PAVLOVIAN CONDITIONING AND TOLERANCE TO ANALGESIA PRODUCED BY ELECTRICAL STIMULATION IN THE BRAINSTEM

by

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ABSTRACT

Pavlovian Conditioning and Tolerance to Analgesia Produced by Electrical Stimulation in the Brainstem

by

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Stimulation of the periaqueductal gray (PAG) of the midbrain produces an analgesia that resembles opiate analgesia. There is considerable behavioral, pharmacological and physiological evidence to suggest a common underlying mechanism for stimulation produced analgesia (SPA) and opiate analgesia. Tolerance to the analgesic effect of opiate drugs has been shown to be subject to the laws of classical conditioning. Specifically, certain aspects of tolerance to opiates have been related to a compensatory physiological response elicited by cues that reliably predict the drug effect. Given the apparent similarity between SPA and opiate analgesia, the present experiments test the hypothesis that tolerance to SPA is also subject to the laws of classical conditioning and show a compensatory physiological response.

Experiment 1 sought to determine if tolerance to SPA can be extinguished. Electrical stimulation of the PAG was delivered for 5 min per day in the presence of distinctive environmental cues. Brain-stimulation increased tail-flick latencies, and tolerance developed over seven daily sessions. Half of the rats
were then given 12 extinction trials which consisted of exposure to the environmental cues, without brain-stimulation. The remaining subjects stayed in their home cages throughout this period. When tested again with brain-stimulation in the experimental environment, animals in the extinction group displayed significant SPA. The home cage group remained tolerant to the PAG stimulation. Reacquisition of tolerance was observed in the extinction group.

Experiment 2 was designed to determine if tolerance to SPA is context specific. Half of the animals were stimulated in a distinctive experimental environment. The remaining animals were stimulated in their colony room. Stimulation of the PAG produced strong analgesia, with tolerance developing over seven daily sessions. On test days all animals were stimulated in the colony room. Animals that developed tolerance in the experimental environment showed significant analgesia when stimulated in the colony room. Animals given an eighth consecutive stimulation session in the colony room remained tolerant to the stimulation.

Experiment 3 examined the hypothesis that tolerance to SPA should be accompanied by the development of a hyperalgesic response to environmental cues. PAG stimulation produced strong analgesia with complete tolerance developing over seven daily sessions. A nonstimulated control group was given daily tail-flick tests and showed a slightly increasing trend in latencies. On the test day half of the stimulated animals were tested without brain-stimulation and a hyperalgesic response was observed. The remaining stimulated rats received an additional
stimulation session and did not show a decreased tail-flick latency.

These results extend the generality of the conditioning model of tolerance to the tolerance that develops to SPA. The SPA paradigm may prove to be a valuable tool for examining physiological substrates of conditioned tolerance.

Dr. Anthony G. Phillips
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INTRODUCTION

Drug tolerance may be defined as a decrease in the pharmacological effect of a given dose of a drug after successive administrations, or to the need for progressively larger doses of a drug to produce the same effects on successive administrations. The usefulness of a particular pharmacological agent is often limited by its relative ability to develop tolerance. Concurrent with the development of tolerance is development of the related phenomenon of dependence. Dependence refers to an altered biological state which requires repeated administration of a drug in order to avoid a characteristic symptom complex known as abstainance syndrome. There is some controversy surrounding the issue of whether tolerance and dependance are manifestations of the same phenomenon. Clearly, the efficacy of a drug as a therapeutic tool should not include perpetual dosage increases. An understanding of the determinants of tolerance could lead to more effective pharmacological treatment regimens as well as increasing our knowledge of neural mechanisms of drug action.

Theories of Drug Tolerance

Despite decades of intense research there is little agreement on the possible mechanisms of drug tolerance. Theories of tolerance may be roughly divided into two categories: physiological theories and behavioral theories (Siegel, 1975). The first category, stresses the direct action of the drug on the biological system. Specifically, this category contains theories that account for tolerance through a mechanism that changes the
reaction of the receptor to the drug, or restricts access of the

drug to the receptor. This view sees the binding of the drug to
the receptor as the critical event. It ignores the fact that the
effect of the molecule-receptor interaction is superimposed on
the normal activity of the nervous system, a system defined by
its plasticity. However, in these theories the nervous system is
treated as a constant.

The second category, the learning theories, contains model
systems that stress the plasticity of the nervous system in its
reaction to the drug-receptor interaction. These theories
emphasize the importance of learned associations to tolerance
development. Although learning theories of tolerance stress the
system on which the action of the drug is superimposed, they do
not exclude the possibility of an important interaction with the
receptor complex.

Despite the divergence of postulated mechanisms, there is
general agreement on those phenomena that should be explained by
a valid theory of tolerance (Hug, 1972). The critical
characteristics are listed below.

**Tolerance Development.** Measurable tolerance can be seen
with as little as one administration of morphine (Kornetsky &
Bain, 1968). After gradual dosage increments, subjects have been
known to tolerate doses hundreds of times the original LD-50
(Seevers & Deneau, 1963; Seevers & Woods, 1953).

**Cross Tolerance.** Subjects tolerant to one drug are tolerant
to pharmacologically similar agents. Occasionally, subjects may
be cross tolerant to dissimilar drugs with similar
**pharmacological effects.**

**Persistence of Tolerance.** Tolerance to morphine has been shown to last up to one year without intervening administrations (Cochin & Kornetsky, 1964; Kornetsky & Bain, 1968).

Theories designed to explain one of these characteristics tend to have trouble with the others. A valid theory must explain all of these phenomena.

**Physiological Theories**

Although all physiological theories of tolerance cannot be listed here, a few are described to give the reader a feeling for the category.

**Metabolic Theories.** Theories of tolerance classified as metabolic theories contend that the relevant effect of the drug is to increase the rate at which the drug is eliminated from the system, thereby reducing availability of the drug to bind to receptors. Metabolic change is supported by differences in distribution of radioactively labeled morphine within the CNS of tolerant and nontolerant dogs and rats (Mule, 1965; Mule, Redman & Flesher, 1967; Mule & Woods, 1962; Mule, Woods & Mellett, 1962). However, these authors point out that the magnitude of differences seen are not sufficient to explain tolerance.

**Immunological theory.** Cochin and Kornetsky (1964) have proposed that tolerance resembles an immune phenomenon, with the drug as an antigen. This theory was conceived to explain the long-lasting nature of tolerance. Serum and tissue passive transfer experiments, in which extracts from tolerant animals are injected into nontolerant animals, have been inconclusive, with
some studies reporting a decrease in sensitivity to morphine (Ungar & Cohen, 1966; Tulunay, Kiran & Kaymakcalan, 1970) while others report no change (Smith & Takemori, 1968) or an increase in sensitivity (Kornetsky & Kiplinger, 1963; Kiplinger & Clift, 1964). At present we can only speculate on the role an immune reaction may play in the induction of tolerance.

**Receptor Modification Theories.** Several theories of drug tolerance propose that the relevant action of the drug is to change the number, structure or sensitivity of the receptors. Collier (1965), for example, contends that drug experience induces the formation of "silent receptors". The drug will then bind to these receptors, leaving less of the drug to bind to "pharmacological" receptors. Binding studies employing radioactive ligands have shown an increase in number of binding sites with drug experience. However, antagonists also increase the number of receptors at much lower doses (Pert, Pasternac & Snyder, 1973) but, of course, do not induce tolerance.

A more recent theory proposes that the binding of the drug molecule to the receptor causes a structural change in the receptor (Tulunay & Takemori, 1974; Snyder & Matthysse, 1975 pp. 65-67). The effect of this change is to increase the affinity of the receptor to as yet unisolated endogenous drug antagonists. Tulunay & Takemori base their theory on shifts in pA2, a reputed comparison of affinity between an agonist and antagonist, after exposure to morphine. In vivo, the definition of pA2 is the negative logarithm of the molar dose of the injected antagonist that reduces the effect of a double dose (i.e. 2 x ED-50) to that
of a single in cerebrospinal fluid of rats, but this substance has yet to be isolated. The use of the pA2 method for theoretical formulation underlines the flaw in physiological theories of tolerance. A critical, implicit assumption of the method, as it is applied in vivo, is that all change takes place at the agonist receptor superimposed on a static system. Again, there is no a priori basis for this assumption.

Learning Theories of Tolerance.

The idea that learning may play an important role in the development of drug dependence has existed for decades. Himmelsbach (1943) first suggested that dependence is conditioned and set dissociation of reward from analgesia and euphoria as a research goal. Wikler and his associates favored a learning theory of dependence in trying to explain many clinical observations, such as high relapse rates and occurrence of abstinence symptoms months after detoxification (Wikler, 1963, 1965, 1972, 1977; Wikler & Pescor, 1967; Wikler, Pescor, Miller & Norell, 1971). These theoreticians viewed dependence and tolerance as separate but related phenomena. When they are seen as manifestations of the same phenomenon the extension to a role for learning in the development of tolerance is direct.

Considerable indirect evidence shows a parallel between learning and development of tolerance. Electroconvulsive shock (Kesner, Priano & DeWitt, 1976; Stolerman, Bunker, Johnson, Jarvik, Krivoy & Zimmerman, 1976), 6-hydroxydopamine lesions (Ayhan, 1972; Blasig, Herz & Gramsch, 1975), and administration of protein synthesis inhibitors (Cohen, Keats, Krivoy & Ungar,
1965; Cox & Ginsberg, 1969; Cox & Osman, 1970; LeBlanc, Matsunga & Kalat, 1976) have all been shown to retard tolerance as well as various learning tasks. Three prominent theories have claimed a role for learning in drug tolerance.

Behavioral Tolerance. Dews (1962) proposed that many of the debilitating effects of a drug could be counteracted by operantly conditioned behavioral patterns. In operant conditioning, performance of a response leads to reinforcement or punishment, thereby increasing or decreasing the likelihood that the response will be performed again (Mackintosh, 1974). In this instance, the observed reduction in drug effect is seen as a consequence of differential reinforcement of more effective patterns of behavior. Dews gives as an example the slowed speech and widened gait of the chronic alcoholic as compensating for the motor effects of alcohol. Experimentally, this phenomenon is seen as an improvement in performance of ethanol-intoxicated rats on a treadmill, only when the animals are allowed to run while intoxicated. A control group, which ran before intoxication, showed less or no tolerance to the ethanol on subsequent tests (Wenger, Tiffany, Bombardier, Nicholls & Woods, 1981; Kalant, LeBlanc & Gibbins, 1976). Dews called this behavioral compensation "behavioral tolerance". Because tolerance is contingent upon performance of the task, this phenomenon has come to be known as contingent tolerance.

This theory seems to account for improved performance on some well-integrated motor tasks, but cannot be an explanation of tolerance to physiological effects, such as tachycardia and
hypothermia, or to analgesia as measured by spinally mediated reflexes, or psychological effects such as drug-induced euphoria.

**Behaviorally Augmented Tolerance.** In a modification of this theory, LeBlanc and his co-workers (LeBlanc, Kalant & Gibbins, 1973) have combined physiological theories with contingent tolerance. They proposed that the function of learning in tolerance development is to accelerate acquisition. As evidence, LeBlanc et al. (1973) showed in the treadmill paradigm that subjects performing the task while intoxicated acquired tolerance faster than controls, but the ultimate degree of tolerance to the motor effects of ethanol is identical for both the experimental and control subjects. They concluded that performance of a behavior may influence the rate of adaptation to a drug, but not the extent of adaptation, and referred to this phenomenon as behaviorally augmented tolerance.

**Conditioning Model of Tolerance.** Recently, attention has focussed on the role that classical conditioning may play in the development of tolerance to certain drug effects. In the classical conditioning experiment, stimuli are presented to the organism without regard to its behavior. A contingency is set up so that one stimulus (conditional stimulus, CS) reliably predicts the occurrence of the second stimulus (unconditional stimulus, UCS). The UCS is selected because it elicits a relevant response, termed the unconditional response (UCR). In Pavlov's (1927) famous experiments, meat paste UCS was chosen because it reliably elicited salivation, the UCR. Pavlov's bell CS does not elicit salivation, prior to pairing with the UCR. The CS comes
to elicit the response, now termed the conditional response (CR), as a function of the CS-UCS pairing. To complete the example, after several pairings of the bell CS and meat paste UCS the bell, by itself, will now elicit salivation.

Siegel (1975) has proposed that narcotic tolerance is the result of learning an association between the systemic effects of the drug and environmental cues that reliably precede these systemic effects. In Siegel’s original model, the drug is designated as the UCS, the effects of the drug are the UCR, the environmental cues are the CS, and a homeostatic compensatory mechanism, opposite in direction to the UCR, is the CR. This homeostatic CR summates with the effect of the drug to counteract its effect. This reduced drug effect is, by definition, tolerance.

A recent interpretation, which brings this theory in line with traditional Pavlovian theory, differentiates between drugs that act on efferent and afferent arms of the nervous system. Drugs that produce tolerance are seen as acting on the efferent arm of a system with negative feedback. Since the direct effect of the drug would not be processed by the central nervous system, the drug cannot be a stimulus. In this case, the drug effect would be the UCS with the organism’s homeostatic reaction to this stimulus as the UCR (Eikelboom & Stewart, 1982). The environmental cues and the homeostatic compensatory mechanism remain as the CS and CR, respectively. As the environmental cues are paired consistently with the drug effect, the cues gain the ability to elicit the compensatory mechanism to counteract the
expected drug effect.

Morphine is considered to be the prototype drug in tolerance studies, primarily because of its clinical relevance (Seevers & Deneau, 1963). Primary support for this Pavlovian model comes from experiments employing the analgesic effect of morphine as a dependent measure. Three lines of direct evidence suggest that Pavlovian conditioning may account for at least some instances of tolerance.

The first line of evidence is the demonstration that conditioned environmental cues presented with a saline placebo can elicit the homeostatic compensatory mechanism. In other words, following pairings, the CS by itself elicits the CR. With morphine tolerant rats this is seen as a hyperalgesic response after presentation of conditional environmental cues (Siegel, 1975). Although a conditional compensatory response has also been shown for the hypothermic effect of alcohol, i.e. hyperthermia, (Crowell, Hinson & Siegel, 1981; Le, Poulos & Cappell, 1979; Mansfield & Cunningham, 1980), Siegel's demonstration with morphine has not been replicated outside his laboratory (Tiffany, Petrie, Baker & Dahl, 1983).

The second line of evidence is the demonstration of context specificity of morphine tolerance. Animals made tolerant to the analgesic effect of morphine give a relatively nontolerant response when tested in a distinct environment. In other words, there is a generalization decrement between the training CS and the dissimilar test CS (Advokat, 1980; Siegel, 1975, 1976). Context specificity of tolerance has also been shown for morphine
induced hyperactivity (Mucha, Volkovskis & Kalant, 1981) and for ethanol’s hypothermic effect (Crowell et al., 1981, Le et al., 1979).

A third line of experiments demonstrate that Pavlovian procedures which normally retard acquisition or maintenance of the CR also retard the acquisition or maintenance of tolerance. Specifically, unpaired presentation of the CS prior to (latent inhibition), or intermittent with (partial reinforcement) CS-UCS pairings will significantly attenuate the rate at which tolerance is acquired (Siegel, 1977). In addition an explicit unpairing of the CS and UCS, that is, presenting both the CS and UCS, but never together, prior to CS-UCS pairing, retards tolerance acquisition to a greater extent than a latent inhibition procedure (Siegel, 1981). Several presentations of the environmental CS without a subsequent analgesic UCS will attenuate an established tolerance. In other words, tolerance to morphine’s analgesic effect can be extinguished (Siegel, 1975, 1976). Animals not receiving these extinction trials over the same time period remain tolerant to the morphine. This extinction effect has also been demonstrated for amphetamine induced anorexia (Poulos, Wilkinson & Cappell, 1981) and for ethanol’s hypothermic effect (Crowell et al., 1981). Theories that consider only the interaction of the drug molecule with the receptor are insufficient to explain the influence that the environmental cues seem to have upon tolerance. Physiological theories of tolerance would predict that equal exposure to the pharmacological agent should produce equal levels of tolerance. The conditioning
theory of tolerance, on the other hand, views the association of predictive cues with the drug effects as a critical event. Obviously, if Pavlovian processes are at play in the development of tolerance, then the "background" activity as well as the activity at the receptor must be taken into consideration in any valid theory of tolerance development.

The conditioning theory of tolerance easily explains the characteristics of tolerance when other theories fail. Because learning represents a relatively permanent change, persistence of tolerance is expected. Administration of diverse agents may be accompanied by similar cues (e.g. identical administration procedures). The homeostatic CR elicited by these cues in tolerant subjects would counteract drugs that may operate through a different mechanism, but have similar effects (i.e. cross tolerance). Because tolerance development seems to follow a learning curve, rate of development is easily explained (Kalant, Poulos & Cappell, 1978; Siegel, in press). There are, however, several phenomena that associative theories of tolerance have yet to explain. First, tolerance has been demonstrated in an ileum excised from morphine-tolerant guinea pigs (Herz, Ziegglansberger, Schulz, Fry & Satoh, 1976). Clearly, environmental influences on this preparation should be minimal. Second, learning theories have difficulty explaining the tolerance of neonates born to drug-tolerant mothers (Siegel, 1978; in press).

With the variety of modes of action of drugs and numerous effects of any particular drug, it is quite likely that no mechanism of drug tolerance is ubiquitous. Separate mechanisms,
both associative and nonassociative, may be responsible for different drug effects (Siegel, in press). In addition, multiple mechanisms may synergize to produce the observed tolerance to any one effect (LeBlanc et. al., 1973).

Recent advances in histological and physiological techniques are now being applied to the study of physiological mechanisms of drug tolerance. However, paradigms used thus far have proved insufficient for studying the physiological substrates of conditioned tolerance (Advokat, 1980). It would be of benefit to explore the substrates of each of the reputed mechanisms.

PROBLEMS WITH MULTIPLE EFFECTS OF DRUGS.

Interpretation of pharmacological experiments in general and tolerance studies in particular has been notoriously difficult, and this is due primarily to the multiple effects of most drugs (Dews, 1962). Peripheral administration of morphine in humans, for example, affects respiratory and pulse rate, vasculature dilation, gastrointestinal motility, hormonal function, fatigue, and activity level, as well as having an analgesic effect (Isbell & White, 1953). Interaction of these many effects of morphine makes it difficult to focus on a particular dependent measure.

Distribution of opioid receptors in the central nervous system and peripheral systems is as varied as the effects of opiate ligands. In-vitro radiolabeled binding and autoradiographic techniques (Atweh & Kuhar, 1977a, 1977b, 1977c; Pearson, Brandeis, Simon & Hiller, 1980; Pert, Kuhar & Snyder, 1976) have located opioid receptors in specific loci from the olfactory bulbs to the spinal cord. Atweh and Kuhar (1983) have
correlated the distribution of opioid receptors in the brain and spinal cord with the multiple effects of opiate administration. For example, microinjection of morphine into the striatum produces the muscular rigidity that high doses of morphine are known to produce. But still, overlap exists: The analgesic effect of morphine is influenced by opioid receptors in the dorsal horn of the spinal cord, substantia gelatinosa of the trigeminal nerve, medial thalamic nuclei, intrathalamic nuclei and periaqueductal gray matter. As pointed out by Dole and his colleagues, the study of tolerance to opiates poses a particularly perplexing problem as many of the multiple responses to the drug are difficult to measure in an isolated system (Snyder & Matthisse, 1975 pp. 65-67). Without an isolated system all results must be interpreted within the context of possible interaction of multiple effects. Two solutions to this problem are evident.

One method of isolating an effect is through local intracranial microinjection of the ligand. The effect of this procedure is to allow only a specific receptor field access to the drug. Since specific loci are correlated with specific actions of opiates, the injection will affect a limited number of systems, thereby limiting the effects of the drug. However, the multiple injections needed for tolerance research are known to produce tissue damage at the injection site. A pilot study using this method proved difficult to interpret due to this damage (Paul & MacCrea, unpublished data). An attempt to extinguish a conditioned tolerance to intracranial microinjection of morphine
into the PAG, resulted in a nonsignificant trend towards successful extinction. It was felt that the damage caused by the repeated injections had lesioned the descending inhibitory mechanism, making it impossible to reproduce the profound effect seen with peripheral injections. This procedure is now being refined by MacCrea and Siegel (personal communication, 1982).

A second possibility involves direct electrical stimulation of the neurons involved in the drug mediated effect.

**Stimulation Produced Analgesia**

Electrical stimulation of the periaqueductal gray (PAG) in the midbrain of rat, (Mayer, Wolfle, Akil, Carder & Liebeskind, 1971; Reynolds, 1969), cat (Liebeskind, Guilbaud, Besson & Oliveras, 1973), monkey (Goodman & Holcombe, 1975; Ruda, Hayes, Dubner & Price, 1976), and man (Hosobuchi, Adam & Linchitz, 1978; Hosobuchi, Rossier, Bloom & Guillemin 1979; Richardson & Akil, 1977) has been shown to produce significant levels of analgesia. Mayer and his colleagues (Mayer & Liebeskind, 1974; Mayer & Price 1976; Watkins & Mayer, 1982) have claimed that there is sufficient evidence to propose a common substrate for stimulation produced analgesia (SPA) and morphine analgesia.

In order to facilitate the relation of SPA to morphine analgesia, a brief overview of the anatomy of the central systems involved in pain perception and opioid analgesia will be given.

**Ascending Pathways.**

**Spinothalamic tract.** The spinothalamic tract has been recognised as a primary pathway of nociceptive information for
over a century (Willis, 1976). Neurons of this tract arise from the dorsal horn of the spinal cord (Giesler, Menetrey, Guilbaud & Besson, 1976), and, after crossing to the contralateral cord, course through the anterolateral quadrant of the spinal white matter (Fig. 1). Studies based on anterograde labeling with horseradish peroxidase have shown these neurons to project to the ventrobasal, medial, and posterior thalamus (Giesler, Basbaum & Menetrey, 1977). Lesions anywhere along the course of the spinothalamic tract may result in deficits in pain perception contralateral and posterior to the lesion (Cassinari & Pagni, 1969; Noordenbosi & Wall, 1976; Spiller & Martin, 1921).

**Spinoreticular Pathway.** Recently a great deal of attention has been given to an important alternative pathway for nociceptive information: the spinoreticular system. Like the spinothalamic tract, neurons of this pathway arise in the dorsal horn, cross, and ascend in the anterolateral funiculi. However, these neurons project to various midbrain structures, particularly the tectum and the PAG (cf. Fields & Basbaum, 1978). Because these brain loci have projections to the limbic system, it has been hypothesized that the spinoreticular pathway carries information related to the aversive-motivational aspects of pain whereas the spinothalamic neurons relay sensory-discriminative information (Melzak & Wall, 1965). Recent evidence suggests that a vast majority of the cells of the anterolateral funiculi project to the midbrain rather than the thalamus (Kelly, 1982).
Figure 1. Afferent pathways involved in nociception and efferent pathways involved in modulation of pain perception (ALF, anterior lateral funiculus; DC, dorsal column; DCN, dorsal column nucleus; DLF, dorsolateral funiculus; ML, medial lemniscus; NI, primary nociceptive sensory neurons; NRM, nucleus raphe magnus; SR, spinoreticular pathway; ST, spinothalamic pathway; T, thalamus).
**Dorsal column afferents.** As large, fast conducting sensory fibers enter the spinal cord, a number ascend immediately in the dorsal column white matter. These axons synapse in the dorsal column nuclei, which projects to the medial lemniscus in the brain stem. It is generally held that this pathway plays a modulatory rather than an information transduction role in the pain system (Fields & Basbaum, 1978).

Efferent Modulation

Although efferent inhibition of spinal reflexes has been studied since the time of Sherrington, only recently has this inhibition been related to afferent sensory transmission (cf Fields & Basbaum, 1978). The discovery that electrical stimulation of various brainstem loci produces inhibition of pain transmission as well as spinally mediated reflexes necessitated the study of efferent modulation of sensory transmission.

The observation that local microinjection of morphine into various brainstem loci inhibited responding to painful stimuli (Jacquet & Lajtha, 1974; Pert & Yaksh, 1974) led to postulation of an efferent inhibitory mechanism for morphine analgesia. Specifically, injection of morphine into the periaqueductal gray (PAG) and sites adjacent to the third ventricle produces an inhibitory effect on ascending nociceptive neurons (Herz, Albus, Metys, Schubert & Teschemacher, 1970; Jacquet & Lajtha, 1974; Lotti, Lomax & George, 1965; Mayer & Murphin, 1976; Pert & Yaksh, 1974; Sharpe, Garnette & Cicero, 1974; Yaksh, Yeung & Rudy, 1976).

The PAG has been shown to be high in opiate binding sites
(Atweh & Kuhar, 1983; Kuhar, Pert & Snyder, 1973). Some PAG cells are immunoreactive for endogenous opioid peptides (Cuello, 1983). Although opiate receptors have been localized in areas of the brain, such as the amygdala and corpus striatum, only opiate injection into the PAG-periventricular region of the midbrain produces analgesia (cf Mayer & Price, 1976; Atweh & Kuhar, 1983).

Among the projections of the PAG are tracts from the dorsal and lateral PAG to the nucleus raphe magnus (NRM; Carlton, Leichnetz, Young & Mayer, 1983) and from the ventral PAG caudally in the dorsolateral funnliculus (DLF) in the spinal cord (Watkins, Griffin, Leichnetz & Mayer, 1981). The NRM, in turn, has major serotonergic and nonserontonergic efferents to the DLF (cf Mayer & Price, 1976). Both the direct PAG and NRM efferents project to regions of the dorsal horn of the spinal cord that are known to contain pain sensitive afferent neurons. Lesions of the DLF abolish the analgesic effect opiate microinjection (Murfin, Bennett & Mayer, 1976) and peripherally administered morphine (Price, Hayes, Bennett, Wilcox & Mayer, 1976). It appears, then, that the analgesic effect of opiates is, at least in part, mediated through an inhibitory system originating in the PAG and decending either through direct spinal projections or relayed via the NRM.

SPA and Opiate Analgesia

Several reviews have gathered convincing physiological, pharmacological, and behavioral evidence to suggest a common neural substrate for SPA and opiate analgesia (Fields & Basbaum, 1978; Mayer & Price, 1976; Watkins et al., 1981). A short
summary of that evidence follows.

Physiological evidence. Perhaps the most striking example of a physiological commonality in the systems of morphine analgesia and SPA is the parallel in their sites of action. The sites at which analgesia is most readily elicited by morphine microinjection are along the PAG and periventricular gray region of the brainstem. Likewise, SPA is most readily elicited at sites extending from the fourth ventricle along the aqueduct and around the third ventricle (Snyder & Matthysse, 1975, p. 95). In addition, both injection of morphine and stimulation of the PAG will alter the spontaneous activity of neurons of the NRM (Behbehani & Fields, 1979; Behbehani & Pomeroy, 1978) and spinal cord sensory interneurons reputed to be involved in pain transmission (Fields & Anderson, 1978; Lovick, West & Wolstencroft, 1978; Oliveras, Benson, Guilbaud & Liebeskind, 1974; Kitahta, Koska, Taub, Bonikos & Hoffert, 1974). Furthermore, lesions of the dorsolateral funiculus of the spinal cord block both morphine analgesia and SPA (Basbaum, Clanton & Fields, 1976).

Stimulation of the PAG in human patients with chronic electrodes causes an increase in opioid peptide metabolites in the cerebrospinal fluid (Hosobuchi et al., 1979). This indicates the involvement of an enkephalinergic synapse in SPA.

Pharmacological Evidence. A primary criterion for attributing an effect to opioid mediation is its reversibility by the specific opiate receptor antagonist, naloxone. It is no surprise, then, that the analgesic effect of intracranial
micrination of opiates into the PAC is antagonized by peripheral naloxone (Malick & Goldstein, 1977). The finding that SPA is at least partially naloxone-reversable lends strong support to Mayer's conclusion (Akil, Mayer & Liebeskind, 1976).

As previously stated, morphine analgesia is thought to be mediated by a spinal serotonergic synapse. Depletion of serotonin by pCPA has a deleterious effect on morphine analgesia (Sugrue, 1979; Tenen, 1968; Vogt, 1974) as well as SPA (Akil & Mayer, 1972; Akil & Liebeskind, 1975). Recently, others have found contradictory data, with depletion of spinal serotonin having little effect on SPA (Johannesson, Watkins, Carlton & Mayer, 1982). The authors suggest parallel descending inhibitory systems, one serotonergic and the other noradrenergic.

Behavioral Evidence. Morphine and PAG stimulation produce a strong suppression of certain spinal reflexes, such as the tail-flick response, which are relatively unaffected by non-narcotic drugs (Crumbach, 1966; Mayer & Liebeskind, 1974). When administered in daily 1 min sessions tolerance develops to the analgesic effect of the stimulation at approximately the same rate as tolerance to morphine (Mayer & Hayes, 1975). Repeated injection of morphine attenuates the analgesic effect of PAG stimulation (Mayer & Hayes, 1975). In other words, morphine and SPA are at least partially cross-tolerant.
Rationale

To summarize, tolerance to the analgesic effect of opiate drugs seems to be controlled by Pavlovian associations between the cues predictive of drug administration and the drug experience. It has been proposed that a homeostatic compensatory response, antagonistic to the drug effect is conditioned to those environmental cues that are predictive of the injection (Siegel, 1975).

The analgesic action of both morphine and electrical stimulation of the PAG appears to be mediated through descending inhibition of spinal nociceptive afferents. Therefore, there is considerable evidence that suggests a common neural substrate for SPA and morphine analgesia.

Given this apparent similarity between the analgesic mechanisms of opiate drugs and SPA, tolerance to SPA should also be controlled by Pavlovian associations. The analgesia that is produced by PAG stimulation is relatively free of the multiple "side effects" that narcotic analgesics are known to produce (Mayer & Price, 1976). A demonstration of conditioned tolerance to SPA will provide a paradigm that is particularly promising in the examination of the physiological mechanisms of these processes. Since no drug is administered, unconfounded biochemical analysis of CNS regions may provide clues to the identity of transmitters that may be involved in conditioned tolerance. Physiological manipulations of brain regions putatively involved in learning the conditional homeostatic response may be made without possible interaction with local
opioid receptors. Finally, comparison to other loci of stimulation promises additional insight into anatomical and physiological aspects of the nociceptive system.

In the morphine analgesia paradigm, conditioned tolerance has been demonstrated with several measures of nociception. Siegel (1975, 1976, 1977, 1978, 1981) has used the hot plate method and a paw pressure analgesiometer. Tiffany and his co-workers (Tiffany & Baker, 1981; Tiffany et al., 1983) have used the flinch/jump electric shock test, and Advokat (1980) has used the tail-flick method of D'Amour and Smith (1941). The formalin writhing test has proved to be relatively unaffected by environmental manipulations (Abbott, Melzack & Leber, 1982). Of these tests, only the tail-flick measure used by Advokat is a spinally mediated nociceptive response. The remainder require coordinated movement to escape the painful stimulus.

The advantage of using a response that is mediated at the level of the spinal cord is threefold. First, spinal reflexes are relatively unaffected by non-opiate analgesics. Use of a spinal reflex then, lends support to the contention that the brain-stimulation is affecting a descending inhibitory opioid system. Second, the use of a spinal reflex eliminates the possibility of operantly conditioned behavioral tolerance as described by Dews (1962). Finally, since a spinal reflex eliminates coordinated motor responses, it may be particularly useful for future examination of physiological mechanisms responsible for conditioned tolerance (Advokat, 1980).

The focused light beam tail-flick of D'Amour and Smith, has
a distinct disadvantage, however. Animals are rigidly restrained, and this may affect nociception. A modification of this method, developed by Gray and Umedely (In Preparation) and used recently by Abbott, et al. (1982) will be used in the present experiments. This method uses relatively unrestrained animals to avoid stress effects. Furthermore, an automatic timing circuit reduces the chance of experimentor bias.

The following experiments provide conclusive evidence that SPA tolerance can be extinguished, is situation specific, and can be accounted for by a conditional homeostatic compensatory mechanism.
EXPERIMENT 1: EXTINCTION OF TOLERANCE TO STIMULATION PRODUCED ANALGESIA

When a CS is followed by a UCS, or an instrumental response by a reinforcing event, the probability of the CR or an instrumental response usually increases. If all else is held constant, the omission of the UCS or reinforcer defines the operation of experimental extinction and usually results in a decrease in the probability of responding (Mackintosh, 1974, p. 405).

A unique prediction of the conditioning model of tolerance is that presentation of an environmental CS not followed by the pharmacological agent should attenuate an established tolerance. In other words, tolerance should be subject to the decremental effect of extinction.

In contrast to this prediction of Conditioning Theory, physiological theories of tolerance would predict that, if experimental and control groups receive a drug on exactly the same schedule, they should develop tolerance to the same degree, regardless of exposure to the cues.

In the morphine analgesia paradigm, repeated pairings of environmental cues with a morphine injection produces tolerance to the drug's analgesic effect. Several presentations of the conditional environmental cues paired with a placebo injection will attenuate this tolerance. Furthermore, subjects that are not exposed to the environmental CS during this extinction phase remain tolerant to the analgesic effect of morphine (Siegel, 1975, 1977, 1978). Clearly, nonassociative models cannot account for the extinction of morphine tolerance by presentation of environmental cues.
As previously noted, the analgesic effect of PAG stimulation develops tolerance with repeated trials. Apparent commonalities between morphine analgesia and SPA suggest that, like morphine, tolerance to SPA should be attenuated by presentation of conditional environmental cues, but not by the time delay alone. Experiment 1 tests this hypothesis.

**Method**

**Subjects.** Twenty-four male, Long-Evans, hooded rats (Canadian Breeding Farms Laboratories, St Constant, Quebec) were housed individually with ad lib access to lab chow (Purina) and water (B.C. Hydro). A light/dark cycle of 12/12 hrs. approximating normal day-night hours, was maintained throughout the experiment. At the time of surgery, animals weighed 300-400g.

**Surgery.** Each animal was anesthetized with sodium pentobarbital (Somnitol, M.T.C. Pharmaceuticals, Hamilton, Ontario, Canada, 65 mg/kg). Using a standard stereotaxic instrument with the incisor bar 5.0 mm above the interaural line, a bipolar electrode (0.250 mm; Plastic Products, Roanoke, Virginia) was aimed at the PAG, lowered, and fixed to the skull with jeweler’s screws and acrylic cement. The coordinates were as follows: 0.6 mm anterior to the interaural line, 0.7 mm lateral to the sagittal suture, and 6.0 mm ventral to the dorsal skull surface. At least 10 days were allowed for recovery from surgery.
Histology. All subjects were sacrificed by carbon dioxide asphyxiation and immediately perfused transcardially with 0.9% NaCl followed by 10% buffered neutral formalin (Luna, 1968, p3). The brains were removed and fixed in the formalin solution for at least 24 hr., frozen and sectioned at 30 microns and stained according to a modified Kluver-Barrera method (Luna, 1968, pp 203-204) for myelin and nerve cells. Electrode placements were confirmed by two independent observers.

Apparatus. Electrical brain stimulation was delivered through 80 cm leads (Plastic Products), attached to commutators and suspended from above to avoid tangling. Two constant current stimulators were programmed to deliver 60 Hz Ac to the electrodes in trains of 200 msec with a 300 msec inter-stimulation interval. Stimulation sessions were carried out in two plexiglas chambers (21.5 x 30.5 x 45.5 cm high). Each chamber was placed in a separate insulated enclosure, ventilated by a small fan.

For analgesia testing, each animal was placed into a small tubular chamber (7.5 cm dia x 21.5 cm length) with an aperture in the rear through which his tail was drawn. Approximately 3 in of the tail was submersed in a 52 °C water bath and the latency to remove the tail was recorded.

Procedure. Baseline tail-flick latencies were recorded over 4 daily sessions; the first 3 measurements were taken in the colony room and the fourth in a separate testing room. On the last baseline day, after analgesia testing, brain stimulation current intensity was determined for each subject in a session
lasting less than 1 min. The current was initially set at 10 ua, and incremented in 2 ua steps. When a motor artifact was displayed the current intensity was reduced by 2 ua and this intensity was used throughout the experiment. A ceiling of 50 ua was maintained.

On each stimulation session, animals were transported to the testing room and placed into one of five chambers in a holding box for 15 min. Sanicel, scented with 10 ml of 10% (vol/vol H2O) almond extract solution (Brooke Bond Inc., Belleville, Ontario), covered the bottom of the chambers. The testing room was illuminated with a dim red light and the ventilation fans provided a distinctive background noise. After 15 min the animals were removed from the holding chamber, connected to the brain stimulator, and placed in the plexiglas stimulation chamber. Trains of electrical stimulation were then delivered for a 5 min period. The analgesic effect of the brain stimulation was determined immediately by measuring tail-flick latency as described above. Animals with tail-flick latencies of at least 150% of baseline scores were considered to be analgesic and were continued into the tolerance phase of the experiment. Sixteen of the twenty-four subjects met this criterion. Tail-flick latencies were measured daily after six additional stimulation sessions.

Animals were then randomly assigned to two groups: EXTINCTION and CONTROL. For the next 12 sessions, animals in the EXTINCTION group (n=8) received a similar treatment to that used in the tolerance phase, with the exception of disconnecting the
brain stimulator from the leads attached to the chronic electrode assembly and leaving the water bath at room temperature (21 °C). This procedure allowed repeated presentation of the cues that had predicted SPA (i.e. extinction trials). Subjects in the CONTROL group (n=8) were left in their home cages throughout this 12 day period.

The extinction phase was followed by 4 daily test sessions in which brain stimulation was again delivered prior to measurement of tail-flick latencies in heated water (52 °C).

Results

Histology. All electrode placements were in, or bordering on, the PAG, 0-2 mm rostral to the third ventricle (Fig. 2).

Stimulation Produced Analgesia. Unless otherwise noted, comparisons are by a mixed design analysis of variance performed by computer using the Statistical Package for the Social Sciences, version 9.

Electrical stimulation of the PAG increased tail-flick latencies from mean baseline scores of 3.05 sec and 2.86 sec to 7.49 sec and 7.29 sec for the EXTINCTION group and CONTROL group respectively (Fig. 3). The comparison for sessions 1 and 2 showed a significant main effect for sessions [F(1,14) = 98.06, p < .001]. Tolerance developed equally for the two groups over the seven stimulation sessions (sessions 2-8), with mean tail-flick latencies dropping to 3.74 sec for the EXTINCTION group and 3.58 sec for CONTROL group animals. The comparison showed a significant sessions effect [F(6,14) = 28.80, p < .001].
Figure 2. Electrode placements of rats in the EXTINCTION group and CONTROL group from Experiment 1. Brain sections were taken from Pellegrino, Pellegrino, and Cushman (1979).
- EXTINCTION
- CONTROL
- NO SPA
Figure 3. Mean tail-flick latencies in seconds for the EXTINCTION and CONTROL groups in Experiment 1. Session 1 represents the final (fourth) baseline session. On sessions 2-8 all rats received pairings of the environmental cues and brain-stimulation. On sessions 9-20 rats in the EXTINCTION group were exposed to the environmental cues but were not stimulated. Rats in the CONTROL group were left undisturbed in their cages during this 12-day period. On test sessions (sessions 21-24) exposure to environmental cues again preceded brain stimulation for all rats.
The graph illustrates the mean tail-flick latency (sec) over sessions for both extinction and control groups. The y-axis represents the latency in seconds, ranging from 0 to 10. The x-axis represents the session number, ranging from 1 to 24. The extinction group (solid line) shows a noticeable decrease in latency over sessions, while the control group (dashed line) shows a less significant decrease. Error bars indicate the variability in the data.
After 12 extinction trials (sessions 9-20) electrical stimulation of the PAG again produced an analgesic effect, increasing the mean tail-flick latency of this group to 5.68 sec. The CONTROL group remained tolerant to the SPA despite 12 days in their home cages, and had a mean tail-flick latency of 3.35 sec after stimulation in session 21. Group differences were reflected in a significant session (8 and 21) by treatment interaction \( F(1,14) = 14.52, \ p < .001 \). Tail-flick latencies of the EXTINCTION and CONTROL groups were significantly different on the first test session (session 21, \( t = 4.20, \ p < .001 \)). Tolerance to SPA was reacquired in the EXTINCTION group after four stimulation sessions, as tail-flick latencies returned to 3.82 sec. This is reflected in a significant sessions by treatment interaction for sessions 21-24 \( F(3,14) = 3.6, \ p = .05 \). A Duncan's Multiple Range test \( Cd(56) = .317k \) confirmed significant differences for comparisons of sessions 21 and 23 \( (p < .05) \), but not for sessions 22 and 24.

**Discussion**

The results of Experiment 1 show that tolerance to the analgesic effect of SPA can be attenuated by an extinction procedure. Repeated stimulation of the PAG induced tolerance to the stimulation, replicating the results of Mayer and Hayes (1975). Subjects presented with the environmental cues which had predicted stimulation for twelve sessions (EXTINCTION group) showed an attenuated tolerance level. That is, their tail-flick latencies were longer than on the last day of the tolerance phase. Subjects remaining in their home cages for the same
twelve day period (CONTROL group) provided a control for any time-dependent attenuation of tolerance. These animals remained tolerant to the PAG stimulation, with tail-flick latencies remaining at pre-delay levels.

It is important to emphasize that the EXTINCTION and CONTROL groups received stimulation on exactly the same schedule throughout this experiment. The only difference between the groups was exposure to the environmental cues during the twelve extinction sessions. Only theories stressing a role for an associative mechanism in tolerance could possibly predict that these extinction trials could attenuate tolerance to SPA.

Although the effect of the extinction trials is robust (p < .001), the analgesic effect of stimulation has not been re-established to pre-tolerance levels. Several explanations are possible. First, it is easy to infer physiological mechanisms to account for at least part of the tolerance that develops to SPA. Such a physiological mechanism would summate with conditioned tolerance, and be relatively unaffected by environmental manipulations. A second possibility is that the number of extinction trials was insufficient to completely attenuate tolerance. A third possibility invokes the concept of generalization decrement. Generalization decrement refers to the decrease in the ability of a stimulus to elicit a CR as the discrepancy between the stimulus and the CS increases. Brain-stimulation is known to have cue properties of its own (Phillips & LePiane, 1978; Phillips & MacDonald, 1979; Stutz & Maroli, 1978). The absence of the stimulation may have altered
the CS during extinction trials, preventing complete extinction. When the full CS (environmental cues + stimulation) is reintroduced on test sessions, an attenuated CR may be elicited during the five min stimulation session. It is impossible to distinguish between these explanations on the basis of the present data.
EXPERIMENT 2: CONTEXT SPECIFICITY OF TOLERANCE TO STIMULATION PRODUCED ANALGESIA

The term "context specificity" implies that a CS-UCS association is dependent upon the environment in which it is formed. A CS that elicits a CR in one environment may not elicit that CR when presented in another environment (e.g. Dweck & Wagner, 1970; Marlin, 1982). Presumably, associations between a CS and features of the environment are formed during conditioning, making the environment part of the CS. When the CS is presented in an environment that is significantly different than the training environment the CS has been changed and a generalization decrement occurs (Rescorla & Wagner, 1972).

Siegel (1975, 1976) has demonstrated that tolerance to morphine's analgesic effect, as tested with the hotplate method and paw pinch analgesiometer and developed in one environment, is attenuated when the drug is given in an alternate environment. Of course, animals tested in the same environment retained tolerance. This effect has been replicated using the radiant heat tail-flick measure (Advokat, 1980) and flinch/jump shock test (Tiffany & Baker, 1981).

Since the analgesic effects of morphine and PAG stimulation may operate by a common mechanism, tolerance to SPA, like tolerance to morphine analgesia, should be dependent upon the context in which it is developed. According to a Pavlovian model, subjects that develop tolerance to SPA in one environment should respond in a relatively nontolerant manner when tested in
an different environment, provided that the two environments are sufficiently distinct. Subjects that develop tolerance and are tested in the same environment should remain tolerant to the brain-stimulation. If the schedule of stimulation is identical for the two groups, physiological theories would predict equivalent tolerance on test sessions, irrespective of the context. Experiment 2 was designed to test these hypotheses.

Method

Subjects. Thirty male, Long-Evans rats were prepared according to the methods described in Experiment 1. At the end of the experiment animals were again sacrificed and brains removed for histological examination.

Apparatus. The two environments used in this experiment were the testing room used in Experiment 1 and the animals' colony room. By using the environment in which the animals live as the final testing environment, novelty was eliminated as a possible cause of analgesia on test sessions.

Stimulation trains were delivered by a single constant current stimulator, programmed as in Experiment 1. The stimulator was carried between the two environments to closely match stimulation parameters.

Apparatus in the separate testing room was identical to that in Experiment 1. Apparatus in the colony room was identical, except that the stimulation chamber was not located in a sound-attenuating enclosure.

Procedure. As in Experiment 1, baseline tail-flick measurements were recorded over four daily sessions with the
first three measurements taken in the colony room and the fourth measurement in the separate testing environment. Stimulation intensities were determined according to the method used in Experiment 1.

Animals were then randomly assigned to two groups: ENVIRONMENT SAME and ENVIRONMENT DIFFERENT. Mean stimulation for the ENVIRONMENT SAME group was 17.7 ua, with a minimum of 10 ua and a maximum of 45 ua. For the ENVIRONMENT DIFFERENT group, mean stimulation intensity was 20.1 ua, with a minimum of 10 ua and a maximum of 41 ua. On each stimulation day, each animal in the ENVIRONMENT SAME group was taken from his cage and immediately attached to the stimulation apparatus. Five min of stimulation trains preceded a tail-flick analgesia test. Subjects were immediately returned to their cages. The colony room was well lighted and access was restricted to maintain quiet.

Animals in the ENVIRONMENT DIFFERENT group were treated as in Experiment 1. Specifically, they were enclosed in the almond-scented holding chamber for fifteen minutes preceding the five minutes of stimulation and a tail-flick test. Only a dim red light illuminated the room, and ventilation fans provided background noise.

Subjects meeting the 150% of baseline analgesia criteria received an additional six sessions of stimulation in their respective environments. Nine animals in each group met this criteria and were continued into the tolerance phase.

The seven-session tolerance phase was followed by four days
of testing, in which all subjects received stimulation in the colony environment. Conditions were identical to those of the ENVIRONMENT SAME group during the tolerance phase. This procedure eliminated the cues that had predicted SPA for the ENVIRONMENT DIFFERENT group while maintaining the cues for the ENVIRONMENT SAME group.

Results

Histology. All but three electrode placements eliciting analgesia were in, or bordering on the PAG. The histology of one subject was lost. All placements not eliciting analgesia were in the PAG (Fig. 4).

Stimulation Produced Analgesia. Mixed design ANOVAs were again used for statistical comparisons. except where noted.

Lack of differences between baseline sessions 3 and 4 (sessions 1 and 2, Fig. 5) were confirmed by a nonsignificant main effect for sessions [F(1,16)=0.247, p>0.5]. The final baseline session was used for the remainder of the statistical comparisons.

Stimulation of the PAG increased mean tail-flick latencies from 3.79 and 3.98 to 6.89 and 7.76 in the ENVIRONMENT DIFFERENT and ENVIRONMENT SAME group, respectively. A significant sessions effect for the comparison of sessions 2 and 3 [F(1,16) = 107.186, p < 0.001] confirmed this analgesic effect. With repeated stimulation in sessions 3 through 9, tolerance developed equally for the two groups. Mean tail-flick latencies dropped to 3.77 for the ENVIRONMENT DIFFERENT group and 3.84 for the ENVIRONMENT SAME group. The comparison showed a significant sessions effect
Figure 4. Electrode placements of rats in the ENVIRONMENT DIFFERENT group and ENVIRONMENT SAME group from Experiment 2. Brain sections were taken from Pellegrino, Pellegrino and Cushman (1979).
- ENVIRONMENT DIFFERENT
- ENVIRONMENT SAME
- NO SPA
Figure 5. Mean tail-flick latencies in seconds for the ENVIRONMENT DIFFERENT and ENVIRONMENT SAME groups in Experiment 2. Session 1 represents the third baseline measurements, which were taken in the colony room. Session 2 represents the final baseline measurements, which were taken in the alternate environment. For sessions 3-9, rats in the ENVIRONMENT DIFFERENT group were administered brain-stimulation in the alternate environment. For these sessions, rats in the ENVIRONMENT SAME group received brain-stimulation in the colony room. On test sessions (sessions 10-13) all rats received stimulation sessions in the colony room.
\[ F(6,96) = 19.98, \ p < 0.001 \], with no main effect for treatment \[ F(1,16) = 0.127, \ p > 0.5 \].

By changing the stimulation environment from the separate room to the colony room to the colony room, the PAC stimulation again produced an analgesic effect, increasing the mean tail-flick latency of the ENVIRONMENT DIFFERENT group to 5.57 sec. The eighth consecutive session of stimulation in the colony room did not change the mean tail-flick latency of the ENVIRONMENT SAME group, which remained at 3.84 sec. A significant sessions by treatment interaction for sessions 9-10 \[ F(1,16) = 10.671, \ p < 0.05 \] confirmed this effect. Since the ENVIRONMENT SAME group’s mean tail-flick latency is identical for sessions 9 and 10, all of this interaction is attributable to the increase in mean tail-flick latency of the ENVIRONMENT DIFFERENT group.

Tolerance to SPA was reacquired in the different group after four stimulation sessions in the new environment. This is confirmed by a significant session by treatment interaction for sessions 10-13 \[ F(3,16) = 3.67, \ p < 0.05 \]. A Duncan’s Multiple Range test \[ Cd(64) = 0.308k \] confirmed significant differences for comparisons on sessions 10, 11, and 12 (p <
Discussion

The results of the second study demonstrate that a change in testing environment is sufficient to attenuate tolerance to SPA. Subjects tested in the same environment, as predicted, do not show this attenuation of tolerance. These results are taken as support for the situational specificity of tolerance to SPA.

Since identical schedules of stimulation were maintained throughout the experiment, theories that contend that administration of the agent is sufficient to produce tolerance fail to predict this pattern of data. The Conditioning Theory of tolerance, on the other hand, maintains that since classically conditioned responses are context dependent, tolerance should also show context dependency (Siegel, 1975, 1976, 1977). Several investigators have demonstrated that tolerance to morphine's analgesic effect is greater when subjects receive all injections in the testing environment. Animals that receive injections in a distinct environment show attenuated tolerance development (Advokat, 1981; Kayan, Woods & Mitchell, 1969; Siegel, 1975, 1976; Tiffany & Baker, 1981). Since Siegel claims that features of the environment serve as the CS it seems more appropriate to term the attenuation of tolerance due to a change in environment as a generalization decrement, rather than context specificity. However, Bardo, Wellman & Hughes (1981) have shown that the features of the testing apparatus seem to be "prepotent" as cues for conditioned tolerance. Since the testing apparatus remains constant throughout this experiment, an argument can be made for labeling the present results as context specificity. In either
case, the present data support a role for associational processes in the acquisition of tolerance.

As in Experiment 1, the data do not show complete attenuation of tolerance. Physiological mechanisms and brain-stimulation as a cue remain as possible explanations. An additional possibility is that, since the same testing apparatus is used (as well as the same experimenter), some CS generalization may occur. Again, the data present no clues to distinguish between these possibilities.
After repeated pairings of a CS and UCS, the CS, which initially elicited no response, often comes to elicit a CR (Pavlov, 1927). Siegel and his associates (Krank, Hinson & Siegel, 1981; Siegel, 1975) paired daily morphine injections (effect is UCS) with environmental cues (CS). Siegel's Conditioning Model of drug tolerance requires the CR to be a homeostatic compensatory mechanism, antagonistic to the analgesic effect of morphine. As a function of CS-UCS pairings, the environmental CS comes to elicit the compensatory CR. The compensatory CR summates with the analgesic action of morphine to produce the attenuated drug effect that defines tolerance (Siegel, 1975). When the environmental cues are then followed by a placebo injection, the compensatory CR is manifest as a hyperalgesic response, relative to subjects receiving an additional injection or noninjected controls (Siegel, 1975). Groups with equivalent unpaired pretest exposure to environmental cues and the drug have eliminated differential pretest assessment experience and environmental novelty as alternative explanations for this effect (Krank et al., 1981).

Compensatory CRs have also been shown for the hypothermic (Siegel, 1978) and locomotor (Mucha et al., 1981) effects of morphine. Curiously, Siegel's conditioned hyperalgesia has not been replicated outside his laboratory (Tiffany et al., 1983).

Considering the many parallels between morphine analgesia
and SPA, environmental cues that have previously been paired with PAG stimulation should elicit a hyperalgesic response. Such a demonstration is critical to a Pavlovian interpretation of tolerance to SPA.

Method

Subjects. Thirty-four male, Long-Evans rats were prepared according to the methods described in Experiment 1. An additional eight rats were left unimplanted. At the end of the experiment, animals with electrodes were sacrificed and brains removed for histological examination.

Apparatus. All apparatus were identical to that used in Experiment 1. The testing room was the only environment. The temperature of the water bath, however, was lowered to 48.5 C. This was done to increase baseline tail-flick latencies and avoid a "floor effect".

Procedure. As in the previous experiments, baseline tail-flick measurements were recorded over four daily sessions with the first three measurements taken in the colony room and the fourth measurement in the separate testing environment.

For thirty of the implanted animals, sessions proceeded exactly as in Experiment 1 through the tolerance development phase. Sixteen animals exceeded the criterion of a two sec increase over baseline tail-flick latency after initial treatment with brain-stimulation. Fourteen animals did not meet this criterion and were excluded from the experiment. The remaining four implanted animals and the eight unimplanted animals served as a nonstimulated control group (NS-CONTROL). Rats in this
group received seven sessions of cue exposure followed by a tail-flick test, corresponding to the tolerance phase.

The tolerance phase of the experiment was followed by a single test session. Half of the stimulated rats (n=8) received an additional stimulation session (S-CONTROL). The remaining stimulated rats (n=8) received a "placebo" stimulation session followed by a tail-flick test (HYPERALGESIA). For animals in the HYPERALGESIA group the session was identical to sessions in the tolerance phase, except that the stimulation leads were disconnected from the stimulator. This allowed presentation of the conditional environmental cues without stimulation. The NS-CONTROL group received an additional tail-flick test session.

Results

Histology. All but two placements eliciting analgesia were found to have electrodes in, or bordering on, the PAG. All placements not eliciting analgesia were within or bordering on the PAG. Placements of the four implanted controls were all within the PAG (Fig. 6). There was no apparent pattern of differences between placements eliciting analgesia and those placements not eliciting analgesia.

Stimulation Produced Analgesia. Decreasing the water bath temperature to 48.5°C had the effect of raising baseline mean tail-flick latencies to 7.78 sec, 8.62 sec, and 7.02 sec for the HYPERALGESIA, S-CONTROL, and NS-CONTROL groups, respectively (Fig. 7, session 1). Stimulation of the PAG increased the mean tail-flick latency of the HYPERALGESIA group to 15.82 sec and the S-CONTROL group to 17.22 sec. By the seventh stimulation session
Figure 6. Electrode placements of rats in the HYPERALGESIA group, S-CONTROL group and implanted NS-CONTROL group animals from Experiment 3. Brain sections were taken from Pellegrino, Pellegrino and Cushman (1979).
• HYPERALGESIA

• S-CONTROL

• NS-CONTROL

• NO SPA
Figure 7. Mean tail-flick latencies in seconds for the HYPERALGESIA, stimulated control (S-CONTROL), and nonstimulated control (NS-CONTROL) groups in Experiment 3. Session 1 represents the final baseline measurements. On sessions 2-8, the HYPERALGESIA and S-CONTROL groups were exposed to environmental cues followed by brain-stimulation. The NS-CONTROL group were exposed to environmental cues but were not stimulated. On session 9, rats in the HYPERALGESIA group were exposed to the environmental cues, but were not stimulated. Rats in the S-CONTROL group were given an additional stimulation session. Rats in the NS-CONTROL group were again exposed to environmental cues, but were not stimulated. The missing value for the NS-CONTROL group on session 6 (-) represents an equipment malfunction.
(Fig. 7, session 8), mean tail-flick latencies had decreased to 9.30 sec in the HYPERALGESIA group and 9.28 sec in the S-CONTROL group.

Across this same seven day period, repeated tail-flick testing did not affect mean latencies of the NS-CONTROL group. A mean tail-flick latency of 9.41 sec did not significantly differ from the session 1 mean of 7.02 sec \( t(7) = 2.32, p > .05 \). There is, however, a trend toward increased tail-flick latency over repeated testing.

A placebo stimulation session decreased the mean tail-flick latency of the HYPERALGESIA group to 5.82 sec, significantly below that group's mean for both the previous session [Duncan's \( Cd(25, 4) = 1.92, p < .05 \)]. An additional stimulation session did not change the S-CONTROL group's mean latency \( X = 10.74 \). A thirteenth consecutive tail-flick session did not change the NS-CONTROL group's mean latency \( X = 7.9 \) sec. A Duncan's multiple range test confirmed significant differences between session 9 scores for the HYPERALGESIA group and both the S-CONTROL \( Cd(254) = 1.89, p < .05 \), and the NS-CONTROL \( Cd(25) = 2.00, p < .05 \).

**Discussion**

The results of Experiment 3 support the prediction that in rats tolerant to the analgesic effect of PAG stimulation, exposure to conditional environmental cues followed by a placebo stimulation session should reveal a hyperalgesic compensatory CR. In this experiment, rats exposed to environmental cues, but not given a stimulation session, were more sensitive to a thermal
stimulus than both a group given an additional stimulation session, and nonstimulated controls. The nonstimulated control group eliminates repeated experience with the testing procedure as an alternative explanation for this effect (Bardo & Hughes, 1978; Hayes & Mayer, 1978; Sherman, 1979). These findings support the contention that tolerance to SPA represents the summation of the analgesic effect of the stimulation and a homeostatic compensatory response.

Although Experiments 1 and 2 support the idea that tolerance to SPA involves associative processes, Experiment 3 provides evidence for a homeostatic compensatory response to an environmental CS. This demonstration is critical to a Pavlovian interpretation of tolerance to SPA.

As previously stated, Siegel's demonstration of a compensatory CR to morphine's analgesic effect has not been replicated outside his laboratory (Tiffany et al., 1983). Therefore, although analgesic agents differ, Experiment 3 represents the first independent demonstration of a hyperalgesic CR.

The existence of drug preparatory CRs suggests a common mechanism for tolerance and dependence (Krank, Hinson & Siegel, 1981; Siegel, in press). When an organism is exposed to cues that predict drug administration, it may "prepare" itself for pharmacological assault (ie. exhibit compensatory CRs). Siegel (1979) and Hinson and Siegel (1980) have suggested that conditional compensatory responses may account for many symptoms of the abstainance syndrome. An understanding of the
Determinants of compensatory CRs may eventually lead to treatment programs for the relief of these symptoms for patients undergoing drug withdrawal.
GENERAL DISCUSSION

The results of the present experiments support the prediction that tolerance to SPA, like tolerance to morphine's analgesic effect, is mediated by a Pavlovian associational mechanism. Specifically, these experiments suggest that SPA tolerance is the result of a classically conditioned homeostatic compensatory response, antagonistic to the analgesic effect of the stimulation. The homeostatic CR summates with SPA to produce the observed reduction in analgesia.

Experiment 1 demonstrated that tolerance to the analgesic effect of PAG stimulation can be extinguished. Repeated presentation of environmental cues associated with SPA attenuates the analgesic effect of the stimulation. A time-delay control group shows no such decrement. Previous experiments (Siegel, 1975, 1977) found that extinction trials attenuated tolerance to morphine's analgesic effect. Neither of these results can be explained by nonassociative theories of tolerance, that hold that the administration of a drug is sufficient to develop tolerance. They are, however, predicted by theories that provide a role for learning in the development of tolerance.

Experiment 2 provided evidence that the development of tolerance to SPA is context specific. A change of testing environment was sufficient to attenuate an established tolerance. Several laboratories have demonstrated the context specificity of tolerance to morphine analgesia (Advokat, 1980; Siegel, 1975, 1977; Tiffany & Baker, 1981). Theories of tolerance that
maintain that repeated administration of the analgesic agent is sufficient to produce tolerance cannot explain this environmental specificity. Since conditional responses are, in many cases, tied to the environment in which they are formed (eg. Dweck & Wagner, 1970; Marlin, 1982), the conditioning theory of tolerance predicts the environmental specificity of tolerance.

A critical tenet of the Conditioning Theory of drug tolerance is that tolerance reflects a homeostatic CR. Experiment 3 demonstrated a hyperalgesic response for SPA tolerant rats that are presented with an environmental CS. With the morphine analgesia paradigm, Siegel and his co-workers (Krank, Hinson & Siegel, 1981; Siegel, 1975) have shown conditional compensatory CRs using a paw-pressure analgesiometer and hotplate test. This effect has not been replicated by others (Tiffany et al., 1983) using the flinch/jump shock test. Despite the difference in analgesic agents, Experiment 3 represents the first independent demonstration of a hyperalgesic CR. Only the Conditioning Theory of tolerance predicts this result. Physiological theories do not allow for a conditional, hyperalgesic response.

Together, these three experiments provide strong evidence that the Conditioning Theory of Tolerance is applicable to the tolerance that develops to SPA. Interpretation of conditioned tolerance according to the stimulus-substitution model proposed by Siegel (1975) has proved awkward. The occurrence of CRs in the opposite direction, as well as in the same direction as the UCRs can not be explained by this model. This issue has been
specifically addressed by Eikelboom and Stewart (1982). These authors propose that CRs opposite in direction to the UCR can be explained by distinguishing between drugs that act on an afferent arm of a regulatory feedback system, and drugs that act on an efferent arm, relative to a central integrator. They argue that drugs having an effect on an afferent or sensory arm of the nervous system show conditional responses in the same direction as the unconditional effect of the drug. In such a situation, the drug is considered to be the UCS and its effect the UCR. Conversely, drugs that have an effect on an efferent arm of the nervous system show a CR in the opposite direction to the drug effect. In this case, since the direct drug effect is not detected by a central integrator, the drug cannot be considered as a stimulus. If a regulatory feedback integrator detects the drug-induced change, then this change is considered to be the UCS, and the regulatory response is the UCR.

Eikelboom and Stewart (1982) propose that there is sufficient evidence to use this model as a psychopharmacological tool. Specifically, agents showing CRs in the same direction as the drug effect should have their effect on the input side of a regulatory integrator, whereas agents showing a compensatory CR affect the output side. According to this model, SPA should be thought of as a Pavlovian conditioning trial, with the analgesic effect of the stimulation as the UCS, and a homeostatic compensatory response, antagonistic to the effect of the stimulation (i.e. hyperalgesia), would then serve as the UCR. Environmental cues that predict the stimulation would be the CS,
and a compensatory mechanism the CR.

On the basis of Eikelboom & Stewart's arguments, tentative anatomical and physiological interpretations can be made of conditioned tolerance to SPA. There is evidence that PAG stimulation activates one or both of two bulbospinal inhibitory pathways (Watkins et al., 1982). One of these pathways is a direct connection between the PAG and the substantia gelatinosa of the spinal cord (Johannesson et al., 1982; Watkins et al., 1982). The other is relayed through the NRM (Bebehanni & Pomeroy, 1978). The consequent increase in output of these inhibitory, bulbospinal, efferent systems would, in turn, cause a decrease in the firing rate of the spinoreticular afferent neurons. It is this effect of the stimulation that should be seen as the UCS. A regulatory integrator would detect a mismatch between sensory input and "setpoint" input (possibly dorsal column afferents, which should not be affected by inhibitory efferents) and produce compensatory output (the UCR). Possible substrates for this homeostatic mechanism include a decrease in the firing rate of either or both of the bulbospinal inhibitory systems or activation of an, as yet, unisolated excitatory efferent system. Evidence for excitatory modulation of nociceptive afferents has recently been found (Larson, 1983). A tryptaminergic system, antagonistic to inhibitory serotonergic neurons, may serve as a "hyperalgesic" modulator in the substantia gelatinosa of the spinal cord. With repeated pairings, environmental cues may come to elicit a decrease in output of the inhibitory pathway, or an increase in output of the
excitatory pathway.

Eikelboom and Stewart's model of conditioned drug-induced physiological responses allows two possibilities for the structure of the regulatory feedback loop. First, it allows the integrator to be located within a closed feedback loop, with the PAG efferent to the integrator (Fig. 8a). In this case the neuroanatomical data would suggest that the integrator may be located between the primary nociceptive afferents and the stimulation site. This may include synapses in the tectum, bulbar reticular formation, and PAG interneurons (Kelly, 1981). An alternative possibility would allow the integrator to be located in the spinothalamic axis with the homeostatic response mediated hormonally through the CSF. For example, beta-endorphin containing neurons have been localized in the mid-line structures of the thalamus, continuing to the PAG (Cuello, 1983). Beta-endorphin is relatively stable in the CSF (Weissman & Meshulam, 1983). If these neurons are found to be activated by painful stimulation and this is accompanied by a rise in CSF beta-endorphin, then the possibility of a long-lasting spinothalamically-mediated analgesia system would be supported. Analgesic agents should induce a decrease in activity of this system. In turn, a decrease in CSF beta-endorphin should be conditioned with repeated SPA or morphine injection.

If the integrator were located efferent to the stimulation, within a closed loop, this model would demand an analgesic CR. As this was not observed, such a location for the integrator can be discounted.
Figure 8. Possible locations of the "integrator," relative to the PAG. In (A), the integrator lies within a closed loop, with feedback attenuating input to the PAG. Tolerance would be seen as a conditioned inhibition of excitatory input to the PAG. In (B), the integrator lies on a feedback loop that is distinct from the PAG-spinal cord analgesia system. The mechanism that is conditioned may be either a decrease in output of a parallel inhibitory system (solid line) or an increase in output of an excitatory, "hyperalgesia" system (broken line).
The second case allowed by the Eikelboom and Stewart model would locate the stimulation on an efferent pathway distinct from the regulatory feedback loop (Fig. 8b). In this case it would be possible for the PAG stimulation to affect direct spinal inhibitory efferents, which induce feedback through the NRM. Alternatively, it is possible that it is the PAG-NRM connection that is stimulated and the regulatory feedback loop returns through the PAG. In this case the integrator could be located anywhere within the feedback loop. If the dorsal column afferents, which feed the NRM through the Medial Lemniscus and possibly by direct input (Fig. 1) are seen as providing information for the setpoint, it becomes likely that the NRM is the integrator, with PAG stimulation exciting a distinct efferent inhibitory system. Replication of the present experiments using NRM and Medial Lemniscus electrode placements may serve to elucidate/confuse these issues. If the NRM is, indeed, the location of the integrator, bimodal results could be expected with electrodes in this area. Placements efferent to the relevant synapses should show compensatory conditioning (i.e. tolerance), whereas afferent placements should show the opposite (i.e. conditioned reverse tolerance or sensitisation). If dorsal column afferent-Medial Lemniscus neurons modulate setpoint, then stimulation of the Medial Lemniscus should produce a hyperalgesic response, with no analgesia. Since the Medial Lemniscus would be on the input side of the integrator, repeated stimulation should produce conditional hyperalgesia. A third possibility in this case is that analgesia resulting from PAG stimulation is
antagonized by direct activation of a hyperalgesia system (Fig. 8b, broken line).

A major obstacle to deliniation of biochemical substrates of conditioned tolerance development has been the multiple effects of pharmacological agents and diversity of receptor localization. The SPA paradigm appears to provide a suitable solution to this problem by demonstrating conditioned tolerance in a relatively isolated system. Using the design of Experiment 3, biochemical comparisons of brain and spinal cord regions before and after exposure to conditional environmental cues may yield a gross picture of transmitters involved in the development of conditioned tolerance. Specifically, a decrease in spinal cord serotonin metabolites for HYPERALGESIA group, relative to CONTROL group rats would point towards a conditioned decrease in NRM output as the CR. A decrease in spinal norepinepherine or norepinepherine metabolites would suggest a closed circuit with a decrease in direct PAG-spinal output as the CR. An increase in tryptamine would suggest a conditioned increase in activity of a putative hyperalgesia system (Larson, 1983). The context specificity design (Experiment 2) would discriminate between tolerance due to conditioning and possible physiological mechanisms. Differences in transmitter and metabolite levels of subjects in the ENVIRONMENT SAME and ENVIRONMENT DIFFERENT groups, after exposure to conditional cues, could be attributed to environment-dependent factors.

An understanding of the biochemical mechanisms involved in the compensatory CR may lead to symptom relief strategies in the
treatment of drug addicts experiencing abstinence syndrome. For example, if a conditional decrease in a serotoninergic inhibitory mechanism is shown to be the substrate of the hyperalgesic CR, administration of a serotonin agonist may relieve the "hypersensativity" experienced during opiate withdrawal.

It would also be advantageous to explore the mechanisms involved in development of conditioned tolerance. Lesion studies, for example, have suggested the involvement of the prefrontal cortex (Kalant et al. 1978) and dopaminergic systems (Blasig et al., 1975) in the development of tolerance to opiates. Deficits in tolerance development resulting from lesions in these areas have been attributed to the loss of tolerance-relevant opioid receptors. Recently, several authors have suggested that these deficits may be attributable to damage in associational circuitry rather than a hardwired tolerance system. Should similar lesions produce an attenuation of conditioned tolerance in the relatively isolated SPA system, a role for local opioid receptors would seem tenuous. Finally, an understanding of the substrates of conditioned tolerance may lead to drug treatment regimens that minimize tolerance development by blocking associations through pharmacological manipulations of relevant cell populations.

The apparent demonstration of Conditioned Tolerance to SPA is unique because it shows this phenomena is a relatively isolated system. More importantly, this paradigm seems to be well suited to physiological and biochemical analysis of the substrates of Conditioned Tolerance. Therefore, these findings
represent an extension of our knowledge of the Conditioned Tolerance phenomena and an impetus for a more detailed, reductionistic investigation.


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