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THE ACQUISITION OF A MALADAPTIVE RESPONSE IN MORPHINE TREATED MICE

by

ARTHUR DAVID CHARAP

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1976

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Chairman of Examining Committee

1/27/76
date

Executive Officer

Dr. J. Goldfarb

Dr. P. Bentley

Dr. S. Diamond

Dr. B. Hine

Supervisory Committee

Mount Sinai Graduate School,
The City University of New York

Abstract

THE ACQUISITION OF A MALADAPTIVE RESPONSE IN MORPHINE TREATED MICE

by

Arthur David Charap

Adviser: Associate Professor S. D. Glick

A majority of naive mice were able to escape from a trough-shaped box, the floor of which delivered a foot-shock. A single injection of morphine significantly impaired escape performance. Tolerance to this effect was not observed after fifteen days of once daily post-injection testing. Analgesic tolerance did, however, occur in mice injected with morphine but not receiving daily testing. The escape impairment observed in the tested mice persisted following cessation of drug administration. No such effects were observed in mice tested prior to, rather than following, daily injection. Control animals tested daily did not habituate to the shock. In mice previously made tolerant to morphine, post-injection testing produced a decline in escape performance and a sustained impairment following discontinuation of the drug. Analgesic tolerance as measured by vocalization was observed to occur in both tested and untested mice.

Increasing the dose of morphine, the number of days of post-injection testing, or shock intensity enhanced the degree and duration of impaired escape behavior. Variations in shock intensity, in the absence of morphine, were not sufficient to produce a sustained escape impairment nor was forced exposure to the shock. Daily testing prior to the beginning of post-injection testing diminished the effect of a single injection of morphine, but

escape performance declined on subsequent days of post-injection testing.

Effects similar to those observed with morphine were obtained with levorphanol but not with propoxyphene or meperidine. Methadone was only effective in generating sustained escape impairment when gradually increasing doses were employed. Chlorpromazine, pentobarbital, and diazepam produced an initial suppression of escape performance, but tolerance developed rapidly.

Results, similar to those observed with shock, were obtained using heat as the noxious stimulus. Simultaneous recording of escape performance and reflex response latencies indicated that escape impairment is produced even though analgesic tolerance, as measured by response latencies, is also present.

The experimental results indicate that administration of phenanthrene narcotic analgesics plus exposure to noxious stimuli interact to produce a maladaptive response, escape failure. The acquisition of this response appears to be independent of morphine's analgesic effects.

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I caught you knockin on my cellar door,

I love ya baby can I have some more,

Ooh, the damage done.

I hit the city and I lost my band,

I watched the needle take another man,

Gone, gone, the damage done.

I sing this song because I love the man,

I know that some of you don't understand,

Milk blood to keep from runnin out.

I've seen the needle and the damage done,

A little part of it in everyone,

But every junkie's like a setting sun.

The Needle and The Damage Done

A song by Neil Young, 1972

Dedicated to Darryl

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INTRODUCTION

Pain

In order to discuss analgesics and analgesia with any facility, one needs a clear understanding of the phenomenon of pain. Unfortunately, pain is a difficult concept to satisfactorily define. For example, if one were to ask a psychophysicist, a psychiatrist or a neurosurgeon for a concise definition of pain, one would likely receive three very different responses.

The issue that has caused such diversity of opinion has centered around the inability to equate the subjective report of pain with objective or physiological measurements of pain. Thus, rather than attempting to define the nature of pain, the present discussion will focus on the central theoretical issues that have contributed to the understanding of this subject.

Specificity Theory

Specificity theory represents the traditional view of pain. This classical approach to the study of pain was first proposed by Descarte. He conceptualized pain pathways as essentially analogous to a "burglar alarm system" or a "transmission line". In this scheme, a stimulus applied to the periphery caused a signal to be sent to the brain where "pain" is perceived and a response is subsequently generated. With only minor refinements, this simplistic explanation of pain has served as the theoretical framework for nearly all physiological experimentation in the field of pain research. This is unfortunate since specificity theory fails to account for a number of crucial observations of pain phenomena. Yet specificity theory has gained such acceptance that it is often presented as fact rather than theory.

Until the 1800's the nature of the "transmission line" between the periphery and the brain was unknown. It was Muller (1842) who first

formulated the doctrine that all sensory information was communicated to the brain via sensory nerves. Muller was uncertain, however, whether the quality of the sensation was a property of the specific nerves or the site of termination of the nerves in the brain.

In the late 1800's neurophysiologists began to study the properties of impulse transmission in nerve fibers. The investigators of the day concluded that the mechanism of transmission, i.e. conduction of the action potential, was essentially equivalent in all fibers. As a result, the Central Nervous System became the focus of attention as the "pain center". At that time physiologists were so convinced of the validity of the "specifistic doctrine" (of a straight-through system from the sensory organ to the brain center responsible for sensation), that it was generally believed that if the "auditory nerve could be connected to the visual cortex, and the optic nerve joined to the auditory cortex, that one could see thunder and hear lightning" (Boring, 1942).

Implicit in Muller's formulation was the notion of a single somasthetic sense or "sense of feeling". This concept of a single sense modality was not accepted by a number of investigators. Von Frey, considered the father of present day specificity theory, expanded on Muller's work and proposed the existence of four major cutaneous sensory modalities: touch, warmth, cold, and pain. Each of these senses was believed to be endowed with a separate projection system to the CNS. Von Frey sought to prove his hypothesis by mapping various areas of skin for different sensory modalities. Previously, anatomists had characterized a number of specialized structures in the skin e.g. pacinian corpuscles, Krauses end bulbs, etc., and Von Frey attempted to correlate these structures with the mapped areas of the skin.

Although Von Frey's findings appeared to support his hypothesis of cutaneous sensation, more recent investigations, as will be discussed, have tended to disprove the proposition that a direct one-to-one relationship exists between the specific end organs and their responsiveness to distinct stimuli (Melzack and Wall, 1962).

Von Frey's contributions essentially ended with a description of sensory events in the peripheral nervous system. Subsequent studies of nerve fibers revealed that specific fiber types existed which could be distinguished by differences in diameter, degree of myelination and conduction velocity (Sinclair, 1967). Consequently, investigations were performed to determine whether discrete fiber types were involved in the transmission of different kinds of sensory information. Based on the assumption that pain sensation required high intensity stimulation and on the observation that the awareness of painful sensation from a particular stimulus was preceded by the awareness of touch or pressure, etc., from the same stimulus, investigators began to study whether the slower fibers were responsible for transmitting "pain" information.

Studies by Burgess and Perl (1967) demonstrated that one class of fibers, A-delta, transmitted impulses only when the skin was damaged by crushing or pinching. Other studies (Bessou and Perl, 1967) showed that small diameter C-fibers fired when the skin was damaged by pressure or heat. Thus, it was reasoned that these two fiber types were the exclusive pathways for pain information (Perl, 1971).

The early description of spinal pathways for pain came initially from pathological studies and later from surgical attempts to relieve intractable pain (Keele, 1957). In studies of patients with damage to

the anterolateral portions of the spinal cord, it was observed that these patients could perform sensory discriminations in areas where they were analgesic to pain. Based on these findings anterolateral cordotomies were performed on patients who were suffering from severe intractable pain. Subsequent histological studies located the origin (central gray matter), course, and termination (thalamus), of this pathway and it is now accepted that the spinothalamic tract carries "pain specific" information in the spinal cord (Milner, 1970).

Head (1910) was the first to propose that the "pain center" in the brain was located in the thalamus. This proposition was based on studies of patients suffering from thalamic infarcts (Dejerine-Roussy Syndrome) who reported that they experienced periods of anesthesia followed by periods of excruciating burning pain, which were refractory to analgesics and cordotomy, but were responsive to thalmotomy. Patients with lesions of other areas of the brain did show similar symptoms i.e. anesthesia or hyperalgesia. Lesions of the frontal cortex did produce changes in affective responses to pain but cortical areas were (in the traditional view) considered to be of secondary importance in the perception of pain. In summary, then, the present form of the specifistic approach to pain is as follows: stimulation of free nerve endings in the periphery cause the firing of either A-delta or C-fibers which synapse in the spinal cord and cause the firing of spino-thalamic fibers which then carry the information to the thalamus, where a final synapse to thalamocortical fibers occurs.

Challenges to Traditional Theories of Pain

Arguments against the specifistic approach to pain are many and

varied. These arguments challenge both the fundamental assumptions of specifistic theory as well as the interpretation of the experimental data in support of it. Melzack (1972) has organised these criticisms into three categories based on the nature of the assumption: anatomical, physiological or psychological.

Anatomical

The anatomical assumptions probably represent the most obvious but the least crucial flaws in specificity theory. Von Frey based his proposition that each receptor type responded to one and only one kind of sensory information on deduction rather than on firm experimental data. For example, he reasoned that since both free nerve endings and areas sensitive to painful stimuli were ubiquitous on the skin surface, that the free nerve endings had to be the pain receptors. Similarly, he reasoned that because in his studies, Ruffini end organs were found in all mapped areas sensitive to heat, that these end organs were specific warmth receptors. However, attempts to replicate these results by other authors (Weddell, 1955; Sinclair, 1967: Melzack and Wall, 1962) have been unsuccessful in that responses to heat stimulation have been found in areas where no Ruffini end organs could be located.

Physiological

The physiological evidence in support of specificity theory relies on the demonstration of pain specific fibers which respond to high intensity stimulation. Critics of traditional theories (Melzack and Wall, 1965) suggest that although specialization exists within the somaesthetic system, there is no direct evidence to indicate that a specific fiber type carries information concerned with distinct kinds of sensation.

The fact that fibers can be found which respond to high intensity stimulation is not sufficient grounds to assume that these fibers carry "pain" information to "pain specific cells". Melzack states this argument as follows: "The view that only the cells that respond exclusively to noxious stimuli subserve pain and that the output of all other cells are no more than background noise is purely a psychological assumption and has no physiological basis. Physiological specialization is a fact that can be retained without acceptance of the psychological assumption that pain is determined entirely by impulses in a straight-through transmission system from the skin to a pain center in the brain." (Melzack, 1973, P. 138)

Psychological

The psychological challenge to specificity theory highlights the crucial issues in understanding and appreciating the complex nature of pain. Implicit in classical theories is the concept that all "pain" originates in, and is therefore dependent on, peripheral stimulation. If this were true, then destruction of peripheral nerve fibers from an area should, in effect, eliminate completely the possibility of perceiving any sensations from that area. Similarly, it would follow that stimulation of an area with "intact" neural and sensory structures should give rise to a report or awareness of sensation in every and all instances. Several kinds of clinical observations clearly indicate that in actuality, this is not the case. For example, in a classic study, Beecher (1966), found that severely wounded soldiers often denied the existence of any pain from their injuries, yet simultaneously they were capable of feeling the sensation of an injection. These patients were presumed to have intact neural structures since upon transfer from the battle scene

they began to suffer pain from their wounds. This classic example of "denial of pain" can not be satisfactorily explained by traditional theories of pain.

A number of studies have been done on patients (Rev. by Melzack, 1973) with phantom limb pain, i.e. amputees who report painful sensations from missing extremities, and studies of patients with causalgias (violent burning sensations in previously injured, but ostensibly healed, tissues). In both these ailments, subjects reported the sensation of pain from body regions which no longer existed or from areas where no apparent direct stimulation had taken place, i.e. spontaneous pain. Once again, specificity theory can provide no adequate explanation for these observations.

Critics of traditional theories also cite reports of patients who have received surgical treatment for the relief of chronic pain. Lesions have been placed at virtually every level of the nervous system from the dorsal roots to the thalamus, yet in many cases (especially causalgias) relief from pain, if it occurs, is often transient. Thus, the perception of pain can persist even following massive damage to the nervous system (Sunderland, 1968).

Another less obvious criticism of traditional theory is the implicit assumption that certain sensations are inherently "painful". It is clear, however, that the nature of the perception of a particular stimulus can be strongly influenced by the affective and/or motivational state of the organism. For example, previous experience and cultural determinants as well as other situational variables such as attention, suggestion, and especially anxiety, have been shown to be able to influence reports of pain sensation. Hill et. al. (1951) performed some interesting experiments

which serve to illustrate this point. They found that a given level of electrical shock or burning heat was perceived as less painful under a "low anxiety" situation (by assuring the subject that he could control the pain producing stimulus) than under normal or "high anxiety" conditions. In addition, they also demonstrated that morphine diminished pain in the "high anxiety" situation but morphine had no demonstrable effect in the "low anxiety" setting.

Role of the Central Nervous System

Examination of the evidence from these and numerous other clinical investigations suggested, that at the very least, so called "higher" or cortical functions played a much greater role in controlling and modifying the quality and intensity of sensation which reached awareness, than classical theorists had attributed to them. Until recently, however, there was no direct anatomical evidence to support the hypothesis that central or cortical systems were involved with primary sensory function.

A crucial advance in this area came from post-mortem studies of patients who had undergone surgical anterolateral cordotomy (Keele, 1957). These investigations revealed a marked discrepancy between the number of degenerating fibers ascending in the spinal cord and the number of fibers entering the thalamus. Subsequent studies, both physiological and anatomical, demonstrated the existence of spino-reticular as well as reticularcortical, and reticular hypothalamic pathways (Truex and Carpenter, 1969) (Mehler et. al., 1960). These studies provided the first anatomical evidence against the traditional "transmission line" approach to pain pathways and, in addition, gave credibility to the suggestion that reticular as well as cortical and limbic structures

could directly influence (via ascending and descending mechanisms) the quality and quantity of somasthetic input.

Modern Concepts of Pain

The weaknesses of specificity theory were not inapparent to early investigators. One of the first and most interesting alternative theories was formulated by Goldscheider (Rev. by Melzack, 1973). He began, initially, by reviewing studies performed on patients with Tabes Dorsalis (neurosyphilis). He noted that one of the major symptoms in these cases were changes in the spatial and temporal perception of somatic input. One of the most peculiar findings was the observation that "mild" stimuli applied over a period of time eventually became extremely painful (temporal summation). In addition, these patients demonstrated extremely long latencies between the application of a stimulus and the onset of pain (sometimes up to one minute). Goldscheider (Noordenboos, 1959) reasoned that a central (dorsal horn) summating process must be directly involved in the sorting out of somatic information. He reasoned that a particular pattern or patterns of nerve impulses were necessary to produce this summation and that at some "critical" level, ordinary or nonnoxious stimulation could evoke painful sensations.

The original "pattern theory" has been modified by a number of authors. Weddell (1955) and Sinclair (1955) suggested that spatial and temporal summating mechanisms would, in themselves, explain all pain phenomena, proposing that non-specific receptors were responsible for generating these impulses. They thus diminished the significance of physiological specificity of the peripheral receptors in the overall scheme of "pain" perception.

Livingston (1943) was the first to propose a central mechanism as a basis for the various pathological pain states, e.g. phantom limb pain and causalgias. He suggested that some disturbance must be occurring which sets up a "reverberating circuit" in neuronal pools of the spinal cord. He reasoned that such circuits were responsible for the maintenance of chronic pain states. One flaw in this and other extensions of pattern theory is that it fails to explain why surgical lesions in the spinal cord very often fail to eliminate chronic pain (White and Sweet, 1969).

Noordenbos (1959) suggested that a specialized input controlling system normally prevents summation from occurring and that destruction of this system leads to pathological pain. This system was hypothesized to be composed of a rapid conducting inhibitory (myelinated, new, epicritic) component and a slower conducting (un-myelinated, old, protopathic) component which together transmit sensory information from the periphery. In pathological pain states, the fast system was said to lose its dominance over the slower system,resulting in hyperalgesia. Thus a ratio of large to small fiber inputs would determine the nature of somatic input (Melzack, 1973).

As previously discussed, it was well acknowledged that a number of psychological variables, e.g. motivation and attention, etc., could strongly influence whether a stimulus was, in fact, "painful". Unfortunately such variables were not accounted for in traditional theories. Sherrington (1906) was probably the first to formally assert that the brain did not process input with "absolute indifference" and he stated that "affective tone is an attribute of all sensation". Even so, until recently cognitive and/or

motivational processes were relegated to a secondary position in pain theories. Such factors, as anxiety or conditioning, were referred to as contributing to the "reaction component" of pain rather than being considered as parallel influences which could interact directly with sensory processes (Melzack, 1973).

Gate-Control Theory

Of all the modern theories of pain, the "Gate-Control Theory" of Melzack and Wall (1965) has received the greatest amount of attention and has provoked the most controversy. The originators of this theory sought to formulate an integrated and comprehensive theory of pain which could account for all the various phenomena otherwise unexplained by previous theories, e.g. the role of temporal summation, physiological specialization, and affective processes, etc.

The "Gate Theory" proposes "that a neural mechanism (T cells) in the dorsal horns of the spinal cord act like a gate which can increase or decrease the flow of nerve impulses from peripheral fibers to the central nervous system. Somatic input is therefore subjected to the modulatory influence of the gate before it evokes pain perception and response. The degree to which the gate increases or decreases sensory transmission is determined by the relative activity in large diameter (A-beta) and small diameter (A-delta and C) fibers and by descending influences from the brain. When the amount of information that passes through the gate exceeds a critical level, it activates the neural areas responsible for pain experience and response", (Melzack, 1973, P. 153).

The "Gate-Control Theory" was initially received with a good deal of enthusiasm because it seemed to provide a satisfactory explanation for a variety of phenomena, e.g. pathological pain states and descending cortical influences, etc. Unfortunately, of late, the "Gate-Theory" has engendered a great deal of criticism (Campbell and Taub, 1973) (Schmidt, 1972) and much of the experimental evidence in support of the theory has been disputed.

The "Gate-Theory" had originally evolved as a consequence of studies (Iggo, 1960) whose goal was to locate small fibers which responded to nociceptive stimuli. These studies were unsuccessful in that very few peripheral fibers conducted impulses following stimulation of nociceptors (free nerve endings). In addition smaller fibers were found to be sensitive to light tactile, but not noxious, stimuli. These results were in apparent contradiction with earlier experiments (Bishop and Heinbecker, 1935) which had demonstrated that direct electrical stimulation of nerve fibers, sufficient to elicit the firing of small nerve fibers, produced pain. The "Gate-Control Theory" with its concept of negative feedback inhibition, was, in essence, an attempt to resolve this apparent paradox (Taub, 1974).

Wall and Sweet (1967) realized the possible clinical implications of a negative feedback system. Using implanted electrodes, they electrically stimulated the peripheral nerves of patients suffering from various kinds of chronic pain. They reported that temporary pain relief was provided by these treatments. No action potential or other electrophysiological data, which could directly substantiate a negative feedback system, was provided in this or other similar studies (Meyer and Field, 1972). Nonetheless, it was claimed that the analgesia produced was a consequence of

direct stimulation of large fibers. The success of "local percutaneous electrical analgesia" and a similar technique involving electrical stimulation of the dorsal columns of the spinal cord for relief of pain (Nashold and Friedman, 1972) were subsequently invoked as "proof" of the validity of the "Gate-Control Theory".

Electrophysiological studies by Taub (1974) and others (Schmidt, 1973) have shown that electrical analgesia appears to occur not by selective stimulation of large fibers, but rather on the contrary, by the blockade of the smaller C and A-delta fibers.

In addition, Taub (1974) found that blockade of tactile pain only occurred at or near levels at which pain was reported from electrical stimulation alone. Taub therefore concluded that electrical analgesia is the result of direct blockade, probably by fatigue, of the firing of sensory fibers, rather than arising by a spinal "gating" mechanism. He further suggested that Dorsal Column Stimulation acts by creating a functional spinal cord transection and not by "jamming" a "gate-control" mechanism.

Summary

It should now be clear that in the field of pain research, there are numerous theories but also disagreement over the experimental results. The "Gate-Control Theory" and other similar modern approaches to the study of pain have served at the very least to stress the importance of cognitive and affective processes in the perception of pain. It should also be evident that any study of pain or related phenomena can not ignore such considerations in their experimental design. Unfortunately, such factors as motivation, and learning, etc. are often neglected in studies purporting to evaluate the nature of pharmacologically produced analgesia. It is this

last point which is particularly relevant to the present study and will be discussed in greater detail.

Analgesic Tests and the Measurement of Pain

> "There are few problems as complex, as frustrating, and yet as challenging as the subject of pain and its measurement. There are few fields in pharmacological literature that can leave the reader feeling as confused as those pertaining to the measurement of pain." (Foldes, et. al., 1964. P. 113).

Analgesic or antinociceptive agents are broadly defined as drugs which diminish the perception of, or response to, noxious stimuli. They are distinguished from general and local anesthetics in that unlike the former, they can relieve pain without producing unconsciousness, and from the latter in that they do not prevent the transmission of nerve impulses from the point of contact to the central nervous system.

Turner (1965) has classified analgesics into three broad categories based on the site of action:

1) Peripheral antipyretics (e.g. salicylic acid) which are analgesic to inflamed tissue where they decrease temperature and relieve edema.

2) Hypothalamic antipyretics (e.g. aminopyrine), which are analgesic to normal and inflamed tissue and also diminish edema.

3) Narcotic analgesics which act on normal and inflamed tissues but do not effect temperature or edema.

Most standard analgesic tests which are employed for evaluating potent narcotic analgesics, e.g. morphine, are with few exceptions, insensitive to non-narcotic agents, e.g. propoxyphene and aspirin, except at toxic doses.

Analgesic Tests

The problems of accurately evaluating analgesic agents in animals and in man has been a perplexing one for pharmacologists. The vast number of pharmacological procedures designed for this purpose over the last fifty

years is an indication of the difficulties inherent in this field.

The strongest obstacles hindering an accurate evaluation of the "pain" relieving qualities of analgesics stem from the arguments presented in the previous section on pain. Since it is evident that pain is a subjective phenomena, therefore any measurement made by an observer can only reflect what is presumed to be the reaction to a "painful" stimulus rather than the "pain" itself. This problem is not merely semantic and is especially significant in animal studies, where it becomes necessary to ask several questions when interpreting experimental data:

1) Does the animal recognize as painful, the stimulus employed?

2) Is the response or lack of response in a subject an indication of analgesia or of some other effect, e.g. neuromuscular blockade?

3) What is the behavioral (motivational state) of the subject with respect to the testing environment, i.e. does the subject receive any reward or positive or negative reinforcement for a particular response? For example, will the production of a particular response by a subject cause termination of a test and therefore of the aversive stimulus?

This last question requires further discussion. In almost all animal experimentation, no matter how sophisticated, the response(s) measured is only the end product of the interaction of many factors. In analgesimetry, this may entail a subtle interplay between the test environment, the physiological and/or the behavioral actions of the drug, and the motivational state of the organism being tested.. For example, Kayan et. al. (1969) demonstrated that prior exposures to a testing situation could influence the response of mice in a procedure for evaluating analgesic tolerance. Similarly, Gebhart and Mitchell (1971) showed that modifica-

tions in the test apparatus alone were sufficient to cause changes in the analgesic response. In another set of experiments, Gebhart et. al. (1972) showed that stress (restraint or noise) could influence subsequent performance in an analgesic test. In addition, Jacob (1963; et. al., 1966) demonstrated that a significant learning component exists even for the supposedly "simple" jumping response commonly measured in the "hot plate" technique. Tamayo and Contreras (1970) found that prior exposure to a testing situation could influence the results of analgesimetry in one kind of test, "tail-pinch," but not in the "hot plate" method.

Thus, as was emphasized in the section on pain, consideration of behavioral variables is of paramount importance in comparing and evaluating studies of analgesia and analgesic agents, especially in the light of the work of Hill et. al. (1952), which has been mentioned, on the influence of anxiety on analgesic measurement. Nonetheless, most standard analgesic tests measure only a small part of what is certainly a most complex phenomena. Yet most authors take for granted that the behavior or behaviors evaluated in their procedure are, in fact, directly measuring pain (or analgesia).

One last point is worth mentioning in this context, the difference between so-called "pathological pain" and "experimental pain". In human studies, it has often been noted that, analgesics (especially non-narcotic agents) are more effective in relieving experimentally induced pain (from pin-prick or radiant heat) than they are in diminishing pathological pain as in cancer etc. This discrepancy is generally ascribed to differences in motivation rather than the source of discomfort, i.e. the normal healthy subjects are said to be cognisant of the transitory nature of the test situation, whereas the actual patients required more relief due to

the chronic nature of their condition (Keele, 1959). This last example serves to reinforce, once again, the importance of the "overall state of the organism" in evaluating analgesia.

Animal Algesimetry

The analgesia tests in common use range from crude to elegant and include procedures which measure the simplest reflexes as well as the most complex learned responses. Part of the reason for the wide variety of analgesic tests is related to the original impetus for the design of such tests, which was, in effect, to provide a simple and reliable means of screening new compounds for analgesic activity. As a consequence, early investigators were not interested in the specificity of these tests for detecting "distinct analgesic properties", but instead they were more interested in finding quantitative methods for selecting compounds with potential for further clinical studies. Thus, these workers, and to a wide extent, most authors in the field did not attempt to ascertain the nature of, or the process(s) underlying the behavior they were measuring.

As a result, standard testing methods employed today, although widely used and referred to, are rather non-specific in that non-analgesic agents elicit comparable results to those obtained by known analgesic agents. Similarly, the relative potencies of some agents may vary from test to test. One example of this was the finding that nalorphine (a partial agonist) demonstrated no analgesic activity as measured by the Tail-Flick test (Winter, 1965) (Woude, 1967), yet this drug was known to have analgesic actions in man and, in addition, it was later found that nalorphine had analgesic activity when measured by another testing procedure (abdominal stretching) (Bentley et. al., 1965).

Another problem encountered in examining the literature in this field

is that the so-called standard testing procedures, e.g. Hot-Plate (mice) and Tail-Flick (rat) are often modified in a variety of fashions to suit the needs of individual investigations. Such modifications introduce another confounding variable as work by Jacob (1962) has clearly demonstrated. He found, as will be discussed below, that the order of potency and relative activity of certain analgesics may vary with changes in stimulus level.

Classification of Analgesic Tests

Analgesic tests can be classified in two general ways, first according to stimulus quality: 1) chemical 2) electrical 3) thermal and 4) mechanical, and second, according to the method of quantification employed. In this respect, procedures can further be subdivided into two categories based on whether or not the stimulus intensity is kept constant.

In one case, constant intensity, the activity of an agent is assessed in relation to the disappearance of a response. In this manner an "all or none" response allows for direct quantitative evaluation, as in the "Tail-Clip" method in which the failure of a mouse to attempt to dislodge a tail clamp (applied at a constant pressure for a fixed amount of time) is regarded as a measure of analgesia. Graded responses may also be used. For example, latencies of response can be measured in the Tail-Clip method, which are then evaluated with respect to standard or control values. Similarly, some directly measurable parameter may be employed,e.g. the number of peritoneal stretching in the intra-peritoneal writhing test.

In the second case drug activity is measured in relation to a stimulus parameter required for the production of a given response. This parameter may involve changes in both duration and intensity of stimulation.

In most standard tests, the stimulus strength is kept constant and response

delay (reaction time) is measured. These tests permit the use of large numbers of subjects, but contain the drawback of introducing arbitrary cut-off times which can skew results.

In situations where the stimulus duration is kept constant, thresholds are measured. One author, (Jacob, 1966), argues that, in practice, stimulus duration is not truly held constant, since it (duration) fluctuates during the time when threshold values are being sought. Similarly, when duration is kept constant, repetition of the stimulus is necessary. These procedures, however, are considered to be more "accurate" than these using constant intensity because they are less influenced by arbitrary "cut off" values.

Tail-Flick Method

Probably the oldest and most prototypical of the standard analgesic tests is the Tail-Flick method originally designed by D'amour and Smith (1941). They used rats as the test animal and a beam of light on the terminal part of the rat's tail as the stimulus. It was found that the normal animal responded in a matter of seconds by removing its tail from the beam of light. The criterion for the action of an analgesic drug was failure to move the tail out of the beam within a specific time limit (Winter, 1965) (Bonnycastle, 1962).

An early modification of this technique was the darkening of the rat's tail in order to intensify the stimulus. One drawback of this and similar modifications was the production of tissue damage, especially in situations where repeated determinations were performed on the same animal, or when the more potent analgesics were being tested,e.g. morphine (Domer, 1971).

A later modification (Bonnycastle and Leonard, 1950) sought to eliminate the problem of tissue damage by using pre-trained rats. It was

found that a training period was necessary before naive rats "learned" to escape the beam. Most rats learned the response following several testing sessions and non-learners were eliminated. Previous observations by Wolff and Hardy (1947) had shown that the stimulus duration necessary to elicit a "pain response" was approximately half that required to produce tissue damage. The authors (Bonnycastle and Leonard, 1950) sought to control the duration of the stimulus in order to prevent tissue damage. This was accomplished by determining the normal reaction time for each rat in an experimental group. The mean reaction time (multiplied by a factor) was then employed as a cut off or "normal allowable period of stimulation" (Bonnycastle, 1962). Proponents of this method claim that this modified Tail-Flick test is sensitive to a broad range of analgesics,i.e. from aspirin to morphine, (Winter, 1965).

A number of authors disagree with these findings. Some investigators feel that the stimulus intensity used in the Bonnycastle procedure is too weak and that this tends to generate broad variations in normal reaction times. Other authors (Winter, 1965) have found that training did not improve the sensitivity of the test. Results of experiments with mild analgesics have been inconsistent and one investigator suggests that responses observed with these drugs are toxic reactions (Hicak, 1964) (Jacob, 1966).

Jacob points out in his review of the subject (1966) that this test (Tail-Flick) is unsatisfactory as a means of evaluating analgesia because not only are the animals restrained, but, in addition, the measured response (tail-flick) involves such a low order of sensorimotor coordination that it can be elicited even in chordotomized animals (Houde, 1967).

Nonetheless, the Tail-Flick test is widely used and a number of new

modifications have been developed. One of these (Janssen et. al., 1963) consists of simply (in place of the radiant heat source) immersing the rat's tail in hot water. In other variations (Nicak, 1964) ultrasound, as well as mechanical and electrical stimuli, are employed. Electrical stimuli appeared to produce more "struggling" than tail-flicking and several authors have ignored the tail-flick, and chosen to record the "struggle" response instead (Ercoli and Lewis, 1945).

Tail-Clip Method

The Tail-Clip method is another of the standard procedures for screening analgesics. It was introduced and extensively studied by Bianchi and Franceschini (1954). In this test, mice are injected with a standard dose of a substance and then the clip, fabricated from an arterial clamp which is enclosed in a rubber casing, is applied to the base of the tail for a fixed length of time (30 seconds). Untreated mice attempt to remove the clip, whereas treated mice are found to be indifferent to it. Control values are obtained by adjusting the clip pressure to a level just sufficient such that all naive control mice attempt to dislodge it (Turner, 1965).

Calculations of potency are made on the basis of the percent of mice which respond to a given dose. Thus, this test fits into the fixed stimulus duration "all or none" category. Some authors claim that this test is an improvement over tests which use thermal stimuli, i.e. hot-plate and tail-flick. They contend that the response to mechanical stimulation requires "higher order" (supraspinal) integration.

One modification of the "Tail-Clip" test was designed by Bianchi and David (1960). Rather than adjusting the clip pressure until a criterion of 100% response was reached, they simply eliminated all mice which did

not respond within 15 seconds. These mice then served as their own controls when retesting occurred following drug administration.

Caudal Compression

Another test, "Caudal Compression", is somewhat more complex than those previously mentioned, (Green and Young, 1951) (Domer, 1971). The apparatus for this procedure is designed to produce a uniformly increasing pressure on a rat's tail. It consists of two syringes connected tip to tip by means of a flexible and portable fluid filled tube. A side arm of the tube is connected to a manometer. The rat's tail is placed between the plunger of one syringe and a stationary surface. In this manner, uniform pressure on the other syringe compresses the tail and also registers on the manometer. Responses are recorded when the rat begins to struggle or when it vocalizes with a squeak. An arbitrary index of analgesia is employed, e.g. when the squeak threshold is double that of a control group.

Hot-Plate Method

Although the tail-flick test is probably the most commonly used procedure for blind screening, the hot-plate test is commonly employed in experiments where tolerance and other pharmacological phenomena are being studied.

The original test was designed by Woolfe and Macdonald (1944). Many variations of this test have evolved over the years, for example: changes in plate temperature, whether constant or varying; size and shape of the test container, the number and durations of exposures to the plate, criterion for response, and treatment of data. In the original method of Woolfe and Macdonald (1944), mice were placed on a heated zinc plate and latencies for any one of a number of "escape oriented" responses were recorded. In

this manner, a large number of animals could be tested and the difference between control and post-treatment latencies were computed.

The hot-plate technique most commonly used today is the procedure of Eddy and Leimbach (1953). In this modification, the temperature of the plate is kept constant by a boiling fluid mixture. The container is a cylinder which allows the subject to walk and to jump. Reaction times are recorded. Latencies of responses such as rapid withdrawal of paws, "dancing", paw licking, and jumping out of the cylinder, are all grouped together.

In the standard Eddy-Leimbach procedure, reaction times were determined at various intervals following drug injection. The criterion for an analgesic response was the number of mice (in groups of 10) which failed to respond within thirty seconds after exposure to the hot plate (Winter, 1965). The sensitivity of this method is claimed to be equivalent to that of the Tail-Flick test (Banziger, 1964).

Although the "Hot-Plate" method has the advantages of being both instrumentally simple and convenient for serial measurements, it has been shown to have a number of practical and theoretical drawbacks. In particular, it is evident that a variety of receptors other than those of "pain" per se, are stimulated (thermic, tactile, visual, etc.) and therefore the observed reactions, e.g. paw licking, jumping, etc. represent complex and probably distinct behaviors in which physical pain may play only a limited or minor role (Bianchi and Franceschini, 1954). Thus it is not surprising that a variety of non-analgesic substances can modify behavior on the hot-plate.

Jacob (1962) sought to analyze the different behaviors of animals on the hot-plate. He found that changes in plate temperature (within the range employed by most investigators) could influence both the order of appear-

ance of various responses, e.g. jumping or licking as well as the frequency and latencies of response. Subsequently, he found that repeated exposure to the hot-plate caused a change in the relative frequencies of different responses, and furthermore, he found that the percentage of animals reacting with a certain response on re-exposure depended on the "plate temperature". When morphine was administered, he observed a differential susceptibility of various behaviors to the action of the drug, which was related to the amount of exposure to the hot-plate prior to morphine administration.

Thus, Jacob (1962) concluded that a number of intervening variables must be taken into consideration when comparing data from experiments performed using different modifications of the test procedure and different response criteria.

Tests of "Mild" Analgesics

A number of tests have been designed to evaluate intermediate or "mild" analgesics, e.g. nalorphine, propoxyphene, etc. The most reliable of these has proved to be the peritoneal writhing test originated by Hendershot and Forsaith (1959). In this procedure, mice are injected intraperitoneally with a heated solution of phenylquinone. The mice are then observed for twenty minutes and the number of "writhes" per mouse is recorded (approximately eighty percent of mice do not writhe and are eliminated from the test population).

On the following day, those mice that did "writhe" are injected with the test drug and twenty minutes later, the phenylquinone is reinjected. The percent of "protection" against writhing produced by a compound at each dose level is computed and ED50's are then calculated. One popular

modification of this test was developed by Okun et. al. (1963). They used benzylquinone which is water soluble and is not an effective inflammatory agent except when given intraperitoneally. Okun et. al. quantitated their results by finding the dose necessary to completely inhibit writhing in 50% of a group of ten mice.

The "writhing" test and other similar tests for mild analgesics, although valuable as screening methods are very non-specific. In fact, antihistaminics, parasympathomimetics, sympathomimetics, adrenergic blocking agents, and a variety of other agents can inhibit writhing at "non-toxic" doses (Turner, 1965).

Problems, similar to those encountered with the "hot-plate" test, are also present in the "writhing" test. The so-called "writhing response" encompasses a wide range of behaviors including stretching, torsion, hind leg retraction, and abdominal scratching, and consequently, authors differ in their interpretation of the significance of the various responses (Winter, 1965).

One additional puzzling finding has been noted wtih regard to the writhing test. Benzylquinone injections were found to have equivalent effects on mice whether they were housed individually or in groups. However, grouped mice showed a greater inhibition of writhing, following injection of narcotic or non-narcotic analgesics, than did mice which were housed individually (Turner, 1965).

Analgesic Tests Employing Electric Shock

Several tests employing an electric shock as an aversive stimulus have been designed. These techniques fall into two categories. The tests in the first category are similar to those previously mentioned in that they

measure some response latency to the noxious stimulus (foot shock or current applied directly to the tail) which is measured in either a free moving or restrained subject. Tests in the second category are more complex and are oriented towards behavorial analysis rather than analgesic screening. In general these tests involve some alteration in a learned response (e.g. escape or avoidance of the noxious stimulus following drug administration) by the performance of a specific learned response.

The reflex changes caused by electrical stimulation of a rat's tail were studied by Carroll and Lim (1960). Thresholds for a variety of withdrawal reactions were calculated. Narcotic analgesics were found to inhibit vocalizations at lower doses and diminish other postural and somatic responses at higher doses.

Similar studies were performed in mice (Wilsen, 1961). An electrode was inserted in the tail of a restrained mouse and the threshold (voltage) for vocalization was measured. The relative number of non-vocalizing mice following drug administration was employed as an index of analgesia.

Surprisingly, no reports of a test which employs a "foot shock" as a stimulus and a jumping reaction as a response, have appeared in the literature.

Analgesia and Learning

In general, there has been little interplay between the disciplines of experimental psychology, e.g. operant conditioning, and the study of analgesia and analgesic reactions per se. This is understandable considering the original goals of most analgesic research, i.e. screening, and the time consuming nature of most behavioral techniques.

The most sophisticated behavioral test devised to measure analgesia was designed by Weiss and Laties (1958). The authors were aware of the need for an analgesic test which could first, measure a more "total" and integrated response to pain rather than a simple reflex latency, and second, could measure accurately a wide range of analgesics, i.e. aspirin to morphine, at analgesic and not toxic doses.

In what has been termed a "fractional escape" situation, rats were trained to press a lever to reduce the intensity of a shock delivered to the floor of an enclosed chamber. Pressing the lever at certain intervals caused the shock levels to stabilize and greater lever pressing rate caused the shock intensity to be decreased. Analgesics were found to act by increasing the "tolerated" shock level (Weiss and Laties, 1958).

The authors (Weiss and Laties, 1970) argue that this test is preferable to standard avoidance procedures, i.e. tests in which diminished performance in an animal previously trained to avoid a noxious stimuli is interpreted as a measure of analgesia, since it had been suggested that the effect of morphine on suppressing avoidance was related to general CNS depression rather than analgesia (Winter, 1965). Weiss and Laties studied varying shock intervals and response-shock reduction ratios. They found that analgesics could raise the tolerated level of shock at doses insufficient to cause any change or impairment in escape behavior. In addition they found that pentobarbital (a non-analgesic sedative) did not alter tolerated shock levels at doses which caused alterations in avoidance behavior. McConnell (1962), however, did not find a qualitative difference between morphine and chlordiazepoxide (a tranquilizer and muscle relaxant known to influence avoidance behavior), i.e. he found that the minimum effective dose necessary for raising the threshold for fractional escape

was the same dose necessary or depressing continuous avoidance.

Avoidance and Screening Tests

Critics of the "fractional escape" method argue that the technique may measure subtle motivational and performance alterations induced by narcotics, but that such parameters do not truly reflect the "sensory components of pain", which are believed to be directly affected by analgesics (Houser and Pare, 1973).

Houser and Pare (1973) have devised a test which they claim overcomes the drawbacks of previous techniques. This method involves the measurement of analgesia using a spatial preference test. Rats were tested daily for 50 minutes in a Plexiglass shuttle box or "tilt cage". Electric shocks (constant current) of gradually increasing magnitude were delivered through a grid floor at each session for 6 days. The animals could escape or avoid the shock by crossing to the opposite side of the apparatus. An aversive threshold was arbitrarily defined as the shock level which an animal was found to avoid 75% of the time. A stable threshold was achieved in all mice by Day six. The authors found that narcotic agonists as well as antagonists could elevate the aversive threshold in a dose dependent fashion. The authors claimed, that unlike other avoidance tests, the results could not be ascribed to sedation because pentobarbital was only able to elevate the aversive threshold at doses which severly hampered the execution of the escape response.

The authors claim that this test is a more satisfactory screening procedure than other avoidance or "titration" methods because the measured response is relatively uncomplicated and therefore more analagous to clinical "pain" stituations, (Houser and Pare, 1973).

Summary

All the foregoing procedures are considered to be "direct measures of analgesia", i.e. they purport to measure a specific diminution in an otherwise pain specific response. Clearly, as with reflex escape responses, this is not the case. Other indirect methods of analgesic testing are also employed. For example, in the Straub-tail test (Aceto et. al., 1969) a particular idiosyncratic response (extensor rigidity of the tail) is found to occur following administration of a particular class of drugs (narcotics). In such tests no noxious stimuli are employed. An unknown compound is simply examined as to whether it can elicit the required response.

As demonstrated by the work of Jacob (1963), Gebhart and Mitchell (1971) and others, there are additional variables introduced into the testing situation when repeated stimulation and drug administration are performed on the same animal. These particular variables (drug-test interactions) are not relevant considering the goals of screening procedures, but are highly significant when one is attempting to evaluate a learned behavior or investigate tolerance phenomena. Such interactions, e.g. behavioral tolerance, will be dealt with in greater detail in the discussion.

Tolerance

The phenomenon of tolerance is one of the most frequently studied in pharmacology. Although much information has been gathered concerning the parameters which govern tolerance, the underlying mechanisms involved have continued to remain obscure.

Tolerance can be defined as a decreased responsiveness to a drug following repeated administration. Thus tolerance can be expressed as either the change in some response parameter upon chronic administration or the quantity of drug necessary to maintain the initial response level (Hug, 1973).

Tolerance may develop very rapidly (minutes or hours) and this is termed acute tolerance or tachyphylaxis, or it may develop over long periods of time and this is termed chronic tolerance.

Cross tolerance denotes the ability of one drug to produce tolerance to another drug, e.g. morphine and meperidine. Cross tolerance occurs between drugs with similar pharmacological actions, i.e. between tranquilizers and barbituates but not between narcotic analgesics and the sedative/tranquilizer drugs.

Tolerance may not occur to every pharmacologic action of a drug, nor does tolerance necessarily develop at equal rates and to an equal degree for each action of a drug.

In experimental studies, investigators have sought to correlate the acquisition, progression, maintenance, and loss of tolerance with a variety of parameters. For example, numerous studies have been performed in order to elucidate the relationship between dose level, interval between drug administration, etc., and the rate of tolerance development.

Tolerance and physical dependence are often considered to be a reflection of the same underlying process. This is because of the observation that physical dependence (defined as withdrawal phenomena which can be elicited upon cessation of drug administration), develops to drugs which are also capable of producing tolerance. In addition, tolerance development has been shown to precede the onset of physical dependence and disappear following withdrawal.

Many authors feel the evidence suggesting a common etiology for tolerance and physical dependence is sufficiently compelling to assume that one process is a reflection of the other. However, tolerance does develop to drugs which are not known to produce physical dependence, e.g. marijuana. Thus, tolerance and dependence could be a result of two parallel but separate processes. Similarly, it is equally possible that different mechanisms could underly tolerance to the various pharmacological actions of a drug. Also, it is possible that a number of mechanisms may be responsible for a single manifestation of tolerance phenomena.

Tolerance to narcotic analgesics can easily be demonstrated both clinically and in laboratory experiments; many, if not all of the original theories proposed to account for tolerance, were based on studies of the action of morphine. Tolerance to the effects of morphine have been demonstrated for a large number of it's actions, e.g. hypothermia, analgesia, locomotor activity in mice (Rethy et.al., 1971) (Rev. by Hug, 1973), etc. However, tolerance has not been demonstrated for such actions as miosis, seizure threshold changes, and alterations in gastrointestinal motility. In addition, intra as well as interspecies variations in absolute as well as relative production of tolerance have been observed (Cochin, 1970). In addition, the great variation in experimental procedures and methods

of data evaluation have produced a good deal of confusion in this area, and may account for some of the conflicting evidence.

For many years a dichotomy was made between acute and chronic tolerance to morphine, based on the original work of Schmidt and Livingston (1933). They found that massive single doses of morphine could confer tolerance to subsequent doses of morphine. They measured a decreased responsiveness to such effects as vomiting, hypotension, incoordination, etc. Martin and Eades (1961) showed that tolerance to analgesia, as well as other actions of morphine, could be demonstrated following a continuous short term infusion. In other experiments (Martin and Eades, 1964), they were unable to demonstrate acute tolerance to the effects of morphine in chronic spinal dogs.

Recent evidence tends to suggest that, at least, in the case of analgesia there is no qualitative difference between the tolerance observed following rapid infusion of high doses and chronic or intermittent administration of small doses of morphine. Thus, within the limits of experimental technology, it appears that tolerance development is a function of both frequency and dosage administered and therefore the degree of exposure of tissues to morphine appears to be the key factor which governs the development and maintenance of tolerance (Hug, 1973).

Tolerance to morphine analgesia can develop to an extraordinary degree, in that previously lethal doses may have almost no discernible effect in a tolerant subject. Yet, tolerance development is never found to be absolute; some effect can always be elicited with a high enough dose unless toxic effects (e.g. convulsions) supervene (Martin, 1970).

Theories of Tolerance and Dependance

The manner by which tolerance and physical dependence occur has long puzzled and intrigued investigators. A sizeable number of theories have been proposed to account for these phenomena; however none of them have gained universal acceptance. Some of these hypotheses differ widely in their approach and postulated mechanisms, whereas others are fairly similar in most key features. Implicit in most of these theories is the concept that the mechanisms of tolerance development simultaneously provide the basis for understanding physical dependence. It should be remembered that although the evidence for a single mechanism of tolerance and dependence is substantial, it is not as yet conclusive. Therefore, it is possible that one theory may accurately account for tolerance development but not at all explain physical dependence.

Marmé (1883) can be credited with proposing the first theory of morphine tolerance and physical dependance. He noted, as many other authors have, that the initial effects of narcotic analgesics are primarily "depressive", i.e. sedation and analgesia, and that these effects diminish relative to the "stimulatory" effects, i.e. changes in gastrointestinal motility, with chronic administration. He devised a unitary hypothesis based on the formation of a morphine derivative with stimulant properties, whose titre rose with chronic morphine administration, thus eventually neutralizing the acute "depressant" effects of morphine. No evidence of a morphine derivative, such as described by Marmé, has been forthcoming but some remnants of the concept of an interaction between distinct stimulatory and depressant systems, is found in more recent hypotheses.

A "Dual-Action" hypothesis of drug dependence was first proposed by

Tatum and Seevers in 1929, to account for cocaine addiction, and was later expanded to include morphine addiction (Tatum and Seevers, 1931). This theory proposes that morphine has a two-fold action on the central nervous system, one action primarily "depressive" and the other "stimulatory". It was postulated that this resulted from their existing two separate receptor sites for morphine, one intracellular and the other extracellular. The extracellular site is said to be responsible for the depressive effects of morphine and is the only site for which tolerance develops (a specific mechanism by which this tolerance takes place is not suggested). As a result, the stimulatory actions of morphine become dominant following long term administration. Withdrawal is described as resulting from the rapid removal of morphine from the membrane site, while morphine, or an active metabolite, is still present intracellularly. This process is said to account for the "hyperexcitability" observed in the abstinence or withdrawal syndrome (Seevers and Deneau, 1968).

The "Dual-Action" theory shares many features (and flaws) in common with the receptor-occupation hypothesis originally proposed by Schmidt and Livingston (1933). Based on observations of acute tolerance to the vascular effects of morphine, they suggested that drug molecules exert their action at the time of occupation of the receptor site and once attached, they produce no effect other than preventing the initiation of a new response, by blocking receptor combination with free drug molecules.

Many facets of morphine action are explained by the latter two theories, such as agonist-antagonist interactions, cross-tolerance, and logarithmic dose-response curves. Recent investigations, however, have indicated that both tolerance and the abstinence syndrome can be observed at intervals long after the cessation of drug administration (Cochin and Kornetsky, 1964; Kornetsky and

Bain, 1968). Therefore, a requisite for the acceptance of the previous theories would be the demonstration of a persistence of morphine or morphine metabolites in tissue after drug administration is discontinued.

Until recently, there has been no evidence that morphine or any of its metabolites lingered in the brain or plasma for any substantial period following termination of drug administration. Using a radioimmunoassay technique specific for morphine (Spector and Parker, 1970), Berkowitz et. al. (1974) determined that, although there is an initial rapid fall in plasma and brain morphine levels ($T_{\frac{1}{2}}$ = 1-2 hours), there is a subsequent gradual fall in plasma morphine levels such that morphine could be detected in plasma 20 days following removal of an implanted morphine pellet. This finding has given new support to the "Dual-Action" theory; however, other investigators (Rev. by Hug, 1973) have observed tolerance to the so called stimulatory effects of morphine. In addition, Cochin (1972) found that the stimulatory effects of morphine, observed during its' administration, do not correspond to the hyperexcitability phenomena observed in withdrawal.

Axelrod's (1956) cellular-adaptation theory approaches the problem of tolerance from another point of view. This theory grew out of evidence that drugs can induce an alteration in the enzymes synthesized for their metabolism. In this manner, continued morphine exposure would induce changes in the receptor proteins or in liver microsomal enzymes known to metabolize morphine.

Initial support for this hypothesis came from evidence (Axelrod, 1956; Cochin and Axelrod, 1959) that the N-demethylase enzyme involved in morphine breakdown shows a decrease in activity with chronic morphine administration. Unfortunately, attempts to isolate altered metabolites or changes in metabolite levels which parallel the time course of tolerance development have been

unsuccessful. In addition, most of the Axelrod's original experiments were performed using methods which are now considered unacceptable and more recent studies using more sophisticated techniques, e.g. radioisotope labeling, have failed to replicate the original results (Cochin, 1972) (Hug, 1973).

Martin (1970) proposes a theory based on the assumption that dependence to morphine is essentially a disease of adaptation to a toxin. He says that this adaptation is a result of "homeostatic redundancy". This theory was devised initially to explain observations that large doses of atropine could block the activity of midbrain reticular systems and that this block could be completely overcome by increasing stimulus strength. It was argued that although all muscarinic synapses were blocked, a secondary non-muscarinic system was still functioning.

Martin (1970) extended his theory to morphine based on studies of the adjustment of animals to the hypothermic properties of morphine. The "redundancy" theory implies that there exist two mor more pathways in the nervous system which are involved in mediating a specific neural function and that these pathways differ in their spectrum of vulnerability to hypertrophy. Martin assumes that morphine interrupts one of these redundant pathways, but not the others. The second part of his hypothesis proposes that the non-susceptible pathways will eventually hypertrophy and bring the system back to homeostatic levels. Subsequently, if morphine were removed from the system, a state of hyperfunction or hyperexcitability would exist, the effects of which would constitute the abstinence syndrome. Martin further refines his theory by suggesting that different levels of tolerance and withdrawal could be related to such factors as the relative functional importance of non-morphine pathways and the relative degree of susceptability to

hypertrophy in nonsusceptible pathways.

Martin's theory is sufficiently broad and schematized to encompass facets of other theories because he does not suggest a specific biochemical or neuropharmacological mechanism that precipitates the hypertrophy of redundant pathways. Since direct evidence for morphine or other drug induced functional and anatomical changes in the nervous system is lacking, Martin's theory remains purely speculative.

Sharpless and Jaffe (1969) proposed a theory of disuse or denervation supersensitivity which is in many ways complimentary to Martin's theory. Both models imply that the development of tolerance and dependence are the extensions of normal compensatory or homeostatic mechanisms by which a cell or physiologic system adjusts to an alteration in its operation.

Emmelin (1961) performed extensive studies on the effects of disuse on cholinergic synapses in the salivary gland. He found that procedures which tended to deprive the gland of normal parasympathetic or sympathetic innervation increased the gland's subsequent sensitivity to acetylcholine or norepinephrine. Sharpless and Jaffe analogize this system to the action of morphine on the CNS. They believe that morphine causes some pre-synaptic inhibition, thereby causing disuse at the post-synaptic site; consequently, there develops increased receptor moieties post-synaptically. Thus, as in Martin's theory, withdrawal is characterized as a manifestation of rebound hyperexcitability, except, in the case of denervation supersensitivity, there is an increase in receptors, whereas in redundancy theory, there is a hypertrophy of neuronal networks.

Direct evidence for increases in receptor moieties has not been forthcoming, but the time course of the development of morphine tolerance appears to correlate

well with Kumelin's data (Jaffe and Sharpless, 1968). In addition, since the theory predicts that disuse hypersensitivity can occur at any type of synapse, it may explain why changes are found in a variety of neurohumoral levels during and after administration of morphine.

At the biochemical level, Goldstein and Goldstein (1961) and Shuster (1961) proposed a theory of homeostasis based on changes in protein synthesis. This "derepression" theory postulates the concept of a morphine induced repression of enzyme synthesis. This enzymatic repression is said to act by inhibiting the actions or synthesis of neurotransmitters required for the proper functioning of the central nervous system (CNS) thus bringing about the effects of sedation, analgesia, etc. Following repeated administration, morphine is said to act at a second site causing the derepression of a system which makes the neurotransmitter synthesizing enzymes. This effect would then increase the amount of neurotransmitter acting in the CNS. Tolerance would occur because more morphine would be required to "depress" the CNS. Alternatively, morphine might also act by increasing the concentration of neurotransmitters by repressing the synthesis of an enzyme that is necessary for the metabolism of the neurotransmitter. When morphine is afterwards withdrawn, the action of the increased amount of neurotransmitter would no longer be counterbalanced by morphine, and central CNS excitation would occur.

Initial support for the "derepression" theory came from studies of the effects of protein synthesis inhibitors on the development of tolerance and physical dependence. A number of studies (Smith et. al., 1961; Loh et. al., 1969) demonstrated that such inhibiters could decrease the agonist effects of morphine and its congeners as well as prevent tolerance development and antagonist induced withdrawal. Such studies, however, are difficult to

evaluate, since protein synthesis inhibitors are highly potent antimetabolites which possess a variety of toxic effects.

All theories of "derepression" postulate the existence of a neurotransmitter whose concentrations are affected by chronic administration of narcotic analgesics. The evidence for the existence of such a compound is inconclusive despite exhaustive efforts (Rev. by Way, 1971) (Rev. by Cochin, 1972). Although changes in levels of serotonin, dopamine, norepinephrine, and acetylcholine, have been observed, such evidence has not been conclusive enough to postulate a causal relationship between these changes and the development of tolerance or dependence.

Cochin and Kornetsky (1968) view the results of the protein synthesis studies from a different perspective; they suggest the possibility that immunological mechanisms might account for tolerance and dependence. In support of their hypothesis, Cochin and Kornetsky cite evidence that there is a delay period between initial contact with morphine and tolerance development and that after the delay period, tolerance to the second dose increases for some time (Kornetsky and Bain, 1968). In addition, Cochin and Kornetsky, (1964) found tolerance to the effects of a single dose of morphine persisted for up to one year following injection. Cochin and Kornetsky argue that these experiments suggest that mechanisms other than alterations in receptor molecules or enzyme induction are responsible for tolerance. They asserted that some sort of immunological response might provide a more satisfactory explanation for their results.

In order to gain further proof, Cochin and Kornetsky (1968) attempted to transfer serum factors from tolerant to non-tolerant animals. The results of these experiments were confounding because the transfer extracts appeared

to increase rather than decrease (as was expected) sensitivity to morphine. As a consequence, Cochin's theory of an immunological basis for tolerance has not gained any degree of acceptance.

Present Investigation

Narcotics addiction is one of the most perplexing problems facing modern society. Much effort has been put forth in the attempt to understand and deal with this complex medical and social phenomenon. Scientific research has contributed to the understanding of some of the pharmacologic aspects of drug addiction, i.e. physical dependance, tolerance, drug self-administration, etc. One aspect of the pharmacology of narcotic drugs, that is still not clearly understood, is the relationship between the administration of these drugs and their influence on the overall behavior and functioning of subjects receiving these substances.

My original interests had been in determining whether "pure" narcotic antagonists, e.g. naloxone, possessed any agonist properties. In particular, I was interested in discovering if narcotic antagonists could influence the development of analgesic tolerance of mice who subsequently received morphine.

Standard analgesic tests usually involve the measurement of some change in reflex latency to a noxious stimulus. These tests are somewhat crude and would have been particularly unsatisfactory for the anticipated needs of this experiment because they are generally insensitive to partial agonists (Glassman, 1971). Consequently, a new test was designed which involved measuring the frequency of escape from a trough-shaped plexiglass box; the floor of which delivered an electric shock.

As expected, a majority of naive mice usually escaped from the box within a few seconds and significantly fewer mice did so following an initial injection of morphine. If classical tolerance were to occur, a significant improvement in escape performance should have been observed on subsequent days. Surprisingly, no improvement was observed in these mice following repeated

daily injections. Even more curious was the observation that these mice remained impaired in their performance for a considerable period of time following cessation of drug administration. When another group of mice were injected with morphine (but not tested daily) for the same number of days as the original group, significant tolerance was observed when the animals were tested following injection on the last day of morphine administration.

These initial results suggested that morphine could induce the learning of an ostensibly maladaptive response, and that this response, once established, could persist even following discontinuation of morphine administration.

The aim of the present study is to describe in depth these phenomena. My experiments have been directed towards two goals. First, I have attempted to determine the nature and degree to which various stimulus parameters influence test results, and second, I have sought to determine the specificity of my findings with respect to various drugs (narcotic and non-narcotic) and to various testing procedures.

This thesis is divided into four sections. In the first section, I have reviewed those aspects of the scientific literature which provide a background, both conceptual and experimental, for the experiments performed. The second section contains a description of the experimental methods and statistical procedures employed for the evaluation of the data. The third section contains a presentation of the results of the experiments followed by the last section, a discussion of these results. This last section will be concerned with relating the results obtained in the present study with those found by other investigators; and finally, I will propose a hypothesis concerning the implications of the present work for the study of drug dependence.

METHODS

Subjects

CF-1 female mice, approximately 90 days old, were used in all experiments. Animals were housed in standard mouse boxes in groups of 16. Food (Purina Lab Chow) and water were provided ad lib. The average weight of a mouse was 20 grams $\pm$ 2 grams.

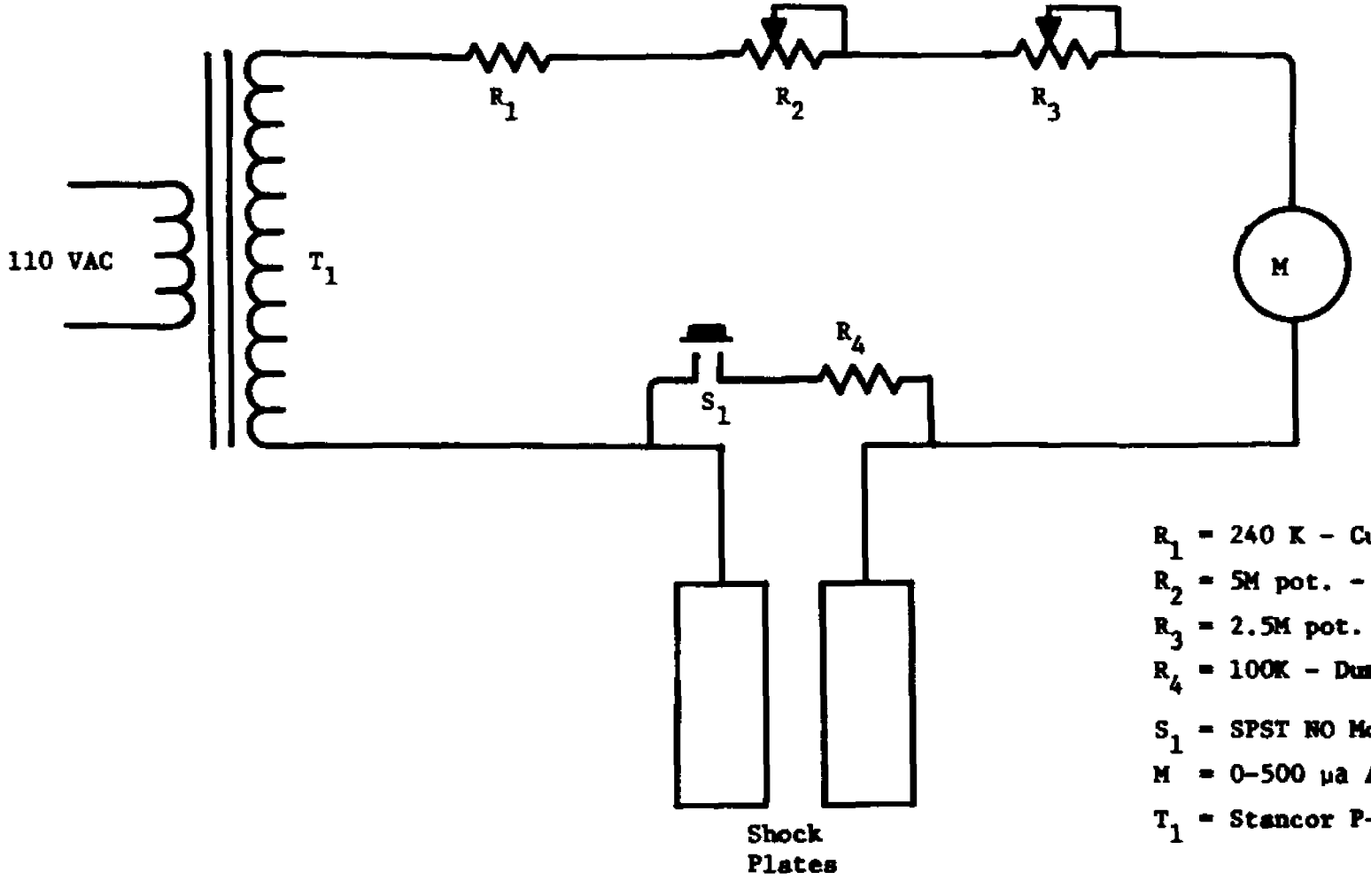
Upon arrival from the supplier, new mice were transferred to their boxes and not employed in any experiments until after at least 48 hours. A 12 hour day-night cycle was maintained in the testing room, which also served as the animal quarters. Cedar chip bedding was used and was changed weekly.

After initial testing all mice were marked for identification purposes using picric acid applied to the fur.

Apparatus

1. Escape Box

The "shock box" consisted of a trough shaped Plexiglass box, 6 inches deep, 10 inches long, 1 1/2 inches wide at the bottom, and 4 inches wide at the top. The top of the box was surrounded by a black plexiglass platform. Parallel steel plates were placed along the floor and bent up to form the inside walls of the box. Because of the narrow floor a mouse always bridged a pair of plates when it stood on the apparatus. A.C. shock was delivered from a constant current source (Diagram). Resistance of the mouse was found to be 10^5 ohms and the shock level was calibrated according to this value. An ammeter was installed in the circuit in order to determine whether the mouse was receiving the shock and to ascertain whether waste matter or debris were shorting the plates.



R_1 = 240 K - Current Limiting Resistor
R_2 = 5M pot. - Course Adj.
R_3 = 2.5M pot. - Fine Adj.
R_4 = 100K - Dummy Resistor
S_1 = SPST NO Momentary
M = 0-500 µa A.C.
T_1 = Stancor P-6011

2. Hot Plate

A hot-plate escape test was constructed in a similar manner to the shock-escape box, except that the bottom of the escape box was replaced by a sheet metal plate. A sheet of asbestos material was placed between the plate and a tray filled with ordinary sand. The sand was used as a damper to prevent fluctuations in temperature on the plate itself. This tray, in turn, was seated on a standard laboratory hot plate.

The hot plate temperature was measured by a surface reading thermometer (PET-Instruments), placed on the sheet metal floor. During the entire testing period the hot plate remained "on" continuously and no testing was done until the floor temperature was found to be constant to within 1° for a 24 hour period prior to any experiment.

Procedures

Testing was performed between 9 a.m. - 1 p.m. Injections were administered intraperitoneally in all instances and were performed using a 26 gauge needle. Injection volume, regardless of dose, was .1 cc. Vehicle, except where indicated, was normal saline. Other solutions were adjusted to the ph of a morphine sulfate solution with a concentration of 5 mg./cc. Morphine solutions were refrigerated and replaced routinely at 60 day intervals.

During the injection-test interval, animals were returned to their boxes. At the time of testing, mice were placed in the apparatus so that contact was made between all four paws and the metal plates. In most experiments, mice were permitted 30 seconds to escape at each trial. Those not escaping within this time limit were graded as non-escapers. The escape latency of those mice escaping was also recorded with an electric stopwatch. Following testing, mice were immediately returned to their boxes.

Selection and Determination of Groups

Determination of initial or baseline performance was accomplished as follows. It was found in preliminary experiments using 6 groups of 16 naive mice, that upon initial testing, ten of sixteen mice per group escaped within the time limit. Thus 10/16 or 62.5% was employed as a population parameter for escape performance in the initial series of experiments. It was found in subsequent experiments, that the 62.5% baseline control value was an accurate population parameter. Variations of one or two mice escaping or not escaping per group were observed occasionally. These groups were adjusted by replacement of a particular mouse in order that all groups had the same 10/16 initial performance. In some studies, 1 or 2 mice died either following morphine administration or from unknown causes. If this occurred on Days 1-5 during the period of drug injection, the mouse was replaced by another with the same baseline (Day 1) performance as the dead mouse. Mice dying on all other days were not replaced. Except where specifically indicated no other selection procedures for groups were employed after Day 1.

Data

In all experiments, the escape latency was recorded for each individual mouse at each testing session. In those experiments where vocalization (squeaking) was studied, a response was recorded when any audible sound was perceived by the observer. In these experiments, mice were not removed from the testing environment until 30 seconds had elapsed.

In some experiments, all types of responses to the noxious stimulus were recorded. In these experiments the latencies for all "stimulus related" behavior, whether vocalization, paw lifting, squeaking, or jumping, etc., were recorded by the observer. In such studies, mice were removed from

the testing apparatus immediately after a response was elicited.

Statistical Analysis

Because the N (16 mice) represented a randomly chosen population and 10 of 16 mice escaped the shock within the criterion time limit, a 62.5% performance level was employed as a population parameter. Performance of mice in subsequent testing situations were compared to their initial performance by employing a Chi^2 test in which 62.5% (10/16) was the expected value. Because there were two classes of results, i.e. escapers and non escapers, there was 1 degree of freedom. It was calculated that an impairment in performance was significant at ($p<.05$) if 5 or fewer mice escaped. One tailed "Sign" tests were performed to determine the direction of change of escape performance both within and between groups of mice (Siegel, 1956).

RESULTS

Introduction

This section contains the experimental results. The majority of the data are presented in graphic form because this format permits the evaluation of trends in escape behavior vis a vis tolerance, as well as absolute levels of performance.

To avoid confusion the use of several terms should be clarified at this point. First, in many experiments reference will be made to a "standard testing regimen" or "schedule". Unless otherwise specified this refers to the testing conditions as described for Group 2 in Table 1. Also, the phrase, "post-injection testing", refers to testing on the same day as injection in contradistinction to "post-morphine" testing which refers to testing on the day after the last of a series of daily morphine injections. Finally, tolerance controls refer to a group of mice tested in the same manner as Group 6 in Table 1 on Days 1 through 16 except that in the majority of experiments tolerance was generally measured after injection on Day 15 rather than on Day 16 (as in Group 6).

Preliminary Experiments

Figure 1 (Groups 1 through 6) represents the escape performance of mice submitted to a variety of treatment schedules designed to evaluate the effects of dosage, sequence of testing and drug injection, the absence of testing during morphine administration, and continuous testing without drug.

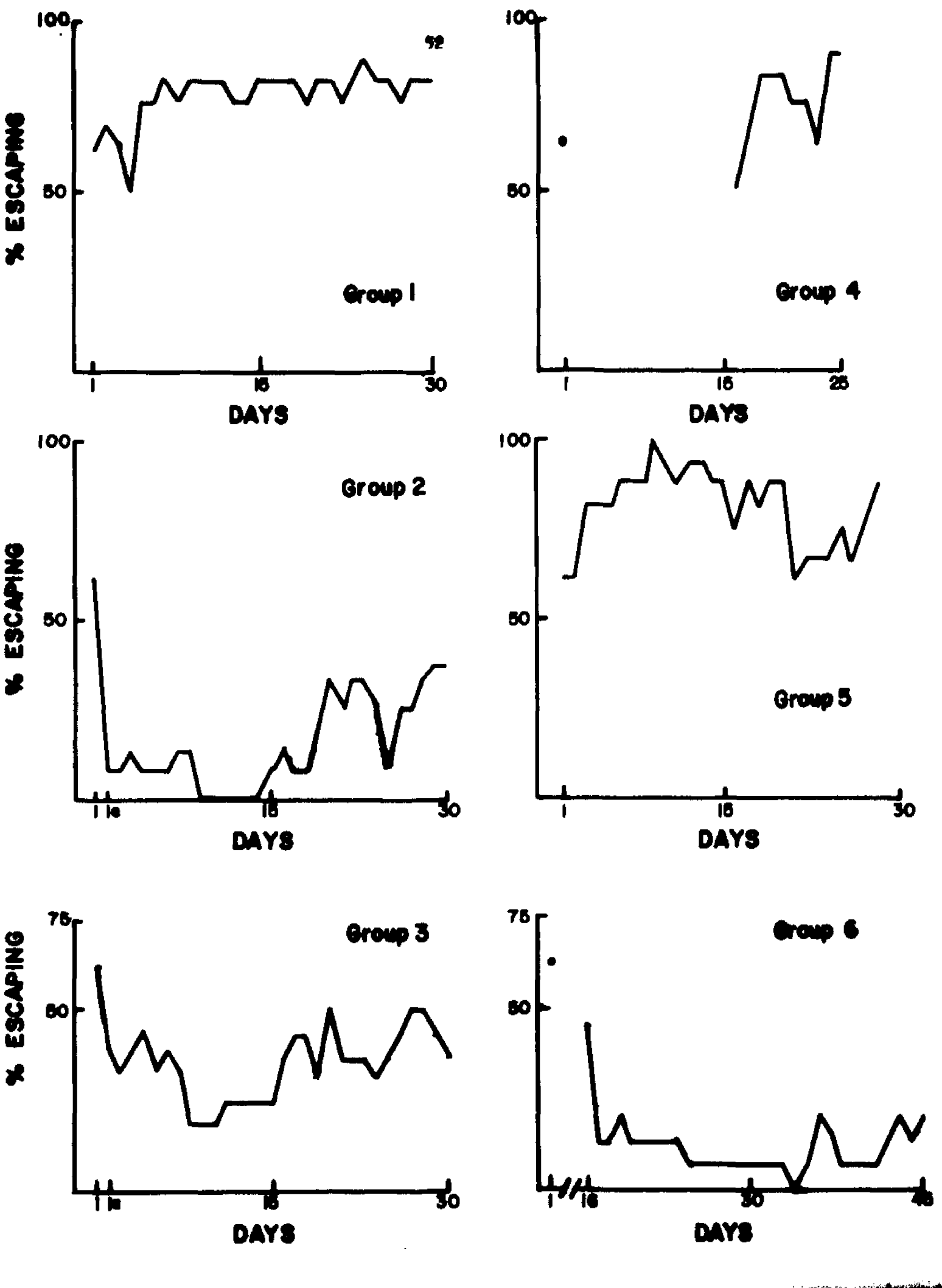
These schedules are outlined in Table 1. The shock level for all groups was 160 ua, with a trial time criterion of 30 seconds. The dose of morphine, when administered, was 25 mg/kg, except Group 3 which received 12.5 mg/kg.

Mice in Group 1 were tested initially on Day 1 and then daily for thirty days thereafter. Mice in Group 2 were tested on Day 1, then injected with morphine, and retested 30 minutes post-injection (Day 1A). This group was then tested daily post-injection for 15 days, followed by 15 additional days of testing without drug administration. A third group of mice (Group 3) received a similar treatment regimen as in Group 2 except that the dose of morphine was 12.5 mg/kg. Mice in Group 4 were injected with morphine following testing on Day 1. They were then injected with morphine for 15 days, followed by an additional 15 days of testing in the absence of drug administration. Group 5 received the same treatment schedule as Group 2 and 3 except that morphine was administered after, rather than prior to, the daily escape trials. Mice in Group 6 were tested on Day 1 then injected with morphine on Days 1 through 30. Post-injection testing was begun on Day 16 and continued until cessation of drug administration on Day 30. These mice were then tested without drug for an additional 15 days.

The escape performance of mice following a single injection of morphine (25 mg/kg) (Figure 1, Group 2, Day 1A) was significantly impaired from their pre-morphine level. Following 15 days of morphine injections and testing, however, these mice demonstrated no improvement in their performance,

Figure 1

Effects of various testing and/or injection sequences on escape behavior in mice receiving morphine. Groups of mice (N=16/group) were treated according to the regimens outlined in Table 1. Shock level was 160 ua at all testing sessions. Days of significant ($p<.05$, Chi^2 test) impairment from control performance (Day 1) were as follows: Group 2 - Days 1A through 28, Group 3 - Days 2, 6, 8 through 15, and 2 of the next 15 days, Group 6 - Days 17 through 45.


% ESCAPING
100
50
92
Group 1
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15
30
DAYS
100
50
Group 4
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25
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Group 2
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Group 5
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DAYS
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75
50
Group 3
1
15
30
DAYS
75
50
Group 6
1
15
30
DAYS

Table 1

Treatment Schedules

GROUPS	Day 1	Days 2-15	Days 16-30	Days 31-45
1	Escape testing	Escape testing	Escape testing	
2	Escape testing followed by morphine[1] and re-testing (Day 1A)	Morphine followed by testing	Escape testing	
3	Escape testing followed by morphine	Morphine followed by testing	Escape testing	
4	Escape testing followed by morphine	Morphine	Escape testing	
5	Escape testing followed by morphine	Escape testing immediately followed by morphine	Escape testing	
6	Escape testing followed by morphine	Morphine	Morphine followed by testing[2]	Escape testing

Table 1

(1) Dosage for Group 3 = 12.5 mg/kg., all other groups received 25 mg/kg.

(2) Post-injection testing began on Day 15 in all experiments except the preliminary study (Figure 1) in which testing was begun on Day 16.

i.e. no evidence of return to their pre-morphine level. In addition, following cessation of morphine treatment, these animals continued to demonstrate a significant impairment in performance for 13 out of 15 daily trials. Results obtained using a smaller dose (12.5 mg/kg) of morphine were comparable but of a smaller magnitude, in that an initial and sustained impairment in performance was observed for the 15 days of post-injection trials. These mice demonstrated only a small impairment in escape performance (2 days) following cessation of morphine. The escape frequency of control animals (Figure 1, Group 1) tested for thirty days fluctuated for the first few days of testing and then stabilized at a constant high level of performance. Mice (Figure 1, Group 4) tested on Day 1 and then receiving morphine for 15 days without testing, showed no impairment when tested without drug on Days 16 through 25. In addition, animals (Figure 1, Group 5) tested on Day 1 and given the same regimen as Group 2 except that the testing was performed before rather than after injection, showed no impairment either during the period of morphine administration (Days 2 through 15) or after (Days 16 through 28). Thus, the impairment in performance demonstrated in Groups 2 and 3 could not be elicited by either morphine (Group 4) or testing (Group 1) alone. Furthermore, the combination of morphine and testing (Group 5) was only effective in producing an impairment in performance during and after drug treatment if the administration of drug preceded escape testing.

In order to ascertain whether any pharmacologic tolerance could be detected during the 15 days of the drug administration period, one group of mice (Group 6) was tested on Day 1 without morphine and then administered morphine for thirty days but not tested for the first 15 of these days. When escape testing was resumed on Day 16, the escape performance of the mice was

Table 2

Comparison of the escape performance in saline and non-saline injected mice. Group 1 (N=16) received daily testing (see Group 1, Table 1); Group 1 (saline) (N=16) was treated similarly except .1cc normal saline was injected 30 minutes prior to testing on Days 2 through 30. Group 2 (N=16) and Group 2 (saline) (N=16) received the same treatment regimen as Group 2 (Table 1) except Group 2 (saline) received .1cc saline injections 30 minutes prior to testing on Days 16 through 30.

Group	Escape Frequency (%)				Days of Significant Impairment *
	Day 1A	Day 15	Day 16	Day 30	
1	-	75.00	75.00	75.00	-
1 (saline)	-	81.25	75.00	81.25	-
2	6.25	6.25	12.50	37.50	13
2 (saline)	6.25	12.50	12.50	43.75	11

*number of days on which post-morphine escape frequency was less than 37.50%.

not significantly different from the Day 1 value. However, upon continued daily post-injection testing, the performance of these mice declined such that by Day 30, the performance was the same as that of Group 2 following their initial injection of morphine (Group 2, Day 1A). In addition, these mice (Group 6) remained significantly impaired for the entire post-morphine testing period (Days 30 through 45).

Saline Controls

Prior to investigating the effects of altering the various parameters employed in the initial experiments, it was necessary to perform a number of control studies.

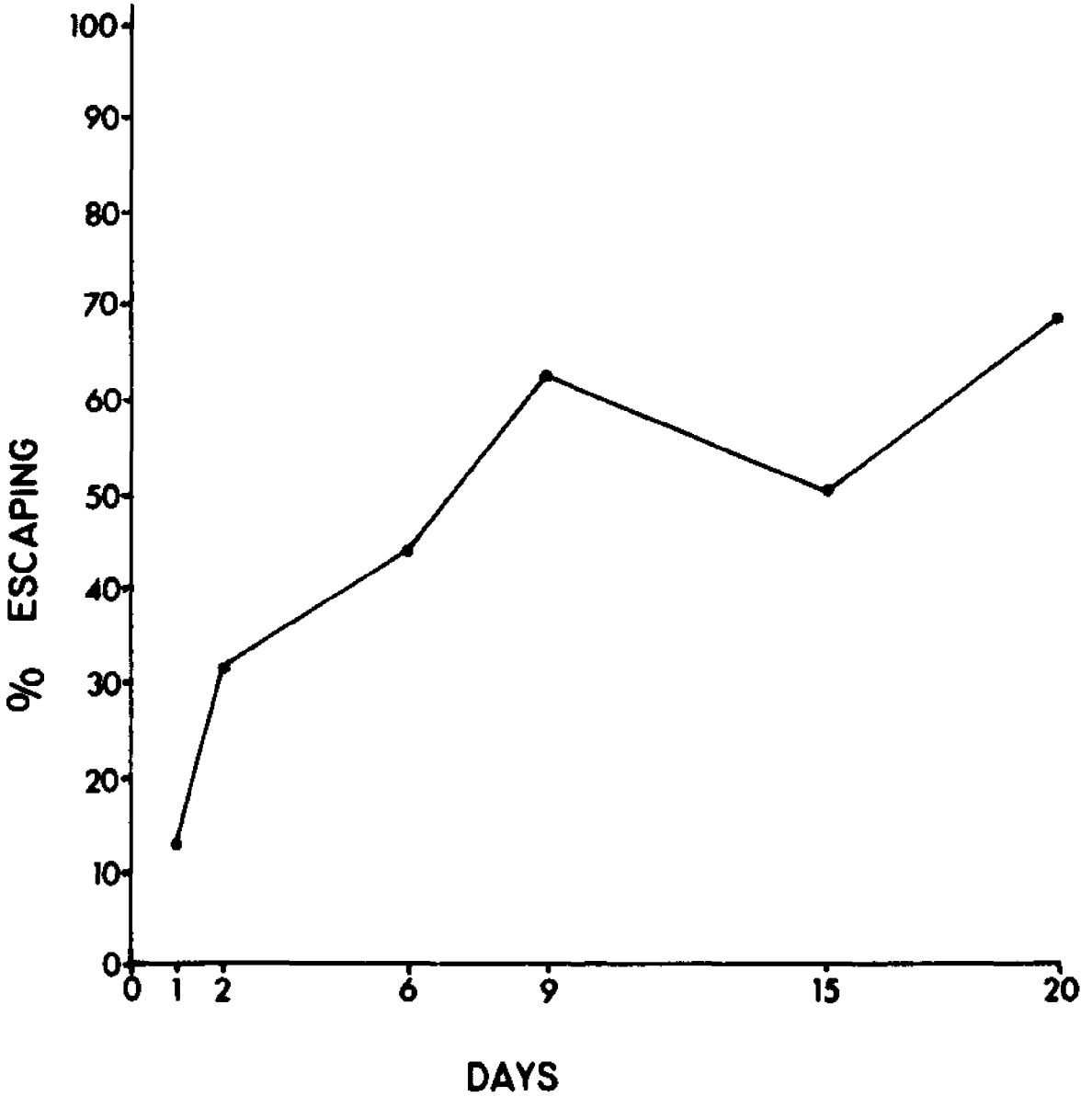
Table 2 represents the data from a comparison study in which the previous experiments (Table 1, Groups 1 and 2) were repeated using saline injections. Mice in Group 1 (saline) received saline injections prior to testing on Days 16 through 30. As indicated in Table 2, no difference in escape performance was observed between the saline and non-saline treated mice in either Groups 1 or 2. Based on these findings, saline controls in subsequent experiments were eliminated.

Tolerance in Untested Mice

In the initial experiments (Figure 1, Group 6), it was found that pharmacologic tolerance to morphine could be demonstrated on the fifteenth day of morphine administration. Additional studies were carried out to determine the extent to which analgesic tolerance developed in mice following various periods of drug administration. Figure 2 illustrates the results of these experiments. Groups of 16 mice were tested on Day 1 and then injected with morphine. Each group was injected daily but not tested again until after injection on Day 1, 2, 6, 9, 15, or 20. Thus, for example, the mice tested

Figure 2

Tolerance to daily morphine injection in untested mice. Six groups of mice (N=16/group) were tested on Day 1 (160 ua) then injected with morphine (25 mg/kg). Mice received morphine for a varying number of days but were not retested until after injection on the final day of drug administration. Significant impairment ($p<.05$, Chi^2 test) from Day 1 (62.5%) control values observed only in 1, 2 and 6 day groups.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1
2
6
9
15
20
DAYS

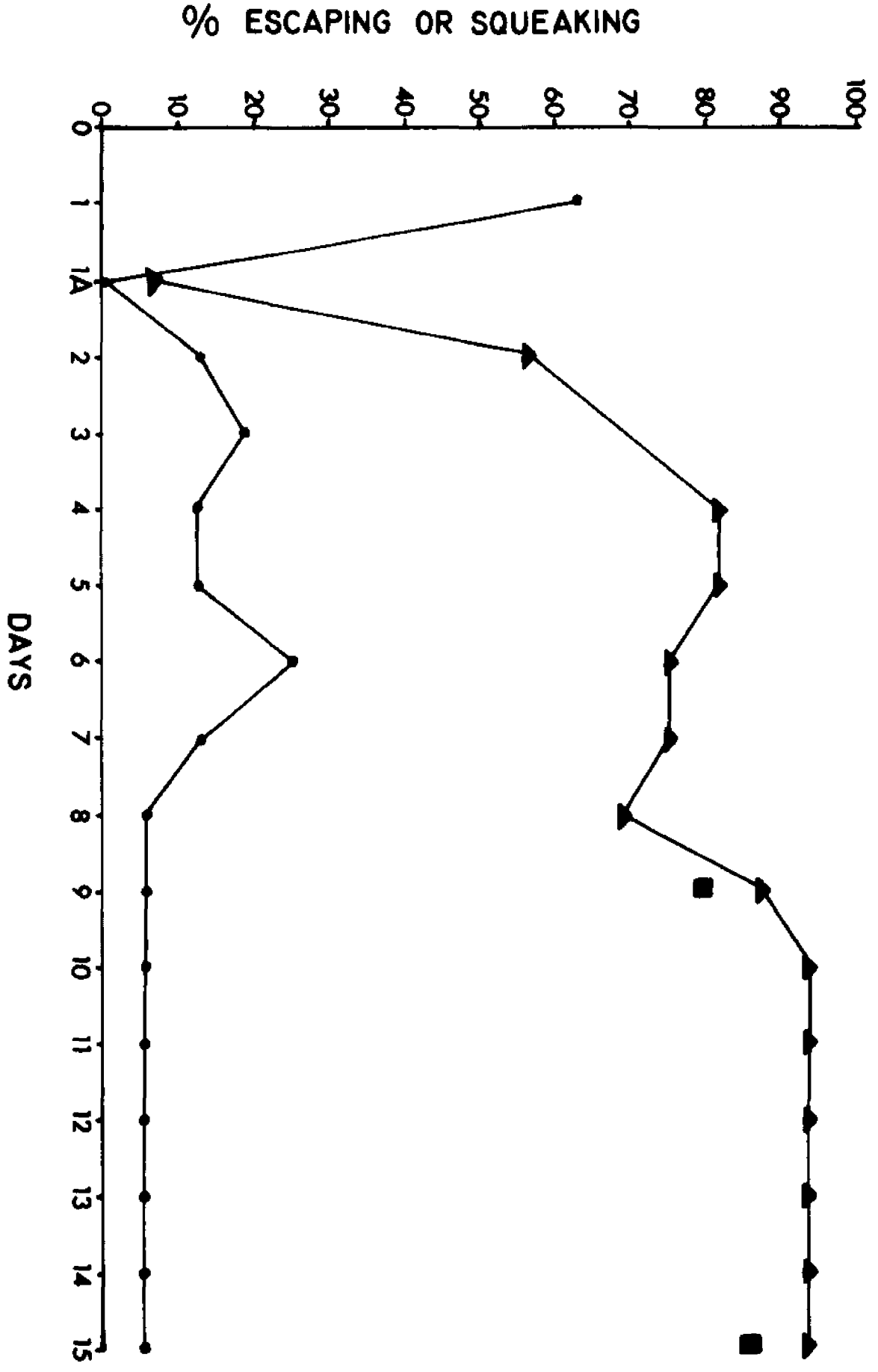
on Day 6 had received testing and injection on Day 1, injections alone on Days 2 through 5, and testing after injection on Day 6. The results demonstrated that the effects of morphine on escape behavior are greatest on Day 1 and then diminish in intensity following daily administration such that, by Day 9, there is no significant difference between the morphine group and Day 1 performance of controls (mice not receiving daily post-injection testing).

Tolerance Measured by Vocalization

The previous experiments suggested that untested mice did become tolerant to the effects of morphine on escape behavior. It was thus necessary to investigate the specificity of the effect of morphine on escape behavior in those animals who were tested following injection. Stated in another manner, the question was, what effect did training have on the perception of the noxious stimulus. In an attempt to answer this question, a group of mice were given the standard treatment schedule (Table 1, Group 2); however, the occurrence of squeaking or vocalization during exposure to the test conditions was recorded as well as the number of mice escaping. Figure 3 illustrates the results of this experiment. It is clear that on initial administration of morphine (Day 1A) there was a marked decrease in the percentage of mice vocalizing in response to the stimulus, paralleling a concomitant fall in escape frequency. However, on subsequent days, the number of mice responding by vocalization increased markedly, whereas the number of mice escaping remained close to that of the Day 1A value. Thus it appeared from these data that mice treated with daily injections and testing failed to escape the noxious stimulus despite the finding that their sensitivity to the stimulus increased profoundly, based on vocalization data, over the period of morphine administration.

Figure 3

Comparison of escape behavior and vocalization frequency in mice receiving daily post-injection testing. A group of mice was tested according to the standard testing procedure (Table 1, Group 2) (160 ua and 25 mg/kg). Escape performance is represented by the ● — ● line; vocalization frequency by the ▲—▲ line. Maximum shock exposure interval = 30 seconds, and mice were not removed after vocalization. Vocalization was measured in two "untested" control groups receiving testing on Day I followed by morphine for 9 and 15 days respectively. Testing was resumed on the final day of morphine administration.


% ESCAPING OR SQUEAKING
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80
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13
14
15

Initial Parametric Studies

Dose Effects

The data in this section represent the results of experiments performed in order to determine the relationship between the dose of morphine administered and various aspects of the escape paradigm.

Two series of nine groups of mice were employed for these experiments. The first nine groups of mice were given the standard treatment schedule (Table 1, Group 2) except that each group received a different daily dose of morphine (0, 7.5, 12.5, 18.75, 25, 37.5, 50, 125, and 250 mg/kg). The second series of nine groups were initially given the same treatment schedule as Group 6, Table 1, Days 1 through 16 except that the groups were tested for tolerance after injection on Day 15 rather than Day 16.

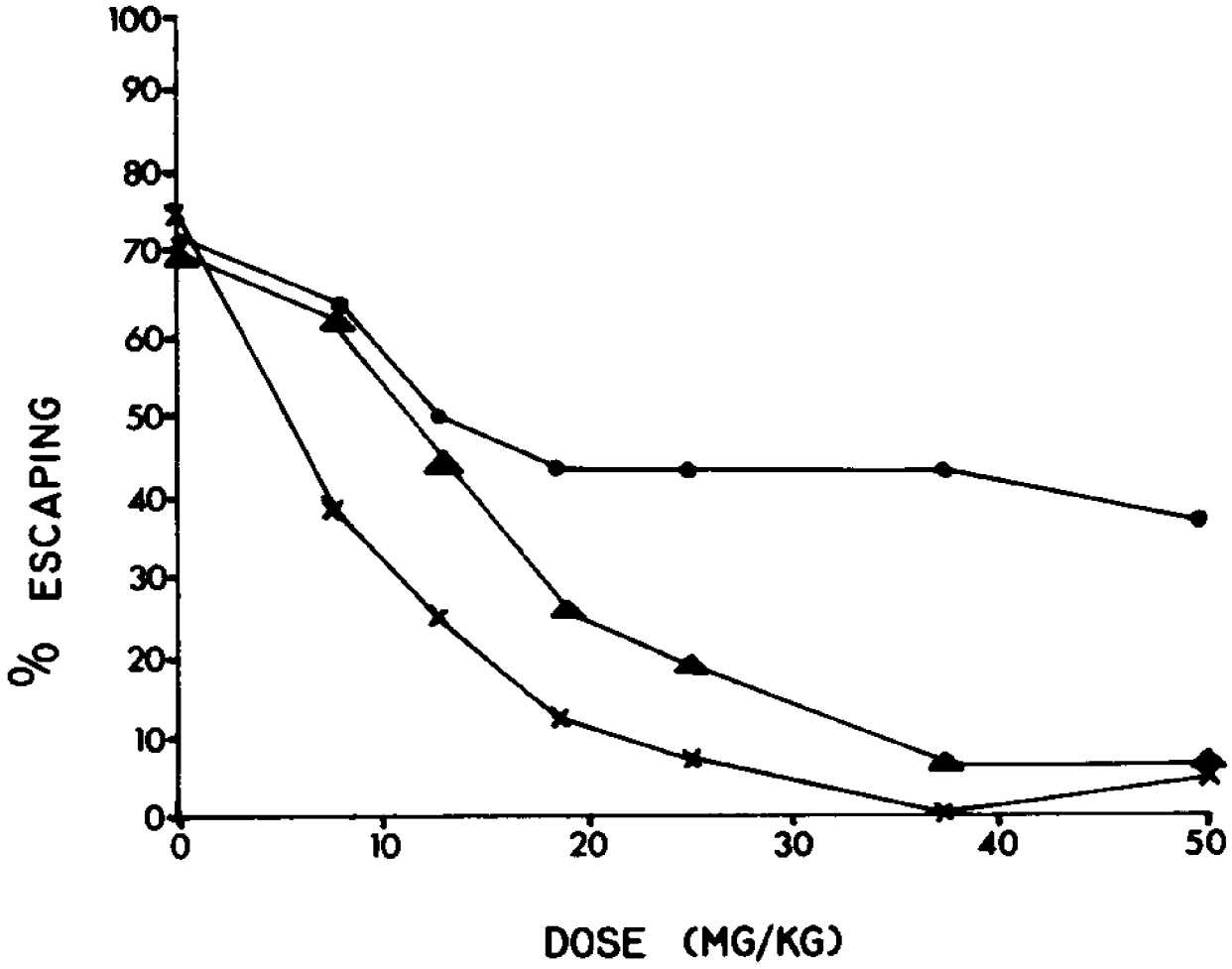
The results of these experiments are illustrated in Figure 4. The results for the 125 and 250 mg/kg groups were not included in this graph because of a high mortality rate which precluded a valid statistical analysis.

A clear dose-response relationship between morphine dose and Day IA performance can be observed. A similar relationship is also observed for the performance of these groups following injection on the last day of morphine administration. If the data from Day 1, Day 15 with testing, and Day 15 without testing are compared, it is evident that for any given dose the degree of tolerance on Day 15 is greater for the untested than for the tested group of mice.

Figure 5 shows the Day 16 (first post-morphine day) performance of the groups mice described above, plotted as a function of daily morphine dose. A clear inverse relationship between dose and escape performance can be observed over the entire range of doses, reaching a maximum at 50 mg/kg.

Figure 4

Escape frequency of mice as a function of (morphine) dose. (X) represents the Day 1 and (●) the Day 15 performance of mice administered varying doses of morphine but otherwise receiving the standard testing procedure (Table 1, Group 2) (160 ua). (▲) represents the performance of untested mice (tolerance controls) after injection on the 15th day of morphine administration. N=16 for all groups except the 50 mg. (tested) group, in which two mice died on Day 2 and were replaced; two more mice died on Day 6 but were not replaced. Escape performance at each dose (except 0) was greater ($p < .05$, Sign test) on D15 (●) in tested groups than in untested (▲) groups.


100
90
80
70
60
50
40
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20
10
0
% ESCAPING
0
10
20
30
40
50
DOSE (MG/KG)

Figure 5

Performance of mice on the day after 15 days of post-injection testing. Groups of mice received the standard testing regimen (Table 1, Group 2) (160 um) at various doses of morphine. The figure shows Day 16 (first post-morphine day) escape performance as a function of daily morphine dose. N=16 for all groups except for the following: 50 mg/kg - two deaths on Day 2, 125 mg/kg - four deaths on Days 1 and 2 (replaced), 250 mg/kg- four deaths on Days 1 and 2 (replaced).

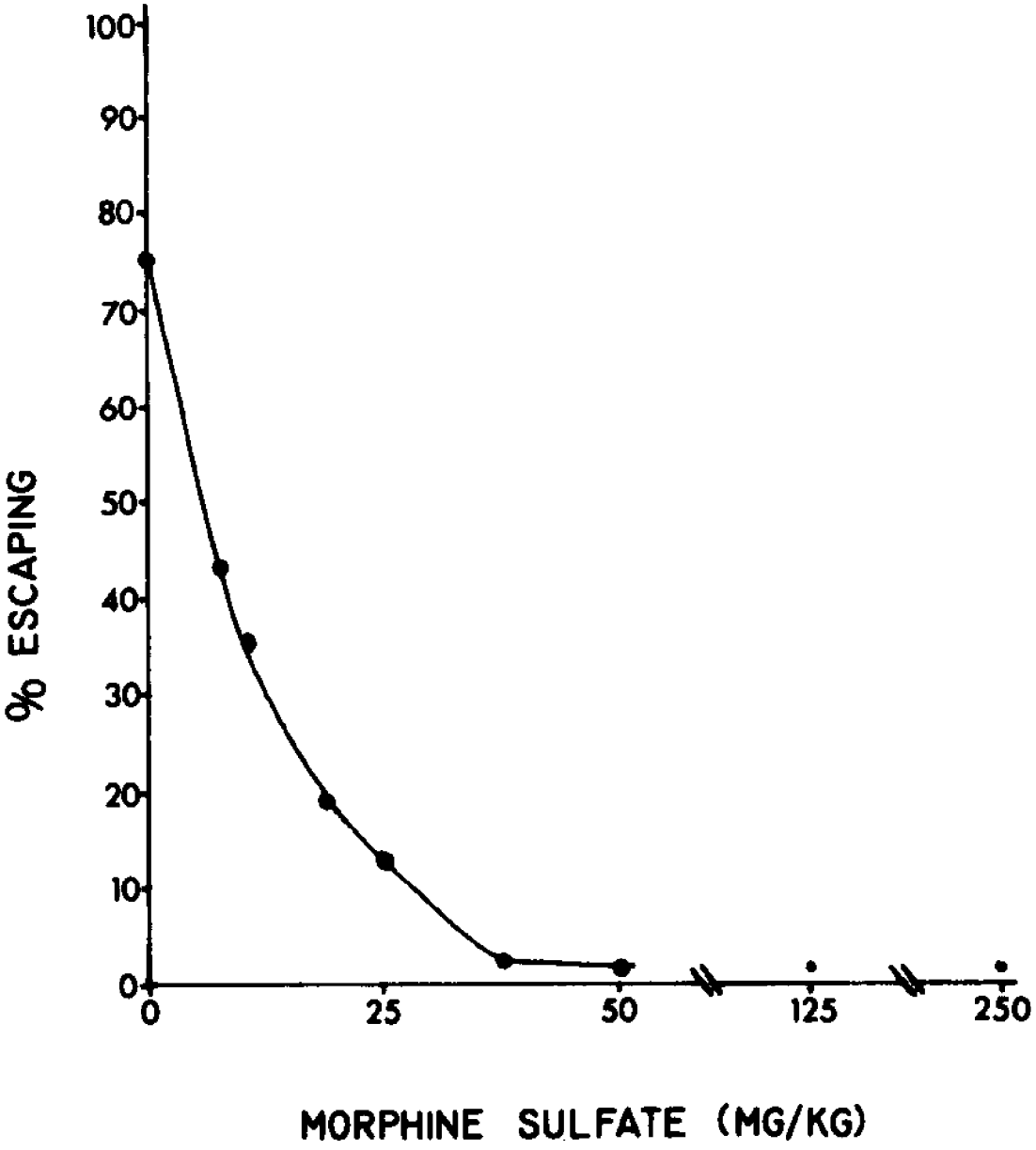

100
90
80
70
60
50
40
30
20
10
0
% ESCAPING
0
25
50
125
250
MORPHINE SULFATE (MG/KG)

Figure 6

Post-morphine escape impairment as a function of daily morphine dose. Groups of mice (N=16/group on Day 1) received the standard testing regimen (Table 1, Group 2) at various doses. The figure illustrates the number of post-morphine days on which the escape performance was significantly diminished ($p<.05$, Chi^2 test) from control (Day 1) performance for each morphine dose.


DAYS OF IMPAIRMENT
15
12
9
6
3
0
0
10
20
30
40
50
125
250
MORPHINE SULFATE (MG/KG)

Thus the Day 16 post-morphine escape frequency correlates well with the values obtained on Days 1 and 15 (Figure 4).

The groups of mice in the previous experiment receiving the standard testing regimen (Figure 1, Group 2) at varying doses were tested for post-morphine escape impairment on Days 16 through 30. Figure 6 illustrates the number of days of post-morphine escape impairment of these mice (≤6/16 mice escaping) as a function of the morphine dose. The results indicate a strong dose-response relationship which reaches a plateau at doses above 37.5 mg/kg, and correlates well with the Day 15 performance of these mice as shown in Figure 4.

Effect of Days

In the initial experiments (Figure 1), all mice received testing and injections for 15 days. It was therefore necessary to determine the relationship between the severity of escape impairment and the number of treatment days.

Figure 7 illustrates the effect of various treatment regimens (0,4,6, 10,12,15,20, and 30 days) on post-injection performance on the last day of drug administration. The results show a clear correlation between post-injection performance and days of exposure to the treatment regimen.

The mice employed in the previous experiment were tested on subsequent days without morphine in order to determine the effect on post-morphine impairment of the various treatment schedules. The results (Figure 8) show the number of days of significant post-morphine impairment as a function of treatment days. These results demonstrate that less than 10 days of treatment (injection and testing) are insufficient to produce continued escape impairment following cessation of morphine. In addition, treatment schedules longer

Figure 1

The effect of varying "Days" of post-injection testing on escape behavior. Groups of mice (N=16/group) were exposed to the standard testing regimen (Table 1, Group 2) except the period (total days) of daily post-injection testing was varied. The figure illustrates the escape frequency on the final day of post-injection testing as a function of the number of testing days. Dose=25 mg/kg and shock level=160 ua for all groups. Escape performance was significantly ($p<.05$, Chi^2 test) impaired from control (62.5%) for "Days" 15, 20, and 30.

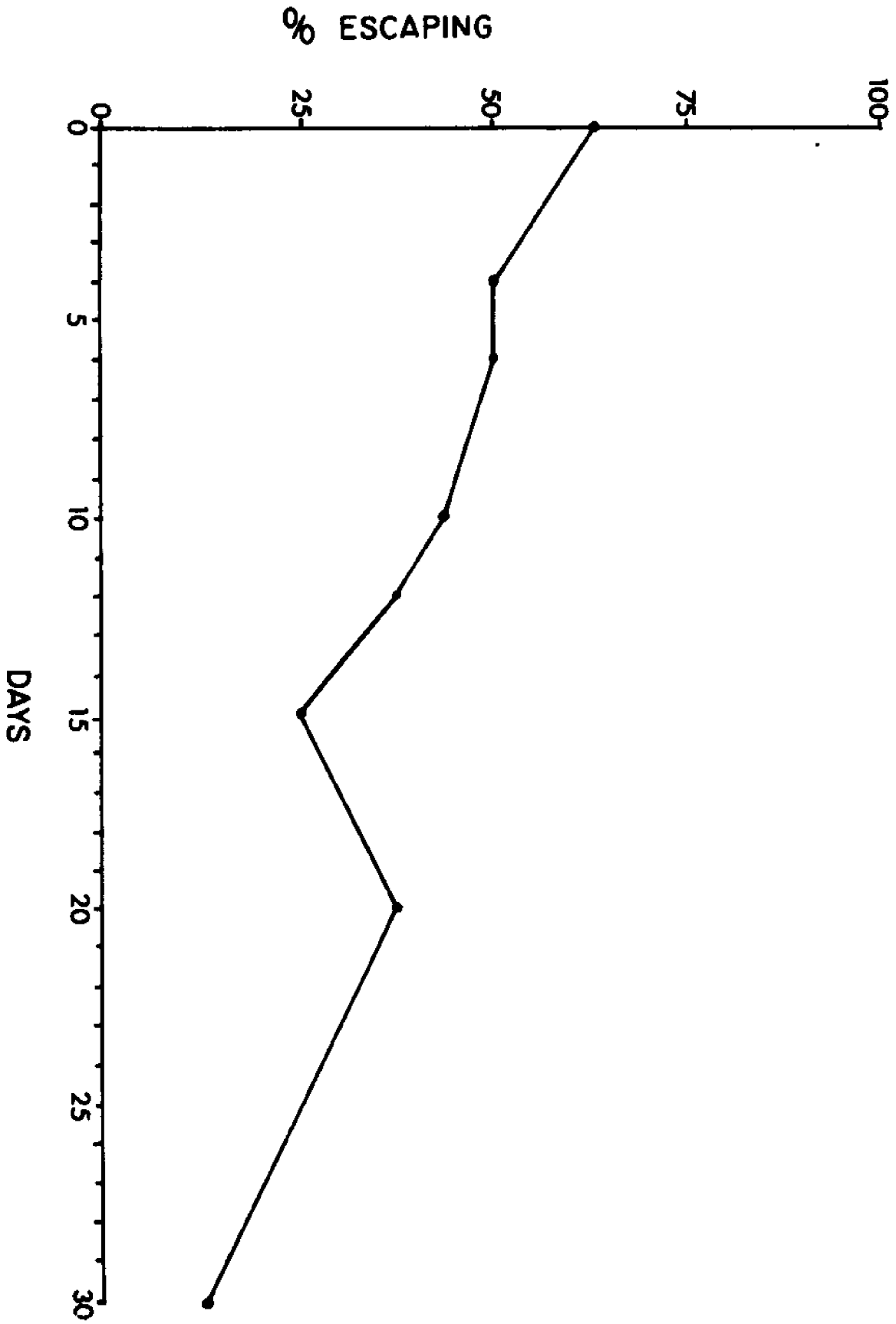
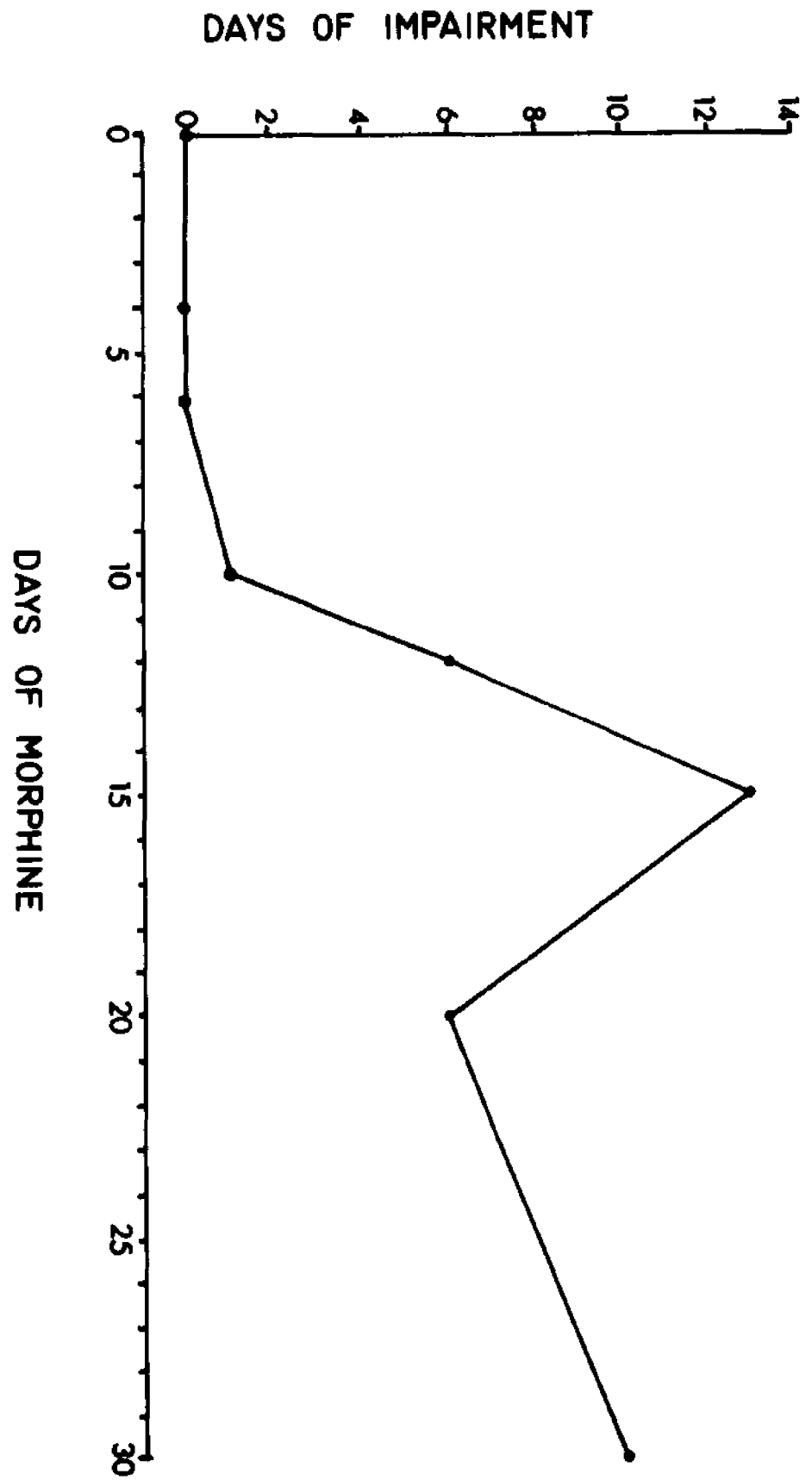

% ESCAPING
0
25
50
75
100
DAYS
0
5
10
15
20
25
30

Figure 8

The effect of varying "Days" of post-injection testing on post-morphine escape impairment. Groups of mice (N=16/group) received the standard testing regimen (Table 1, Group 2) except that the period (total days) of daily post-injection testing was varied. The figure expresses the number of days on which post-morphine escape frequency was significantly ($p<.05$, Chi^2 test) impaired from control (Day 1). Dose of morphine= 25 mg/kg and shock level=160 ua for all groups. No relapse (i.e. return to significant impairment) was found, when mice were tested for 15 additional days until Day 45.


DAYS OF IMPAIRMENT
0
2
4
6
8
10
12
14
DAYS OF MORPHINE
0
5
10
15
20
25
30

than 15 days did not further increase the number of impairment days.

Shock Intensity

The decision to use the shock intensity (160 ua) in the preliminary experiments was determined empirically. It was found that at this current level, less than 100% of mice escaped when not receiving morphine and, in addition, some fraction of mice in each group escaped when morphine was administered (acutely). Thus, 160 ua was a convenient shock intensity because it provided for an adequate range of improvement in mice not receiving drug while simultaneously allowing room for measuring performance fluctuations in mice receiving drug.

In a preliminary experiment, a shock-response curve was generated for groups of naive mice (no previous exposure to the testing apparatus). The results of this experiment are represented in Figure 9. A linear shock-escape curve was obtained in the 70-200 ua range. Threshold for escape was between 40 and 60 ua and maximal responding could be observed at levels above 200 ua.

Having established a dose-response relationship for shock intensity and escape frequency, further experiments were undertaken in order to determine to what extent the original results observed at 160 ua were reproducible when different shock intensities were employed at various stages in the testing procedure.

In the first experiments, two shock levels, (105 and 200 ua), were used because these levels represented the extremes of the linear part of the dose-response curve. Two groups of mice were used for each shock level.

Two groups of mice received the same treatment schedules as did Group 1 in Table 1, except that the shock intensity at each testing session was 105 or 200 ua respectively. Similarly, the other two groups of mice received

Figure 9

Acute shock-response curve for naive mice. Nine groups (N=16/group) of naive mice were tested at different shock levels. The escape performance of these mice are plotted as a function of shock intensity. A standard maximum shock interval of 30 seconds was employed.

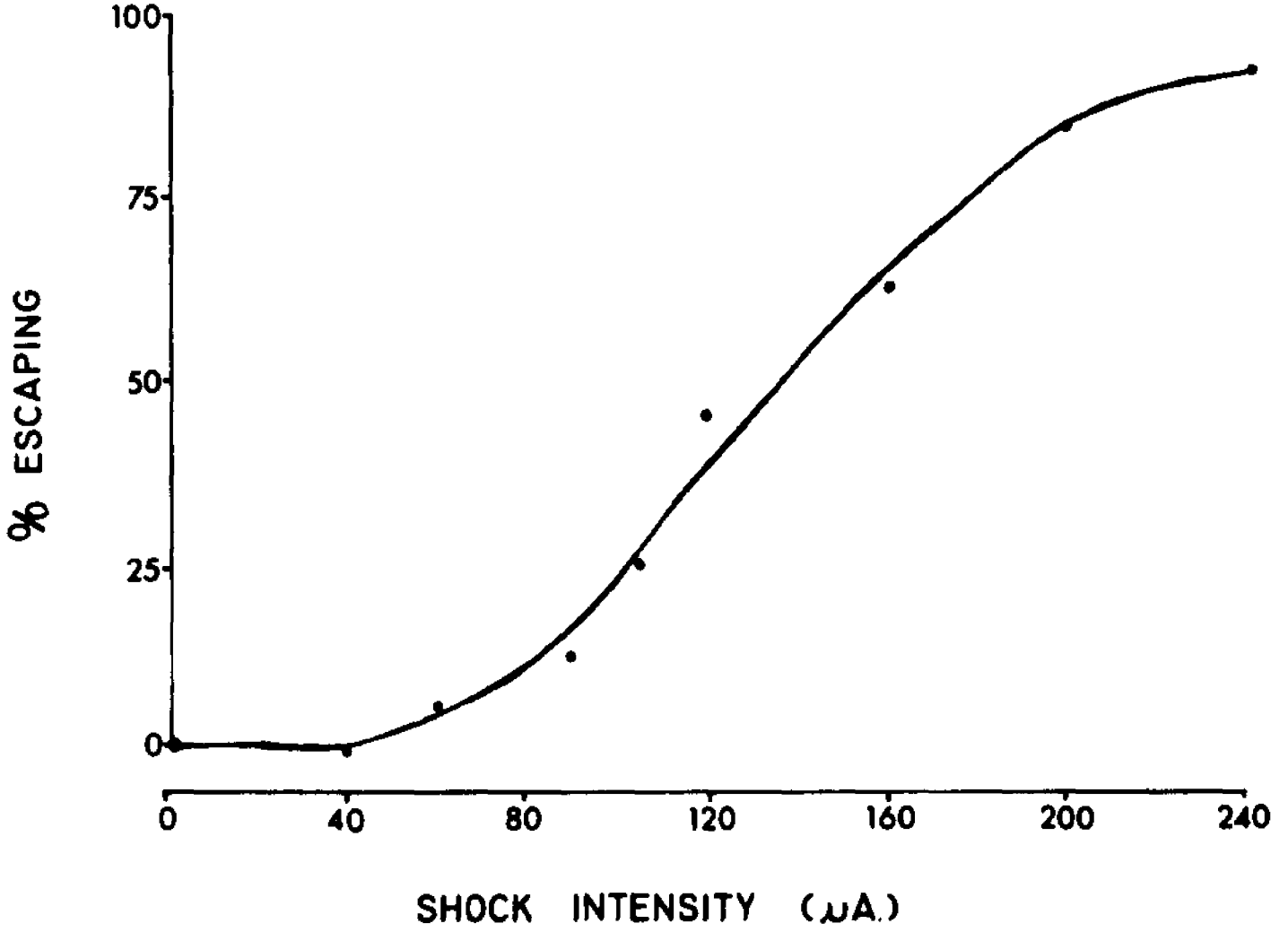
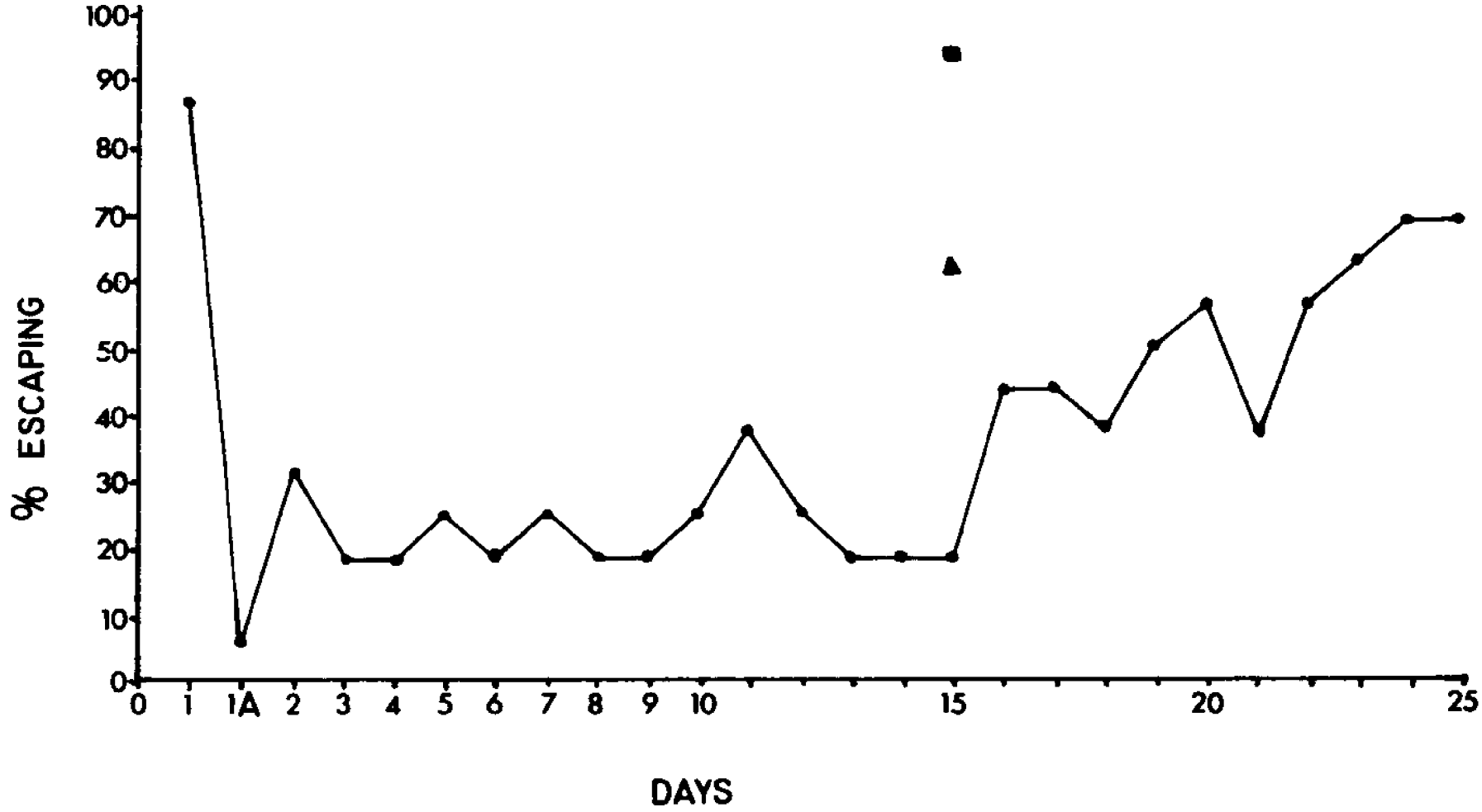

100
75
50
25
0
% ESCAPING
0
40
80
120
160
200
240
SHOCK INTENSITY (μA.)

Figure 10

Escape performance of mice tested at 200 ua. A group (N=16) of mice (●) received the standard testing regimen (Table 1, Group 2) except that shock intensity was 200 ua at each trial. Morphine dose was 25 mg/kg. Another group (■) of mice (N=16) were tested daily at 200 ua. Also one group (▲) of mice (N=16) was tested on Day 1 then injected on Day 1 and for fourteen days thereafter but was not retested until after injection (tolerance control) on Day 15. Days of significant ($p<.05$, Chi^2 test) impairment from Control (Day 1) were as follows: Days 1A-19, and 21.


% ESCAPING
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0
0 1 1A 2 3 4 5 6 7 8 9 10 15 20 25
DAYS

the standard testing procedure (Table 1, Group 2) except that different shock intensities were substituted at all testing periods. Two additional groups were also included. These mice received the same treatment as those in Table 1, Group 6, Days 1 through 15 except that different shock levels were employed, i.e. they were tested for tolerance on Day 15 of drug administration.

The results of these experiments are illustrated in Figures 10 and 11. The Day 15 performance of a control group (tested daily at 200 ua) is also shown in Figure 10. The performance of mice receiving morphine and post-injection testing at 200 ua on Day 1 (Figure 9, Day 1A) were significantly decreased from their baseline pre-drug level. On subsequent days, although some fluctuations were observed, the escape behavior of these mice remained impaired during the period of testing and morphine treatment. Similarly, there were a number of post-impairment days although these mice returned to within baseline values by Day 24.

The results using the lower shock intensity (105 ua) are represented in Figure 11. It is clear from the graph that low shock levels, coupled with morphine administration, produced a high degree of escape impairment. Of note, is the performance of the tolerance control group on Day 15. This group demonstrated a level of performance greater than the experimental group (testing and morphine). This finding is consistent with results observed at higher shock levels.

As demonstrated in the previous experiments (Figure 11, Days 1 through 15), control mice tested at 105 ua shock levels showed a sustained low level of performance during the 15 day testing period. Similar results were observed at 90 ua shock intensity. The performance of these latter mice resembled the Day 1 through 15 performance of mice in the original

Figure 11

Escape performance of mice tested at 105 ua. A group (N=16) of mice (●) received the standard testing regimen (Table 1, Group 2) except that a 105 ua shock was employed at all testing sessions. Morphine dose was 25 mg/kg. Another group (▲) of mice (N=16) was tested daily at 105 ua. Also, one group (■) of mice (N-16) was tested on Day 1 then injected with morphine on that day and for fourteen days thereafter, but was not retested (tolerance control) until after injection on Day 15. Performance of mice on Days 5-15 was less ($p<.05$, Sign test) than performance on Days 1A-4.

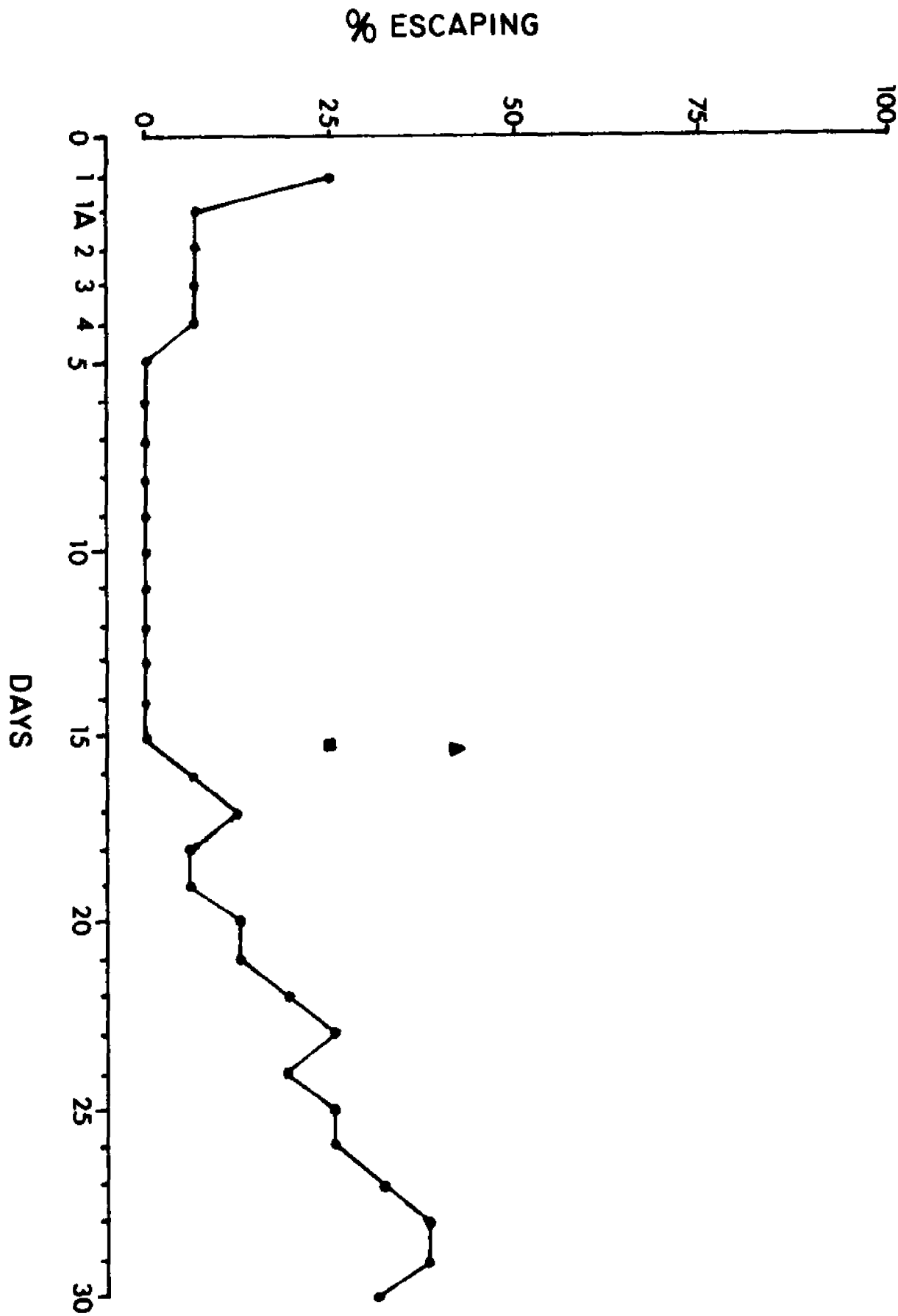
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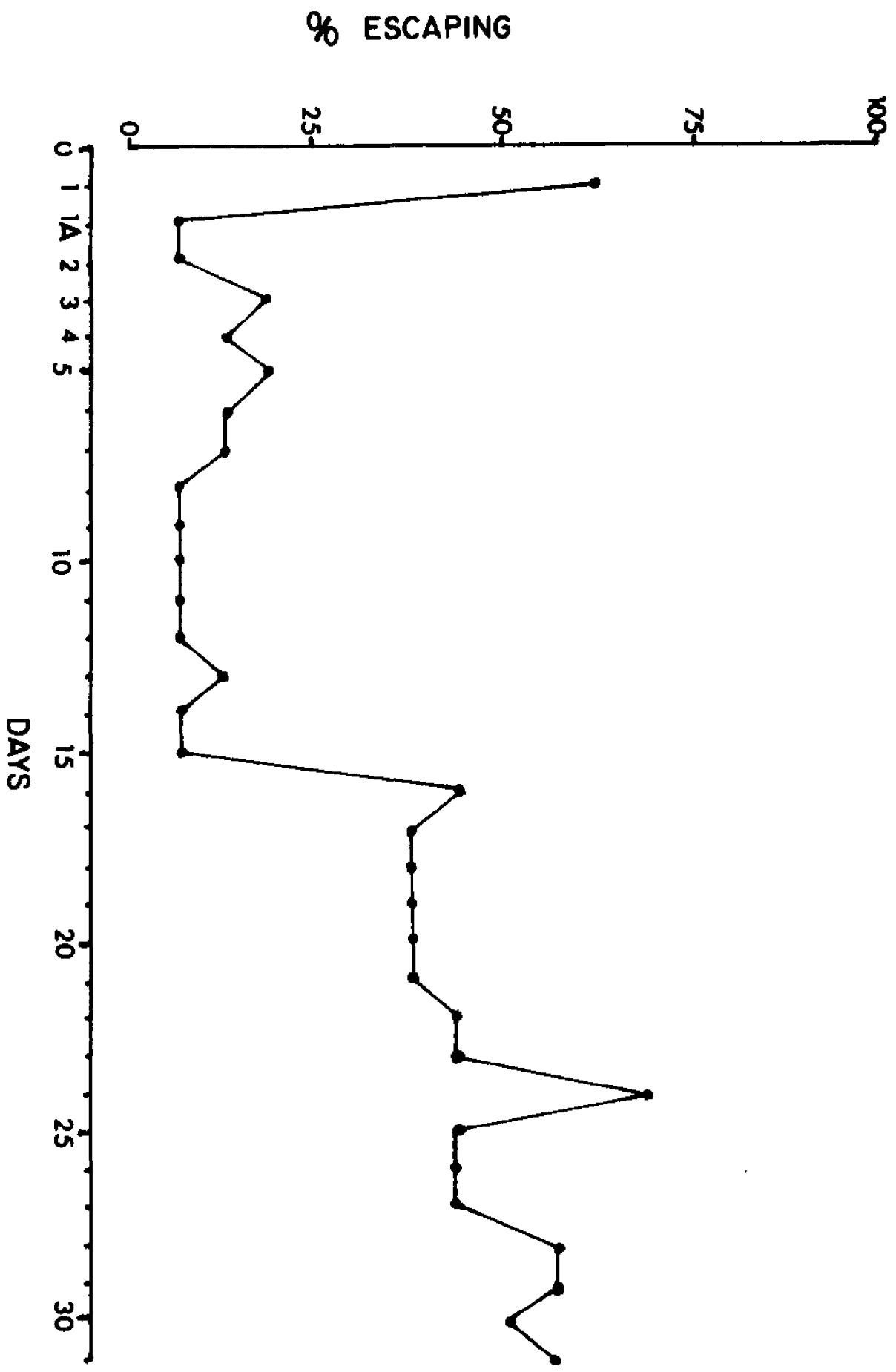
Figure 12

Escape performance of mice tested daily at 90 ua without morphine. A group of mice (N=16) were tested at 160 ua on Day 1; then retested on Day 1 and for fourteen days thereafter at 90 ua. On Days 16 through 31, the shock level was changed to 160 ua. Maximum shock-exposure interval was 30 seconds on all days. Days of significant impairment ($p<.05$, Chi^2 test) from control (Day 1) were Days 1A through 15.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experiments (Figure 1, Group 2) receiving daily post-injection testing. Consequently, an experiment was designed in order to determine whether mice which had undergone testing at a shock level near threshold for 15 days would show an impairment in baseline performance, when the shock was increased to 160 ua on Day 15. Such an impairment would suggest that one effect of morphine might be to lower the perceived shock intensity and that post-morphine impairment was somehow related to the mice having been exposed to a stimulus of subthreshold intensity.

Figure 12 illustrates the performance of a group of mice that was tested at 160 ua on Day 1 and then retested at 90 ua on Day 1 and for 14 days thereafter. On Day 16 the shock was increased to 160 ua and these mice were tested for an additional 15 days. The behavior of the mice during the first 15 was similar to that observed with morphine. There was an initial and sustained decrease in escape performance during the days when the shock level was 90 ua but when the shock level was returned to 160 ua (Day 16), performance immediately improved and there were no days when a significant residual impairment was observed. However, it is noteworthy, that the performance of these mice on Days 16 through 30 was below the level that would have been expected if the mice had received no testing during days 1A through 15 (Figure 1, Group 4).

Because the results of the previous experiment were not completely satisfactory in resolving the question of the specific role of continued exposure to the shock stimulus in generating impaired performance, several additional experiments were performed. In the first, groups of mice were tested at 160 ua on Day 1 and then retested on Day 1 and for 15 days thereafter on schedules of gradually increasing shock levels. The purpose of these experiments was to

better simulate the conditions in which there is development of tolerance to morphine. Figure 13 is a representative sample of this series of experiments. The choice of daily change in shock increment was determined on an empirical basis such that day to day fluctuations in performance were kept to a minimum. The group of mice in Figure 13 received a testing schedule whereby a shock level of 138 ua was reached by Day 15. On Day 16, the shock intensity was changed to 160 ua. On this day, the mice retained no impairment and a marked improvement in performance was observed on Days 17 through 30.

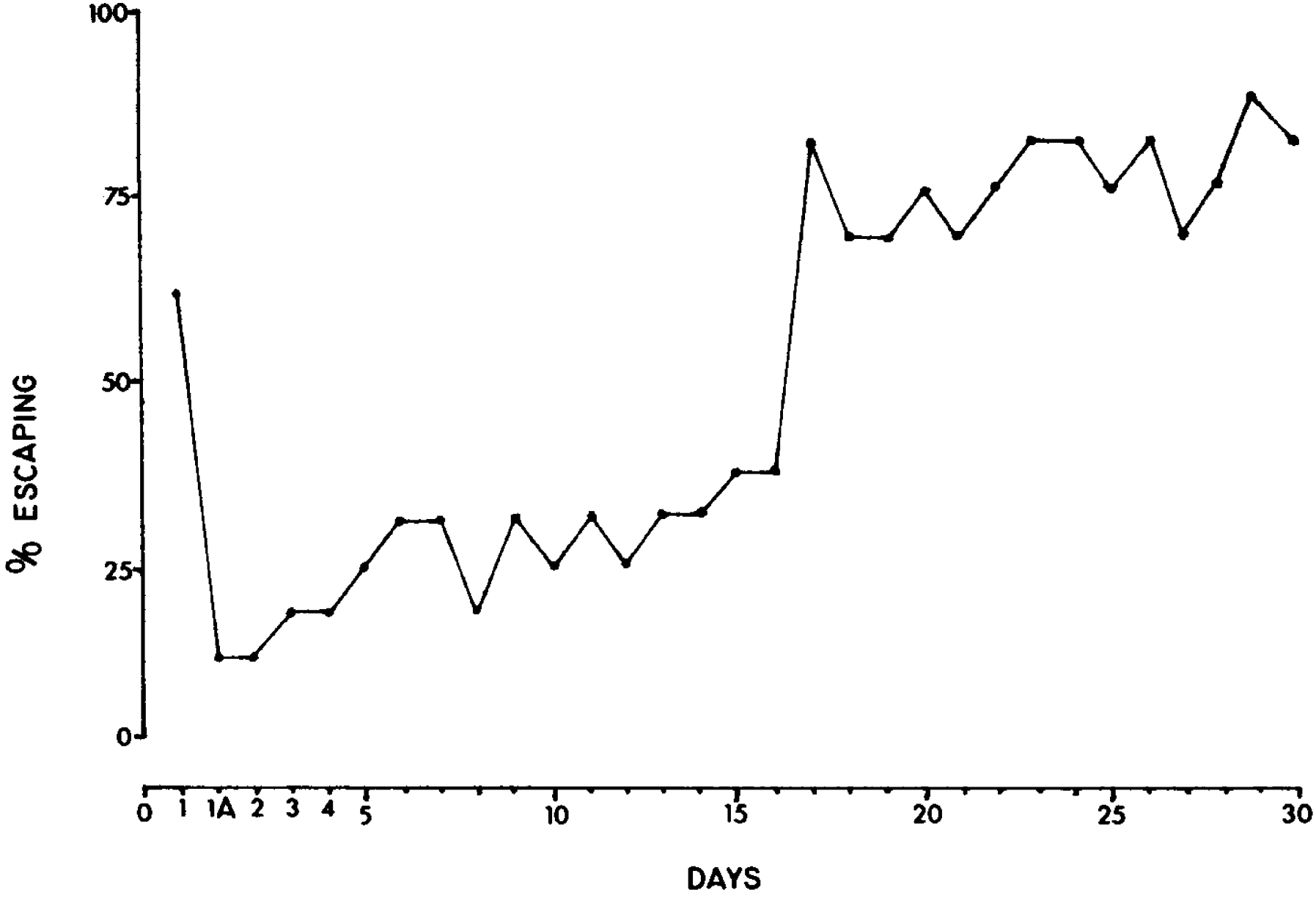
Although numerous treatment schedules over varying periods of days were tried, all attempts at increasing shock levels, without simultaneously increasing to some degree, escape performance, were unsuccessful. Additional experiments using various shock schedules including groups that were tested on Day 1 at low shock levels, i.e. no pretreatment at 160 ua, were carried out. The results of these experiments can be summarized as follows: Groups of mice subjected to treatment regimens consisting of various schedules of shock intensities, demonstrated either no impairment or a non-significant short term residual escape impairment when retested at the control shock value (160 ua).

Figure 14 presents the data from a series of experiments in which groups of mice were tested at 160 ua on Day 1 and then retested after morphine on Days 1 through 15 at shock levels of 0, 70, 90, 105, 120, 160 and 200 ua respectively. The group receiving "0" shock intensity was injected and then placed in the testing apparatus for 30 seconds without current being administered across the plates. The post-morphine impairment days were plotted as a function of the Day 1 through 15 shock level.

The results (70-200 ua) show a direct relationship between the degree of escape impairment observed following cessation of morphine administration and

Figure 13

Escape performance of mice tested at gradually increasing shock levels. A group of mice (N=16) were tested at 160 ua on Day 1, then retested immediately on Day 1 at 90 ua. Shock levels were gradually increased over the next fourteen days. A shock level of 138 ua was employed on Day 15 and the shock intensity was increased to 160 ua on Days 16 through 30. Days of significant impairment ($p<.05$, Chi^2 test) from control (Day 1) = Days 1 through 14.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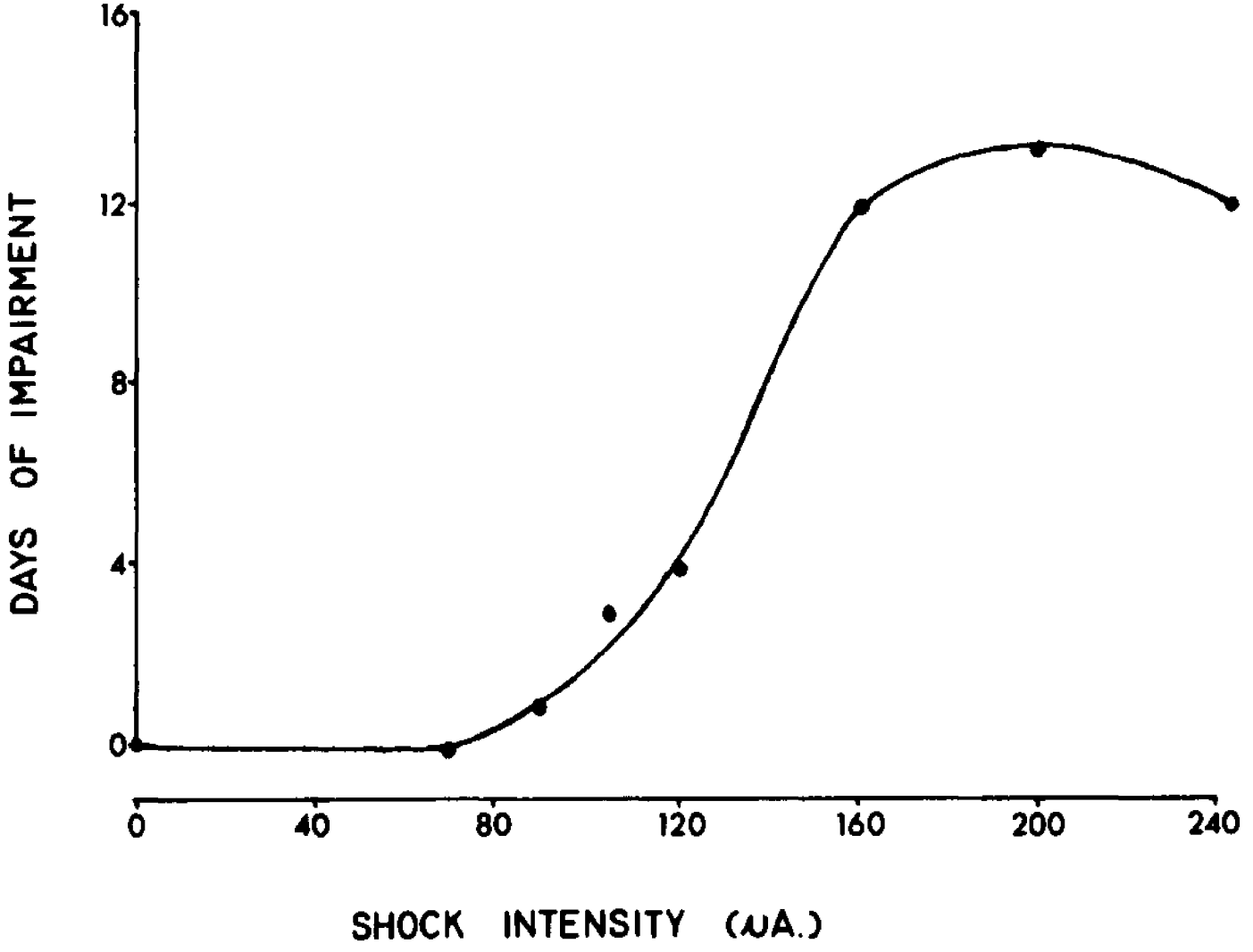
DAYS

Figure 14

Post-morphine impairment as a function of shock intensity. Eight groups* of mice (N=16) were tested according to the standard testing procedure (Table 1, Group 2) on Day 1. Post-injection testing on Day 1A through 15 was performed at various (0-240 ua) shock intensities. On Days 16 through 30 all groups were tested at 160 ua. Dose of morphine was 25 mg/kg and shock exposure interval was 30 seconds (maximum). The figure illustrates the number of days of significantly ($p<.05$, Chi^2 test) impaired post-morphine escape performance as a function of shock intensity.

* "0" group was placed in the shock apparatus (Days 1A through 15) 30 minutes after injection but no current was administered across the plates.


16
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8
4
0
DAYS OF IMPAIRMENT
0
40
80
120
160
200
240
SHOCK INTENSITY (μA.)

the intensity of shock to which the mice were exposed during the period of morphine administration. These results demonstrated that a prerequisite for post-morphine impairment was both morphine administration and superthreshold shock intensity.

Shock Exposure

In the previous experiments, regardless of the shock level employed, mice were permitted 30 seconds to escape. This arbitrary cut-off was chosen after preliminary control experiments indicated that there was a less than 5% probability that a mouse which had not escaped within 30 seconds, would so do if allowed an additional 90 seconds. These findings were based on studies of two groups of mice tested daily with and without drug administration. Additional experiments, however, were necessary in order to determine what effect, if any, different periods of shock exposure had on influencing the development and maintenance of impaired escape behavior.

The first group of experiments was designed to study the effects of various shock exposure times during morphine administration (i.e. Days 1A through 15 in Group 2, Figure 1) on post-morphine escape impairment. Exposure time on Day 1 and Days 16 through 30 was kept at 30 seconds. The results are presented in Table 3. Comparisons were made of Day 15 performance, Day 16 performance, number of post-morphine impairment days, and the mean escape frequency on all days of morphine administration.

Increased exposure time could have had two opposing influences on the acquisition of escape impairment. First, increased exposure time could have afforded the subjects greater time to escape and thus improve performance both during and after morphine. The results in Table 4 suggest a different explanation: that in fact, the increased exposure time worked to impair

Table 3

Escape performance of mice exposed to varying maximum shock exposure durations. All groups (N=16/group) were tested according to the standard testing procedure (Table 1, Group 2).

Exposure Duration (seconds)	Escape Frequency (%)			Days of Significant Impairment**
	Day 15	Mean*	Day 16	
30	12.50	6.25	12.50	12
5	12.50	6.25	31.25	1
15	12.50	31.25	31.25	2
60	0	6.25	6.25	12
120	6.25	6.25	6.25	14

*average post-injection escape frequency for Days 1A through 15.

**number of days on which post-morphine escape frequency was less than 37.50%.

performance rather than to improve it. This is evidenced by the increased impairment days at longer exposure times, and more clearly, by the decrease in impairment days at shorter exposures. The effect of varying exposure times, however, was not reflected in the performance of mice during the time morphine was administered. Thus although the initial control impairments demonstrated that there was no significant change in the escape performance of drug treated and untreated mice with respect to increased shock exposure, the present experiments show, however, that the degree of daily shock exposure directly influences post-morphine escape performance.

In one further experiment, post-morphine impairment data were compared using various shock-exposure intervals on post-morphine testing days. The results (Table 4) showed that recovery was more rapid with increased exposure time. Thus, exposure facilitates the acquisition of escape impairment, but inhibits the maintenance of impairment after cessation of drug.

Pre-Morphine Testing

In all the previous experiments, mice were permitted only a single exposure to the testing apparatus prior to morphine administration, i.e. Day 1. A series of experiments were designed in order to examine the effects of daily testing, prior to morphine administration, on the development of tolerance and the establishment of residual or post-morphine impairment. Groups of mice were tested for a varying number of days (0, 4, 8, 12, and 15) before beginning the standard testing schedule (Table 1, Group 2). For example, Figure 15 represents the escape performance of a group of mice tested in the escape apparatus for eight days. Beginning on Day 9, they then received morphine and post-injection testing for 15 days, followed by an additional 15 days of testing without morphine.

Table 4

Escape performance of mice exposed to varying maximum shock durations during the post-morphine testing period. All groups (N=16/group) were tested according to the standard testing procedure (Table 1, Group 2) through Day 15.

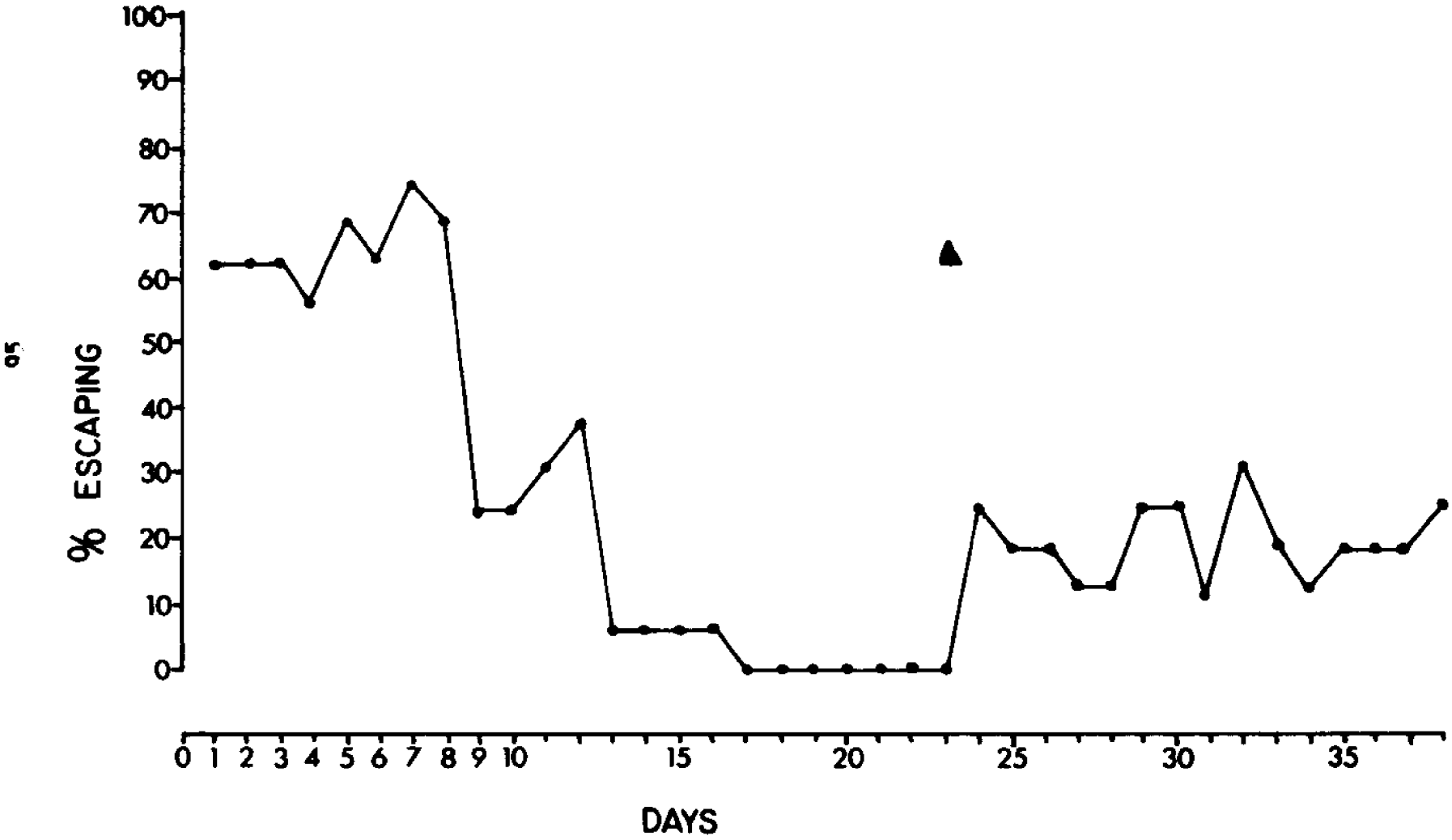
Exposure Duration (seconds)	Escape Frequency (%) Day 16	Days of Significant Impairment*
30	12.50	12
5	12.50	14 **
15	18.75	12
60	18.75	6
90	25.00	2
120	25.00	3

*number of days on which post-morphine escape frequency was less than 37.50%.

**number of impairment days were greater ($p<.05$, Sign test) at "5 seconds" than at other exposure intervals.

Figure 15

Escape performance of mice receiving eight days of trials prior to daily post-injection testing. A group (•) of mice (N=16) received escape trials (160 ua) once a day for eight days prior to exposure to the standard testing regimen (Table 1, Group 2). Morphine dose was 25 mg/kg and shock exposure interval (maximum) was 30 seconds at all testing sessions. Another group (▲) received eight days of pre-testing then 15 days of morphine injection but were not retested until after injection on the final day of drug administration (Day 23). Days of significant impairment ($p<.05$, Chi^2 test) from control (Day 1) were Days 9 through 11, 13 through 38. Performance on Day 13-23 was significantly ($p<.01$, Sign test) less then on Day 9.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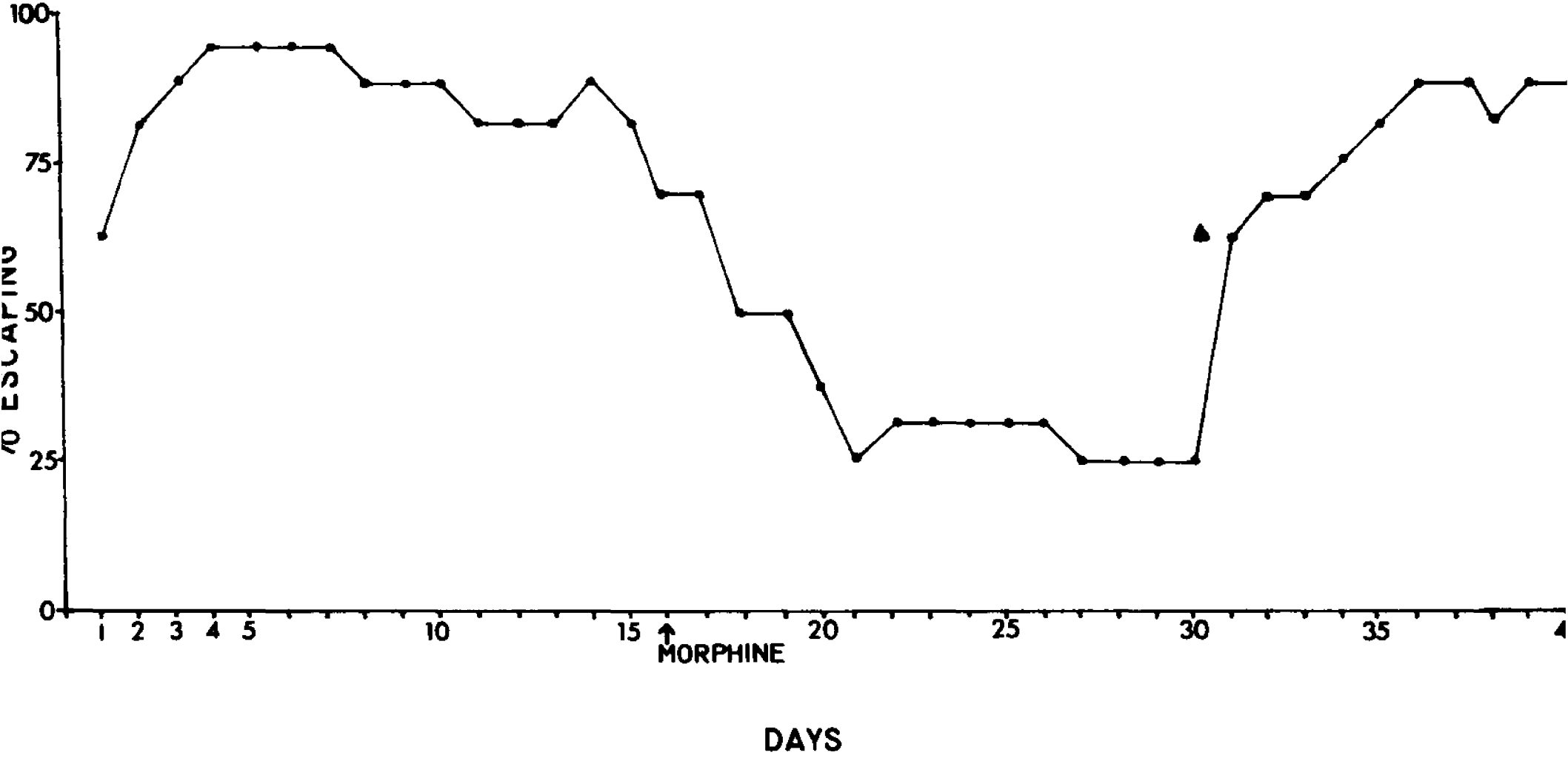
DAYS

Figure 16

Escape performance of mice receiving 15 days of trials prior to daily post-injection testing. A group (•) of mice (N=16) received escape trials once a day for 15 days prior to exposure to the standard testing regimen (Table 1, Group 2). Morphine dose was 25 mg/kg and shock exposure interval (maximum) was 30 seconds at all testing sessions. Another group (▲) received 15 days of pre-morphine testing then 15 days of morphine injection but were not retested until after injection on the final day of drug administration (Day 30). Days of significant ($p<.05$, Chi² test) impairment from control (Day 1) were Days 21 through 30. Performance on Days 17-30 was significantly ($p<.01$ Sign test) less than on Day 16.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MORPHINE
DAYS

A series of control groups of mice were tested as above, for 4, 8, 10, 12, and 15 days prior to receiving morphine. However, these groups were not retested until after injection on the last day of morphine administration. Thus the Day 15 morphine (Testing Day 23, Figure 15) performance served as a basis for comparing tolerance levels.

The data from these experiments are presented in Table 5. There is, as might be expected, a strong correlation between the performance of mice on Day 1 of morphine administration and the number of previous testing days. A similar relationship can be observed between pre-morphine testing days and performance on Day 15 of morphine administration and Day 16 (following cessation of morphine administration). Similarly, there is a strong correlation between pretesting days and the observed level of tolerance in initial mice on Day 15 of morphine administration. In addition, as might be predicted from the previous data, there is an inverse relationship between pretesting days and the number of post-morphine impairment days.

The most pertinent finding in these studies can be found by comparing the Day 1 and Day 15 morphine performance for each pre-morphine testing interval. In each case the Day 15 performance was less than the Day 1 performance. This effect is best illustrated in Figures 15 and 16. Figure 16 represents the performance of the group receiving 15 days of pretesting. Post-morphine testing was ended on Day 40. In both graphs there is a significant progressive decrease in performance during the period of morphine administration. Also of note is the finding that despite up to eight days of pretesting there is no observable decrease in post-morphine escape impairment.

Table 5

Escape performance of mice tested prior to morphine administration. Groups of mice (N=16/group) were tested for varying number of days prior to exposure to either the standard testing procedure (Table 1, Group 2) or (Tol. groups): 15 days of morphine administration, with retesting only after injection on Day 15 (of morphine administration).

Number of Pretesting Days	Escape Frequency (%)			Days of Significant Impairment**
	Day 1A*	Day 15[1]	Day 16	
Control***	6.25	6.25	18.75	12
Control*** (Tol)	-	43.75	-	-
4	25.00	0	25.00	12
4 (Tol)	-	43.75	-	-
8	25.00	0	25.00	15
8 (Tol)	-	50.00	-	-
12	43.75	25.00	31.25	1
12 (Tol)	-	62.50	-	-
15	68.75	25.00	62.50	0
15 (Tol)	-	62.50	-	-
20	68.75	37.50	75.00	0
20 (Tol)	-	75.00	-	-

* Day 1A = first day of morphine administration.

** number of days on which post-morphine escape frequency was less than 37.50%.

*** pre-testing only on Day 1.

(1) comparison of tested groups shows that Day 1A escape frequency was greater ($p<.05$, Sign test) than Day 15 escape frequency.

Recovery

The experiments described so far have concentrated on examining the influence of various parameters such as dose, shock intensity, etc. on the acquisition and maintenance of escape impairment as measured by daily testing. The following experiments will be concerned with the effects of various changes in the continuous daily testing regimens on tolerance development, post-morphine impairment and recovery (return to Day 1 values).

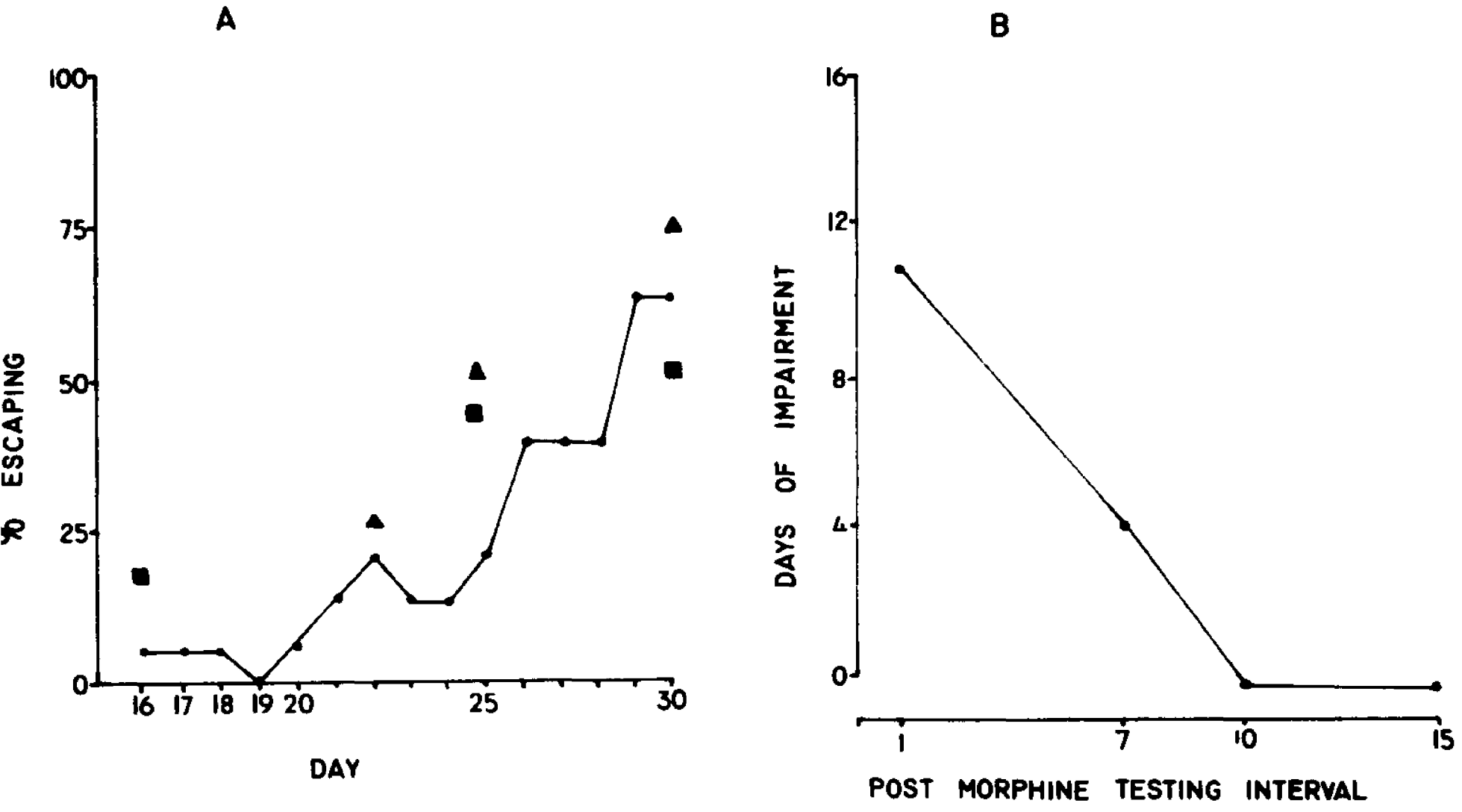
In the first set of experiments, one control group was exposed to the standard 30 day testing regimen (Table 1, Group 2). Other groups of mice received the equivalent schedule through Day 15. These mice were not tested until either 7, 10 or 15 days later. For comparison, several additional experimental groups were also included. One group received the standard testing schedule (Table 1, Group 2) through Day 16, i.e. one day's testing following cessation of morphine, but were not retested again until Day 22. Another group received similar treatment but was not retested until Day 30. With the exception of the latter group, all the experimental groups received 15 continuous days of testing once post-morphine testing was begun. Thus, the purpose of this experiment was to determine whether absence of daily testing after cessation of morphine administration had any effect on the maintenance, or alternatively, the recovery from, morphine induced escape impairment.

The results are represented graphically in Figure 17. Examination of the data on the performance of the untested mice shows that daily testing has a small and not significant effect on maintaining escape impairment. Similarly, Figure 17B shows that the spontaneous improvement in the untested group is permanent as reflected by the strong correlation between the post-morphine interval and the number of days of significant impairment.

Figure 17

A. Comparison of the effects of continuous and discontinuous post-morphine testing. Six groups (N=16/group) of mice were tested according to the standard procedure (Table 1, Group 2) through Day 15. One group (•) received daily post-morphine testing through Day 30. Two groups (■) were tested on Day 16 (same escape frequency in both groups); one tested again on Day 25 and the other on Day 30. Three groups (▲) were tested either on Days 22, 25 or 30. Dose of morphine = 25 mg/kg, shock level = 160 ua, maximum shock exposure interval = 30 seconds.

B. The number of post-morphine impairment days expressed as a function of the day on which post-morphine testing began. Four groups of mice (N=16/group) received the standard testing regimen (Table 1, Group 2) through Day 15. Post-morphine testing (15 days total) was begun on either Day 1, 7, 10, or 15. Morphine dose = 25 mg/kg, shock level = 160 ua, and maximum shock exposure interval = 30 seconds. Days of impairment of mice beginning post-morphine testing on Days 10 and 15 was significantly ($p < .05$, Chi^2 test) less than control (11/16).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POST MORPHINE TESTING INTERVAL

Thus irrespective of the post-morphine testing schedule, recovery from post-morphine impairment was complete and sustained after 15 days.

Relapse

Having established in the previous experiments the time course of recovery from post-morphine impairment, it then became necessary to study the phenomenon of relapse, i.e. whether and under what conditions mice previously displaying an escape impairment, could be induced to redevelop this impairment.

The effects of varying a number of parameters were examined in these experiments in order to determine their influence on subsequent relapse. In the first of these experiments, groups of mice were tested according to the standard schedule (Table 1, Group 2) through Day 30. Retesting of these mice was then begun either on Day 30, 60, 90, or 120. All these groups were then subjected to the same regimen as before (Table 1, Group 2). A parallel series of experiments were also carried out. Groups of mice were tested in the standard fashion through Day 16 but not retested again until either Day 30, 60, 90, or 120.

The results are summarized in Table 6. They indicate that there was no significant effect of prior treatment or intertrial interval on any parameter of relapse studied.

Because in these and subsequent studies, there seemed to be little effect of prior treatments on any parameter of relapse, a modified relapse treatment schedule was tried. In these studies, lower doses of morphine and lower shock intensities were substituted in the period of relapse testing in order to provide a more sensitive means of detecting subtle changes in relapse performance. The results were inconclusive in that there was no significant effect of various pretreatments on subsequent relapse performance either between

Table 6

Escape performance of mice on re-exposure to the standard testing procedure. Groups of mice (N=16/group) were tested according to the standard testing procedure (Table 1, Group 2) until either Day 15 (final day of morphine administration) or Day 16 (D16). A "Day 15" and a "Day 16" group were then re-exposed to the standard testing procedure on either Day 30, 60, 90 or 120.

First Re-Exposure Day	Escape Frequency (%) on Retesting			Days of Significant Impairment**
	Day 1	Mean*	Day 16	
Control***	62.50	12.50	18.75	12
Day 30	62.50	12.50	18.75	11
D16	68.75	6.25	18.75	10
Day 60	75.00	6.25	18.75	10
D16	81.75	6.25	25.00	11
Day 90	62.50	0	18.75	11
D16	62.50	12.50	25.00	10
Day 120	81.75	25.00	18.75	10
D 16	56.75	12.50	18.75	10

* Average escape frequency for Days 1A through 15.

** number of days on which post-morphine escape frequency was less than 37.50%.

*** group tested according to standard testing procedure (Table 1, Group 2).

experimental groups or between any experimental groups and controls.

Intertrial (Day) Intervals

In previous experiments, once morphine treatment was begun, i.e. Day 1A, it was continued on a daily basis until the beginning of the post-morphine testing period. Consequently, a number of experiments were performed in an attempt to assess the effect of varying the intertrial interval.

Groups of mice were tested on Day 1 and 1A as usual (Table 1, Group 2) but were then begun on various schedules of alternating (1, 3, 5, and 7 days) post-injection testing. Thus the group on the alternating 3-day schedule was tested after injection on Day 1, then not tested or injected for two days, then retested on the third day (after injection), etc. Each group was tested as such until 15 morphine plus testing days were accumulated. These mice were then tested without morphine daily for 15 consecutive days.

Groups of control mice were tested according to the various interval schedules daily (without morphine). Also, tolerance control groups received morphine on the same schedules as the experimental groups but were not tested until after injection on either the 5th or the 15th testing day respectively. Thus for each experimental group, a tolerance value was obtained in the beginning and at the end of the morphine treatment schedule.

The results of these experiments are summarized in Table 7. The first two columns compare the performance of tested and untested mice on Days 5 and 15 respectively. "Days" refer to testing days, not actual days. Similarly, the values in the third column represent the mean number of mice escaping over the 15 testing plus injection days.

The results show several noteworthy trends. First, there is an increase in the level of tolerance on Days 5 and 15 as the intertrial interval increases.

Table 7

Escape performance of mice tested at various intertrial intervals (days). All groups of mice (N=16/group) were tested on Day 1 and retested after injection on Day 1A. Subsequent post-injection testing was continued on alternating (1, 3, 5 and 7) day schedules until 15 post-injection days accumulated; at which time standard (Table 1, Group 2, Days 16 through 30) post-morphine testing was performed. Corresponding tolerance control groups were tested after injection either on Day 5 or 15. Shock level = 160 ua.

Intertrial Interval Days	Escape Frequency (%)			Days of Significant Impairment**
	Day 5	Day 15[1]	Mean*	
Control***	25.00	12.50	12.50	12
Control Tol.	37.50	50.00	-	-
1	25.00	6.25	12.50	11
1 Tol.	43.75	62.50	-	-
3	37.50	12.50	25.00	7
3 Tol.	50.00	62.50	-	-
5	43.75	15.00	31.25	5
5 Tol.	50.00	68.75	-	-
7	58.75	25.00	37.50	0
7 Tol.	68.75	75.00	-	-

* average post-injection escape frequency for Days 1A through 15.

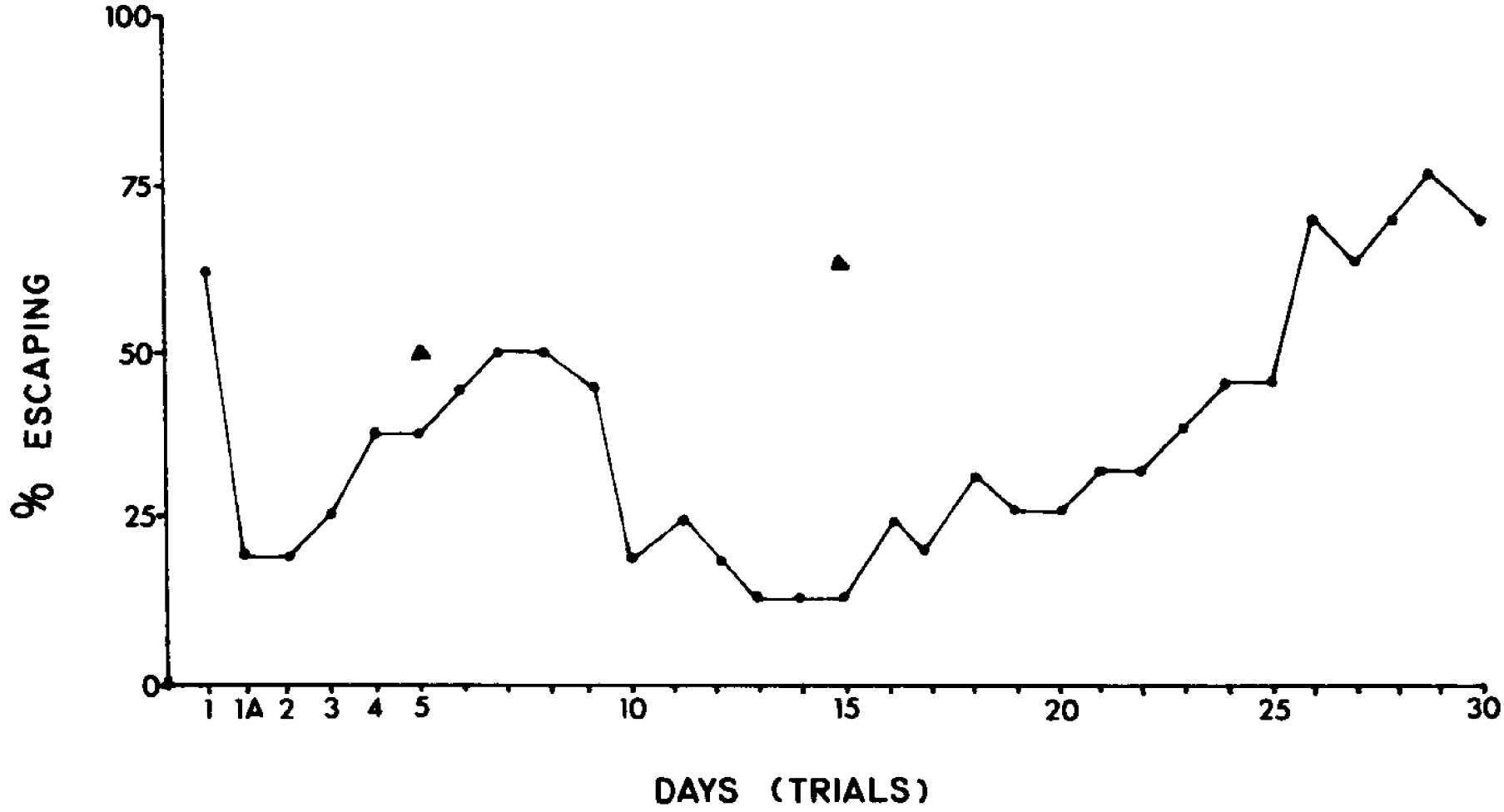
** number of days on which post-morphine escape frequency was less than 37.50%.

*** group tested according to standard testing procedure (Table 1, Group 2).

(1) comparison of all tested groups shows that escape frequency on Day 5 was greater ($p<.05$, Sign test) than on Day 15.

Figure 18

Escape performance of mice tested on an alternating (3 day) schedule. A group of mice (N=16) received the standard testing procedure (Table 1, Group 2) except the days of post-injection testing were spaced 3 days apart (i.e. mice were tested on Day 1A, not tested or injected for 2 days, then tested after injection on the following day - Day 2). Post-morphine testing (Day 16 through 30) was performed on a daily basis. Two additional groups (▲) were tested and injected on Day 1 and received injections, but no testing, according to the alternate (3 day) schedule. Post-injection testing was begun on either Day 5 or Day 15 respectively. Significant ($p<.05$, Chi^2 test) post-morphine impairment from control (Day 1) was observed on Days 1A, 2, 3, and 10 through 22. The escape frequency on Days 10-15 was less ($p<.05$, Sign test) than Day 9.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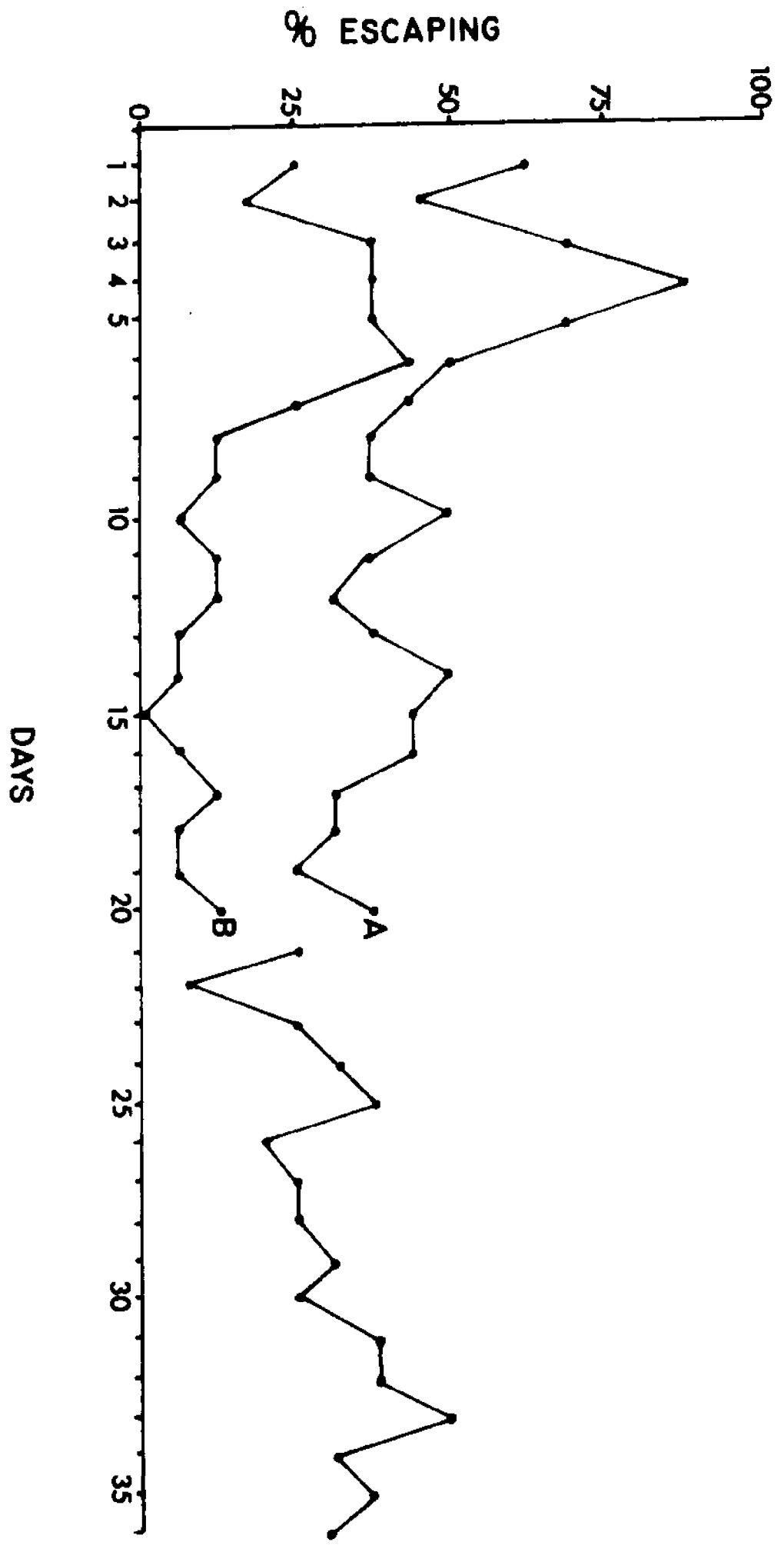
DAYS (TRIALS)

Yet despite this decrease in post-morphine impairment and increasing mean response frequency, there is no significant change in the Day 15 performance of the tested groups.

Examination of Figure 18, which illustrates the performance of the group receiving post-injection testing every third day, provides some insights into this apparent paradox. Unlike any of the other testing regimens, these mice showed an increase in escape performance over the first eight days of testing. However, on subsequent days, they demonstrated a decrease in escape frequency, returning to baseline (Day 1A) levels by the fifteenth injection day. Similar results were found in the "5 and 7" day groups, suggesting that tolerance develops during the intertrial interval so as to antagonize the effect of repeated testing during the first eight injection days.

Figure 19

Escape performance in mice tested before and after daily morphine injection. A group of mice (N=16) were tested before (A) and after (B) injection on each day of twenty days of morphine administration. Morphine dose = 25 mg/kg, shock level = 160 ua, maximum shock interval = 30 seconds. Post-morphine testing was performed on a daily basis on Days 21 through 36. Days of significant ($p<.05$, Chi^2 test) impairment from control (Day 1) were as follows: (A) - Days 1, 2, and 7 through 20; (B) - Days 12, 17, 18, and 19 and post-morphine Days 21 through 24, 26 through 30 and 35.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Further Parametric Investigations

Daily Testing Before and After Morphine Administration

With the exception of Days 1 and 1A, all experiments described to this point have been conducted on a once-daily testing basis. An experiment was designed to determine the effects of daily testing before and after morphine injection on escape behavior. A group of mice were tested as usual on Day 1, but on Days 2 through 20 they received testing 30 minutes prior to injection. They were then retested 30 minutes after injection. Thus two escape values were obtained for each day of morphine administration.

The results are presented in Figure 19. To avoid confusion both Day 1 values are presented together rather than as Day 1 and 1A as in previous figures. The results show several trends. First, through Day 4, there was little or no alteration in the pre-injection performance of the mice as compared to groups of mice tested daily with pre-injection testing (Figure 1, Group 5) or without it (Figure 1, Group 1). The performance of mice after injection for the first seven days was somewhat higher than that of mice exposed to the standard testing regimen. However, on Days 8 through 20, the post-injection performance of these mice reached the expected values for mice receiving the standard testing procedure (Table 1, Group 2). In addition, there was a clear and sustained fall (after Day 4) in pre-injection performance to a level below that observed in mice tested daily (Figure 1, Group 5) pre-injection. Pre- and post-injection testing was carried out past Day 15 until Day 20 in order to determine if the pre- and post-injection performance would eventually overlap.

This was not the case. However, despite the apparent negative effect of pre-injection testing on post-injection performance, (at least during the first seven days) there was still 10 days of significant post-morphine escape impairment.

Injection-Testing Interval

In all the experiments with morphine described so far, there was a post-injection-to-testing interval of thirty minues. Several acute and chronic experiments were designed to evaluate the possible influence of the injection-testing interval on various parameters of the escape paradigm.

Table 8 contains the results of experiments comparing the effects of various post-injection intervals on escape frequency. The performance of mice acutely (Day 1A) and chronically (Day 15) tested at various times after morphine injection did not vary significantly between the control (30 minutes) and the experimental groups at 15 and 60 minutes. The group tested at 120 minutes performed in a similar fashion to mice receiving low doses of morphine i.e. they had slightly better performance on Day 15 and fewer impairment days.

Habituation Controls

In the original experiments, injected but not tested control groups, were not exposed to the testing apparatus. Animals were simply returned to their boxes immediately following injection. Consequently, several experiments were carried out to determine what effect, if any, habituation to the testing environment, i.e. placing mice in the testing apparatus without any current across the plates, might have on the test results.

In the first experiments, habituation controls were performed for Group 4 in Table 1. A group of mice were tested on Day 1, then placed in the shock apparatus with no current applied for 30 seconds, for 15 consecutive days. Testing at 160 ua was begun again on Day 16. A similar experiment was

Table 8

Escape performance of mice tested at various post-injection testing intervals. Groups of mice (N=16/group) were tested according to the standard testing procedure (Table 1, Group 2) except that varying (15, 60, 120 minutes) post-injection testing intervals were substituted.

Post-Injection Testing Intervals (minutes)	Escape Frequency (%)		Days of Significant Impairment*
	Day 1A	Day 15	
30 (control)	6.25	6.25	12
15	12.50	12.50	10
60	12.50	12.50	11
120	25.00	25.00	6

* number of days on which post-morphine escape frequency was less than 37.50%.

performed for the tolerance control group, (Table 1, Group 6). In this experiment, mice were habituated to the apparatus for 30 seconds, one half hour following injection on Days 1 through 14.

In both these experiments, there was no observable difference between the habituated and non-habituated groups with regard to escape performance. Also, although occasionally, one or two mice climbed out of the box within the 30 second time limit, there was no evidence of spontaneous escape jumping in the habituation controls.

A number of additional habituation experiments were performed. Except for one experiment, no difference was observed between habituated groups. The exception was found in a comparison study between two groups of mice tested for recovery from escape impairment. One group of mice received the standard testing procedure through Day 16 and was not retested again until Day 30 and again on Day 60. The experimental group received the same schedule except that they were placed in the escape apparatus without shock for 30 seconds on Days 17 through 29 and Days 31 through 59.

The experiments on recovery (Figure 17) showed that there was no effect of testing on sustaining or diminishing post-morphine escape impairment. However, in this experiment, the habituated group had a sustained impairment on Day 30 and on Day 60 (3/16 mice escaping on Days 16 and 30, 5/16 escaping on Day 60).

Forced and Delayed Shock Exposure

The role of habituation to the testing apparatus in affecting escape behavior was explored in the previous section. A further series of experiments was subsequently designed to investigate other factors relating to the interaction of the testing environment and escape behavior.

In the first experiment, one group of mice was tested as usual on Day 1 but upon retesting after injection on Day 1 and on all subsequent days, the mice were placed in the testing apparatus for 10 seconds prior to turning "on" the electric current. Thus, the mice were permitted a 10 second delay period in which to avoid the shock. Another group was tested daily without morphine, but was also permitted a 10 second delay period prior to stimulus onset.

The results were as follows: there was no difference between the "delay" group and the control group (Figure 1, Group 2) as measured by any of the parameters of escape performance. In addition, there was no evidence of avoidance learning i.e. escaping prior to stimulus onset, either during or after the period of morphine administration. Two of sixteen mice did learn to "avoid" the shock in the group receiving daily testing, one beginning on Day 10 and one on Day 12.

The experimental data presented so far have suggested that morphine and some degree of shock intensity were necessary to generate impaired escape performance. The question still remained as to what specific effect morphine had in facilitating suppression of escape behavior. If the effect of morphine was to, in essence, "paralyze" the mice, then forcing the mice to endure the stimulus should have the same effect as morphine. A simple experiment was designed to test this hypothesis. A clear plastic top was fitted to the escape apparatus, in such a fashion that the mice were forced to stay in contact with the charged plates at all times. A group of mice was tested using this apparatus on Days 1A through 15, then tested for impairment on subsequent days.

During Days 1A through 15, the mice were observed to squeak and jump in the same manner as moderately "tolerant" morphine treated control mice.

However, no post "forced shock" impairment was observed. Moreover, these mice escaped at a frequency of 87.5% on Day 16 and never dropped below this level on subsequent days.

Escalating Dose Levels

Previous experiments have shown that tolerance to morphine induced escape impairment does not occur in daily tested mice, although it does develop over the same period in untested mice. It might be suggested that the development of pharmacological tolerance to morphine in tested mice facilitates a gradual desensitization to the stimulus in the tested mice. An experiment was devised in order to investigate this possibility. Two groups of mice were tested as usual (Table 1, Group 2 and Table 1, Group 6, Days 1-15), except that beginning on Day 2 and continuing through Day 15, gradually increasing (in increments of 25 mg/kg per day) doses of morphine were administered. By Day 15, the dose was 250 mg/kg. The experimental group was then tested for escape performance without morphine for an additional 15 days.

The results are presented in Figure 20. It is clear from the daily performance of the mice receiving morphine, that increasing dosage, which should negate any interaction of tolerance on desensitizing the mice to the shock, has little effect on performance during or after morphine. The low escape frequency on Day 15 in the control group argues against the possibility that tolerance developed to the high dose of morphine.

Specially Selected Groups

Because of the initial and repeated observation that 10 of 16 naive mice escaped from the shock apparatus under the original testing conditions, random groups of mice were used in all experiments.

In this final set of experiments, only mice that had successfully escaped

Figure 20

The escape performance of mice exposed to daily gradually increasing doses of morphine. A group (●) of mice (N=16) received the standard testing regimen (Table 1, Group 2) except that the dose was gradually increased over Days 1A through 15 (25 - 250 mg/kg.). A second group (N=16) of mice (▲) received the same dose schedule as above except post-injection testing was not performed until Day 15. Shock level = 160 ua, maximum exposure interval = 30 seconds. Days of significant impairment ($p<.05$, Chi^2 test) from control (Day 1) were Days 1A through 21.

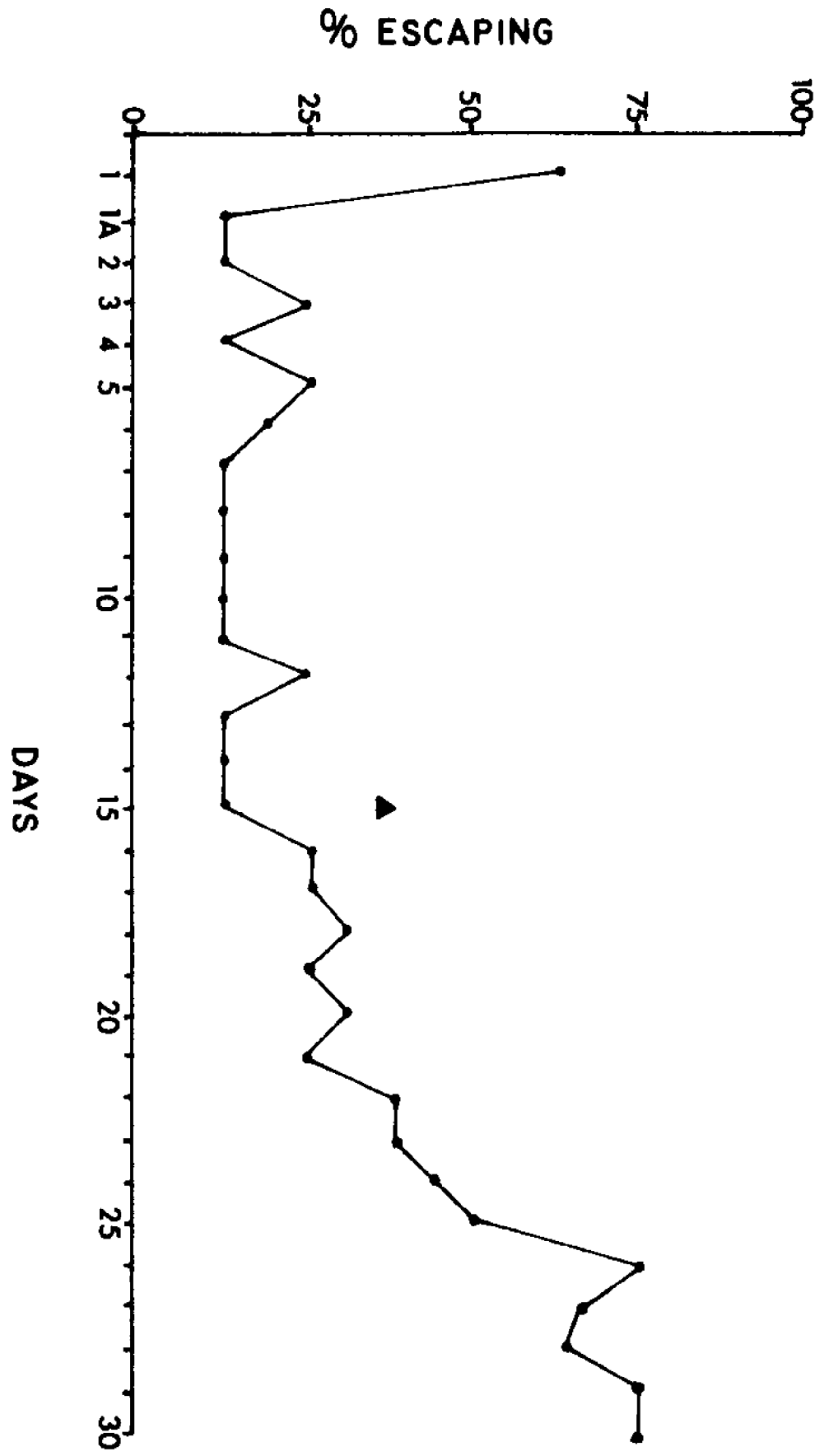
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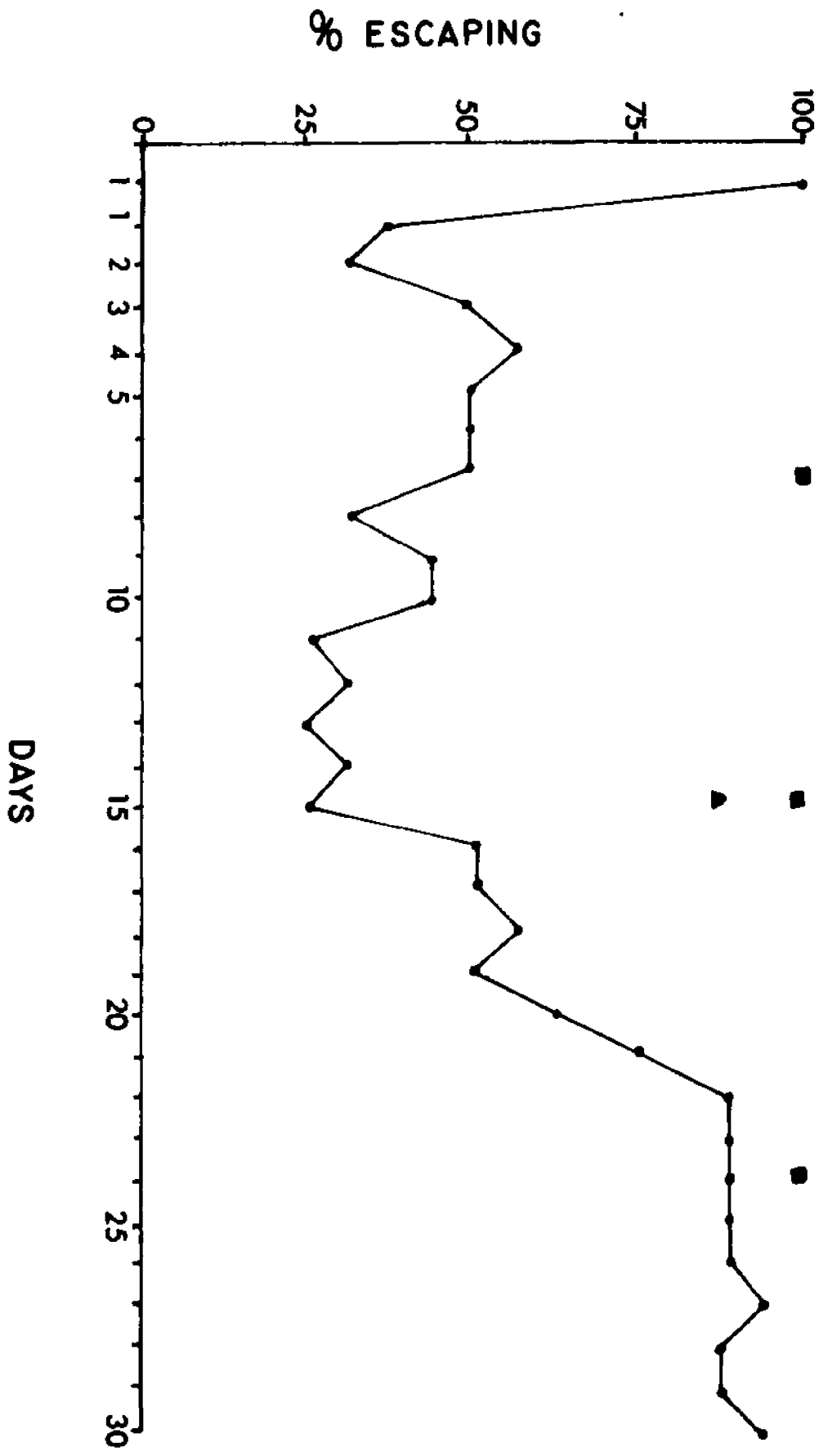
Figure 21

The escape performance of selected (100% escaping) groups of mice. Naive mice were tested; only those escaping were selected, such that three groups (N=16) of 100% "escapees" were formed. One group (●) received the standard testing regimen (Table 1, Group 2). The second group (■) received daily testing without morphine, and third (▲) received daily morphine injections (25 mg/kg) but no testing until after injection on Day 15.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on Day 1 were employed. Three groups of (16) mice were subjected to the treatment schedules outlined in Table 1 for Groups 1, 2, and 6 (through Day 15). The results are illustrated in Figure 21.

Due to the selection process, Chi^2 analysis could not be done on the experimental group because the Chi^2 test is only applicable to random populations. However, the performance of these mice although higher than that observed with random populations, were parallel to the original experimental groups in most aspects of escape performance. There was an initial rise in escape frequency over the first five days of drug administration, but the escape performance eventually fell to approximately Day 1A levels by Day 15. In addition, there was a sustained escape impairment after cessation of morphine administration followed by a gradual return to control levels on subsequent days.

Drugs Other Than Morphine

The experiments presented so far have been concerned with quantitatively defining the original phenomena observed with morphine. The experiments presented in this section will deal with the specificity of these phenomena with respect to different classes of drugs. Four types of drugs were studied: narcotic analgesics, barbituates, major tranquilizers and minor tranquilizers.

Narcotic Analgesics

Levorphanol

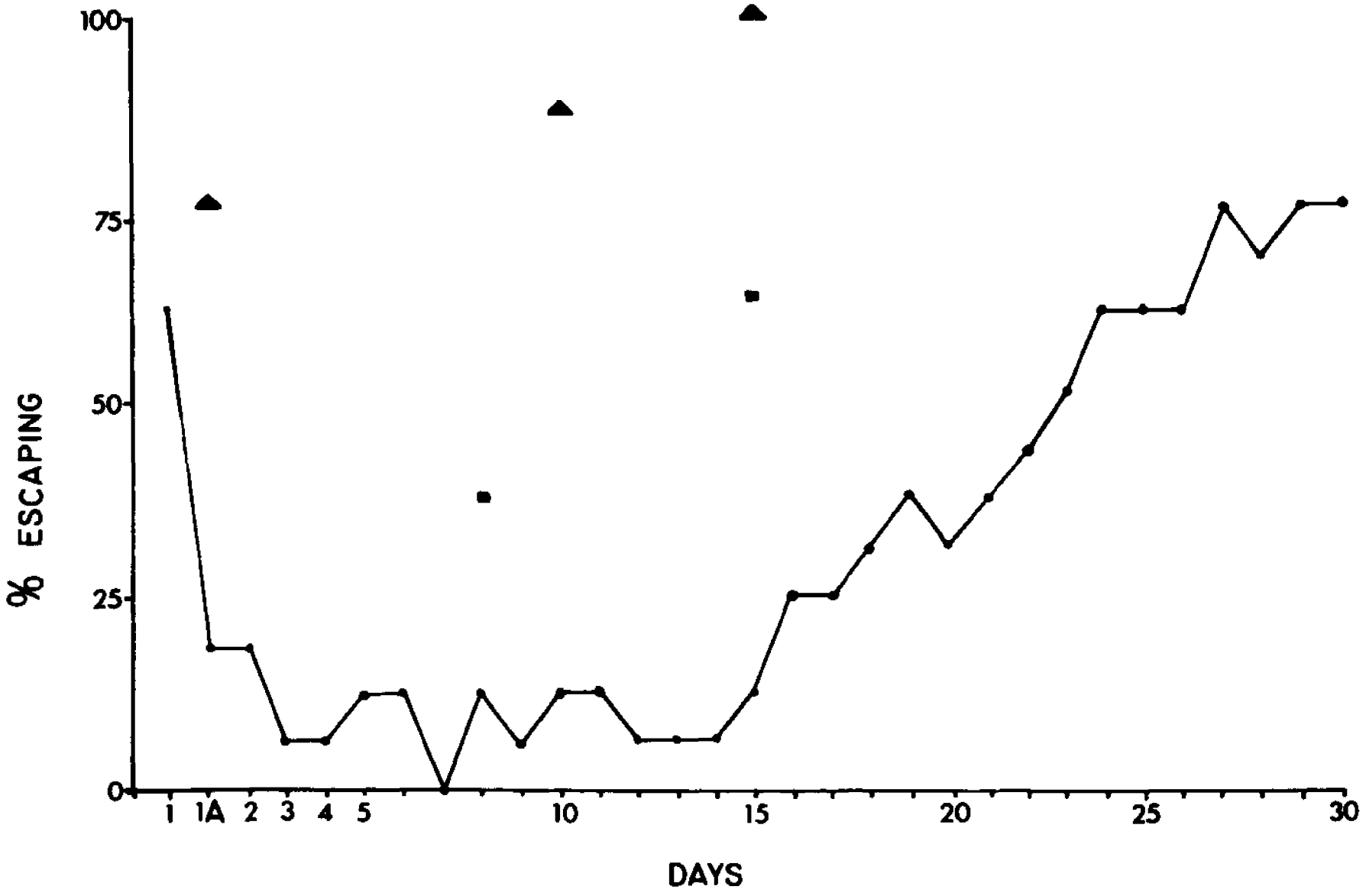
Preliminary testing of mice at various doses showed that a 12.5 mg/kg injection of levorphanol produced an initial Day 1 escape impairment similar to that observed with morphine at 25 mg/kg. Two groups of mice were subsequently tested with levorphanol. One group received the standard testing procedure (Table 1, Group 2), and the other received the standard tolerance schedule (Table 1, Group 6, Day 1 through 15).

Two additional groups were also included. The first group was tested for tolerance on Day 0 instead of Day 15. The second group received the standard testing regimen (Table 1, Group 2) except that they received dextrorphan, (25 mg/kg), a non-analgesic optical isomer of levorphanol.

The results are illustrated in Figure 22. The behavior of the mice receiving the standard testing regimen with levorphanol shows all of the pertinent features of similarly exposed morphine treated mice. There is a marked and sustained low level performance during morphine administration, even though tolerance was simultaneously demonstrated in the untested group. There is also a four-day post-morphine residual impairment. The group receiving dextrorphan elicited none of these features and behaved similarly to uninjected

Figure 22

The escape performance of mice receiving levorphanol and dextrorphan. Two groups (N=16/group) of mice received the standard testing (160 ua) regimen (Table 1, Group 2) except that levorphanol (12.5 mg/kg) was administered instead of morphine in one group (●) and dextrorphan (25 mg/kg) in the other (▲). Two additional groups (■) received daily levorphanol (12.5 mg/kg) injection but no post-injection testing until either Day 8 or 15. All testing was performed 30 minutes after injection. Days of significant ($p<.05$, Chi^2 test) impairment from control (Day 1) were Days 1A through 18 and 20.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controls.

Meperidine

A range of doses of meperidine from 10 to 150 mg/kg was tried in an attempt to approximate the performance of mice receiving morphine. However, at doses sufficient to produce a sustained low level of impairment, there was a greater than 25% mortality rate . In a final attempt to overcome this obstacle, additional mice were added to each group to make up for any deaths. Unfortunately, the daily mortality rate was so high as to preclude a valid interpretation of the results.

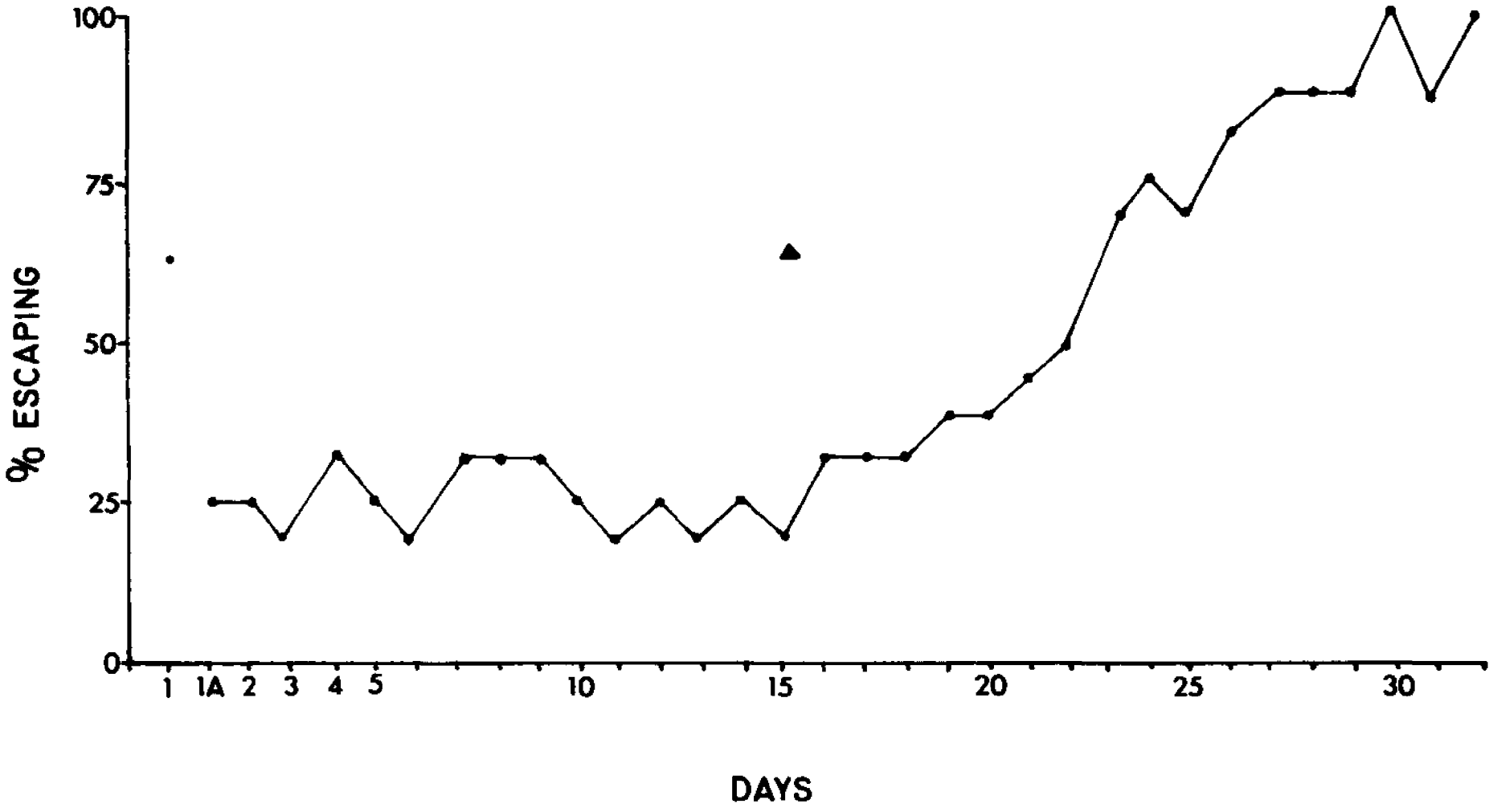
Methadone

The same problem of a high mortality rate accompanying low escape frequencies was encountered with methadone. However, unlike meperidine, with methadone the mortality rate fell markedly on successive days of drug administration. Thus, a method was devised to overcome the initial high death rate observed with doses of methadone which were effective in producing low escape frequencies. Two groups of mice were tested on Day 1 then injected for two days with methadone at 2.5 mg/kg, then for two days at 5.0 mg/kg. On the fifth day, methadone was administered at 7.5 mg/kg. One group of mice was then tested one hour later and following injection for 14 days thereafter. The second group received 14 additional injections at 7.5 mg/kg and were tested for tolerance after injection on the 15th day of injection at the high dosage.

The results are presented in Figure 23. For clarity, the graph begins on Day 14 which is the first day of post-injection testing. Although the

Figure 23

Escape performance of mice receiving methadone. A group of mice (N=16) were tested on Day 1, then received two days of daily methadone (2.5 mg/kg) injections followed by two days of injections at 5.0 mg/kg. Daily post-injection (7.5 mg/kg) testing was begun on the following day (Day 1A). A second group (N) was tested on Day 1, received the same dose schedule as above, but no testing until after injection on Day 15. All testing was performed 60 minutes after injection. Days of significant impairment ($p < .05$, Chi^2 test) from control (Day 1) were Days 1A through 18.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effects of methadone were not as marked as with morphine or levorphanol, the general features of the original phenomena were still observable. There was no evidence of tolerance in the tested group as compared to the untested group and there were three consecutive days of post-morphine impairment followed by a gradual improvement in performance on subsequent days.

Propoxyphene

At the shock intensity of 160 ua, no dose of propoxyphene was effective in significantly suppressing escape behavior. At high doses (greater than 100 mg/kg) the majority of mice either died in convulsions or, if not, they successfully escaped from the testing apparatus.

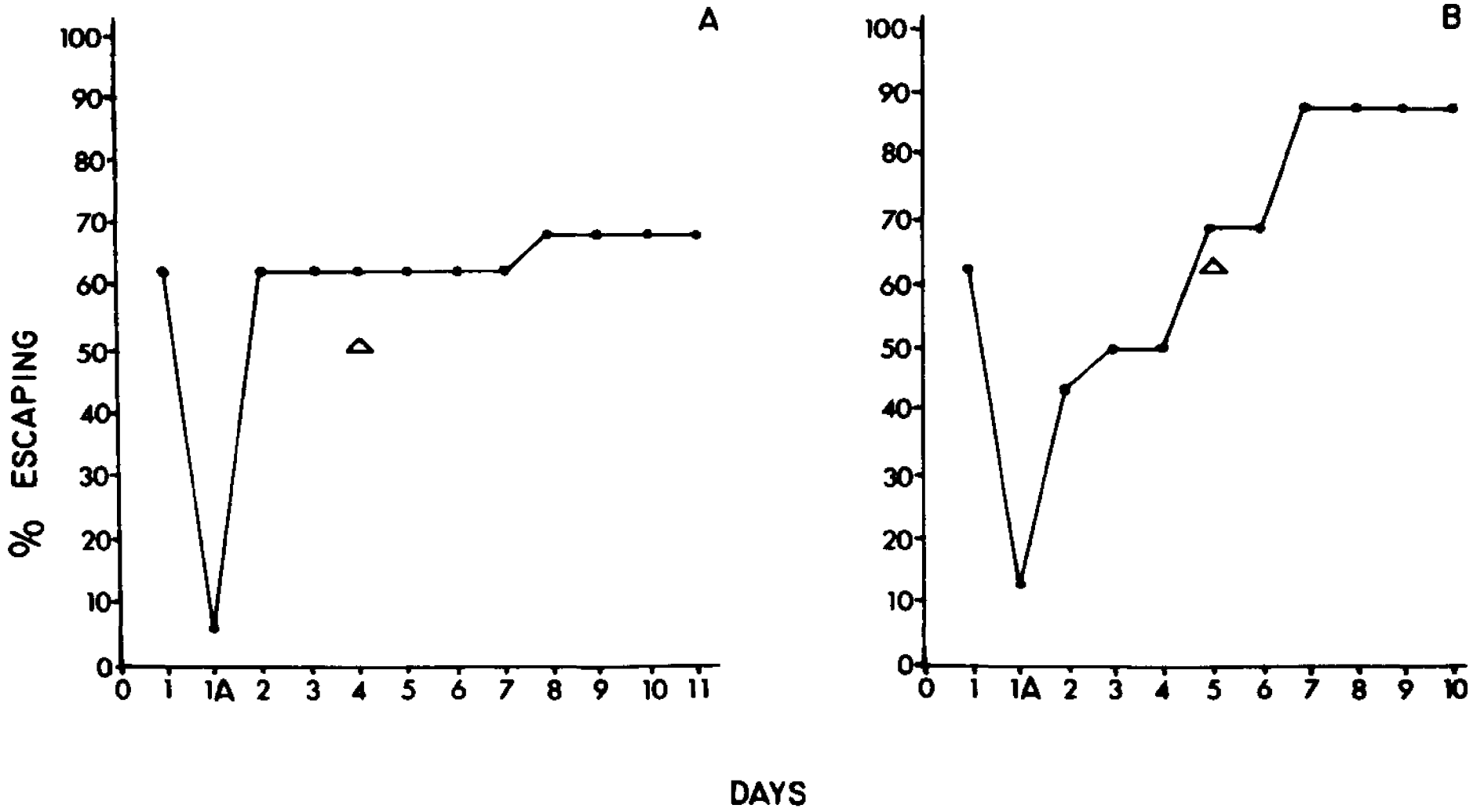
Barbiturates and Tranquilizers

Chlorpromazine administered acutely at a dose of 40 mg/kg was found to effectively suppress escape behavior (on Day 1A) to a level equivalent to that observed with morphine (25 mg/kg). A similar effect was also obtained with diazepam at 12.5 mg/kg. Groups of mice were tested according to the standard procedure. For each drug, one group received daily post-injection testing while the other was tested for tolerance, without prior (except Day 1) testing, on Day 5 of drug administration.

The results are presented in Figure 24. The results for both chlorpromazine (A) and diazepam (B) are similar in many respects. With both drugs, tolerance developed rapidly in both the tested and untested groups. In addition, and in contrast to morphine, the tested groups developed a greater degree of tolerance than the untested groups. Tolerance to diazepam proceded at a more gradual rate in the tested group, but their eventaul performance was even greater than that observed with chlorpromazine.

Figure 24

Escape performance of mice receiving chlorpromazine (A) and diazepam (B). Two groups of mice (N=16/group) received the standard testing regimen (Table 1, Group 2) except that one group received chlorpromazine (40 mg/kg) and the other diazepam (12.5 mg/kg). Two additional groups (▲) were injected as above but were not tested until after drug administration on Day 5. All testing was performed 30 minutes after injection at 160 ua. Days of significant ($p<.05$, Chi^2 test) impairment from control (Day 1) were observed on Day 1A (both A and B) only.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Pentobarbital

After preliminary testing of mice with a broad range of doses of pentobarbital, a marked impairment in escape performance could only be found at doses approaching the LD. 50. In addition, on subsequent testing days, tolerance rapidly developed similar to that observed with diazepam (Figure 24B).

Naloxone

A number of experiments were performed to determine whether administration of naloxone, a narcotic antagonist, could modify the response of animals to shock in the presence and in the absence of morphine administration. Initially, it was found that naloxone, in doses (20 mg/kg) well above those used in most studies, did not produce any signs of withdrawal (jumping or shaking) in mice having previously received morphine (25 mg/kg) for up to 90 days. Daily naloxone injections prior to testing had no effect on the return to baseline escape performance of mice previously exposed to the first 15 days of the standard testing paradigm. Daily naloxone injections (10 minutes prior to testing) alone produced no effect on escape performance. Naloxone administered 20 minutes after morphine injection counteracted the effects of morphine and consequently blocked the development of escape impairment.

Hot Plate

The initial experiments were concerned with studying the effects of stimulus parameters on the escape behavior of mice exposed to shock following the administration of various agents. Irrespective of the parameter or agent studied, the stimulus mode was electric shock applied to the feet. This section deals with the results of experiments using a qualitatively different stimulus, heat.

A variety of experiments were carried out in order to select the stimulus conditions which could best reproduce the results observed when the standard testing procedure (Table 1, Group 2) was employed using shock as the stimulus. In preliminary experiments, it was observed that temperatures which produced a 62.5% escape frequency in naive mice, were insufficient to detect any tolerance in morphine treated mice (i.e. in groups not tested daily). Thus a temperature of 60°C was eventually chosen for use in these experiments even though the Day 1 (naive) performance of mice at this temperature was 87.5% escaping.

In the first experiments, one group of mice was exposed to the standard testing procedure substituting the hot plate for the shock apparatus. Another group was tested for tolerance in the standard manner on Day 15. A third group (Figure 25) was tested daily on the hot plate without morphine. The performance of the latter group fluctuated over the first eight days of testing but improved and continued at a sustained high level over the remaining testing period.

The results from the experimental group are illustrated in Figure 26. It is clear that the behavior of morphine treated mice on the hot plate parallels in many aspects, the performance of mice when electric shock is used

Figure 25

Escape performance of mice tested daily on the hot-plate. A group of mice (N=16) were tested on the heated plate (60°C.) for 15 days with a maximum stimulus exposure of 30 seconds at each trial.

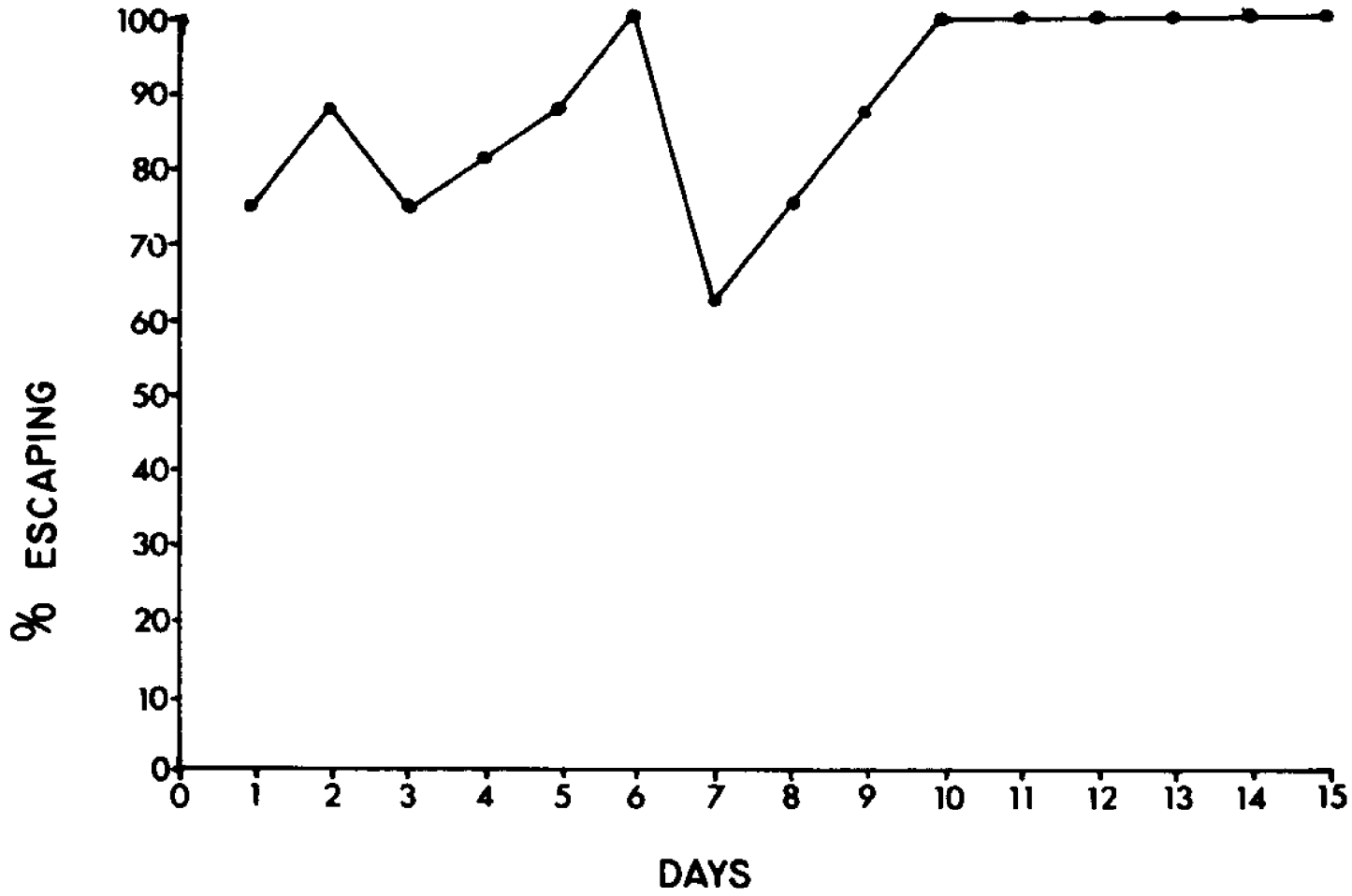
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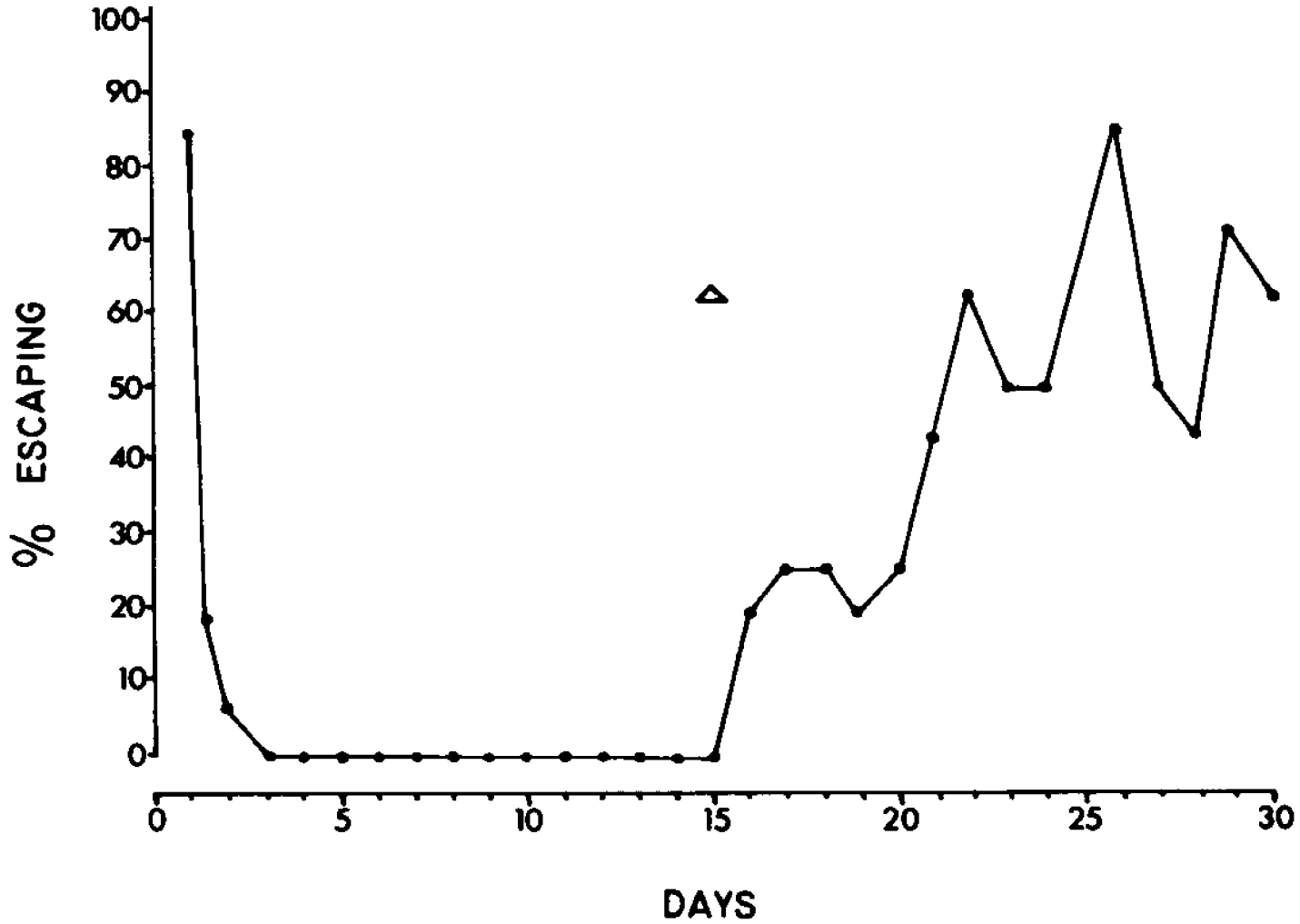
Figure 26

Escape performance of mice tested after morphine injection on the hot-plate. A group of mice (N=16) received the standard testing regimen (Table 1, Group 2) except a heated plate (60°C.) was substituted for foot-shock. Maximum heat exposure at each trial was 30 seconds. A second group (▲) of mice (N=16) was tested on Day 1 and injected with morphine (25 mg/kg) daily for 15 days, but were not tested until after injection on Day 15. Days of significant impairment ($p<.05$, Chi^2 test) from control (Day 1) were as follows: Days 1A through 24, 27 and 28.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as the stimulus. There is an initial and sustained impairment in performance of mice tested daily after morphine injection. In addition, the escape frequency of the group tested after injection on Day 15 following daily injection was significantly higher than the experimental group. Also there were 10 days of significant escape impairment following cessation of morphine.

Most authors studying analgesia on the hot plate measure latencies to "escape oriented" responses such as squeaking, paw lifting or jumping (not necessarily escaping). In order to compare escape behavior and "reflex" responses to heat, a group of mice were exposed to the standard testing regimen (Table 1, Group 2) for 15 days. Data were collected on escape frequency and on latencies of "reflex" responses. Latencies to all responses whether squeaking or actual escaping, were evaluated equally. The results are presented in Figure 27.

Figure 27A illustrates the mean daily response latency over the 15-day period. It is evident from the results that a marked increase in response latencies occurs on Day 1A. On successive days, however, latencies rapidly diminished, eventually falling below the baseline Day 1 mean latency. The response latencies of the untested group on Day 15 were not significantly different from those of the tested group. The corresponding escape performance of these groups is shown in Figure 27B. It is clear, from a comparison of the two graphs, that an escape impairment develops even though there is a simultaneous decrease in daily mean response latencies.

In a final experiment with the hot plate, two groups of mice were tested according to the standard testing schedule (Table 1, Group 2) through Day 15. One group, however, (Table 9, HTP-SHK) was tested on the hot plate through Day 15 then tested in the shock apparatus through Day 30. Similarly the second group (SHK-HTP, Table 9) was tested through Day 15 with shock as the

Figure 27

Comparison of the escape behavior and reflex latencies of morphine treated mice on the hot-plate. One group of mice (N=16) received the standard testing regimen (Table 1, Group 2) on the hot-plate; both escape frequency and reflex response latencies were recorded. Two additional groups of mice (▲) received daily injections but no testing until after injection on either Day 7 or 15. Mean response latencies are represented in (A) and escape performance in (B).

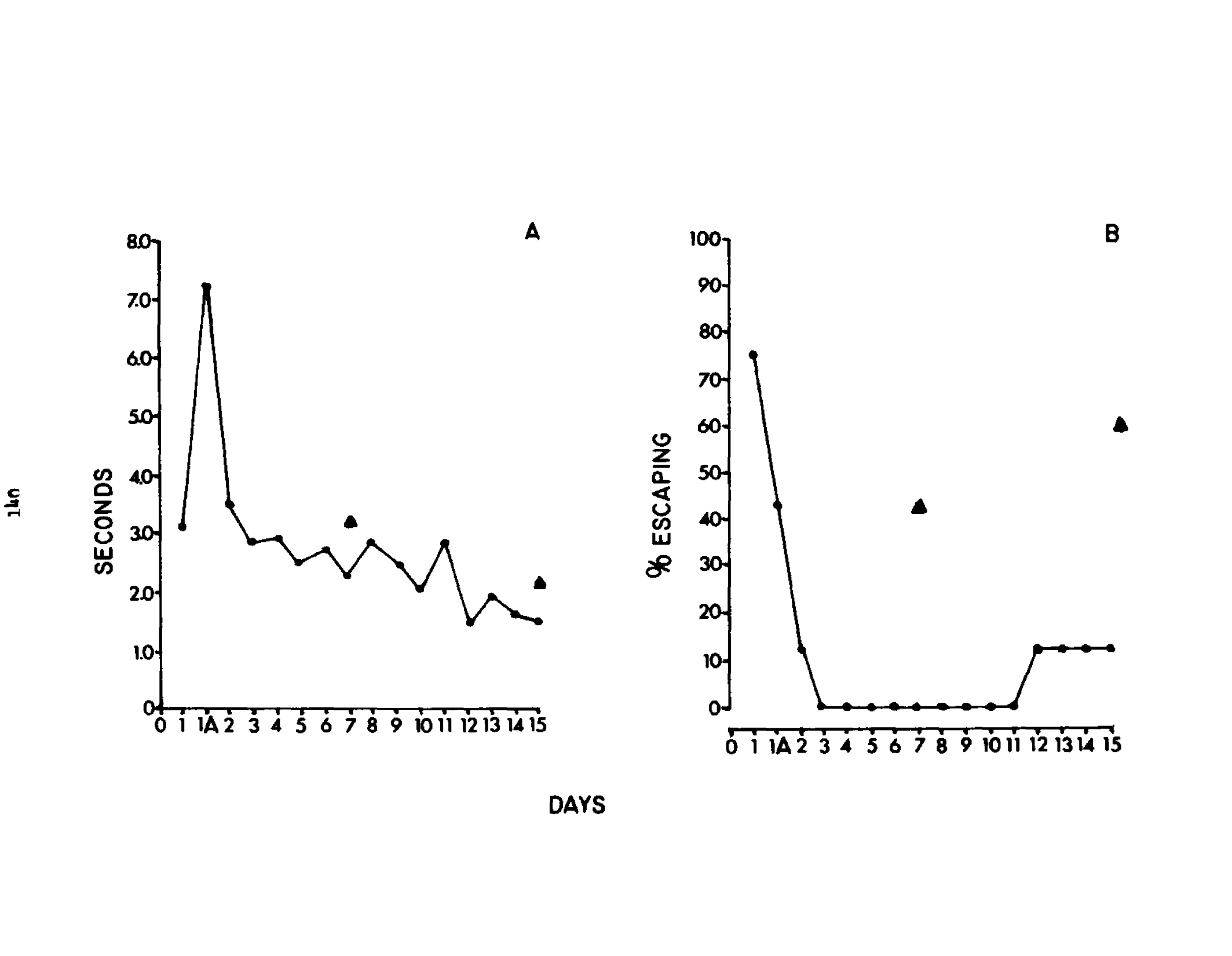
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Table 9

Escape performance of mice switched from the hot-plate to the shock apparatus and from the shock apparatus to the hot-plate. Groups of mice (N=16/group) were tested through Day 15 according to the standard testing procedure (Table 1, Group 2) either in the shock apparatus (SHK) or on the hot-plate (HTP), then switched to the alternative testing stimulus on Days 16 through 30. Control groups were not switched.

Testing Sequence	Escape Frequency (%)		Days of Significant Impairment*
	Day 15	Day 16	
HTP→SHK	0	6.25	9
HTP→HTP (Control)	0	18.75	9
SHK→HTP	12.50	12.50	10
SHK→SHK (Control)	6.25	18.75	10

* number of days on which post-morphine escape frequency was less than 37.50%.

stimulus, then through Day 30 on the hot plate.

The data presented in Table 9 also include the results of control experiments i.e. 30 days of shock and 30 days on the hot plate. It is clear from the data that mice can be tested with morphine, with either stimulus, and still demonstrate an escape impairment when tested on the alternative modality.

DISCUSSION

Introduction

The development of tolerance to the analgesic actions of morphine has been demonstrated in a variety of testing situations (Rev. by Hug, 1973). Kavan et. al. (1960) sought to determine whether repeated testing or exposure to the testing environment could influence tolerance development. Using the hot-plate technique, they found that tolerance developed more rapidly in subjects having had experience in the testing apparatus than in subjects with limited exposure to the hot-plate. Subsequent studies by Adams et. al. (1969) confirmed these finding. Tamayo and Contreras (1970), in contrast, found that experience could influence the development of tolerance to morphine analgesia when a different test was employed, the tail-pinch procedure. They, however, found that repeated testing had no effect on tolerance development with the hot-plate. There have been no reports in the literature of repeated testing or exposure to the testing environment interfering with tolerance development.

As expected, tolerance to the effects of morphine on escape behavior was observed in "untested" mice in the initial experiments. However, unexpectedly, tolerance was not observed in mice receiving the same drug regimen plus daily post-injection testing. An additional unexpected finding was that the impairment in escape behavior persisted after cessation of morphine administration.

Five major categories of experiments were performed to characterize and delineate these phenomena: 1) preliminary experiments including tolerance

and saline controls; 2) parametric studies of the original paradigm; 3) modifications of the original paradigm by altering intertrial interval, days of pre-morphine testing, etc.; 4) extension of studies to drugs other than morphine; and 5) studies of escape performance of mice on the hot-plate as compared to that observed in the shock-escape paradigm.

Preliminary Experiments

In the initial series of experiments, a majority of naive mice were found to escape the noxious stimulus. On subsequent daily testing, the performance of these mice remained at a similar level. Thus, mice repeatedly exposed to the testing environment did not habituate to the aversive stimulus. In a control group of mice, injection with saline prior to escape testing did not alter escape performance. When a group of mice was injected with morphine (25 mg/kg) immediately following initial testing on Day 1, and then retested thirty minutes later, there was a significant decrease in escape performance. This result was expected because 25 mg/kg of morphine has been shown to be an effective analgesic dose in mice (Cochin and Kornetsky, 1964). After 15 days of daily post-injection testing, there was no significant improvement in escape performance. Thus, tolerance to the initial escape depressing effect of morphine was not observed. The simplest explanation of this finding is that the duration of drug treatment (15 days) was insufficient to produce tolerance. This possibility was rendered unlikely by the following observation. Mice (Figure 1, Group 6) were tested on Day 1 and then injected with morphine on 15 subsequent days, but were not retested until after injection on Day 16. These mice were found to be tolerant to morphine on Day 16. Thus, the testing procedure was sufficiently sensitive to detect tolerance to morphine after 15 days of drug administration. The results suggest that repeated exposure to the testing situation could influence the production, or at least the detection, of tolerance.

In order to determine whether impaired tolerance development required that morphine be administered before testing, a group of mice (Figure 1, Group 5) was tested on Day 1, then injected with morphine but not retested,

On Days 2 through 15, these mice were tested 30 minutes prior to, rather than following, morphine injection. The escape performance of this group on Day 15 was equivalent to that observed in mice tested daily without morphine treatment. These results indicate that post-injection testing is required for the production of impaired escape performance.

Escape Impairment Following Cessation of Morphine

It was expected that discontinuation of morphine administration in mice receiving daily post-injection testing would lead to recovery from the drug effect. Recovery, however, was not observed on the day after the last morphine administration. When post-morphine testing was continued, recovery of escape performance was not observed on 13 of 15 subsequent days. These findings might have been accounted for on the basis of the continued presence of morphine (in mice) following discontinuation of drug administration. This hypothesis was rejected since mice tested on Day 1, then injected on Day 1 and for 15 days thereafter, showed no escape impairment 24 hours after the last drug treatment or on subsequent days. Similarly, no post-morphine impairment was observed in mice (Group 5) who had received pre-injection testing for 15 days. In summary, these results showed that post-morphine impairment could not be elicited by morphine and testing, unless testing occurred following morphine administration. Furthermore, the above findings indicated that daily post-injection testing was required for the production of impaired escape performance both during and following morphine administration.

The preliminary data suggested that the interaction between morphine and escape testing resulted in the learning of a maladaptive response (failure to escape the shock). The preliminary experiments, however, did not reveal the time course of the acquisition of the learned response, since

performance deficits approached a maximum by Day 2 of drug administration. Two additional preliminary experiments clearly illustrated that daily training and morphine injection did interact to produce a progressive decline in escape performance. In the first, a group of mice (Figure 1, Group 3) received the same regimen as Group 2 except that the dose of morphine was reduced by half to 12.5 mg/kg. The Day 1 effect of morphine was less than that observed with the higher dose, however, there was a gradual decline in performance on subsequent days. There was also a proportionately greater Day 15 escape performance and decreased number of post-morphine impairment days with the smaller dose of morphine. In the second experiment, mice received morphine (25 mg/kg) alone for 15 days. Although these mice demonstrated significant tolerance when tested on Day 16, their performance steadily declined on subsequent days such that the performance of these mice on the final day of drug administration was indistinguishable from that observed in the original paradigm (Figure 1, Group 2). Similarly the escape impairment observed in these mice persisted following discontinuation of morphine administration on Day 30.

In summary, the results of these preliminary experiments suggested that morphine, when administered before testing, could induce the acquisition of an ostensibly inappropriate and maladaptive response to a noxious stimulus. Moreover, this response, once learned, persists following cessation of drug administration.

Tolerance In Untested Mice

In the initial experiments, the time course of the development of pharmacologic tolerance was not explored. This was subsequently determined (Figure 2) and shown to be gradual and progressive over the 15 day period.

Tolerance Measured By Vocalization

Most investigators studying morphine analgesia, measure responses other than escape, such as latency of paw lifting or vocalization. Vocalization frequency and escape performance were simultaneously recorded in a group of mice exposed to the standard testing regimen. Tolerance, as measured by vocalization frequency (Figure 3), proceeded at a rate similar to that observed for escape behavior in untested mice (Figure 2). Vocalization frequencies, in control groups tested after injection on either the 9th or the 15th day of drug administration, were not significantly different from those in the tested group. The demonstration of pharmacologic tolerance, as measured by vocalization in tested animals, strongly suggests that, at least on the later days of drug administration, morphine does not act by inhibiting the perception of the noxious stimulus.

Parametric Studies

An unexpected interaction between morphine and escape behavior was observed in the preliminary experiments. These experiments were performed under a limited set of stimulus and testing conditions. Before attempting to explore the implications of the original findings, it was necessary to define the relationship between the experimental parameters involved.

Dose

Groups of mice were subjected to the standard testing regimen except that individual groups received different doses of morphine. An inverse relationship was found to exist between morphine dose and 1) post-injection escape performance on Day 1; 2) the post-injection escape performance on Day 15, and 3) the Day 16 post-morphine escape performance. These results indicated a clear dose-response relationship between morphine dose and escape impairment both at the beginning, during, and immediately following morphine administration. A comparison of Day 15 escape performance between "untested" controls and tested groups for each dose showed that escape performance was invariably higher in the "untested" groups.

A positive correlation was also found between daily morphine dose and total days of post-morphine impairment. Thus the degree of post-morphine escape impairment appeared to parallel the escape impairment observed during drug administration.

Days

Experiments were performed to determine the relationship between the number of days of post-injection testing and subsequent post-morphine escape performance. The performance of mice on the day after the last morphine

administration was found to be inversely related to the number of previous post-injection testing days (Figure 7). A minimum number of six treatment days was necessary to produce a sustained (greater than 1 day) post-morphine impairment. A maximum duration of impairment was produced by 15 days of post-injection testing. An extension of the number of morphine-plus-testing days did not increase the number of post-morphine impairment days. One explanation for the plateau in the treatment days vs. impairment days curve might be that, at longer treatment intervals, mice became sufficiently tolerant to morphine such that their sensitivity to the shock was equivalent to that of untreated (no morphine) mice. The performance of Group 6 in Figure 1 argues against this hypothesis. These mice received morphine alone for 15 days, at which time tolerance was observed. Performance of these mice, however, deteriorated on 15 subsequent days of post-injection testing. These results suggested that the ability of morphine (25 mg/kg daily) to negatively effect escape performance continued even after pharmacologic tolerance developed.

Shock Intensity

Varying the shock intensity employed in the escape paradigm might have had conflicting effects. For example, increased shock intensity could, as might be predicted, improve escape performance. Conversely, increased shock intensity might (as observed with "Dose" and "Days") facilitate the learning of the impaired escape response, thus diminishing escape performance. In order to delineate the effects of changes in shock intensity, experiments were performed in which the shock level was altered at various points in the original paradigm.

For naive mice (Figure 9), a direct relationship was found between the

number of mice escaping on Day 1 and shock intensity. When a high shock level (200 ua) was employed throughout the standard testing period, the trends in escape performance were equivalent to those observed at the standard shock level (160 ua): there was an initial and sustained fall in escape performance on Days 14 through 15 which was followed by a gradual return to baseline performance when morphine was discontinued. Similar results, a sustained escape impairment during morphine administration with a subsequent gradual return to baseline performance following discontinuation of morphine administration, were observed in another group of mice tested at 105 ua.

In additional experiments, the standard 160 ua stimulus was employed on Days 1, and 16 through 30, but the shock level on the intervening test days was varied in different groups of mice. The results indicated that a monotonic relationship existed between the extent of post-morphine impairment measured at a standard shock (160 ua) and the shock level employed during morphine administration. These results serve as additional evidence that morphine and shock are both necessary to produce the phenomenon of post-morphine impairment. As in the studies of other parameters, the duration of post-morphine impairment could not be extended past 15 days even with the highest shock intensity.

In the preliminary experiments, shock alone (at a fixed intensity) did not produce an impairment in escape performance. Part of the analgesic action of morphine, however, is often attributed to its ability to alter the perception of noxious stimuli. Morphine has been shown to be analgesic on Day 1 and tolerance has been shown to develop over the fifteen day testing period. Thus, it might be suggested that, as analgesic tolerance developed, mice become

desensitized to the foot shock as a consequence of repeated exposure to gradually increasing perceived shock levels. Post-morphine escape impairment could, therefore, be postulated to have resulted from a gradual habituation to foot shock. To test this possibility, various regimens of low shock intensities and gradually increasing shock levels were employed in an attempt to reproduce the findings with morphine and shock. No such manipulation of shock intensities generated a sustained residual escape impairment. Similarly, later experiments using "forced" exposure to shock were also unsuccessful in that mice never habituated to the shock, i.e. they escaped immediately on Day 16 when the restraining cover was removed. The failure of testing at low shock intensities with morphine (Figure 12), or gradually increasing shock intensities (Figure 13) without morphine, to produce a subsequent escape impairment, adds further evidence that the mechanism by which morphine produces escape impairment is not simply by diminishing the perception of the noxious stimulus. This finding, coupled with the observation that tolerance to escape suppression develops to morphine, in untested mice, as well as to vocalization in tested mice, indicated that shock alone is inadequate to produce escape impairment and that a unique interaction occurs when morphine treated mice are tested for escape behavior.

Shock Exposure

Varying the maximal duration of shock exposure per test trial could be postulated to have conflicting effects on escape behavior. For example, increasing shock exposure, while possibly enhancing the acquisition of the impaired response, might, by increasing the trial period (greater than 30 seconds), permit more mice to escape. This would lead to a measurement of an improvement rather than an impairment in escape performance. A similar

potential for ambiguity could be postulated to exist for experiments conducted with shorter durations of shock exposure. For example, decreasing shock duration, by diminishing total exposure to the shock, might inhibit the development of impaired escape behavior. Alternatively, by reducing the shock duration, fewer mice might be observed to escape. Furthermore, altering shock duration in the post-morphine period might have different or opposite effects than a similar change in shock duration during the period of morphine administration.

Prolongation of daily shock exposure duration, by as long as 90 seconds, was not found to significantly effect the number of naive mice escaping the shock. There was also no significant difference in escape performance between groups of mice tested daily (without morphine) for 15 days with a standard 30-second trial and groups allowed a greater maximum shock exposure. There was no significant effect of varying exposure times during morphine administration on post-injection performance. However, the duration of post-morphine impairment was found to be positively correlated with the length of maximum shock exposure during morphine administration. This finding is probably a simple dose effect, i.e. the greater the prior shock exposure the greater the impairment. In contrast, in experiments in which the maximum exposure duration was altered in the post-morphine testing period, recovery of escape performance was more rapid with greater exposure times. Thus, shock exposure appears to facilitate the acquisition of escape impairment, but inhibits the maintenance of this effect after cessation of drug administration.

Pre-Morphine Testing

The experimental results discussed above indicate that morphine, in

the presence of a noxious stimulus, can induce the learning of an apparently maladaptive response, failure to escape the noxious stimulus. In most learning studies, including those involving morphine, the subject acquires an ostensibly adaptive response. In a typical experiment, a subject is deprived of food or water, then placed in a "Skinner Box" in which the subject learns to perform a task (e.g. press a lever) in order to receive a reward (food or water).

To study the effects of a drug on such behavior, two kinds of experiments can be performed. First, the drug can be administered during the period of conditioning and its effects on acquisition of a behavior can be evaluated. Second, the drug can be administered to subjects after a behavior has been learned. In the latter experiments, one studies the effects of the drug on performance.

Experiments, as described above, differ in several crucial aspects from those performed in the present investigation. A good example for comparison is a study by Herman et. al. (1972) on the effects of multiple injections of morphine on shuttle-box behavior in the rat. This study is representative of the majority of the investigations on the behavioral effects of morphine and it is particularly relevant to this discussion because both the doses employed (5 and 20 mg/kg) and the time course (13 days) approximate the parameters of the present investigation.

Herman et. al. (1972) attempted to show the time course and pattern of tolerance to morphine as reflected by the initial disruption and subsequent recovery of a previously learned conditioned avoidance response. The testing apparatus was a shuttle-box which consisted of two chambers equipped with grid floors separated by a partition. A rat was placed in one chamber and

a light (conditioned stimulus) was turned on 10 seconds prior to the onset of the delivery of a 500 ua foot shock (unconditioned stimulus). If the rat entered the adjoining chamber prior to shock, an avoidance response was recorded. If the rat entered the adjoining chamber after the onset of the unconditioned stimulus, an escape response was recorded. As in the present study, naive subjects successfully escaped the noxious stimulus without difficulty. Rats were tested 25 times a day until a criterion of 90% avoidance (of 25 trials) was achieved. One group of these rats then received morphine prior to avoidance training on 13 additional consecutive days. Another group received only morphine without further testing. Despite the apparent methodological similarity of this experiment to those in the present study, Herman et. al. found that tolerance to morphine-induced disruption of avoidance behavior did occur (in both tested and untested groups) at all doses within the 13 day testing period. The greatest disruption of avoidance occurred on the first day at the higher (20 mg/kg) dose. However, even at this dose, no change in escape behavior was found on any day of morphine administration.[1]

The explanation of the apparent discrepancy between the results of Herman et. al. (1972) and the present study centers around crucial differences

[1]The question of whether morphine can produce a disruption of avoidance behavior at doses which do not effect escape behavior is the subject of much controversy in the literature (Herz, 1960; Bannerjee, 1971). The suggestion is that if morphine can produce avoidance impairment without escape impairment, then morphine's action on behavior is primarily "anxiolytic" rather than related to its analgesic properties. This theory grew out of an earlier finding that tranquilizers (phenothiazines) have been shown to produce a disruption in avoidance behavior without effecting escape behavior (Verhave, et. al., 1959). Domino et. al., 1965 , however, found in his experiments on the acquisition of conditioned pole jumping, that avoidance behavior could not be disrupted without simultaneously effecting escape behavior. The controversy remains unresolved because of the incomparability of the experimental designs and the failure of the authors to do exhaustive parametric investigations.

in the experimental methods employed. Herman et. al. were interested in measuring changes in avoidance behavior. Since avoidance behavior, unlike escape behavior (either in the shuttle-box or escape-box employed in this study), is a learned response, a great number of trials were necessary to achieve the criterion of 90% avoidance. In addition, on every post-injection testing day, rats received 25 trials. Perhaps, because Herman et. al. (1972) like others, chose to examine avoidance behavior rather than the spontaneously elicited escape behavior, testing conditions (e.g. dose, etc.) were employed, such that changes in elicited behavior were minimized.

In the present investigation, mice in the standard paradigm (Figure 1) received only one exposure to the testing apparatus (Day 1) prior to morphine and were permitted (except on Day 1) only one trial per testing day. In one experiment (Table 5), however, mice received varying numbers of days of testing prior to morphine administration. The results of this experiment indicated that, with greater than 15 days of pretesting, a marked decline in post-morphine impairment could be observed. Similarly, there was an improvement in Day 1, Day 15 (tolerance and experimental) and Day 16 (post-morphine) escape performance with increased pre-morphine testing. Thus, it seems likely that if mice were exposed to multiple trials for several days before and then during morphine administration, little effect of morphine on escape behavior would be observed on Day 1 or any other day of morphine administration. The differences between the present results and those of other investigators studying the interaction of narcotic drugs and learning can probably be attributed to such differences in experimental design and methodology.

The Conditioned Emotional Response

The failure of mice to escape the foot shock both during and especially

after morphine administration has been referred to above as a maladaptive response. This is because it is difficult to conceive of a situation (using the present experimental design) in which failure to escape could be viewed as an adaptive response. It is evident from the vocalization and the tolerance studies that mice both perceive the stimulus as "aversive" and are capable (in terms of locomotor ability) of escaping the shock. It is unlikely that mice learned to associate failure to escape with the delivery of a reward in the form of morphine injection, since there was no contingent relationship between the administration of the drug and exposure to the stimulus, i.e. morphine injection preceeded, not followed, testing.

The acquisition of an apparently maladaptive response is not a finding unique to this investigation. Estes and Skinner (1941) subjected rats to an inavoidable noxious stimulus, e.g. foot-shock, preceded by a tone. After repeated trials, upon presentation of the tone, the rats began to elicit a variety of responses including "freezing", urination and defecation. These "anticipatory" responses were collectively termed a Conditioned Emotional Response (CER). Estes and Skinner suggested that the acquisition of the CER represented the learning of an "anxiety reaction". When the CER procedure was superimposed on a baseline of operant responding, presentation of the pre-shock stimulus was followed by a fall in the response rate. This disruption of responding was termed conditioned suppression.

Brady (1955), using a modification of the Estes-Skinner procedure, trained rats to press a lever for a water reward. When the response rates stabilized, he administered a series of conditioning trials in which a clicking noise was presented for three minutes prior to the onset of a foot shock. He observed a suppression in responding as well as "freezing", defecation and crouching. Brady and Hunt (1955) initially used this technique to

evaluate the effects of electroconvulsive shock and cortical lesions on restoring operant behavior.

Since the conditioned suppression observed in the Estes-Skinner procedure was proposed to be an objective and quantitative measure of "anxiety", it engendered substantial interest from pharmacologists who sought to use this method to investigate the "anti-anxiety" properties of drugs (Kelleher and Morse, 1968). Hill et. al. (1954) found that morphine restored operant responding in rats exposed to a modified version of the Estes-Skinner procedure. Subsequently, Lauener (1963) found that neither morphine nor phenothiazines, effectively restored suppressed responding. He did find, however, that chlordiazepoxide, meprobamate and most barbituates could antagonize conditioned suppression. The discrepancy between the results in these studies was attributed to variations in testing procedures and training schedules (Kelleher and Morse, 1968).

Punished Responding

Conditioned suppression can be viewed as a maladaptive response because the decrease in operant responding only suspends delivery of the reward but does not prevent or diminish the aversive stimulus. In contrast, another technique was developed in which suppression of responding can be viewed as an adaptive rather than a maladaptive response. In this procedure, referred to as punishment suppressed responding, a response previously associated with a positive reinforcement (reward) becomes additionally associated with an aversive stimulus, thereby creating a "conflict" situation. Failure to respond in this paradigm is clearly an adaptive response because the delivery of the "punishment" is contingent upon the response rate of the subject. In the punishment suppressed responding procedure, avoidance of the

aversive stimulus is passive in that the stimulus is only delivered following some response by the subject as distinguished from "active avoidance" learning techniques in which the subject must perform some behavior in order to delay the onset of the aversive stimulus.

In contrast to the inconsistencies observed with the Estes-Skinner procedure, pharmacological studies of punishment suppressed behavior have been highly reproducible (Kelleher and Morse, 1964). Geller et. al. (1962) initially studied the effects of chlorpromazine and chlordiazepoxide on punished responding in the rat. They trained food deprived rats to press a lever for a food reward on a variable-interval schedule of reinforcement.[1] After lever pressing rates stabilized, a tone was introduced which signalled a period of continuous reinforcement (CRF), i.e. each lever press produced a reward. Thus, periods of low reinforcement (VI) were alternated with periods, during the tone, of high reinforcement (CRF). After a number of sessions under these conditions, a punishment contingency was added such that rats not only received a reward (food) but were punished (foot shock) for responding during the tone. Geller et. al. (1962) found that, over a wide range of shock levels, chlordiazepoxide, meprobamate and various barbiturates restored responding suppressed by the tone. Phenothiazines, however, did not restore suppressed responding. In subsequent studies, Geller et. al. (1963) found that morphine, like phenothiazines, did not restore suppressed responding. Moreover, they found that morphine further suppressed the already diminished response rate. In summary then, these early studies of the effects of drugs on conditioned suppression showed that the minor tranquilizers such

[1] In operant conditioning, various schedules of reinforcement are employed. These schedules can be classified according to ratio, i.e. the number of responses necessary to produce a reward, or according to interval, i.e. the period between a prior reward and the time when a response will again produce a reward. In a variable interval (VI) schedule, this period is varied about some mean time interval.

as chlordiazepoxide and meprobamate were effective in restoring responding suppressed by punishment (Geller et. al. 1962, 1963). As discussed previously, major tranquilizers, e.g. phenothiazines and morphine have been shown to be effective in decreasing escape and avoidance responding (CAR) (Verhave, et. al. 1960). Such results were initially interpreted (Herz, 1960) as indicating that chlorpromazine (and morphine) attentuate the effects of noxious stimuli through a reduction of the "fear" or "anxiety" which was assumed to underly such behavior. This interpretation was inconsistent, however, with the finding that morphine and the major tranquilizers were ineffective in influencing suppressed responding (Geller et. al., 1963).

One of the more puzzling and interesting findings in the punished responding experiments of Geller (1963) was the observation that morphine was ineffective in restoring suppressed responding. Morphine has always been considered to act (see introduction) by relieving the "anxiety", as well as the sensory and perceptual aspects, of pain. Clinically, administration of narcotics is often based on the assumption that such drugs have anxiolytic as well as analgesic properties (Hill, 1952). Geller (1963) concluded from his studies of morphine and punished responding that the effects of minor tranquilizers, e.g. meprobamate and chlordiazepoxide, were unrelated to analgesia. Geller did not explain the apparent paradox that morphine is a clinically effective "anti-anxiety" agent but is ineffective in restoring responding suppressed by punishment.

More recent studies have shown that the interaction of morphine as well as other drugs with suppressed responding is more complicated than originally outlined by Geller (1962, 1963). Djahanguiri et. al. (1966) were the first to demonstrate that morphine could increase the rate of operant responding.

They submitted cats to prolonged daily low dose (.2 mg/kg/day) treatments of morphine and found an improvement in food-reinforced responding. In subsequent experiments,,Thompson et. al. (1970) observed that the change in response rates of rats following morphine administration varied with different reinforcement schedules (fixed interval, varied interval, fixed ratio, varied ratio) as well as dose. In addition, these authors found that the nature of the response to morphine varied with the baseline performance of the subjects on the individual schedules. This finding was in agreement with earlier observations (Dews and Morse, 1961) with other agents, that the effect of drugs on the response rate of schedule-induced behavior depended to a considerable degree on the response rates prior to drug administration.

In later experiements, Holtzman and Jewett (1972) found that the effect of morphine on conditioned avoidance responding in the rat could be qualitatively altered with changes in shock intensity. They found that at a "high shock intensity" (1300 ua), morphine increased avoidance responding, while at lower shock intensities (800 ua), morphine decreased avoidance responding.

In another study, Stitzer (1974) examined the influence of shock intensity (and frequency) on the key-peck responding of pigeons (for food). Pigeons trained to peck at a key for a food reward simultaneously received electric shock during the fixed ratio component of an alternating (fixed interval and fixed ratio) schedule of reinforcement. Shock intensities and frequencies were adjusted to produce either a moderate or severe response suppression during the punishment component. Chlorpromazine reduced punished responding at both shock levels while increasing rates during unpunished intervals. Morphine, however, increased rates of responding both during punished and unpunished intervals at moderate shock intensities and frequencies.

At shock levels which severely diminished response rates, morphine had qualitatively similar effects to chlorpromazine.

In summary, the studies of the effects of morphine on punished and avoidance behavior confirmed the earlier findings of Dews (1958) and others, that the interactions of drugs and behavior can be influenced both qualitatively and quantitatively by variations in dose, baseline performance and schedules of reinforcement. In addition, these studies showed that even when the above variables are accounted for, interspecies' differences may still be observed, e.g. Dimahanguiris' (1966) studies with cats.

Motivational interpretations have assumed, a priori, that conditioned avoidance (i.e. behaviors which terminate or delay the presentation of a noxious stimulus) and behavior which is suppressed by the presentation of a noxious stimulus, both reflect common components of "fear" and "anxiety" (Kelleher and Morse, 1968). Once one is not bound by this unitary hypothesis, it becomes possible to view the drug studies as having demonstrated that "anxiety" represents a complex combination of behaviors or motivational states each influenced in a different fashion by psychoactive drugs and experimental conditions.

The purpose of this discussion of conditioned avoidance and emotional responses is to provide a theoretical and experimental framework, upon which a plausible explanation for the behavior described in the present investigation can be formulated. Although it is clear that the CER and punishment experiments differ strikingly from those presented in this work, some comparisons are, however, valuable in helping to evaluate the nature of the interaction of morphine and escape behavior described in the present study.

Many differences between the punishment experiments and the present investigation can be cited. For example, in the escape paradigm, no conditioned

stimulus, i.e. no stimulus associated with a punishment (shock), is presented. More importantly, in the escape testing procedure, there is no punishment contingency for escaping or not escaping; there is no clearly defined conflict involved in the testing procedure. Furthermore, in the CER and punishment procedures, drugs were administered to subjects already eliciting suppressed responding in an attempt to antagonize the suppression. In contrast, in the present investigation, drugs were introduced immediately following initial exposure to the testing apparatus (Day 1A). In addition, no prior training was necessary since mice spontaneously escaped from the noxious stimulus.

Conditioned Emotional Response and Escape Behavior Following Morphine Administration

As previously described, the decline in response rate in the punishment suppression paradigm represents a form of passive avoidance learning which is clearly adaptive; although reward presentation is postponed, the noxious stimulus is also avoided. No such adaptive significance can be attributed to the fall in response rate observed on the conditioned suppression paradigm since the decline in response only decreases reward presentation, but does not effect delivery of the (non-contingent) foot-shock. Several features of the acquisition of impaired escape performance in mice receiving morphine in the shock-escape paradigm can be analogized to the CER and conditioned suppression paradigms. The failure of mice to escape the shock on the first days of morphine administration can be explained on the basis of analgesic actions of the drug. Similarly, the disruption of operant responding at the time of presentation of an aversive stimulus can be ascribed to a direct action of the electric shock. Untested mice (receiving morphine) were found to escape the shock on days when tested mice did not do so. Also, tolerance

rapidly developed to morphine induced vocalization suppression in tested mice. Thus, the failure of (tested) mice to escape the shock on subsequent days of morphine administration (and on post-morphine days) can not be attributed to analgesia or to some motor impairment. Similarly, subjects in the conditioned suppression experiments are not inhibited from responding after presentation of the conditioned stimulus either by immediate punishment or by a "direct" disruption of performance via presentation of an aversive stimulus. Thus, the failure of mice to escape the shock in the present study and the response suppression in the CER studies can therefore be viewed as representing the acquisition of an obstensibly maladaptive response. Furthermore, the return to baseline escape responding after repeated post-morphine trials can be compared to the extinction (return to baseline rates) of response suppression observe when the tone was no longer paired with a noxious stimulus.

One other finding, although difficult to quantitatively evaluate, suggests that the CER and the failure to escape in the shock apparatus, are at least in some ways analogous behavioral states. In the CER, subjects are usually observed to defecate, "freeze", crouch and otherwise remain immobile, during the presentation of the conditioned stimulus. In the present investigation, mice failing to escape the noxious stimulus were observed to behave in much the same fashion as subjects in the CER. Mice crouched, frequently defected and urinated, but made little attempt to escape, either by jumping or climbing the walls of the box.

If one wishes to postulate that the CER and the failure of mice to escape under morphine are the result of analgous behavioral processes, one must explain how morphine and shock interact to produce suppression of escape

behavior. One common denominator between the CER experiments and shock-escape paradigm is that in both situations, subjects fail to respond in an adaptive fashion under what can be termed "stress" conditions. The stress in the CER and escape experiments can be attributed to the anticipation of the noxious stimulus.

Stress is a broad and general term, usually defined differently according to the context in which it is discussed. The "stress" reaction has been shown to encompass a multiple of physiological responses. Selye and others have contributed greatly to the study and understanding of the physiological manifestations of stress and the nature of the actions of various agents in modifying the bodies' response to stress producing conditions. Selye (1960) proposed the term "General Adaptation Syndrome" (GAS) to describe the variety of autonomic and endocrine responses observed in prolonged exposure to various kinds of stress. The first phase of the GAS was called the "alarm reaction", consisting primarily of greatly increased adrenal cortical and sympathetic nervous system function. If the stress continued, a longer phase of "resistance to stress" occurred in which the organism endured the stress as best it could. Eventually a "stage of exhaustion" ensued in which the organism lost the ability to withstand the stress (Selye and Heuser, 1955).

Exposure to stress has been shown to produce striking changes in the activity of the endocrine system, especially the hypothalalamic-pituitary-adrenal axis (Selye, 1960). Numerous studies have been performed to evaluate the effects of various stimuli on physiological parameters of stress such as ACTH release and ascorbic acid depletion of the adrenals (Liddle, et. al., 1962). In this regard, Selye (1936) in his earliest experiments, employed morphine to evoke an "alarm reaction" thereby bringing about increased adrenal cortical

activity. In subsequent investigations, the ability of single doses of morphine to stimulate the release of corticosteroids (in dogs and mice) was well documented, (Rev. by Sloan, 1971). However, morphine has also been shown to produce the opposite effect (decreased ACTH and urinary corticoids) in chronically treated subjects (man, mouse, and rat) (Rev. by Sloan, 1971). An even more curious finding is the observation that morphine can antagonize the adrenal response to an additional or superimposed stressful stimulus, e.g. exposure to cold or pitressin injection (Briggs and Munson, 1955).

The mechanism of the interaction between morphine and stress has been studied by several authors (Rev. by George, 1971). Based on various lesion studies, which demonstrated that the effects of morphine on adrenal function were blocked by hypophysectomy, it is now believed that the action or actions of morphine on the adrenals is initiated in the CNS and mediated via the hypothalamic-pituitary neurosecretory system. The nature and site of this action, however, remains unknown (Rev. by Sloan, 1971).

Most researchers studying drug-stress interactions have concentrated on the modification of physiological parameters of stress by drugs. A few authors, however, have studied this problem from a different point of view: the alteration of drug responses by stress. In one early study, Friend and Harris (1948), observed that adrenalectomy diminished the analgesic effect of morphine. In contrast other authors showed that the chronic administration of steroid preparations could decrease the analgesic response to morphine (Winter and Flataker, 1951). Of related interest is one study by Rupe et. al. (1963) on the stress modification of the response (sleeping time) of rats to hexobarbital. In initial experiments, the authors found that rats previously stressed by hind-leg ligation showed a significantly decreased

response to barbiturates. The authors then sought to determine whether this modification of drug response was mediated through the pituitary-adrenal system. When they administered ACTH to produce a release of adrenal corticosteroids, the response (decrease in sleeping time) was enhanced. Next, based on the previously described findings of Briggs and Munson (1955) that morphine blocked the adrenal response to stressful stimuli, Rupe et. al. chronically pretreated rats with morphine as a means of producing a "functional hypophysectomy". Chronic administration of morphine as well as adrenalectomy were found to block the effects of a stress (hind-leg traction) on hexobarbital sleep time, i.e. sleeping time was increased. Thus, these authors used morphine administration to block the normal physiological response to stress and then studied the effect of this interaction on the actions of a second drug.

The studies described above indicated that morphine could influence the physiological response to stress and, conversely, that stress could modify the response to drugs. Such findings may be analogized to the previously discussed behavioral investigations of Dews and Morse (1958) and Stitzer (1974). These authors found that the baseline performance level of a subject could influence, both qualitatively and quantitatively, the responses observed when a pharmacologic agent was introduced. The obvious question raised is whether authors studying behavioral correlates of stress have observed drugs to produce one kind of behavior in an unstressed subject, and a different or altered response in a "stressed" subject. With regard to morphine, the answer to the question is that most authors have been concerned with determining whether the drug could restore previously impaired performance as in the CER.

Jacob (1963) was one of the few authors to directly investigate the effects of morphine on learning under "stressful" conditions. His findings are particularly relevant to the present investigation because his experiments were designed (unlike the CER and other operant studies) to evaluate subtle changes in spontaneously elicited behaviors with an emphasis on changes in acquisition of learned tasks as well as on disruptions of baseline performance. Jacob (1963) employed three different experimental methods to study the effects of morphine on "adaptation" and "learning" . Using a modification of the hot-plate technique (Woolfe and MacDonald, 1944), he found that untreated mice, on initial exposure, licked their paws and then eventually escaped (jumped) from the stimulus. On subsequent days, the jump reaction took precedence over "licking". Jacob concluded that the replacement of the "lick response" by the "jump" reaction represented the substitution of an escape response by an avoidance response. He then found that morphine prevented this change in response at doses that were sub-analgesic; "licking times" (latencies) were not altered at doses which inhibited the jump response. Jacob concluded from these findings that morphine possesses "an inhibitory action on the adaptation of animals to a stressful situation".

In a second series of experiments, Jacob (1963) employed a "forced swimming test" previously used to investigate the "anti-fatigue" properties of different substances. In this procedure, exhaustion times were measured in subjects forced to continue swimming for long periods. Mice, on re-exposure to the test, were found to have increased exhaustion times. The effect of morphine in naive subjects was to prolong exhaustion times. However, morphine diminished the normally occurring increase in exhaustion times in mice previously exposed to the test. Jacob decided that the swimming test

involved no "noxious" stimuli and he therefore concluded that the results were not related to the analgesic action of morphine.

In a third series of experiments Jacob studied the effects of morphine on the performance of mice on an elevated maze learning task. Mice were initially trained to complete the maze and jump (or climb) off a platform in order to receive a food reward. In later trials mice were fed ad. lib. before testing, and the food reward was discontinued. Subjects continued to complete the maze. Jacob found that morphine (12.5-50 mg/kg) increased the number of errors and the running time in the maze. The largest increase was found in "final delay", the time between finishing the maze and climbing from the platform. Jacob interpreted this last result as constituting a disruptive effect of morphine on a conflict situation which involved neither "painful" nor "stressful" stimuli.

In summary, Jacob (1963) postulated from his three experiments:

> "Summarizing the results obtained with the three tests, one is tempted to consider that they all point to the property of morphine to depress the ability of the animals to adapt themselves to a situation characterized by an unusual environment, which need not be a physically nocieptive one. This activity of morphine is one of the most important of its properties as it appears with lower dosages than its physical analgesic properties, than its obvious actions on motricity or sensory motor coordination and than its effects on 'retaining' previous experience. In each case, the effect of morphine is to depress the reactivity regardless of the immediate consequence of this depression, whether detrimental (hot plate test) or beneficial (swimming test) or apparently neutral (maze test); in each case, however, the final result of morphinization is detrimental as the 'learning' of the inhabitual environment (or adaptation to it), is depressed".

Jacob's (1963) work can be criticized on many levels. Both his conclusions and his interpretations of the experimental data are open to question. In

his hot plate experiments, Jacob made a number of assumptions. He claimed that since the licking response was replaced by a jump response following successive exposure to the stimulus, that the jump response represented a learned (conditioned) response. He provided no direct evidence for this supposition, referring only to previous studies which indicated that this substitution of responses could be blocked by electroconvulsive shock between trials. An alternative explanation might be that "licking", like jumping, was a learned behavior and that the failure of a dose of morphine to alter licking times may indicate only that licking had become a conditioned response. Thus, because Jacob failed to adequately prove that the jump response was a conditioned avoidance phenomenon, his conclusion that morphine, by selectively inhibiting jumping, acted specifically to block adaptation to stressful stimulus, was invalid. Jacob further asserted that the failure of a specific dose of morphine to alter licking times, while simultaneously increasing jumping times, represented sufficient evidence that failure to jump was unrelated to a motor or sensory impairment. An alternative interpretation of this finding might be that the licking response required less motor coordination than the jump response. Thus, the failure of morphine-treated mice to execute a jump may reflect an ataxic effect rather than a specific stress-related phenomenon.

Jacob also failed to consider an additional factor in evaluating his data. Recent studies (Rev. by Overton, 1971) as will be discussed later, have shown that tasks learned in a "no-drug" condition may fail to transfer to the drug state or vice-versa (Hill et. al., 1971). This phenomenon, termed "state-dependent" learning, may account for the failure to observe the putative learned response (jump) as compared to the unlearned response

(lick) in morphinized subjects.

Similar criticisms, as outlined above, may be applied to Jacobs' (1963) swimming and maze studies, in that he again made assumptions and interpretations based on limited and tenuous data. For example Jacob failed to exclude the possibility of a state-dependent effect in the swimming test. In these experiments, morphine was found to increase exhaustion times in naive mice but produced the opposite effect in mice previously exposed to the test. Thus, it was possible that the observed changes in exhaustion times were not related to a modification of a stress response, but represented, instead, the failure of a learned response to transfer from a "drug" to a "no-drug" condition. Jacob further maintained that morphine's action in the swimming test is unrelated to its analgesic properties in that "no physical pain can be postulated in this test". He, however, provided no experimental data in support of this questionable assumption.

Again in the maze-learning experiments, Jacob over-interpreted his data, failing to adequately evaluate alternative hypotheses. He claimed that because a food reward was terminated at some unspecified time prior to morphine administration, that hunger can be eliminated as a possible motive for mice completing the maze. Based on this unvalidated supposition, he concluded that the maze is a "neutral test" and consequently that morphine's effects can not be attributed to an effect on hunger or on the gastrointestinal system. In addition, Jacob assumed, without direct evidence, that morphine's effects on maze performance were unrelated to locomotor ataxia or sensory disorientation.

In summary, Jacob's (1963) study suffers from two main flaws. First, he fails to provide any details of his experimental methods. Second, and

more importantly, Jacob overlooked or failed to adequately consider, in his experimental design and his evaluation of the data, possible alternative interpretations of his results. Jacob's conclusions that morphine had a specific detrimental effect on learning or adaptation to a change in environment, are therefore difficult to accept due to his over-interpretation of the data. Nonetheless, Jacob's investigation is noteworthy in that he, at least, attempted to inquire into some of the less obvious, and more difficult areas of drug behavior interaction to experimentally explore.

The methodological problems encountered in Jacob's (1963) study point out the difficulties involved in isolating the specific manner by which a drug may act to modify behavior. For example, Jacob argued that the swim test did not involve "pain" and, therefore, that morphine's effect on subjects exposed to this test was unrelated to analgesia. Such an assumption is difficult if not impossible to prove. The disruption of the performance of a response by a drug can be produced by any one or a combination of effects of a drug, e.g. ataxia, sensory disorientation, or analgesia. To assert that a response is specifically related to one of these effects, it is necessary to control for the alternative possibilities. This problem is especially difficult with analgesics. Since pain is a subjective response, it is impossible to determine with certainty that a particular test does not involve painful stimuli. The problem, as outlined above, of determining the mechanism of a drug-behavior interaction, was encountered in the present investigation.

The experiments thus far discussed have led to the following observations. A majority of naive mice were found to successfully escape a noxious stimulus and they continued to do so on repeated daily testing. A single injection of morphine decreased the number of mice escaping. Tolerance was found to

develop to this effect in untested mice but not in mice receiving daily post-injection testing. Although there was an eventual improvement in performance, the impairment in escape behavior observed in mice receiving daily post-injection testing, persisted after cessation of morphine administration. The duration and degree of this effect was shown to vary with dosage, days of treatment, and shock intensity. Since escape impairment could be produced in subjects with demonstrated analgesic tolerance, the ability of morphine to generate this response appeared to be independent of its analgesic actions. Thus, the interaction of morphine and escape testing appeared to cause the acquisition of an ostensibly maladpative response.

Since there were no reports in the literature of a similar effect involving morphine and/or escape behavior, it was necessary to search for a comparable paradigm to explain these phenomena. The Conditioned Emotional Response (CER) appeared to be analagous in some respects to the behavior observed in the present study. It was suggested that a common feature in both the CER and the shock-escape paradigm was the failure of a subject to respond appropriately in a "stressful" stiuation. The literature relating to the interactions of stress and morphine was reviewed. It was found that morphine could influence several of the physiological parameters of the stress response and that stress could also modify the response to morphine. These studies were, however, difficult to relate to the present investigation for several reasons, e.g. acute and chronic morphine produced differing effects on the stress response. Finally Jacob's (1963) study of the interaction of morphine and learning was discussed. Jacob's findings were in agreement with those of the present study, however, the validity of his conclusions were questioned

on the basis of flaws in his experimental design.

In reviewing the data summarized above, it can be postulated that impaired escape performance represents a unique interaction between morphine and the stress of exposure to a noxious stimulus. Direct supportive evidence for this hypothesis is, however, lacking and therefore the mechanisms underlying the observed phenomena remain unknown at this time.

Further Parametric Studies

The following section contains a discussion of experiments performed to further delineate the parameters and experimental conditions which governed the production of the original phenomena.

Relapse and Recovery

A number of experiments were performed to determine the relationship between modifications in the post-morphine testing schedule and recovery (i.e. return to baseline Day 1 performance from the residual escape impairment). The data, Figure 16, indicate that mice recover from the post-morphine escape impairment at approximately the same rate whether testing was or was not performed on a continuous basis after Day 16. These findings suggest that recovery occurs independently of the number of post-morphine trials, i.e. testing per se does not seem to inhibit or facilitate recovery.

Experiments were performed to determine whether prior exposure to the standard testing regimen, i.e. 15 days of post-injection testing followed by 15 days of testing alone, influenced escape performance when mice were subsequently re-exposed to the standard testing regimen. No difference in escape behavior was observed between the initial and "relapse" performance of mice receiving two consecutive exposures to the standard testing procedure.

In summary, the results of the recovery and relapse experiments suggest that once daily morphine and shock exposure are terminated, a return to baseline performance occurs regardless of whether or not daily testing is performed. In addition, recovery does not appear to influence subsequent redevelopment of escape impairment on re-exposure to the standard testing procedure.

Intertrial Interval

In the standard testing procedure, mice were tested once a day for

fifteen days. In one series of experiments, groups of mice were tested-injected and retested as usual on Day 1, and the total number of post-injection days was held constant at fifteen days. The intertrial interval, however, was varied from once every other day to once every seven days. Post morphine testing was performed on a standard (daily) basis. One unexpected finding in these experiments was the apparent tolerance development (escape performance improved from Day 1 levels) observed on the first days of morphine administration. Surprisingly, despite this improvement in performance during the early days of drug administration, performance steadily declined on the latter days of drug administration. A possible explanation for this finding might be that the increased intertrial intervals allowed for more rapid development of analgesic tolerance to morphine. It is also possible that the acquisition of impaired escape behavior was facilitated by the relative proximity of the trials in the standard daily testing regimen. Whatever the cause of this peak in escape performance, of greatest significance is the decline in performance observed on the final days of drug administration.

The parametric studies described so far have indicated that certain modifications in the standard testing procedure can interfere with the production of impaired escape performance. First, pre-morphine testing was found to diminish the Day 1 morphine effect as well as reducing the duration of post-morphine impairment. Similarly, increasing the interval between testing days as in the previous experiments, also diminished the initial effect of injection and testing on performance. Also, in the preliminary experiments (Figure 1, Group 6) a prior course of daily morphine injections was found to produce an improvement in performance when post-injection testing was subsequently begun. Similar results, an interference in the initial effect

of morphine on escape performance, were observed in another experiment. A group of mice was tested and injected as usual on Day 1, but received pre-injection as well as post-injection testing on subsequent days. The escape performance (post-injection) of these mice on the first days of drug administration was greater than that observed with the standard testing procedure. On subsequent days, however, both pre- and post-injection performance showed a steady decline to a level equivalent to that observed with the standard testing regimen. These findings indicate that a number of modifications in the original paradigm (pre-morphine testing, prior morphine treatment, increased intertrial interval, and pre and post-injection testing) can diminish the initial response to morphine. It is possible that such modifications only affect analgesia induced escape suppression. This hypothesis would explain the decline in performance observed, in all these groups, on the final days of drug administration.

Post-injection testing was found to produce impaired escape behavior in mice previously made tolerant to the analgesic effects of morphine. Thus, morphine's ability to produce escape impairment appears to be independent of its analgesic properties. Furthermore, these results indicated that the finding that morphine disrupts escape performance, is neither fortuitous nor dependent on a restricted range of testing conditions.

Escalating Dose Levels

Experiments have been described, e.g. escalating shock levels and forced exposure to shock, where the purpose was to attempt to reproduce the experimental results without the use of morphine. Toward a similar goal, experiments were performed to determine if the dosage of morphine could be manipulated such that a post-morphine impairment would not occur. It could be

argued that the combination of a dose of morphine, which decreased in effectiveness with analgesic tolerance, and daily trials interacted such that morphine facilitated a progressive desensitization to foot-shock. In order to test this hypothesis, a group of mice was exposed to escalating doses of morphine over the fifteen day testing period. A tolerance control group received the same dose schedule, but no testing until after injection on Day 15. Tolerance was not observed to the final dose of morphine on Day 15 in the control group, suggesting that morphine was still analgesic. Similarly, vocalization was noted less frequently in the experimental group than in groups tested at a constant dose. The above findings suggested that morphine remained analgesic throughout the testing period. The post-morphine performance of the experimental group was equivalent to that observed in standard (25 mg/kg day) groups, providing additional evidence that morphine's ability to produce impaired escape behavior is independent of it's analgesic action.

Delayed Testing

In conditioning experiments, subjects learn to avoid rather than escape an aversive stimulus. In the previous experiments mice were not permitted the opportunity to avoid the shock. It is conceivable, then, that mice could have learned to avoid the shock. Also, it has been suggested that one similarity between the CER and the present paradigm was the observation of crouching, "freezing" and other manifestations of "anxiety". In the CER, such responses were observed to occur prior to the onset of the noxious stimulus. The standard testing procedure did not allow for the detection of such "anticipatory" responses. In addition, it is conceivable that foot-shock has two actions in the escape paradigm. During the initial days of drug administration, shock and morphine may initiate the learning of the

maladaptive response. On subsequent days, however, shock may simply act to immobilize the mice. If the above hypothesis were true, mice placed on the plates, prior to the onset of shock, should be able to avoid the shock. Consequently, experiments were designed to investigate the effect of delaying the onset of foot-shock. One group of mice received daily testing for thirty days but were placed on the plates ten seconds prior to the onset of shock on Days 2 through 30. A second group received the standard testing regimen (Table 1, Group 2) except that mice were placed on the plates ten seconds prior to shock onset on Days 14 through 15. Mice were observed on each day to determine: 1) if the subjects learned to avoid the shock in the ten second pre-shock interval; 2) if mice crouched or "froze" before and/or after shock onset; 3) if a post-morphine impairment developed in the experimental group.

Only a few of the control mice and none of the morphine treated mice learned to avoid the shock. Crouching and "freezing" during the delay period was observed in the morphine treated mice, but not in the control group. Moreover, these responses became more evident on the final days of drug administration. The post-morphine impairment observed in the "delay" group was indistinguishable from that observed in mice exposed to the standard testing procedure. The finding that mice demonstrated CER-like responses, but did not avoid the shock suggests that they became conditioned to respond in an impaired fashion and that the shock alone did not produce immobilization.

Studies with Other Pharmacologic Agents

The experiments thus far discussed have described phenomena produced by the interaction of morphine and an escape task. The following section will discuss the specificity of these phenomena with regard to various classes of drugs.

Narcotic Analgesics

In all the parametric studies, post-injection testing took place 30 minutes after injection. The results in Table 9 clearly show that the effects of morphine on all the key features of the escape paradigm remain essentially the same at post-injection intervals of 15 to 60 minutes, and change only quantitively at 120 minutes.

In all studies with other drugs an attempt was made to choose a dose and an injection-testing interval which would produce approximately the same performance on Day 1A as observed with morphine 25 mg/kg, at 30 minutes after injection.

Levorphanol was chosen for study because its clinical usage and spectrum of action is similar to that of morphine. Levorphanol is approximately 3 times more potent than morphine in its analgesic action (Lasagna, 1964), but has an equivalent duration of action. A dose of 12.5 mg/kg was found to produce effects equivalent to 25 mg/kg of morphine on Day 1A. The results are quite clear in that the escape performance of mice, both "tested and tolerance controls", was similar in all aspects to that observed in the original paradigm (Figure 1-Groups 2 and 6) with morphine.

When mice were injected with dextrorphan, the analgesically inactive optical isomer of levorphanol, no effect on escape performance was observed. Similarly, propoxyphene, a "mild" analgesic, with a structural resemblence

to methadone, was also ineffective in suppressing escape behavior except at toxic and near-lethal doses.

Meperidine is an analgesic agent of the phenylpiperidine series used clinically in place of morphine. Its spectrum of actions is similar to that of morphine; however, it is 1/10th as potent an analgesic. Meperidine is known to possess a number of side-effects generally ascribed to its atropine-like actions (Lasagna, 1964). In the present investigation, no dose of meperidine (less than the LD 50) could suppress Day 1A escape performance to the level observed with morphine 25 mg/kg.

Similar problems (high mortality rate at doses which produce a criterion level of response inhibition) were observed with methadone, an analgesic with with potency in between that of morphine and meperidine, but having a longer time of onset and duration of action.

It was noted in a comparative study of the effects of morphine, meperidine and methadone on the CER, that the slope of dose response curves were "flatter" for morphine than for meperidine or methadone (Hill et. al., 1966). Thus it is possible that the relatively smaller ratio between the LD 50 and the ED 50 found in Hill's study might account for the high mortality rate. It is also possible that the failure to inhibit escaping at doses well below the LD 50 may represent a distinguishing feature of synthetic (non-phenanthrene) narcotic analgesics, although the evidence presented here is far from conclusive. In the studies with methadone, pretreatment with low doses and the use of replacement mice (on Days 1 and 2) was necessary in order to produce results which resembled those observed with morphine (25 mg/kg).

Major and Minor Tranquilizers

As has been previously discussed, major tranquilizers (phenothiazines)

and minor tranquilizers (benzodiazepines) have differing effects on avoidance and escape behavior. Clinically, minor tranquilizers are employed as sedative-hypnotics or antianxiety agents. Major tranquilizers are primarily used in the treatment of the psychoses. These agents do, however, share some common effects, analgesia and sedation.

As discussed in previous sections, a variety of behavioral techniques have been employed to both identify and distinguish between major tranquilizers (or neuroleptics) and minor tranquilizers. The clinical efficacy of the minor tranquilizers is generally held to be related to the difference between the "calmative" or "antianxiety" actions of these agents and their toxic (hypnotic or ataxic) effects. A number of behavioral models have been developed to experimentally determine these ratios. One commonly employed method is a modification of the standard conditioned avoidance procedure, designed by Heise and Boff (1962). In this technique, animals are trained to postpone an electric foot shock by pressing a lever at a regular rate, and in case of avoidance failure, to terminate a shock by pressing a second lever. Depressant drugs of all kinds tend to lower the rate of lever pressing, increase the number of shocks delivered, and to some extent, produce escape failure (failure to terminate the shock). For each compound the minimum effective dose (MED) necessary to significantly increase escape failure and the MED necessary to increase avoidance failure, is determined and a ratio of the two values is computed. This "dose range ratio" (Heise and Boff, 1962) is said to reflect the range of doses over which a drug has a measurable depressant action without causing complete inability to respond.

High "dose range ratios" were obtained with the benzodiazepine class

of drugs. Lower ratios were observed with phenothiazines, and the lowest ratios were found with barbiturates (Zbinden and Randall, 1967). Such ratios clearly distinguished between neuroleptics and minor tranquilizers on a quantitative basis. A more complicated procedure (discrete trial trace avoidance) was developed by Heise and McConnell (1961). Rats were trained to respond to a 5-second noise stimulus by pressing a lever. Failure to respond was followed by a 5-second noise coupled with shock. Interposed between the warning sound and the shock was a 5-second silent "gap" (Heise and McConnell, 1961). Subjects were trained to a criterion of 90% avoidance (responding during the initial tone). When these subjects were administered compounds of the benzodiazepine class, the number of responses in the "gap" period markedly increased. Chlorpromazine, in contrast, had a "markedly different effect" (Zbinden and Randall, 1967) in that subjects failing to respond to the initial warning stimulus almost never responded during the "gap" period, while still maintaining the ability to escape the stimulus (terminate the shock). These results were interpreted as having demonstrated a distinct action of benzodiazepines (and phenobarbital) on lengthening the latency of response to a "warning" stimulus, whereas chlorpromazine was said to have "a selective blocking effect on the response to a discreet warning stimulus" (Zbinden and Randall, 1967).

In summary, "anti-anxiety" drugs of the benzodiazepine class reduced "passive avoidance", i.e. the tendency to refrain from a response with potentially aversive consequences. In contrast, the phenothiazines failed to inhibit "passive avoidance" in conflict situations but, on the other hand, were effective in reducing "active avoidance" responses which postponed aversive stimuli.

Drugs representative of each of these three classes of agents were employed in the present study. As with other agents, e.g. levorphanol, an attempt was made to find doses which best approximated the results observed on Day 1A with morphine (25 mg/kg).

No dose of pentobarbital was effective in suppressing Day 1A performance to the levels observed with morphine, i.e. less than 25% of mice escaping, without a mortality rate of greater than 50%. This result is not surprising considering that barbiturates have been shown to lack analgesic properties in some testing situations (Price and Dripps, 1970). Mice failing to escape the shock were observed to be ataxic, i.e. they were unable to exhibit a righting reflex. Yet, these mice when no longer ataxic on subsequent days, did escape successfully. Tolerance could only be overcome by increasing the barbiturate dose to such a level that the majority of mice died.

Although chlorpromazine and diazepam are clearly distinguishable in complex avoidance procedures, in the present investigation their effects were essentially identical. In both cases rapid tolerance developed in tested and untested groups. Unlike the barbiturates, mice treated with chlorpromazine (40 mg/kg) and diazepam (12.5 mg/kg) did not appear ataxic on Day 1A, i.e. righting responses were unaffected, suggesting that analgesia rather than locomotor impairment produced the escape failure. This result is not surprising because both phenothiazines and benzodiazepines have been reported to exhibit mild analgesic activity (Sternbach et. al., 1964; Leme et. al., 1961; Cicero et. al., 1974) in the dose ranges employed in this investigation. Morphine, neuroleptics and minor tranquilizers, therefore, share sedative, and to some extent, analgesic actions. Sustained escape failure, however, could only be produced by phenanthrene narcotic analgesics suggesting that

this phenomenon is independent of the drugs' analgesic action. The observation that subjects receiving pentobarbital, even though obviously ataxic on Day 1, escaped rapidly once locomotor ability was regained on subsequent days, adds further evidence that forced exposure to the stimulus, e.g. by restraint or incapacitation, was insufficient to produce an escape impairment.

Hot-Plate Studies

In order to prove that the interaction of morphine and a noxious stimulus was not limited to a specific stimulus mode, it was necessary to design experiments in which all of the features of the standard testing procedure were retained, except that electric shock would no longer be the stimulus. The "hot-plate" was chosen for these experiments because using the hot-plate made it possible to compare the experimental results in this study with those of other investigators.

The standard procedure for measuring the effects of analgesic agents on the "hot-plate" has been described in the "INTRODUCTION". Since the "hot-plate" technique was originally designed to compare and identify analgesic drugs, and the responses measured were considered to be "reflex" in nature and therefore "unlearned", little thought was given to controlling for the possibility that experience in the hot-plate could influence the experimental results. Thus, when investigators began to study the development of tolerance to the analgesic properties of narcotics, it was not considered necessary to determine whether repeated exposure to the hot-plate could influence the experimental results.

A possible drug-test interaction was considered by Cochin and Kornetsky (1964) in a study of single dose tolerance to morphine. These authors acknowledged the possibility that prior exposure to the hot-plate might influence response latencies. They, however, found no significant difference in the (acute) post-injection latencies of rats tested for four days prior to exposure as compared to rats with no prior exposure to the apparatus. In a later experiment, two groups of rats were injected with morphine, but only

one group received post-injection testing. When both groups of rats were re-injected with morphine two months later, no significant difference in response latencies were observed. Cochin and Kornetsky concluded from these findings that measurements of tolerance to morphine were not influenced by a drug-test interaction and they remarked "practice makes no difference in the latency of response to heat as measure by the hot-plate".

Because other authors (Overton 1964, 1966) reported that drug-test interactions did occur with other depressant drugs, Kayan et. al. (1969) decided to examine, in detail, the original findings of Cochin and Kornetsky (1964). Kayan et. al. (1969) performed experiments employing the hot-plate technique of Johannesson and Woods (1964). They found a significant difference between the reaction times of rats repeatedly tested on the hot-plate as compared to naive controls. More importantly, they found that the rate of tolerance development to morphine was less rapid in rats not tested after injection, than in rats that had been tested following injection. Adams et. al. (1969) found that exposure to the plate facilitated tolerance development regardless of whether subjects were tested with the hot-plate at 55° C. or at the ambient temperature of 25°C. Thus, exposure to the test environment was sufficient to alter responsiveness. In addition, Adams et. al. found that exposure, acquired prior to administration of morphine, did not influence the response to a subsequent dose of morphine. Thus, Cochin and Kornetsky's (1964) original observation was confirmed on this point.

Gebhart and Mitchell (1971) confirmed the earlier findings of Kayan et. al. (1969) and, in addition, found that exposure to the cylinder surrounding the hot-plate, as well as to the ambient temperature, could enhance the rate of tolerance development. These authors suggested that a possible drug-stress interaction could account for these effects since rats were observed

to defecate and urinate when exposed to the plate at the ambient temperature, or to the testing cylinder. Gebhart and Mitchell (1971) concluded that there are two types of tolerance development to the analgesic effects of morphine in the hot-plate procedure. One kind of tolerance was said to develop via a drug-test interaction (behavioral tolerance). The second type, pharmacologic tolerance, was said to develop independently of exposure to the testing apparatus.

In later experiments, Gebhart et. al. (1971) sought to determine whether some aspect of "learning" was associated with the drug-test interaction on the hot-plate. The authors found that removing subjects from the plate following a response (lifting of the rear paw) significantly decreased response latencies as compared to control subjects (not removed from the plate after responding). Thus, the authors concluded that the rats "learned" to improve their response latency when this action was reinforced by immediate removal from the noxious stimulus. Tested and reinforced rats showed a significantly greater degree of tolerance than untested and non-reinforced rats. However, no significant difference in response latencies was found between tested and reinforced groups and tested and non-reinforced groups. Gebhart et. al. (1971) therefore concluded that although learning could influence response latency, learning per se. did not contribute to the acquisition of behavioral tolerance in tested, as compared to untested, chronically injected rats.

It is worth noting, that in the series of studies on the hot-plate, Adams, Kavan and the other authors gave equal weight (latency values) to jump responses and paw lifting. No mention was made of the relative frequency of such behaviors, nor was any reference made as to whether the relative frequencies of such responses shifted during the course of morphine administration. It would be interesting to find out whether these authors found a relative

diminution in jump (escape) responses following repeated post-injection exposure to the hot-plate.

In a related experiment, Gebhart et. al. (1972) investigated the effects of "stress" on the development of tolerance to morphine using the hot-plate. Three types of stressful stimuli were employed: restraint, auditory, and swimming. Two series of experiments were carried out. In the first series, there were three major groups: 1) a tested group (testing once before and once after drug administration); 2) a non-tested group (receiving drug but, not tested on the heated plate until the fifth of five days of daily morphine injection); and 3) a "swim stress" group, receiving injection followed by a 15 minute exposure to a cylinder filled with water, but not tested on the hot-plate until the final day of drug administration.

The four groups comprising the second experiment consisted of 1) a "restraint stress" group (rats were enclosed in a towel for sixty seconds) but otherwise treated the same as Group 3 in the first series, and 2) an "auditory stress" group, treated as above except that rats were exposed (60 seconds) to a high intensity tone (110 dbs.). The third and fourth groups were equivalent to the tested and non-tested groups in the first series of experiments.

Gebhart et. al. (1972) found no evidence that a "drug-stress" interaction was operative in the development of analgesic tolerance to morphine as measured by the hot-plate. They found that the latencies of tested morphine-treated subjects were significantly shorter than the values observed in either the stressed or unstressed (as well as untested) groups. The authors did find that a significant degree of tolerance did develop in one untested control group (although to significantly smaller levels than observed in the tested group. Curiously, tolerance was never observed in any of the stressed

groups. In other words, it appeared that tolerance developed to a lesser extent in the stressed groups than in the untested controls. The authors (Gebhart et. al., 1972) did not explore this finding any further but concentrated instead on their observation that tested rats developed a level of tolerance significantly greater than that observed in any of the other groups.

Gebhart and Mitchell (1972) autopsied the stressed subjects and found consistent pathological changes in the gastric mucosa. No such changes were observed in the tested groups. Thus, the authors concluded from these post-mortem examinations that testing itself did not constitute a "stressful" experience, at least in comparison to the other types of stress employed.

Gebhart et. al. (1972) concluded from these experiments and from their earlier work (1971) that neither stress nor learning (via reinforcement) was involved in the drug-test interactions observed on the hot-plate. The authors proposed that the behavioral tolerance observed on the hot-plate reflected a more "general phenomenon" involving an "adaptive" change "brought about due to the necessity of task-performance while under the influence of the drug". Thus, they postulated, since "non-tested" animals are not required to perform a task while under the influence of a drug, tolerance to the functional impairment induced by the drug develops slowly, or not at all.

In more recent experiments, Kayan et. al. (1973) investigated the possibility that extensive pre-drug experience on the hot-plate could influence post-injection latencies. The earlier experiments of Cochin and Kornetsky (1964) and Adams et. al. (1960) had not reported any effect of limited (4-5 days) pre-drug testing on acute post-injection performance. In their investigation, Kayan et. al. (1973) found that 11 days of repeated testing caused a diminution in the analgesic response to a single dose of morphine.

The authors described this phenomenon as "initial behavioral tolerance". This "initial behavioral tolerance" was observed consistently in subjects tested daily, but only infrequently in subjects tested weekly (same total testing trials). Thus, Kayan et. al. (1973) concluded that the intertrial interval influenced the subsequent demonstration of behavioral tolerance. A similar effect of intertrial interval was observed to influence the development of "chronic behavioral tolerance"; differential rates of tolerance development were observed in subjects receiving repeated post-injection testing as compared to those subjects receiving weekly testings.

In summary, the series of studies by Adams, Kayan, Gebhart, and others showed that one aspect of tolerance development to the analgesic effects to morphine can be attributed to a drug-test interaction. This interaction was demonstrated by the finding that tolerance developed more rapidly in tested (post-injection) subjects than in non-tested subjects. The term "behavioral tolerance", originated by Thompson and Schuster (1968), was applied to this phenomenon to distinguish it from "pharmacologic" tolerance, i.e. tolerance observed in untested subjects.

The finding of a drug-test interaction such as behavioral tolerance is not unique to morphine or to the hot-plate technique. Behavioral tolerance has been referred to by Overton (1964) as a form of "state-dependent learning". "State-dependent learning" refers to the ability of drugs to act as discriminative stimuli. Drugs can act as cues, e.g. subjects have been shown to learn to make a particular response in the "drug-state" and a different response in the "no drug-state" or vice-versa. It is possible that administration of morphine influences response latencies on the hot-plate not only via its analgesic effects, but also by producing some change in sensory

discrimination. Thus, through repeated exposure to the testing environment, following morphine administration, subjects might learn to adapt to the "Drug-altered" state. The development of behavioral tolerance can, therefore, be viewed as an adaptation to changes in discriminative cues which were brought about by administration of the drug.

Overton (1971) performed extensive studies of discriminative learning on a T-Maze (mice learn to escape a shock by entering one of two arms of a T-shaped maze). He found that a variety of drugs were relatively effective in acquiring discriminative control of stimuli. Those drugs exerting the strongest control included the barbiturates and benzodiazepines. Moderately active drugs included morphine as well as various compounds known to act on cholinergic and adrenergic systems. Phenothiazines were found to be relatively inactive in controlling discriminative behavior.

Overton (1971) attempted to determine the mechanisms underlying the drug-induced modification of discriminative stimuli termed "state-dependent" or "dissociative" learning. He suggested that since "centrally" acting drugs are more effective in producing state-dependent effects, than drugs acting at the periphery, it was unlikely that the phenomenon was directly related to an alteration of sensory cues. However, he also stated that "there is no direct evidence to either support or negate the hypothesis that drugs acquire response control by virtue of some central process that is independent of sensory control" (Rev. by Overton, 1971).

In the present study, the effect of repeated exposure to the foot shock was to diminish tolerance to morphine, as measured by escape performance. This finding is in direct conflict with those of Kayan et. al. (1969), who found that post-injection testing facilitated the measurement of tolerance

on the hot-plate. The results of the studies on vocalization (Figure 3) provided the first clue as to the possible nature of this paradox. There was a daily increase in vocalization frequency concomitant with a sustained low level of escape performance. Consequently, in performing the hot-plate experiments, two values were recorded: the escape frequency, and the latencies as measured by the technique of Johannesson and Woods (1964).

In experiments with repeated daily testing of non-drugged animals (Figure 22), mice were found to successfully escape the heated-plate in much the same fashion as they did in the shock apparatus. Thus, mice did not habituate to the heat stimulus. The performance of tested and injected mice, exposed to the standard testing procedure (Figure 21), was clearly analogous to the performance of mice in the shock experiments. A sustained post-injection escape impairment was observed throughout the period of drug administration, yet tolerance was observed in an untested group. In addition, there was a period of post-morphine impairment with recovery to baseline values by Day 30.

When mean response latencies were recorded (Figure 23 A and B), it became clear that escape behavior and reflex latencies responded differently to repeated morphine administration. Mean response latencies following the first morphine injection were longer than pre-morphine control values. Response latencies declined on successive days of drug administration, approximating pre-morphine values by the 3rd and 4th day. The mean latencies in the control (untested) groups on Days 7 and 15 were not significantly different from the values obtained in the experimental group. Therefore, there was no evidence for behavioral tolerance when measuring reflex-latencies. This finding clearly indicates that similar results to those observed in

other studies (Kayan et. al., 1969) can be obtained using the present testing methodology; if one measure reflex latencies rather than escape behavior. Thus, the results of previous "hot-plate" studies (Kayan et. al., 1969; Adams et. al., 1969; etc.) can be satisfactorily reconciled with the results of the present study on the basis of the nature of the response measured.

In summary, the results of the hot-plate experiments show that the original findings are not limited to one stimulus mode. In addition, it was found that mice receiving post-injection testing with one stimulus (either shock or heat) exhibited a post-morphine impairment when subsequently tested using the other stimulus.

Summary and Conclusions

The present experiments demonstrate that mice, receiving daily injections of phenanthrene narcotic analgesics followed by exposure to a foot-shock, acquire a maladaptive response (escape failure). The acquisition of this response is not dependent on the analgesic action of morphine because it occurs in mice previously made tolerant to the analgesic properties of the drug. Once acquired, demonstration of this response did not require the presence of the drug since escape failure was found to persist following discontinuation of the drug. Impairment in escape performance required interaction of drug and testing since it could not be produced by shock alone, drug alone, or by forced exposure to the shock. Non-narcotic analgesics, barbiturates and major and minor tranquilizers produced an initial escape suppression, but performance rapidly returned to pre-drug values. The phenomena were not restricted to a single stimulus mode since results similar to those with shock were observed when "heat" was employed as the noxious stimulus.

In total, the experimental results suggested that narcotic analgesics can interact with an aversive stimulus, through a unique and as yet unknown mechanism, such that subjects (once capable of an appropriate response), learn to perform in a maladaptive fashion. The question now becomes: what is the implication of these phenomena to the understanding of drug action and to the problem of drug addiction? The results in this study serve to reinforce the findings in previous investigations that the behavioral state of an organism, as determined by its environment and prior experience, can influence the response to a drug. Thus, morphine can be said to possess analgesic action and possibly anti-anxiety effects under certain conditions, but in other situations, e.g. repeated exposure to stress, the drug may

potentiate the fear or anxiety associated with some experience.

The abuse liability of narcotic analgesics has been ascribed to the combination of such drugs' analgesic and euphoriant properties and their ability to produce physical dependence. These being the most obvious pharmacological concomitants of drug addiction, little consideration has been given to the possible effects of chronic drug administration on the behavior and judgement of the addict. The findings in the present study suggest that the interaction of the drug and experience may influence the behavior of the addict. It is possible that a drug-behavior interaction, similar to that observed with mice in the present investigation, in the addict, leads to the performance of inappropriate or self-destructive behavior.

No tolerance to the ability of narcotic analgesics to produce maladaptive responding was observed in the present investigation. Moreover, impaired performance was found to persist following discontinuation of the drug. The above observations suggest that whatever the nature of the drug-behavior interaction, the resulting alteration of function is long lasting. Chronic drug administration may, therefore, produce a disorganization in functioning or judgement which then contributes to the difficulties encountered in successfully treating the addict. The possibility that chronic drug abuse can interfere with cognitive processes should, therefore, be considered in the design of strategies (e.g. methadone maintenance) for the treatment of drug addiction.

The experiments presented in this thesis were performed employing a specific strain (CF-1) of mice. In order to give further support to the contention that the observations in the present investigation represent a general phenomenon, it will be necessary to demonstrate these phenomena in other species of animals as well as different strains of mice.

A number of additional experiments might be valuable in providing further insight into the mechanisms by which morphine interacts with the aversive stimulus. For example, it would be interesting to determine whether simultaneous administration of benzodiazepines or other "anti-anxiety" agents with morphine, counteracted the normally observed morphine-induced suppression of escape behavior. It would also be interesting to discover if these "anti-anxiety" drugs could, in the post-morphine period, influence the return to base-line performance. If benzodiazepines did counteract morphine-induced escape impairment, this finding would lend support to the hypothesis that this interaction is analagous to the CER. Similarly, further quantification of the "CER-like" responses observed in tested animals, might also provide additional evidence that the escape impairment represents a form of CER.

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