eliciting the b-process. In this sense the model is comparable to Siegel's compensatory response hypothesis. As such it is open to the same criticisms.

There is, however, one fundamental difference between the compensatory response hypothesis and the opponent process model that allows the latter to account for a phenomenon that the former cannot. Specifically, the opponent process model can account for those demonstrations of tolerance that do not appear to be attributable to the operation of a Pavlovian mechanism. It is clear that not all demonstrations of the development of morphine tolerance are environment-specific. Demonstrations of tolerance in vitro are the most obvious examples (Ehrenpreis, Light & Schonbuch, 1972; Schulz, Seidl, Wuster & Herz, 1982), but non-environment-specific tolerance has also been observed in vivo (see Baker & Tiffany, 1985; Kesner & Baker, 1981; for reviews). Such demonstrations have been termed "dispositional" tolerance in order to distinguish them from environment-specific tolerance, and also to indicate that the probable mechanism underlying the effect involves a physiological substrate that is unaffected by environmental manipulations. The opponent process model can readily account for dispositional tolerance because the b-process is assumed to grow in strength primarily as a function of the number of morphine presentations. As such, tolerance should be observed even if no single environment is consistently paired with morphine delivery. In contrast, since Siegel's compensatory response hypothesis requires that tolerance should be observed only in those

instances where the environment elicits a compensatory CR, his hypothesis cannot account for demonstrations of dispositional tolerance.

In fairness, it should be noted that Siegel (1978) acknowledges that not all instances of tolerance can be accounted for by the Pavlovian mechanism he has proposed. His emphasis is on explaining the environmental specificity of some forms of tolerance development. As a consequence, demonstrations of dispositional tolerance are not fatal for his hypothesis. Nevertheless, the opponent process model possesses more explanatory power than Siegel's hypothesis because it can, at least theoretically, accomodate demonstrations of dispositional tolerance.

To summarize, both Siegel and Solomon attribute the development of environment-specific tolerance to the conditioning of a compensatory or opponent CR that counteracts the direct, unconditioned effects of morphine. Thus, both theories make identical predictions regarding the development of environment-specific tolerance. The opponent process model, however, is better equipped to accomodate demonstrations of dispositional tolerance.

The Novelty Hypothesis: Both the compensatory response hypothesis and the opponent process model invoke Pavlovian conditioning as the mechanism underlying the development of environment-specific tolerance. Not all theories of the effect have assumed that this mechanism is operative, however. In this and the next section two models of environment-specific tolerance that do not invoke Pavlovian conditioning as their modus operandi are reviewed.

Bardo and Hughes (1979) suggested that environment-specific tolerance to morphine may reflect the habituation of an endogenously mediated analgesia produced by the novelty of the analgesia testing apparatus. That is, animals administered analgesia tests for the first time display reduced sensitivity to nociceptive stimulation because the novelty of the testing conditions elicits a novelty- or stress-induced analgesia. As animals acquire more experience with the test procedure, however, the novelty-induced analgesia habituates, resulting in a heightened sensitivity to pain.

In order to test this hypothesis, Bardo and Hughes (1979) exposed animals to a hot-plate apparatus maintained at ambient temperature. One group was exposed following an injection of morphine, while a second group received saline prior to exposure. Two other groups also received morphine and saline, respectively, but were not exposed to the hot-plate cues. Following the exposure phase of the experiment all groups were administered tests for analgesia on a 49.5° C hot-plate. It was found that animals previously exposed to the plate were hyperalgesic during the test for analgesia relative to animals experiencing the plate for the first time. Moreover, this effect occurred independently of whether the animals were previously exposed under morphine or saline, as well as whether tests for analgesia were conducted under morphine or saline. These results suggest that morphine need not be paired with a particular environment in order for increased pain sensitivity to be observed. All that is required is that animals be made familiar with the test apparatus.

Both Kayan et al. (1969) and Sherman (1979) have reported evidence

consistent with the novelty hypothesis. However, habituation to novelty can account for only part of the increased pain sensitivity observed during the development of environment-specific tolerance. First, environment-specific tolerance has been observed in the absence of any pre-exposure to the testing apparatus (Siegel, Hinson & Krank, 1978; 1981). Second, in much environment-specific tolerance research all animals are equally familiarized with the testing apparatus, but only animals that receive such exposure under the influence of morphine display maximal levels of tolerance (e.g., Adams et al., 1969). Finally, the novelty hypothesis cannot explain demonstrations of the extinction of tolerance following non-reinforced (i.e., non-drugged) exposures to the test apparatus (Siegel, 1975; 1977; Siegel et al., 1980). Since animals administered extinction trials receive more exposure to the test apparatus than non-extinguished animals, the novelty hypothesis would predict that extinction should result in more, not less, tolerance.

The Habituation Hypothesis: The most recent theory of situation-specific tolerance has been advanced by Baker and Tiffany (1985; see also Kesner & Baker, 1981; for an earlier formulation). Noting that the behavioral manifestations of both habituation and tolerance are characterized by a reduction in response magnitude to a stimulus over repeated stimulus presentations, Baker and Tiffany argue that the two processes may be identical.

The particular model of habituation adopted by Baker and Tiffany was originally developed by Wagner (1976). Wagner assumed that the magnitude of responding promoted by a stimulus is determined by the

degree to which it is processed in short-term memory (STM). The level of stimulus processing is determined by the extent to which the stimulus is already primed or represented in STM. That is, a surprising stimulus is assumed to generate more processing than an expected stimulus because the latter is already primed in STM.

According to Wagner, there are two ways in which a stimulus can be primed in STM, self-generated and associative priming. Self-generated priming refers to those instances where a stimulus is presented at a time when it is already represented in STM, for example, by a prior, but recent, presentation of the stimulus. Associative priming occurs not by the presentation of the stimulus itself, but rather by the presentation of a second stimulus that predicts or has been paired with delivery of the first stimulus. For example, if a tone has been paired with shock administration, subsequent presentation of the tone alone will suffice to elicit a representation of shock in STM.

Baker and Tiffany assume that self-generated priming and associative priming are the mechanisms underlying the development of dispositional and environment-specific tolerance, respectively. If, for example, a second administration of morphine follows a first before the effects of the first administration have dissipated, then the effect of the second administration will be diminished. Thus, the development of dispositional tolerance is presumed to be an inverse function of the interval between morphine administrations and a direct linear function of the dose administered; the shorter the interval or the higher the dose, the greater the development of dispositional tolerance. This relationship between administration interval, dose and

dispositional tolerance, however, is assumed to occur only in those instances where there are no reliable cues that signal drug delivery. When reliable pre-drug signals exist, dispositional tolerance is overshadowed by environment-specific tolerance due to associative priming. In this case the drug signal primes a representation of drug in STM before the drug is administered. As a consequence, when the drug is administered it will exert a smaller effect because it has already been primed by the pre-drug signal.

Unlike dispositional tolerance, associatively primed, environment-specific tolerance is assumed to be acquired more rapidly with long inter-administration-intervals and smaller doses. This occurs because longer intervals and smaller doses permit more accurate discrimination of reliable drug signals. Thus, those conditions that would be expected to optimize the development of dispositional tolerance (short inter-administration intervals, high doses) are precisely those that would be detrimental to the development of environment-specific tolerance.

Baker and Tiffany have reviewed the morphine tolerance literature and have shown convincingly that much of the evidence is consistent with the habituation model. These authors themselves note, however, that there are limitations to the model. Most problematic is that the model cannot account for CRs that are antagonistic to unconditioned drug effects. Indeed, on the basis of research conducted within their own laboratory (Tiffany et al., 1983; Zelmer, Tiffany & Baker, 1984; cited in Baker & Tiffany, 1985), Baker and Tiffany explicitly question the validity of those demonstrations of conditioned hyperalgesia that

have been reported (Krank et al., 1981; Siegel, 1975). They were unable, however, to provide a satisfactory account of the discrepant results emanating from their own and Siegel's laboratories. They offered the possibility that Siegel's results may be the result of a drug-stress interaction, or possibly attributable to conditioned activity effects (see Mucha, Volkovskis & Kalant, 1981; Tiffany, et al., 1983), without explaining in detail how these hypotheses can account for Siegel's data.

Nevertheless, the theoretical advantage of the habituation model lies in the possibility that a single mechanism may be able to account for dispositional and environment-specific tolerance. It is worth noting, however, that the attempt to integrate both dispositional and environment-specific tolerance within an habituation framework does not convey a theoretical advantage over models that invoke Pavlovian conditioning as one of a number of mechanisms mediating tolerance development. For example, in an earlier formulation of the habituation model, Kesner and Baker (1981) suggested that both Pavlovian and habituative processes were involved in tolerance development. Dispositional tolerance was attributed to habituation, while environment-specific tolerance was attributed to Pavlovian conditioning. Moreover, Tiffany and Baker (1985) acknowledge that such a "two-process" model of morphine tolerance can account for the available data as readily as the habituation model. Further research will be required, therefore, to determine if environment-specific tolerance is best attributed to a Pavlovian or an habituation mechanism.

Foundation of a New Hypothesis: The conclusion to be drawn from the review of the four models of environment-specific tolerance just completed is that Pavlovian conditioning very probably plays a role in the development of the effect. The major theoretical challenge to this claim was provided by the habituation model proposed by Baker and Tiffany (1985), and, as noted, above, many of the predictions of the model are consonant with those that invoke Pavlovian conditioning as the operative mechanism. It is not clear, however, that the homeostatic models suggested by Siegel (1975; 1976; 1977) or Solomon (1977; 1980) correctly characterize the influence of Pavlovian conditioning on environment-specific tolerance. Demonstrations of CRs that are opposite in direction to drug UCRs are critical to the unequivocal support of these models; yet the evidence for these is inconsistent. Moreover, neither model provides a mechanism through which the conditioning of compensatory or opponent CRs may occur.

Eikelboom and Stewart (1982) have argued that a coherent explanation of the Pavlovian conditioning of drug-induced physiological responses must first take into consideration what facets of the drug experience constitute the UCS and the UCR. It has been well demonstrated, for example, that morphine can induce either excitatory or depressant effects depending on the response under investigation (see Eikelboom & Stewart, 1982; for review). In fact, with some response systems, for example, those controlling body temperature (Cox, Ary, Chesarak & Lomax, 1976; Gunne, 1960) and locomotor activity (Babbini & Davis, 1972; Sloan, Brooks, Eisenman & Martin, 1962; Vasko & Domino, 1978), a single administration of morphine can produce

biphasic, depressant/excitatory, effects. These results make it difficult, in many cases, to identify which effect constitutes the UCR to morphine. Should the UCR be considered the depressant or the excitatory effect? The correct identification of the UCR to morphine is particularly crucial to models of environment-specific tolerance that assume the operation of a homeostatic Pavlovian mechanism. Because the CR is presumed to antagonize the UCR, the nature and direction of the UCR must be specified before it can be determined if, indeed, the CR opposes the UCR.

It could be argued that the foregoing discussion is irrelevant to investigations of the analgesic effect of morphine for the simple reason that morphine administration always unconditionally induces analgesia. This generalization, however, like most, is incorrect. Although rare, there have been reports that, under certain conditions, morphine elicits an unconditioned hyperalgesia (Jacquet & Lajtha, 1973; Kayan, Woods & Mitchell, 1971). These results raise the possibility that the conditioned hyperalgesia demonstrated by Siegel and his associates is a CR that mimics a hyperalgesic UCR.

Eikelboom and Stewart suggest that the identification of the UCR must be made on the basis of the manner in which a drug affects the response system controlling the response in question. Fortunately, the system mediating an organism's ability to regulate pain sensitivity is beginning to be defined. Thus, examining the manner in which morphine affects this system should provide some clues into the manner in which Pavlovian conditioning affects the development of situation-specific tolerance to morphine analgesia.

The Endogenous Pain Control System: The very fact that morphine induces analgesia suggests the existence of an endogenous mechanism for the perception and modulation of nociceptive stimulation. It was not until Reynold's (1969) demonstration that electrical stimulation of the periaqueductal gray (PAG) attenuates pain responsivity, however, that the details of the endogenous pain control system (EPCS) began to be elucidated. In particular, Reynold's demonstration spurred a considerable research effort that has, for the most part, identified the neural circuitry involved in the endogenous control of pain.

Basbaum and his associates have made several reviews of the relevant literature (Basbaum, 1984; Basbaum & Fields, 1978; 1984; Fields & Basbaum, 1978; see also Mayer & Price, 1976), and have proposed a tentative model of the EPCS. The EPCS is a three-tiered system comprising the dorsolateral funiculus (DLF) of the spinal cord, as well as a number of diverse nuclei located within the mid-brain and the rostral medulla (see Figure 1). The neuronal circuits are constructed such that they form a negative feedback loop. Thus, nociceptive information is carried from the periphery to the spinal cord and is then relayed, via the nucleus reticularis gigantocellularis, to the PAG in the mid-brain. The PAG innervates various nuclei within the rostral medulla (e.g., the nucleus raphe magnus, the nucleus reticularis magnocellularis) which in turn project fibers to the DLF. These latter fibers, when activated, inhibit the transmission of the nociceptive signal, thereby inducing analgesia.

One of the first questions that arose from the discovery of the EPCS is whether this system is involved in the mediation of the

Figure 1: The endogenous pain control system. (See text for details. Abbreviations: DLF: dorsolateral funiculus; E: enkephalin; 5-HT: serotonin; NRM: nucleus raphe magnus; PAG: periaqueductal gray; RGC: nucleus reticularis gigantocellularis; RMC: nucleus reticularis magnocellularis; SP: substance P. Adapted from Basbaum & Fields, 1978).

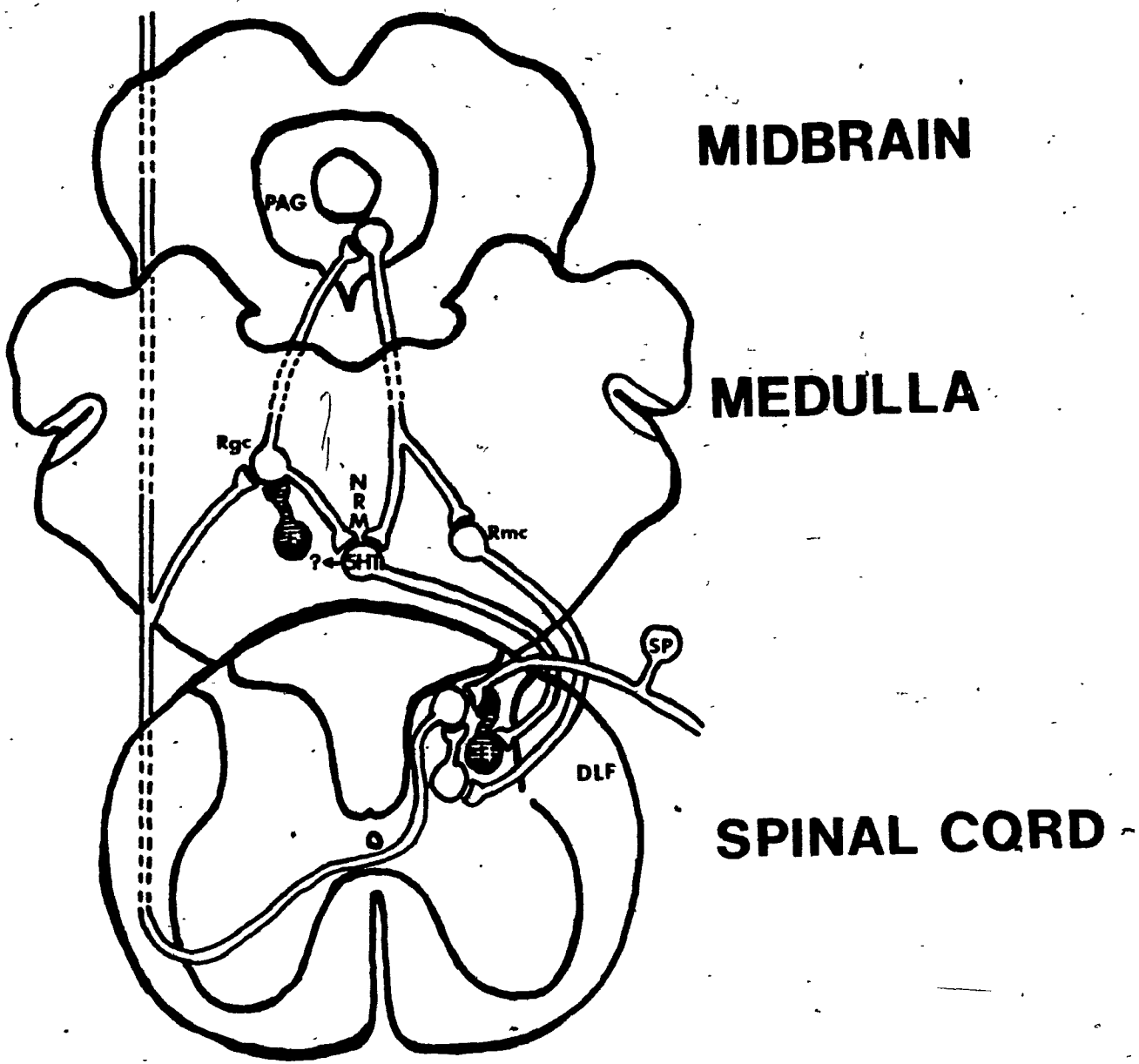

Figure 1

analgesic effect of morphine. It soon became evident that it was. First, morphine administration into the PAG (Lewis & Gebhart, 1977; Yaksh, Yeung & Rudy, 1976), the raphe magnus (Azami, Lleweln & Roberts, 1982) and intrathecal administration of morphine directly into the spinal cord (Yaksh & Rudy, 1976) was shown to produce analgesia. Second, administration of the opiate antagonist naloxone blocks the antinociceptive effect of morphine microinjected into a number of brain nuclei or the spinal subarachnoid space (see Yaksh & Rudy, 1978; for review). Third, all of the critical neuronal loci of the EPCS have been found to be rich in opiate receptors (Atweh & Kuhar, 1977a; 1977b; Pert, Kuhar & Snyder, 1976). Finally, cross-tolerance between morphine- and stimulation-produced analgesia has been observed (Mayer & Hayes, 1975).

There appear to be at least two sites where morphine exerts its analgesic effects (Basbaum, 1984). Both sites are located on the efferent arm of the system. First, exogenous morphine is assumed to directly activate descending inhibitory neurons originating in the PAG and the rostral medulla. This suggestion is suported by the observation that the analgesic effect of systemic morphine is abolished by spinal cord transection (Satoh & Takagi, 1971). However, as noted above, the finding that morphine produces analgesia when injected into the spinal cord (Yaksh & Rudy, 1976) indicates a direct spinal action. There is some evidence that the supraspinal site is dominant (Barton, Basbaum & Fields, 1980), but Yeung and Rudy (1980) have demonstrated that the overall analgesic effect of morphine most likely results from an interaction between spinal and supraspinal sites.

A second question that has received extensive attention is the identification of the neurotransmitters or neuromodulators responsible for the analgesia resulting from the activation of the EPCS. The discovery of endogenous, opiate-like peptides (i.e., met- and leu-enkephalin, Hughes et al., 1976; beta-endorphin, Li & Chung, 1976) possessing analgesic activity (Beluzzi et al., 1976; Bradbury, Feldberg, Smyth & Snell, 1976), appeared to suggest that endogenous analogues to morphine were responsible, and raised the possibility that the pain-inhibitory function of the EPCS is mediated by a single transmitter. It was soon demonstrated, however, that naloxone administration did not always produce complete reversal of stimulation-produced analgesia (Akil, Mayer & Liebeskind, 1976), suggesting that the EPCS consisted of two components: a naloxone-sensitive, opiate-mediated component, and a naloxone-insensitive, non-opiate component. The neurotransmitter mediating the non-opiate component of the EPCS has yet to be identified, although there is some evidence to suggest that norepinephrine is involved (see Basbaum & Fields, 1978; Basbaum, Moss & Glazer, 1983; for review).

The third, and perhaps most important, issue that has been investigated has involved the identification of the conditions that normally activate the EPCS. Noting that "pain inhibits pain" (Melzack, 1975), and that exposure to noxious peripheral stimulation is the most effective way of activating the nuclei in the rostral medulla (Guilbaud, Peschanski, Gautron & Binder, 1980), Basbaum and Fields (1978) suggested that pain itself may be one of the factors activating the EPCS. Support for this hypothesis came from subsequent

demonstrations that exposure to painful electric foot- or tail-shock elicits profound and long-duration analgesia as assessed by a variety analgesia tests (Chesher & Chan, 1977; Drugan, Moye & Maier, 1982; Hayes, Bennett, Newlon & Mayer, 1978; Hyson, Ashcraft, Drugan, Grau & Maier, 1981; Lewis, Cannon & Liebeskind, 1980; Lewis, Sherman & Liebeskind, 1981; Madden, Akil, Patrick & Barchas, 1977; Maier, Drugan & Grau, 1982; Maier, Sherman, Lewis, Terman & Liebeskind, 1983; Terman, Shavit, Lewis, Cannon & Liebeskind, 1984; Watkins, Cobelli, Faris, Aceto & Mayer, 1982). Pain is not unique, however, in its ability to induce endogenously mediated analgesia. It has been shown that exposure to a variety of non-painful stressors, such as centrifugal rotation, injection of intraperitoneal hypertonic saline (Hayes et al., 1978), restraint (Amir & Amit, 1978), hypoglycemia (Bodnar, Kelly & Glusman, 1979), novelty (Bardo & Hughes, 1979; Sherman, 1979) and exposure to a bright and noisy environment (Tiffany et al., 1983), reduce pain sensitivity.

Recently, it has also been demonstrated that, in addition to nociceptive stimulation or stressors themselves, exposure to environmental signals predictive of pain or stress can activate the EPCS (Chance & Rosecrans, 1979a; 1979b; Fanselow, 1984; Fansleow & Baackes, 1982; Hayes et al., 1978; Oliverio & Castellano, 1982; Sherman, Strub & Lewis, 1984; Watkins, Cobelli & Mayer, 1982). That is, exposure to cues previously associated with stressors that in themselves activate the EPCS can elicit analgesia in the absence of the stressor, an effect that has become known as "conditioned autoanalgesia" (Chance, 1980).

It has been shown that the analgesic potency of morphine is enhanced during those instances where the EPCS has been previously activated, suggesting that the antinociception resulting from the activation of the EPCS can synergize with morphine's analgesic actions (Colpaert, Niemegeers & Janssen, 1978; Colpaert, Niemegeers, Janssen & Maroli, 1980; Sherman, Procter & Strub, 1982; Sherman et al., 1984). In each of these studies the analgesic potency of morphine was potentiated by administering analgesia tests in the presence of cues capable of eliciting conditioned autoanalgesia or by exposing animals to nociceptive stimulation prior to morphine administration and the assessment of analgesia.

Although it is clear that, once activated, the EPCS can enhance morphine analgesia, it is worthwhile considering the complimentary question of the effect of morphine administration upon the activation of the EPCS. Consider, for example, animals that are exposed to nociceptive stimulation some time after they are administered morphine. Given that morphine is an analgesic, it should reduce the intensity of the nociceptive stimulation, and as a result the pain-induced activation of the EPCS that would normally occur in the absence of morphine would be reduced. Not only would the unconditioned activation of the EPCS be inhibited, but, as a result, the development of conditioned autoanalgesia to the cues predictive of pain would be attenuated as well.

These considerations suggest an alternative account of the influence of Pavlovian conditioning on the development of environment-specific morphine analgesic tolerance. Specifically, they imply that

the the effect is not attributable to the conditioning of a conditioned compensatory or opponent response, but rather it depends upon the degree to which morphine administration reduces the unconditioned (and, as a consequence, the conditioned) activation of the EPCS. Consider, for instance, two groups of animals that are exposed to nociceptive stimulation in a distinctive environment. One group is exposed under morphine, the other under saline. When both groups are subsequently administered analgesia tests under saline, the morphine-exposed group will appear hyperalgesic relative to the saline-exposed group because of morphine attenuation of the development of conditioned autoanalgesia. Moreover, when both groups receive morphine in the distinctive environment, the morphine-exposed group should appear more tolerant to the drug, because, as noted above, conditioned autoanalgesia summates with morphine-induced analgesia.

The Present Experiments: The foregoing discussion can be summarized more succinctly: The development of environment-specific tolerance to morphine analgesia will vary, at least in part, with the extent to which the environment recruits or activates the EPCS. When recruitment is minimal, as in the scenario outlined above, tolerance will appear maximal. The first series of experiments to be reported in this thesis was carried out in order to test this prediction. The effect of morphine administration on the development of conditioned autoanalgesia was examined. Animals were administered either morphine or saline and then exposed to varying intensities of nociceptive stimulation. On the test days, animals were administered either saline or morphine in the environment associated with nociceptive stimulation

and then given tests for conditioned autoanalgesia. It was predicted that animals exposed to nociceptive stimulation under morphine would show increased sensitivity to pain during analgesic testing relative to animals exposed to nociceptive stimulation under saline.

In the second series of experiments, a corollary of the hypothesis was investigated. Specifically, the hypothesis predicts that the level of conditoned autoanalgesia, and therefore the analgesic potency of morphine, will be enhanced in those circumstances where the recruitment of the EPCS is increased. One way of attempting this was to administer the opiate receptor blocker naloxone prior to nociceptive stimulation. It was thought that naloxone administration might interfere with the pain-inhibitory effects of the EPCS, thereby causing animals to experience more pain (Amir & Amit, 1978; Bonnet, Alpert & Klinerock, 1978; Carmody, Carroll & Morgans, 1979; Coderre & Rollman, 1983; Jacob, Tremblay & Colombel, 1974). This increased pain would in turn lead to a greater recruitment of the EPCS over repeated exposures to nociceptive stimulation and to a greater conditioned autoanalgesic response, which would be expected to manifest itself as an environment-specific analgesia when tests were conducted without opiate receptor blockade.

Chapter 1

In the present series of experiments the effect of morphine administration on the development of conditioned autoanalgesia was examined. As stated in the introduction, it was predicted that animals exposed to nociceptive stimulation under the influence of morphine would display lowered levels of conditioned autoanalgesia than animals exposed to pain under saline.

Experiment 1

In Experiment 1 animals were administered either morphine or saline and were then exposed to either no shock, 1 mA shock for 45 sec, or 2.5 mA shock for 180 sec. The shock parameters were selected in order to vary both the intensity and the duration of the nociceptive stimulus. Previous research has indicated that the unconditioned analgesia elicited by these two different sets of parameters is mediated by different endogenous pain control substrates. Forty-five sec of 1 mA shock appears to elicit an opiate-mediated analgesia, at least insofar as it is blocked by prior administration of naloxone (Sherman et al., 1984; Terman, et al., 1984). Exposure to 2.5 mA shock for 180 sec is not antagonized by naloxone (Lewis et al., 1980), suggesting mediation by a non-opiate substrate. The use of these different parameters permitted the determination of whether morphine-attenuation of conditioned autoanalgesia was dependent upon the substrate activated by the nociceptive stimulus.

Method

Subjects: In this and subsequent experiments the subjects were experimentally naive, male Wistar rats obtained from Charles River

Breeding Farms, St. Constant, Quebec. The rats weighed 275-300 g upon arrival at the laboratory. They were individually housed, provided with constant access to food and water, and were maintained on a 12:12 light/dark schedule. All procedures were conducted during the light phase of the cycle. In the present experiment 48 rats were used.

Apparatus: The apparatus was located in a test room illuminated by three 25 W, red light bulbs and maintained at a constant 22° C temperature. Analgesia was assessed by a hot-plate apparatus, which consisted of a 20.3 x 20.3 x 38.1 cm clear plexiglas chamber mounted on a .6 cm thick, 26.7 x 30.5 cm piece of sheet metal. A hinged, wire mesh top mounted on the top of the chamber prevented the animals from escaping from the chamber. The plate temperature was controlled by immersing the sheet metal in a water bath heated by a Haake E2 Immersion/Open Bath Circulator.

Shock was administered in eight operant conditioning chambers measuring 19.5 x 29 x 23 cm (inner dimensions). The side walls were made of stainless steel and the front and back walls and the ceiling were constructed of transparent plexiglas. The floor was constructed of stainless steel bars. A lever, food cup and two plastic light covers were located on the right side wall. These objects were not used in the present experiment. The chambers were located in sound and light attenuating chests. Both the fan and houselight of the chests were disconnected, providing a dark and quiet environment. Shocks were administered by eight Grason Stadler scrambled shock generators (Model E1064GS). Shock administration was controlled by an ISAAC 91A (Cyborg Corporation) laboratory interface connected to an Apple II+

microcomputer located in an adjacent room.

Morphine or saline was administered by subcutaneous injection in the dorsal surface of the neck. In this and subsequent experiments the morphine dose was 5 mg/kg dissolved in a dose/ml/kg volume.

Procedure: All animals were handled in the morning (10-11:00) and afternoon (16-17:00) for five days prior to the start of the experiment. In addition, the rats were weighed during their morning handling.

Habituation Days: The first three days of the experiment were used to habituate the animals to the procedures in order to minimize the influence of stress-induced analgesia elicited by the novelty of the testing procedure (cf. Bardo & Hughes, 1979; Sherman, 1979). The rats were transported to the test room and placed on the plate surface which was maintained at ambient temperature (22° C), for 60 sec. The animals were subsequently placed in the shock boxes, but received no shock. They were then removed and placed on the plate for 60 sec at 30, 45 and 60 min following the initial placement into the shock boxes. During the interval between plate exposures the animals were returned to the shock boxes. After the last exposure animals were returned to their home cages. No injections were administered during these days.

Conditioning Days: On the first conditioning day, the rats were assigned to one of six groups (N=8) differing with respect to drug treatment and shock condition. Three groups of rats were administered morphine in the test room and saline in the home cage (groups RM-M/HC-S). Three groups received the reverse drug treatment; saline was injected in the test room and morphine in the home cage (groups RM-

S/HC-M). Within each drug treatment condition, one of the groups was administered 1 mA shock for 45 sec (moderate shock), a second group received 2.5 mA shock for 180 sec (strong shock), while the third group was not exposed to any shock (no shock).

The conditioning days of the experiment were similar to the habituation days. Following transportation to the test room animals were exposed to the ambient temperature plate for 60 sec. They were then administered their test room injection and placed in the shock chambers. Shock administration for the groups assigned to the moderate shock condition began 28 min, 15 sec following placement in the shock boxes. In the strong shock condition shock administration began 26 min following placement in the shock boxes. This procedure was adopted to insure that shock termination occurred at the same time for both shock conditions. Groups assigned to the no-shock condition were merely placed in the shock chambers without shock. Ambient temperature plate exposures (60 sec) were conducted at 30, 45 and 60 min following placement in the shock chambers. The animals were then returned to their home cages where they received their home cage injection 4-6 hours later. Except for days where analgesic testing was conducted (see below), conditioning was conducted over four consecutive days.

Analgesia Testing Days: On the analgesia test days the water temperature of the hot-plate bath was 50° (± 0.2° C). The animals were placed on the surface of the plate and the latency to perform a hind-paw lick or a jump response was recorded to the nearest 0.1 sec with a stopwatch. The animals were removed from the plate chamber immediately following either of these responses. A jump response was defined as

any vertical movement that lifted the animal's entire body at least three inches from the surface of the plate (as indicated by a marker taped onto the hot-plate chamber).

The procedure followed during the analgesia testing days was identical to that on the conditioning days with the exceptions that no shock was administered in the shock chambers and no home cage injections were administered. The hot-plate test conducted prior to injection in the test room and prior to placement in the shock boxes constituted the baseline trial. The baseline trial was conducted to determine if any conditioning occurred to general cues such as transportation to the test room or the test room itself. Tests occurring 30, 45 and 60 min following placement into the shock chambers constituted the post-CS trials.

The first two analgesia testing days were conducted after the first and fourth conditioning days. Following the second analgesia testing day the animals were given a two day respite during which time they remained undisturbed in their home cages. They were then administered a third analgesia testing day. On the first three test days all animals were administered saline. The day following the third saline test day all animals were injected with 5 mg/kg morphine in the test room prior to placement in the shock boxes and post-CS testing.

Results

Figure 2 shows the mean response latencies on the post-CS trials for the six groups on the three saline and the morphine test days. For purposes of analysis the data from the three post-CS trials were collapsed. Inspection of Figure 2 shows that exposure to both moderate

Figure 2: Mean response latencies (± SEM) for groups previously injected with morphine in the test room and saline in the home cage (RM-M/HC-S) or saline in the test room and morphine in the home cage (RM-S/HC-M) during the three saline and the morphine test days of Experiment 1. Prior to the test days animals were exposed to either no shock (left panel), moderate shock (center panel) or severe shock (right panel).

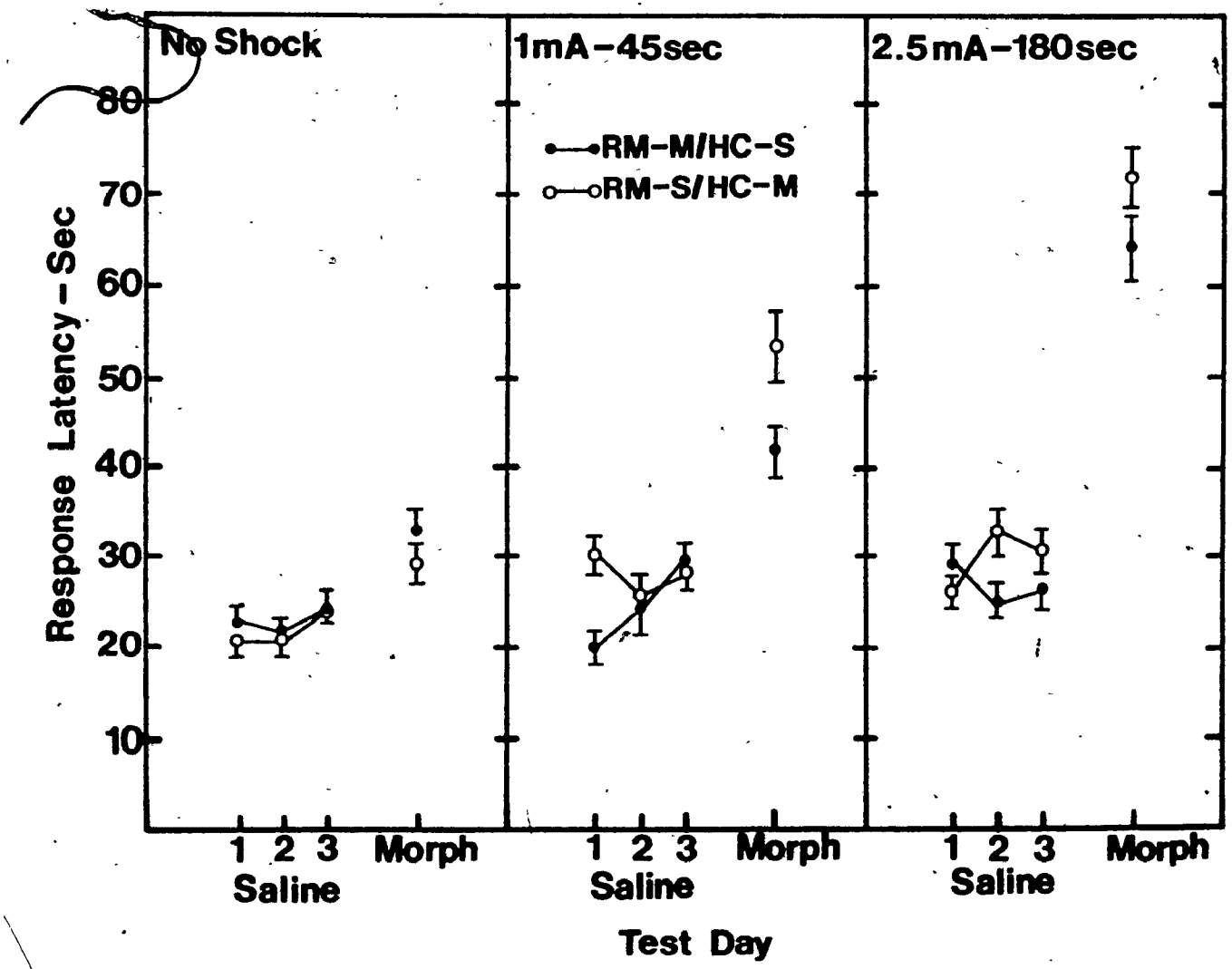
No Shock
1mA-45sec
2.5mA-180sec
RM-M/HC-S
RM-S/HC-M
Response Latency-Sec
80
70
60
50
40
30
20
10
1 2 3 Morph
Saline
Test Day

Figure 2

and severe shock increased latencies relative to non-shocked animals. This observation was confirmed by a split-plot analysis of variance (drug treatment x shock condition x test days), which yielded a significant main effect of shock condition, $F(2,42) = 22.91$, $p < .001$. It is also clear from Figure 2 that the magnitude of the conditioned autoanalgesia within the two shock conditions varied as a function of the drug treatment administered during conditioning days and drug treatment at the time of testing. This was confirmed by a significant drug treatment x shock condition x test days interaction, $F(6,126) = 2.25$, $p < .05$. Tests for simple main effects (Kirk, 1968, pp. 179-182) were conducted between groups for each shock condition for each test day. No significant differences were obtained between the non-shocked groups on any test day, $Fs(1,168) \leq 1.13$, $ps > .05$. Moreover, although the response latencies for group RM-S/HC-M-2.5 mA-180 sec were longer than those for group RM-M/HC-S-2.5 mA-180 sec on saline tests 2 and 3 and on the morphine test day, these differences were not statistically significant, $Fs(1,168) \leq 3.14$, $ps > .05$. In contrast, group RM-S/HC-M-1 mA-45 sec exhibited significantly longer latencies than group RM-M/HC-S-1mA-45 sec on the first test day, $F(1,168) = 5.89$, $p < .025$. The difference occurred only on the first saline test, no significant differences between these groups occurred on the second or third saline tests, $Fs < 1.0$. However, when these groups were subsequently tested under morphine the longer latencies initially observed in group RM-S/HC-M-1mA-45 sec reappeared, $F(1,168) = 7.38$, $p < .01$.

The analysis of variance performed on the baseline scores resulted in significant main effects for test days, $F(3,126) = 3.99$, $p < .01$,

and a significant shock condition x test days interaction, $F(6,126) = 3.89$, $p < .002$. Pairwise comparisons, conducted using Tukey's Wholly Significant Difference test, revealed that animals in the strong shock condition exhibited significantly longer latencies than non-shocked and moderate shocked animals on the second saline test day. However, no other differences between shock conditions were obtained for any other test day. These results suggest that some conditioning of autoanalgesia occurred to the general transportation or test room cues in the strong shock condition, but that this conditioning was weak and transient.

Discussion

The results of Experiment 1 replicate previous reports that the environment in which animals are exposed to nociceptive stimulation elicits analgesia when animals are exposed to the environment without nociceptive stimulation (Chance, 1980; Chance & Rosecrans, 1979a; 1979b; Fanselow, 1984; Fansleow & Baackes, 1982; Oliverio & Castellano, 1982; Sherman et al., 1984; Watkins et al., 1982). The results of Experiment 1 also provide partial support for the hypothesis that morphine attenuates the development of conditioned autoanalgesia. Animals administered 1 mA shock for 45 sec under the influence of morphine displayed lower response latencies on both the first saline test day and the morphine test day. However, there were no differences between the groups on saline test days 2 and 3. These results suggest that morphine attenuated conditioning early in training, but upon further exposure to shock the level of conditioned autoanalgesia in animals shocked under morphine approached the level seen in animals

shocked under saline. Thus, some degree of autoanalgesia was conditioned in morphine-shocked animals.

The results from the morphine test day, however, suggest that animals previously shocked under saline and then exposed to the CS under morphine displayed significantly greater analgesia than animals previously shocked under morphine. It is as if the manifestation of the greater degree of conditioned autoanalgesia in the saline-shocked group is accentuated when testing is conducted under morphine. The absence of a difference on saline test days 2 and 3 may simply reflect a ceiling effect. That is, a 50° hot plate test may have been insensitive to any subtle difference in autoanalgesia between saline- and morphine-shocked animals. During the morphine test day, however, the ceiling is lifted, and the difference between groups reappears. Sherman et al. (1984; Experiment 2) have shown that animals exposed to shock did not display longer latencies than non-shocked controls when tests for conditioned autoanalgesia were conducted under saline and the plate temperature was 52.5°, but they did show increased latencies if the temperature of the plate was reduced to 51.0°. When tests for conditioning were conducted under morphine, however, the temperature of the hot-plate was irrelevant. In their experiments animals exposed to shock were more analgesic than non-shocked animals at both temperatures.[2]

Consider now the results from the 2.5 mA for 180 sec shock condition, where morphine administration consistently failed to attenuate conditioned autoanalgesia. This failure may simply reflect the severity of the shock to which these animals were exposed. The

dose of morphine administered may have been insufficient to significantly reduce the aversiveness of this form of shock. Therefore morphine would not attenuate conditioned autoanalgesia.

Alternatively, these results are consistent with the possibility that morphine may attenuate autoanalgesia in those cases where the shock parameters activate only the opiate component of the endogenous pain-suppression mechanism (i.e, the moderate shock condition; see Sherman et al., 1984; Terman et al., 1984), but not in those instances where shock elicits a non-opiate mediated analgesia (i.e., the severe shock condition; see Lewis et al., 1980; 1981; Terman et al., 1984). The results of Experiment 1 do not permit the determination of whether shock severity or the analgesic substrate activated by shock is the crucial variable, although it should be possible to discriminate between these two possibilities. If morphine failed to attenuate the development of conditioned autoanalgesia produced by a more severe form of shock, but one that elicited an opiate-mediated analgesia (see Terman et al., 1984), this would suggest that shock severity, rather than the antinociceptive substrate activated by shock, is of primary importance.

Finally, there were no significant differences in response latencies between morphine- and saline-treated animals within the no shock condition. This result suggests that morphine administration alone is insufficient to produce (apparent) conditioned hyperalgesia in animals habituated to the experimental procedures. Rather, it suggests that morphine-treated animals will display hyperalgesia only in those cases where they (and their saline controls) are exposed to some form

of stress or nociceptive stimulaton. This issue will be elaborated on further in the General Discussion.

Experiment 2

Experiment 2 was conducted to determine whether morphine administration would attenuate the conditioned autoanalgesia produced by a weaker form of shock; specifically, 1mA shock for 15 sec. In addition, a few minor modifications were incorporated in the design of Experiment 2. First, because the effects observed during the baseline trials of Experiment 1 were weak and transient, and because these trials may have made it difficult for animals to distinguish between CS trials and non-CS trials, no baseline trials were administered in Experiment 2. Second, only two post-CS hot-plate tests were administered in the present experiment. Finally, shock was administered for seven days, rather than four as in Experiment 1.

Method

Subjects and Apparatus: The subjects were 48 rats maintained as in Experiment 1. The apparatus was identical to that employed in Experiment 1.

Procedure: As in Experiment 1, the animals were handled twice daily for five days prior to the start of the experiment.

Habituation Days: The habituation days of Experiment 2 were similar to those of Experiment 1 with the exceptions that no baseline exposure was administered and the animals received only two post-CS exposures. The exposures occurred 30 and 45 min following placement into the shock boxes.

Conditioning Days: On the first conditioning day the animals were randomly assigned to six groups (N=8). Three of the groups (RM-M/HC-S) received morphine in the test room and saline in the home cage, the

other three (RM-S/HC-M) received the reverse drug treatment. Within each drug treatment condition, one group was never exposed to shock (no shock), the second group was exposed to 1 mA shock for 45 sec (moderate shock), while the third group received 1 mA shock for 15 sec (mild shock). For animals receiving moderate shock, shock administration began 28 min, 15 sec following placement into the shock boxes. Shock administration for the mild shock condition began 28 min, 45 sec following placement into the shock boxes. Shock was administered for seven days.

Analgesia Testing Days: The analgesia test days were similar to those of Experiment 1, with the exceptions already noted. Analgesia testing days, during which all animals received saline in the test room, were conducted following the first, fourth and seventh conditioning days. A morphine test day (5 mg/kg) was conducted on the day following the third saline test day.

Results

Figure 3 shows the mean response latencies for the six groups on the three saline and the morphine test days of Experiment 2. As in Experiment 1, the data from the two post-CS trials were collapsed for the purpose of data analysis. The analysis of variance (drug treatment x shock condition x test days) revealed that exposure to shock produced conditioned autoanalgesia, $F(2,42) = 8.52$, $p < .002$. However, as in the previous experiment, the magnitude of the conditioned autoanalgesia varied as a function of drug treatment and test day, as indicated by a significant three way interaction, $F(6,126) = 3.27$, $p < .01$. Tests for simple main effects, conducted between groups at each shock condition

Figure 3: Mean response latencies (± SEM) for groups previously injected with morphine in the test room and saline in the home cage (RM-M/HC-S) or saline in the test room and morphine in the home cage (RM-S/HC-M) during the three saline and the morphine test days of Experiment 2. Prior to the test days animals were exposed to either no shock (left panel), mild shock (center panel) or moderate shock (right panel).

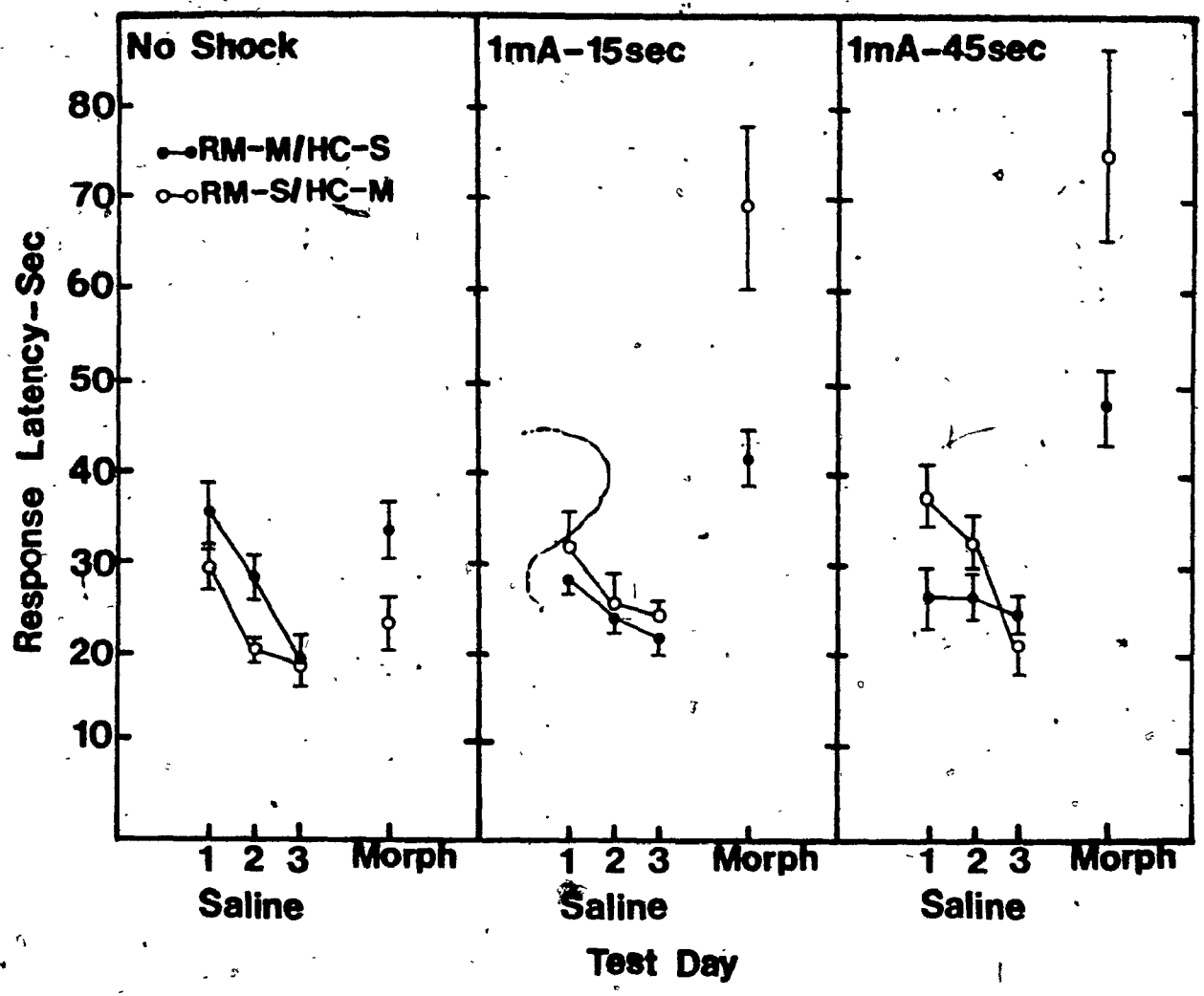
No Shock
1mA-15sec
1mA-45sec
RM-M/HC-S
RM-S/HC-M
Response Latency-Sec
80
70
60
50
40
30
20
10
1 2 3 Morph
Saline
Test Day

Figure 3

for each day, revealed the nature of this variability. Although group RM-M/HC-S-NO SHOCK displayed longer latencies than group RM-S/HC-M-NO SHOCK on saline tests 1 and 2 and on the morphine test day, these differences were not significantly different, $\underline{F}s(1,168) \leq 3.10$, $\underline{p} > .05$. Nor were there any significant between-group differences on the three saline test days at the 1 mA for 15 sec shock condition, $\underline{F}s < 1.0$. On the morphine test day, however, animals previously administered this form of shock under saline displayed significantly longer latencies than did animals shocked under morphine, $\underline{F}(1,168) = 22.14$, $\underline{p} < .001$. As in Experiment 1, group RM-S/HC-M-1mA-45 sec exhibited longer latencies than group RM-M/HC-S-1mA-45 sec on the first saline test day, $\underline{F}(1,168) = 4.06$, $\underline{p} < .05$, but not on the second or third saline test days, $\underline{F}s(1,168) \leq 1.06$, $\underline{p}s > .05$. However, when tested under morphine, animals previously shocked under saline again showed longer latencies than animals previously shocked under morphine, $\underline{F}(1,168) = 14.72$, $\underline{p} < .001$, a finding that also replicates that of Experiment 1.

Discussion

As in Experiment 1, animals exposed to the moderate shock condition under saline displayed longer latencies than morphine-shocked animals on the first saline test day and the morphine test day. These findings add further support to the argument that morphine administration attenuates the conditioned autoanalgesia elicited by this form of shock. More interesting, however, were the results from animals exposed to the mild shock condition. Although there was no evidence for morphine-attenuation of conditioned autoanalgesia within

this shock condition on the three saline test days, on the morphine test day animals previously shocked under saline displayed significantly longer latencies than animals shocked under morphine. As argued previously, these results may reflect a ceiling effect. That is, it is possible that saline-shocked animals possessed stronger levels of conditioned autoanalgesia relative to morphine-shocked animals, but that the hot-plate test employed prevented the manifestation of this difference until the animals were tested under morphine.

Experiment 3

In the majority of experiments demonstrating the development of situation-specific morphine analgesic tolerance, the nociceptive stimulus to which animals are exposed is not electric footshock, but thermal stimulation as applied by either the hot-plate or the tail-flick test. Therefore, Experiment 3 was conducted to determined if exposure to thermal stimulation would produce conditioned autoanalgesia and whether or not morphine administration would attenuate the development of conditioned autoanalgesia. Animals were exposed to either an ambient temperature, 22° C cold-plate, or a 50°, 52° or 54° C hot-plate. Tests for conditioned autoanalgesia were conducted using both the tail-flick and the hot-plate tests.

Method

Subjects: The subjects were 64 rats maintained as in the previous experiments.

Apparatus: The hot-plate apparatus was identical to that used in the previous experiments. Tail-flick testing was conducted with an acrylic restrainer (Harvard Bioscience, Catalog No. 52-0916). Following injection in the test room the animals were isolated in separate 30.5 x 20.3 x 15.2 cm wooden boxes, which were lined with Beta-chip and covered by steel grid tops.

Procedure: Twice a day for five days preceding the experiment the animals were handled and acclimatized to being inserted and restrained in the restrainer. Because in most studies of situation-specific morphine analgesic tolerance animals are not habituated to the testing procedure, no habituation to the test room or hot-plate apparatus was

given in the present experiment.

Conditioning Days: On the first conditioning day the animals were randomly divided into eight groups (N=8), differing with respect to drug treatment and the temperature of the hot-plate. Four groups (RM-M/HC-S) received morphine (5 mg/kg) in the test room and saline in the home cage, while the remaining four groups received the reverse drug treatment (RM-S/HC-M). Within each drug treatment condition, one of the groups was exposed to an ambient temperature, 22° cold-plate; the remaining three groups were exposed to the plate immersed in a 50°, 52° or 54° water bath, respectively.

The animals were transported to the test room and immediately administered their test room injection. They were placed in the wooden boxes for 30 min and then exposed to the plate apparatus. All exposures to the plate were of 45 sec duration. For animals receiving hot-plate exposures, the latency to lick the hind paw or to make a jump reponse was recorded to the nearest 0.1 sec with a stopwatch. The animals were then transported to their home cages where they received their home cage injections 4-6 hours later. Four conditioning days were administered.

Analgesia Testing Days: On the test days for conditioned autoanalgesia all animals were injected with saline and 30 min later received either a tail-flick or a hot plate test. During the tail-flick test days the animals were first exposed to the ambient temperature cold-plate for 45 sec. They were then inserted into the restrainer and the distal 5 cm of the tail was immersed into 50° water. The latency to completely withdraw the tail from the water was measured to the nearest 0.1 sec.

Following tail-flick testing the animals were returned to their home cages.

Three tail-flick testing days were conducted. The first two test days occurred following the first and the fourth conditioning days. The third test day was conducted three days following the second test day. During the respite between the second and third test days the animals remained undisturbed in their home cages.

Three days following the third tail-flick test day, the animals were administered a hot-plate test under saline. The temperature of the hot-plate water bath was 50° C and the latency to perform a hind-paw lick or a jump response was measured to the nearest 0.1 sec.

Results

Conditioning Days: Figure 4 presents the mean response latencies for the six groups receiving hot-plate exposures during the four conditioning days of the experiment. The analysis of variance (drug treatment x hot-plate temperature x conditioning days) yielded significant main effects for hot-plate temperature, $F(2,42) = 38.29$, $p < .001$, reflecting the fact that higher temperatures produced shorter latencies, and for drug treatment, $F(1,42) = 32.24$, $p < .001$, indicating that morphine tended to elevate latencies. However, it is clear from Figure 4 that the analgesic potency of morphine varied as a function of plate temperature and conditioning days, as confirmed by a significant three way interaction, $F(6,126) = 3.10$, $p < .01$. Tests for simple main effects conducted over days for each group at each plate temperature revealed that the latencies for morphine-exposed animals declined over days, $Fs(3,126) \geq 8.51$, $p < .001$, indicating tolerance to

Figure 4: Mean response latencies (± SEM) for groups injected with morphine in the test room and saline in the home cage (RM-M/HC-S) or saline in the test room and morphine in the home cage (RM-S/HC-M) and exposed to either a 50° (left panel), 52° (center panel) or 54° (right panel) hot-plate during the four conditioning days of Experiment 3.

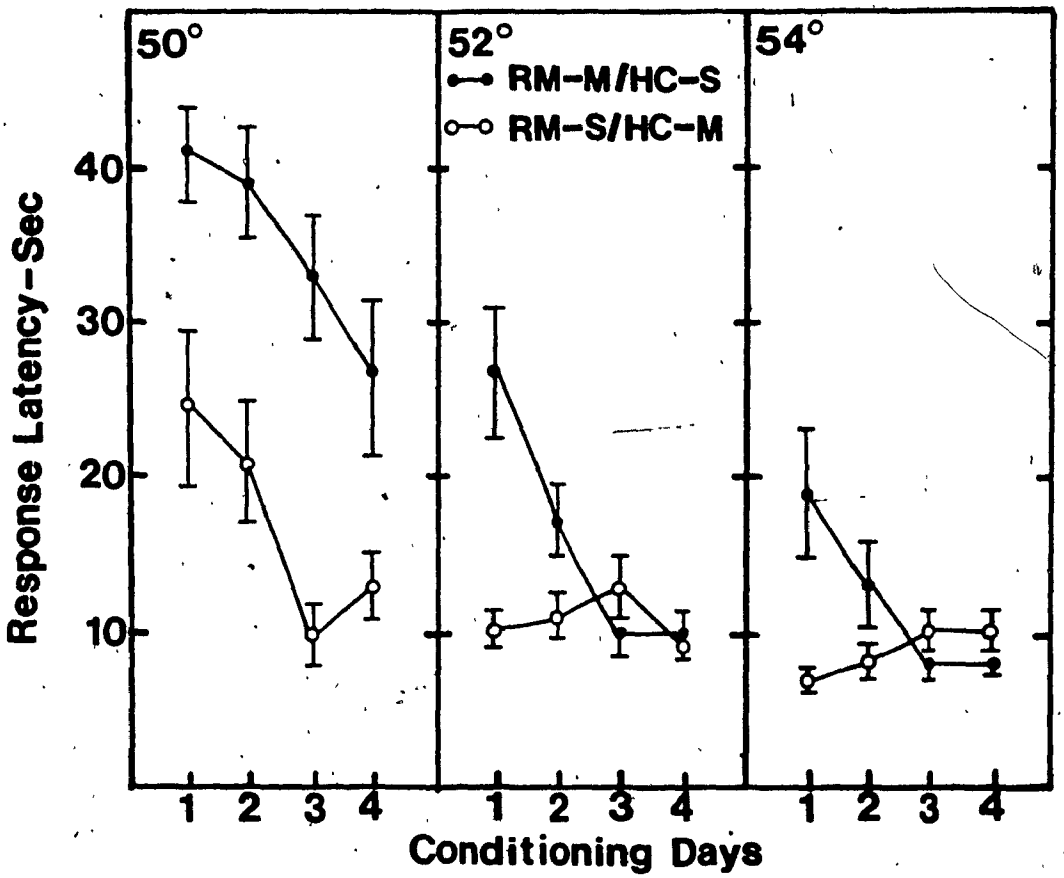
50°
52°
54°
RM-M/HC-S
RM-S/HC-M
Response Latency-Sec
40
30
20
10
1 2 3 4
1 2 3 4
1 2 3 4
Conditioning Days

Figure 4

morphine analgesia. Among saline-exposed animals, only group RM-S/HC-M-50° displayed a significant decline over days, $F(3,126) = 9.18$, $p < .001$.

Tests for simple main effects were also conducted between groups at each plate temperature for each day. On the first conditioning day morphine significantly elevated latencies at each plate temperature, $Fs(1,168) \geq 10.31$, $ps < .005$. The latencies of morphine-treated animals on the 50° plate temperature were consistently longer than those of saline-treated animals, $Fs(1,168) \geq 12.84$, $ps < .001$, but at the 52° and 54° plate temperatures there were no significant differences between morphine- and saline-exposed animals over conditioning days 2-4, $Fs(1,168) \leq 2.72$, $p > .05$.

Tail Flick Testing Days: Figure 5 shows the mean tail-flick latencies for the eight groups for each of the three tail-flick test days conducted under saline. The analysis of variance (conditioning drug treatment x conditioning hot-plate temperature x tail-flick test days) yielded a significant main effect of conditioning drug treatment, $F(1,56) = 4.25$, $p < .025$, indicating that animals that had previously been administered saline in the test room displayed longer tail-flick latencies than those that had received morphine. This effect occurred on all three test days as indicated by a non-significant drug treatment x test days interaction, $F(2,112) = 1.18$, $p > .05$. The longer latencies exhibited by animals previously administered saline in the test room appeared to be independent of the hot-plate temperature during conditioning, neither the drug treatment x plate temperature or the drug treatment x plate temperature x test days interactions were

Figure 5: Mean tail-flick latencies (± SEM) for groups previously injected with morphine in the test room and saline in the home cage (RM-M/HC-S) or saline in the test room and morphine in the home cage (RM-S/HC-M) and previously exposed to either a 22° (left panel), 50° (left-center panel), 52° (right-center panel) or 54° (right panel) hot-plate during the three tail-flick test days of Experiment 3.

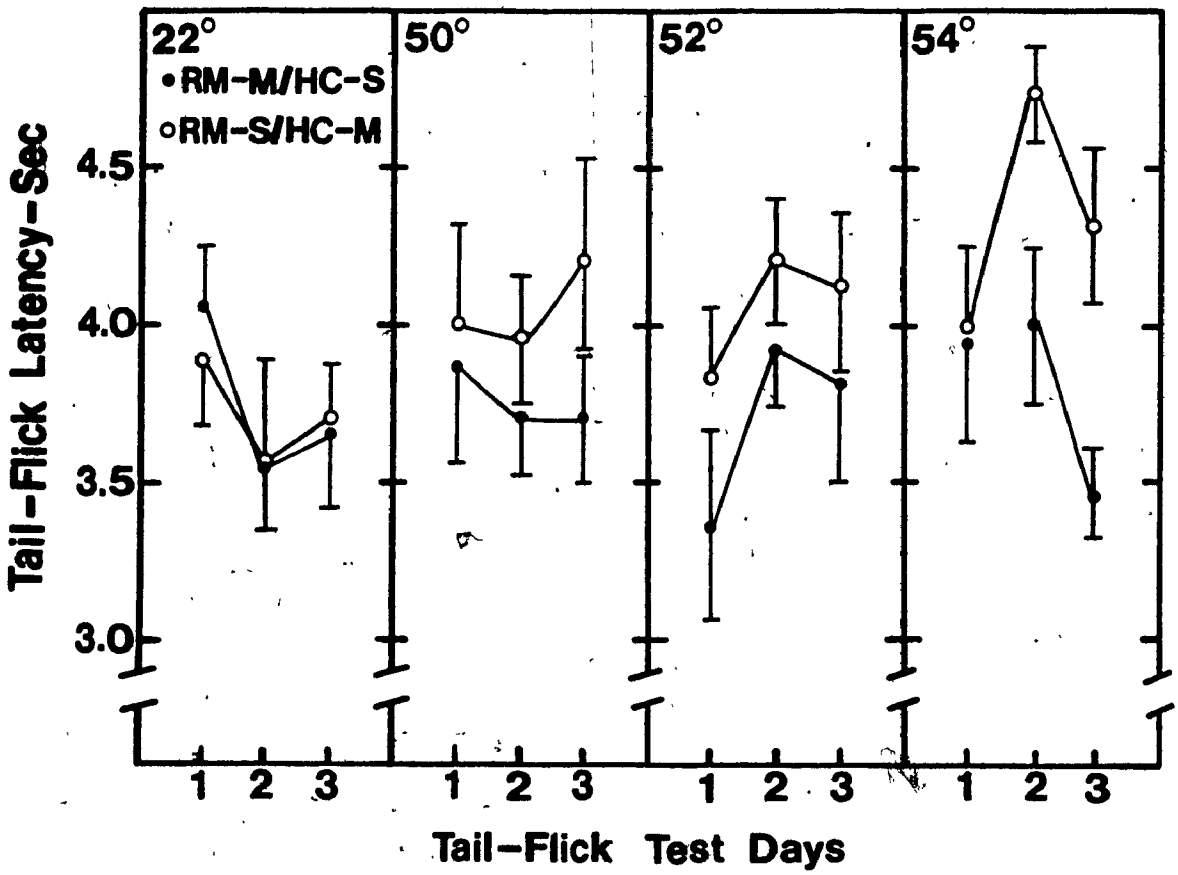
22°
•RM-M/HC-S
○RM-S/HC-M
50°
52°
54°
Tail-Flick Latency-Sec
4.5
4.0
3.5
3.0
1 2 3
1 2 3
1 2 3
1 2 3
Tail-Flick Test Days

Figure 5

significant, Fs < 1.0. However, inspection of Figure 5 clearly shows that the longer tail-flick latencies of animals administered saline during the conditioning days occurred only in the groups exposed to the hot-plate; animals previously exposed to the 22° plate under saline displayed tail-flick latencies on the test days that were not different from those animals previously administered morphine when exposed to the plate.

Hot-Plate Test Day: Figure 6 shows the mean response latencies for the eight groups on the hot-plate testing day of the experiment when all animals received saline injections and were tested on the 50° hot plate. Inspection of Figure 6 reveals that animals previously exposed to the 52° and 54° hot-plate under saline during the conditioning days exhibited longer latencies than animals previously exposed to these temperatures under morphine. No differences in latencies between saline- and morphine-treated animals exposed to the 22° and 50° temperatures were observed. The analysis of variance (conditioning drug treatment x conditioning plate temperature) yielded significant main effects for drug treatment, $F(1,56) = 11.63$, $p < .002$, indicating that animals receiving saline in the test room during conditioning generally displayed longer latencies than morphine-treated animals, and for plate temperature, $F(3,56) = 11.85$, $p < .001$, reflecting the longer latencies in animals previously exposed to the 52° and 54° plate temperatures. In addition, the drug treatment x plate temperature interaction was significant, $F(3,56) = 11.41$, $p < .001$. Tests for simple main effects, conducted between groups for each plate temperature, revealed no significant differences in saline- or morphine-exposed animals at

Figure 6: Mean response latencies (± SEM) for groups previously injected with morphine in the test room and saline in the home cage (RM-M/HC-S) or saline in the test room and morphine in the home cage (RM-S/HC-M), as a function of the hot-plate temperature during the conditioning phase, for the hot-plate test day of Experiment 3.

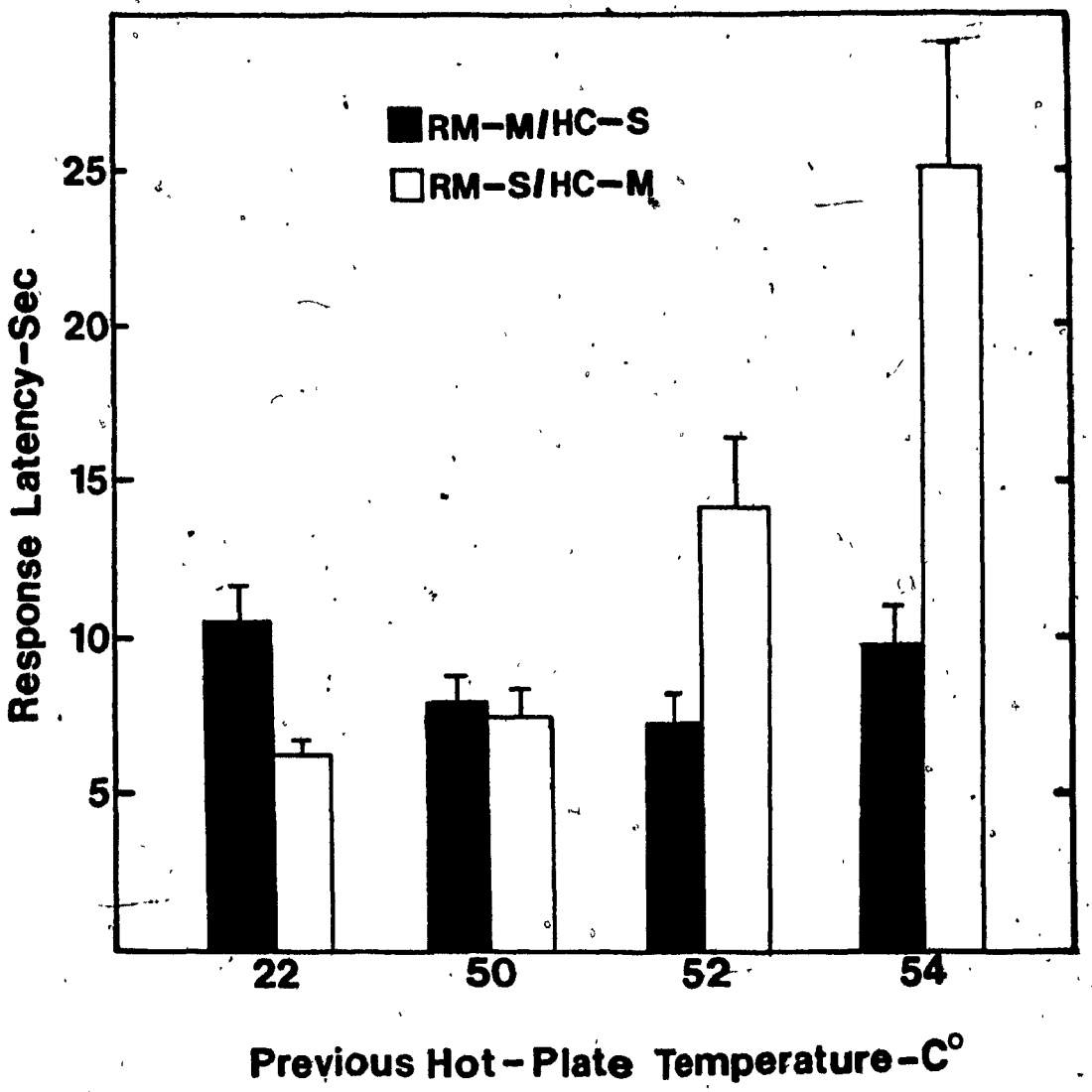
RM-M/HC-S
RM-S/HC-M
Response Latency-Sec
25
20
15
10
5
22
50
52
54
Previous Hot-Plate Temperature-C°

Figure 6

either the 22° or the 50° plate temperatures, $Fs(1,56) \leq 2.76$, $ps > .05$. In contrast, animals previously exposed to either the 52° or 54° plates under saline exhibited significantly longer latencies than animals that had experienced these temperatures under morphine, $Fs(1,56) \geq 7.17$, $ps < .01$.

Discussion

The results of Experiment 3 show that, as was found with electric footshock, exposure to thermal stimulation can elicit conditioned autoanalgesia (see also Sherman et al., 1982, for related findings). More importantly, the results also demonstrate that morphine administration attenuates the conditioned autoanalgesia elicited by this type of nociceptive stimulus. When tested under saline, animals previously exposed to the hot-plate under morphine displayed shorter latencies than animals exposed under saline. This effect occurred whether tests for conditioned autoanalgesia were conducted using the tail-flick or the hot-plate test.

The results of Experiment 3 also demonstrate that the greater the intensity of the noxious stimulus to which animals are exposed during conditioning, the greater the extent to which morphine attenuated the development of conditioned autoanalgesia. Differences between the morphine- and saline-treated groups only occurred when the nociceptive stimulus was sufficiently intense for conditioning of autoanalgesia to be evident among saline-treated animals. Examination of Figures 5 and 6 shows that morphine-attenuation of conditioned autoanalgesia, as assessed by the tail-flick and hot-plate tests, became more pronounced as the temperature of the plate was increased. Figure 6 shows that, as

assessed by the hot-plate test, little, if any, conditioning of autoanalgesia was evident among animals exposed to the 50° hot-plate. Both morphine- and saline-treated animals exposed to this plate temperature had latencies similar to those of animals previously exposed to the 22° plate.

General Discussion

The results of the three experiments presented here suggest that administration of morphine prior to exposure to nociceptive stimulation can attenuate the development of conditioned autoanalgesia. Furthermore, this effect occurs when animals are exposed to either electric shock (Experiments 1 and 2) or to thermal stimulation (Experiment 3). These results suggest that one of the mechanisms underlying situation-specific tolerance to morphine analgesia, and the putative hyperalgesia that has been reported to occur in the same environment when tests for analgesia are conducted under saline, may be the attenuation by morphine of the development of situation-specific or conditioned autoanalgesia.

The results of the present experiments may also explain why some investigators have (Krank et al., 1981; Siegel, 1975), while others have not (Abbott et al., 1982; Morris, et al., Note 1; Tiffany et al., 1983; Sherman, 1979) found evidence for "conditioned hyperalgesia" when tests for analgesia are conducted under saline. The present experiments suggest that evidence for "hyperalgesia" in morphine-treated animals will depend upon the degree to which autoanalgesia is conditioned in animals exposed to a stressful or nociceptive environment under saline, which in turn is dependent upon the intensity of nociceptive stimulation. Evidence for this is found, for example, on the hot-plate test day of Experiment 3 (see Figure 6), where there was no difference in latencies between animals previously administered morphine and those previously administered saline at the the 50° hot-plate temperature. Animals exposed to this temperature showed

latencies similar to those of animals exposed the 22° plate, suggesting that exposure to the 50° hot-plate failed to promote substantial levels of conditioned autoanalgesia.

In addition, however, little evidence for "hyperalgesia" will be observed in the test environment in animals previously administered morphine if the intensity of the nociceptive stimulus experienced during the conditioning trials is too severe. In this case the dose of morphine used may be ineffective in reducing the intensity of the pain, and therefore will not attenuate conditioned autoanalgesia. When tests for analgesia are conducted under saline, animals previously exposed to noxious stimulation under morphine will show latencies similar to those exposed under saline; in this case, however, both groups should display latencies that are longer than those of animals never exposed to nociceptive stimulation. This possibility is supported by the results from animals exposed to the strong shock condition (2.5 mA for 180 sec) in Experiment 1. In this shock condition both morphine-treated and saline-treated animals exhibited longer latencies than non-shocked animals on the saline test day.

The present results also suggest that the intensity of nociceptive stimulation used in tests conducted under saline to assess conditioned autoanalgesia will affect the appearance of "hyperalgesia." Specifically, if the nociceptive stimulation of the analgesia test is too intense for conditioned autoanalgesia to manifest itself, then the demonstration of "hyperalgesia" will be masked. For example, in Experiment 2, there was no evidence for attenuation of conditioned autoanalgesia (i.e., hyperalgesia) in animals previously exposed to the

mild shock (1 mA for 15 sec) condition under morphine. It will be remembered, however, that on the morphine test day these same animals showed considerably less analgesia than those previously exposed to mild shock under saline. As stated earlier, conditioned autoanalgesia may not manifest itself when pain tests are conducted under saline, but when tests are conducted under morphine the two sources of analgesia synergize to produce a stronger analgesic effect (Sherman et al., 1984).

The hypothesis that situation-specific tolerance to morphine analgesia and evidence for "conditioned hyperalgesia" arise from the attenuation by morphine of the UCS for conditioned autoanalgesia (i.e., nociceptive stimulation), thereby attenuating the development of conditioned autoanalgesia, suggests that exposure to nociceptive stimulation is crucial for their development. In none of the experiments were there any differences in response latencies between morphine- and saline-treated animals that were not explicitly exposed to nociceptive stimulation. There are several studies in the literature, however, in which evidence for "conditioned hyperalgesia" (Krank, et al., 1981) or situation-specific tolerance to morphine analgesia (Adams et al., 1969; Gebhart & Mitchell, 1971; 1972; Siegel et al., 1981; Tiffany & Baker, 1981; Tiffany et al., 1983) was found in animals given repeated morphine injections but not exposed to nociceptive stimulation. Because the animals were never exposed to nociceptive stimulation, it would appear difficult to argue that the situation-specific tolerance to morphine analgesia or "conditioned hyperalgesia" observed in morphine-treated animals arose because

morphine rendered them analgesic to noxious stimulation and therefore blocked the conditioning of autoanalgesia.

Recall, however, that the more general implication of the hypothesis is that tolerance to the analgesic effect of morphine will be more pronounced under any circumstances where the activation of endogenous pain control mechanisms is prevented or reduced. It has been argued that animals previously exposed to nociceptive stimulation under morphine are relatively less analgesic in tests under either saline or morphine than animals previously exposed under saline because morphine prevented the activation of the endogenous analgesic substrate. However, in the introduction it was noted that exposure to nociceptive stimulation is only one way to activate endogenous pain control mechanisms. Exposure to non-painful stressors can also activate the EPCS. It is possible that morphine administration may interfere with the ability of these stressors to activate endogenous pain control mechanisms, perhaps through its anxiolytic actions (Amir, Brown, & Amit, 1980; Beecher, 1956). It has been well demonstrated, for example, that animals exposed to a stress-inducing environment under the influence of morphine appear more tolerant to the analgesic effect of morphine compared to animals exposed to the environment under the influence of saline (Adams et al., 1969; Gebhart & Mitchell, 1971; 1972; Tiffany et al., 1983). These demonstrations of a morphine-stress interaction are consistent with the hypothesis that the tolerance observed in animals exposed to stress under the influence of morphine is attributable to morphine-attenuation of the activation of endogenous pain control mechanisms by stress. It should be noted, however, that

although this hypothesis may account for the demonstrations of morphine analgesic tolerance when animals are not exposed to nociceptive stimulation, it is not clear that it can account for the one report of "hyperalgesia" observed under these circumstances (Krank et al., 1981). Tiffany et al. (1983) failed to observe any differences between animals exposed to stress under morphine and those exposed under saline when tests for analgesia were conducted under the influence of saline. Thus, further research is needed to determine whether morphine attenuation of stress-induced analgesia can account for "hyperalgesia" when animals are not exposed to nociceptive stimulation.

Chapter 2

The results of Experiments 1-3 supported the hypothesis that morphine would attenuate the development of conditioned autoanalgesia. The experiments reported below investigated the corollary of the hypothesis that the administration of naloxone prior to exposure to nociceptive stimulation would, by increasing the perceived intensity of pain, enhance the development of conditioned autoanalgesia.

Experiment 4

In Experiment 4, the effect of the repeated administration of three doses of naloxone, 0.5, 2.0 and 10.0 mg/kg, upon the development of conditioned autoanalgesia was examined. Animals were injected with either naloxone or saline in the distinctive test environment and then administered a nociceptive test. Following the last day of naloxone administration all animals were tested for conditioned autoanalgesia following an injection of either saline or morphine. As stated in the introduction, it was predicted that animals tested under the influence of naloxone would appear hyperalgesic relative to animals given repeated tests under saline, but would display reduced sensitivity to pain when testing was conducted in the absence of opiate receptor blockade.

Method

Subjects and Apparatus: The subjects were 84 rats maintained as in the previous experiments. The hot-plate apparatus was identical to that employed in the previous experiments. Naloxone HCl (Endo laboratories) was administered by sub-cutaneous injection in the dorsal neck area. Three doses of naloxone were administered, 0.5, 2.0 and 10.0 mg/kg.

Naloxone was dissolved in 0.9% saline in a mg/ml/kg volume.

Procedure: For five days prior to the experiment per se, animals were handled in the morning (10:00-11:00) and afternoon (16:00-17:00) for approximately one min. The animals were also weighed during their morning handling. The day after the last handling day the experiment proper began.

Naloxone Treatment Phase During the eight days of the naloxone treatment phase, the rats were transported to the test room, injected with either naloxone or saline and then placed in the wooden boxes. The experiment was run in two replications. In the first replication, the interval between injection in the distinctive room and hot-plate testing was 15 min. An N of 6 subjects per group was employed. In the second replication ~~the~~ injection-test-interval was 30 min and groups consisted of 8 rats each. Following the appropriate injection-test-interval, each animal was placed on the hot-plate surface and the latency to perform a hind-paw lick was recorded to the nearest 0.1 sec with a timer (Lafayette Instrument Co.). The water temperature of the hot-plate bath was 48.5 (±.2)°C. This low temperature was used because it appears to provide a more sensitive analgesic test (see O'Callaghan & Holtzman, 1975). If no response was observed within 90 sec the test was terminated and a paw-lick latency (PLL) of 90 sec was recorded. Following the hot-plate test all animals were returned to their home cages where, between two and four hours after the hot-plate test, the animals were administered a second injection. In each replication, six groups of animals were employed. The groups differed with respect to the dose of naloxone received and the environment in which naloxone was

administered. Groups RM-N/HC-S-0.5 mg/kg, RM-N/HC-S-2 mg/kg and RM-N/HC-S-10 mg/kg were administered 0.5, 2.0 and 10.0 mg/kg naloxone, respectively, in the test room and saline in the home cage. Groups RM-S/HC-N-0.5 mg/kg, RM-S/HC-N-2 mg/kg and RM-S/HC-N-10 mg/kg received the reverse drug treatment; saline was administered in the test room and naloxone was injected in the home cage. Except for a two day respite between days 4 and 5, the naloxone testing phase was conducted on successive days.

Saline and Morphine Test Phase: The day following the last naloxone treatment day constituted the first saline test day. On the test day all animals were administered saline in the distinctive room and no injection was given in the home cage. The general procedure remained identical to that followed during the naloxone administration phase in all other respects. A second saline test day was conducted one week following the first. During the interval between the first and second saline test days the animals remained undisturbed in their home cages and no drugs were administered. The one week interval was selected to insure that any residual traces of naloxone would be eliminated from the body. On the day following the second test day, all animals were administered a hot-plate test under the influence of 5 mg/kg morphine. Again, the general procedure followed on this day was identical to that followed during the saline test days.

Results

Since preliminary statistical analyses revealed no significant main effect for the injection-test-interval, nor any significant interaction between the injection-test-interval and any other factor,

the data from both replications were combined to yield an N of 14 per group.

Naloxone Treatment Phase Figure 7 shows the mean PLLs for the six groups over the eight days of the experiment in which naloxone was administered. Inspection of Figure 7 reveals that there was no support for the prediction that animals administered hot-plate tests while under the influence of naloxone would display a hyperalgesic response. To the contrary, naloxone-tested animals appeared analgesic relative to their saline controls, although the magnitude of the effect was dependent upon the dose administered. With the exception of days 6 and 7, rats receiving 0.5 mg/kg naloxone in the test room displayed longer PLLs than their saline controls. Administration of 2 mg/kg prior to nociceptive testing failed to produce any marked or consistent effect. By far the most pronounced analgesia was exhibited in animals injected with 10 mg/kg naloxone in the test room, particularly from days 4-8.

A split plot analysis of variance, with drug treatment (RM-N/HC-S and RM-S/HC-N) and dose as between subject factors and days as a within subject factor, confirmed these observations. The main effects for both drug administration, $F(1,78) = 14.13$, $p < .005$, and days, $F(7,546) = 22.01$, $p < .0001$, were significant, which reflected the findings that, respectively, naloxone-tested animals displayed longer PLLs than saline-tested animals and that the PLLs generally decreased over repeated tests. Although the main effect for dose was not significant, $F < 1.0$, the drug treatment x dose x days interaction was significant, $F(14,546) = 2.58$, $p < .002$, indicating that latencies did vary as a function of dose. Tests for simple main effects, conducted between

Figure 7: Mean paw-lick latencies (± SEM) for groups injected with naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) during the eight days of the naloxone treatment phase of Experiment 4. Groups were administered either 0.5 (left panel), 2 (center panel) or 10 mg/kg (right panel) naloxone.

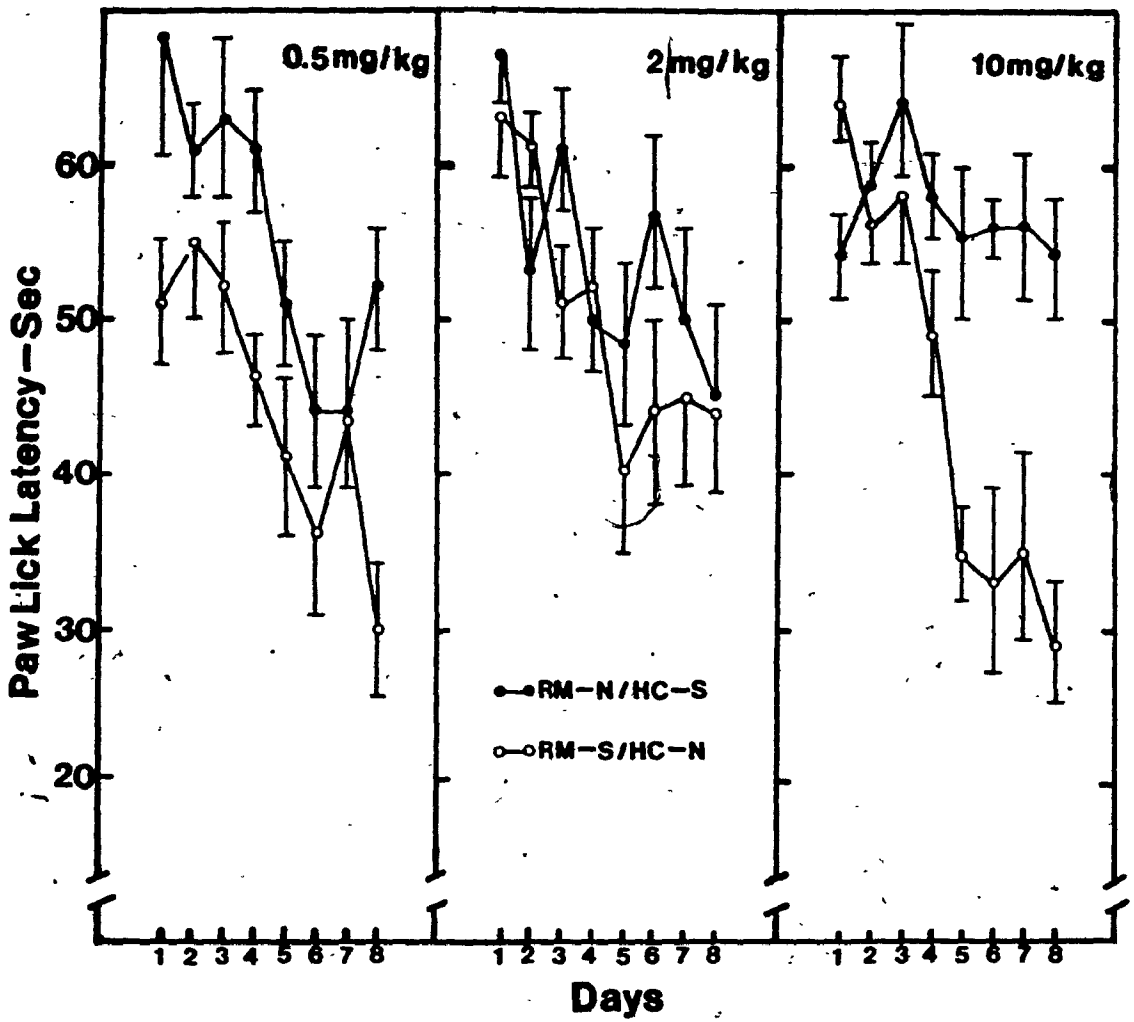
0.5mg/kg
2mg/kg
10mg/kg
Paw Lick Latency—Sec
60
50
40
30
20
RM—N/HC—S
RM—S/HC—N
1 2 3 4 5 6 7 8
Days

Figure 7

groups at each dose for each day, revealed the nature of this variation. At the 10 mg/kg dose the mean PLLs for naloxone-tested and saline-tested animals did not differ from days 1-3, $\underline{F}s(1,624) \leq 2.20$, $\underline{p}s > .05$, but from days 4-8 the PLLs for group RM-N/HC-S-10 mg/kg were significantly longer than those for group RM-S/HC-N-10 mg/kg, $\underline{F}s(1,624) \geq 9.60$, $\underline{p}s < .01$. The longer PLLs in group RM-N/HC-S-0.5 mg/kg were significantly different from group RM-S/HC-N-0.5 mg/kg only on days 1, 4 and 8, $\underline{F}s(1,624) \geq 5.44$, $\underline{p}s < .05$. No significant differences were observed between naloxone-tested and saline-tested animals at the 2 mg/kg dose, $\underline{F}s(1,624) \leq 3.79$, $\underline{p}s > .05$. Tests conducted for each group over days revealed that, with the exception of group RM-N/HC-S-10 mg/kg ($\underline{F} < 1.0$), all groups showed a general decrease in PLLs over days, $\underline{F}s(7,624) \geq 2.40$; $\underline{p}s < .05$.

Saline and Morphine Test Phase Figure 8 shows the mean PLLs for the six groups over the three test days. With the exception of group RM-N/HC-S-2 mg/kg on the second saline test day, the groups previously receiving naloxone in the distinctive room displayed elevated latencies relative to their respective saline control groups. The analysis of variance (previous drug treatment x previous naloxone dose x test day) performed on these data revealed significant main effects for previous drug treatment, $\underline{F}(1,78) = 14.15$, and for test days, $\underline{F}(2,156) = 34.16$, $\underline{p}s < .001$. The main effect for previous drug treatment indicates that animals previously exposed to the hot-plate under naloxone were analgesic when tested under saline or morphine compared to animals previously given hot-plate tests under saline. The test days effect reflects the general tendency for latencies to decline from the first

Figure 8: Mean paw-lick latencies (± SEM) for groups previously administered naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) as a function of previous naloxone dose during the two saline test days (left and center panels) and the morphine test day (right panel) of Experiment 4.

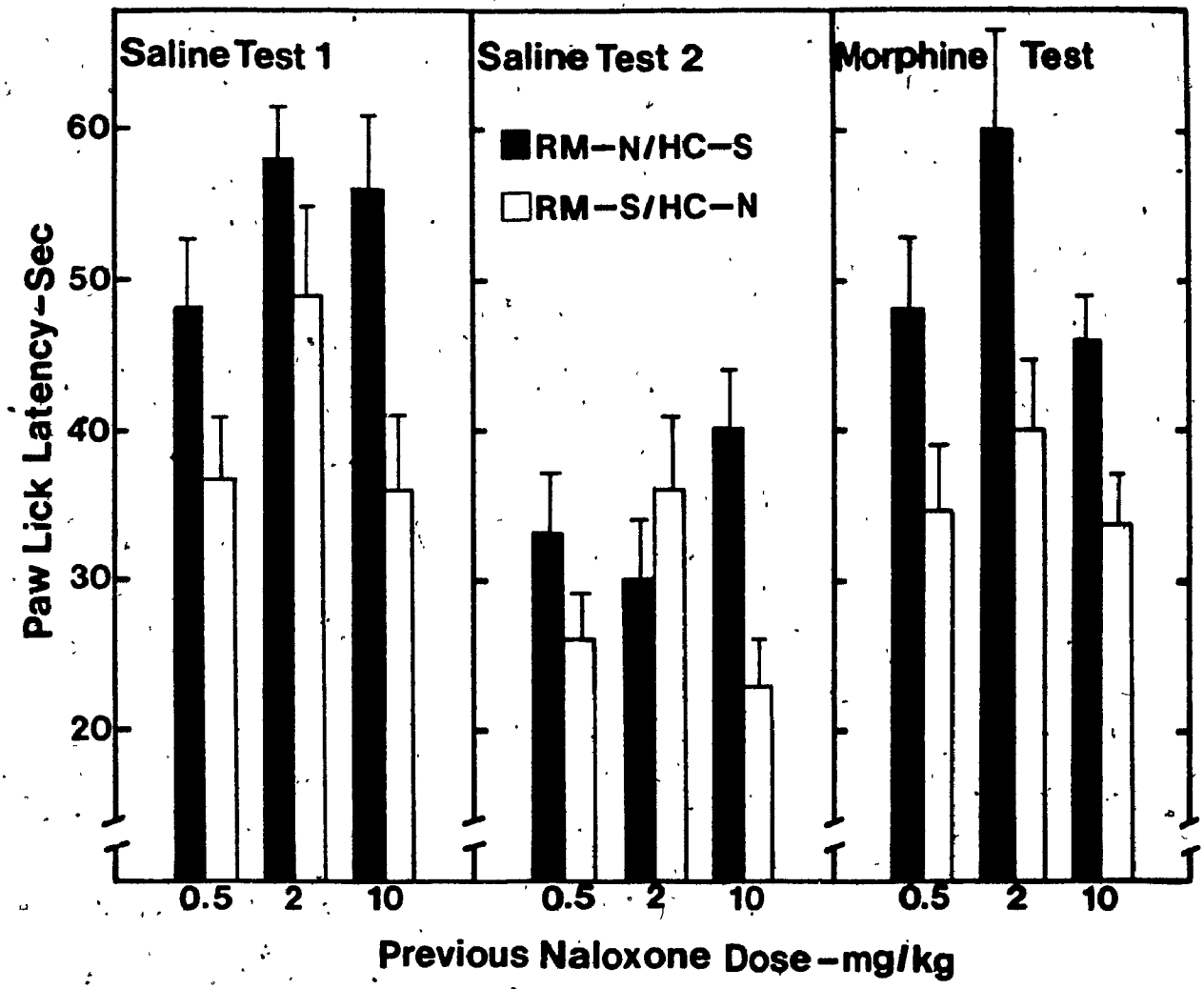
Saline Test 1
Saline Test 2
Morphine Test
RM–N/HC–S
RM–S/HC–N
Paw Lick Latency–Sec
60
50
40
30
20
0.5
2
10
0.5
2
10
0.5
2
10
Previous Naloxone Dose–mg/kg

Figure 8

saline test to the second, as well as the increase in PLLs when the animals were tested under morphine. The analgesia did not vary as a function of previous naloxone dose; neither the main effect for dose, $F(2,78) = 2.37$, the dose x group interaction, $F < 1.0$, the dose x test days interaction, $F(4,156) = 1.10$, or the dose x drug treatment x test days interaction, $F(4,156) = 2.24$, were significant ($ps > .05$).

Discussion

Experiment 4 yielded three main findings. First, the administration of naloxone prior to nociceptive testing did not produce a hyperalgesic response, as was initially predicted, but instead increased latencies relative to animals tested under saline (but that received naloxone injections in the home cage). The analgesic effect observed following repeated naloxone administration was a function of the dose administered; the strongest effect occurred at the 10 mg/kg dose with less robust effects occurring at the lower doses. Second, animals previously administered hot-plate tests while under the influence of naloxone displayed stronger levels of conditioned autoanalgesia when tested under saline than did animals always given hot-plate tests under saline. Third, the greater conditioned autoanalgesia observed in naloxone-tested animals summated with the analgesic effect of morphine. Unlike the analgesia observed during the naloxone administration phase of the experiment, the greater conditioned autoanalgesia and the synergism with morphine analgesia displayed during the test phase of the experiment were independent of the dose of naloxone administered.

Although it was not possible to identify the mechanism involved in

the naloxone-induced analgesia from the design of Experiment 4, two possibilities exist. First, it is possible that the analgesia was opiate mediated. According to this view, the repeated administration of naloxone might have caused some change in the functioning of the opiate component of the EPCS; for example, a greater release of endogenous opiate ligands or a change in opiate receptor affinity or number. Lahti and Collins (1978) have shown that chronic opiate receptor blockade results in prolonged increases in brain opiate binding sites, an effect that is manifested behaviorally as an enhancement of morphine's analgesic potency (Tang & Collins, 1978). This explanation of the results, however, is questionable. In the Tang and Collins study the increase in morphine's analgesic potency was demonstrated after termination of opiate receptor blockade, whereas the analgesia observed in the present experiment developed during naloxone administration. Furthermore, the blockade regimen used in the present experiment can hardly be considered chronic.

A more likely alternative is that the analgesia was mediated by the non-opiate component of the EPCS. This alternative is suggested by the finding that the highest dose of naloxone used (10 mg/kg) produced the strongest effect. Secondly, the fact that, contrary to expectation, naloxone did not produce hyperalgesia also argues in favor of a non-opiate mediation of the analgesia displayed when animals are tested under the influence of naloxone. A non-opiate mediated analgesia, should, at least initially, be insensitive to naloxone administration, therefore no evidence of hyperalgesia would be obtained. The issue of the opiate-non-opiate nature of the naloxone-

induced analgesia is directly addressed in Experiment 9.

Although it is probable that the analgesia observed when animals were tested under naloxone was mediated by the non-opiate component of the EPCS, it does not necessarily follow that the enhanced conditioned autoanalgesia observed was also controlled by this substrate. Indeed, inspection of the results from the groups administered 0.5 and 2 mg/kg naloxone in the test room suggests that the substrate mediating the conditioned autoanalgesia may be quite distinct from that controlling the naloxone-induced analgesia. Both groups exhibited a pronounced conditioned autoanalgesia when tested under saline or morphine despite the fact that the naloxone-induced analgesia observed at the 0.5 and 2.0 mg/kg doses was weak or absent. This result might suggest that, unlike the non-opiate mediated, naloxone-induced analgesia, the conditioned autoanalgesia may be, at least in part, mediated by the opiate component of the EPCS. The majority of studies that have investigated the substrate underlying conditioned autoanalgesia have found it to be mediated primarily by an opiate-substrate (Devries, Chance, Payne & Rosecrans, 1979; Fanselow, 1984; Fanselow & Baackes, 1982; Fanselow & Bolles, 1979; Oliverio & Casellano, 1982; Sherman et al., 1984; 1982; Watkins et al., 1982; but see Chance & Rosecrans, 1979a, b; Hayes et al., 1978; for exceptions). Furthermore, it appears that the conditioning of opiate-mediated autoanalgesia occurs even in those instances where the unconditioned analgesia is mediated primarily by a non-opiate mechanism (Watkins et al., 1982), or when an unconditioned, opiate-mediated analgesia is prevented from occurring by prior opiate receptor blockade (Fanselow, 1984; Fanselow & Baackes,

1982). It is possible, therefore, that in the present experiment the opiate antinociceptive mechanism was activated (and therefore conditioned) when animals were tested under naloxone, but that the analgesic effects of recruitment of the opiate system were blocked by naloxone. When testing was conducted under saline or morphine, however, the opiate component was conditionally activated without inhibition, resulting in an opiate-mediated conditioned autoanalgesia.

Although this argument may account for the finding that all three doses of naloxone employed in this study enhanced the development of conditioned autoanalgesia, it does not explain why the analgesia that developed during naloxone administration was most pronounced in animals administered the highest dose of naloxone. The most likely possibility is that the development of analgesia under naloxone is dependent upon the degree to which the opiate component is antagonized during exposure to nociceptive stimulation. The General Discussion offers a model to explain how this possibility may occur.

Experiment 5

In Experiment 5, the effect of varying the hot-plate temperature upon the development of naloxone-induced analgesia and conditioned autoanalgesia was examined.

Method

Subjects and Apparatus: The subjects were 36 rats maintained as in the previous experiments. The apparatus was identical to that used in Experiment 4.

Procedure: As in Experiment 4, all animals were handled twice daily for the first five days following arrival at the laboratory.

Naloxone Administration Phase The rats were randomly assigned to six groups (N=6). For days 1-8 of the experiment, three of the groups were administered naloxone (10 mg/kg in a 10 mg/ml solution) in the test room and saline in the home cage (groups RM-N/HC-S). The remaining three groups received saline in the test room and naloxone in the home cage (groups RM-S/HC-N). The groups also differed with respect to the temperature of the plate during hot-plate testing. One group from each drug administration condition was tested on a plate immersed in a 48.5° C bath, the bath temperature for the second group was 52.0° C, while that for the third group was 56.0° C. A 30 min interval elapsed between injection in the test room and the hot-plate test. As in Experiment 4, a two day rest period, during which the animals were left undisturbed in their home cages, occurred between days 4 and 5.

Saline and Morphine Test Phase The test phase of Experiment 5 was identical to that of Experiment 4.

Results

Naloxone Treatment Phase: Figure 9 presents the mean PLLs for the six groups over the first eight days of the experiment. As in Experiment 4, animals tested on the 48.5° hot-plate following administration of 10 mg/kg naloxone displayed longer PLLs than saline-tested animals, beginning on day 5. No differences between naloxone- and saline-tested animals were observed at the higher two plate temperatures. The analysis of variance (drug treatment x plate temperature x days) revealed significant main effects for plate temperature, $F(2,30) = 350.70$, and for days, $F(7,210) = 25.29$ ($ps < .0001$), as well as a significant plate temperature x days interaction, $F(14,210) = 8.56$; $p < .0001$. Tests for simple main effects conducted over days at each plate temperature revealed that the PLLs declined over days in animals tested on the 48.5° C plate, $F(7,210) = 25.09$, $p < .001$, and the 52.0° C plate, $F(7,210) = 6.96$, $p < .01$, but not in animals tested on the 56.0° C plate, $F(7,210) = 1.93$, $p > .05$. However, Figure 9 shows that the decline in latencies among animals tested on the 48.5° plate occurred mainly among saline-tested animals. Naloxone-treated animals tested at this temperature displayed relatively constant latencies over days 2-8 following a decline in latencies from day 1. The main effect for drug treatment only approached significance, $F(1,30) = 3.65$, $p < .07$. In spite of this weak overall effect of drug treatment, Figure 9 shows that, on the last four days of testing, naloxone-treated animals tested on the 48.5° C plate had considerably longer latencies than did saline-treated animals tested at that temperature, a finding which replicates that seen in

Figure 9: Mean paw-lick latencies (± SEM) for groups receiving naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) for each of the three plate temperatures during the eight days of the naloxone treatment phase of Experiment 5.

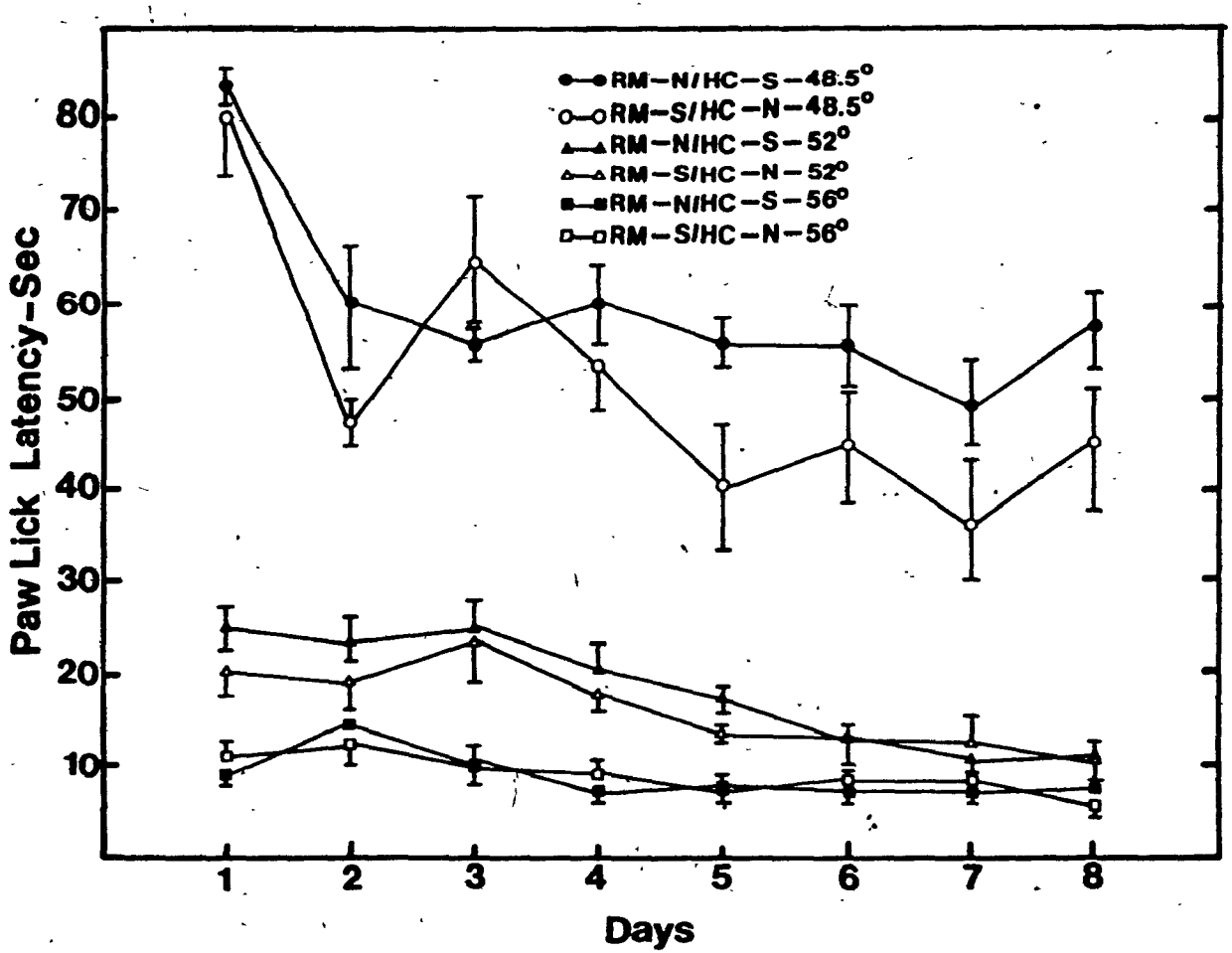
RM-N/HC-S-48.5°
RM-S/HC-N-48.5°
RM-N/HC-S-52°
RM-S/HC-N-52°
RM-N/HC-S-56°
RM-S/HC-N-56°
Paw Lick Latency-Sec
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
Days

Figure 9

Experiment 4. In order to statistically confirm this observation, Scheffe's test (Kirk, 1968) was employed. It was found that although the latencies of the groups tested on the 48.5° C plate did not differ on the first four test days combined, $F < 1.0$, over the last four days the combined mean PLL of the naloxone-tested animals were significantly longer than animals tested under saline, $F(7,240) = 11.68$, $p < .001$.

Saline and Morphine Test Phase: Figure 10 presents the PLLs for the six groups for the two saline test days and the morphine test day. On all three days group RM-N/HC-S-48.5° exhibited longer latencies than group RM-S/HC-N-48.5°. No differences were observed at the higher plate temperatures on any day. A previous drug treatment x plate temperature x test days analysis of variance confirmed these observations. The main effects for previous drug treatment, $F(1,30) = 4.68$, $p < .05$, and plate temperature, $F(2,30) = 112.88$, $p < .0001$, were significant as was the drug administration x plate temperature interaction, $F(2,30) = 3.36$, $p < .05$. Tests for simple main effects revealed that group RM-N/HC-S-48.5° exhibited longer PLLs than group RM-S/HC-N-48.5°, $F(1,30) = 11.30$, $p < .01$. No differences between groups were observed at either the 52.0° or 56.0° plate temperatures, $Fs < 1.0$.

Discussion

In this experiment, as in Experiment 4, naloxone-induced analgesia was evident during the last four days of testing, and administration of naloxone prior to nociceptive testing resulted in greater conditioned autoanalgesia when hot-plate tests were given under saline or morphine. However, these effects were evident only in animals tested on the

Figure 10: Mean paw-lick latencies (± SEM) for groups previously injected with naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) for each of the three plate temperatures during the two saline test days (left and center panels) and the morphine test day (right panel) of Experiment 5.

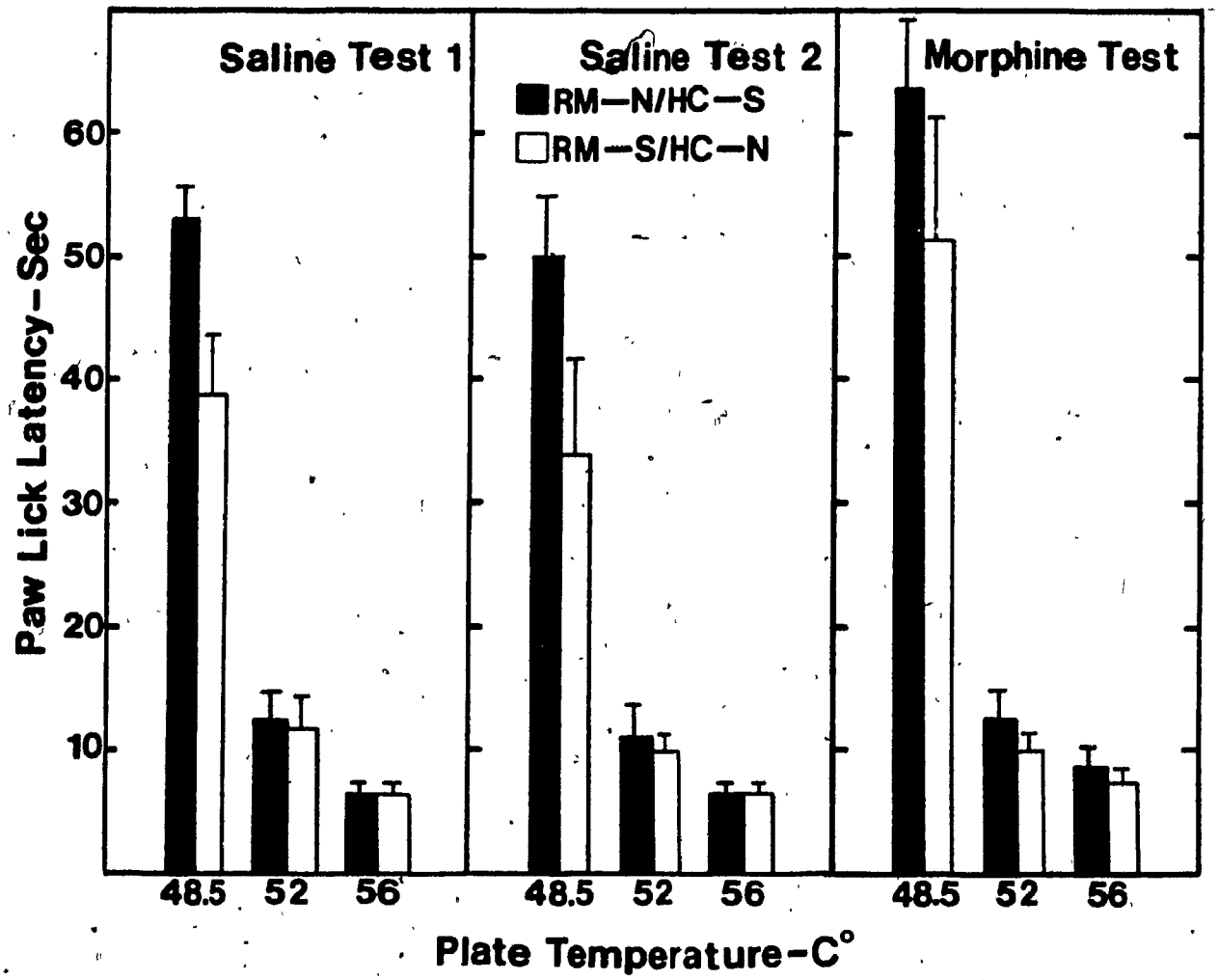
Saline Test 1
Saline Test 2
Morphine Test
RM—N/HC—S
RM—S/HC—N
Paw Lick Latency-Sec
60
50
40
30
20
10
48.5
52
56
48.5
52
56
48.5
52
56
Plate Temperature-C°

Figure 10

lowest plate temperature (48.5°). No evidence for naloxone-induced analgesia or potentiation of conditioned autoanalgesia was evident at the 52.0° or 56.0° C plate temperatures. The fact that no differences were obtained at the hotter plate temperatures suggests that the potency of the naloxone-induced analgesia and the conditioned autoanalgesia was insufficient to inhibit the effects of more intense levels of nociceptive stimulation, and adds further support to the argument that the intensity of the nociceptive stimulus during tests for analgesia is crucial to the demonstration of certain analgesic effects. Effects might very well have been observed if, following tests on the 52.0° or 56.0° plates, these animals had been administered hot-plate tests at the 48.5° temperature.

Experiment 6

There exists considerable evidence to suggest that analgesia-inducing manipulations vary in effectiveness depending on the analgesia test employed. For example, Dennis and Melzack (1980) reported that administration of the serotonin precursor L-tryptophan produces analgesia as assessed by the tail-flick and formalin tests, but not on the hot-plate test (see also Dennis, Melzack, Guttman & Boucher, 1980). Thus, it appears that one of the critical variables involved in the determination of the analgesic effectiveness of various manipulations is the type of analgesia test employed. Given this finding, Experiment 6 examined whether naloxone would induce analgesia as assessed by the tail-flick test. Furthermore, as in Experiment 5, the effects of different intensities of nociceptive stimulation on the development of the naloxone-induced analgesia was investigated.

Method

Subject: The subjects were 32 rats maintained as in the previous experiments.

Apparatus: The apparatus used in the previous experiments was also employed here. In addition, however, a white plastic bottle, 15 cm long and 5.25 cm wide (inner diameter) with the bottom of the bottle removed, was used to restrain the animals during tail-flick testing. The rat was inserted into the bottle through the open-ended bottom. In addition, the rat could protrude its head through a small, semicircular opening at the front of the bottle.

Procedure: Twice a day for the five days preceding the experiment, the animals were handled and acclimatized to being inserted and restrained

in the bottle.

The tail-flick test consisted of inserting the rat into the bottle, immersing the distal 5 cm of the rats' tail into heated water, and measuring the latency to completely withdraw the tail from the water to the nearest 0.1 sec. Animals were assigned to one of four groups (n=8). Two were tested at 45° C (± 0.2°) water temperature and two at 50°. At each temperature one group (RM-N/HC-S) received naloxone (10mg/kg) in the test room and saline in the home cage and the other group (RM-S/HC-N) received saline in the test room and naloxone in the home cage.

Tail-flick testing under the drug treatment regimen was conducted for eight days. On day 9 all groups were administered tail-flick tests (at their respective water temperatures) under saline. One week following the first saline test a second saline test was administered. The interval between injection in the distinctive room and testing was 30 min throughout the duration of the study.

Results

Naloxone Testing Phase: Figure 11 shows the mean tail-flick latencies from the first eight days of the experiment. A drug treatment x water temperature x days analysis of variance was performed on the data. Naloxone administration prior to testing induced analgesia at both temperatures as indicated by a significant main effect for drug treatment, $F(1,28) = 6.31$, $p < .02$, and a non-significant drug treatment x water temperature interaction, $F < 1.0$. The analgesia was evident throughout the testing period; neither the drug treatment x days nor the drug treatment x days x water temperature

Figure 11: Mean tail-flick latencies (± SEM) for groups administered naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N), tested at either the 45° (left panel) or the 50° (right panel) water temperature during the eight days of the naloxone treatment phase of Experiment 6.

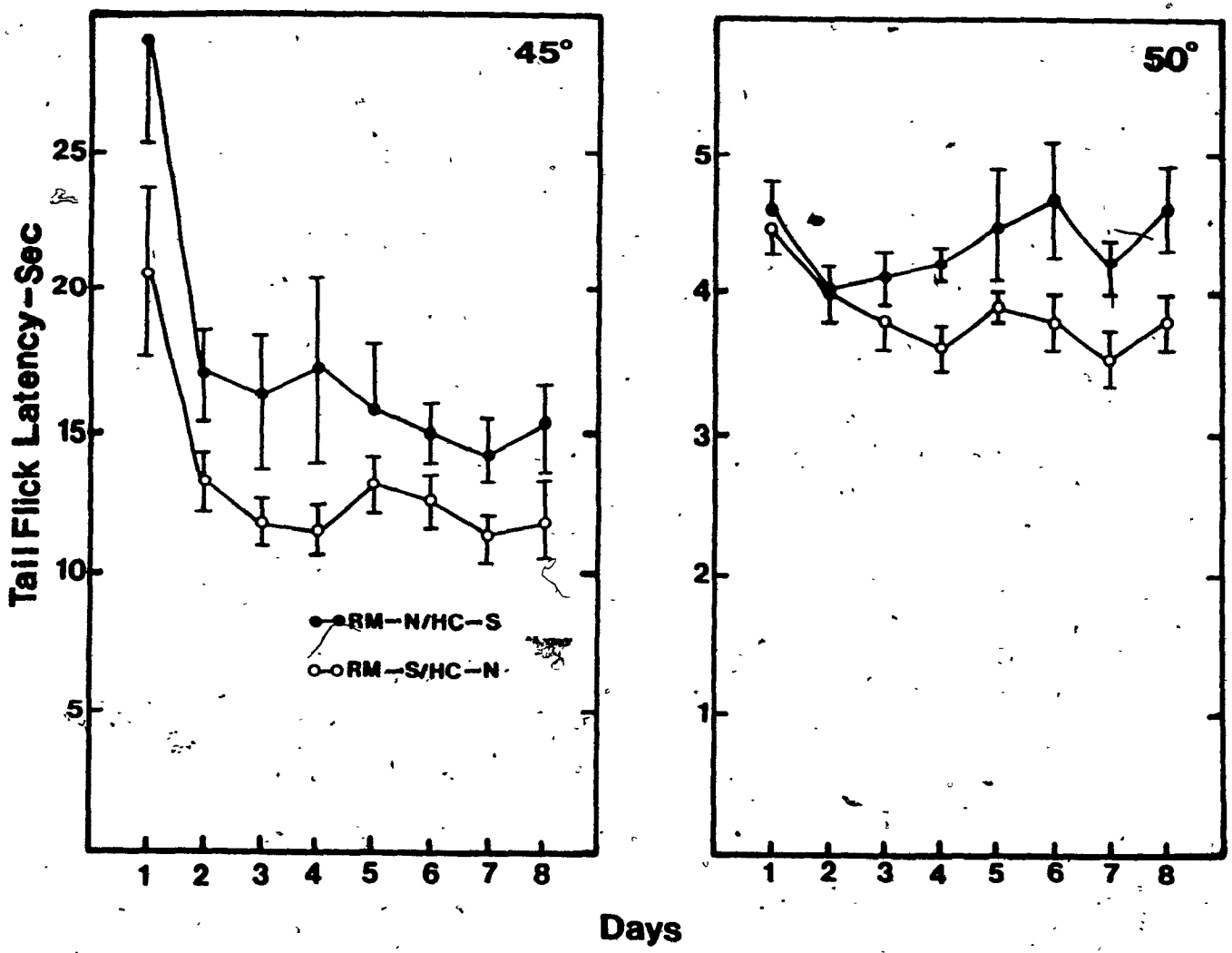
45°
50°
Tail Flick Latency-Sec
25
20
15
10
5
5
4
3
2
1
RM-N/HC-S
RM-S/HC-N
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
Days

Figure 11

interactions were significant (Fs < 1.0). Both the main effect for days, $F(7,196) = 14.54$, and the days x water temperature interaction, $F(7,196) = 12.17$, were significant (ps < .0001), reflecting the fact that animals tested at the 45° temperature showed a substantial decrease in tail-flick latencies over days while the latencies for the animals tested at the 50° temperature remained relatively constant.

Saline Test Phase: Figure 12 shows the mean tail-flick latencies during the two saline test days. Animals previously tested under naloxone exhibited longer tail-flick latencies on the saline test days than animals previously tested under saline. This effect occurred irrespective of water temperature. These observations were confirmed statistically. The main effect for previous drug treatment was significant, $F(1,28) = 6.41$, $p < .02$, but neither the main effect for water temperature or test days, nor any of the interactions, were significant (Fs < 1.0).

Discussion

The results of Experiment 6 show that, as in the hot-plate test, the administration of 10 mg/kg naloxone prior to tail-flick testing induced analgesia and led to greater conditioned autoanalgesia when tests were conducted under saline.

Unlike in the previous studies, where evidence for naloxone-induced analgesia developed only after repeated administration and testing, in the present study naloxone-treated animals displayed longer latencies from the first day of testing. This raises the possibility that the analgesia cannot be attributed to naloxone administration per se, but rather to different basal levels of analgesia in the different

Figure 12: Mean tail-flick latencies (± SEM) for groups previously administered naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N), tested at either the 45° (left panel) or the 50° (right panel) water temperature, during the two saline test days of Experiment 6.

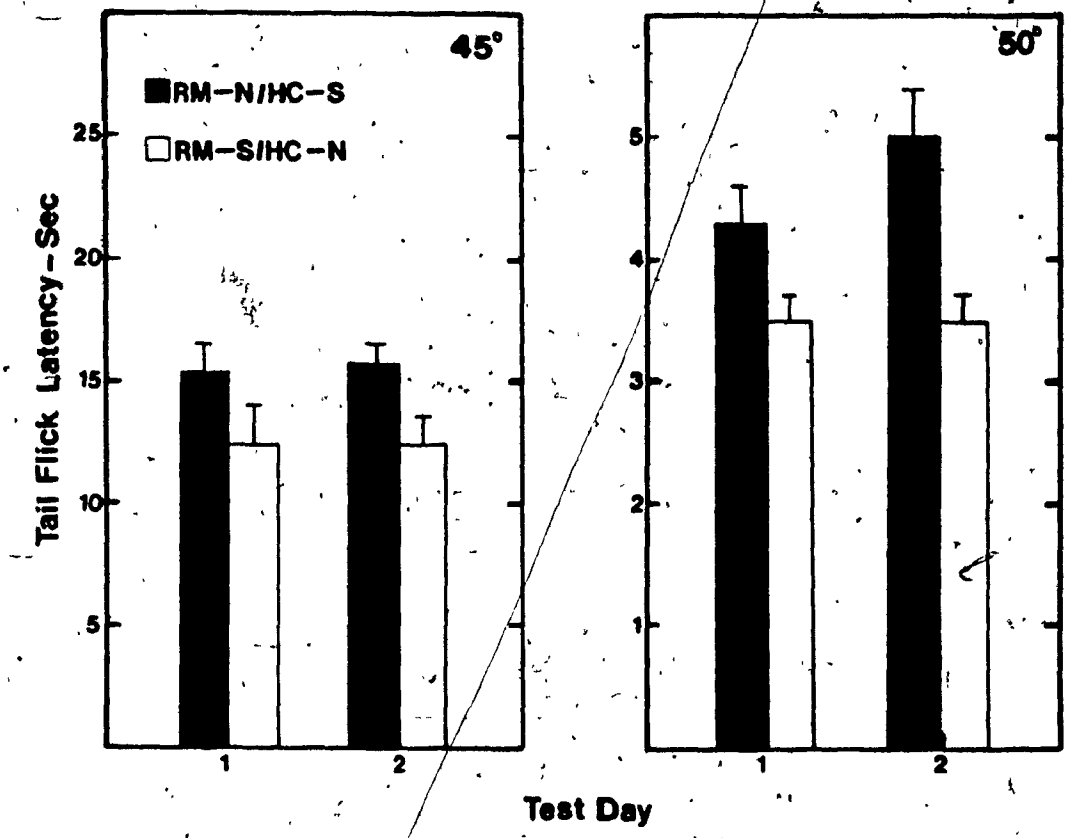
45°
50°
RM-N/HC-S
RM-S/HC-N
Tail Flick Latency-Sec
25
20
15
10
5
5
4
3
2
1
1
2
1
2
Test Day

Figure 12

groups of animals. While there is no direct evidence to contradict this argument, an alternative explanation for why naloxone-treated animals had longer latencies from the first day of testing can be suggested. It will be recalled that in this experiment all animals were familiarized with the testing bottle before the experiment. This familiarization should have reduced the influence of stress-induced analgesia produced by novelty in saline-treated animals. As discussed earlier, Bardo and Hughes (1979) have demonstrated that animals familiarized with the testing apparatus display shorter response latencies than animals exposed to the testing apparatus for the first time. The finding from the present experiment that, in animals familiarized with the testing apparatus, naloxone produced longer latencies from the first day of nociceptive testing raised the possibility that the naloxone-induced analgesia resulted from an interaction with novelty/stress-induced analgesia. This possibility was investigated in the next experiment.

Experiment 7

In the introduction, it was argued that recruitment of the EPCS would be increased in animals tested under naloxone. The increased recruitment of the EPCS was hypothesized to arise from increased pain perception brought about by blockade of opiate receptors mediating the effect of the opiate antinociceptive component. This greater recruitment of the EPCS would in turn result in a greater conditioned autoanalgesia. While the results of the experiments reported to this point indicate that opiate receptor blockade did, in fact, potentiate the development of conditioned autoanalgesia, they did not provide evidence for increased pain perception following naloxone administration. Repeated administration of naloxone prior to nociceptive testing led to the development of analgesia that was evident while the animals were under the influence of naloxone. According to the argument, longer response latencies should have been observed only when animals were tested in the absence of opiate receptor blockade.

It is therefore important to note that the differences in PLLs that were found between animals tested under naloxone and animals tested under saline emerged mainly as the result of a decline, over repeated tests, in the latencies of the saline-tested animals; the PLLs of naloxone-treated animals tended to decline less rapidly. This result suggests that naloxone might attenuate the habituation of stress-induced analgesia caused by the novelty of the test environment (Bardo & Hughes, 1979). According to this argument, animals administered naloxone might be analgesic relative to saline-treated animals because

they continue to display novelty/stress-induced analgesia at a time when the saline-treated animals have habituated to the test apparatus.

The present experiment assessed the effect of naloxone administration on the habituation of novelty/stress-induced analgesia. The design employed was similar to that employed by Bardo and Hughes (1979). Animals were administered either naloxone or saline in the test room and were then exposed to an ambient temperature (22 $\pm$ 1° C) cold-plate. A third group of animals was not exposed to the testing apparatus. If naloxone-induced analgesia resulted, at least in part, from continued stress-reactivity, then animals given cold-plate exposures under naloxone should display, in subsequent hot-plate tests, latencies of similar magnitude to non-habituated animals, and that both of these groups should appear analgesic relative to animals given cold-plate exposures under saline.

In addition to determining the effects of naloxone administration on stress-reactivity, Experiment 4 provided an opportunity to assess directly whether exposure to nociceptive stimulation, in itself, contributed to the development of the analgesia observed ~~in~~ naloxone-tested animals. Following the habituation phase of the experiment, a second phase was initiated in which all animals were given repeated nociceptive tests on the 48.5° C hot-plate. During this phase the three original groups were divided in half; one subgroup received naloxone prior to nociceptive testing, and the other was injected with saline. If animals given hot-plate tests under naloxone developed analgesia, even after they had been habituated to the plate cues, then it could be concluded that nociceptive stimulation in itself

contributes to the elicitation of the analgesia seen in naloxone-treated animals. Because analgesia due to novelty/stress should be minimal in animals previously familiarized with the apparatus, any analgesia which did develop could be attributed to the effects of nociceptive stimulation.

Method

Subjects and Apparatus: The subjects were forty-eight rats maintained as in the previous experiments. The apparatus used was identical to that in Experiments 4 and 5.

Procedure: The animals were handled twice daily for five days prior to the start of the experiment.

The experiment consisted of two phases; habituation and hot-plate testing.

Habituation Phase: The animals were randomly divided into three groups (N=16), differing with respect to the manner in which the animals were exposed to the non-functional hot-plate apparatus. Animals in group HAB-NAL were transported to the test room, injected with naloxone (10 mg/kg) and 30 min later were placed on the 22° C cold-plate for 60 sec. They were then returned to their home cages, where, three to five hours later, they were injected with saline. Animals in group HAB-SAL were administered saline in the test room and naloxone in the home cage. Animals in group NO HAB were never exposed to the hot-plate apparatus; remaining in their home cages and receiving no injections throughout the duration of this phase. In order to attempt to equate for the effects of handling, the animals were picked up for approximately one min at the times when animals in the other groups were given their test

room and home cage injections.

The habituation phase lasted for eight days and was followed by a saline test day in which all animals were given a test for analgesia on the 48.5° hot-plate.

Hot-Plate Testing: This saline test day was followed by an eight day hot-plate testing phase during which all animals were given one nociceptive test per day on the 48.5° C hot-plate. Half of the animals in each of the three original groups were administered naloxone in the test room and saline in the home cage, while for the remaining animals the drug treatment was reversed. Note that for half of the animals that had previously received habituation to the plate cues, the drug treatment administered during the hot-plate testing phase was the same as that received during the habituation phase (groups HAB-NAL/RM-N/HC-S and HAB-SAL/RM-S/HC-N), while for the remaining half the drug treatment administered during the hot-plate testing phase was the opposite from that given during the habituation phase (groups HAB-NAL/RM-S/HC-N and HAB-SAL/RM-N/HC-S). Following the last day of this phase, all animals were given a second saline test day.

Results

Saline Test Day: Figure 13 shows the mean PLLs (± SEM) for groups HAB-NAL, HAB-SAL and NO-HAB on the saline test day that followed the eight habituation days. The analysis of variance revealed a significant effect of habituation treatment, $F(2,45) = 29.84$, $p < .0001$. Animals that had received naloxone in the test room during habituation and animals that had not been habituated to the hot-plate apparatus both exhibited longer PLLs than animals that were habituated under saline

Figure 13: Mean paw-lick latencies (± SEM) for the group previously habituated to the plate under naloxone, the group previously habituated under saline, and the non-habituated group during the first saline test day of Experiment 7.

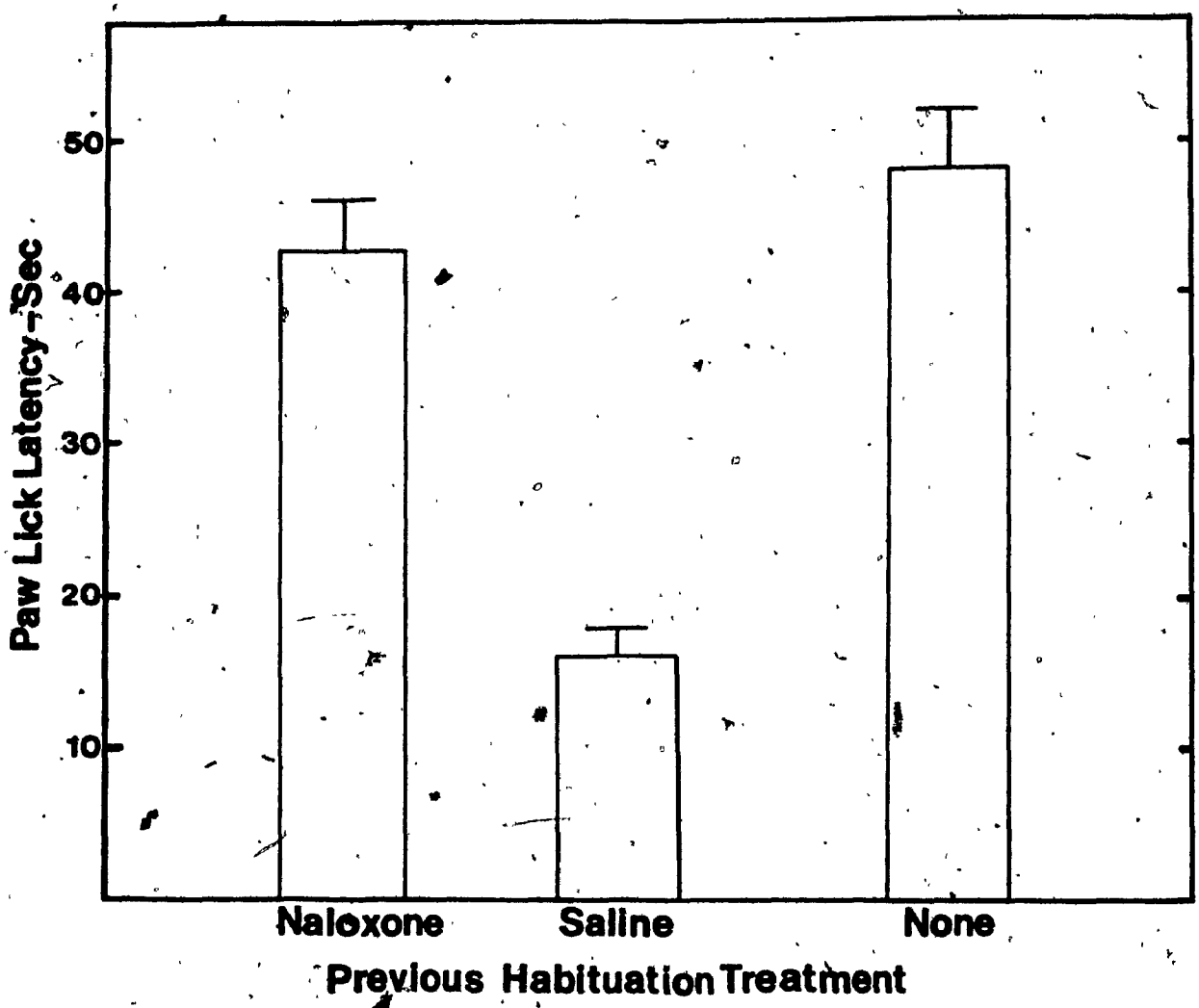
Paw Lick Latency-Sec
50
40
30
20
10
Naloxone
Saline
None
Previous Habituation Treatment

Figure 13

(Tukey's tests). The PLLs for naloxone-habituated animals did not differ from non-habituated animals.

Hot-Plate Testing Phase: Figure 14 shows the mean PLLs for the six groups during the hot-plate testing phase of the experiment. The analysis of variance (previous habituation treatment x hot-plate testing drug treatment x days) yielded significant habituation treatment x days, $F(14,294) = 2.07$, $p < .025$, and hot-plate drug treatment x days, $F(7,294) = 7.15$, $p < .0001$, interactions.

As in previous experiments, animals tested on the hot-plate under naloxone had longer PLLs than saline-tested animals. More interesting, however, were the differences between groups as a function of previous habituation treatment. Animals previously habituated to the apparatus under naloxone showed no significant changes in PLLs over days, $F(7,294) = 1.58$, $p > .05$, whereas the latencies for animals that received no habituation treatment decreased over days, $F(7,294) = 5.90$, $p < .01$. Inspection of Figure 14, however, shows that this decrease in latencies occurred primarily among animals receiving hot-plate tests under saline; on day 8, the latencies for animals that were tested under naloxone were similar to those for day 1. The PLLs for animals habituated under saline also varied as a function of days, $F(7,294) = 5.00$, $p < .01$; reflecting the fact that the latencies for these animals increased on days 2 and 3 and then stabilized over days 4-8. However, even here this trend was more descriptive of animals given hot-plate tests under saline. The latencies of animals tested under naloxone were longer than they had been on day 1 throughout the hot-plate testing phase.

Figure 14: Mean paw-lick latencies (+ SEM) for groups injected with naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) during the eight days of the hot-plate testing phase of Experiment 7. The left panel shows the latencies for animals previously habituated to the plate under naloxone, the center panel for animals previously habituated under saline, and the right panel for animals not previously habituated to the plate.

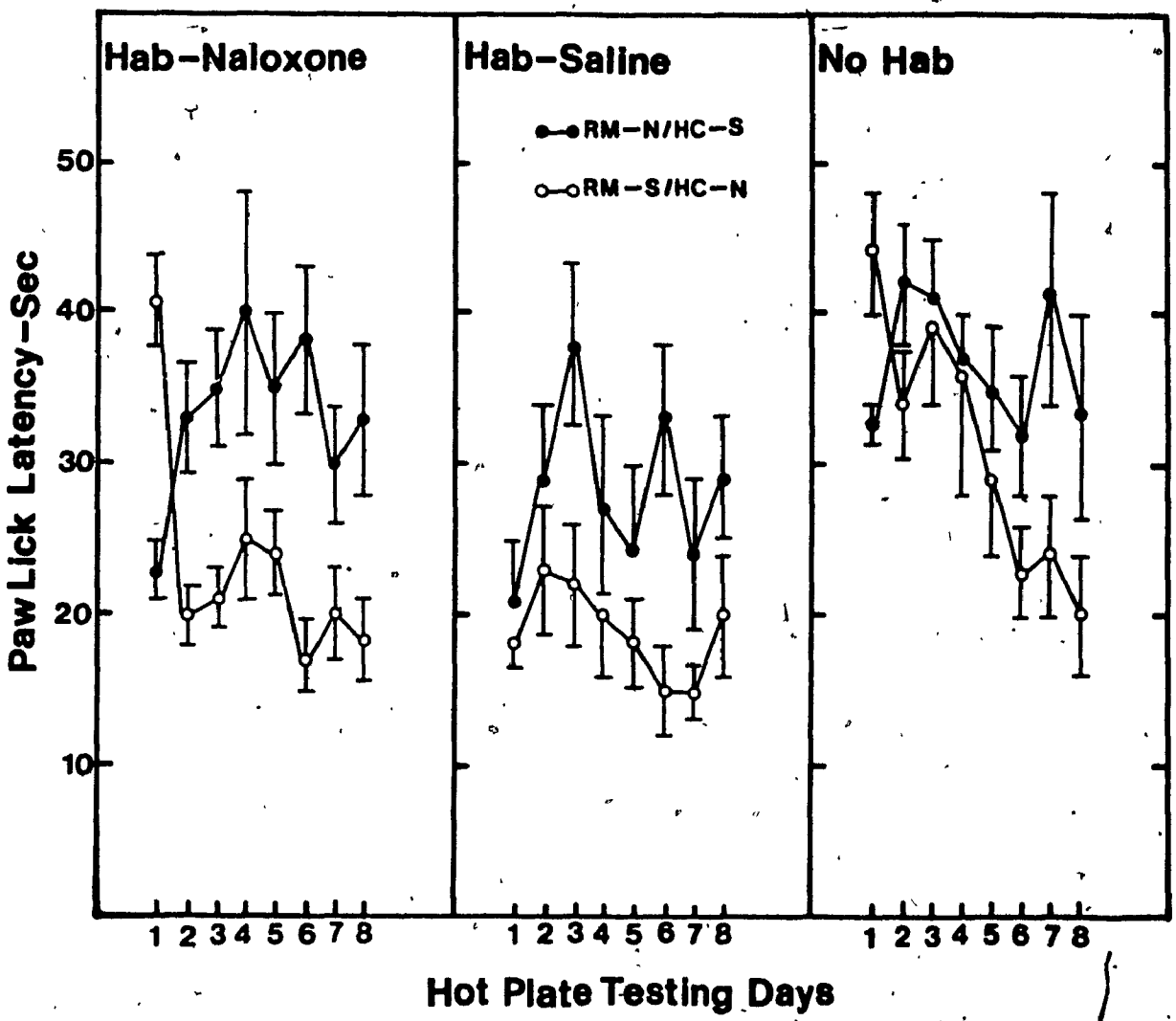
Hab–Naloxone
Hab–Saline
No Hab
RM–N/HC–S
RM–S/HC–N
Paw Lick Latency–Sec
50
40
30
20
10
1 2 3 4 5 6 7 8
Hot Plate Testing Days

Figure 14

Tests conducted to determine the nature of the hot-plate drug treatment x days interaction revealed that animals tested under naloxone exhibited significantly longer PLLs than saline-tested animals on all 8 days, $\underline{F}s(1,336) \geq 5.22$, $\underline{p}s < .05$. The latencies of naloxone-tested animals tended to increase over days, $\underline{F}(7,294) = 3.50$, $\underline{p} < .01$, particularly in animals previously habituated to the hot-plate (i. e., groups HAB-NAL/RM-N/HC-S and HAB-SAL/RM-N/HC-S). In contrast, the latencies of saline-tested animals decreased over days, $\underline{F}(7,294) = 7.90$, $\underline{p} < .01$, particularly among animals previously not habituated to the hot-plate cues or habituated to the plate under naloxone (groups NO HAB/RM-S/HC-N and HAB-NAL/RM-S/HC-N).

Figure 15 shows the results from the saline test day that followed the hot-plate testing phase of the experiment. The analysis of variance (previous habituation treatment x hot-plate testing drug treatment) revealed a significant main effect for hot-plate testing drug treatment, $\underline{F}(1,42) = 16.02$, $\underline{p} < .001$, reflecting the fact that animals previously given hot-plate tests under naloxone exhibited longer PLLs than animals previously tested under saline. Neither the main effect for previous habituation treatment, $\underline{F}(2,42) = 2.53$, $\underline{p} > .05$, nor the habituation treatment x hot-plate testing drug treatment interaction ($\underline{F} < 1.0$) was significant.

Discussion

In this experiment animals that either were not habituated to the plate cues prior to testing or were habituated under naloxone had longer PLLs than animals habituated under saline. These results

Figure 15: Mean paw-lick latencies (+ SEM) for groups previously receiving naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) as a function of previous habituation treatment during the second saline test day of Experiment 7.

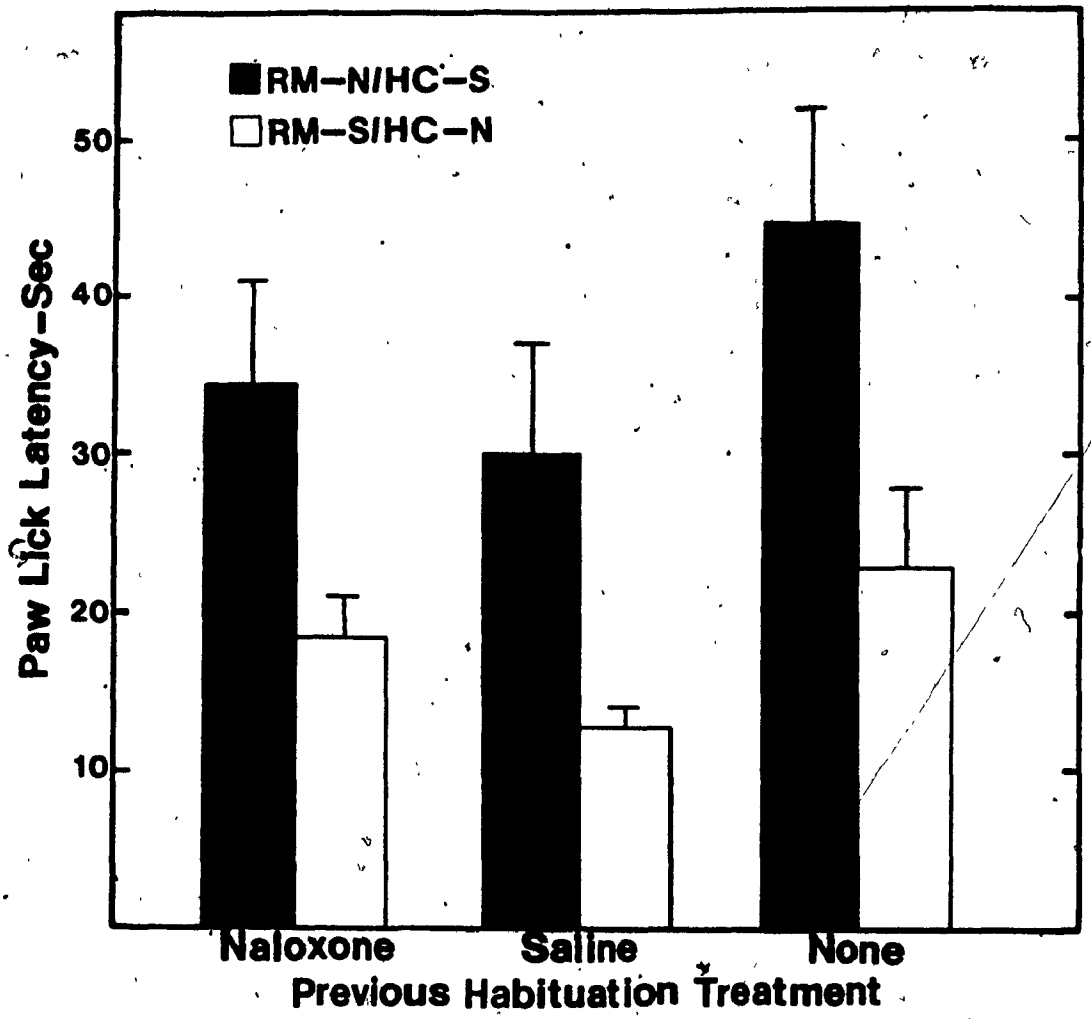
RM–N/HC–S
RM–S/HC–N
Paw Lick Latency–Sec
50
40
30
20
10
Naloxone
Saline
None
Previous Habituation Treatment

Figure 15

support the hypothesis that the analgesia observed when animals are tested under naloxone arises, in part, from the fact that habituation of novelty/stress is attenuated by naloxone administration.

Although naloxone-attenuation of the habituation of novelty/stress contributed to the naloxone-induced analgesia, the results from the hot-plate testing phase of the experiment suggest that exposure to nociceptive stimulation also contributed to the development of the effect. It was found that animals exposed to the plate during the habituation phase of the experiment and then exposed to nociceptive stimulation under the influence of naloxone during the hot-plate testing phase (i.e., groups HAB-NAL/RM-N/HC-S and HAB-SAL/RM-N/HC-S) displayed increased latencies over days. An increase in latencies would be expected if naloxone administration prior to nociceptive testing caused an increased recruitment of the EPCS. However, it is not clear that the greater recruitment of the EPCS results from naloxone-produced changes in pain sensitivity since in this experiment, as in Experiments 4-6, no evidence for hyperalgesia was obtained. Exactly how exposure to nociceptive stimulation results in the greater recruitment of the EPCS, therefore, remains unanswered.

One finding in this experiment that differed from that of the previous experiments is that during the hot-plate testing phase animals tested under naloxone actually showed increases in latencies over trials, particularly in animals previously exposed to the plate (It was noted earlier that in the previous experiments the difference between naloxone- and saline-tested groups arose primarily from the decline in PLLs over trials in saline-tested animals). One explanation for this

finding might be that in this experiment animals were familiarized with the testing procedure prior to being given nociceptive tests. It is interesting to note in this regard that in the animals not habituated to the plate prior to hot-plate testing (NO HAB), the pattern of results was similar to that seen in previous experiments; saline-treated ~~animals~~ showed a decrease in latencies over trials whereas naloxone-treated animals showed little change.

Experiment 8

The effects of pre-exposure to the hot-plate apparatus on the development of analgesia under naloxone was studied further in Experiment 8. In this experiment animals were administered either naloxone or saline and then pre-exposed to either a 48.5° C hot-plate, an ambient temperature cold plate or were not exposed to the plate. Following this pre-exposure phase the animals were tested for analgesia under morphine.

Experiment 5 also addressed another possible interpretation of the findings obtained with naloxone. Because in all the experiments reported thus far, animals were removed from the hot-plate immediately following a paw lick, it would have been possible for animals to learn that paw-licking leads to immediate escape from the hot-plate. Perhaps naloxone interferes with the learning of this instrumental escape contingency. The more naloxone interferes with the learning of this contingency, the more analgesic animals tested under naloxone would appear relative to saline-treated controls. In order to control for this possibility, in the present experiment all animals were kept on the hot-plate surface for 90 sec regardless of when they first paw-licked.

Method

Subjects and Apparatus: The subjects were forty-eight rats maintained as previously described. The apparatus was identical to that used in Experiments 4, 5 and 7.

Procedure: Following five days of twice daily handling, the animals were randomly divided into six groups (N=8). Three of the groups were

given naloxone (10 mg/kg) in the test room and saline in the home cage, the remaining three groups were given saline in the test room and naloxone in the home cage. Within each drug treatment condition, one group was exposed during the first eight days of the experiment either to a plate immersed in a 48.5° (± .2°) C bath, (groups RM-N/HC-S-HP and RM-S/HC-N-HP), to an ambient temperature (22 ± 1° C) cold-plate (groups RM-N/HC-S-CP and RM-S/HC-N-CP), or to none of the hot-plate apparatus cues (groups RM-N/HC-S-NP and RM-S/HC-N-NP). In order to control for the effects of handling, animals not exposed to the hot-plate were instead placed in a 36.3 x 35 x 25 cm wooden box, with a wire mesh floor. All daily exposures, whether to the hot-plate, the cold-plate, or the wooden box, were of 90 sec duration.

On day 9 all groups were given hot-plate (48.5°) tests following administraton of 5 mg/kg morphine. During the morphine test the animals were left on the hot-plate surface for 120 sec regardless of when they first licked their paws.

Two days following the morphine test day, all animals were given nociceptive tests on the 48.5° hot-plate for five days. During this phase the different groups were given the same drug treatments as they had been given during the first eight days of the experiment. The animals were kept on the hot-plate surface for 90 sec during this phase. The interval between injection in the test room and nociceptive testing was 30 min throughout the study.

Results

Plate Exposure Phase: Figure 16 shows the mean PLLs for groups RM-N/HC-S-HP and RM-S/HC-N-HP, the two groups that received hot-plate

Figure 16: Mean paw-lick latencies (± SEM) for the two groups exposed to the 48.5° hot-plate during the eight days of the plate exposure phase of Experiment 8. Group RM-N/HC-S-HP was administered naloxone in the test room and saline in the home cage; group RM-S/HC-N-HP received saline in the test room and naloxone in the home cage.

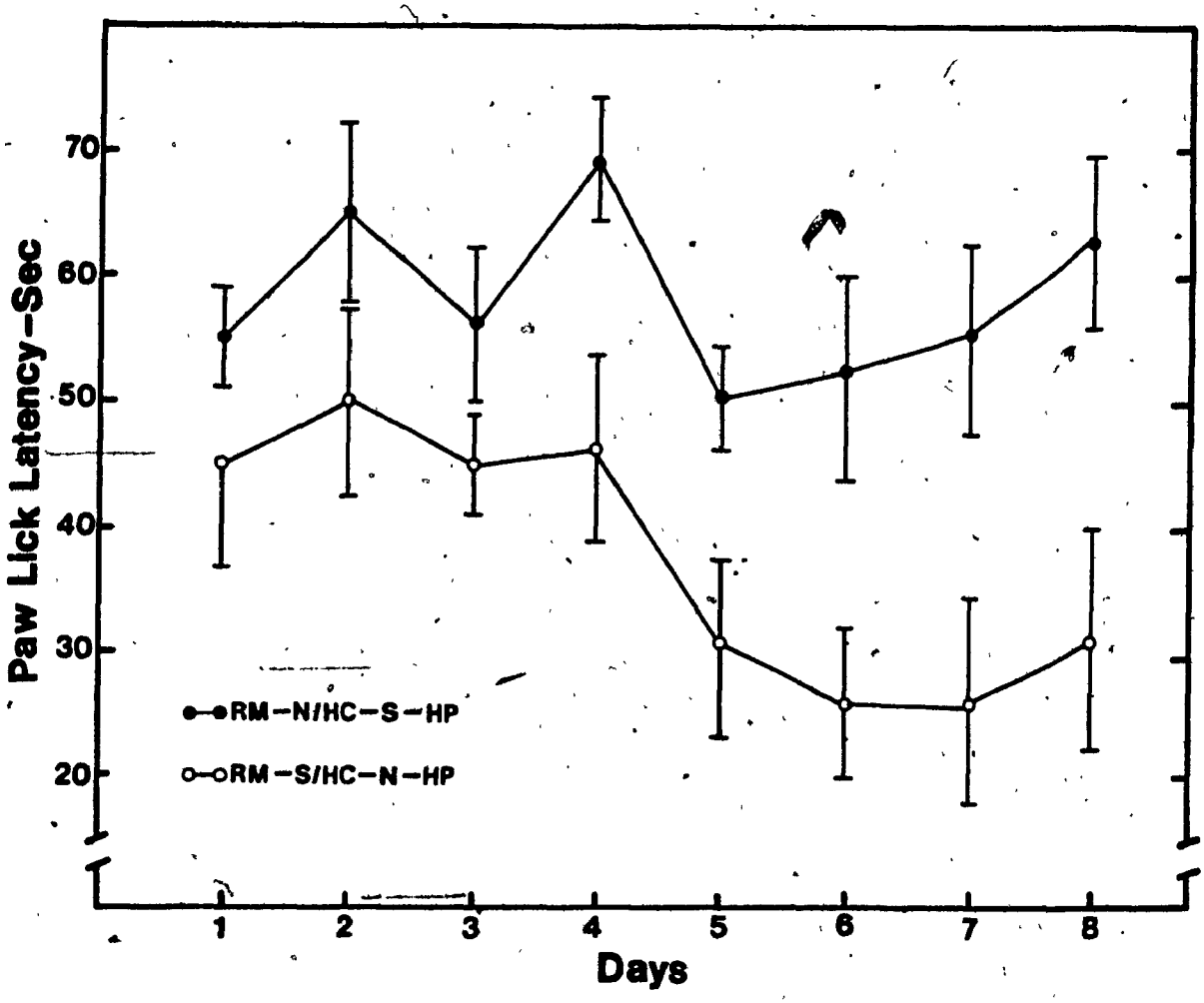
Paw Lick Latency–Sec
70
60
50
40
30
20
RM–N/HC–S–HP
RM–S/HC–N–HP
1
2
3
4
5
6
7
8
Days

Figure 16

tests during the plate exposure phase of the experiment. A drug treatment x days analysis of variance revealed significant main effects for drug treatment, $F(1,14) = 7.08$, $p < .02$, reflecting the longer PLLs in naloxone-tested animals, and days, $F(7,98) = 6.26$, $p < .0001$; reflecting the overall tendency for PLLs to decrease over days, particularly in saline-tested animals. The drug treatment x days interaction was not significant, $F(7,98) = 1.78$, $p > .05$.

Morphine Test Day: Figure 17 shows the results from the morphine test day. Prior to the morphine test day one animal in group RM-S/HC-N-NP died. Therefore the mean PLLs for this group are based on N=7. A between-subjects analysis of variance (previous drug treatment x previous plate exposure) revealed that the main effect for previous plate exposure was significant, $F(2,41) = 11.22$, $p < .0001$. Animals never exposed to the plate exhibited significantly longer latencies than either hot- or cold-plate exposed animals (Tukey's tests). However, Figure 17 shows that the overall difference between animals exposed to the hot-plate and those not exposed to the plate was mainly due to the low latencies displayed by the group that had been exposed to the hot-plate under saline, group RM-S/HC-N-HP. Group RM-N/HC-S-HP exhibited latencies of comparable magnitude to non-exposed animals. The overall latencies between hot- and cold-plate exposed animals did not differ. The main effect for drug treatment was also significant, $F(1,41) = 4.93$, $p < .05$, but the drug treatment x plate exposure interaction was not, $F(2,41) = 2.18$, $p > .05$. These results suggest that animals previously administered naloxone in the test room were analgesic relative to their respective saline control animals

Figure 17: Mean paw-lick latencies (± SEM) for groups previously injected with naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) as a function of previous plate exposure during the morphine test day of Experiment 8.

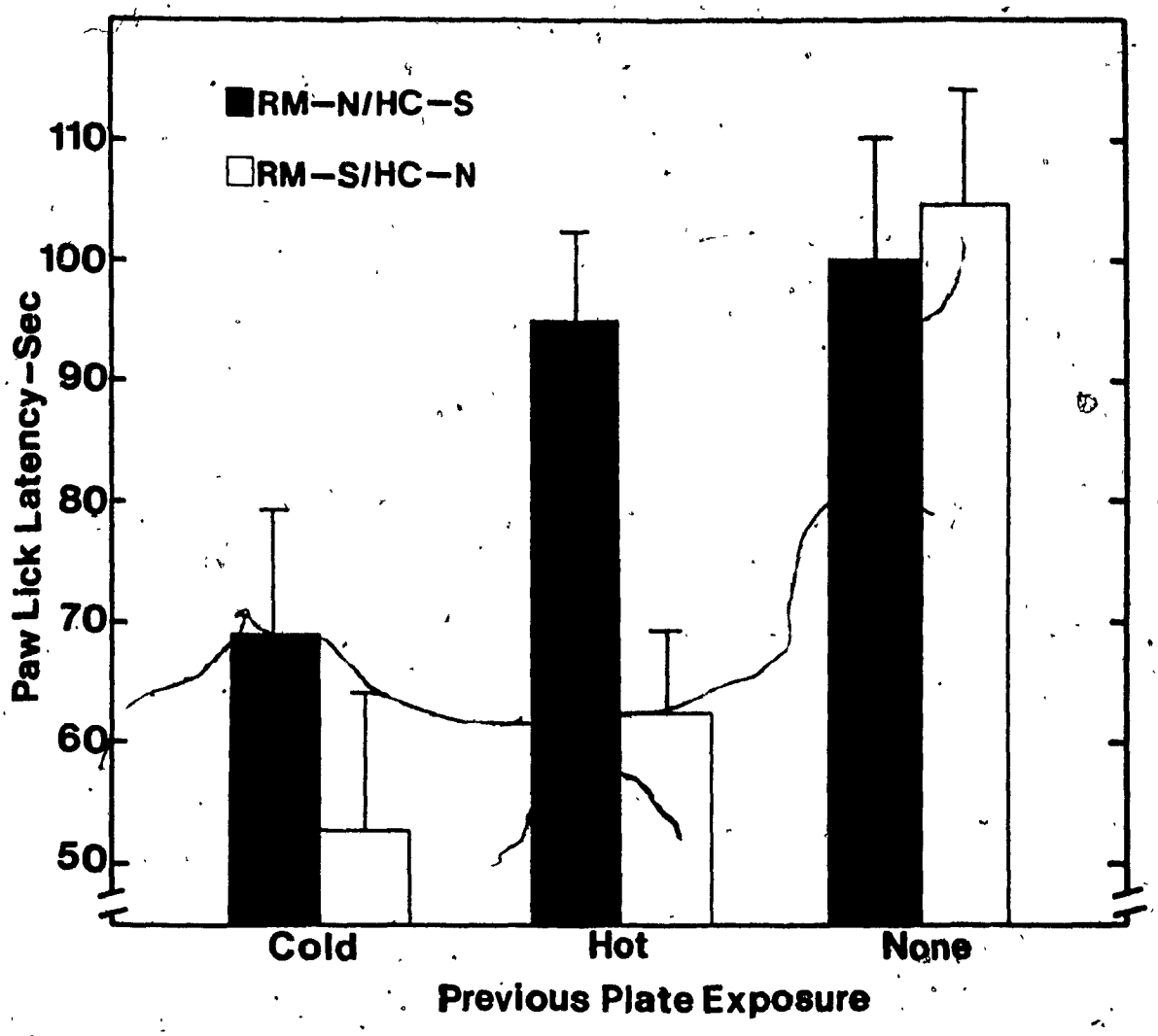
RM–N/HC–S
RM–S/HC–N
110
100
90
80
70
60
50
Paw Lick Latency–Sec
Cold
Hot
None
Previous Plate Exposure

Figure 17

independent of the type of plate exposure previously administered. However, Figure 17 clearly shows that the analgesia induced by naloxone was most evident in animals previously exposed to the hot-plate. Naloxone-treated animals exposed to the cold-plate displayed only slightly longer latencies than their saline controls, whereas the latencies for group RM-N/HC-S-NP were higher than those for group RM-S/HC-N-NP.

Hot-Plate Testing Phase: Figure 18 shows the mean PLLs for the six groups on days 10-14 of the experiment, the five days in which all animals were tested on the 48.5° hot-plate. The analysis of variance (drug treatment x previous plate exposure x days) yielded a significant drug treatment x plate exposure x days interaction, $F(8,164) = 2.84$, $p < .03$. Tests for simple main effects conducted between groups at each plate exposure for each day revealed that naloxone administration produced longer latencies in animals previously exposed to either the hot- or cold-plate on all five days, $Fs(1,205) \geq 4.19$, $ps < .05$. In contrast, significant differences between naloxone- and saline-tested animals not previously exposed to the plate emerged only from day 12 on, $Fs(1,20$

-- Tests conducted for each group over days revealed that PLLs increased in naloxone-treated animals previously exposed to the hot- or cold-plate, $Fs(4,164) \geq 6.54$, $p < .01$, but not in animals not previously exposed to the plate, $F(4,164) = 2.30$, $p > .05$. The only significant change over days among saline-tested animals was a reduction in PLLs in group RM-S/HC-N-NP, $F(4,164) = 4.55$, $p < .01$.

Discussion

The results of the present experiment demonstrate that

Figure 18: Mean paw-lick latencies for the groups receiving naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) during the five days of the hot-plate testing phase of Experiment 8. The left panel shows the latencies for groups previously exposed to the 22° cold-plate, the latencies for groups previously exposed to the 48.5° hot-plate are displayed in the center panel, and those for the groups not previously exposed to the plate cues are shown in the right panel.

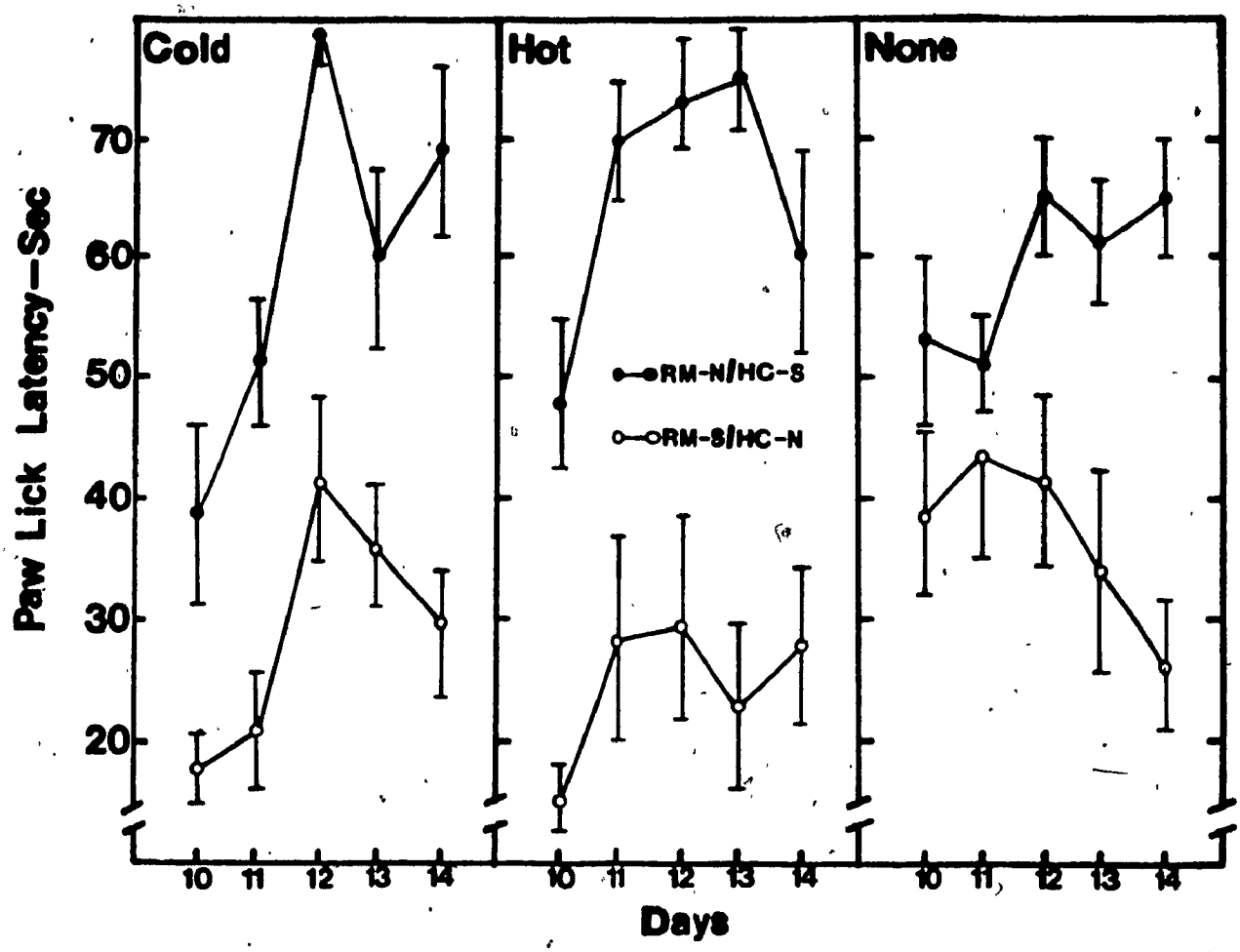
Cold
Hot
None
Paw Lick Latency—Sec
70
60
50
40
30
20
RM-N/HC-S
RM-S/HC-N
10
11
12
13
14
Days

Figure 18

the analgesic effect of morphine is most marked in animals never previously exposed to the test apparatus or in animals previously exposed to the hot-plate under naloxone. The fact that animals pre-exposed to the cold-plate displayed lower latencies in the morphine test, regardless of whether they were exposed under naloxone or saline, suggests that some degree of habituation to novelty/stress does occur under naloxone. The fact that group RM-N/HC-S-HP exhibited latencies comparable to non-pre-exposed animals supports the idea that exposure to nociceptive stimulation under naloxone counteracts the decrease in latencies attributable to novelty/stress.

The results from the hot-plate testing phase of the experiment do suggest, however, that habituation to the novelty of the apparatus is less complete under naloxone than under saline; for inspite of the fact that the latencies for the group that was pre-exposed to the cold-plate under naloxone (RM-N/HC-S-CP) were only slightly longer than the saline control group on the morphine test day, this group displayed longer latencies than group RM-S/HC-N-CP on all five days of the hot-plate testing phase. Naloxone-tested animals not previously exposed to the plate were analgesic relative to their saline control group only from the third day.

Three further observations can be made about the result of Experiment 8. Note that groups RM-N/HC-S-HP and RM-N/HC-S-CP displayed increased latencies over the hot-plate testing phase of the experiment, whereas group RM-N/HC-S-NP did not. This finding adds further support for the earlier suggestion that animals must have some prior experience with the plate apparatus before increases in latencies will be

observed. Note also that the increase in latencies over tests supports our contention that exposure to nociceptive stimulation contributes to the naloxone-induced analgesia. Finally, the finding that naloxone produced analgesia in spite of the fact that paw-licking did not result in immediate removal from the plate surface suggests that the analgesia is not the result of naloxone interfering with the learning of the escape contingency.

Experiment 9

Because it seemed reasonable to conclude that pain-induced analgesia contributed to the effects seen in these experiments, the next step was to determine which component of the endogenous pain control mechanism was involved. Lewis and his associates (Lewis et al., 1980; 1981) have demonstrated that administration of inescapable footshock can elicit either opiate- or non-opiate-mediated analgesia depending on the temporal characteristics of shock administration. Prolonged, intermittent shock elicits an opiate-mediated analgesia, as indicated by the fact that it is blocked by prior naloxone administration and shows cross-tolerance with morphine. Brief, continuous shock appears to elicit a non-opiate-mediated analgesia in that it is neither blocked by naloxone nor shows cross-tolerance with morphine.

In Experiment 9 these findings were used in an attempt to determine more directly which component of the endogenous pain control system mediated the naloxone-induced analgesia seen in the previous experiments. Rats were injected with either naloxone or saline, administered either prolonged, intermittent shock, brief, continuous shock, or no shock, and then received tail-flick tests for analgesia. If the naloxone-induced analgesia was opiate-mediated then naloxone should enhance the analgesia produced by prolonged, intermittent shock. If, on the other hand, the analgesia was mediated by the non-opiate component, then naloxone should potentiate the analgesia produced by brief, continuous shock.

Method

Subjects: The subjects were forty-eight rats maintained as in the previous experiments.

Apparatus: The analgesia testing apparatus was identical to that used in Experiment 6. Shock was administered in the operant conditioning chambers used in Experiments 1-3.

Procedure: Animals were handled and acclimatized to the tail-flick testing bottle twice daily for five days prior to the start of the experiment.

The general procedure involved transporting the animals to the test room, and immediately administering two tail-flick tests (spaced approximately 2 min apart). These constituted the baseline trials. The animals were then injected with either naloxone or saline, and placed in the shock chambers for 20 min. They were then removed and administered tail-flick tests at 1, 5 and 12 min following removal. These tests constituted the post-shock trials. The water temperature was 50° (± .2°) C. During the intervals between tests the animals were not returned to the shock chambers but were placed in the wooden holding boxes used previously.

Six groups (N=8) of animals were employed. Three groups were injected with naloxone (10 mg/kg) in the test room and saline in the home cage, while the remaining three groups received the reverse drug treatment. The groups also differed with respect to shock condition. Groups RM-N/HC-S-PROLONGED SHOCK and RM-S/HC-N-PROLONGED SHOCK received 1 sec pulses of ~~2.5~~ mA shock every 5 sec for 20 min, begining immediately upon placement into the shock chambers. Groups RM-N/HC-S-

BRIEF SHOCK and RM-S/HC-N-BRIEF SHOCK were administered 2.5 mA shock continuously for 180 sec. In this shock condition shock administration began 17 min after the animals were placed in the shock chambers, thereby insuring that the interval between the termination of shock administration and tail-flick testing was identical for both shock conditions. Groups RM-N/HC-S-NO SHOCK and RM-S/HC-N-NO SHOCK were simply placed in the shock chambers, without shock, for 20 min.

Shock was administered for 7 days. Following the last shock administration day, all animals were given two test days. The procedure for the test days was identical to that for the shock administration days with the exception that no shock was administered. On the first test day all animals received saline. On the second test they were injected with 5 mg/kg morphine.

Results

Naloxone Treatment and Shock Administration Phase: Separate analyses of variance were performed on the baseline and post-shock tail-flick latency scores. For purposes of analysis the data from the two baseline and from the three post-shock trials were collapsed. The analysis of variance (drug treatment x shock condition x days) performed on the baseline data revealed a significant main effect for drug treatment, $F(1,42) = 5.15$, $p < .03$, indicating that prior to drug injection animals that received naloxone in the test room exhibited marginally longer tail-flick baseline latencies ($M = 5.84$) than saline-treated animals ($M = 5.28$). In addition, the shock condition x days interaction was significant, $F(12,252) = 1.90$, $p < .05$. Tukey's tests revealed the baseline latencies of non-shocked animals increased over

days. The baseline latencies for brief- and prolonged-shocked animals showed initial increases over days 2-6, but on day 7 the latencies had returned to levels observed on day 1.

Figure 19 shows the mean post-shock tail-flick latencies for the six groups on the first and last day of shock administration. The analysis of variance performed on the post-shock data revealed a significant drug treatment x shock condition x days interaction, $F(12,252) = 3.22$, $p < .001$. On day 1, exposure to both brief and prolonged shock produced analgesia; Tukey's tests revealed that saline-treated animals administered either brief or prolonged shock exhibited significantly longer latencies than non-shocked animals. However, tests for simple main effects conducted between groups at each shock condition revealed that naloxone-administration blocked the analgesia produced by prolonged shock (Figure 19, center panel); group RM-N/HC-S-PROLONGED SHOCK exhibited shorter latencies than group RM-S/HC-N-PROLONGED SHOCK, $F(1,294) = 17.89$, $p < .001$. This blockade appeared to be complete, since Tukey's tests revealed that the PLLs for naloxone treated, prolonged shocked animals not not significantly differ from either naloxone- or saline-treated animals not exposed to shock. Naloxone did not produce any significant effects in non-shocked or brief shocked animals on day 1, $Fs < 1.0$.

Several features of the results from the last day of shock administration merit attention. First, tolerance developed to the analgesia produced by exposure to prolonged shock. This conclusion was supported by the finding that there was a sginificant decrease in the latencies of saline-treated animals exposed to prolonged shock,

Figure 19: Mean tail-flick latencies (± SEM) for groups receiving naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) during the first and last day of shock administration of Experiment 9. The left panel shows the latencies for groups which were not shocked, the center panel the latencies for groups receiving prolonged, intermittent shock, and the right panel the latencies for groups administered brief, continuous shock.

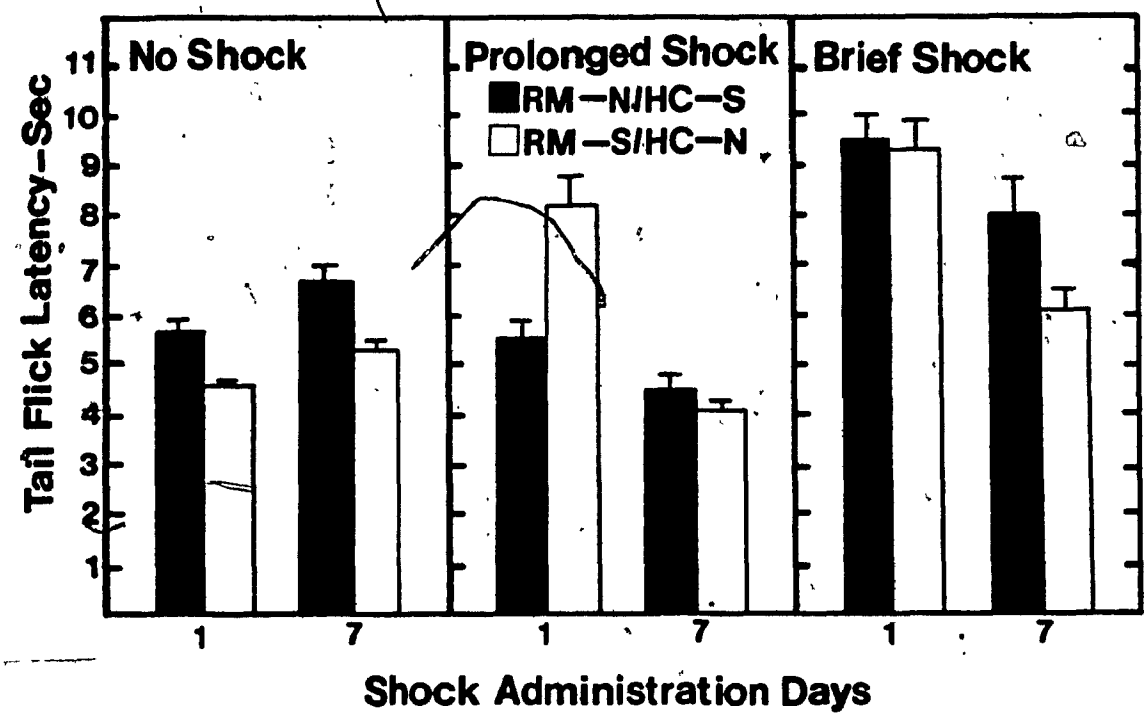
No Shock
Prolonged Shock
RM—N/HC—S
RM—S/HC—N
Brief Shock
Tail Flick Latency—Sec
11
10
9
8
7
6
5
4
3
2
1
1
7
1
7
1
7
Shock Administration Days

Figure 19

$F(6,252) = 22.39$, $p < .0001$. Moreover, by day 7 there was no significant difference between saline- and naloxone-treated animals within this shock condition ($F < 1.0$). Second, the latencies of both saline- and naloxone-treated animals receiving brief shock also showed a decrease over days, $Fs(6,252) \geq 4.40$, $ps < .01$, but this decrement was clearly of a lower magnitude than that seen in prolonged shocked animals. Third, and most importantly, naloxone administration enhanced the analgesia produced by brief shock. On day 7, group RM-N/HC-S-BRIEF SHOCK displayed significantly longer latencies than group RM-S/HC-N-BRIEF SHOCK, $F(1,294) = 9.10$, $p < .001$. Finally, as in the previous studies, naloxone administration elicited analgesia in animals merely given tail-flick tests. On day 7, group RM-N/HC-S-NO SHOCK exhibited longer latencies than its saline control group, $F(1,294) = 4.85$, $p < .05$.

Saline and Morphine Test Days: No significant main effects or interactions were obtained for the baseline latencies on the saline and morphine test days, indicating that the baseline latencies were not affected by either previous drug treatment or previous shock condition on either of the test days. The analysis of variance conducted on the trials following exposure to the shock chambers revealed a significant previous drug treatment x previous shock condition x test day interaction, $F(2,42) = 5.09$, $p < .02$. The results from the test days are shown in Figure 20. Consider first the results from the morphine test day. It is clear from Figure 20 that morphine administration elevated tail-flick latencies in all animals in comparison to the latencies exhibited on the saline test day. This observation was

Figure 20: Mean tail-flick latencies (± SEM) for groups previously administered naloxone in the test room and saline in the home cage (RM-N/HC-S) or saline in the test room and naloxone in the home cage (RM-S/HC-N) during the saline and morphine test days of Experiment 9. The left panel shows the latencies for the groups not previously exposed to shock, the center those for groups previously receiving prolonged, intermittent shock, and the right panel displays the latencies for groups previously administered brief, continuous shock.

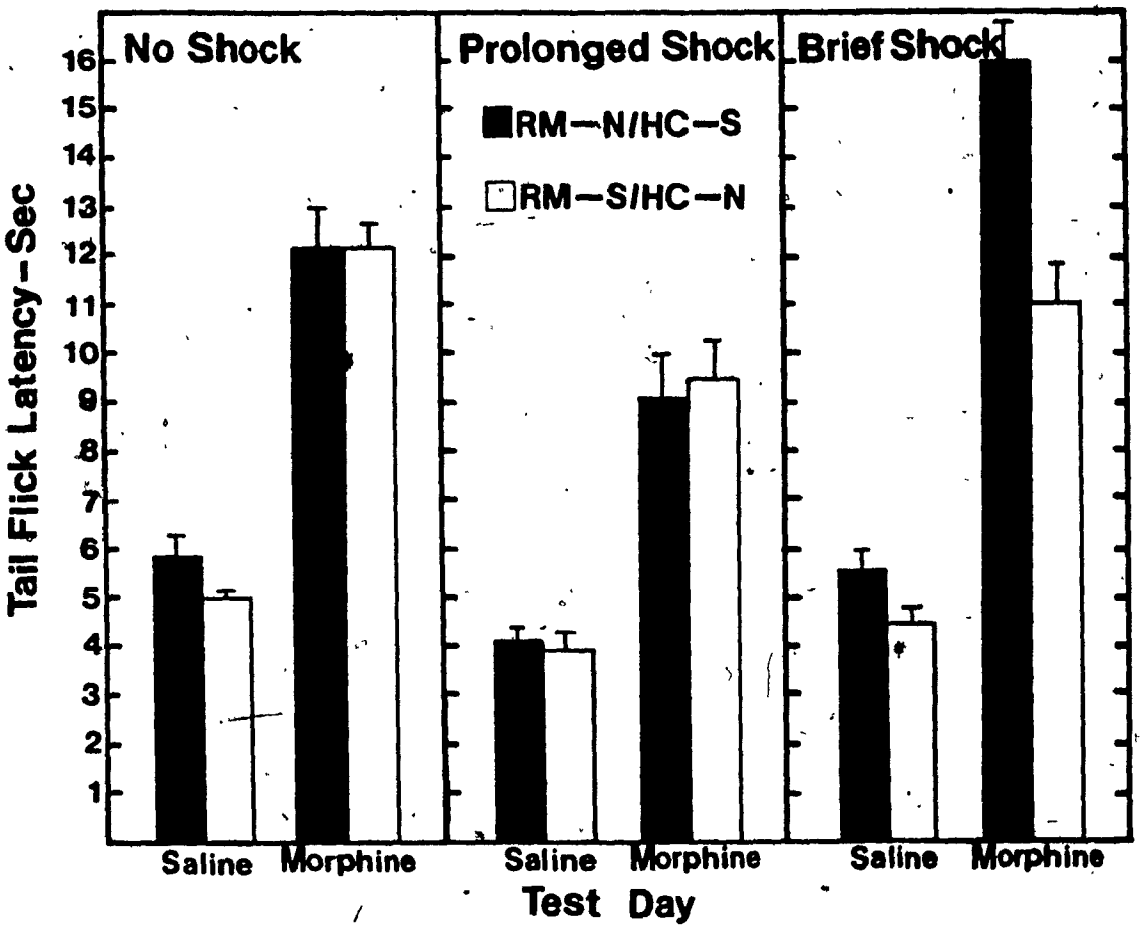
No Shock
Prolonged Shock
Brief Shock
RM—N/HC—S
RM—S/HC—N
Tail Flick Latency—Sec
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Saline
Morphine
Saline
Morphine
Saline
Morphine
Test Day

Figure 20

confirmed statistically by test for simple main effects conducted between test days for each group, $Fs(1,42) \geq 29.23$, $ps < .001$. Although morphine administration induced analgesia in all animals, the magnitude of the effect was dependent upon both prior drug treatment and shock condition. Note that animals previously exposed to prolonged shock exhibited shorter latencies than either non-shocked or brief shocked animals on the morphine test day (Tukey's tests). This result suggests that partial cross-tolerance developed between prolonged shock-induced and morphine analgesia. Note, as well, that the magnitude of the morphine analgesia was in part dependent upon the nature of the previous drug treatment. animals previously given brief shock while under the influence of naloxone (group RM-N/HC-S-BRIEF SHOCK) exhibited markedly longer latencies than animals receiving brief shock under saline (group RM-S/HC-N-BRIEF SHOCK), $F(1,84) = 25.64$, $p < .001$, suggesting that the combination of naloxone and brief shock led to a conditioned autoanalgesia that summated with the analgesic effect of morphine to a greater extent than exposure to brief shock alone. No significant differences were found between naloxone- and saline-treated animals in either of the other two shock conditions, $Fs < 1.0$.

On the saline test day no significant differences were found between non-shocked, prolonged shocked and brief shocked animals, $F(2,84) = 1.63$, $p > .05$. Nor were there any significant differences between naloxone-treated and saline-treated animals within any of the shock conditions, $Fs < 1.0$.

Discussion

The most important result of Experiment 9 was the finding that

naloxone administration enhanced the analgesia produced by exposure to brief shock (non-opiate mediated), but antagonized the analgesia elicited by prolonged shock (opiate mediated). Taken together, these results provide substantial support for the hypothesis that the analgesia observed in animals tested under naloxone is mediated by the non-opiate component of the EPCS.

The results from the morphine test day reveal that, in animals previously exposed to brief shock under naloxone, the conditioned autoanalgesia summated with morphine to produce the longest latencies. On the other hand, animals exposed to prolonged shock, known to elicit an opiate-mediated analgesia (Lewis et al., 1980; 1981) had the shortest latencies when tested under morphine. This finding suggest that the repeated elicitation of opiate-mediated pain suppression led to tolerance both to endogenous opiate- (see Figure 19, day 7) and to morphine- (see Figure 20, morphine test day) induced analgesia.

It has been argued that although the unconditioned analgesia observed during naloxone administration is mediated by the non-opiate component of the EPCS, this need not imply that the opiate component is not recruited. One question that arises is why tolerance to the opiate component does not develop in this instance. Why does activation of the non-opiate component spare the opiate component from tolerance development? Since the development of tolerance to the opiate component appears to be the result of a depletion of central met- and leu-enkephalin (Madden et al., 1977; McGivern, Mousa, Couri & Berntson, 1983), it follows that the activation of the non-opiate component must somehow retard this process. It is possible that the non-opiate

component inhibits the release of these opiate peptides, but not necessarily the activation of the opiate component.

One troubling aspect of the data from Experiment 9 is that, on the saline test day, we failed to obtain statistical support for the potentiation of conditioned autoanalgesia by previous naloxone treatment. This failure is particularly puzzling given the fact that we did find evidence for naloxone-potentiation of conditioned autoanalgesia as assessed by the tail-flick test in Experiment 6. The reason for the failure to replicate is unclear. It is worth noting, however, that the procedures employed in the two experiments were not identical. For instance, in the present experiment two baseline and three post shock tail-flick tests per day were administered, whereas in Experiment 6 animals received only one daily analgesic assessment. Exactly why or how these differences may have influenced the results cannot be directly determined.

General Discussion

The results of Experiments 4-9 support the prediction that the development of conditioned autoanalgesia will be enhanced in animals that are exposed to nociceptive stimulation while under the influence of naloxone. However, the precise reason for the facilitory effect of naloxone administration on the development of conditioned autoanalgesia remains to be discovered. It was initially predicted that the enhancement of conditioned autoanalgesia by naloxone administration would result from the increased perception of pain resulting from opiate receptor blockade. However, there was no evidence for hyperalgesia in any of the experiments. To the contrary, the unanticipated effect of naloxone administration was to raise pain thresholds. On the other hand, the results of Experiment 7 and 8 did provide evidence that exposure to nociceptive stimulation plays a role in the development of both the analgesia observed when animals are exposed to nociceptive stimulation under naloxone and the resultant potentiation of conditioned autoanalgesia. In these experiments animals exposed to nociceptive stimulation under naloxone displayed longer latencies than saline-treated animals even after they been habituated to the hot-plate apparatus.

A second factor implicated in the development of the naloxone-induced analgesia was the attenuation of the habituation of stress-induced analgesia produced by the novelty of the hot-plate apparatus. This was demonstrated most clearly in Experiment 7, where animals exposed to the cold-plate under naloxone displayed latencies of comparable magnitude to non-exposed animals, and latencies

significantly longer than animals exposed to the plate under saline.

It is possible that these two factors interact to produce the naloxone-induced and potentiated conditioned autoanalgesia observed when animals are exposed to nociceptive stimulation under the influence of naloxone. That is, naloxone may, indeed, increase pain sensitivity, but evidence for such hyperalgesia is masked during the initial exposures to nociceptive stimulation by novelty/stress-induced analgesia. However, the results from the morphine test day of Experiment 8 suggest that some habituation of novelty/stress occurs among animals administered naloxone. Examination of Figure 17 shows that animals exposed to the cold-plate under naloxone displayed lower latencies than animals exposed to the hot-plate under naloxone when both groups were tested under morphine. It may be, therefore, that as the novelty/stress-induced analgesia begins to diminish through habituation, exposure to nociceptive stimulation begins to exert is effect; thereby maintaining, or perhaps even increasing (see hot-plate testing phases of Experiments 7 and 8, Figures 14 and 18), the longer latencies observed in naloxone-treated animals. The results of Experiments 7 and 8 provide some support for this possibility. In these experiments, naloxone-treated animals exhibited increases in latencies after they had been exposed to the plate apparatus. An increase in latencies would be expected if, following the attenuation of novelty/stress-induced analgesia, naloxone caused increased recuitment of the EPCS by increasing the perceived intensity of nociceptive stimulation. No such increases were observed in any of the other experiments, and it is worth remembering that in these

experiments the animals were not previously familiarized with the testing procedure.

Experiments 4-9 also demonstrate that the analgesic potency of morphine varies as a function of the degree to which the EPCS is activated. First, an enhancement of morphine analgesia was observed when naloxone administration potentiated the development of conditioned autoanalgesia. Alternatively, morphine's analgesic potency was reduced when nociceptive testing was conducted under conditions where the activation of the EPCS was attenuated. It was argued previously that one of the conditions responsible for the attenuated recruitment of the EPCS is when the development of conditioned autoanalgesia is inhibited. Experiments 4-9 suggest two other conditions where attenuated recruitment of the EPCS may occur. The first is when the ability of a stressor to recruit the EPCS is diminished, as, for example, when habituation to novelty/stress-induced analgesia occurs. As noted above, Experiment 8 demonstrated that the analgesic effect of morphine was reduced in animals exposed to the cold-plate. The second condition is when a stressor activates an endogenous pain control substrate that in itself displays tolerance. This conclusion is supported by the results of Experiment 9, where it was seen that animals that showed tolerance to the opiate-mediated analgesia produced by exposure to prolonged shock had shorter latencies when tested under morphine than animals that had received either no shock or brief shock.

A final issue that requires discussion concerns the nature of the substrate mediating the analgesia observed in the present experiments. Several features of the present results suggest that the analgesia that

is evident in animals given nociceptive tests under the influence of naloxone is mediated by a non-opiate mechanism. In itself, the fact that the magnitude of the analgesia is greatest in animals treated with high doses of naloxone makes it unlikely that it is opiate-mediated. More important, however, are the results of Experiment 9, where it was shown that when the shock parameters were manipulated in a way known to elicit primarily non-opiate-mediated analgesia, naloxone-treated animals displayed effects similar to those seen in the previous experiments.

It is important to point out here, however, that although the analgesia observed in animals tested under naloxone is non-opiate mediated, this does not imply that the opiate pain suppression mechanism cannot be recruited. Under naloxone, the effects of the recruitment of the opiate mechanism are merely blocked. Recognition of this possibility is important for an understanding of the nature of the mechanism controlling the conditioned analgesia shown in these experiments. The results of the present experiments, and those of several others (Devries et al., 1979; Fanselow, 1984; Fanselow & Baackes, 1982; Fansleow & Bolles, 1979; Oliverio & Castellano, 1982; Sherman et al., 1984; Watkins et al., 1982) suggest that the conditioned autoanalgesia observed is, at least in part, opiate mediated. In Experiment 4, for example, when tests for conditioning were given under morphine, conditioned autoanalgesia was evident in animals previously administered 0.5 and 2.0 mg/kg, even though at these doses there was little evidence for non-opiate-mediated analgesia when animals were tested under naloxone.

There is, then, an apparent discrepancy between the source of the analgesia observed in animals given nociceptive tests under naloxone and the analgesia apparent in tests for conditioned autoanalgesia, a finding that appears difficult to explain (see also Watkins et al., 1982). But recall the point made earlier that naloxone would block the expression of analgesia by the opiate component but not necessarily the recruitment of the system. It is well documented that Pavlovian conditioning occurs when the expression of the unconditioned response is prevented, all that is required is that the response be recruited (see Eikelboom & Stewart, 1982; Mackintosh, 1974; pp. 79-80).

The idea that the analgesic effect observed during opiate receptor blockade might be non-opiate mediated and the conditioned autoanalgesia, at least in part, opiate-mediated, could be further accomodated by the assumption that collateral inhibition exists between the opiate and non-opiate components of the EPCS (Akil & Watson, 1980; Kirchgessner, Bodnar & Pasternak, 1982). According to this hypothesis, activation of one of the components inhibits activation of the other. Kirchgessner et al. (1982) have provided evidence consistent with this hypothesis, showing that intracerebroventricular administration of naloxazone antagonizes morphine-induced analgesia, but potentiates the non-opiate-mediated analgesia produced by cold water swims. Conversely, administration of D-phenylalanine, an anti-enkephalinase, potentiates morphine analgesia (Alleva, Castellano & Oliverio, 1980) but antagonizes cold-water-swim-induced analgesia (Bodnar, Lattner & Wallace, 1980).

The collateral inhibition model can account for the results of the

present experiments with the assumption that both components of the EPCS are normally activated by nociceptive tests such as those used in the present experiments. Under naloxone, however, the effectiveness of the opiate component is reduced and the non-opiate component released from inhibition by the opiate component (see Girardot & Holloway, 1984, for similar reasoning). Thus, the analgesia observed under naloxone treatment would be mediated primarily by the non-opiate component. Note, again, however, that although naloxone administration might release inhibition of the non-opiate component, there is no reason to assume that it should interfere with the activation of the opiate component. Thus, when animals are tested under saline or morphine, the test environment could conditionally activate the opiate component, resulting in the opiate-mediated properties of the observed conditioned analgesia.

This reasoning may also account for why exposure to shock confined to the hind paws, which elicits a primarily non-opiate-mediated analgesia, appears to give rise to a conditioned opiate-mediated analgesia (Watkins et al., 1982; see also Experiment 9 of the present manuscript) In this instance, exposure to hind-paw shock would activate the non-opiate component more strongly than the opiate one. Thus, the degree of inhibition of the opiate component by the non-opiate component would be stronger than the inhibition of the non-opiate component produced by the opiate component, resulting in a predominantly non-opiate-mediated analgesia. The finding that prior naloxone administration enhances the analgesia produced by exposure to brief shock (Experiment 9) is also consistent, since, as argued above,

naloxone administration would block the opiate-mediated inhibition of the non-opiate component, resulting in the potentiation of the non-opiate-mediated analgesia.

The validity of the collateral inhibition explanation of the present results rests upon the critical assumption that the testing procedures employed in these experiments activate, perhaps in addition to the non-opiate mechanism, the opiate antinociception mechanism. As noted above, there appear to be two factors that elicit the analgesia observed in naloxone-tested animals; novelty-induced stress and exposure to nociceptive stimulation. In order to validate the collateral inhibition hypothesis, therefore, it would be necessary to demonstrate that both these factors can activate the opiate mechanism.

Bardo and Hughes (1979) have reported that the analgesia elicited by exposure to a novel hot-plate apparatus was not reversed by naloxone administration. Tiffany et al. (1984) similarly observed a lack of effect of naloxone administration on the analgesia elicited by exposure to a bright, noisy environment. These results imply a non-opiate mediation of environmental novelty/stress-induced analgesia. A problem common to both these studies, however, is that naloxone was administered after the animals were exposed to the stress-inducing environment. It has been shown that naloxone can prevent stress-induced analgesia, but the drug cannot reverse the effect once it has been elicited (Watkins & Mayer, 1982). More direct evidence for the non-opiate mediation of environmental novelty/stress-induced analgesia was provided by Tiffany et al. (1984) who showed that the analgesia elicited by a stressful environment is not correlated with increased

levels of central met- or leu-enkephalin. On the other hand, Tiffany et al. report that such analgesia displays cross-tolerance with morphine. Moreover, in Experiment 8 of the present investigation, animals preexposed to the cold plate displayed less analgesia than non-exposed animals when tested for analgesia under morphine. These latter two findings suggest that the opiate component may be involved in the mediation of environmental novelty/stress-induced analgesia. Thus, although current evidence favors the conclusion that novelty stress elicits a non-opiate-mediated analgesia, there are some data which are consistent with the argument that the opiate mechanism may be involved.

Evidence concerning the substrate activated by nociceptive stimulation favors the idea that the testing procedures used in these experiments activate the opiate component. The experiments of Lewis et al. (1980; 1981) would appear to suggest that brief exposure to nociceptive stimulation activates the non-opiate substrate, while more prolonged exposure elicits an opiate-mediated analgesia. If this is the case, the brevity of the nociceptive stimulation employed in the present studies would force the conclusion that the substrate normally activated by the testing regimen is non-opiate. More recent evidence, however, has challenged the hypothesis that the temporal characteristics of nociceptive stimulation constitute the critical factor controlling which component is activated. Rather, it now appears that it is the severity of nociceptive stimulation which is crucial (Bolles & Fanselow, 1982; Fanselow, 1982). Low severity stimulation activates the opiate substrate, while more intense levels activate the non-opiate substrate. According to this argument, then,

brief shock elicits non-opiate mediated analgesia because it represents a more severe form of nociceptive stimulation than prolonged shock (Fanselow, 1984). Terman et al. (1984) have recently reported evidence consistent with this argument. They report that administration of 2.5 mA footshock for one to two minutes elicits a naltrexone-reversible analgesia, while the analgesia induced by more severe shock (2.5 mA for three to five minutes) is naltrexone-insensitive. Furthermore, they demonstrated that by holding the duration of shock constant, they could induce differentially-mediated analgesia by manipulating current intensity. Low intensity shock (1-2 mA) elicited opiate-mediated analgesia; higher intensities (3-4 mA) produced non-opiate analgesia (see also Fanselow, 1984). On the basis of these latter results, therefore, it is possible that the nociceptive stimulation employed in the present studies would activate the opiate substrate. With the exception of Experiment 9, the levels of nociceptive stimulation employed were of a relatively low intensity.

Conclusion

The results of the experiments reported in this thesis are consistent with the hypothesis that the development of environment-specific tolerance to the analgesic effect of morphine will covary, at least in part, with the degree to which the EPCS is activated during analgesia testing. First, the development of tolerance will appear maximal in those circumstances where the activation of the EPCS is inhibited. This was supported by the results of Experiments 1-3, where it was shown that the analgesic potency of morphine was reduced when the development of conditioned autoanalgesia was inhibited. Second, morphine will exert its strongest effect when the EPCS is activated in collaboration with morphine administration. Experiments 4-9 demonstrated that the administration of naloxone prior to exposure to nociceptive stimulation enhanced the development of conditioned autoanalgesia, and that the enhanced conditioned autoanalgesia synergized with morphine-induced analgesia to produce a stronger effect (see also Sherman et al., 1984).

It would appear that the hypothesis summarized above provides the best interpretation of these results. Neither Siegel's (1975; 1976; 1977) compensatory response hypothesis, Solomon's (1977; 1980) opponent process model, Bardo and Hughes's (1979) novelty hypothesis, nor Baker and Tiffany's (1985) habituation model can account for all of the results reported in this thesis. For example, in Experiments 1-3 there were no differences in latencies between morphine- and saline-treated animals that were not exposed to nociceptive stimulation when tests for analgesia were conducted under morphine or saline. According to the

four hypotheses, exposure to nociceptive stimulation is not presumed to influence the development of tolerance. All that is required is that animals experience morphine in a distinctive environment (e.g., Siegel, Solomon, Tiffany and Baker) or become familiarized with the analgesia testing procedure (Bardo and Hughes). In Experiments 1-3, however, the degree of experience with both morphine in the test environment and the analgesia testing procedure was identical for animals exposed and not exposed to nociceptive stimulation, yet tolerance was only observed when animals were exposed to nociceptive stimulation. Thus, these results support the hypothesis that morphine administration attenuated the development of conditioned autoanalgesia, which resulted in a lowered analgesic effect of morphine.

It is possible that Siegel's, Solomon's and Tiffany and Baker's hypotheses can account for the results of Experiments 4-9; because in this case naloxone potentiated the development of conditioned autoanalgesia in the absence of nociceptive stimulation (e.g., Experiment 7). Thus, these results are consistent with the possibility that that the environment in which naloxone was administered elicited a conditioned analgesic response that counteracted the unconditioned hyperalgesic effect of naloxone (Siegel and Solomon) or that tolerance to the hyperalgesic effect of naloxone developed via associatively primed habituation (Baker and Tiffany). The problem with these interpretations is that there was no evidence for naloxone-induced hyperalgesia (It was noted before that the the hypothesis presented in this thesis may be able to account for the absence of evidence for hyperalgesia). Secondly, the habituation model proposed by Baker and

Tiffany cannot account for the enhancement of conditioned autoanalgesia resulting from naloxone administration. According to this model, the only effect that should have been observed was tolerance to the unconditioned hyperalgesic effect. The model does not provide a mechanism that would account for the analgesia-inducing effect of naloxone administration. Finally, it is not evident that the compensatory or opponent response interpretations provide anything more than a description of the results obtained following naloxone administration, since they, too, fail to identify a mechanism through which naloxone-induced analgesia can occur. The advantage of the present hypothesis is that it does specify, at least tentatively, a mechanism through which naloxone can exert the effects obtained in Experiments 4-9.

Limitations of the Hypothesis: Although it is likely that the development of environment-specific tolerance is in part dependent upon the degree to which the EPCS is activated during analgesia testing, it is not clear that this hypothesis can account for all of the manipulations that have been shown to affect the rate of tolerance development. For example, the available literature suggests that as the dose of morphine administered is increased, the degree of tolerance attributable to the environment-drug contingency is reduced (for review, see Baker & Tiffany, 1985; Kesner & Baker, 1981). Assuming that larger doses of morphine would inhibit the activation of the EPCS to a greater extent than low doses, the present hypothesis would have to predict that large doses should result in a potentiation of the rate of tolerance development. One possibility is that large doses of

morphine may produce an unconditioned hyperalgesic effect by activating sites that smaller doses would not (Jacquet & Lajtha, 1973; but cf. Kayan et al., 1971), thereby resulting in an increased activation of the EPCS. Alternatively, it is possible that large doses of morphine may exert their effects not on the activation of the EPCS, but rather on the effectiveness of the EPCS once activated. For example, high doses may result in a lowered availability of the receptor for either exogenous or endogenous opiate agonists. A large dose of morphine would be expected to occupy a greater number of opiate receptors for a longer period of time, thereby reducing the number of available receptors for endogenous opiates or a second, subsequent administration of morphine (cf. Axelrod, 1956; Seevers, 1958).

Secondly, it remains to be determined whether the hypothesis can accomodate demonstrations of dispositional tolerance. For example, Tiffany and Baker (1981) reported that tolerance to the analgesic effect of morphine developed in animals administered morphine in the home cage. The animals in this study, however, all received extensive experience with the analgesia testing procedure prior to the induction of tolerance. Thus, it would appear that these results are attributable to the diminution of stress-induced analgesia. In this regard it is noteworthy that several investigators have failed to observe evidence for dispositional tolerance in animals naive with regard to the test procedure (Kayan & Mitchell, 1972; Siegel, 1975; 1976; Siegel et al., 1978). In fact, Kayan and Mitchell (1972) have shown that a single administration of morphine results in significant tolerance to a subsequent administration if the first administration

was followed by exposure to the hot-plate, but no tolerance developed if the first administration occurred in the absence of exposure to the hot-plate (see also Advocat, 1981).

It may be, therefore, that dispositional tolerance reflects the attenuation of stress-induced analgesia in animals familiarized with the testing procedure. This possibility would be consistent with the hypothesis that the development of tolerance is in part dependent upon the degree to which the EPCS is activated. The attenuation of stress-induced analgesia would lead to a lowered activation of the EPCS, which in turn would result in a lowered analgesic effect when tests for analgesia are conducted under the influence of morphine.

Footnotes

[1] This argument should not be considered as having been experimentally verified. In fact, the available data suggest that, at least for morphine, the argument is not correct. The pyretic response elicited by a CS paired with low doses of morphine typically mimics the hyperthermic UCR elicited by morphine (Eikelboom & Stewart, 1979; Sherman, 1979; see also Baker & Tiffany, 1985; Eikelboom & Stewart, 1982; for reviews). The only implication to be drawn from the above argument is that, at least intuitively, the logic of homeostasis is applicable to the conditioning of pyretic responses.

[2] The fact that Sherman et al. obtained evidence for conditioned autoanalgesia under saline using a hotter plate temperature than that employed in the present study would appear to contradict the argument that the absence of morphine-attenuation of conditioned autoanalgesia on saline test days 2 and 3 is attributable to a ceiling effect. However, the method employed by Sherman et al. to heat their hot-plate differed from that employed here. It may be, therefore, that the plate temperatures are not directly comparable. In addition, it could be argued that the procedures employed by Sherman et al. promoted more robust autoanalgesia than the procedures employed here; they exposed their animals to nine days of shock, whereas only four were employed in the present experiment. In addition, in the present experiment the conditioning days were interspersed with analgesia test days, whereas Sherman et al. shocked their animals on consecutive days before assessing conditioned autoanalgesia.

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