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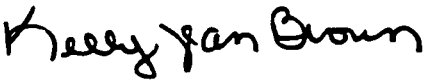

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ABSTRACT

Title of Dissertation: Examination of Acute Sensitivity to Morphine and Morphine Self-Administration Following Physical and Environmental Stressors in Fischer-344 and Lewis Female Rats

Kelly Jean Brown, Doctor of Philosophy, 1997

Dissertation directed by: Neil E. Grunberg, Ph.D.

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The present experiments examined the effects of different environmental conditions on acute morphine sensitivity and morphine self-administration in two genetically diverse inbred strains of rats. Fischer-344 and Lewis rats were subjects because they are related to the commonly used Sprague-Dawley strain, but differ from each other in behavioral and biological responses to opioids and environmental conditions. It was hypothesized that differential behavioral and biological responses to morphine under the various environmental conditions would be strain dependent. In Experiment 1, behavioral and biological effects of acute morphine injections were examined in 96 Fischer-344 and 96 Lewis rats that were either group housed, individually housed, or group housed and immobilized. F-344 rats were more sensitive to morphine's analgesic and locomotor effects, whereas Lewis rats were more sensitive to morphine's effects on body temperature, vertical movements, and rotarod performance. Strain-dependent environmental effects on the acoustic startle response, vertical activity, speed, and body temperature also were found. Further, environmental conditions interacted with strain of

rat to produce differential effects of morphine on hot-plate and speed of locomotion. Specifically, F-344 rats had different responses to morphine under the different environmental conditions, whereas for Lewis rats the responses to morphine did not change on these measures. In addition to behavioral changes, strain-dependent environmental influences on peripheral and central morphine levels also were found. In Experiment 2, the effects of the same environmental conditions on subsequent morphine self-administration were examined in 30 Fischer-344 and 30 Lewis rats. Morphine self-administration of F-344 rats was more affected by the different environmental conditions than was the self-administration behavior of Lewis rats. In addition, the effect of the specific environmental condition was strain dependent. Specifically, grouped F-344 rats tended to self-administer more than did individually housed or immobilized F-344 rats. In contrast, differences between Lewis rats were a result of individually housed rats consuming more than the grouped or immobilized Lewis rats. Corticosterone, a biochemical index of stress, did not appear to mediate the changes in morphine's behavioral and biological effects or differences in morphine self-administration found between the different environmental conditions. These findings suggest that the interaction between genotype and environmental conditions are important variables to consider when addressing issues of drug sensitivity and pharmacokinetics. If these findings generalize to humans, then certain populations may be particularly responsive to different environmental influences significant enough to produce changes in behavioral, physiological, and biological responses to drugs and consequently alter one's susceptibility to tolerance, toxicity, or addiction liability.

Examination of Acute Sensitivity to Morphine and Morphine Self-Administration Following Physical and Environmental Stressors in Fischer-344 and Lewis Female Rats

by

Kelly Jean Brown

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Department of Medical and Clinical Psychology
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INTRODUCTION

The use and abuse of illicit drugs are self-destructive behaviors that affect individual health and that can affect the broader society. Clinical data and human reports suggest that individuals differ in their vulnerability to initiate, maintain, and relapse to drug-taking behavior. Factors that contribute to an individual's propensity to use and abuse drugs include biological and environmental variables. Human studies and animal experiments indicate that there is a genetic predisposition for drug abuse (Shuster, 1990). Genetic effects on stressor-induced behavioral, neurochemical, and hormonal changes also have been noted (Anisman & Zacharko, 1992; Parsons, 1988). Further, it is reported that stress plays an important role in sensitizing drug responses and mediating drug-seeking behaviors (Brown, Klein, Rahman, & Grunberg, 1995a; Shaham, Alvares, Nespor, & Grunberg, 1992; Shaham & Stewart, 1994). However, little research has examined how genetic factors and stress interact to affect drug responses and drug-seeking behaviors.

Among commonly abused drugs, the opioids, a group of drugs that have long been considered to be the gold standard of addictive drugs, are among today's leading causes of accidental drug overdoses. Opioids are the number one choice of prescribed medication for the relief of severe pain. In addition, initiation of opioid use is relatively common among the general public. A subset of this population becomes addicted and continues on from use to abuse. In addition, illicit drug trafficking and use of opioids as recreational drugs also occurs in the United States.

It is important to examine how stressors affect behavioral and biological responses to opioids and how these responses may influence voluntary opioid intake and opioid

addiction. Factors that contribute to variability in stress-induced effects include the type of stressor (e.g., social, physical, psychological) and the psychobiological make-up of the individual. Animal models are useful to examine such questions because they provide investigators with strict control over environmental conditions and a variety of genetically homogeneous populations to study. Consequently, the investigator can control the type of stressor to which the animal will be exposed and can partial out effects related to environmental influences and those effects related to biological differences.

This research examined the effects of group housing, individual housing, and immobilization on acute morphine behavioral and physiological sensitivity and morphine self-administration in Fischer-344 and Lewis female rats. Individual housing and immobilization have been shown to produce reliable increases in corticosterone levels, a biochemical indicator of stress, as compared to group housed and no stress control female rats (Brown & Grunberg, 1995; Kant, Lenox, Bunnell, Mougey, Pennington, & Meyerhoff, 1983). The F-344 and Lewis strains of rats were chosen as subjects for several reasons. They are genetically divergent and differ on a number of behavioral, biochemical, and electrophysiological responses to morphine (George, 1991a; George, Porrino, Ritz, & Goldberg, 1991; Guitart, et al.,1993). Further, the Lewis rats originated from Wistar stock which is an outbred strain of rat commonly used in behavioral research and in the examination of opioid self-administration. In addition, the commonly used outbred Sprague-Dawley rat is the maternal strain for Lewis and F-344 rats. Because both strains have a genetic background common to a widely used outbred strain of rat, the data collected on these rats may provide generalizable information pertinent to a more

diverse population. In addition, the F-344 and Lewis rats are currently being bred together to produce a new recombinant inbred line of rats which will allow for the furthering of genetic research in these strains. These strains, therefore, provide a useful comparison in which behavioral and physiological changes in response to morphine following stress manipulations can be investigated as a function of genetic differences. In addition, findings with these two well-characterized strains provide a useful beginning to address the broader issue of genotype and differential responses to drugs and environment.

Female rats were used for several reasons. First, female rats have a higher avidity for opioids than do male rats as indexed by higher amounts of morphine and fentanyl self-administration in operant and home cage oral self-administration paradigms (Alexander, Coambs, & Hadaway, 1978; Brown, et al., 1995a; Hadaway, Alexander, Coambs, & Beyerstein, 1979; Klein, Popke, & Grunberg, 1996). Secondly, female rats have been shown to either increase or decrease opioid self-administration in response to different types of stressors, whereas male rats have only been shown to increase drug self-administration or show no differences in comparison to a non-stressed control group. Specifically, female rats self-administer more fentanyl following mild electric footshock than they do following a no-stress period (Klein, Shaham, Alvares, & Grunberg, 1993). In contrast, however, female rats self-administer less fentanyl when stressed by individual housing in comparison to non-stressed group housed females (Brown, et al., 1995a). Consequently, female rats provide a subject population that allows for a broad examination of the effects of stress on opioid self-administration.

Experiment 1 examined effects of immobilization stress, individual housing, and

group housing on acute behavioral and physiological sensitivity to morphine. Experiment 2 examined effects of immobilization stress, individual housing, and group housing on subsequent morphine oral self-administration (SA). It was hypothesized that group housing, individual housing, and immobilization stress would interact with the different genotypes to produce differences in acute sensitivity to subcutaneous morphine injections and voluntary morphine self-administration between F-344 and Lewis rats.

This report begins with a review of the literature that is relevant to the proposed experiments. Specifically, this paper provides information on genetic contributions to drug addiction. Next, stress is discussed as a general concept and in terms of its effects on drug self-administration and drug sensitization. Genetic variability in stress responses also is reviewed. Then the prevalence of opioid use, opioids' actions, and the rewarding effects of opioids are addressed with a specific emphasis on morphine. In addition, this section includes a section focusing on strain variability in behavioral and biological effects of opioids. Finally, behavioral and biological comparisons between the Fischer-344 and Lewis rat strains are described. These reviews are followed by an overview of the proposed experiments, a list of specific hypotheses with their rationales, a detailed description of the methods and procedures, a statistical overview, results, and discussion of findings.

Genetic Influences on Drug Addiction

Factors that contribute to an individual's propensity to use or abuse a drug include environmental and pharmacological variables. These factors alone, however, do not explain individual differences in drug use patterns within a given population exposed to

similar environmental conditions. Consequently, a comprehensive examination of drug addiction must also include organismic variables, such as genetic variables.

Human Reports of Drug Abuse Vulnerability

Individuals differ in their vulnerability to use and abuse licit and illicit drugs. Clinical data and human reports suggest that genetic and environmental influences are involved in differentially predisposing individuals to begin drug-taking behavior. In recent surveys of people aged 12 years or older, 29% of the individuals reported never trying tobacco cigarettes and 17% reported never drinking alcohol (Uhl, Elmer, LaBuda, & Pickens, 1995). Because these two licit substances are easily accessible, these data suggest that not all individuals who have access to a drug will seize the opportunity to use it. Similarly, statistics released by the National Institute of Drug Abuse (1991) suggest that only 16% of individuals with access to heroin report using it.

Individual differences also occur in patterns of drug use and in the likelihood of its continuation. For instance, the pattern of drug use within an individual's life span can often be described by an inverted-U shaped function, with drug use beginning in the early teen years, peaking during the late teens and early twenties, and then declining shortly afterward (Kandel & Raveis, 1989). Unfortunately, however, some individuals continue to use and abuse licit and illicit drugs past their twenties. Specifically, 93% of alcohol drinkers, 60% of tobacco smokers, 19% of heroin users, and 8% of hallucinogen users report continue use of these substances well into their late thirties (Raveis & Kandel, 1987). In addition, only some individuals who abuse one drug will continue on to be a polydrug user (Jaffe, 1990).

Lending support to these reports are familial studies, and population human genetic studies provide evidence for a genetic predisposition for drug abuse. Through a convergence of data from family studies, twin studies, and adoption studies, it has been determined that genetic and environmental variables influence drug use patterns. There are, however, limitations to studying such a complex behavior in a human population. Problems inherent in studying genetic vulnerabilities to drug use in humans include: diagnostic imprecision, assortive mating, etiological heterogeneity, inability to perform invasive procedures, ethical considerations in examining initial drug exposure, and lack of strict environmental control. These restraints have led to a great increase in the use of animal models to complement human studies and extend the examination of genetic influences on drug-taking behavior.

Genetic Animal Models to Examine Drug Use

Most drugs used and abused by humans also serve as positive reinforcers for animals. For example, animals will self-administer opioids (e.g., morphine, etonitazene), psychomotor stimulants (e.g., cocaine, amphetamine, nicotine), sedative-hypnotics (e.g., pentobarbital, ethanol), benzodiazepines (e.g., chlordiazopoxide), and arylcycloalkylamines (e.g., phencyclidine) (George & Goldberg, 1989). Consequently, animal models allow for the examination of genetic and environmental influences on drug use vulnerability. Specifically, animal models can be used to examine drug-related phenotypes, such as initial sensitivity, dependence, tolerance, sensitization, and conditioned drug effects.

Most animal studies of addictive drugs have been performed in rats and mice

because of the vast number of genetically defined strains available and the ease with which transgenic mice can be produced. Many of these specified populations of rats and mice remain stable over years and across laboratories (Crabbe & Phillips, 1990). In addition, the similarities between the mouse and human genome increase the likelihood of finding analogous sites in humans (Uhl et al., 1995). Further, rats and mice allow for various drug measures (i.e., sensitivity, dependence) to be correlated with neurochemical, neurophysiological, and neuroanatomical mechanisms in the various, well-characterized rodent strains.

Use of Inbred Strains to Assess Vulnerability to Drug Abuse

Most of the progress in the analyses of genetic contributions to drug use vulnerability has come from inbred and outbred correlations, comparisons between selectively bred animals, and cross-breeding experiments at the behavioral and biochemical levels of analysis (Crabbe & Li, 1995). Selective lines have been successfully bred for specific responses to ethanol, opioids, benzodiazepines, barbiturates, nicotine, and cocaine. Unfortunately, selective lines have been bred for only some drug responses (e.g., sensitivity, preference). For example, no lines to date have been bred for increased or decreased tolerance to any drug (Crabbe & Li, 1995). In addition, because selection experiments deal with finite numbers of animals, inbreeding is often a concern and may result in a genetic drift in which both trait-relevant and trait-irrelevant genes are forced into a state of homozygosity by chance rather than by the mechanism of selective mating of those animals exhibiting extreme phenotypic responses. This genetic drift increases the likelihood of finding other genetically-related differences between the lines that are not the

result of the genes related to the selectively bred trait (Crabbe et al., 1990). In order to verify differences found between selectively bred lines, many researchers examine similar phenotypes in inbred strains of animals as a complementary pharmacogenetic technique.

In contrast to selectively bred lines, the particular alleles that are fixed in a given inbred strain are done so by chance. An inbred strain is developed after 20 or more generations of brother-sister mating (Crabbe & Phillips, 1990). The resulting animals are virtually genetically identical and homozygous at all gene loci and are analogous to monozygotic twins. Consequently, when members of an inbred strain differ on a given measure the variability is contributed solely to the environment. Similarly, when environmental conditions are held constant and differences are found between mean responses of different inbred strains, the variability is solely attributable to genetic differences. By comparing several inbred strains that show a range of responses to a particular drug, correlations can be made between the mean phenotypic values for each strain and genotype (Crabbe et al., 1990). Because the genetic uniformity of inbred strains is so stable across time and laboratories, data sets on battery of tests are cumulative and can be pooled (Crabbe & Li, 1995). Over 100 commercially available inbred strains of rats and mice are currently available of which include Fischer-344 and Lewis rats.

Stress

Stress is a process in which environmental or psychological events threaten an organism's safety and leads to a response directed at alleviating its potentially harmful effects (Baum, Grunberg, & Singer, 1982). Over the last 80+ years, stress has been regarded as a response (Canon, 1914), a nonspecific syndrome (Selye, 1955), a specific

patterning of hormonal responses (Mason, 1975), and a process involving appraisal and coping (Lazarus, 1966). In addition, stress also has been differentiated in terms of its length (i.e., acute vs chronic) and its qualitative dimension (i.e., physical, psychological, social). Despite these discrepancies, stress is a useful construct to use in examining the link between biobehavioral responses to environmental stimuli and subsequent health changes.

Stress causes responses of the autonomic nervous system, as well the hypothalamic-pituitary-adrenal cortical axis (Grunberg & Singer, 1990). Catecholamine release is increased (e.g., norepinephrine, epinephrine) from the adrenal medulla and nerve terminals, and corticosteroid release is increased from the adrenal cortex in response to an increase of adrenocorticotropic hormone released from the pituitary gland. The catecholamines act to increase blood pressure, heart rate, respiration, peripheral vasoconstriction, and blood flow to active muscles, whereas the corticosteroids act to increase energy mobilization. These changes are often described as preparing the organism for "fight or flight" as first coined by Cannon who discovered an increase of epinephrine in the bloodstream and a preparation of tissue in cats frightened by barking dogs (Cannon & de la Paz, 1911).

When measuring a stress response or validating that a stressor was effective, it is important to use a multilevel assessment approach. This approach should include behavioral, physiological, and biochemical analyses of the organism. There may be changes in some but not all of these systems and a pattern can emerge (Mason, 1975). Alternatively, in the case of a nonspecific stress syndrome (Selye, 1955), the stress

response does not necessarily move in parallel across the different systems involved. Some common stress-induced changes in rodents include elevated corticosterone levels, increased latency to tail flick, disruption in exploratory patterns, changes in body temperature, and altered acoustic startle responses (ASR) and prepulse inhibition (PPI) (Acri, 1994; Cabib, Kempf, Schleef, Mele, & Puglisi-Allegra, 1988; Jørgenson, Fasmer, Berge, Tveiten, & Hole, 1984; Kant, et al., 1983).

All types of stressors, however, do not produce the same changes and many of these measures can be either decreased or increased depending upon the type and duration of the stressor. For example, immobilization stress has been reported to produce hypothermia (Jørgenson, et al., 1984) in mice, whereas cohort removal stress produces hyperthermia (Groenink, van der Gugten, Zethof, van der Heyden, & Olivier, 1994). In addition, the ASR is unaffected by a social defeat stressor (Miczek, 1991), is decreased by footshock (Leitner, 1988) and tailpinch (Sorenson & Swerdlow, 1982) stressors, and is increased by immobilization and observation stressors (Acri, 1994). Similarly, PPI is decreased by cold swim (Leitner, 1989) and is increased by immobilization and observation stressors (Acri, 1994).

With regard to the effects of stressor duration, acute exposure to inescapable footshock caused a decline in norepinephrine and an increase in corticosterone, whereas chronic exposure to this same stressor results in increased norepinephrine and corticosterone levels above controls (Irwin, Ahluwalia, Zacharko, & Anisman, 1986). Similarly, behavioral activation and analgesia produced by acute footshock exposure disappear following prolonged exposure to the stressor (Menendez, Andres-Trelles,

Hidalgo, & Baamonde, 1993; Prince & Anisman, 1984). These studies suggest that a multilevel assessment of the stress response is valuable to measure behavioral and biological changes.

Genetic Variability in Stress Responses

There also is considerable individual variability in the quality and quantity of stress responses in rats (Parsons, 1988). Highly individualized behavioral responses to different situations have been observed within single outbred and inbred strains of rats (Cools, Brachten, Heeren, Willemen, & Ellenbroeck, 1990; Fokkema, Smit, van der Gugten, & Koolhaas, 1988; Koolhaas, Fokkema, Bohus, & van Oortmerssen, 1986; Pradhan, Arunasmitha, & Udaya,1990). Differences between strains of rats in behavioral, physiological, and biochemical responses to stressors also have been reported (McCarty, Gilad, Weise, & Kopin, 1979; Ray & Barrett, 1975). Further, rats selectively bred for different behavioral responses to novel environments (e.g., Roman High and Low Avoidance, Maudsley Reactive and Non-Reactive) also display different behavioral, physiological, and biochemical responses to different stressors (Benešouvá, Beneš, Fraňková, & Tikal, 1977; Blizard, 1971; Gentsch, Lichsteiner, Driscoll, & Feer, 1982). Experiments using inbred rat strains that differ in their catecholamine responses to stress indicate that changes in plasma catecholamine levels after stress are closely related to observed strain differences in behavioral responses to stress (McCarty & Kopin, 1978). In addition, strain variability in the adaptation to stress as measured with a variety of different biochemical measures have been reported (Stone & McCarty, 1983). Mouse models suggest that genetic factors influence vulnerability to stressor-induced changes and that

there is an underlying polygenetic architecture (Harshfield & Simmel, 1979; Shanks & Anisman, 1993; Shanks, Griffiths, & Anisman, 1994).

Stress and Drug Self-Administration

Clinical reports and epidemiologic data indicate that stressful events are positively correlated with drug initiation in adolescents (Wills, 1986) and with alcohol consumption by alcoholics (O'Doherty, 1991). In addition, cigarette smokers smoke more under stress (Rose, Ananda, & Jarvik, 1983), and stressful life events are associated with maintenance of opioid use among opioid addicts and relapse to opioid abuse among formerly abstaining opioid addicts (O'Doherty, 1991; Kosten, Rounsaville, & Kleber, 1986). Animal models of stress-induced drug self-administration provide support for these observations. Commonly used and biochemically validated stressors such as uncontrollable footshock, immobilization, and tail-pinch all have been reported to increase ethanol and amphetamine self-administration in rats (Piazza, Deminière, Le Moal, & Simon, 1990; Pohorecky, 1990). In addition, less commonly used, non-physical stressors such as environmental noise, chronic social isolation, and crowding have been reported to increase the self-administration of ethanol, amphetamine, and barbital in rats and mice (Hannon & Donlon-Bantz, 1975; Mollenauer, Bryson, Robison, Sardo, & Coleman, 1993; Roske, Baeger, Frenzel, & Oehme, 1994; Zimmerberg & Brett, 1992). Further, Ramsey and Van Ree (1993) reported that emotional (i.e., observation stress) but not physical stress (i.e., hot plate, footshock) enhanced intravenous cocaine self-administration in rats.

With regard to opioids, male rats self-administer greater amounts of intrathecal, intracerebroventricular, intravenous, or oral opioids during or following a physical stressor

(Dib, 1985; Dib & Duclaux, 1982; Shaham, et al., 1992; Shaham, Klein, Alvares, & Grunberg, 1993; Shaham & Stewart, 1994). Similarly, social isolation or individual housing has been reported to increase the initiation of opioid self-administration, to increase maintenance levels, and to increase the amount of opioid self-administration during relapse in comparison to group housed animals (Alexander, Beyerstein, Hadaway, & Coambs, 1981; Alexander, Coambs, & Hadaway, 1978; Bozarth, Murray, & Wise, 1989; Marks-Kaufman & Lewis, 1984). These studies manipulating environmental conditions, however, did not evaluate the effects of the housing conditions on other behavioral, physiological, or biochemical measures. Based on these experiments examining opioid self-administration using either potentially painful physical stressors or non-physical environmental manipulations without stressor validation, the evidence is equivocal as to whether stress is playing a role and if rats are self-administering greater amounts of opioids under these conditions just to alleviate pain or provide them with something to do.

Recent experiments provide support for the hypothesis that opioid self-administration is increased by stress *per se*. First, it has been shown that temporal and conditioning factors are important in determining whether opioid self-administration will increase in response to immobilization stress. Specifically, the pairing of the stressor with the drug self-administration period resulted in increased opioid self-administration compared with a condition in which exposure to the stressor and the drug self-administration period were explicitly not paired (Shaham, 1993). Second, it has been reported that rats exposed to repeated predictable footshock will self-administer more

fentanyl than will rats exposed to the identical amount of unpredictable footshock across initiation, maintenance, and relapse periods (Klein, Popke, & Grunberg, in press). Also, using a biochemically validated environmental stressor indicating that male rats are stressed when crowded and female rats are stressed when individually housed (Brown & Grunberg, 1995), female rats decreased opioid self-administration following the environmental stressor, whereas no differences in fentanyl consumption was found between the stressed and non-stressed males (Brown, et al., 1995a). It is important to note that although the results of this last experiment are opposite of findings based on previous studies using environmental manipulations, the self-administration period and procedure are substantially different. In addition, although crowding has been found to produce elevated corticosterone levels in male rats for up to 15 days (Brown & Grunberg, 1995), individually housed and crowded male rats in this experiment did not have statistically different corticosterone levels at the end of the experiment which lasted over 50 days (Brown, et al., 1995a)

In conjunction with reports that stressors increase opioid self-administration, it has been reported that greater plasma corticosterone levels in response to footshock stress are positively correlated with early fentanyl self-administration (Klein, et al., in press). There also is a main effect of sex in that female rats self-administer greater amounts of opioids than do male rats, regardless of stress or housing conditions (Alexander, et al., 1981; Alexander, et al., 1978; Brown, et al., 1995; Klein, et al., in press). Further, female rats have higher baseline levels of corticosterone than do male rats (Brown & Grunberg, 1995; Kant, et al., 1983). Finally, evidence of oral and intravenous SA of corticosterone

solutions at plasma levels comparable to those induced by stress suggests that corticosterone has reward potential (Deroche, Piazza, Deminière, Le Moal, & Simon, 1993; Piazza, Deroche, Deminière, Maccari, Le Moal, & Simon, 1993). Therefore, corticosterone, or regulators of glucocorticoid release, such as adrenocorticotropin hormone, corticotropin-releasing hormone, and corticotropin-releasing factor, may mediate opioid consumption. Changes in corticosterone levels in response to stress, as opposed to absolute corticosterone values, may influence the drug's rewarding effects and drug self-administration.

Stress-Induced Drug Sensitization

One reason that stress increases drug consumption in humans and animals may be that the actions of the drug become more rewarding under stressful conditions. Evidence that stress increases drug effects or produces sensitization, which may be involved in drug reward, is growing. For example, chronic stress has been reported to enhance apomorphine-induced stereotypic climbing in mice (Cabib, Puglisi-Allegra, & Oliverio, 1984), and tail-pinch, restraint, and repeated exposure to intermittent footshock stressors are interchangeable with amphetamine, morphine, and ethanol in their ability to produce behavioral sensitization to the drugs' locomotor effects (Antelman, Eichler, Black, & Kocan, 1980; Badiani, Cabib, & Puglisi-Allegra, 1992; Deroche, Piazza, Casolini, Maccari, Le Moal, & Simon, 1992; Hahn, Zacharko, & Anisman, 1986; Leyton & Stewart, 1990; Roberts, Lessov, & Phillips, 1995; Shaham & Stewart, 1995). In addition, stress can potentiate the analgesic and hypothermic effects of morphine (Martin, Pryzbylik, & Spector, 1977; Sherman, Strub, & Lewis, 1984).

Critical to the production of stress-induced morphine and amphetamine sensitization is an intact adrenal gland (Deroche, Piazza, Casolini, et al., 1992; Deroche, Piazza, Le Moal, & Simon, 1993). Corticosterone administration alone can increase behavioral responses to amphetamine (Deroche, Piazza, Maccari, Le Moal, & Simon, 1992; Pauly, Robinson, & Collins, 1993) and severity of acute withdrawal from ethanol (Roberts, Crabbe, & Keith, 1994). Further, the glucocorticoid receptor antagonist RU 38486 prevents ethanol sensitization suggesting a direct role for corticosteroids in this process (Roberts, et al., 1995).

There also are individual differences with regard to the effects of stress on drug sensitization. Stimulatory effects of morphine and the self-administration of corticosterone can be predicted by corticosteroid reactivity to novelty stress within an outbred rat strain (Deroche, Piazza, Le Moal, et al., 1993; Piazza, et al., 1993). Further, there are strain differences in the effects of stress to increase amphetamine-induced locomotor activity in mice (Anisman & Cygan, 1975).

Besides sensitizing the locomotor effects of amphetamine and opioids, stress can augment the toxic effects of cocaine and fentanyl (an opioid agonist). Specifically, 58% male rats died in response to daily injections of cocaine following restraint stress compared with 17% that died in response to the daily injections alone (Pudiak & Bozarth, 1994). Similarly, in our own laboratory, several stressed female rats died following 6-hour fentanyl drug self-administration despite self-administering lower doses than non-stressed female rats (Brown, et al., 1995a). These results suggest that stress and increases in corticosterone modulate drug effects but that the direction and intensity of these effects

are dependent upon the drug and other individual difference factors including the animal's corticosteroid response based on novelty of stimulus, sex, and strain.

Opioids

It is estimated that there are approximately 600,000 opioid addicts in the United States and nearly 2,000,000 opioid abusers. Although these numbers reflect a relatively low prevalence of opioid dependence, historical experience suggests that the vulnerability to dependence is relatively high. In addition to recreational use, opioids are commonly prescribed as effective analgesics in medical situations. Consequently, it is particularly important to distinguish between people who are genetically predisposed to opioid dependence from those who do not possess this vulnerability. With regard to abuse liability, human clinical research with opioids is focused on the development of effective analgesics with reduced abuse liability (Bigelow & Preston, 1995). The majority of this research, however, is conducted on prior or current opioid drug users because of ethical considerations in giving naive subjects access to a potentially addictive substance. In addition, the findings that stem from this research are directed towards the probability of drug dependence occurring within a larger population and are not concerned with the subsample of highly vulnerable people *per se*.

Properties and Actions of Morphine and Chemically Related Opioids

Opiates refer to drugs that derive from opium which is obtained from the milky droppings from the unripe seed capsules of the poppy plant, *papaver somniferum*. Once obtained, this juice is dried and powdered to make opium which contains more than 20 distinct alkaloids. These alkaloids can be divided into two distinct chemical classes known

as the phenanthrenes and the benzylisoquinolines. The principle and largest constituent phenanthrene is morphine which was first isolated in 1806 by Sertürner (Jaffe & Martin, 1990). The term opioid is more inclusive and applies to all naturally occurring and synthetic opioid peptides with morphine-like effects. The endogenous opioid peptides include three families: the enkephalins, the dynorphins, and the β-endorphins.

The structure of morphine was determined more than a century ago and is shown in Figure 1. Many semisynthetic opioid derivatives are made by relatively simple modifications to the morphine molecule. Morphine is extensively metabolized in the liver and undergoes significant hepatic first-pass metabolism following oral administration. It is mainly conjugated with glucuronic acid to form both active and inactive metabolites and eliminated by glomerular filtration mainly as morphine-3-glucuronide (Jaffe & Martin, 1990). Morphine is well-absorbed from subcutaneous and intramuscular sites as well as from mucosal surfaces of the nose and gastrointestinal tract. The half-life of morphine is approximately 2.0 hours and the half-life of morphine-6-glucuronide (an active metabolite) is somewhat longer. Morphine has a pKa of 9.85 (Vožeh & Schmidlin, 1987) and is highly soluble in water in the form of morphine-sulfate (Merck Index, 1983).

Receptors at which opioids such as morphine act are located in the brain, spinal cord, adrenal medulla, and the gastrointestinal tract. With the discovery of stereospecific high affinity binding sites, it has been possible to trace many opiate drug effects via their interaction with specific binding sites and their mediation of endogenous opioids. The most well characterized receptor types and subtypes include μ_1, μ_2, δ, and κ, although ϵ and λ receptor types have been characterized (Cox & Werling, 1991). The μ receptor

mediates analgesia, miosis, bradycardia, and hypothermia. More specifically, the μ_1 receptor has been proposed to mediate analgesia and the μ_2 receptor has been proposed to mediate respiratory depression. In addition, the μ receptor and its subtypes have been the most extensively studied to determine its involvement in appetitive and motivational effects. These studies suggest that the μ receptor, or more specifically the μ_2 subtype, plays an active role in the reward-mediated behaviors and may do so through the mediation of dopaminergic pathways (Suzuki, Funada, Narita, Misawa, & Nagase, 1993). Delta receptor sites have been associated with seizures and reward and the κ sites also are thought to mediate analgesic effects and produce dysphoria (Jaffe & Martin, 1990). The μ, δ, and κ receptors are most often found on the presynaptic nerve terminals and appear to function primarily by exerting inhibitory modulation of synaptic transmission in the CNS and the myenteric plexus. In addition, the receptors appear to be coupled to guanine nucleotide-binding regulatory proteins (G proteins). Morphine acts as a full agonist at the μ and κ receptor sites.

Morphine's mood changing, mental clouding, and sleep inducing effects were the basis for its name that was derived from the Greek god Morpheus, the god of dreams and the father of Hypnos (Jaffe & Martin, 1990). In addition to these effects, morphine and other opioids produce analgesia, respiratory depression, decreased gastrointestinal motility, nausea, vomiting, changes in body temperature, the inhibition of the release of gonadotrophin-releasing hormone and corticotropin-releasing factor, and hypotension. Morphine was once referred to as "God's own medicine" by Sir William Osler and remains the standard by which new analgesics are measured. Morphine-like drugs are used

therapeutically to provide symptomatic relief of pain, cough, and diarrhea and is thought to relieve suffering by altering the emotional component of the painful experience as well as by producing analgesia.

Reinforcing Effects of Morphine and Chemically Related Opioids

The rapid intravenous injection of an opioid has been described by addicts as producing a warm flushing of the skin and sensations in the lower abdomen similar in intensity and quality to sexual orgasm (Jaffe, 1990). As with many of the other effects produced by opioids, significant tolerance to this euphorogenic effect develops, especially following chronic and continuous use. Consequently, to achieve this "rush" or euphoric high the dose of the drug must be constantly increased. In addition, 8-12 hours following the last injection, many negative consequences start to occur including lacrimation, rhinorrhea, yawning and sweating. If another dose of the opioid is not taken, then this physical withdrawal syndrome continues to worsen and includes restlessness, dilated pupils, increased heart rate and blood pressure, anorexia, gooseflesh, muscle spasms, irritability, insomnia, vomiting, diarrhea, and tremor. Without treatment or administration of an opioid, this abstinence syndrome can last for 7 to 10 days.

These positive (i.e., rush) and negative (i.e., relief from withdrawal) reinforcing effects of opioids are likely to be responsible for the initiation and maintenance of opioid use and the development of the drug addiction process (Bozarth, 1994; Cox & Werling, 1991). In addition, two other mechanisms by which drug addiction can occur by means of the opioid's positive or negative reinforcing effects have been suggested. First, there is some evidence indicating that the termination of chronic opioid use decreases

dopaminergic activity and impairs the ability of natural reinforcers to activate this system and that opioid use restores normal dopaminergic activity. Second, some reports suggest that the positive reinforcing effects of opioids are enhanced with repeated exposure similar to the sensitization of its stimulatory effects (Bozarth, 1994).

The positive reinforcing effects of opioids appear to involve the mesolimbic dopaminergic pathway, arising from cell bodies in the mesenphalic ventral tegmental area (VTA), projecting anteriorly through the medial forebrain bundle (MFB) to the nucleus accumbens (NA) and olfactory tubercle (OT) (Cox & Werling, 1991). μ and δ agonist opioids activate the dopaminergic system by inhibiting neurons that tonically inhibit dopaminergic neurons in the VTA (Jaffe, 1990). Opioids produce behavioral (Joyce & Iversen, 1979), electrophysiological (Matthews & German, 1984), and neurochemical (Di Chiara & Imperato, 1988) changes indicative of activation in this dopaminergic pathway. Further, the destruction of the dopamine-containing cell bodies in the VTA disrupts the acquisition of intravenous heroin self-administration, indicating an important role of this pathway in initial drug reward processes (Bozarth & Wise, 1986). It also is important to note that stress activates the VTA system as well and may cross-sensitize positive reinforcement, thereby providing one possible mechanism by which stress may be positively related to opioid use (Grunberg, 1994). Separate from opioid's positive reinforcing effects, the physical dependence-producing effects of opioids involve the periventricular gray region (Bozarth, 1994). Opioid infusions into the periventricular brain region are not positively reinforcing to drug naive subjects but do produce signs of physical dependence once the drug is removed.

Genetic Variability in the Behavioral and Physiological Effects of Opioids

Measures of opioid sensitivity extensively examined for genetic differences in rodents include behavioral activation (e.g., Brase, Loh, & Way, 1977; Eidelberg, Erspamer, Kreinick, & Harris, 1975; Gwynn & Domino, 1984a; Moskowitz, Terman, Carter, Morgan, & Liebeskind, 1985), analgesia (e.g., Belknap, Lamé, & Danielson, 1990; Bonnet & Peterson, 1975; Kasson & George, 1984), and thermoregulation (e.g., Belknap, Noordewier, and Lamé, 1989; Kasson & George, 1984; Muraki & Kato, 1986). Opioid sensitivity varies across different strains and these differences are partially under genetic control. In addition, strain differences exist in the effects of opioids on respiratory rate (Muraki & Kato, 1986) and feeding (Gosnell & Krahn, 1993). Although some pharmacokinetic differences have been reported among the different strains of mice tested (Brase et al., 1977; Gwynn & Domino, 1984b), it is clear that these factors alone are not responsible for the reported differences because of the disparity in sensitivity among the different behaviors. Specifically, the effects of opiates on locomotor activity and analgesia are negatively correlated (Castellano & Oliverio, 1975; Oliverio & Castellano, 1974), indicating that they cannot be regulated by the same pathway. In contrast, morphine-induced hypothermia and respiratory depression are positively correlated (Muraki & Kato, 1986), suggesting that common mechanisms may be involved. Further, a single gene has been located that enhances opioid sensitivity to increased locomotor activity and decreased body temperature in C57BL/6 mice (Katz & Doyle, 1980). In general, the results of these studies suggest that the effects of opioids on different behavioral and physiological measures are likely to involve multiple neurophysiological systems and

appear to be regulated by more than one gene.

In addition to strain differences in the actions of opioids, there is empirical evidence that genetic differences exist in the rewarding effects of opioids. Nichols and Hsiao (1967) were the first to separate stock Sprague-Dawley rats into "addiction susceptible" rats (i.e., highest quartile) and "addiction resistant rats" (i.e., lowest quartile) and then to randomly inbreed them among their respective groups. Strains differed in their susceptibility to prefer morphine in the F_1, F_2, and F_3 generations with the addiction susceptible line drinking more of the morphine solution than did the addiction resistant line. These results were partially supported some years later (Rönnbäck, 1989), at which time it was found that inbred Sprague-Dawley rats vary in morphine preference over a control liquid diet from a few percent to nearly 80 percent. This study also reported that rats labeled as "morphine-preferring" showed increases in their preference for morphine from the F_1 to F_2 generations.

In addition to these studies involving the selective breeding of rats for morphine consumption and preferences, it also has been established that some inbred rat strains differ in opioid preference and consumption. Specifically, Lewis rats have a greater preference for etonitazene (George, 1991a; Suzuki, George, & Meisch, 1992), morphine, and codeine (Suzuki, Otani, Koike, & Misawa, 1988) than do Fischer-344 rats in studies involving liquid and food morphine-laced diets using home cage and operant procedures. Similarly, food-deprived Wistar rats increase their preference for etonitazene over water, whereas food-deprived Sprague-Dawley rats decrease their drug intake by 50% and never exceed a preference for the drug over water (Carroll, Pederson, & Harrison, 1986).

Maudsley Reactive rats have a higher preference for morphine as compared with Maudsley Non-Reactive rats (Satinder, 1977; Satinder, 1982).

Fischer-344 and Lewis Inbred Rat Strains

The development of inbred animal strains provides investigators with the opportunity to examine questions on populations chosen for specific characteristics. The Lewis and Fischer-344 (F-344) are two of over 100 inbred strains of rats currently available and the outbred Sprague-Dawley strain is the maternal strain for both inbred strains. The Lewis and F-344 strains of rats are divergent on a number of behavioral, electrophysiological, and neurochemical responses to opioids and stimulant drugs. In addition, they differ in basal corticosteroid profiles and in their neuroendocrine and behavioral syndromes in response to stress and corticotrophin releasing hormone (CRH). Summaries of the behavioral, physiological, neurochemical, and biochemical differences between F-344 and Lewis rats are presented in Tables 1 and 2. Consequently, these two strains of rats are particularly useful to examine the relationship between the stress reactivity and subsequent drug use while systematically examining genetic and environmental influences separately and as they interact.

Drug Responsivity

Lewis and F-344 rats differ in their responses to acute and chronic administrations of stimulants and morphine. Lewis rats are more sensitive to the locomotor-enhancing effects of cocaine and methamphetamine than are F-344 rats (Camp, Browman, & Robinson, 1994; George, 1991a) and show greater sensitization to the locomotor effects of repeated cocaine and methamphetamine injections than are the F-344 rats (Camp, et

al., 1994; Kosten, Miserendino, Chi, & Nestler, 1994). Further, Lewis rats display greater cocaine conditioned place preference than do F-344 rats (Kosten et al., 1994), although reports are not consistent as to whether Lewis rats are more susceptible to cocaine-induced conditioned taste aversion (Glowa, Shaw, & Riley, 1994, Kosten, et al., 1994). The Lewis rats also are more sensitive to the acute analgesic effects of morphine evaluated by the hot plate method (Suzuki, Otani, & Misawa, 1988). In contrast, F-344 rats are more sensitive to the lethal effects of cocaine (George, 1991b) and the locomotor-enhancing effects of amphetamine than are the Lewis rats (George, et al., 1991). It is important to note that following acute injections of methamphetamine and cocaine, Lewis rats have higher plasma and brain levels of these drugs than do F-344 rats, suggesting that pharmacokinetic differences may play a role (Camp, et al., 1994). In addition, these results suggest that sensitivity to a drug's reinforcing effects may be under different genetic control than its lethal and aversive effects and that the locomotor effects of stimulant drugs may work at different sites.

One experiment examining the effects of chronic morphine self-administration in Lewis and F-344 rats suggests that they exhibit differences in the development of tolerance to opioid-induced stupor and slow-wave sleep (Mayo-Michelson & Young, 1992). Specifically, a reduction of morphine-induced stupor occurred across seven days for Lewis and F-344 rats but the rate of reduction was greater for the Lewis rats. In contrast, F-344 rats exhibited a tolerance to morphine's increase in latency to slow-wave sleep by day seven, whereas Lewis rats did not develop tolerance to this measure. These results suggest that Lewis and F-344 rats develop tolerance to morphine in different

behaviors.

With regard to drug self-administration and preference, Lewis rats consume more etonitazene, cocaine, alcohol, morphine, codeine, and sedatives than do F-344 rats in studies involving liquid and food-laced diets using home cage and operant procedures (George, 1990; George, 1991; Suzuki, George, et al., 1992; Suzuki, Koike, Yanaura, George, & Meisch, 1987; Suzuki, Otani, Koike, et al., 1988). Further, studies involving Lewis and F-344 among various other strains of rats and mice indicate that there is a moderate but insignificant relationship between ethanol reinforcement and ethanol preference and virtually no relationship between the propensity to self-administer ethanol and its neurosensitivity (George, 1990). In general, these data suggest that there is a underlying genetic determinant of drug self-administration behavior across drug classes in Lewis and F-344 rats. This difference may reflect variations in the drugs' reinforcing effects, differences in ability to discriminate or condition to the drug stimulus, or differences in the development of tolerance or sensitization that can occur with chronic exposure.

Following chronic opioid administration through infusion or pellet, Lewis and F-344 rats display a different behavioral and electrophysiological abstinence profile in response to naloxone challenge. Lewis rats displayed a greater number of wet-dog shakes, diarrhea, and body position than did the F-344 rats (Mayo-Michelson & Young, 1992). Lewis rats also have been reported to lose more weight in comparison to F-344 rats during opioid withdrawal (Gonzalez & Altshuler, 1978). These data suggest that Lewis rats are more physically dependent upon the morphine than are F-344 rats. In

contrast, F-344 rats exhibited a greater percent increase in EEG peak frequency and decrease in total power, the summation of all absolute power spectral density values across the entire frequency band range, after the naloxone injection than did Lewis rats. Because morphine-tolerant rats display reduced EEG spectral power, these data suggest that F-344 rats are more physically dependent on morphine than are Lewis rats (Mayo-Michelson & Young, 1992). Interestingly, it also has been reported that the F-344 rats show milder withdrawal symptoms to pentobarbital (Suzuki, et al., 1987) in comparison with Lewis rats but greater withdrawal signs following chronic ethanol, barbital, or diazepam administration (Suzuki, Lu, Motegi, Yoshii, & Misawa, 1992; Suzuki, Motegi, Otani, Koike, & Misawa, 1992). These results suggest that genetics may play a role in producing qualitatively different opioid abstinence syndromes in addition to quantitative differences in physical dependence *per se*.

An extensive amount of research has examined neurochemical differences between Lewis and F-344 rat strains. Research on these two strains of rats has revealed that drug-naive Lewis rats have higher levels of tyrosine hydroxylase and four other phosphoproteins, and lower levels of three neurofilament proteins in the mesolimbic dopamine system than do drug-naive F-344 rats (Beitner-Johnson, Guitart, & Nestler, 1991; Beitner-Johnson, Guitart, & Nestler, 1993; Guitart, Beitner-Johnson, Marby, Kosten, & Nestler, 1992). Further, the basal extracellular levels of the dopamine metabolites DOPAC and HVA were lower in the nucleus accumbens of Lewis than F-344 rats (Strecker, Eberle, & Ashby, 1995). In addition, chronic morphine administration increased enzyme immunoreactivity and decreased neurofilament protein levels in the

ventral tegmental area (VTA) of F-344 rats but had no effect in Lewis rats (Guitart, et al., 1992). Further, levels of adenylate cyclase and cyclic AMP-dependent protein kinase activity were higher in the nucleus accumbens and locus coeruleus of Lewis rats compared with F-344 rats, whereas $G_{i\alpha}$ and G_{β} protein levels were lower in Lewis rats (Guitart, et al., 1993). Differences in response to morphine also were evident in that morphine increased levels of adenylate cyclase and cyclic-AMP dependent protein kinase in the nucleus accumbens of F-344 rats only, but increased the enzyme levels in the locus coeruleus of both strains (Guitart et al., 1993).

In response to stimulants, it has been reported that Lewis rats had a greater enhancement of extracellular dopamine in the ventral striatum compared with F-344 rats (Camp, et al., 1994). There were, however, no differences in ligand affinity and receptor density of dopamine transporters and dopaminergic D_1 and D_2 receptors in striatal tissue of these two strains (George, et al., 1991). In contrast, cocaine-induced increases in extracellular dopamine in the nucleus accumbens did not differ in Lewis and F-344 rats, and Lewis rats had a smaller peak dopamine elevation and a slower return to basal dopamine levels (Strecker, et al., 1995). These studies suggest that mesolimbic tyrosine hydroxylase, neurofilament protein levels, and extracellular dopamine release may mediate some aspects of drug reinforcement and that Lewis and F-344 rat strains provide a useful model to examine these and other biochemical differences that may contribute to individual genetic differences in vulnerability to drug addiction.

Stress Responsivity

The Lewis and F-344 rats not only make a good model to examine the effects of

stress on drug self-administration because of their differences in drug sensitivity and preference, but also because they differ with respect to their biochemical and behavioral responsiveness to stressors. Specifically, F-344 have significantly greater increases in plasma ACTH and corticosterone in response to observation, open field, restraint, swim, and ether stress as compared with the Lewis rats (Sternberg, et al., 1992). F-344 rats also have a significant increase in corticosterone levels following acoustic startle which does not occur in Lewis rats (Glowa, Geyer, Gold, & Sternberg, 1992). In addition, only the F-344 rats have an increase in CRH mRNA expression in the paraventricular nucleus (PVN) of the hypothalamus following restraint or ether stress. Behaviorally, the Lewis rats were less able to stay afloat during swim stress and produced significantly fewer fecal boli than did F-344 rats during restraint stress. In addition, stressed Lewis rats were more active in the periphery, crossed more outer squares, and groomed less than did stressed F-344 rats (Glowa, Sternberg, & Gold, 1992; Sternberg, et al., 1992). These stress-induced behaviors are consistent with earlier reports that Lewis rats respond significantly less in terms of the number of anticipatory responses to conditioned stimuli (Katzev & Mills, 1974).

Similar to different stressors, the direct administration of CRH also causes strain-dependent behavioral changes (Glowa, Sternberg, et al., 1992). Specifically, CRH reduced the total activity in an open field more in Lewis than in F-344 rats. In addition, inner and outer square crosses were reduced in F-344 rats, whereas only outer square crosses were reduced in Lewis rats. Further, CRH increased grooming in Lewis rats but had no significant effect on F-344 rats. Similarly, an inverse relationship has been

reported between the acoustic startle response and HPA reactivity in that Lewis rats that showed no changes in corticosterone levels following an acoustic startle displayed the greatest behavioral response (Glowa, Geyer, et al., 1992). In addition, this relationship was further supported by a negative correlation between corticosterone levels and acoustic startle amplitudes within the F-344 strain.

In addition to differences in response to stress and CRH, there also are strain differences in diurnal basal corticosterone levels. Specifically, although there are no differences in basal corticosterone levels during the rats' inactive period, F-344 rats display a diurnal rise in basal corticosterone levels during their active cycle that is not evident in Lewis rats (Dhabhar, McEwen, & Spencer, 1993). F-344 rats also express significantly higher absolute corticosteroid-binding globulin levels in plasma, spleen, and thymus. Further, the total basal CRH content per hypothalamus are lower in F-344 rats than in Lewis rats reflecting chronic depletion in response to their hyper-responsiveness to a variety of situations (Sternberg, et al., 1992).

OVERVIEW AND MAJOR HYPOTHESES

General Overview

Clinical data and human reports suggest that genetic and environmental influences are involved in differentially predisposing individuals to initiate, maintain, and relapse to drug-taking behaviors. It is well established that there is a genetic component to drug responses and stress responses, and that stress plays an important role in mediating drug-taking behaviors. Little work, however, has examined how genetic factors and stress interact to affect drug responses and consequent drug-seeking behaviors. Comparing the effects of stress on morphine sensitivity and morphine self-administration in two inbred strains of rats is a good approach to begin this examination.

The objective of this research was to examine the effects of two stressors (e.g., immobilization and individual housing) in comparison with a no-stress condition (e.g., grouped housing) on acute morphine behavioral and physiological sensitivity and morphine self-administration in F-344 and Lewis female rats. Because it has been clearly established that the F-344 and Lewis rat strains differ on a number of behavioral and biological responses to opioids and stress independently, this animal model is particularly suitable to the examination of stress reactivity on morphine sensitivity and self-administration.

This work also allows for the direct comparison of different housing conditions and/or different stressors on the effects of morphine sensitivity and avidity. This comparison is important because behavioral, biochemical, and physiological effects can vary in response to different stressors and housing conditions. Grouped housing,

individual housing, and immobilization were the environmental treatment conditions (e.g., no-stress, stress) because they represent different types of stressors. Specifically, individual housing is an environmental stressor for female rats, yet it is commonly used as the housing condition for experimental animals under study for individual behaviors including drug self-administration. In contrast, immobilization stress is a commonly used stress procedure because it produces a reliable increase in corticosterone, a biochemical index of stress, in a short period of time.

By using female rats as subjects, this research also examined effects of housing conditions and stress on morphine self-administration on both ends of the continuum. Specifically, female rats have been found either to increase or decrease opioid self-administration in response to different types of stressors, whereas male rats have only been reported to increase drug self-administration or show no differences in comparison to a non-stressed control group. In addition, physical (e.g., mild electric footshock) and environmental stressors (e.g., individual housing) produce changes in opioid self-administration in female rats, whereas only physical stressors have been reported to opioid self-administration in male rats.

Major and Minor Hypotheses

The major and minor hypotheses of this dissertation research are listed below. A rationale for each hypothesis is provided. Hypotheses are separated by experiment. There were a total of 15 major and 8 minor hypotheses. In general, morphine is hypothesized to affect the behavioral responses of Lewis and F-344 rats on all responses except acoustic startle responses and prepulse inhibition. In addition, the different stressful environmental

conditions are hypothesized to sensitize morphine's effects on different behaviors. Further, Lewis rats are hypothesized to show greater responses on more complex tasks and be more sensitive to the pharmacological effects of morphine on a range of behaviors including morphine self-administration as compared to F-344 rats. In contrast, F-344 rats are hypothesized to have higher increases in corticosterone in response to the stressful environmental conditions than are Lewis rats.

Experiment 1 examined behavioral and physiological responses to acute morphine administration with and without exposure to two different stressors and in two strains of rats. There were 11 major hypotheses and 6 minor hypotheses for Experiment 1. Major hypotheses are listed first followed by minor hypotheses.

Major Hypotheses:

Major Hypothesis 1: It was hypothesized that morphine administration would decrease rotarod performance in F-344 and Lewis rats.

Rationale: Rotarod performance requires balance which morphine's central nervous system depressant effects are likely to diminish.

Major Hypothesis 2: It was hypothesized that Lewis rats would be more sensitive to the effects of morphine on rotarod performance than would F-344 rats.

Rationale: Previous reports have indicated that Lewis rats are generally more sensitive to various drugs than are F-344 rats (Camp et al., 1994; Kosten, et al., 1994).

Major Hypothesis 3: It was hypothesized that immobilization and individual housing stressors would increase analgesia in Lewis and F-344 rats.

Rationale: It has been reported that different types of stressors increase analgesia (Jørgenson, et al., 1984; Menendez, 1993).

Major Hypothesis 4: It was hypothesized that Lewis rats would exhibit more analgesia (i.e., have a greater latency to hind-paw lick on a hot plate) than would F-344 rats at baseline.

Rationale: Pilot data indicated that Lewis rats demonstrated a higher latency to lick the hind paw on a hot plate.

Major Hypothesis 5: It was hypothesized that Lewis rats would be more sensitive to the analgesic effects of morphine than would F-344 rats.

Rationale: Previous reports indicated that Lewis rats were more sensitive to the acute analgesic effects of morphine (Suzuki, Otani, & Misawa, 1988).

Major Hypothesis 6: It was hypothesized that morphine administration would affect locomotor activity (e.g., total distance, horizontal activity, speed) dose-dependently in an inverted-U shaped function (e.g., 5 mg/kg < 10mg/kg >20mg/kg).

Rationale: Morphine has been reported to increase locomotor activity at low and medium doses (5 mg/kg, 10mg/kg) and to decrease locomotor activity at higher doses (20 mg/kg) (Babbini & Davis, 1972).

Major Hypothesis 7: It was hypothesized that Lewis rats would be more sensitive to the effects of morphine on locomotion.

Rationale: It has been reported that Lewis rats are more sensitive to the locomotor-inducing effects of various drugs (e.g., Camp, et al., 1994).

Major Hypothesis 8: It was hypothesized that immobilization and individual housing stressors would sensitize F-344 and Lewis rats to the effects of morphine on various measures in the behavioral test battery.

Rationale: It has been reported that exposure to intermittent footshock stressor is interchangeable with amphetamine, morphine, and ethanol in their ability to produce behavioral sensitization to the drugs' locomotor effects (Antelman, Eichler, Black, & Kocan, 1980; Badiani, Cabib, & Puglisi-Allegra, 1992; Deroche, Piazza, Casolini, Maccari, Le Moal, & Simon, 1992; Hahn, Zacharko, & Anisman, 1986; Leyton & Stewart, 1990; Roberts, Lessov, & Phillips, 1995; Shaham & Stewart, 1995). In addition, stress has been reported to potentiate the analgesic and hypothermic effects of morphine (Martin, Pryzbylik, & Spector, 1977; Sherman, Strub, & Lewis, 1984).

Major Hypothesis 9: It was hypothesized that morphine administration would produce hypothermia in F-344 and Lewis rats.

Rationale: Based on morphine's pharmacological hypothermic actions, it is hypothesized that morphine will decrease body temperature in Lewis and F-344 rats.

Major Hypothesis 10: It was hypothesized that Lewis rats would have higher brain and serum morphine levels following an acute morphine injection compared to F-344 rats.

Rationale: It has been reported that acute injections of methamphetamine and cocaine result in higher plasma and brain levels of these drugs in Lewis than in F-

344 rats suggesting that pharmacokinetic differences exist between these strains of rats (Camp, et al., 1994).

Major Hypothesis 11: It was hypothesized that immobilized F-344 rats would have higher corticosterone levels than would individually housed F-344 rats, whereas immobilized Lewis rats would have lower corticosterone levels than would individually housed Lewis rats.

Rationale: F-344 rats have been reported to show higher increases in corticosterone following restraint stress in comparison to open field activity, whereas Lewis rats have higher corticosterone levels in response to open field in comparison to restraint stress (Sternberg, et al., 1992).

Minor Hypotheses:

Minor Hypothesis 1: It was hypothesized that morphine administration would dose-dependently increase the latency of F-344 and Lewis rats to lick their hind paw on a hot plate.

Rationale: Morphine is a pharmacological analgesic and, therefore, should decrease nociceptive responses in Lewis and F-344 rats.

Minor Hypothesis 2: It was hypothesized that Lewis rats would have higher acoustic startle responses (ASR) and show more prepulse inhibition (PPI) than would F-344 rats regardless of drug condition.

Rationale: Glowa, Geyer, et al. (1992) have reported that Lewis rats display greater ASR than do F-344.

Minor Hypothesis 3: It was hypothesized that F-344 rats would exhibit more

horizontal locomotor behavior (i.e., horizontal counts, distance, speed) than would Lewis rats regardless of stress or drug condition, whereas Lewis rats would exhibit more thigmotaxis than would F-344 rats.

Rationale: Previous reports have indicated that F-344 rats exhibit higher baseline locomotor activity (George, 1991b) but that Lewis rats cross more outer squares than inner squares in a locomotor chamber (Glowa, Sternberg, et al., 1992).

Minor Hypothesis 4: It was hypothesized that thigmotaxis (i.e., wall hugging), an index of anxiety, would be decreased by morphine administration.

Rationale: Morphine is a pharmacological anxiolytic and, therefore, should decrease wall hugging in Lewis and F-344 rats.

Minor Hypothesis 5: It was hypothesized that immobilized and individually-housed F-344 and Lewis rats would have higher levels of corticosterone than would grouped subjects.

Rationale: Immobilization and individual housing have been reported to increase corticosterone levels in female rats compared with group housed, crowded, or non-stressed control females (Brown & Grunberg, 1995; Kant., et al., 1983).

Minor Hypothesis 6: It was hypothesized that F-344 rats would have higher levels of corticosterone following immobilization and individual housing than would immobilized and individually-housed Lewis rats.

Rationale: With regard to strain differences, corticosterone responses have been reported to be significantly lower in Lewis than in F-344 rats in response to open-field stress, restraint stress, swim stress, and ether (Dhabhar, et al., 1993;

Sternberg, et al., 1992).

Experiment 2 examined morphine oral self-administration with and without two different stressors in two strains of rats. There were 4 (#s12-15) major and 2 (#s 7-8) minor hypotheses for Experiment 2.

Major Hypotheses:

Major Hypothesis 12: It was hypothesized that Lewis rats would orally self-administer more morphine than would F-344 rats.

Rationale: Previous reports have indicated that Lewis rats are more likely to self-administer opiates than are F-344 rats (George, 1990; George, 1991; Suzuki, George, et al., 1992; Suzuki, Otani, Koike, et al., 1988).

Major Hypothesis 13: It was hypothesized that individually housed F-344 and Lewis rats would self-administer more morphine than would group housed F-344 and Lewis rats.

Rationale: Physical stressors (i.e., immobilization, electric footshock) have been reported to increase oral opiate self-administration in male and female rats (Klein, et al., 1993; Shaham, et al., 1992; Shaham, et al., 1993).

Major Hypothesis 14: It was hypothesized that immobilization stress would decrease morphine self-administration in F-344 and increase morphine self-administration in Lewis rats compared with their respective group housed conspecifics.

Rationale: Previous reports indicate that F-344 rats have higher increases in corticosterone following various stressors compared with Lewis rats (Sternberg, et

al., 1992). In addition, it has been reported that F-344 rats have smaller increases in corticosterone following open field activity in comparison to restraint stress. Immobilization stress, in contrast, has been reported to increase corticosterone levels in female F-344 rats (Sternberg, et al., 1992), comparable to corticosterone levels reported in individually-housed female Wistar rats (Brown & Grunberg, 1995). Further, we have previously found that individually-housed females self-administer less fentanyl than do non-stressed crowded females (Brown, et al., 1995). These high levels of corticosterone are believed to increase the sensitivity to the opioid's effects and consequently result in less drug being necessary to produce the same effect. Because Lewis rats do not exhibit extremely high increases in corticosterone levels in response to stress, their sensitivity to the drug will not be has high and they will continue to increase their self-administration of the drug in the presence of both stressors as compared to the non-stressed Lewis rats.

Major Hypothesis 15: It was hypothesized that immobilized F-344 rats would have higher corticosterone levels than would individually housed F-344 rats, whereas immobilized Lewis rats would have lower corticosterone levels than would individually housed Lewis rats.

Rationale: F-344 rats have been reported to have higher increases in corticosterone following restraint stress in comparison to open field activity, whereas Lewis rats have higher corticosterone levels in response to open field in comparison to restraint stress (Sternberg, et al., 1992).

Minor Hypotheses:

Minor Hypothesis 7: It was hypothesized that immobilized and individually housed F-344 and Lewis rats would have higher levels of corticosterone than would non-stressed control subjects.

Rationale: Immobilization and individual housing have been reported to increase corticosterone levels in female rats compared with grouped housed or crowded females (Brown & Grunberg, 1995; Kant., et al., 1983).

Minor Hypothesis 8: It was hypothesized that F-344 rats would have higher levels of corticosterone following immobilization and individual housing than would immobilized and individually-housed Lewis rats.

Rationale: With regard to strain differences, corticosterone responses have been reported to be significantly lower in Lewis than in F-344 rats in response to open-field stress, restraint stress, swim stress, and ether (Dhabhar, et al., 1993; Sternberg, et al., 1992).

Experiment 1

Overview

The purpose of Experiment 1 was to examine effects of immobilization, individual housing, and group housing on acute behavioral and physiological sensitivity to morphine. Subjects included 96 Fischer-344 and 96 Lewis female rats. Subjects were randomly assigned to one of three experimental conditions: (1) one 20 minute immobilization period; (2) two days of individual housing; or (3) continuous group housing prior to baseline. Within each experimental condition subjects (n=8 per cell) were randomly assigned to receive a single subcutaneous dose (i.e., 0 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg) of morphine sulphate. The experimental design is presented in Table 3. During a no-injection baseline phase and following the injection each subject was run through a test battery including behavioral and physiological measures. Specifically, the test battery was comprised of rotarod, hot-plate, acoustic startle response (ASR), prepulse inhibition (PPI), body temperature and various measures of locomotor activity (i.e., distance traveled, speed, horizontal movement, vertical movement, thigmotaxis). These particular measures were included because they are simple and non-invasive and they have been used frequently in empirical evaluations of stress responses and responses to opiates. The order of the test battery (see Table 4) was designed to minimize any effects of one measure on another based on pilot work and previous experience with these measures. It is noteworthy that a similar test battery consisting of respiratory rate, startle responses, Y-maze activity, heart rate, and body temperature obtained identical results as when the same measures were used individually (Marks, Romm, Bealer, & Collins, 1985). After the

behavioral measures on the testing day, trunk blood was collected for assay of corticosterone and serum morphine levels. In addition, whole brains were collected for assay of morphine brain levels. Serum and brain morphine levels were measured to assess possible pharmacokinetic differences between F-344 and Lewis rats in response to stress.

Methods

Subjects and Non-stress Housing Conditions

Subjects included 96 Fischer-344 and 96 Lewis female rats purchased from the National Cancer Institute (Rockville, MD) at approximately 4-5 weeks of age (200 g). Sample size was based on reports in the literature and in our laboratory using these behavioral measures. Animals were group housed in same-strain groups of four in polypropylene cages (47 X 37 X 19 cm) with absorbent recycled paper (Cell-Sorb Plus ™) and stainless steel wire-bar lids with slotted feeders. This housing condition was chosen based on a previous finding that female rats have higher corticosterone levels, a biochemical index of stress, when individually housed than when grouped or crowded in numbers of four or greater (Brown & Grunberg, 1995). Subjects remained undisturbed, except for routine maintenance, in this condition for approximately four weeks to keep the age of the animals consistent with previous reports of oral opiate self-administration in rats (Brown, et al., 1995a; Shaham, et al., 1992). The housing room was maintained at 23-25 °C, 50% relative humidity, and a 12-hour light/dark cycle (lights on at 0700hrs). Food (Harlan Teklad 7001) and water were readily accessible at all times.

Drug

Morphine-sulphate (Mallinckrodt Inc., St. Louis, MO) was dissolved in

physiological saline and was injected at doses of 0.0 mg/kg, 5.0 mg/kg, 10.0 mg/kg, and 20.0 mg/kg subcutaneously. Drug solutions were made based on weight of drug expressed as the salt. The injection volume of the morphine and saline was 1.0 ml/kg body weight. These doses were based on work in the literature and represent commonly used low, medium, and high doses.

Stress Manipulation

Immobilization. Subjects in the restraint stress condition remained group housed (nonstressful housing) in numbers of four in their home cages (47 X 37 X 19 cm) until the experimental drug testing phase. Immediately before the drug testing period, animals were removed from their home cages and were restrained in a commercially available finger-like restraint apparatus (Centrap Cage, Fisher Scientific) for a period of 20 minutes. This type of limited immobilization is a reliable stressor in various strains of rats, including Lewis and F-344 rats, as indexed by increases in corticosterone levels (Raygada, Shaham, Nespor, Kant, & Grunberg, 1991; Dhabhar, et al., 1993).

Individual Housing. Two days before the experimental drug testing phase, subjects were individually housed in their home cages (44 X 23 X 20 cm). Previous research in our laboratory has reported that female rats individually housed for 18 hours per day before being transferred to a second individually housed condition have higher corticosterone levels than do female rats that are grouped or crowded for 18 hours a day before being transferred to an individually-housed condition (Brown & Grunberg, 1995). This procedure reliably increases corticosterone levels in the individually housed rats following 8 and 15 days of this manipulation. In addition, two days of individual housing

versus group housing produces significant differences in opiate self-administration in female rats (Brown, et al., 1995a).

Behavioral and Physiological Test Battery

Rotarod. Motor coordination, balance, and strength was assessed in a 4-lane Omnitech Rotarod performance machine (Omnitech Electronics, Columbus, Ohio) using a modified procedure based on previous findings from our laboratory (Rahman, Grunberg, & Mueller, in press). This measure provided an additional index of movement to assess the effects of morphine on locomotion. Animals were placed on the still rod and rotation speed was gradually increased in 2-4 rpm increments until a 20 rpm maximum was reached. Time to reach maximum speed was 1 minute at which time animals had an additional 1 minute to maintain themselves on the bar at 20 rpm. Amount of time the animal remained on the beam was recorded in seconds with a maximum of 130. Animals did not receive any punishment for falling off the rod and remained on the bottom of the test chamber until all animals completed their trial. All rats were trained on this task for 10 trials prior to testing. During testing, all animals received two trials as described above and the best of the two scores was analyzed for between-subjects differences.

Hot Plate. The hot plate test assesses nociception as judged by the latency to react to a thermal stimulus and is mediated by processes within the central nervous system (Tjølson, Rosoland, Berge, & Kjell, 1991). Changes in antinociception occur in response to stress and opioid injections (e.g., Belknap, et al., 1990; Menendez, et al., 1993). Latency to react to a thermal stimulus was measured in a hot plate analgesiometer (Omnitech) and testing parameters were based on previous findings from our laboratory

(Rahman, et al., in press) as well as pilot data. The hot plate analgesiometer was heated to 51 °C and animals were placed in the chamber with the lid on. Rats were removed from the chamber once the animal lifted and licked the hind-paw or for a maximum time of 90 seconds. Each animal received two trials with a 2 minute rest period between each trial. The time it took the animal to perform the end-point behavior was recorded in seconds with a maximum of 90. The average of the two scores was recorded and analyzed for between-subjects differences.

Acoustic Startle Response/Prepulse Inhibition. Acoustic startle responses and prepulse inhibition were measured using a four-station acoustic startle system (Coulbourn Instruments, Allentown, PA) based on published reports from our laboratory (e.g., Acri, Brown, Saah, & Grunberg, 1995). These measures reflect the animal's innate defensive reaction to an environmental stimulus (Davis, 1984) and provide a sensitive behavioral measure of stress reactivity capable of predicting subsequent opioid self-administration (Brown, Klein, Rahman, & Grunberg, 1995b). Animals were enclosed in 8 x 8 x 16 cm open air cages that restrict locomotion but do not restrain the animal. Cages were placed on one of four platforms in a sound-attenuating test chamber. Background noise within the test chamber was 56dB from a ventilating fan. Startle eliciting acoustic stimuli consisted of 20 ms noise bursts of 112 dB. Prepulse inhibition trials consisted of a 20 ms, 1 kHz pure tone of 68 dB preceding the startle eliciting stimuli by 100 msec. Trials with no stimuli and trials with a prepulse tone alone also were presented. The subject's movement in response to the stimuli presentation was measured as voltage change by a strain gauge system incorporated in each platform. Movement was converted to grams of

body weight change following analog to digital conversion. Responses were recorded by an interfaced microcomputer as the maximum response occuring within 200 msec of the onset of the startle eliciting stimulus. One test session consisted of a 2-minute quiet adaptation period followed by random presentations of 8 no-stimulus trials, 8 prepulse alone trials, 8 startle trials, and 8 tone + startle trials with an inter-trial interval range of 10-20 seconds. Animals received one acclimation session 3-5 days before baseline or test data were collected. The mean of the 8 no stimulus trials, startle trials and 8 tone + startle trials was automatically calculated. Before statistical analyses were done, all acoustic startle measures were derived by subtracting the mean of the no stimulus trial (i.e., the body weight measured on the acoustic startle platform) from the animal's mean score on the startle alone and tone + startle trials. This calculation controls for differences in random activity and body weight. PPI was then calculated for each animal by subtracting the corrected mean of the tone + startle trials from the corrected mean of the startle trials.

Body Temperature. Body temperature was measured using a Bailey Instruments (Model #Bat-8; Saddle Brook, New Jersey) rectal probe. The probe was lubricated with peanut oil and was inserted approximately 2.5 cm into the rectal cavity. Body temperature was displayed and recorded in degrees Celsius. Body temperature changes occur in response to stress and opioid injections (e.g., Belknap, et al., 1989; Jørgenson, et al., 1984).

Locomotor Activity. Locomotor activity was measured using an Omnitech Digiscan infrared photocell system (Model RXYZCM (16 TAO); Omnitech Electronics, Columbus Ohio). Animals were placed in a clear Plexiglas chamber (40 X 40 X 30 cm)

one at a time for 30 minutes. Fifteen pairs of infrared photocells are located every 2.5 cm from left to right and from front to back in a plane 2 cm above the floor of the chamber to measure horizontal movement. An additional 15 pairs of infrared photocells are located every 2.5 cm from left to right 10.5 cm above the floor of the chamber to measure vertical movement. Dependent variables included total distance (an index of locomotion not confounded by repetitive occlusions of a single beam as a result of grooming or other behaviors unrelated to locomotion), horizontal activity, vertical activity (an indirect assessment of rearing activity), speed, and thigmotaxis (wall hugging behavior) . Horizontal and vertical activity, total distance traveled, margin time, center time, and time in motion were automatically calculated based on beam breaks in two minute time periods and transferred to a personal computer via an Omnitech analyzer (Model DCM-8-BBU). Speed was calculated as total distance traveled divided by time in motion. Thigmotaxis (wall hugging) was calculated as center time divided by margin time. Analyses were performed on total scores for each dependent variable calculated by adding together the scores recorded every two minutes during the 30 minute time period. Animals received one acclimation session 3-5 days before baseline or test data were collected. Locomotor activity is sensitive to changes in behavioral activation in response to opiates (e.g., Babbini & Davis, 1972).

Corticosterone Levels

Animals were sacrificed by decapitation without anesthesia at the end of the experiment and trunk blood was collected in non-treated tubes. Blood was centrifuged (1500 X g) for 20 minutes at 4°C. Serum was frozen and stored at -70°C in separate

microtubes until assayed for corticosterone using a standard radioimmunoassay kit (ICN Biomedicals).

Serum Morphine Levels

Serum morphine levels were measured using a coated tube RIA kit (Diagnostic Products Corporated). Animals were sacrificed by decapitation without anesthesia at the end of the experiment and trunk blood was collected in non-treated tubes. Blood was centrifuged (1500 X g) for 20 minutes at 4°C. Serum was frozen and stored at -70°C in separate microtubes until assayed for serum morphine levels. The serum of animals receiving the 10 or 20 mg/kg dose of morphine was initially diluted 1:3 with zero diluent. However, the initial assay revealed that several levels were still outside the range of the standard curve. In these cases the serum was assayed again with the serum of animals that received the 10 or 20 mg/kg diluted 1:5 and the serum of animals that received the 5 mg/kg dose diluted 1:3. The interassay reliability was r= +.896.

Brain Morphine Levels

At the time of sacrifice, brains were removed and stored at -70°C until assayed using a coated tube RIA kit (Diagnostic Products Corporated). Brains were homogenized in a 1:1 weight to volume solution of physiological saline. The homogenates were centrifuged at 35,000 X g for 20 minutes at 4 °C and aliquots of the supernatant were assayed.

Procedure

For logistical purposes, 24 Lewis and 24 Fischer rats arrived at the Laboratory Animal of Medicine, USUHS, weekly for a period of four weeks. Table 5 presents the

ordering schedule. Upon arrival, all animals were group housed in same strain numbers of four for a period of three weeks. During this time animals were undisturbed except for routine maintenance. Food and water were readily available. During the fourth week, subjects were gentled, acclimated to the acoustic startle procedure, rectal probe, hot plate, and locomotion chamber, and given 10 practice trials on the rotarod behavioral task as described above. Experimental manipulations began on the fifth week of subjects' arrival for each separate purchase order. Within each purchase order group, subjects were randomly assigned and equally distributed across experimental stress and drug dose conditions between strains. For example, among the 24 Lewis rats from the first purchase order, eight rats were randomly assigned to each of the three stress conditions and within each stress condition, two rats were randomly assigned to each of the four drug dose conditions. Animals were run through the test battery in groups of four, and eight animals per strain were run each day. Baseline testing occured between 1000 and 1200 hours and testing occurred between 1200 and 1400 hours.

Baseline Testing

Baseline testing consisted of running each animal through the test battery following removal from their group housing condition. Eight subjects were tested each day in groups of four designated as Group A and Group B. Group A preceded Group B by 40 minutes on all tests. Four Lewis and four Fischer-344 rats were run each day. The test battery consisted of two rotarod performance trials, two hot-plate trials, an acoustic startle and prepulse inhibition session, a body temperature measurement, and 30 minutes in a locomotion chamber. The test battery took one hour to complete for each group. Table 4

presents the order and timing of the test battery and the running of Groups A and B. Immediately following the test battery, animals were taken back to their home room and placed in their home cage if in the grouped housing or immobilization condition or in an individual cage if in the individually housed condition.

Drug Testing

Drug testing was identical to baseline testing except that animals were injected with one of four drug doses immediately following their respective stress condition and 10 minutes before being run through the test battery (see Table 4). Eight subjects were tested each day in groups of four designated as Group A and Group B. Group A preceded Group B by 45 minutes on all tests. The drug testing session was separated from the baseline session by one day. Four Lewis and four Fischer-344 rats were run each day and the stress and drug dose conditions were randomly dispersed across days. Following the test battery, all subjects were immediately sacrificed and blood and whole brains were collected and assayed.

Statistical Analyses

Experiment 1 was a mixed factorial design with strain (2), stress (3), and drug dose (4) being the between-subjects factors and test day (e.g., baseline, drug) being the within-subject factor. Dependent variables included rotarod performance (seconds), body temperature (degrees centigrade), hot plate analgesia (seconds), acoustic startle response, prepulse inhibition, speed of locomotion (cm/sec), distance traveled (cm), thigmotaxis (center time/wall time), horizontal movement, vertical movement, plasma corticosterone levels (ng/ml), serum morphine levels (ng/ml), and brain morphine levels (ng/g). Each

test day was analyzed separately.

To determine if there were baseline differences between the strains, stress conditions, or drug groups, univariate three-way ANOVAs were conducted on the three main effects with all two-way and three-way interactions dropped from the equation to use a conservative statistical plan that optimized clarity of findings. When significant baseline differences were present, the dependent variables were analyzed by univariate three-way ANCOVAs with baseline measures used as a covariate and the three-way interaction dropped from the equation. When baseline differences were not present, dependent variables were analyzed by univariate three-way ANOVAs with the three-way interaction dropped from the equation. Because locomotor data consisted of five related measures (e.g., distance traveled, horizontal movement, vertical movement, speed, thigmotaxis) a multivariate analysis of covariance, using baseline performance as the covariate, was performed with all five measures before separate univariate ANOVAs or ANCOVAs were conducted. This MANCOVA was conducted to insure that significant findings found with univariate analyses were not the result of chance and to allow for the relationship among the various aspects of locomotion.

Further, separate two-way ANOVAs (or ANCOVAs when appropriate) were conducted for both strains and for each drug dose when necessary for a better understanding of the data. Tukey HSD or Dunnett post-hoc analyses were conducted to determine differences among specific groups depending on the nature of the question. All significance tests were two-tailed and evaluated at $\alpha = 0.05$.

Results

Behavioral and Physiological Test Battery

Rotarod. Rotarod performance was used as a behavioral measure of coordination and balance. Figures 2 and 3 present the amount of time (seconds) on the rotating rod for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in performance levels between the strains, stress conditions, or drugs groups, a three-way ANOVA was conducted. This analysis revealed a significant main effect of strain [$F(1,186)=73.146$, $p<.05$]; F-344 rats stayed on the rotating bar significantly longer than did Lewis rats. There were no significant differences in baseline performance between stress conditions [$F(2,186)=0.189$, n.s.] or drug groups [$F(3,186)=0.452$, n.s.].

Performance during testing was analyzed with a three-way ANCOVA, using baseline performance as a covariate, taking into account all main effects and two-way interactions. Table 6 provides a listing of these results. The significant main effect of strain persisted during testing [$F(1,174)=73.201$, $p<.05$], with the F-344 rats performing better than the Lewis rats regardless of stress or drug exposure. A significant main effect of drug also was revealed [$F(3,174)=19.784$, $p<.05$] with morphine exposure decreasing rotarod performance. Stress alone had no significant effect on rotarod performance [$F(2,174)=0.396$, n.s.] and there were no significant interactions.

Because of significant strain differences, separate two-way ANOVAs were conducted for F-344 and Lewis rats. Table 7 provides a listing of these results. These

analyses indicated that there were significant effects for drug for F-344 [$F(3,85)=12.165$, $p<.05$] and Lewis [$F(3,85)=6.760$, $p<.05$] rats. Dunnett post-hoc analyses using the 0 mg/kg dose as the control group indicated that F-344 rats showed a decrease in rotarod test performance following 20 mg/kg or morphine whereas Lewis rats showed performance decrements following both 10 and 20 mg/kg doses of morphine (see Figure 4). There were no significant stress or stress x drug dose interactions for F-344 or Lewis rats.

Hotplate. Latency to lick the hind-paw on a hotplate was used as a behavioral measure of nocioception. Figures 5 and 6 present the latency to lick the hind paw (seconds) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in nocioception between the strains, stress conditions, or drugs groups a three-way ANOVA was conducted on these three main effects. This analysis revealed a significant main effect of strain [$F(1,186)=7.031$, $p<.05$]; Lewis rats remained on the hotplate significantly longer than did F-344 rats before licking their hind paw. There were no significant differences in baseline nocioception between stress conditions [$F(2,186)=2.319$, n.s.] or drug groups [$F(3,186)=1.314$, n.s.].

Nocioception during testing was analyzed with a three-way ANCOVA, using baseline performance as a covariate, taking into account all main effects and two-way interactions. No main effect of strain [$F(1,174)=1.314$, n.s.] was found, although strain interacted with stress [$F(2,174)=11.927$, $p<.05$] and drug dose [$F(3,174)=9.113$, $p<.05$]. The strain x stress interaction is illustrated in Figure 7. In addition, for a clearer

presentation of the strain x drug interaction, percent change scores from control (0 mg/kg) for F-344 and Lewis rats are presented in Figure 8. Main effects of stress [$F(2,174)=5.577$, $p<.05$] and drug [$F(3,174)=243.239$, $p<.05$] also were revealed with stress decreasing latency to hind-paw lick and drug dose increasing hind-paw lick latency. No significant stress x drug interaction [$F(6,174)=0.669$, n.s.] was found.

Because of significant strain interactions with stress and drug dose, separate two-way ANOVAs were conducted for the F-344 and Lewis rats. With regard to F-344 rats, the main effect of stress persisted [$F(2,85)=12.772$, $p<.05$] as did the main effect for drug dose [$F(3,85)=93.940$, $p<.05$]. In contrast, there was no main effect of stress for Lewis rats [$F(2,84)=1.164$, n.s.]. Similar to F-344 rats, the main effect of drug was significant [$F(3,84)=226.485$, $p<.05$] for Lewis rats. No stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats, respectively ([$F(6,85)=1.164$, n.s.]; [$F(6,84)=1.642$, n.s.]).

To further understand the significant strain x dose and strain x stress interactions, separate two-way ANCOVAs, using baseline hot plate performance as a covariate, were conducted at each drug dose. Table 8 provides a listing of these results. In summary, significant strain x stress interactions were revealed at the 0 mg/kg [$F(2,41)=3.425$, $p<.05$], 5 mg/kg [$F(2,43)=3.376$, $p<.05$], and 10 mg/kg [$F(2,41)=6.691$, $p<.05$] doses. Tukey HSD post-hoc analyses indicated that immobilized F-344 rats receiving 10 mg/kg of morphine had a shorter latency to lick their hind-paw than did grouped or individually housed F-344 rats receiving the same drug dose. In addition, the 5 mg/kg immobilized F-344 rats had a shorter latency to lick than did the 5 mg/kg grouped F-344 rats. No

significant differences were found between stress conditions for Lewis rats at either the 5 or 10 mg/kg doses.

Acoustic Startle Response. The acoustic startle response (ASR) measures an animal's innate reflex to an auditory startling stimulus and was measured to provide a behavioral measure of stress reactivity. Figures 9 and 10 present the amplitude of the animals response in grams of weight change (movement weight - still weight) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in ASR between the strains, stress conditions, or drugs groups a three-way ANOVA was conducted on these three main effects. This analysis revealed no baseline strain differences [$F(1,186)=0.125$, n.s.]. There was, however, a significant difference in baseline ASR between drug conditions [$F(3,186)=5.827$, $p<.05$] and marginal difference between stress conditions [$F(2,186)=2.673$, $p=.07$] indicating that random assignment to treatment conditions did not create equal differences between groups on this variable.

ASR during testing was analyzed with a three-way ANCOVA, using baseline responses as a covariate, taking into account all main effects and two-way interactions but eliminating the three-way interaction. Table 9 provides a list of these results. No main effect of strain [$F(1,174)=2.428$, n.s.] nor stress [$F(2,174)=1.911$, n.s.] was found. The main effect of drug dose remained significant [$F(3,174)=3.051$, $p<.05$], with animals receiving the 20 mg/kg morphine dose responding significantly less than animals in all other drug condition (Tukey HSD). There were no significant interactions.

To test the *a priori* hypotheses that there would be strain differences in ASR,

separate two-way ANCOVAs with baseline ASR as a covariate, were conducted on each strain. Table 10 provides a list of these results. In summary, the only significant finding was a main effect of stress in F-344 rats [$F(2,84)=4.387$, $p<.05$]. Tukey HSD post-hoc analysis indicated that the individually housed F-344 rats had significantly higher ASR than did immobilized F-344 rats (See Figure 11).

Prepulse Inhibition. Prepulse inhibition (PPI) is a measure of the dampening or lessening of the ASR when a non-startle eliciting tone immediately precedes a startle eliciting stimulus. This measure is thought to reflect processes involving sensorimotor gating. Figures 12 and 13 present the amplitude of the animals response in grams of weight change (movement weight - still weight) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in PPI between the strains, stress conditions, or drugs groups a three-way ANOVA was conducted on these three main effects. This analysis revealed no baseline strain differences [$F(1,186)=0.396$, n.s.] or differences between assigned drug conditions [$F(3,186)=1.970$, n.s.]. There was, however, a significant difference in baseline PPI between stress conditions [$F(2,186)=6.024$, $p<.05$] indicating that random assignment to stress conditions did not create equal differences between groups on this variable.

PPI during testing was analyzed with a three-way ANCOVA, using baseline responses as a covariate, taking into account all main effects and two-way interactions but eliminating the three-way interaction. Table 11 provides a list of these results. A marginal main effect of strain was revealed [$F(1,174)=3.434$, $p=.07$] as was a significant main effect

of drug dose [F(3,174)=2.702, p<.05] with the 20 mg/kg morphine dose decreasing PPI. No significant main effect of stress was found [F(2,174)=0.852, n.s.] and there were no significant interactions.

Because of the marginally significant main effect of strain, separate two-way ANCOVAs, with baseline PPI as a covariate, were conducted for the F-344 and Lewis rats. Table 12 provides a list of these results. In summary, the only significant finding was a main effect of dose in F-344 rats [F(3,84)=6.029, p<.05]. Tukey HSD post-hoc analysis indicated that F-344 rats receiving 20 mg/kg of morphine had significantly less PPI than did F-344 rats receiving 0 or 5 mg/kg doses (See Figure 14).

Body Temperature. Body temperature was recorded to measure physiological changes in response to stress and morphine. Figures 15 and 16 present body temperatures (degrees Centigrade) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in body temperature between the strains, stress conditions, or drugs groups, a three-way ANOVA was conducted. This analysis revealed a significant main effect of strain [F(1,186)=372.413, p<.05]; F-344 rats had significantly higher core body temperatures than did Lewis rats. There also was a significant differences in baseline body temperatures between stress conditions [F(2,186)=3.573, p<.05]. There was no significant difference between drug groups [F(3,186)=1.330, n.s.].

Body temperature during testing was analyzed with a three-way ANCOVA, using baseline temperature as a covariate, taking into account all main effects and two-way interactions. Significant main effects of strain [F(1,174)=57.076, p<.05], drug

[F(3,174)=22.670, p<.05], and stress [F(2,174)=3.036, p=.05] were found. In addition, strain interacted with dose [F(3,174)=12.389, p<.05] and a marginally significant strain x stress interaction [F(2,174)=2.541, p=.08] was revealed. Figure 17 presents the body temperatures for grouped, individually housed, and immobilized F-344 and Lewis rats collapsed across drug condition. No significant stress x drug interaction [F(6,174)=1.599, n.s.] was found.

Because of significant strain differences and a significant strain x drug dose interaction, separate two-way ANCOVAs, with baseline body temperature as a covariate, were conducted for the F-344 and Lewis rats. These results are listed in Table 13. With regard to F-344 rats, the main effect of stress was not present [F(2,84)=0.989, n.s.] although the significant main effect of drug dose [F(3,84)=10.049, p<.05] remained. In contrast, both main effects of stress [F(2,83)=3.576, p<.05] and drug dose [F(3,83)=21.869, p<.05] were significant for Lewis rats. No stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats.

To further understand the significant strain x dose interaction, Dunnett T post-hoc analyses using the 0 mg/kg dose as the control group indicated that F-344 rats showed increases in body temperature following all three morphine doses as compared to the saline group. In Lewis rats, however, a different pattern emerged with the 5 mg/kg dose increasing temperature, the 10 mg/kg dose producing no difference in body temperature as compared to the saline controls, and the 20 mg/kg dose producing a decrease in body temperature (See Figure 18).

Locomotor Activity. Locomotor activity consisted of five related but separate

measures. To insure that significant findings on any univariate analysis were not the result of chance due to the number of analyses conducted, a multivariate analysis of covariance was conducted including all five measures using baseline measures as covariates. Between-subjects factors included strain, stress, and drug dose taking into account all main effects and two-way interactions. Dependent variables included total distance, horizontal activity, vertical activity, speed, and thigmotaxis. The test statistic chosen to evaluate multivariate differences was Pillai's trace because of its high power and robustness for finding correct significance (SPSS, Inc., 1990).

The MANCOVA revealed significant main effects of strain [$F(5,160)=3.638$, $p<.05$], stress [$F(10,322)=2.453$, $p<.05$], and drug dose [$F(15,486)=13.179$, $p<.05$]. Additionally, significant strain x stress [$F(10,322)=2.286$, $p<.05$] and strain x dose [$F(15,486)=4.337$, $p<.05$] interactions were found along with a marginally significant stress x dose [$F(30,820)=1.448$, $p=.06$] interaction. Each dependent variable was then analyzed in separate analyses of covariance followed by post-hoc tests when appropriate to examine effects of strain, stress, and drug dose in relation to specific hypotheses.

Total Distance. Total distance is a measure of centimeters moved in a locomotor chamber and is used as a reflection of lethargy. Figures 19 and 20 present the total distance moved (centimeters) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in locomotion between the strains, stress conditions, or drugs groups a three-way ANOVA was conducted on these three main effects. This analysis revealed no significant differences between strains

[F(1,186)=0.785, n.s.] or stress conditions [F(2,186)=0.476, n.s.]. There was, however, a significant main effect for drug dose [F(3,186)=7.028, p<.05].

Distance traveled during testing was analyzed with a three-way ANCOVA, using baseline distance as a covariate, taking into account all main effects and two-way interactions. These results are listed in Table 14. Significant main effects of strain [F(1,173)=16.536, p<.05] and drug dose [F(3,173)=17.352, p<.05] were found. In addition, strain significantly interacted with dose [F(3,173)=4.229, p<.05]. There was no significant main effect of stress [F(2,173)=1.109, n.s.] or significant interactions with stress.

Because of significant strain differences and a significant strain x drug dose interaction, separate two-way ANCOVAs, using baseline distance traveled as a covariate, were conducted for the F-344 and Lewis rats. Table 15 provides a list of these results. The main effect of drug dose was significant for F-344 [F(3,84)=8.282, p<.05] and Lewis [F(3,82)=11.913, p<.05] rats. Similarly for F-344 and Lewis rats, the main effect of stress was not present. In addition, no stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats.

To further examine the significant strain x dose interaction, Dunnett-T post-hoc analyses using the 0 mg/kg dose as the control group were conducted. These results indicated the 10 mg/kg and 20 mg/kg doses of morphine produced significant decreases in distance traveled compared to the 0 mg/kg dose for F-344 and Lewis rats. Although the 5 mg/kg dose did not significantly differ from the 0 mg/kg dose for either strain, the data showed that the 5 mg/kg dose produced an increase in distance traveled as compared to

the 0 mg/kg dose for F-344. In contrast, the 5 mg/kg dose decreased distance traveled in Lewis rats as compared to the 0 mg/kg dose (See Figure 21). In addition, to more clearly see the strain x dose interaction, the same data are presented in Figure 22 as percent change from control (0 mg/kg).

Horizontal Activity. Horizontal activity is a measure of the number of beams along a horizontal plane an animal breaks while moving freely in a locomotor chamber. Figures 23 and 24 present the total number of beams broken for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in horizontal activity between the strains, stress conditions, or drugs groups a three-way ANOVA was conducted on these three main effects. This analysis revealed no significant differences between strains [$F(1,186)=0.212$, n.s.] or stress conditions [$F(2,186)=0.216$, n.s.]. There was, however, a significant main effect for drug dose [$F(3,186)=6.878$, $p<.05$].

Horizontal activity during testing was analyzed with a three-way ANCOVA, using baseline horizontal activity as a covariate, taking into account all main effects and two-way interactions. These results are listed in Table 16. Significant main effects of strain [$F(1,173)=13.818$, $p<.05$] and drug dose [$F(3,173)=39.326$, $p<.05$] were found. In addition, strain significantly interacted with drug dose [$F(3,173)=7.258$, $p<.05$]. There was no significant main effect of stress [$F(2,173)=0.089$, n.s.] or significant interactions with stress.

Because of significant strain differences and a significant strain x drug dose

interaction, separate two-way ANCOVAs, with baseline horizontal activity as a covariate, were conducted for the F-344 and Lewis rats. These results are listed in Table 17. The main effect of drug dose was significant for F-344 [$F(3,84)=22.471$, $p<.05$] and Lewis [$F(3,82)=15.335$, $p<.05$] rats. Similarly for F-344 and Lewis rats, the main effect of stress was not present and there were no significant interactions for either strain.

To further examine the significant strain x dose interaction, Dunnett-T post-hoc analyses using the 0 mg/kg dose as the control group were conducted. These results indicated the 10 mg/kg and 20 mg/kg doses of morphine produced significant decreases in horizontal activity compared to the 0 mg/kg dose for F-344 and Lewis rats (See Figure 25).

Vertical Activity. Vertical activity is a measure of the number of beams along a vertical plane an animal breaks while moving freely in a locomotor chamber and is indirect measure of rearing behavior. Figures 26 and 27 present the number of vertical beams broken by Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in vertical activity between the strains, stress conditions, or drug groups a three-way ANOVA was conducted on these three main effects. This analysis revealed a significant main effect of strain [$F(1,186)=5.428$, $p<.05$]; F-344 rats broke significantly more vertical beams than did Lewis rats. There was no significant differences in baseline vertical activity between stress conditions [$F(2,186)=1.017$, n.s.]. However, there were significant differences between drug groups [$F(3,186)=6.036$, $p<.05$].

Vertical activity during testing was analyzed with a three-way ANCOVA, using baseline vertical activity as a covariate, taking into account all main effects and two-way interactions. The main effect of strain [$F(1,173)=5.755$, $p<.05$] remained with F-344 rats continuing to display more vertical activity than did Lewis rats. In addition, strain significantly interacted with stress [$F(2,173)=4.860$, $p<.05$] and drug dose [$F(3,173)=4.583$, $p<.05$]. A significant main effect of drug dose [$F(3,173)=23.062$, $p<.05$] with increasing drug doses decreasing vertical activity also was found. A marginally significant main effect of stress [$F(2,173)=2.645$, $p=.07$] also was revealed with individually-housed rats breaking more vertical beams than did grouped or immobilized rats. No significant stress x drug interaction [$F(6,173)=0.496$, n.s.] was found.

Because of significant strain interactions with stress and drug dose, separate two-way ANCOVAs, with baseline vertical activity as a covariate, were conducted for F-344 and Lewis rats. These results are listed in Table 18. With regard to F-344 rats, the main effect of stress persisted [$F(2,84)=4.459$, $p<.05$] as did the main effect for drug dose [$F(3,84)=12.776$, $p<.05$]. In contrast, there was no main effect of stress for Lewis rats [$F(2,82)=1.448$, n.s.] (See Figure 28). Similar to F-344 rats, the main effect of drug was significant [$F(3,82)=18.194$, $p<.05$] for Lewis rats. No stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats.

To further examine the significant strain x dose interaction, Dunnett-T post-hoc analyses using the 0 mg/kg dose as the control group indicated that Lewis rats showed decreases in vertical activity following all three morphine doses as compared to the saline group. In F-344 rats, however, a different pattern emerged with only the 10 mg/kg and 20

mg/kg doses producing a decrease in vertical activity as compared to the saline controls (See Figure 29).

Speed. Speed is a measure of locomotive activity calculated as total distance traveled divided by the number of total seconds in motion. Figures 30 and 31 present the speed (cm/s) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in speed between the strains, stress conditions, or drug groups a three-way ANOVA was conducted. This analysis indicated that there were no significant differences in baseline speed between F-344 and Lewis rats [$F(1,186)=0.987$, n.s.] or between the randomly assigned stress groups [$F(2,186)=0.081$, n.s.]. However, there were significant differences between drug groups [$F(3,186)=9.289$, $p<.05$].

Speed during testing was analyzed with a three-way ANCOVA, using baseline speed as a covariate, taking into account all main effects and two-way interactions. Significant main effects of strain [$F(1,171)=23.960$, $p<.05$], stress [$F(2,171)=4.716$, $p<.05$], and drug [$F(3,171)=11.646$, $p<.05$] were found. In addition, strain interacted with dose [$F(3,171)=8.011$, $p<.05$] and a marginally significant strain x stress interaction [$F(2,171)=2.675$, $p=.07$] was revealed. Further, a significant stress x drug interaction [$F(6,171)=4.807$, $p<.05$] was found.

Because of significant strain differences and a significant strain x drug dose interaction, separate two-way ANCOVAs, with baseline speed as a covariate, were conducted for the F-344 and Lewis rats. A significant main effect of stress emerged for F-344 [$F(2,83)=4.033$, $p<.05$] and Lewis [$F(2,81)=4.671$, $p<.05$] rats. No one stress

condition was significantly different from any other stress condition in F-344 rats, whereas no stress grouped Lewis rats had a significantly higher speed rate than did individually housed Lewis rats (Tukey HSD) (See Figure 32). In addition, a main effect of drug was significant for F-344 [$F(3,83)=11.771$, $p<.05$] and Lewis [$F(3,81)=2.655$, $p=.05$] rats (See Figure 33). Among F-344 rats, the rats in the 20 mg/kg morphine condition had a significantly higher rate of speed than did F-344 rats in the 0, 5, or 10 mg/kg dose conditions which did not significantly differ from one another (Tukey HSD). Further, a stress x drug dose interaction was revealed for F-344 [$F(6,83)=4.048$, $p<.05$] but not for Lewis rats [$F(6,81)=1.553$, n.s.]. Tukey HSD post-hoc analyses indicated that non-stressed grouped F-344 rats had a higher speed rate than did individually housed or immobilized rats in the 20 mg/kg dose condition but that F-344 rats in the three different stress conditions did not differ from one another at the 0, 5, or 10 mg/kg dose conditions (Tukey HSD) (See Figure 30).

Thigmotaxis. Thigmotaxis is used as a measure of anxiety as measured by how much time is spent in the center of the locomotor chamber as opposed to the wall. It was calculated as the ratio of center time over margin time (seconds). A larger number is indicative of less anxiety as operationalized as more time spent in the center or away from the wall. Figures 34 and 35 present thigmotaxic behavior (center/wall time in seconds) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. To determine if there were baseline differences in degree of thigmotaxis between the strains, stress conditions, or drug groups, a three-way ANOVA was conducted. This analysis indicated that there was a

significant main effect of strain [$F(1,186)=29.593$, $p<.05$], with F-344 rats displaying less wall hugging behavior than did Lewis rats. There was no significant main effect of stress [$F(2,186)=1.043$, n.s.]. However, there was a significant main effect of drug dose [$F(3,186)=3.964$, $p<.05$].

Thigmotaxis during testing was analyzed with a three-way ANCOVA, using baseline responses as a covariate, taking into account all main effects and two-way interactions. These results are listed in Table 19. No main effect of strain [$F(1,168)=0.088$, n.s.] nor stress [$F(2,168)=1.545$, n.s.] was found. The main effect of drug dose remained significant [$F(3,168)=4.734$, $p<.05$], with wall hugging behavior decreasing with higher morphine doses (See Figure 36). Further, there was a significant strain x drug dose interaction [$F(3,168)=3.164$, $p<.05$]. There were no significant interactions with stress.

To test the *a priori* hypotheses that there would be strain differences in thigmotaxis and because of the significant strain x drug interaction, separate two-way ANCOVAs with baseline thigmotaxis as a covariate, were conducted on each strain. Table 20 provides a list of these results. In summary, the only significant finding was a main effect of drug dose in F-344 rats [$F(3,81)=5.003$, $p<.05$]. Tukey HSD post-hoc analysis indicated that F-344 rats receiving the 20 mg/kg dose of morphine showed significantly less wall hugging behavior than did F-344 rats receiving 0, 5, or 10 mg/kg of morphine.

Serum Morphine Levels

Serum morphine levels were measured to validate subjects' exposure to morphine

and to evaluate any pharmacokinetic differences as a result of strain differences or stress conditions. Figures 37 and 38 present serum morphine levels (ng/ml) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. Serum morphine levels were analyzed with a three-way ANOVA, taking into account all main effects and two-way interactions. No main effect of strain [$F(1,175)=1.300$, n.s.] nor stress [$F(2,175)=0.126$, n.s.] was found, although strain interacted with stress (See Figure 39) [$F(2,175)=3.436$, $p<.05$] and drug dose (See Figure 40) [$F(3,175)=6.255$, $p<.05$]. A main effect of drug dose [$F(3,175)=235.357$, $p<.05$] also was revealed with serum morphine levels increasing with drug dose. No significant stress x drug interaction [$F(6,175)=0.341$, n.s.] was found.

Because of significant strain interactions with stress and drug dose, separate two-way ANOVAs were conducted for the F-344 and Lewis rats. These results are listed in Table 21. With regard to F-344 rats, the main effect of stress was no longer significant [$F(2,85)=1.195$, n.s.], whereas the main effect of drug dose [$F(3,85)=153.940$, $p<.05$] persisted. In contrast, the main effect of stress for Lewis rats was marginally significant [$F(2,85)=2.331$, $p=.09$] along with a significant main effect of drug [$F(3,85)=93.504$, $p<.05$]. All drug doses significantly differed from all other drug doses for F-344 and Lewis rats (Tukey HSD). No stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats.

To further examine the strain x stress and strain x dose interactions, separate two-way ANOVAs were conducted for each drug dose. These results are listed in Table 22. There was a significant main effect of strain at the 0 mg/kg [$F(1,42)=15.133$, $p<.05$.] dose

with Lewis rats having higher morphine serum levels than did F-344 rats. In addition, at the 20 mg/kg dose, there also was a main effect of strain [$F(1,41)=8.182$, $p<.05$]. However, at the this drug dose, F-344 rats had higher serum morphine levels than did Lewis rats. These two main effects of strain at different drug doses with relative values in the opposite direction may account for the overall significant strain x dose interaction (See Figure 40). Further, there was a significant strain x stress interaction at the 10 mg/kg dose [$F(3,42)=3.991$, $p<.05$].

Brain Morphine Levels

Brain morphine levels were measured to validate subjects' exposure to morphine and to evaluate any pharmacokinetic differences in central brain levels as a result of strain differences or stress conditions. Figures 41 and 42 present brain morphine levels (ng/g) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. Brain morphine levels were analyzed with a three-way ANOVA, taking into account all main effects and two-way interactions. These results are listed in Table 23. No main effect of strain [$F(1,175)=2.166$, n.s.] or stress [$F(2,175)=1.618$, n.s.] was found. There was, however, a main effect of drug dose [$F(3,175)=378.711$, $p<.05$], with brain morphine levels significantly increasing with each successive drug dose (Tukey HSD). In addition, there was a significant strain x stress interaction [$F(2,175)=3.776$, $p<.05$] (See Figure 43). Specifically, there were progressive increases in brain morphine levels from the grouped to the individually-housed to the immobilized Lewis rats, whereas there were no differences in brain morphine levels among F-344 rats in the three different experimental conditions.

Because of the significant strain x stress interaction, separate two-way ANOVAs were conducted for F-344 and Lewis rats. These results are listed in Table 24. With regard to F-344 rats, the main effect of stress was not significant [$F(2,85)=0.210$, n.s.] whereas a significant main effect of drug dose [$F(3,85)=169.000$, $p<.05$] was found. In contrast, significant main effects of stress [$F(2,85)=6.480$, $p<.05$] and dose [$F(3,85)=223.741$, $p<.05$] were found for Lewis rats. All drug doses significantly differed from all other drug doses for F-344 and Lewis rats (Tukey HSD). No stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats.

To further examine the strain by stress interaction and main effect of dose, separate two-way ANOVAs were conducted for each drug dose. These results are listed in Table 25. There was a significant main effect of strain at the 5 mg/kg [$F(1,44)=4.147$, $p<.05$.] dose with F-344 rats having higher morphine brain levels than did Lewis rats. In addition, at the 10 mg/kg dose, there was a main effect of stress [$F(2,42)=3.669$, $p<.05$] with the hypothesized levels of increasing stress (e.g., group housing<individually housed<immobilized) showing increases in brain morphine levels. Separate one-way ANOVAs for each strain at the 10 mg/kg dose revealed a significant main effect of stress for Lewis rats [$F(2,23)=5.200$, $p<.05$] but not F-344 rats [$F(2,25)=0.884$, n.s.] at this dose. Tukey HSD post-hoc analysis indicated that no-stress Lewis rats had significantly lower brain morphine levels than did immobilized Lewis rats at the 10 mg/kg dose (See Figure 42).

Corticosterone Levels

Corticosterone (CCS) is a biochemical index of stress and was measured to

evaluate subjects' response to the different stress conditions. Figures 44 and 45 present corticosterone levels (ng/ml) for Fischer-344 and Lewis rats, respectively, separated by drug dose within the grouped, individually housed, and immobilized experimental conditions. Corticosterone levels were analyzed with a three-way ANOVA, taking into account all main effects and two-way interactions. Main effects of strain [$F(1,175)=169.647$, $p<.05$] and drug dose [$F(3,171)=92.203$, $p<.05$] were found, with F-344 having significantly higher levels than did Lewis rats and corticosterone levels increasing with morphine on board. There was, however, no main effect of stress [$F(2,175)=2.223$, n.s.]. In addition, there were significant strain x stress (See Figure 46) [$F(2,175)=4.410$, $p<.05$] and strain x dose interactions [$F(3,175)=13.797$, $p<.05$]. No stress x dose interaction [$F(6,175)=1.100$, n.s.] was found.

Because of the significant strain x stress interaction, separate two-way ANOVAs were conducted for F-344 and Lewis rats. These results are listed in Table 26. With regard to F-344 rats, the main effect of stress was marginally significant [$F(2,85)=2.703$, $p=.07$], whereas the main effect of drug dose [$F(3,85)=61.703$, $p<.05$] was significant. Significant main effects of stress [$F(2,84)=4.492$, $p<.05$] and dose [$F(3,84)=30.023$, $p<.05$] were found for Lewis rats. For F-344 rats the 5, 10, and 20 mg/kg subjects had significantly higher corticosterone levels than did subjects receiving 0 mg/kg of morphine (Tukey HSD). Similarly for Lewis rats, subjects receiving 5, 10, or 20 mg/kg had significantly higher corticosterone levels than did Lewis rats receiving 0 mg/kg morphine (Tukey HSD). In addition, however, Lewis rats receiving 20 mg/kg of morphine also had significantly higher levels of corticosterone than did Lewis rats receiving 5 mg/kg of

morphine (Tukey HSD) (See Figure 47). No stress x drug dose interaction was revealed for the F-344 or Lewis strain of rats.

To further examine the strain x stress and strain x dose interactions, separate two-way ANOVAs were conducted for each drug dose. These results are listed in Table 27. There was a significant main effect of strain at the 5 mg/kg [$F(1,42)=94.888$, $p<.05$], 10 mg/kg [$F(1,42)=134.810$, $p<.05$], and 20 mg/kg doses [$F(1,42)=205.719$, $p<.05$.], with F-344 rats having higher corticosterone levels than did Lewis rats. In addition, there was a significant strain x stress interaction [$F(2,41)=10.949$, $p<.05$] at the 20 mg/kg dose. Separate one-way ANOVAs for each strain at the 20 mg/kg dose revealed significant main effects of stress for F-344 [$F(2,20)=5.477$, $p<.05$] and Lewis rats [$F(2,20)=6.594$, $p<.05$] at this dose. Tukey HSD post-hoc analysis indicated that no-stress F-344 rats had significantly greater corticosterone levels than did individually housed F-344 rats 20 mg/kg dose (See Figure 44). In contrast, no-stress Lewis rats had significantly lower levels of corticosterone than did individually housed Lewis rats (Tukey HSD) (See Figure 45).

Confirmation of Major Hypotheses

Major Hypothesis 1. The 20 mg/kg dose of morphine significantly decreased rotarod performance in F-344 and Lewis rats in comparison to subjects receiving a saline injection confirming the hypothesis that morphine administration would decrease rotarod performance in both strain of rats.

Major Hypothesis 2. F-344 rats showed a significant decrease in rotarod performance following a 20 mg/kg injection of morphine, whereas Lewis rats showed significant performance decrements following both the 10 mg/kg and 20 mg/kg injection of morphine

confirming the hypothesis that Lewis rats would be more sensitive to the effects of morphine on rotarod performance.

Major Hypothesis 3. The hypothesis that immobilization and individual housing stressors would increase analgesia in F-344 and Lewis rats was not confirmed. In contrast, immobilization stress decreased latency to hind-paw lick in comparison to the grouped and individually housed subjects.

Major Hypothesis 4. The hypothesis that Lewis rats would exhibit more analgesia at baseline as measured by a greater latency to lick their hind-paw on a hot plate was confirmed.

Major Hypothesis 5. The hypothesis that Lewis rats would be more sensitive to the analgesic effects of morphine was not confirmed. Specifically, analgesia was increased in F-344 and Lewis rats at the same morphine doses.

Major Hypothesis 6. The hypothesis that morphine administration would affect the total distance traveled, horizontal activity, and speed of F-344 and Lewis rats dose-dependently in an inverted-U shaped function was not confirmed. Specifically, distance traveled and horizontal movement were both decreased at the 10 mg/kg and 20 mg/kg doses of morphine for F-344 and Lewis rats. Further, speed was increased only at the 20 mg/kg dose for F-344 rats alone. The 5 mg/kg dose produced no significant changes in these behaviors for both or either strain alone.

Major Hypothesis 7. The hypothesis that Lewis rats would be more sensitive to the locomotor (e.g., distance traveled, horizontal activity, and speed) effects of morphine was not confirmed. In contrast, F-344 rats alone showed a significant increase in speed at the

20 mg/kg dose which was not effective in significantly changing the locomoting speed of Lewis rats. Both strains were similarly affected at the 10 mg/kg and 20 mg/kg doses on distance traveled and horizontal activity.

Major Hypothesis 8. The hypothesis that immobilization and individual housing would sensitize F-344 and Lewis rats to the effects of morphine on some behavioral measures was not confirmed. In contrast, data suggest that any interaction that these experimental conditions did have with morphine was to diminish the effect of morphine alone. Specifically, immobilization shortened the morphine-induced increase in latency to hind-paw lick (hot plate) at the 5 mg/kg and 10 mg/kg doses. In addition, the morphine induced increase in speed at the 20 mg/kg dose for grouped subjects was diminished to saline control levels in individually housed and immobilized subjects given 20 mg/kg of morphine. It is noteworthy that both of these experimental condition by drug interactions affected F-344 rats but not Lewis rats.

Major Hypothesis 9. The hypothesis that morphine administration would produce hypothermia in F-344 and Lewis rats was not supported. Specifically, morphine increased the core body temperature of F-344 rats at the 5 mg/kg, 10 mg/kg, and 20 mg/kg doses compared with the saline control group. In contrast, the 5 mg/kg dose increased the body temperature of Lewis rats, whereas the 10 mg/kg dose had no effect, and the 20 mg/kg dose decreased body temperature of Lewis rats.

Major Hypothesis 10. The hypothesis that Lewis rats would have higher brain and serum morphine levels than would F-344 rats following acute morphine injections was not confirmed. With regard to serum morphine levels, the only significant strain difference

occurred following the 20 mg/kg morphine dose where F-344 rats had higher serum morphine levels than did Lewis rats. Similarly, F-344 rats had significantly higher brain morphine levels following the 5 mg/kg morphine dose.

Major Hypothesis 11. The hypothesis that immobilized F-344 rats would have higher corticosterone levels than would individually housed F-344 rats, whereas immobilized Lewis rats would have lower corticosterone levels than would individually housed Lewis rats was not confirmed. Specifically, there was a trend for the immobilized rats in both strains to have higher corticosterone levels in comparison to individually housed rats of their respective strain.

Confirmation of Minor Hypotheses

Minor Hypothesis 1. Morphine dose-dependently increased the latency of F-344 and Lewis rats to lick their hind paws on the hot plate test confirming Minor Hypothesis 1.

Minor Hypothesis 2. The hypothesis that Lewis rats would have higher ASR and show more PPI than would F-344 rats, regardless of stress or drug condition, was partially confirmed. Specifically, there was a trend for the Lewis rats showing more PPI on the day of testing than did F-344 rats. There were no significant differences between strains at baseline for ASR or PPI or at testing for ASR.

Minor Hypothesis 3. The hypothesis that F-344 rats would exhibit more horizontal locomotor activity (e.g., horizontal counts, distance, speed) than would Lewis rats regardless of stress or drug condition, whereas Lewis rats would exhibit more thigmotaxis than would F-344 rats was partially confirmed. Specifically, F-344 rats exhibited more horizontal activity as measured through distance traveled, horizontal counts and speed

than did Lewis rats on test days, although there were no baseline differences on any of these variables. Further, Lewis rats exhibited more thigmotaxis than did F-344 rats at baseline and this same trend held for testing although no longer statistically significant.

Minor Hypothesis 4. The 10 mg/kg and 20 mg/kg doses of morphine significantly reduced thigmotaxis in comparison to baseline and the saline control group confirming the hypothesis that morphine would decrease wall-hugging behavior.

Minor Hypothesis 5. The hypothesis that immobilized and individually housed F-344 and Lewis rats would have higher levels of corticosterone than would grouped subjects was partially confirmed. Specifically, there was a non-significant trend in the Lewis rats with the individually housed rats having higher corticosterone levels than grouped rats and the immobilized rats having higher corticosterone levels than grouped and individually housed rats. In contrast, the F-344 grouped rats had higher corticosterone levels than the individually housed or immobilized rats although this difference was not significant.

Minor Hypothesis 6. Individually housed and immobilized F-344 rats had higher corticosterone levels than did individually housed and immobilized Lewis rats confirming Minor Hypothesis 6.

Discussion

The purpose of Experiment 1 was to examine effects of group housing, individual housing, and immobilization on behavioral and physiological sensitivity to an acute morphine injection in two genetically diverse inbred strains of rats. This examination was of interest because it brought together two aspects of drug addiction that independently have been shown to influence drug use: (1) some people have a biological predisposition

to abuse drugs, and (2) an individual's environment plays a role in the initiation, maintenance, and relapse to drug-taking behavior. In addition, with regard to the environment and its effects on subsequent drug behavior, it has either been operationalized as a specific type of stressor (i.e., mild electric footshock) or a different kind of housing condition (i.e., enriched vs. deprived). By using two different kinds of housing conditions (e.g., group vs. individual) and two different types of potential stressors (e.g., individual housing and immobilization), this experiment also allows for the examination of environmental effects in its broader sense. Further, because it has been clearly established that the F-344 and Lewis rat strains differ on a number of behavioral and biological responses to opioids and stress independently, this animal model is particularly suitable to the examination of stress reactivity on morphine sensitivity. Morphine and stress sensitivity were examined on behavioral, physiological, and biological levels. In addition, many different aspects of performance were measured including motor coordination, nocioception, innate reflexive responses, sensorimotor gating, and measures of activity.

The results of Experiment 1 are summarized in Table 28. With regard to the behavioral tests, five hypotheses were confirmed, two were partially confirmed, and five were not supported. It is noteworthy that among the five hypotheses that were not confirmed, the results were opposite of what was predicted. In general, all five measures comprising the behavioral test battery (e.g., rotarod, hot plate, ASR, PPI, locomotion) were sensitive to differences to at least one of the three independent manipulations (e.g., strain, housing/stress, drug dose).

The first measure of the test battery was rotarod performance which consisted of

two 2-minute trials during which time the rat tries to balance itself on a rotating rod that reaches a maximum speed of 20 rpm. Although given a minimum of 10 practice trials, it was clear that Lewis rats did not perform as well as F-344 rats. Because rats did not receive any incentive for staying on the rod, however, it is ambiguous as to whether or not the Lewis rats could not stay on for the maximum 130 seconds or did not persist on the rod. During the initial practice/habituation trials, F-344 and Lewis rats that stayed on the rod the longest were more agitated as indexed by more audible vocalizations than were rats that fell or jumped off the rod earlier in the trial. Consequently, although the rats that stayed on the rod habituated to the task, it is possible that Lewis rats more quickly learned that is was less aversive to jump off early. This explanation could account for the extreme variability of scores for Lewis rats during baseline as compared to the scores of F-344 rats. The rotarod task was sensitive enough to pick up a significant decrease in performance for both strains receiving 20 mg/kg of morphine in comparison to rats receiving saline confirming Major Hypothesis 1. In addition, Lewis rats had a decrement in performance following the 10 mg/kg dose of morphine, indicating that Lewis rats were more sensitive to the effects of morphine on rotarod performance than were F-344 rats confirming Major Hypothesis 2.

The second behavioral measure consisted of two 90-second maximal trials on a hot plate. As hypothesized based on prior pilot data, Lewis rats exhibited less sensitivity to pain during baseline as indexed by a longer latency to lift and lick their hind-paw in comparison to F-344 rats confirming Major Hypothesis 4. This strain difference, however, did not hold during testing because of a higher sensitivity to morphine on this

behavior in F-344 compared to Lewis rats. This finding is opposite to Major Hypothesis 5 based on Suzuki, et al. (1988) who reported that Lewis rats were more sensitive to the analgesic effects of morphine. It is noteworthy, however, that Suzuki and colleagues (1988) based their reports on ED_{50} values that may be more sensitive in detecting changes in pain sensitivity at lower drug doses. Specifically, they reported an ED_{50} for Lewis rats at 3.86 mg/kg. Because the lowest dose used in the present experiment was 5.0 mg/kg, it is possible that this strain differences in sensitivity to the analgesic effects of low doses of morphine was missed and that this strain difference is reversed at higher doses as reported in the present experiment.

In the present experiment, both strains had a dose-dependent decrease in pain response confirming Minor Hypothesis 1 and validating that the morphine doses were accurate and effective. However, when latency was computed as a percent of control (saline group), it became clear that F-344 rats display a greater increase in latency to lift and lick their paw compared to Lewis rats. Specifically, at the 5 mg/kg dose, F-344 rats showed nearly a 100% increase over control, whereas Lewis rats showed half this increase. Similarly, at the 10 mg/kg dose, F-344 rats increased their latency by 200% over control, whereas Lewis rats had yet to show an increase reaching 100% over control. Both strains reached a ceiling effect at the 90 second mark at the 20 mg/kg dose.

In addition to strain differences in baseline nocioception and in response to morphine, the hot plate measure also was successful in detecting strain specific changes in pain perception in response to the different experimental conditions with and without morphine on board. Specifically, immobilized F-344 rats displayed a shorter latency to

lick and lift their hind-paw than did grouped or individually housed F-344 rats. This same pattern occurred across all drug dose conditions and was significant at the 5 and 10 mg/kg doses. The Lewis rats, in contrast, had no significant differences between experimental conditions.

Following the hot plate, subjects were exposed to acoustic stimuli to measure their startle reflex (ASR) and prepulse inhibition (PPI). Unexpectedly, there were no baseline strain differences on either measure. In partial support of Minor Hypothesis 2, however, there was a trend for Lewis rats to have more PPI than did F-344 rats during testing regardless of stress or drug condition. The ASR results are in contrast to those reported by Glowa and colleagues (1992) who found a 3-fold higher ASR in Lewis rats compared with F-344 rats. It is noteworthy, however, that there were several differences between the two paradigms including the age of the subjects, the number of test sessions, the number of trials, and the startle apparatus, all of which have been shown to alter ASR and PPI (Acri, et al., 1995; Davis, 1984; Kaltwasser, 1990). Another important difference is that ASR and PPI testing occurred in the middle of a test battery in the present experiment, whereas it occurred as one isolated event in the experiment performed by Glowa et al. (1992). Although it has been shown in mice that other measures preceding ASR testing had no carry over effect, it may be that rats are more likely to habituate to chronic handling which could then, in turn, affect their overall startle response. In addition, the animals in the present experiment were gentled for three days, given practice trials on several of the other measures prior to the beginning of the experiment, and given an habituation session in the ASR apparatus that replicated the actual baseline and test

session.

This experiment also revealed that the 20 mg/kg dose of morphine was effective in decreasing ASR and PPI compared with subjects receiving saline, 5 or 10 mg/kg of morphine. This finding is consistent with other reports indicating that lower doses of morphine (2.5-10mg/kg) did not alter ASR compared to a saline control group (Davis, 1979; Miczek, 1991). With regard to experimental housing condition, there was no overall effect, but F-344 rats appeared to be sensitive to the individually-housed condition. Specifically, although the group housed and immobilized F-344 rats did not significantly differ from each other on ASR, the individually housed F-344 rats had ASR higher than grouped or immobilized subjects with this difference being significant between the immobilized and individually housed animals. Housing condition was not an important factor on this variable for Lewis rats.

To measure a physiological response to morphine and experimental conditions, the core body temperature of the rats was taken. This measurement occurred as part of the test battery immediately following reactivity to acoustic stimuli. This measure proved to be sensitive to strain differences, morphine's biphasic effects, stress conditions, and interactions among these variables. F-344 rats had higher core body temperature than did Lewis rats. In addition, morphine also had a significant effect on body temperature, although the direction of this effect was strain specific. In particular, F-344 rats increased body temperature compared to saline levels at the 5, 10, and 20 mg/kg doses which is the normal effect of low morphine doses. In contrast, Lewis rats had a hyperthermic response at the 5 mg/kg dose, had no change from saline control levels at the 10 mg/kg dose, and

had a hypothermic response at the 20 mg/kg dose. Considering morphines biphasic dose effect in that higher doses of morphine induce hypothermia, these results suggest that Lewis rats are more sensitive to the effects of morphine on this dependent measure. Interestingly, the body temperature of Lewis rats also was affected by the experimental condition in that grouped rats had higher body temperature than did individually housed which had higher body temperature than did immobilized rats.

Locomotor activity was the last behavior to be assessed in the test battery and was comprised of five separate measures: total distance traveled, horizontal activity, vertical activity, speed, and thigmotaxis. Because correlational analyses as well as a factor loading analysis (statistics not shown) indicated that distance traveled, horizontal activity, and vertical activity are highly related variables they will be discussed as a unit. Results from speed and thigmotaxis will then be reviewed.

Although total distance is measured in centimeters and horizontal activity is measured as the number of beams broken, they gave nearly identical results, suggesting that there was little if any repetitive behavior breaking a single beam because of excessive grooming or other behavior unrelated to locomotion. Both measures indicated the F-344 rats were more active than were Lewis rats. Additionally, both strains were affected by the 10 and 20 mg/kg doses of morphine, as indexed by a decrease in locomotor behavior as compared to saline control animals. It is important to note, however, that like the effects of morphine on hot plate responding, strain differences in response to morphine on locomotor activity are not as striking until the change in behavior is expressed as a percent of control. Specifically, at the 10 and 20 mg/kg doses the distance traveled by F-344 rats

is decreased to only 15-25% of saline animals, whereas Lewis rats are still traveling at nearly 40% of control levels. Similar values are obtained with horizontal counts. These results do not support Major Hypothesis 7 that predicted Lewis rats to be more sensitive to the locomoting effects of morphine. Because Lewis rats are more sensitive to the locomotor-enhancing effects of cocaine and methamphetamine (Camp, et al., 1994), whereas F-344 rats are more sensitive to the locomotor-enhancing effects of amphetamine (George, et al., 1991) and morphine, these data suggest that the locomotor-enhancing effects of drugs are not uniform and may act at different levels or pathways. Further, these data suggest that F-344 and Lewis differ at these sites.

In addition to the strain differences and morphine effects on distance and horizontal activity, results obtained from the measure of vertical activity indicate that housing condition also is a contributing factor with F-344 rats. Specifically, individually housed F-344 rats performed more vertical movements than did grouped or immobilized F-344 rats. Similar to the effects of housing on ASR, these data suggest that housing condition may be important in regulating some aspects of activity in F-344 but not Lewis rats. With regard to morphine dose, the vertical behavior of Lewis rats was significantly depressed at the 5, 10, and 20 mg/kg doses compared to saline control animals, whereas F-344 rats showed the same decrease as before with only the 10 and 20 mg/kg doses. These data indicate that Lewis rats may be more sensitive than are F-344 rats to morphine on this particular measure of activity.

Speed was affected by the strain of rat, experimental condition, and dose of morphine. F-344 rats were again more active than Lewis rats. The particularly interesting

effects, however, occur at the 20 mg/kg dose of morphine for the F-344 rats alone. At this dose, F-344 rats show a significant increase in behavior over any other saline or drug group. This increase in behavior, however, also is dependent upon the experimental condition with the individual housing and immobilization appearing to dampen morphine's locomotor-enhancing effects. Specifically, within the group of F-344 rats receiving the 20 mg/kg dose, the grouped rats are faster than the individually-housed F-344 rats which are faster than the immobilized rats. The fact that all four drug conditions are indistinguishable from one another in the immobilized condition further supports the idea that the experimental condition is interacting with the high dose of morphine to mask the drug's speed-enhancing effects. In contrast to F-344 rats, the speed of Lewis rats was affected by housing condition alone regardless of drug condition. Specifically, the individualized Lewis rats were slower than the grouped or immobilized Lewis rats which did not differ from one another.

Thigmotaxis or wall-hugging behavior is an index of anxiety (Simon, Dupuis, & Costentin, 1994). As hypothesized, Lewis rats spent more time near the margins than in the center of the chamber than did F-344 rats at baseline. This strain difference, however, was eliminated during testing because of a decrease in wall-hugging behavior in both strains receiving a 10 or 20 mg/kg dose of morphine.

From the behavioral data in can be inferred that morphine and experimental condition altered the performance of both or either strain on a number of different actions. In order to confirm that morphine and stress levels were being manipulated, however, actual serum and brain levels as well as serum corticosterone levels were measured. These

measures provide direct information to validate that the morphine injections were accurate and to assess whether the different experimental conditions differed in their ability to cause the animals stress as indexed by higher levels of corticosterone.

Serum and brain morphine levels validated that morphine injections were successful and revealed that the different doses were accurate in creating a dose-dependent effect. In contrast to Major Hypothesis 10, however, Lewis rats did not have higher serum or brain morphine levels than did F-344 rats. In fact, where strain differences were found, F-344 rats had higher levels than did Lewis rats. Specifically, F-344 rats had higher serum morphine levels following the 20 mg/kg dose and higher brain morphine levels following the 5 mg/kg dose than did Lewis rats. Because it has been previously reported that male Lewis rats have higher plasma and brain methamphetamine and cocaine levels than do male F-344 rats 40 and 120 minutes following an acute injection of the respective drug, the current findings suggest three possibilities. First, strain differences in pharmacokinetics are drug specific; second, sex differences in pharmacokinetic processes interact with strain differences and are, therefore, not generalizable between male and female rats; and third, the experimental manipulations in the present experiment altered the metabolism or availability of the drug, thereby masking and/or reversing the expected strain differences in drug levels.

This third explanation for why Lewis rats did not have higher morphine levels than did F-344 rats is particularly interesting because the morphine levels of F-344 and Lewis rats were oppositely affected by the experimental conditions. With regard to F-344 rats, serum morphine levels were lowest in the grouped subjects and highest in the immobilized

subjects with the levels of the individually housed subjects in between. In contrast, serum morphine levels were highest in the grouped Lewis rats, and lowest in the immobilized Lewis rats with the levels of the individually housed subjects in between. These within strain differences, however, were not statistically significant.

The experimental conditions also differentially affected the brain morphine levels of F-344 and Lewis rats. Brain morphine levels did not differ between experimental conditions for F-344 rats, whereas the brain morphine levels of Lewis rats were significantly altered. Specifically, immobilized Lewis rats had the highest levels of brain morphine, whereas grouped Lewis rats had the lowest brain morphine levels. This same trend was found across all three morphine doses and reached significance at the 10 mg/kg dose. It is important to note that the experimental conditions produced opposite affects in serum and brain morphine levels of Lewis rats in that serum morphine levels were lowest and brain morphine levels were highest in the immobilized rats, whereas the opposite was true in the grouped rats.

Stressors alter the activity of the hypothalamic-pituitary-adrenal (HPA) and corticosterone release is one biochemical product from this process indicating its activation. Further, F-344 and Lewis rats differ considerably in the responsivity of their HPA axis to different stressors with F-344 rats being generally hyper-responsive and Lewis rats being hypo-responsive. The overall strain difference in corticosterone levels observed in this experiment reflect this difference. The pattern of corticosterone levels for each strain by experimental condition, however, was not as predicted.

Based on previous work using female Wistar rats (Brown & Grunberg, 1995) and

work conducted on female F-344 and Lewis rats (Sternberg, et al., 1992), it was predicted that group housing would be less stressful than individual housing for both strains and that both housing conditions would be less stressful than immobilization for F-344 rats whereas Lewis rats would be more stressed by individual housing than immobilization. The results of the corticosterone data for both strains, however, are as follow: 1) F-344: grouped ≥ immobilized ≥ individually, and 2) Lewis: grouped ≤ individual housing ≤ immobilized. Not only was the pattern of corticosterone levels not as expected, but it also was surprising that the overall stress effect was significant for Lewis rats which have lower than normal hypothalamic-pituitary-adrenal (HPA) axis functioning yet was only marginally significant for F-344 rats with generally high HPA axis functioning.

Another possible reason for the discrepancy in corticosterone data is that the morphine, which increased corticosterone levels in both strains, reached a ceiling thereby eliminating any further increase as a result of the stressor. Because the same pattern holds true when just the saline injected animals are considered, these data suggest that this explanation was not the case. In addition, the difference in corticosterone levels between the saline injected F-344 and Lewis rats is negligible with the largest difference occurring between the group housed rats. Another alternative for the small between-stress condition differences is that the injection and the test battery itself masked the effects of the experimental conditions alone. Although this is not necessarily a common phenomena, it may be that F-344 rats are too reactive and therefore have elevated corticosterone levels in response to small events and Lewis rats have such a poor functioning HPA axis that more intense events are necessary to produce elevations above a certain level. Further

research using either another no-stress control group receiving no other treatment besides the stress manipulation or taking pre- and post-corticosterone levels is necessary.

Because changes in behavior occurred between the different experimental conditions, regardless of little or no differences in corticosterone levels, these data suggest that stress *per se* may not being playing a role. In addition, there was no indication of stress sensitizing F-344 or Lewis rats to the behavioral effects of morphine. In fact, many of the changes in behavior, with and without morphine on board, did not correspond with the changes in corticosterone. However, increases in corticosterone are merely an index of stress and not the definition. Stress can alter many biological systems in conjunction with the HPA axis including the regulation of endogenous opioid peptides (Amir, Brown, & Amit, 1980), dopaminergic pathways (Kalivas & Stewart, 1991), and hepatic drug metabolism (Matamoros & Levine, 1996). The opposite changes in serum and brain morphine levels that occurred between the different experiment conditions provides evidence to support the idea that some underlying biological change is taking place.

When the behavioral, biochemical, and pharmacological data are examined in relation to one another, there does not appear to be a simple relationship. It was expected that stress, as indexed by higher corticosterone levels, would sensitize F-344 and Lewis rats to the effects of morphine on behavior. However, because the differences in corticosterone levels between the different experimental conditions were not large, stress-induced changes in morphine sensitivity were not found. Although morphine's analgesic and speed-enhancing affects were altered in F-344 rats by the experimental conditions, these changes did not coincide with differences in corticosterone, serum morphine, or

brain morphine levels. The fact that just F-344 rats were affected, however, suggests that there may be strain differences in pharmacodynamic plasticity. As with the corticosterone levels, the magnitude of change in the serum and brain morphine levels between the different experimental conditions, were not large enough to produce significant behavioral changes.

In general, F-344 rats appear to be more active than are Lewis rats and morphine in doses ranging from 5-20 mg/kg produces decreases in simple (e.g., horizontal activity) as well as more complex behaviors (e.g., prepulse inhibition) in both strains of rats. It is noteworthy, however, that the particular behaviors being influenced are strain-dependent. In addition, environmental influences are not general but also differ between strains. Specifically, F-344 rats display more behavioral differences between the environmental conditions, whereas Lewis rats show greater differences in physiological measures between the environmental manipulations. Further, the effects of morphine on behavior are altered by the environmental manipulations in F-344 rats only.

To summarize, the results of Experiment 1 suggest that responsiveness to morphine can be altered by the genotype of the animal and the environmental circumstances preceding the event. Even more importantly, these data imply that the genotype of the animal is important in determining what effect the environment (e.g., housing, stress) will have on altering morphine's pharmacological actions and availability. Based on these findings, it follows that genotypic differences also may exist in the effects of the environment on initiating morphine use. To test this hypothesis, a follow-up experiment was conducted to examine the effects of the same experimental conditions

(i.e., group housing, individual housing, immobilization) on subsequent morphine self-administration in drug naive F-344 and Lewis rats.

Experiment 2

Overview

Results from Experiment 1 suggest that environmental conditions interact with strain of rat to produce differential effects of morphine on hot-plate, speed of locomotion, and serum and brain morphine levels following an acute parenteral injection. To extend these findings, the present experiment was conducted to determine if environmental and genetic factors would influence the self-administration of morphine as well. Specifically, Experiment 2 examined the effects of group housing, individual housing, and immobilization on subsequent morphine oral self-administration (SA) in a home cage paradigm. Subjects included 30 Fischer-344 and 30 Lewis female drug naive rats. Subjects were randomly assigned to one of three experimental conditions: (1) immobilization; (2) individually housing; or (3) no-stress. The experiment was divided into seven phases: (1) baseline water consumption; (2) experimental water consumption (3) morphine (0.5 mg/ml) SA following 18-hour water access period; (4) morphine (0.25 mg/ml) SA following 18-hour water access period; (5) morphine (0.25 mg/ml) SA following 18-hour no water access period; (6) morphine (0.25 mg/ml) SA following 18-hour water access period; and (7) quinine (87.5 mg/ml for Lewis; 162.5 mg/ml for Fischer-344) SA following 18-hour water access period. The experimental design and timeline are presented in Tables 29 and 30. During the 6-hour morphine and quinine SA periods, subjects received access to the morphine or quinine solution with or without a separate water bottle in an individual cage immediately following immobilization, individual housing, or removal from their home cage. The morphine and quinine phases

of the experiment consisted of two or four no-choice consumption days (i.e., only morphine or quinine available) followed by one day of choice consumption (i.e., choice between morphine or quinine and water). At the end of the experiment, subjects were decapitated and trunk blood was collected and assayed for corticosterone.

Methods

Subjects and Non-stress Housing Conditions

Subjects included 30 Fischer-344 and 30 Lewis female rats purchased from the National Cancer Institute (Rockville, MD) at approximately 4-5 weeks of age (200 g). Animals were group housed in same-strain groups of five in polypropylene cages (47 X 37 X 19 cm) with absorbent wood chip contact bedding (Pine-Dri) and stainless steel wire-bar lids that have slotted feeders. This housing condition was chosen based on a previous finding that female rats have higher corticosterone levels, a biochemical index of stress, when individually housed than when grouped or crowded in numbers of four or greater (Brown & Grunberg, 1995). Subjects remained undisturbed, except for routine maintenance, in this condition for five weeks or until they reached two months of age to keep the age of the animals consistent with previous reports of opiate self-administration (Brown, et al., 1995a; Shaham, et al., 1992). The housing room was maintained at 23-25 °C, 50% relative humidity, and a 12-hour light/dark cycle (lights on at 0700hrs). Food (Harlan Teklad 7001) was readily accessible at all times.

Drug

Morphine-sulfate (Mallinckrodt, St. Louis, Missouri) concentrations of 0.25 mg/ml and 0.5 mg/ml and quinine-hemi sulfate concentrations of 68.5 µg/ml and 162.5 µg/ml (Sigma,

St.Louis, Missouri) were dissolved in tap water with the drug weight expressed as its salt. Analyses of both compounds revealed the purity of the morphine-sulfate to be 99% (Dave Darwin, NIDA Addiction Research Center, personal communication) and the purity of the quinine-sulfate to be 94% (Sigma). These solutions were presented to the animals in inverted 500 ml Plexiglas bottles with rubber stopper tops and nonleaking metal spouts. These concentration were based on previous work conducted in our laboratory (Shaham, 1993; Shaham et al., 1992). The quinine concentrations were piloted separately for F-344 and Lewis rats and were matched based on behavioral data to the bitter taste of the 0.25mg/ml morphine-sulfate concentration so that preference for either solution would be similar.

Stress Manipulation

Immobilization. Subjects in the restraint stress condition remained group housed in numbers of five in their home cages (47 X 37 X 19 cm) for approximately 18 hours a day. Immediately before the 6-hour self-administration period, animals were removed from their home cages and restrained in a commercially available finger-like restraint apparatus (Centrap Cage, Fisher Scientific) for a period of 20 minutes. This type of limited immobilization is a reliable stressor in various strains of rats, including Lewis and F-344 rats, as indexed by increases in corticosterone levels (Raygada, et al., 1991, Dhabhar, et al., 1993).

Individual Housing. Subjects in the individually housed condition were individually housed in their home cages (44 X 23 X 20 cm) for 18 hours a day before the 6-hour self-administration period. Previous research in our laboratory has reported that

female rats individually housed for 18 hours per day before being transferred to a second individually housed condition have higher corticosterone levels than do female rats that are grouped or crowded for 18 hours a day before being transferred to an individually housed condition (Brown & Grunberg, 1995). This procedure reliably increased corticosterone levels in the individually housed rats following 8 and 15 days of this manipulation. In addition, two days of individual housing versus group housing produces significant differences in opiate self-administration in female rats (Brown, et al., 1995a).

Corticosterone Levels

Animals were sacrificed by decapitation without anesthesia at the end of the experiment. Trunk blood was collected in non-treated tubes and were centrifuged (1500 X g) for 20 minutes at 4°C. Serum was frozen and stored at -70°C in separate microtubes until assayed for corticosterone using a standard radioimmunoassay kit (ICN Biomedicals).

Procedure

Water Consumption

Six-hour water (1000-1600 hours) consumption was evaluated prior to morphine presentation (Phases I and II). This period consisted of four days. On the first two days, all animals were taken from the group housed condition and were transferred to an individual condition for 6-hours a day at which time no stress baseline water consumption was evaluated for all subjects. On days three and four, water consumption was evaluated following each animal's respective stress condition (i.e., no stress, immobilization, 18-hour individual housing). The position of the water bottle was switched every day to avoid a

conditioned place preference. This phase served to acclimate the subjects to the experimental procedure and to collect data to use as a covariate in the analysis of morphine consumption.

Morphine Consumption

Morphine consumption was evaluated across four different cycles (Phases III - VI). Phase III consisted of two no-choice drug days and one drug/water choice day. On no-choice drug days, animals had access to a 0.5 mg/ml morphine solution only during the 6-hour (1000-1600 hrs) time period following the subject's respective experimental condition. On drug/water choice days, the subjects was presented with a choice between the 0.5 mg/ml morphine solution and water. Phase IV was identical to Phase III except that the morphine concentration was dropped to 0.25 mg/ml to increase the drug consumption behavior. During Phase V, the morphine concentration remained at 0.25 mg/ml. In addition, the number of no-choice drug days was increased to four days and animals did not have access to water for 18 hours preceding the 6-hour SA period. Phase VI was identical to Phase V except that subjects were once again given access to water during the 18 hours preceding the SA period.

Drug and water bottles were switched every day to avoid conditioned place preference. This modified procedure was based on Brown et al. (1995a) and Shaham et al. (1992). Both studies have reported that this procedure produces reliable differences between non-stressed and stressed animals using either the immobilization or housing stressor with morphine or fentanyl.

Quinine Replacement

Following Phase VI, the 0.25 mg/ml morphine-sulfate solution was replaced with a 68.5 µg/ml quinine solution for the Lewis rats and a 162.5 µg/ml quinine solution for the F-344 rats. Quinine consumption was be evaluated for one cycle consisting of four no-choice drug days and one drug/water choice day. On no-choice drug days, animals had access to the 68.5 µg/ml or 162.5 µg/ml quinine solution only during the 6-hour (1000-1600 hrs) time period following the subject's respective experimental condition. On drug/water choice days, the subjects were presented with a choice between the quinine solution and water. Drug and water bottles were side-switched every day to avoid side preference. This procedure is conducted to control for the palatability effects of the bitter morphine solution and the effects of stress on non-specific taste reactivity.

Statistical Analyses

Experiment 2 was a mixed factorial design with strain (2) and stress (3) being the between-subjects factors and phase (7) being the within-subject factor. Dependent variables included water consumption, morphine consumption, percent morphine consumption, quinine consumption, percent quinine consumption, and plasma corticosterone levels. Morphine consumption was calculated as mg/kg to adjust for body weight differences between the strains. Water and quinine consumption were analyzed in milliliters. It is important to note that on the first day that a new bottle was introduced some bottles would excessively leak because of a defective top or a poor fit. In these cases in which the cage was noted as being wet at the end of the 6-hour access period and the post-weight of the bottle was more than two standard deviations higher than the mean,

the mean value of the group was given in place of the lost data point.

Mean baseline water consumption, mean no-drug treatment water consumption, and corticosterone levels were analyzed by univariate two-way ANOVAs. Morphine consumption and quinine consumption were analyzed within phase across no-choice and choice days by two-way repeated-measure ANCOVAs with treatment water consumption as a covariate. Absolute morphine consumption and percent morphine consumption on choice days also was analyzed across phases. In addition, the correlation between plasma corticosterone levels and mean morphine consumption on choice days also was determined. Quinine consumption was compared to morphine consumption by using multiple paired t-tests to compare the percentage of quinine consumed in the presence of water to (Choice Day 6) to the percentage of morphine consumed in the presence of water (Choice Days 1-5). T-tests were two-tailed and evaluated at a Bonferroni adjusted alpha of $p<.01$.

Mauchley's test of sphericity was done before any repeated-measures analyses were conducted. In cases where the assumption of sphericity was violated, Greenhouse-Geiser epsilon was used to adjust the *F* statistic. Tukey HSD post-hoc analyses were conducted to determine differences among specific groups on specific days. All significance tests were two-tailed and evaluated at $\alpha= 0.05$ except where specified.

Results

Water Consumption (Phases I & II)

Phase I. Figure 48 presents a two-day average of six-hour water consumption during baseline for grouped F-344 and Lewis rats prior to their random assignment to

their respective treatment conditions. A two-way ANOVA revealed a significant main effect of strain [$F(1,54)=44.100$, $p<.05$] with Lewis rats drinking significantly more water than did F-344 rats. There was no main effect of stress [$F(2,54)=0.892$, n.s.] or strain x stress interaction [$F(1,54)=0.901$, n.s.], indicating that F-344 and Lewis rats were successively assigned to treatment conditions with similar means.

Phase II. Figure 49 presents a two-day average of six-hour water consumption during treatment for grouped, individually housed, and immobilized F-344 and Lewis rats. A two-way ANOVA revealed significant main effects of strain [$F(1,54)=74.907$, $p<.05$] and stress [$F(2,54)=13.535$, $p<.05$] with Lewis rats drinking more than did F-344 rats. A strain x stress interaction [$F(2,54)=4.266$, $p<.05$] also was found. Because of the significant strain difference and the strain x stress interaction, separate one-way ANOVAs were conducted for F-344 and Lewis rats. These analyses indicated a significant main effect of stress for Lewis rats [$F(2,27)=10.996$, $p<.05$] but not for F-344 rats [$F(2,27)=2.607$, $p=.09$]. Tukey HSD post-hoc analyses indicated that immobilized Lewis rats drank significantly more water than did grouped or individually housed Lewis rats ($p<.05$).

Morphine Consumption (Phases III-VI)

Phase III. Figure 50 presents the amount of morphine consumed, expressed in mg/kg to account for body weight differences, by grouped, individually housed, and immobilized F-344 and Lewis rats during Phase III. A repeated-measures analysis, using treatment water consumption as a covariate, revealed a significant within subject time x stress interaction [$F(10,265)=2.337$, $p<.05$] as well as time x strain x stress interaction

[F(10,265)=2.983, p<.05]. A main effect of time [F(5,265)=2.107, p=.07] and time x strain interaction [F(5,265)=2.046, p=.07] were marginally significant. These results indicate that there was a fluctuation in drug consumption during this phase of the experiment.

The between-subjects analysis indicated that there was no main effect of strain [F(1,53)=0.893, n.s.] or stress [F(2,53)=1.062, n.s.]. There was, however, a significant strain x stress interaction [F(2,53)=4.117, p<.05]. Separate repeated-measures analyses, using treatment water consumption as a covariate, were conducted for F-344 and Lewis rats. This analysis of F-344 rats indicated a marginally significant within subject time x stress interaction [F(10,130)=1.850, p=.06] but no main effect of time [F(5,130)=0.418, p=.09]. The between-subjects stress effect was significant [F(2,26)=4.323, p<.05] with the grouped F-344 rats generally consuming more morphine than did individually housed F-344 rats. For Lewis rats, the within-subject main effect of time [F(5,130)=2.438, p<.05] and time x stress interaction [F(10,130)=3.157, p<.05] were significant. There was no between-subjects main effect of stress [F(2,26)=2.378, n.s.]. Tukey HSD post-hoc analyses indicated that individually housed Lewis rats consumed significantly more morphine than did grouped or immobilized Lewis rats during two of the six days of this phase.

Phase IV. Figure 51 presents the amount of morphine consumed, expressed in mg/kg to account for body weight differences, by grouped, individually housed, and immobilized F-344 and Lewis rats during Phase IV. A repeated-measures analysis, using treatment water consumption as a covariate, revealed a marginally significant within

subject time x stress interaction [F(4,106)=2.023, p=.10] as well as time x strain x stress interaction [F(4,106)=2.447, p=.05]. No main effect of time [F(2,106)=0.606, n.s.] or time x strain interaction [F(2,106)=0.931, n.s.] was found. These results indicate that drug consumption was fairly consistent across these three days.

The between-subjects analysis indicated a marginally significant main effect of strain [F(1,53)=3.680, p<.05] with a trend of F-344 rats consuming more morphine than did Lewis rats. There was no main effect of stress [F(2,53)=1.003, n.s.] or strain x stress interaction [F(2,53)=2.252, n.s.]. Separate repeated-measures analyses, using treatment water consumption as a covariate, were conducted for F-344 and Lewis rats. This analysis of F-344 rats indicated that there was no within-subject effect of time [F(2,52)=0.161, n.s.] or time x stress interaction [F(4,52)=0.710, n.s.]. The between-subjects stress effect also was not significant [F(2,26)=1.719, n.s.]. For Lewis rats, the within subject main effect of time was not significant [F(2,52)=0.709, n.s.]. There was, however, a marginally significant time x stress interaction [F(4,52)=2.523, p<.05]. There was no between-subjects main effect of stress [F(2,26)=0.786, n.s.].

Phase V. Figure 52 presents the amount of morphine consumed, expressed in mg/kg to account for body weight differences, by grouped, individually housed, and immobilized F-344 and Lewis rats during Phase V. A repeated-measures analysis, using treatment water consumption as a covariate, indicated that there were no significant within-subject effects. Specifically, there was no main effect of time [F(4,212)=1.343, n.s.] or time x strain [F(4,212)=0.921, n.s.], time x stress [F(8,212)=0.130, n.s.], or time x strain x stress [F(8,212)=0.693, n.s.] interactions. These results indicate that morphine

consumption was stable over time during this phase.

The between-subjects analysis indicated that there was a main effect of strain [$F(1,53)=14.888$, $p<.05$] with F-344 rats consuming significantly more than Lewis rats. There was no main effect of stress [$F(2,53)=1.761$, n.s.] or strain x stress interaction [$F(2,53)=2.158$, n.s.]. Separate repeated-measures analyses, using treatment water consumption as a covariate, were conducted for F-344 and Lewis rats. This analysis of F-344 rats indicated a marginally significant within-subject main effect of time [$F(4,104)=0.835$, $p=.09$] but no time x stress interaction [$F(8,104)=0.835$, n.s.]. The between-subjects stress effect was significant [$F(2,26)=4.591$, $p<.05$] with the grouped F-344 rats consuming more morphine than did individually housed or immobilized F-344 rats on four of the five days of this phase. There was no within-subject main effect of time [$F(4,104)=0.381$, n.s.], time x stress interaction [$F(8,104)=0.285$, n.s.], or between-subjects main effect of stress [$F(2,26)=0.320$, n.s.] for Lewis rats.

Phase VI. Figure 53 presents the amount of morphine consumed, expressed in mg/kg to account for body weight differences, by grouped, individually housed, and immobilized F-344 and Lewis rats during Phase VI. A repeated-measures analysis, using treatment water consumption as a covariate, revealed a significant within-subject time x strain interaction [$F(4,212)=4.235$, $p<.05$] as well as a marginally significant main effect of time [$F(4,212)=2.028$, $p=.09$]. There was no time x stress [$F(8,212)=1.129$, n.s.] or time x strain x stress [$F(8,212)=1.095$, n.s.] interaction. These results indicate that there was a fluctuation in drug consumption during this phase of the experiment.

The between-subjects analysis indicated that there was a main effect of strain

[F(1,53)=5.731, p<.05] with F-344 consuming a more morphine than did Lewis rats and a significant strain x stress interaction [F(2,53)=4.345, p<.05]. There was no main effect of stress [F(2,53)=0.713, n.s.]. Separate repeated-measures analyses, using treatment water consumption as a covariate, were conducted for F-344 and Lewis rats. This analysis of F-344 rats indicated a significant within-subject time x stress interaction [F(8,104)=2.405, p<.05] but no main effect of time [F(4,104)=1.558, n.s.]. The between-subjects stress effect was significant [F(2,26)=3.705, p<.05] with the immobilized F-344 rats consuming significantly more morphine than did grouped and/or individually housed F-344 rats on three of five days of this phase (Tukey HSD). For Lewis rats, the within-subject main effect of time [F(4,104)=1.067, n.s.] and time x stress interaction [F(8,104)=0.620, n.s.] were not significant. In addition, there was no between-subjects main effect of stress [F(2,26)=0.955, n.s.].

Morphine Consumption (Choice Days)

Figure 54 presents the amount of morphine consumed, expressed in mg/kg to account for body weight differences, by grouped, individually housed, and immobilized F-344 and Lewis rats on choice days only. A repeated-measures analysis, using treatment water consumption as a covariate, revealed a significant within-subject main effect of time [F(4,212)=4.775, p<.05]. There were no time x strain, [F(4,212)=1.076, n.s.], time x stress [F(8,212)=1.019, n.s.], or time x strain x stress [F(8,212)=0.631, n.s.] interactions. The main effect of time was a result of changes in morphine consumption across the different phases as the drug concentration was lowered and water availability was manipulated.

The between-subjects analysis indicated that there was a main effect of strain [$F(1,53)=13.118$, $p<.05$] with F-344 consuming more morphine than did Lewis rats. In addition, there was a main effect of stress [$F(2,53)=3.094$, $p=.05$] as well as a significant strain x stress interaction [$F(2,53)=4.374$, $p<.05$]. Separate repeated-measures analyses, using treatment water consumption as a covariate, were conducted for F-344 and Lewis rats. This analysis of F-344 rats indicated that there was no significant within-subject main effect of time [$F(4,104)=1.821$, n.s.] or time x stress interaction [$F(8,104)=0.961$, n.s.]. The between-subjects stress effect, however, was significant [$F(2,26)=6.226$, $p<.05$] with the grouped F-344 rats generally consuming more morphine than did individually housed or immobilized F-344 rats. For Lewis rats, the within-subject main effect of time [$F(4,104)=4.372$, $p<.05$] was significant. The time x stress interaction [$F(8,104)=0.708$, n.s.] was not significant. In addition, there was no between-subjects main effect of stress [$F(2,26)=2.365$, n.s.].

Figure 55 presents percent of morphine or quinine (Choice Day 6) consumed out of total fluid consumption (morphine or quinine + water), by grouped, individually housed, and immobilized F-344 and Lewis rats on choice days only. Repeated-measure analyses were conducted on morphine choice days only (Choice Days 1-5). The *F* statistic was adjusted using Greenhouse-Geiser epsilon in some cases where the assumption of sphericity has been violated based on Mauchley's test of sphericity. A repeated-measures analysis, using treatment water consumption as a covariate, revealed a significant within-subject main effect of time [$F(4,189)=11.338$, $p<.05$] and time x strain interaction [$F(4,189)=2.760$, $p<.05$]. There were no time x stress [$F(7,189)=1.295$, n.s.] or time x

strain x stress [$F(7,189)=0.652$, n.s.] interactions. The main effect of time was a result of changes in morphine consumption across the different phases as the drug concentration was lowered and water availability was manipulated.

The between-subjects analysis indicated that there was a main effect of strain [$F(1,53)=7.650$, $p<.05$] with F-344 consuming a higher percentage of morphine in their overall fluid consumption than did Lewis rats. In addition, there was a significant main effect of stress [$F(2,53)=6.153$, $p<.05$] as well as a significant strain x stress interaction [$F(2,53)=7.052$, $p<.05$]. Separate repeated-measures analyses, using treatment water consumption as a covariate, were conducted for F-344 and Lewis rats. This analysis of F-344 rats indicated that there was a significant within-subject main effect of time [$F(4,104)=4.172$, $p<.05$] but no time x stress interaction [$F(8,104)=0.634$, n.s.]. In addition, the between-subjects stress effect was significant [$F(2,26)=6.226$, $p<.05$] with the grouped F-344 rats consuming a significantly higher percentage of morphine than did individually housed and/or immobilized F-344 rats on three out of five choice days (Tukey HSD). For Lewis rats, the within-subject main effect of time [$F(3,81.12)=10.087$, $p<.05$] was significant. The time x stress interaction [$F(6,81.12)=1.282$, n.s.] was not significant. Further, there was a between-subjects main effect of stress [$F(2,26)=4.007$, $p<.05$] with the individually housed Lewis rats generally consuming a higher percentage of morphine than did grouped or immobilized Lewis rats. Tukey HSD post-hoc analysis revealed that individually-housed Lewis rats consumed a higher percentage of morphine than did immobilized Lewis rats on the second choice day.

Quinine Replacement (Phase VII)

Figure 56 presents the amount of quinine consumed (ml) by grouped, individually housed, and immobilized F-344 and Lewis rats during Phase VII. A repeated-measures analysis, using treatment water consumption as a covariate, revealed a significant within-subject time x stress interaction [$F(8,212)=2.397$, $p<.05$] and a time x strain x stress interaction [$F(8,212)=2.443$, $p<.05$]. There was no main effect of time [$F(4,212)=1.916$, $p<.05$] or a time x strain interaction [$F(4,212)=1.847$, n.s.]. These results indicate that there was some fluctuation in quinine consumption over time during this phase of the experiment. The between-subjects analysis indicated that there was no main effect of strain [$F(1,53)=0.088$, n.s.] or stress [$F(2,53)=0.560$, n.s.] and that there was no strain x stress interaction [$F(2,53)=1.094$, n.s.].

To compare quinine consumption to morphine consumption, the last day of the quinine phase (e.g., Choice Day 6-quinine/water) was compared to each of the morphine/water choice days (e.g., Choice Days 1-5) using multiple paired t-tests (See Figure 55). These analyses revealed that, in general, F-344 and Lewis rats consumed more quinine in the presence of water than they did morphine in the presence of water. Specifically, individually housed F-344 rats had a higher percentage of quinine consumption on Choice Day 6 than morphine consumption on Choice Days 4 [$t(9)=7.400$, $p<.01$] and 5 [($t(9)=4.521$, $p<.01$) as did the immobilized F-344 rats on Days 4 [$t(9)=3.441$, $p<.01$] and 5 [$t(9)=4.344$, $p<.01$]. For Lewis rats, grouped and immobilized rats showed higher percentage on Choice Day 6 in comparison to Choice Days 3 [$t(9)=4.947$, $p<.01$; $t(9)=3.281$, $p<.01$], 4 [$t(9)=7.099$, $p<.01$; $t(9)=5.719$, $p<.01$], and 5

[t(9)=6.804, p<.01; t(9)=4.282, p<.01], respectively. Similarly, individually-housed Lewis rats had a higher percentage on Choice Day 6 than on Choice Days 4 [t(9)=7.990, p<.01] and 5 [t(9)=4.106, p<.01].

Corticosterone Levels

Corticosterone (CCS) is a biochemical index of stress and was measured to evaluate subjects' response to the different stress (e.g., housing) conditions. Figure 57 presents corticosterone levels (ng/ml) for grouped, individually housed, and immobilized Fischer-344 and Lewis rats. A two-way ANOVA revealed a significant main effect of stress [F(2,54)=72.110, p<.05] and a significant strain x stress interaction [F(2,54)=6.141, p<.05]. There was no main effect of strain [F(1,54)=0.764, n.s.]. Separate one-way ANOVAs conducted for F-344 and Lewis rats revealed significant main effects of stress for both strains [F(2,27)=52.473, p<.05; F(2,27)=21.267, p<.05], respectively. Further, Tukey HSD post-hoc analyses revealed that immobilized rats of both strains had significantly higher levels of corticosterone than did grouped or individually housed rats or their respective strains. In addition, immobilized F-344 rats had higher corticosterone levels than did immobilized Lewis rats [t (18)=3.802, p<.05].

Confirmation of Major Hypotheses

Major Hypothesis 12. The hypothesis that Lewis rats would orally self-administer more morphine than would F-344 rats was not confirmed. In contrast, F-344 rats consumed more morphine per kilogram of weight than did Lewis rats during Phases IV, V, and VI. In addition, F-344 rats consumed a higher percentage of morphine than did Lewis rats during choice days.

Major Hypothesis 13. Individually-housed Lewis rats consumed more morphine per kilogram of body weight (0.5 mg/ml concentration) during Phase III and a higher percentage of morphine in the presence of water on choice days than did grouped Lewis rats partially confirming Hypothesis 13. In contrast to Hypothesis 13, grouped F-344 rats consumed more morphine than did individually-housed F-344 rats during Phases III and V. In addition, grouped F-344 rats consumed more morphine per kilogram of body weight and in percent of choice for morphine than did individually housed F-344 rats.

Major Hypothesis 14. In partial confirmation of Major Hypothesis 14, immobilized F-344 rats consumed less morphine per kilogram of body weight (0.25 mg/ml concentration) than did grouped F-344 rats during Phase V (18-hour water deprivation) and on choice days, and consumed a lower percentage of morphine in the presence of water on choice days. In contrast to Hypothesis 14, however, immobilized F-344 rats consumed more morphine per kilogram of body weight (0.25 mg/ml concentration) than did grouped F-344 rats during Phase VI (18-hour access to water). With regard to Lewis rats, morphine consumption did not differ between the immobilized and grouped rats during any phase disconfirming the hypothesis that immobilization stress would increase morphine drug self-administration in Lewis rats compared with group-housed rats.

Major Hypothesis 15. Immobilized F-344 and Lewis rats had higher corticosterone levels than did individually housed F-344 and Lewis rats partially confirming the hypothesis that immobilized F-344 rats would have higher corticosterone levels than would individually housed F-344 rats, whereas immobilized Lewis rats would have lower corticosterone levels than would individually-housed Lewis rats.

Confirmation of Minor Hypotheses

Minor Hypothesis 7. Immobilized F-344 and Lewis rats had significantly higher corticosterone levels than did grouped F-344 and Lewis rats partially confirming Minor Hypothesis 7. Further, individually housed F-344 and Lewis rats had higher although not significantly different corticosterone levels than did grouped F-344 and Lewis rats.

Minor Hypothesis 8. Immobilized F-344 had higher corticosterone levels than did immobilized Lewis rats partially confirming Minor Hypothesis 8. In opposition, however, there were no significant differences in corticosterone levels between individually housed F-344 and Lewis rats.

Discussion

The purpose of Experiment 2 was to extend the findings of Experiment 1 by examining the effects of group housing, individual housing, and immobilization on morphine self-administration in female F-344 and Lewis rats. As with other behaviors, different environmental conditions as well as stressors have been shown to modify opioid self-administration. The home cage procedure used in this experiment was chosen because it has been reported to produce reliable and consistent differences in opioid self-administration in response to different housing conditions, stressors, and sex-differences.

Although findings were not robust, a few trends were found during different phases of the experiment. A summary of the between-subjects results are listed in Table 31. In general, the morphine self-administration of F-344 rats was more affected by the different experimental conditions than was the self-administration behavior of Lewis rats. In addition, the effect of the specific experimental condition was strain dependent.

Specifically, grouped F-344 rats tended to self-administer more than did individually housed or immobilized F-344 rats. In contrast, differences between Lewis rats were a result of the individually-housed rats consuming more than the grouped or immobilized Lewis rats. These same trends also held true for F-344 and Lewis rats when morphine self-administration was measured as a percent of overall fluid consumption on choice days which is a better index of morphine avidity. It is important to note, however, that mean morphine preferences ranged from 25-57% for F-344 rats and 16-55% for Lewis rats when water was available for the 18-hour no drug period suggesting that avidity for morphine was low for both strains.

Although previous reports indicate that Lewis rats generally consume more and show a greater preference for morphine, as well as other drugs, than do F-344 rats, the results of this experiment were in the opposite direction. Specifically, F-344 rats consumed more morphine (mg/kg) and showed a greater preference for morphine than did Lewis rats, regardless of experimental condition. The most parsimonious explanation for this reversal is that the intake of morphine was too low (e.g., 8-18 mg/kg dose over 6 hours) to produce any pharmacological effects and that strain differences in drug consumption were a result of differences in taste perception of the bitter tasting solution. Specifically, F-344 rats consumed more morphine than did Lewis rats because they experienced less aversion to the bitter taste. This explanation is partially supported by the behavioral observation that several Lewis rats would push their morphine bottle up through the slotted lid hindering their access to the drug.

In an attempt to increase drug initiation and circumvent the taste issue, several

changes were made during difference phases of the experiment. The first change was to decrease the morphine concentration from 0.50 mg/ml to 0.25 mg/ml to decrease the bitter taste of the solution. This decrease in concentration, however, did not result in an increase in amount of liquid consumed. Consequently, lower doses of morphine were ingested and the likelihood of the subjects experiencing any pharmacological effects was small.

The second change involved water-depriving subjects for 18-hours prior to receiving access to the morphine to increase their thirst drive and motivation to drink. In conjunction with this change, the number of no-choice days per cycle was increased from two to four days to increase drug exposure time. Following this manipulation, subjects increased their dose of morphine consumption to as high as 25 mg/kg for both strains. Although it is difficult to determine whether this was enough to produce pharmacological affects, there was some change in drug consumption once subjects were no longer water deprived. Specifically, immobilized F-344 rats now consumed more than the grouped or individually housed F-344 rats. No changes in morphine consumption occurred in Lewis rats. In addition, F-344 rats continued to consume more morphine than did Lewis rats.

The data from the quinine phase suggest that the strain differences in morphine consumption were influenced by more than just differences in taste perception. Because the quinine solution was matched to the taste of morphine independently for each strain, it would be expected that there would be no differences between morphine and quinine consumption for either strain if taste was the only factor. In other words, F-344 rats would consume more quinine, as they did morphine, than would Lewis rats. This,

however, did not occur. In fact, F-344 and Lewis rats consumed equal amounts of the bitter-tasting but pharmacologically inactive liquid. Further, there was a slight increase in preference for quinine during the choice day as compared with preference for the 0.25 mg/ml concentration of morphine suggesting that subjects increased their consumption to achieve the pharmacological effect previously paired with the bitter taste of morphine.

Another possible reason for the unexpected reversal of drug consumption between F-344 and Lewis rats is that different aspects of drug-taking behavior are being measured in the different experiments. Specifically, in previous experiments examining oral drug self-administration, the drug self-administration period follows a training or induction period phase that insures that all subjects involved have consumed enough of the drug to have significant pharmacological consequences. Under these induction schedules, the F-344 and Lewis rats may receive vasts amount of the drug which may be having different effects as a result of the different underlying neurochemistry of the two strains. Specifically, because Lewis rats have the brain chemistry of a drug-tolerant animal the high induction dose may be rewarding, whereas for F-344 rats the same drug dose may be aversive. Consequently these experiments may actually be measuring drug maintenance behavior following two very different drug experiences as opposed to drug initiation under similar histories.

To determine whether drug consumption was influenced by stressor experience, corticosterone levels were measured. These data indicate that F-344 and Lewis rats were similarly affected on this measure by the different experimental conditions. Specifically, individually housed rats had slightly higher corticosterone levels than did group housed

rats and immobilized rats had significantly higher corticosterone levels than did group or individually housed rats. Although immobilized F-344 rats consumed more morphine than did group or individually housed during the last morphine consumption phase, mean drug consumption on choice days was not significantly associated with corticosterone levels for F-344 ($r=-.228$) or Lewis ($r=.020$) rats. These results suggest that stress, as indexed by an increase in corticosterone, did not affect drug self-administration for either strain.

In general, F-344 rats consumed more morphine than did Lewis rats across phases of the experiment. In addition, environmental influences played a greater role in affecting morphine self-administration in F-344 than in Lewis rats. Specifically, grouped F-344 rats tended to self-administer more morphine than did individually housed or immobilized F-344 rats. In contrast, only water consumption and percent morphine preference differed between environmental conditions for Lewis rats. Further, there were no strain differences, environmental effects, or strain by environment interactions during the quinine phase. It is noteworthy, however, that the environmental conditions similarly affected the corticosterone levels of F-344 and Lewis rats. These results suggest that environmental influences play a greater role in affecting morphine initiation in F-344 rats than in Lewis rats.

GENERAL DISCUSSION

Experiment 1 and Experiment 2 examined effects of strain and environmental conditions or stress on morphine sensitivity. Experiment 1 used a test battery comprised of behavioral, physiological and biochemical measures. Experiment 2, used a home cage self-administration paradigm. The results of these experiments indicate that genotype is an important moderating variable in the relationship between the environment and drug sensitivity as well as the environment and drug self-administration. Stress, as indexed by elevated corticosterone levels, however, did not appear to be the underlying mechanism for these effects.

Experiment 1 revealed that the behavior of F-344 with and without morphine was affected by the environmental manipulation on the hot plate and two measures of locomotion (e.g., vertical activity, speed). Specifically, pain sensitivity was higher in immobilized rats (e.g., decreased latency of hot plate), vertical activity and acoustic startle responses were higher in individually housed rats, and speed was higher in grouped rats. In addition, Experiment 2 indicated that morphine preference was highest in group-housed F-344 rats. With regard to Lewis rats, body temperature and speed were affected by environmental conditions. Specifically, body temperature was lower in immobilized rats and speed was higher in grouped rats. Further, morphine preference was highest in individually-housed Lewis rats. Prepulse inhibition and rotarod performance were not influenced by environmental conditions for either strain.

Four major conclusions can be reached from these results. First, environmental influences on behavior, morphine sensitivity, and morphine self-administration are strain-

dependent. Second, environmental effects do not cause general behavioral changes but appear to be behavior specific. Third, more behaviors of F-344 rats are affected by environmental manipulations than are the behaviors of Lewis rats. Fourth, environmental influences on morphine self-administration are not obviously associated with changes in pain sensitivity, locomotor behaviors, startle reflexes, or body temperature.

The mechanisms by which any of these behavioral changes occur is still unclear. One purpose of these experiments was to examine stress levels, as indexed by increases in corticosterone, in response to the different environmental conditions. The basic assumption being that stress would alter the sensitivity to morphine (Experiment 1) which would subsequently lead to differences in morphine self-administration (Experiment 2). The corticosterone data, however, suggest that this relationship is not the mechanism underlying environmentally-altered behavioral changes, changes in morphine sensitivity, or morphine self-administration. Future experiments should consider examining corticotropin releasing hormone as another possible mechanism.

In Experiment 1, individually-housed F-344 rats had lower corticosterone levels than did immobilized F-344 rats which had lower levels than did grouped rats. In contrast, grouped Lewis rats lower corticosterone levels than did individually housed rats which had lower levels than did immobilized rats. No one group, however, was significantly different from any other group for either strain. With these data in mind, it could be that stress decreased ASR and vertical activity in F-344 rats and body temperature in Lewis rats. However, correlations between corticosterone levels and these measures for the saline control animals indicate that there was no significant relationship between stress and ASR

(r=-.006) or vertical activity (r=-.359) for F-344 rats or between stress and body temperature (r=.169) for Lewis rats.

The corticosterone data in Experiment 2 are much clearer in their differentiation between environmental conditions. Specifically, immobilized F-344 and Lewis rats had significantly higher corticosterone levels than did grouped or individually housed F-344 and Lewis rats. As in Experiment 1, however, the self-administration data do not support the hypothesis that stress altered morphine preference in either strain.

The morphine serum and brain data from Experiment 1, however, do suggest that the environment causes some biological changes that alter morphine availability peripherally as well as centrally. In addition, the effects of the environmental conditions on morphine levels also were strain-dependent. With regard to F-344 rats, grouped rats had lower serum morphine levels than did individually housed rats which had lower levels than immobilized rats. Brain morphine levels, however, were not affected by the different conditions. In contrast, grouped Lewis rats had higher serum morphine than did individually-housed rats which had higher levels than did immobilized rats. Further, the brain morphine levels were altered. Specifically, grouped Lewis rats had lower levels than did individually housed rats which had lower levels than immobilized rats.

One possible reason for these strain differences in morphine levels is that the environmental conditions may be affecting the strains at two different levels. Changes in morphine levels occur only in the serum of F-344 rats suggesting changes in enzymatic functions in the periphery such that morphine metabolism is quicker in grouped animals than in the individually housed or immobilized rats. However, with Lewis rats the picture

appears to be more complex because changes in morphine levels are found in the serum and brain and in a complementary fashion. In other words, when morphine levels are lowest in the serum they are highest in the brain. Consequently, although changes in the metabolism of morphine is probable as with F-344 rats, changes also may be occurring at a central level such that more morphine is actually reaching the brain area before being metabolized. Future research examining the functioning of hepatic enzymes in F-344 and Lewis rats under these various housing conditions would help lead to the understanding of these differences. In addition, further experiments also should consider differences in environmental influences on receptor number and function, conformational changes in functioning receptors, and drug affinity for different sites of action.

In summary, the results of the present two experiments suggest that environmental influences on behavior, morphine sensitivity, brain and serum morphine availability, and corticosterone levels are strain-dependent. Specifically, environmentally-induced changes in behavior, including drug self-administration, and morphine sensitivity are more pronounced in F-344 rats. In contrast, environmental conditions appear to influence physiological and biological changes in Lewis rats.

A continued examination of these findings should begin with a replication of the results. This is particularly important for two reasons. First, because of the complexity of the test battery and the number of behaviors examined, some of the findings are equivocal and may be spurious given the number of statistical analyses conducted and consequent likelihood of experimental wise error. Second, some of the results are in opposition to previous reports using the same strain of rats. Consequently, future studies should be kept

simple, examining a fewer number of behaviors on any given subject. This change may be useful in minimizing any confounding effects one behavior may have on another. In addition, some of the behaviors appear to be of little value in the examination of strain interactions with pharmacological and environmental manipulations and may be eliminated, while the addition of other behaviors may prove useful in creating a more theoretical framework by which to work (e.g., simple vs. complex behaviors; physiological vs behavioral). In addition because of within strain variability on several of the behavioral parameter, future experiments should assign subjects to particular treatment conditions based on baseline performance levels.

In general, the current findings suggest that the interaction between genotype and environmental conditions are important variables to consider. These results are in agreement with genetic research on personality as assessed by self-report questionnaires, twin studies, and more recent sibling adoption designs (Schmitz, Saudino, Plomin, Fulker, & Defries, 1996). Specifically, these data suggest that environmental variance is attributable to the nonshared environment rather than the shared environment. In other words, salient environmental influences in development make children growing up in the same family different, not similar.

The present results may have clinical relevance to issues of drug sensitivity and initiation. If these findings generalize to humans, then certain populations may be particularly responsive to different environmental influences significant enough to produce changes in behavioral, physiological, and biological responses to drugs. The assessment of these environmental influences, however, may be difficult to conduct and its relationship

with stress levels is still unclear.

Awareness of these moderating variables in hospital settings where drugs are prescribed for relief or in institutions where addiction is already being fought may be useful to predict or to manipulate certain outcome variables. For example, someone may begin to show different responses to their drug regimen or show a trough in their steady-state drug levels if placed in a private room as opposed to a community floor. Similarly, some people may show fluctuations in responses in their normal drug regimen following an acute but intrusive procedure. Not only are these changes important to monitor for the patients immediate comfort but also to avoid circumstances of physical dependence and the initiation of drug self-administration.

In order to better understand the interaction between individual differences and environmental influences on drug responsivity, future research is necessary. Through the use of quantitative behavioral genetics, gene mapping techniques, and multilevel assessment test batteries, it may be possible to eventually identify the genetic markers that influence biobehavioral responses to environmental conditions, drugs, and their interactions.

TABLES

Table 1. Experiment 1. Introduction: Summary of Behavioral Differences between Fischer-344 and Lewis Rats

Behavioral

- Lewis > Fischer-344
  - acoustic startle response
  - activity in periphery of open-field
  - thigmotaxis
  - preference for drugs of abuse (self-administration)
  - sensitivity to acute analgesic effects of morphine
  - sensitivity to locomotor-enhancing effects of cocaine and methamphetamine
  - sensitization of locomotor effects of repeated cocaine and methamphetamine injections
  - cocaine conditioned place preference
  - behavioral opioid abstinence syndrome (e.g., number of wet-dog shakes, diarrhea episodes, abnormal posture, weight loss)
  - withdrawal from pentobarbital
  - stress-induced swimming decrement

- Fischer-344 > Lewis
  - locomotor activity
  - sensitivity to lethal effects of cocaine
  - sensitivity to locomotor-enhancing effects of amphetamine
  - withdrawal from ethanol, barbital, diazepam
  - stress-induced defecation and grooming

Table 2. Experiment 1. Introduction: Summary of Physiological, Neurochemical, and Biochemical Differences between Fischer-344 and Lewis Rats

Physiological

- Lewis > Fischer-344
  - rate of morphine-induced stupor
  - plasma and brain levels of stimulant drugs following acute injection

- Fischer-344 > Lewis
  - electrophysiological opioid abstinence (e.g., percent increase in EEG peak frequency, decrease in total power)
  - tolerance to morphine-induced increase in latency to slow-wave sleep

Neurochemical

- Lewis > Fischer-344
  - drug-naive levels of tyrosine hydroxylase in VTA and four other phosphoproteins in mesolimbic dopamine system
  - morphine-induced levels of adenylate cyclase and cAMP dependent protein kinase in the Nac and LC
  - enhancement of extracellular dopamine in ventral striatum in response to stimulants

- Fischer-344 > Lewis
  - drug-naive levels of three neurofilament proteins in mesolimbic dopamine system
  - levels of basal extracellular dopamine metabolites DOPAC and HVA in Nac
  - morphine-induced levels of tyrosine hydroxylase in Nac
  - morphine-induced levels of $G_{i\alpha}$ and G_{β} in Nac and LC
  - morphine-induced increases in enzyme immunoreactivity in VTA and adenylate cyclase and cyclic-AMP dependent protein kinase in Nac
  - morphine-induced decrease in neurofilament protein levels in VTA
  - cocaine-induced peak dopamine elevation with faster return to basal levels

Biochemical

- Lewis > Fischer-344
  - total basal CRH content per hypothalamus

- Fischer-344 > Lewis
  - biosynthesis or release of corticotropin releasing hormone
  - responsive in adrenocorticotropic hormone and corticosterone responses to a variety of stressors
  - stress-induced hypothalamic paraventricular CRH mRNA expression in response to restraint
  - corticosteroid-binding globulin in plasma, spleen, and thymus

Table 3. Experiment 1. Methods: Experimental Design

Strain (2)	X Stress Condition (3)	X Drug Dose (4)
Lewis (N=96)	No stress (n=32)	0.0, 5.0,10.0, & 20.0 mg/kg (n=8 per dose)
	Immobilization (n=32)	0.0, 5.0,10.0, & 20.0 mg/kg (n=8 per dose)
	Individual Housing (n=32)	0.0, 5.0,10.0, & 20.0 mg/kg (n=8 per dose)
Fischer-344 (N=96)	No stress (n=32)	0.0, 5.0,10.0, & 20.0 mg/kg (n=8 per dose)
	Immobilization (n=32)	0.0, 5.0,10.0, & 20.0 mg/kg (n=8 per dose)
	Individual Housing (n=32)	0.0, 5.0,10.0, & 20.0 mg/kg (n=8 per dose)

Table 4. Experiment 1. Methods: Ordering and Timing of Injections, Test Battery, and Corticosterone Assessment on Baseline and Testing Days

Time	Group	Procedure
(1140)*	A	Immobilize appropriate animals
1150	B	Home room
(1200)	A	Injection
1010 (1210)	A	Rotarod (2 2-minute trials)
1015 (1215)	A	Hot Plate (2 90-second trials)
1025 (1225)	A	ASR/PPI (1 10-minute session)
1035 (1235)	A	Body Temperature (1 measure)
1040 (1240)	A	Locomotion (1 30-minute session)
(1245)	B	Injection
1050 (1255)	B	Rotarod (2 2-minute trials)
1055 (1300)	B	Hot Plate (2 90-second trials)
1105 (1310)	B	ASR/PPI (1 10-minute session)
1110	A	Home room
1115 (1320)	B	Body Temperature (1 measure)
1120 (1325)	B	Locomotion (1 30-minute session)
(1330)	A	Sacrifice
(1415)	B	Sacrifice

* Times designated in parentheses refer to afternoon A/B group.

Table 5. Experiment 1. Methods: Experimental Timeline

Wk	Arrival Timeline	Experimental Timeline
1	Group 1: 24 Lewis and 24 Fischer-344	Group 1: House in same-strain groups of four
2	Group 2: 24 Lewis and 24 Fischer-344	Group 2: House in same-strain groups of four
3	Group 3: 24 Lewis and 24 Fischer-344	Group 3: House in same-strain groups of four
4	Group 4: 24 Lewis and 24 Fischer-344	Group 1: Acclimate to test battery Group 4: House in same-strain groups of four
5		Group 1: Conduct baseline and experimental protocol Group 2: Acclimate to test battery
6		Group 2: Conduct baseline and experimental protocol Group 3: Acclimate to test battery
7		Group 3: Conduct baseline and experimental protocol Group 4: Acclimate to test battery
8		Group 4: Conduct baseline and experimental protocol

Table 6. Experiment 1. Results: Listing of three-way ANCOVA results on rotarod performance during testing.

Strain	F(1, 174)=73.201, p<.05
Stress	F(2, 174)=0.396, n.s.
Drug Dose	F(3, 174)=19.784, p<.05
Strain X Stress	F(2,174)=2.324, p=.10
Strain X Drug Dose	F(3,174)=1.407, n.s.
Stress X Drug Dose	F(6,174)=0.738, n.s.

Table 7. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on rotarod performance.

	Stress	Dose	Stress X Dose
F-344	F(2,85)=1.678, n.s.	F(3,85)=12.165, p<.05	F(6,85)=0.215, n.s.
Lewis	F(2,85)=1.053, n.s.	F(3,85)=6.760, p<.05	F(6,85)=1.226, n.s.

Table 8. Experiment 1. Results: Listing of two-way ANCOVA results at each drug dose on hot plate latency.

	Strain	Stress	Strain X Stress
0 mg/ml	F(1,41)=6.588, p<.05	F(2,41)=0.800, n.s.	F(2,41)=3.425, p<.05
5 mg/ml	F(1,43)=2.733, p=0.11	F(2,43)=1.574, n.s.	F(2,43)=3.376, p<.05
10 mg/ml	F(1,41)=8.044, p<.05	F(2,41)=2.744, p=0.08	F(2,41)=6.961, p<.05
20 mg/ml	F(1,40)=4.488, p<.05	F(2,40)=3.383, p<.05	F(2,40)=2.585, p=0.09

Table 9. Experiment 1. Results: Listing of three-way ANCOVA results on acoustic startle response during testing.

Strain	F(1, 174)=2.428, n.s.
Stress	F(2, 174)=1.911, n.s.
Drug Dose	F(3, 174)=3.051, p<.05
Strain X Stress	F(2,174)=1.383, n.s.
Strain X Drug Dose	F(3,174)=0.152, n.s.
Stress X Drug Dose	F(6,174)=0.280, n.s.

Table 10. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on acoustic startle response.

	Stress	Dose	Stress X Dose
F-344	F(2,84)=4.387, p<.05	F(3,84)=1.854, n.s.	F(6,84)=0.660, n.s.
Lewis	F(2,83)=0.709, n.s.	F(3,83)=1.454, n.s.	F(6,83)=0.782, n.s.

Table 11. Experiment 1. Results: Listing of three-way ANCOVA results on prepulse inhibition during testing.

Strain	F(1, 174)=3.434, p=.07
Stress	F(2, 174)=0.852, n.s.
Drug Dose	F(3, 174)=2.702, p<.05
Strain X Stress	F(2,174)=0.464, n.s.
Strain X Drug Dose	F(3,174)=1.735, n.s.
Stress X Drug Dose	F(6,174)=0.536, n.s.

Table 12. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on prepulse inhibition.

	Stress	Dose	Stress X Dose
F-344	F(2,84)=0.917, n.s.	F(3,84)=6.029, p<.05	F(6,84)=0.987, n.s.
Lewis	F(2,83)=0.472, n.s.	F(3,83)=1.275, n.s.	F(6,83)=0.378, n.s.

Table 13. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on body temperature.

	Stress	Dose	Stress X Dose
F-344	F(2,84)=0.989, n.s.	F(3,84)=10.049, p<.05	F(6,84)=0.999, n.s.
Lewis	F(2,83)=3.576, p<.05	F(3,83)=21.869, p<.05	F(6,83)=1.758, n.s.

Table 14. Experiment 1. Results: Listing of three-way ANCOVA results on total distance traveled during testing.

Strain	F(1, 173)=16.536, p<.05
Stress	F(2, 173)=1.109, n.s.
Drug Dose	F(3, 173)=17.352, p<.05
Strain X Stress	F(2,173)=0.367, n.s.
Strain X Drug Dose	F(3,173)=4.229, p<.05
Stress X Drug Dose	F(6,173)=0.612, n.s.

Table 15. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on total distance traveled.

	Stress	Dose	Stress X Dose
F-344	F(2,84)=0.417, n.s.	F(3,84)=8.282, p<.05	F(6,84)=0.818, n.s.
Lewis	F(2,82)=1.401, n.s.	F(3,82)=11.913, p<.05	F(6,82)=0.526, n.s.

Table 16. Experiment 1. Results: Listing of three-way ANCOVA results on total horizontal activity during testing.

Strain	F(1, 173)=13.818, p<.05
Stress	F(2, 173)=0.089, n.s.
Drug Dose	F(3, 173)=39.326, p<.05
Strain X Stress	F(2,173)=0.585, n.s.
Strain X Drug Dose	F(3,173)=7.258, p<.05
Stress X Drug Dose	F(6,173)=0.511, n.s.

Table 17. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on horizontal activity.

	Stress	Dose	Stress X Dose
F-344	F(2,84)=0.190, n.s.	F(3,84)=22.471, p<.05	F(6,84)=0.825, n.s.
Lewis	F(2,82)=0.748, n.s.	F(3,82)=15.335, p<.05	F(6,82)=0.615, n.s.

Table 18. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on vertical activity.

	Stress	Dose	Stress X Dose
F-344	F(2,84)=4.459, p<.05	F(3,84)=12.776, p<.05	F(6,84)=0.945, n.s.
Lewis	F(2,82)=1.448, n.s.	F(3,82)=18.194, p<.05	F(6,82)=1.038, n.s.

Table 19. Experiment 1. Results: Listing of three-way ANCOVA results on thigmotaxis during testing.

Strain	F(1, 168)=0.088, n.s.
Stress	F(2, 168)=1.545, n.s.
Drug Dose	F(3, 168)=4.734, p<.05
Strain X Stress	F(2,168)=0.792, n.s.
Strain X Drug Dose	F(3,168)=3.164, p<.05
Stress X Drug Dose	F(6,168)=0.869, n.s.

Table 20. Experiment 1. Results: Listing of two-way ANCOVA results for each strain on measure of thigmotaxis.

	Stress	Dose	Stress X Dose
F-344	F(2,81)=1.308, n.s.	F(3,81)=5.003, p<.05	F(6,81)=1.017, n.s.
Lewis	F(2,80)=1.095, n.s.	F(3,80)=1.364, n.s.	F(6,80)=0.681, n.s.

Table 21. Experiment 1. Results: Listing of two-way ANOVA results for each strain on serum morphine levels.

	Stress	Dose	Stress X Dose
F-344	F(2,85)=1.195, n.s.	F(3,85)=153.940, p<.05	F(6,85)=0.645, n.s.
Lewis	F(2,85)=2.331, p=.09	F(3,85)=93.504, p<.05	F(6,85)=1.111, n.s.

Table 22. Experiment 1. Results: Listing of two-way ANOVA results at each drug dose on serum morphine levels.

	Strain	Stress	Strain X Stress
0 mg/ml	F(1,42)=15.133, p<.05	F(2,42)=0.174, n.s.	F(2,42)=0.620, n.s.
5 mg/ml	F(1,44)=0.013, n.s.	F(2,44)=0.928, n.s.	F(2,44)=0.092, n.s.
10 mg/ml	F(1,42)=2.076, n.s.	F(2,42)=0.503, n.s.	F(2,42)=3.991, p<.05
20 mg/ml	F(1,41)=8.182, p<.05	F(2,41)=0.017, n.s.	F(2,41)=0.674, n.s.

Table 23. Experiment 1. Results: Listing of three-way ANOVA results on brain morphine levels.

Strain	F(1,175)=2.166, n.s.
Stress	F(2,175)=1.618, n.s.
Drug Dose	F(3,175)=378.711, p<.05
Strain X Stress	F(2,175)=3.776, p<.05
Strain X Drug Dose	F(3,175)=0.750, n.s.
Stress X Drug Dose	F(6,175)=0.622, n.s.

Table 24. Experiment 1. Results: Listing of two-way ANOVA results for each strain on brain morphine levels.

	Stress	Dose	Stress X Dose
F-344	F(2,85)=0.210, n.s.	F(3,85)=169.000, p<.05	F(6,85)=0.700, n.s.
Lewis	F(2,85)=6.480, p<.05	F(3,85)=223.741, p<.05	F(6,85)=1.219, n.s.

Table 25. Experiment 1. Results: Listing of two-way ANOVA results at each drug dose on brain morphine levels.

	Strain	Stress	Strain X Stress
0 mg/ml	F(1,42)=0.490, n.s.	F(2,42)=0.150, n.s.	F(2,42)=0.768, n.s.
5 mg/ml	F(1,44)=4.147, p<.05	F(2,44)=2.642, p=0.08	F(2,44)=1.821, n.s.
10 mg/ml	F(1,42)=0.029, n.s.	F(2,42)=3.669, p<.05	F(2,42)=2.720, p=0.08
20 mg/ml	F(1,41)=1.158, n.s.	F(2,41)=0.008, n.s.	F(2,41)=1.554, n.s.

Table 26. Experiment 1. Results: Listing of two-way ANOVA results for each strain on corticosterone levels.

	Stress	Dose	Stress X Dose
F-344	F(2,85)=2.703, p=.07	F(3,85)=61.703, p<.05	F(6,85)=0.814, n.s.
Lewis	F(2,84)=4.492, p<.05	F(3,84)=30.023, p<.05	F(6,84)=0.943, n.s.

Table 27. Experiment 1. Results: Listing of two-way ANOVA results at each drug dose on corticosterone levels.

	Strain	Stress	Strain X Stress
0 mg/ml	F(1,42)=0.359, n.s.	F(2,42)=1.250, n.s.	F(2,42)=0.178, n.s.
5 mg/ml	F(1,44)=94.888, p<.05	F(2,44)=0.985, n.s.	F(2,44)=0.604, n.s.
10 mg/ml	F(1,42)=134.810, p<.05	F(2,42)=2.601, p=0.09	F(2,42)=3.103,p=0.06
20 mg/ml	F(1,41)=205.719, p<.05	F(2,41)=0.655, n.s.	F(2,41)=10.949,p<.05

Table 28. Experiment 1. Discussion: Summary of Results

Strain Differences:

Fischer-344 > Lewis	*Lewis > Fischer-344*
-rotarod performance	-thigmotaxis
-pain sensitivity	
-body temperature	
-distance/horizontal activity/vertical activity	
-speed	
-corticosterone levels	

Morphine Effects:

Decrease	*Increase*	*Biphasic*
-rotarod performance	-speed	-body temperature
-pain sensitivity	-serum morphine levels	
-ASR/PPI	-brain morphine levels	
-distance/horizontal activity/vertical activity	-corticosterone levels	
-thigmotaxis		

Strain Differences in Morphine Sensitivity:

Fischer-344 > Lewis	*Lewis > Fischer-344*
-analgesia	-rotarod performance decrement
-decrease in distance	-hypothermia
-decrease in PPI	-decrease in vertical activity

Strain Differences in Environmental Influences:

Fischer-344	*Lewis*
-pain sensitivity (IM>GH,IH)	-body temp (GH>IH>IM)
-ASR (IH>GH, IM)	-speed (IH<GH,IM)
-vertical activity (IH>GH,IM)	-corticosterone (IM>IH,GH)
-serum morphine levels (IM>IH>GH)	-serum morphine levels (GH>IH>IM)
	-brain morphine levels (IM>IH>GH)

Strain Differences in Environmental Influences on Morphine Sensitivity

Fischer-344	*Lewis*
-Speed: 20 mg/kg enhancement in GH dampened in IH & IM	-none
-Analgesia: 5 &10 mg/kg analgesia in GH & IH ⇓ in IM	

Table 29. Experiment 2. Methods: Experimental Design

Strain (2)	**X**	**Condition (3)**
Lewis (N=30)		No stress (N=10)
		Immobilization (N=10)
		Individual Housing (N=10)
Fischer-344 (N=30)		No stress (N=10)
		Immobilization (N=10)
		Individual Housing (N=10)

Table 30. Experiment 2. Methods: Experimental Timeline

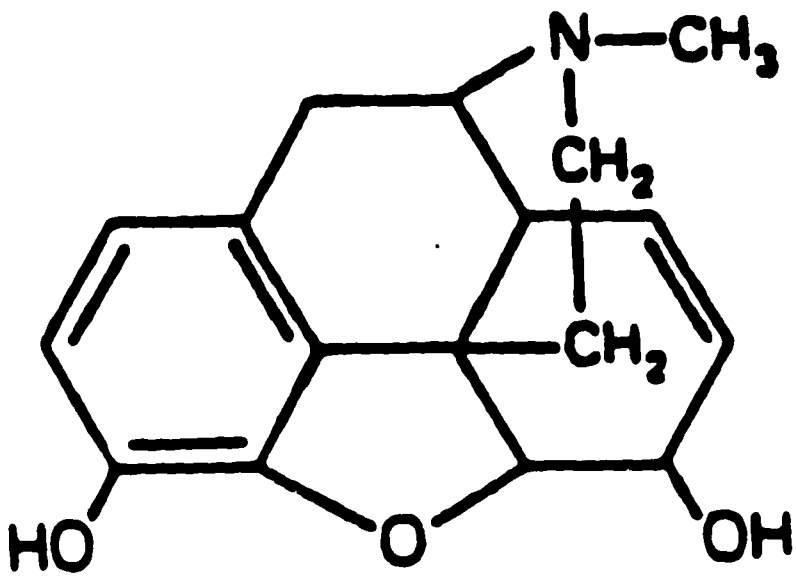
Phase	Conditions	Days
Water Consumption		
I	all subjects group housed	2
II	subjects grouped, individually housed, or immobilized	2
Morphine Consumption		
III	0.5 mg/ml concentration	two 3-day cycles
IV	0.25 mg/ml concentration	one 3-day cycle
V	0.25 mg/ml concentration following 18-hour water deprivation	one 5-day cycle
VI	0.25 mg/ml concentration	one 5-day cycle
Quinine Replacement		
VII	taste matched to 0.25 mg/ml morphine concentration for each strain 68.5 µg/ml -- Fischer-344 162.5 µg/ml -- Lewis	one 5-day cycle

Table 31. Experiment 2. Discussion: Summary of Results

Phase	Conditions	Between-Subjects Effects
Water Consumption		
I	all subjects group housed	L > F
II	subjects grouped, individually housed, or immobilized	L > F; L: IM > GH,IH
Morphine Consumption		
III	0.5 mg/ml concentration	F: GH > IH,IM
IV	0.25 mg/ml concentration	F > L
V	0.25 mg/ml concentration following 18-hour water deprivation	F > L F: GH > IH,IM
VI	0.25 mg/ml concentration	F > L F: IM > GH,IH
Choice Days	mg/kg	F > L F: GH > IH,IM
Choice Days	percent morphine consumption	F > L F: GH > IH,IM L: IH > GH, IM
Quinine Replacement		
VII	taste matched to 0.25 mg/ml morphine concentration for each strain 68.5 µg/ml -- Fischer-344 162.5 µg/ml -- Lewis	no differences

FIGURES

Figure 1. Chemical structure of morphine



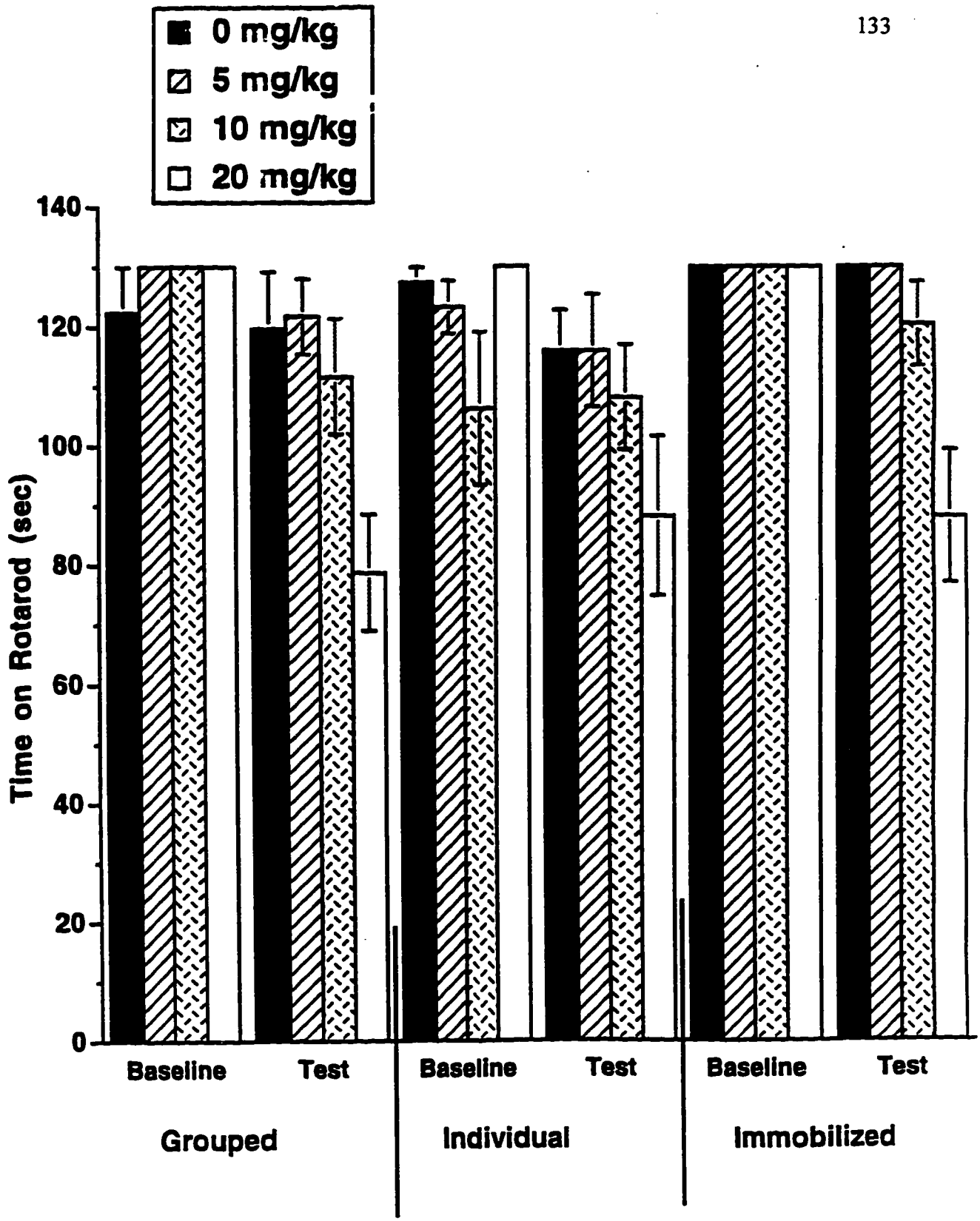


Figure 2. Experiment 1. Amount of time on rotating rod for F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

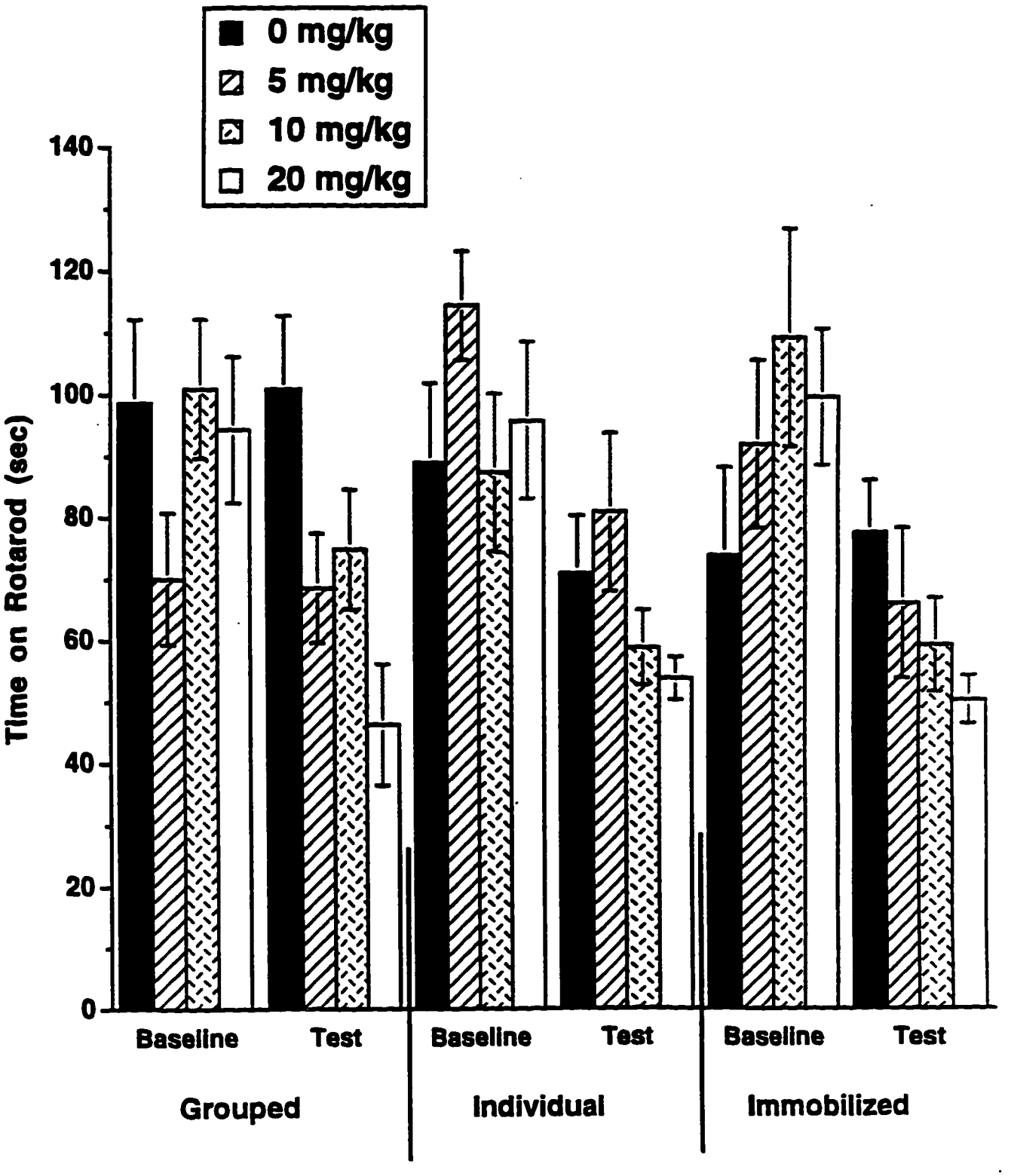
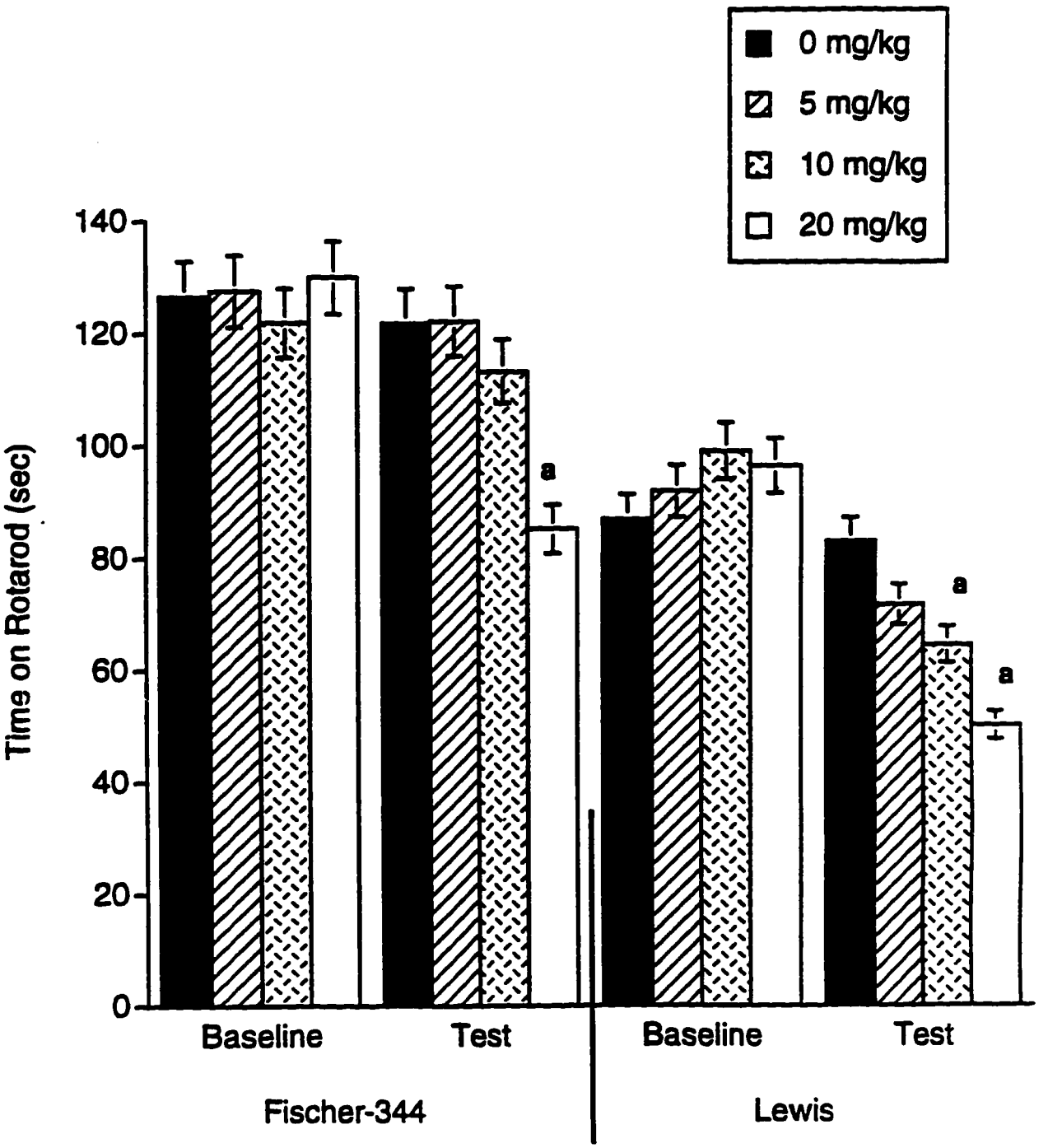


Figure 3. Experiment 1. Amount of time on rotating rod for Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from 0 mg/kg dose group on test day for respective strain (Dunnett, $p<.05$).

Figure 4. Experiment 1. Amount of time on rotating rod for F-344 and Lewis rats during baseline and testing separated by drug dose (means and standard errors).

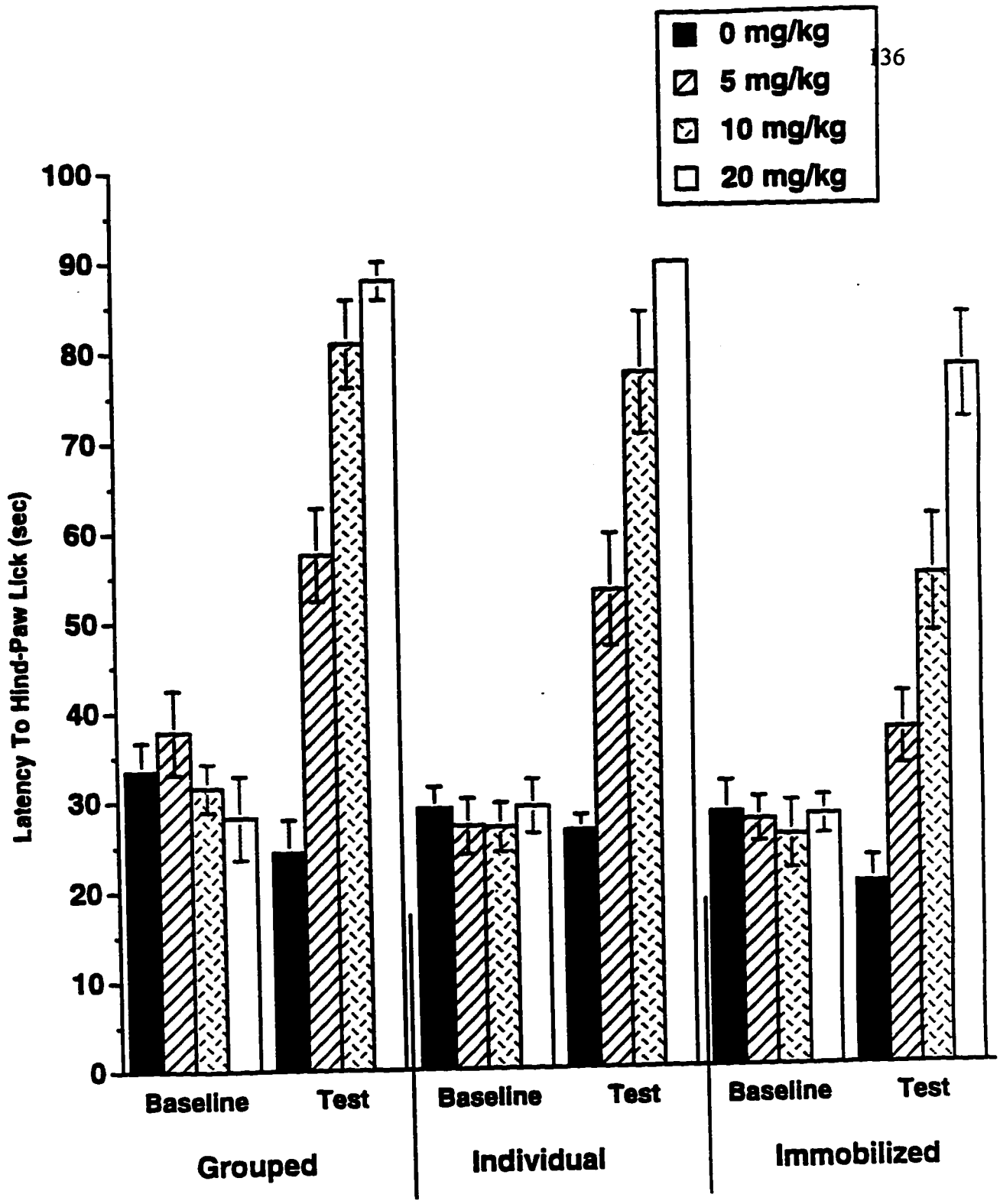


Figure 5. Experiment 1. Latency to hind-paw lick on hotplate for F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

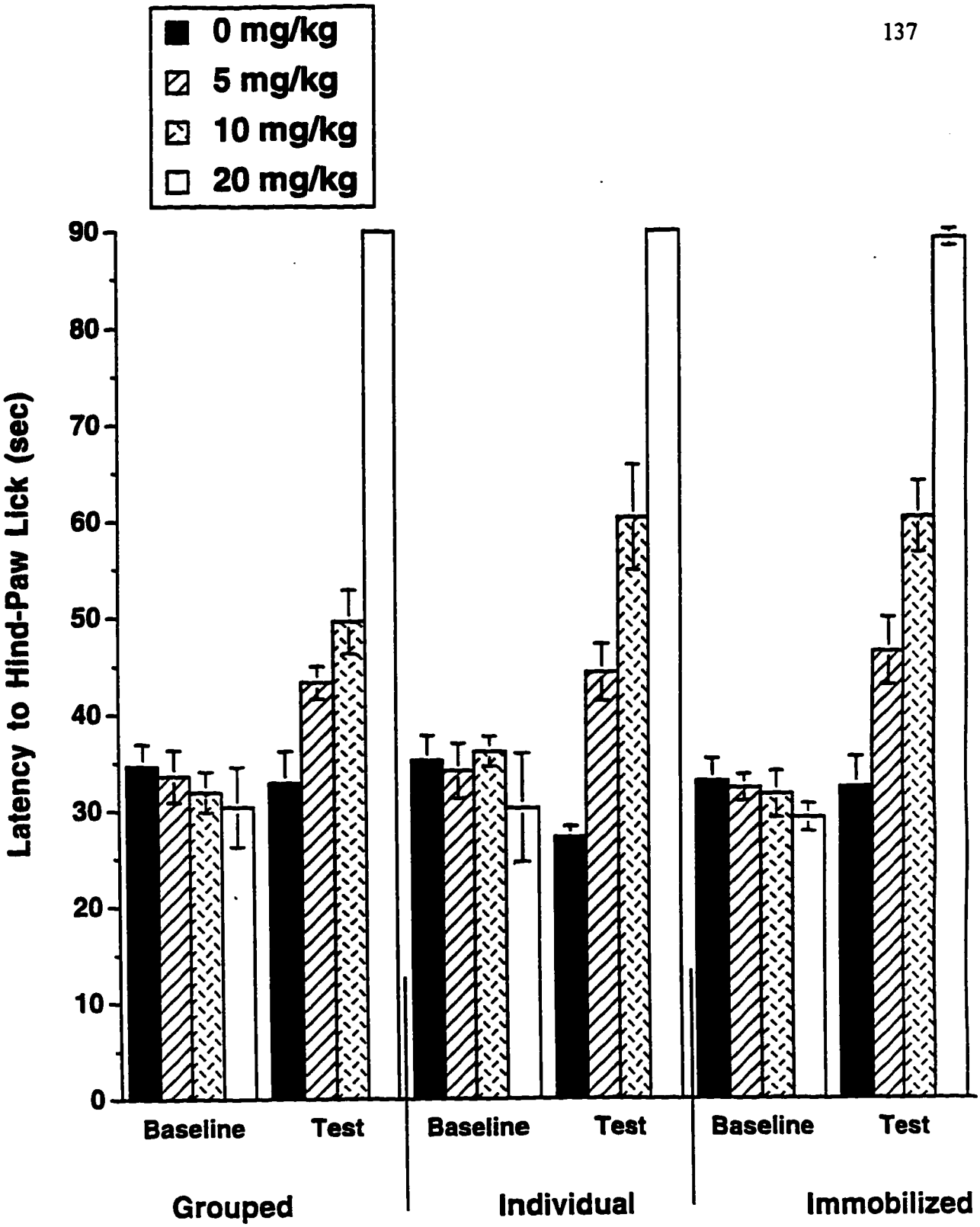


Figure 6. Experiment 1. Latency to hind-paw lick on hotplate for Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

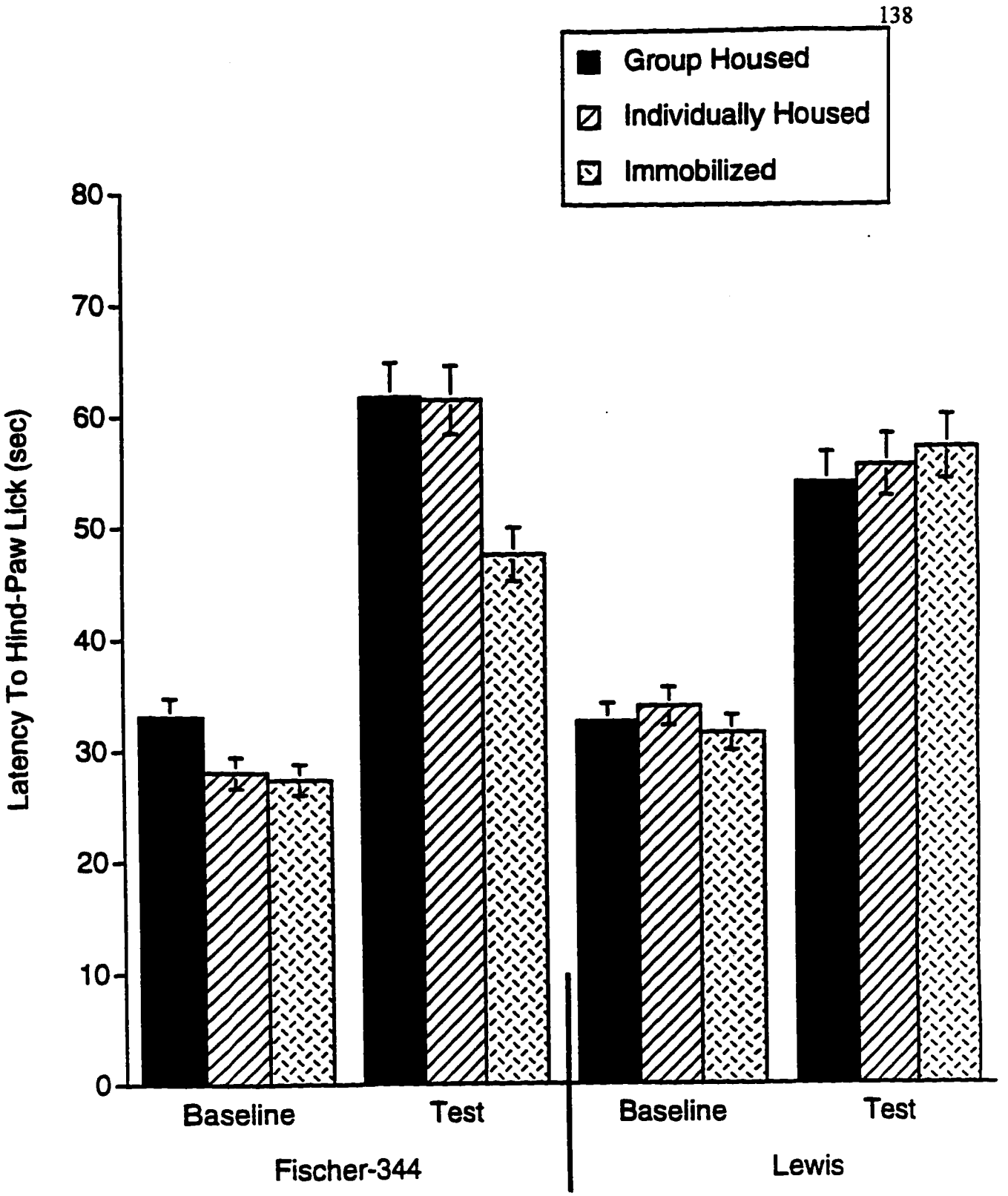


Figure 7. Experiment 1. Latency to hind-paw lick on hotplate for F-344 and Lewis rats during baseline and testing separated by experimental condition (means and standard errors)

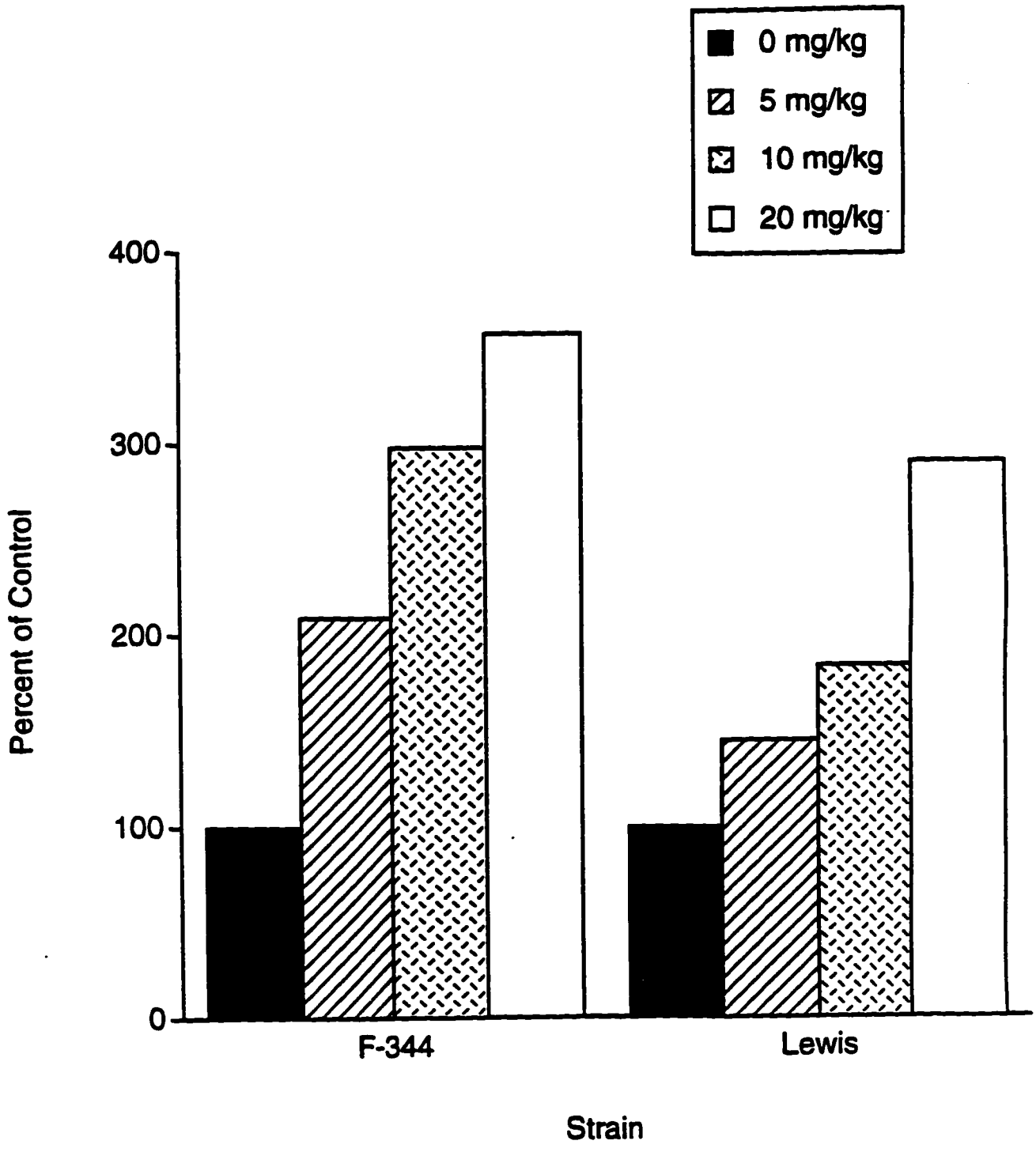


Figure 8. Experiment 1. Latency to hind-paw lick on hotplate presented as percent of control (0 mg/kg) for F-344 and Lewis rats during testing separated by drug dose.

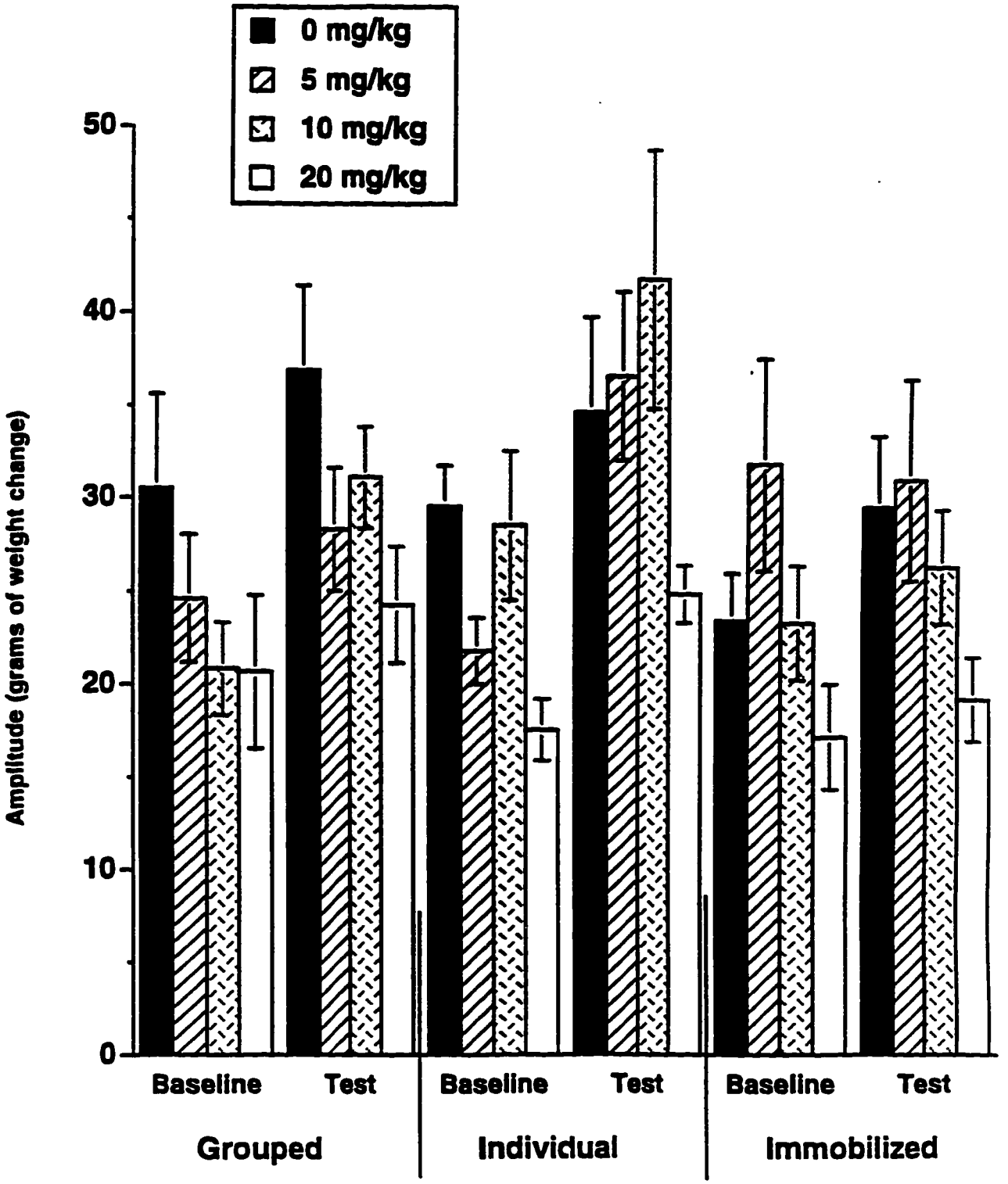


Figure 9. Experiment 1. Startle amplitude for F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

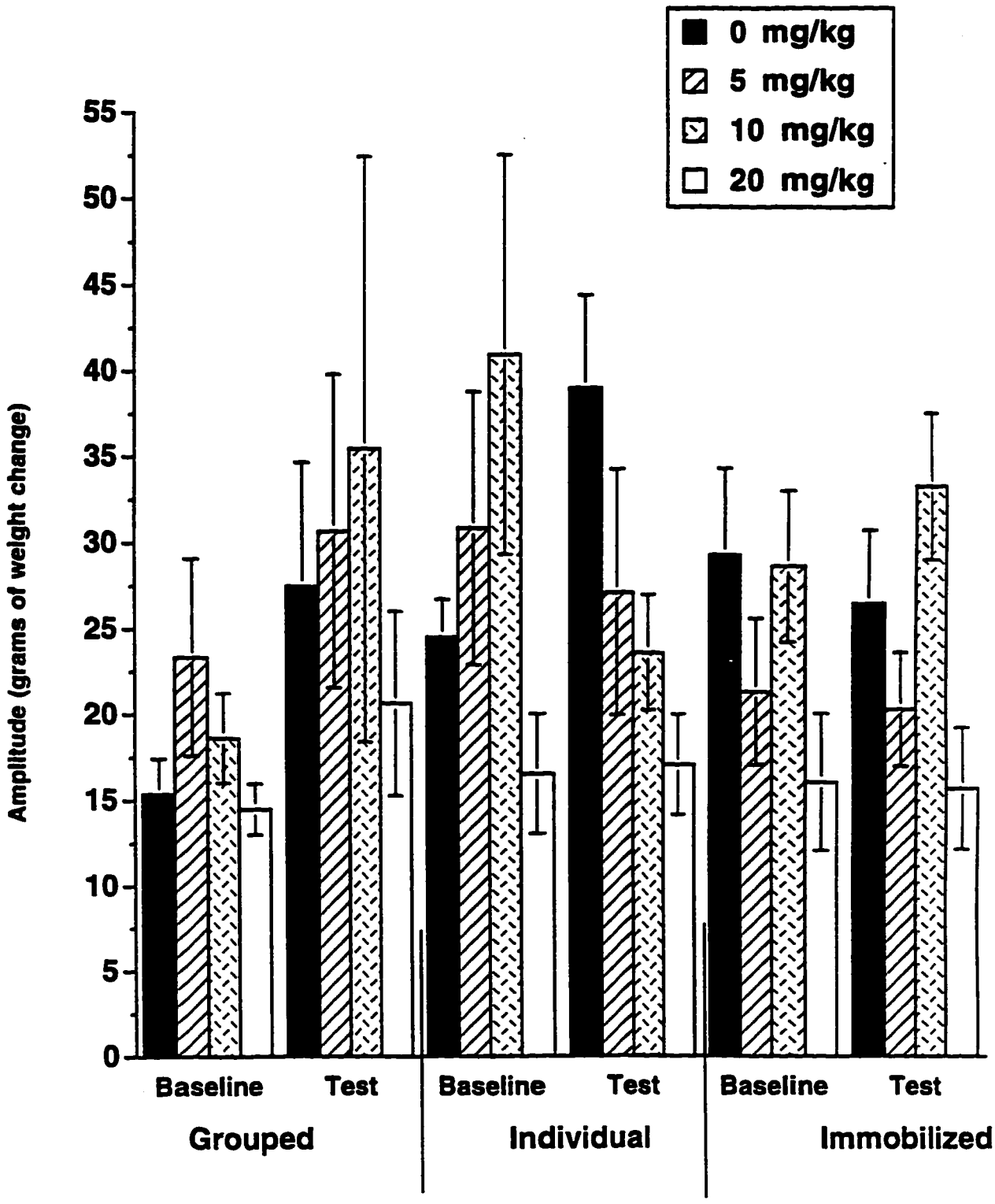
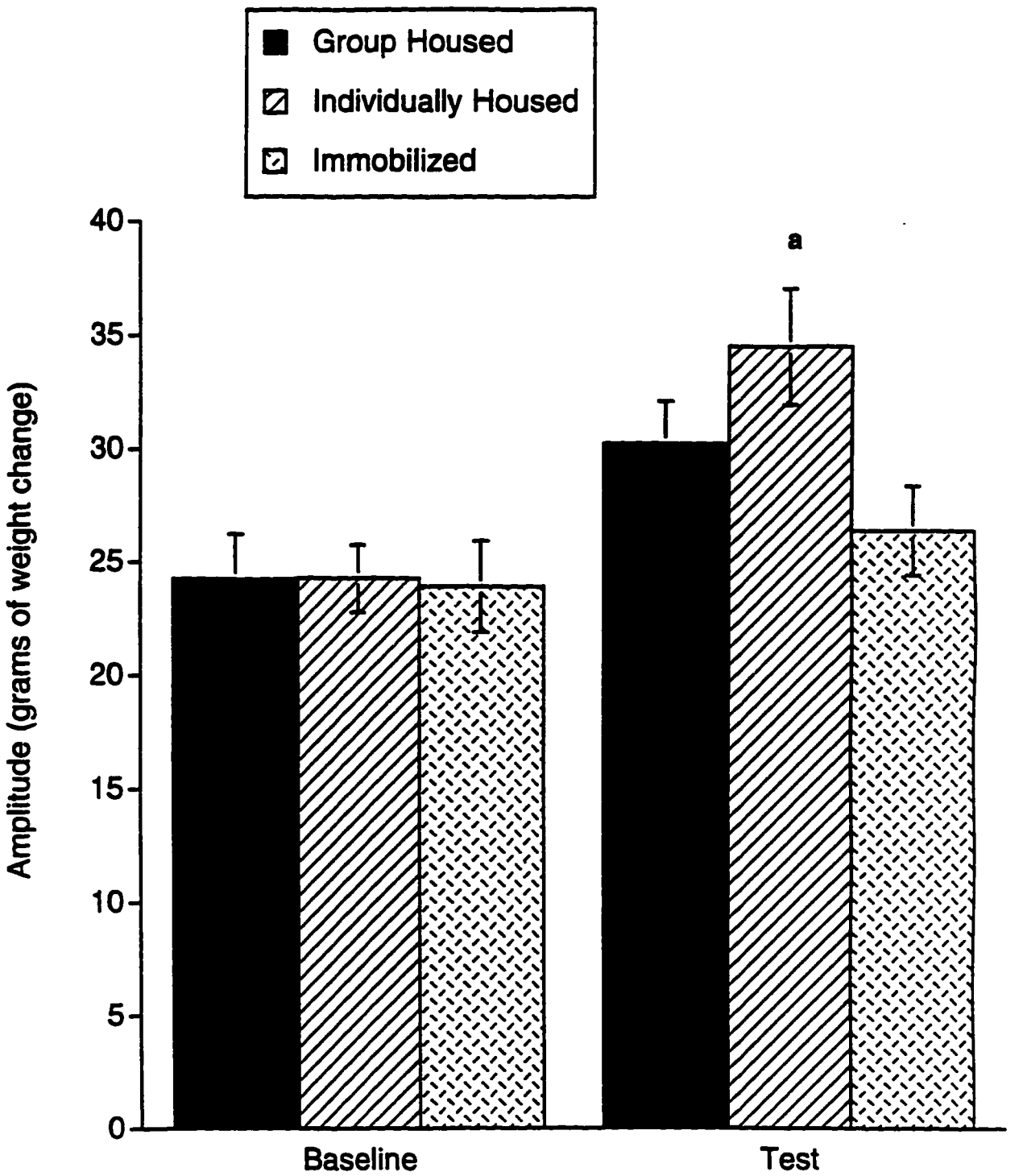


Figure 10. Experiment 1. Startle amplitude for Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from immobilized group on test day (Tukey HSD, $p<.05$).

Figure 11. Experiment 1. Startle amplitude for F-344 rats during baseline and testing separated by experimental condition (means and standard errors).

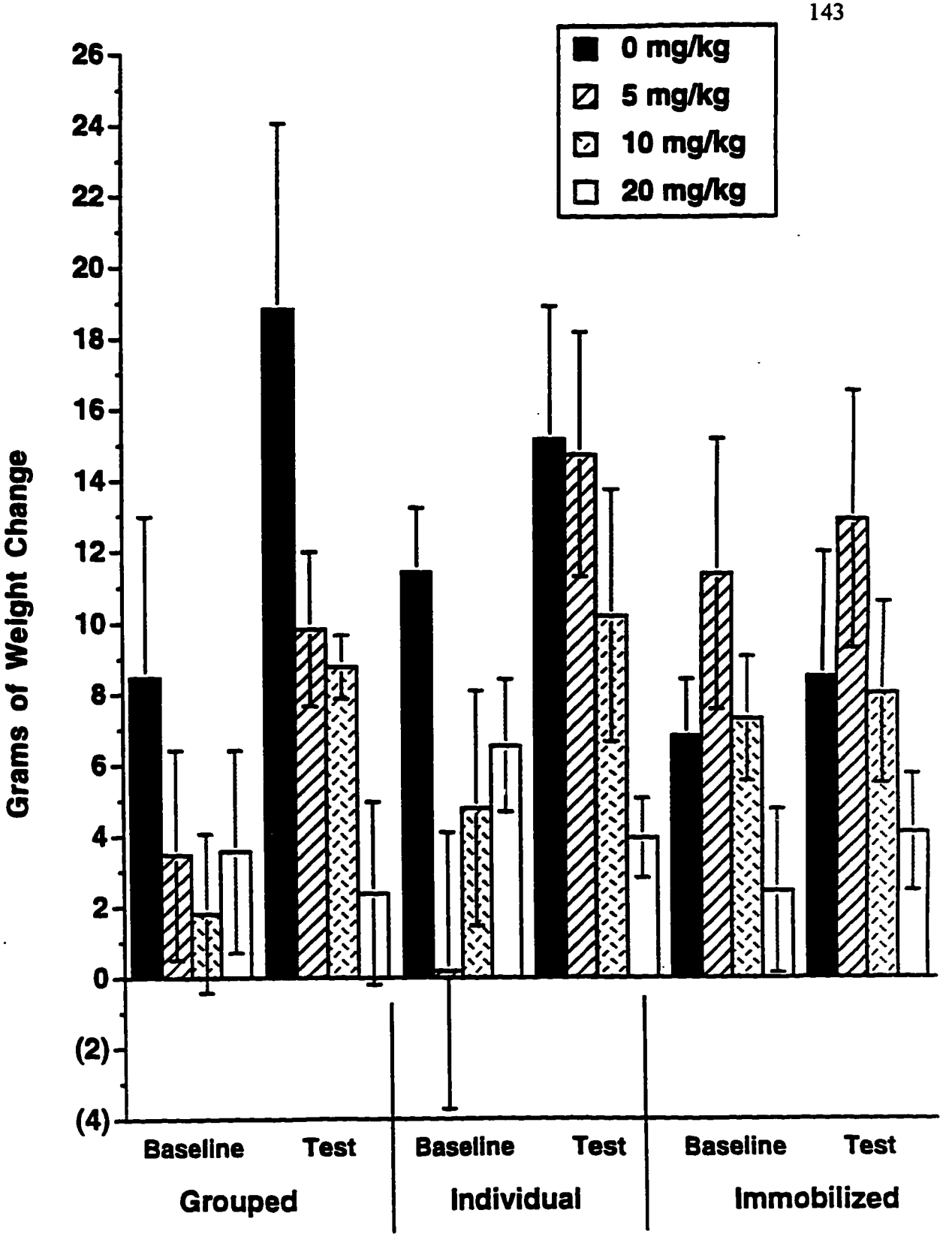


Figure 12. Experiment 1. Amount of inhibition for F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

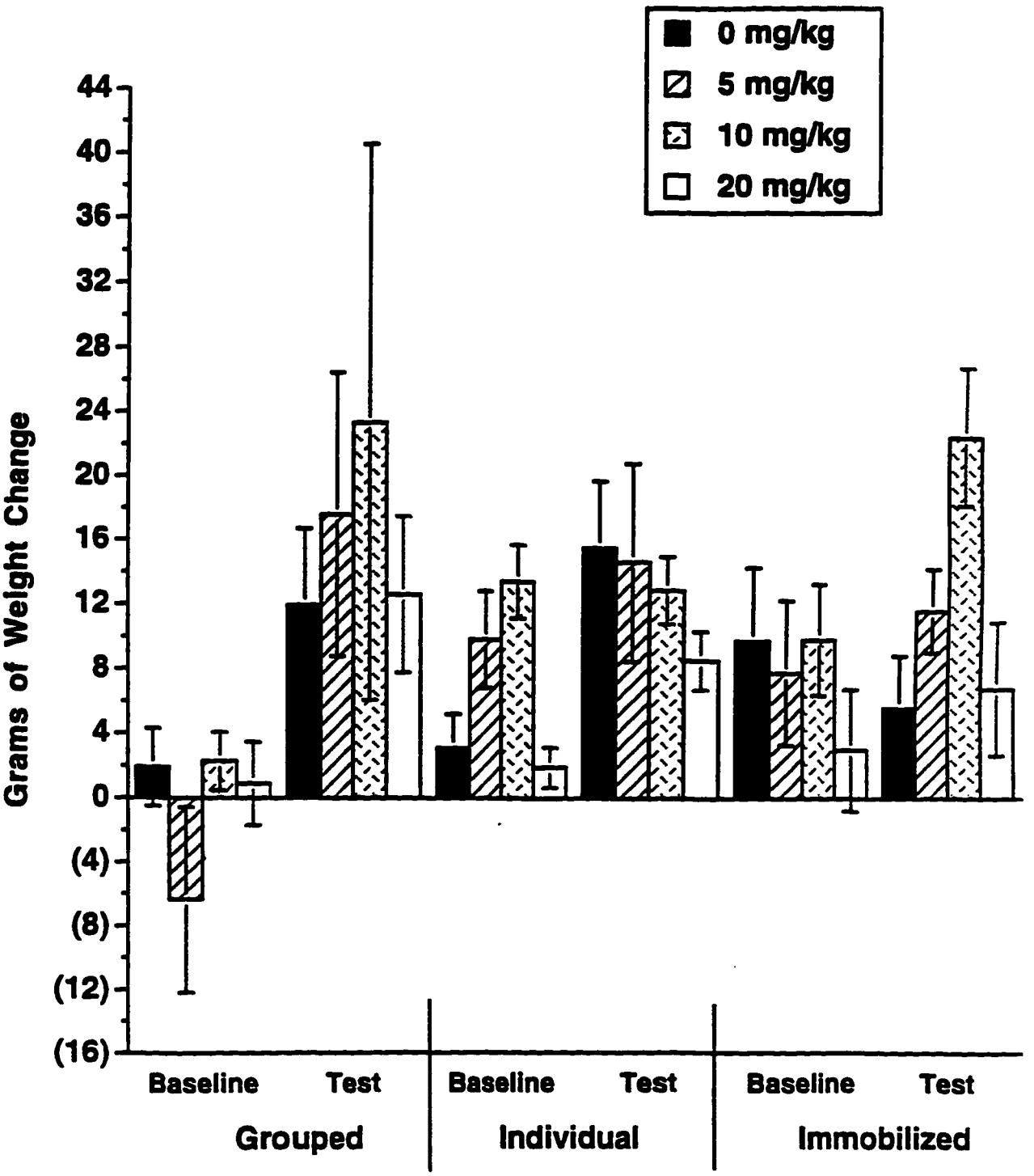
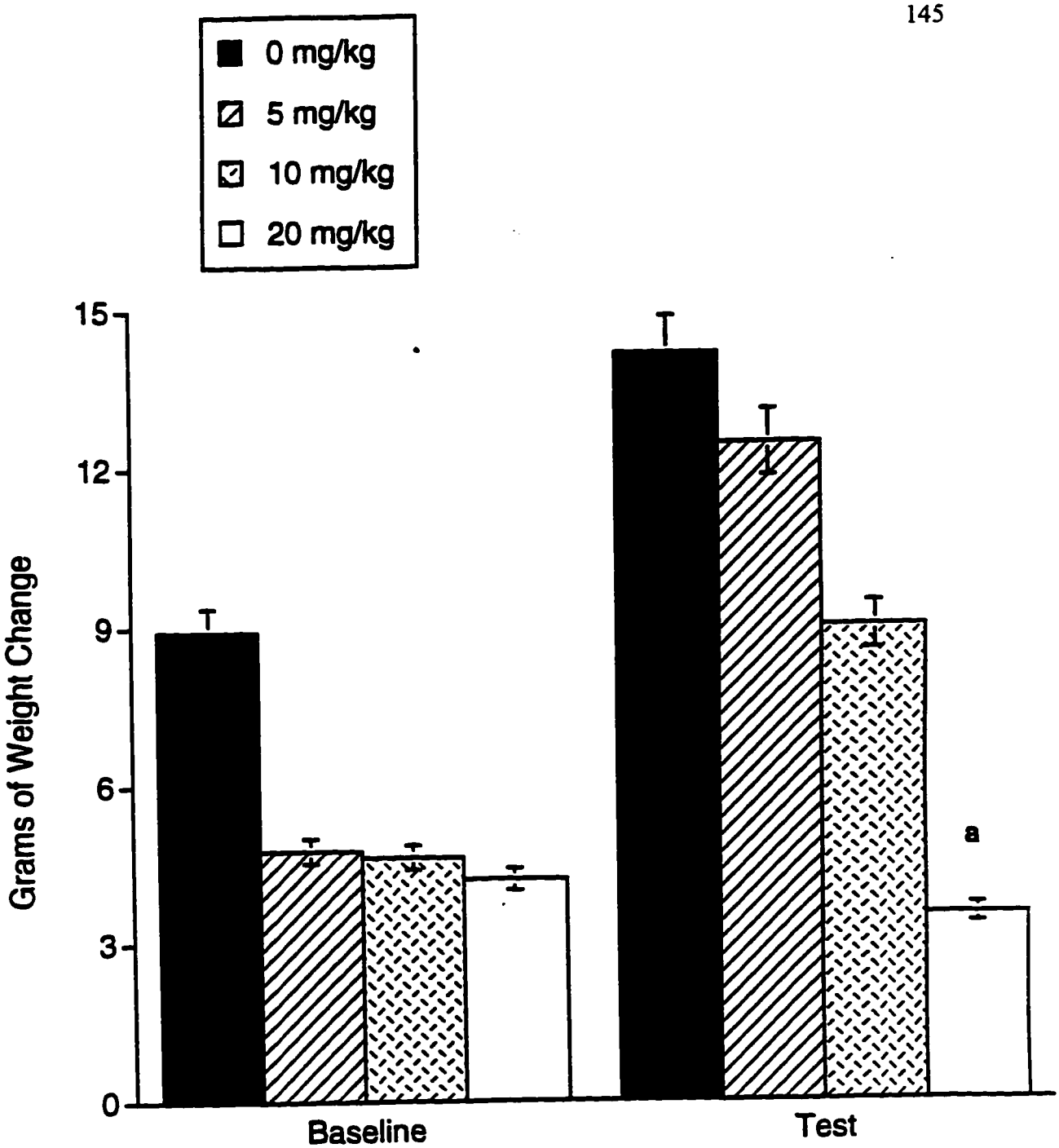


Figure 13. Experiment 1. Amount of inhibition for Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from 0 or 5 mg/kg subjects on test day.

Figure 14. Experiment 1. Amount of inhibition for F-344 rats during baseline and testing separated by drug dose (means and standard errors).

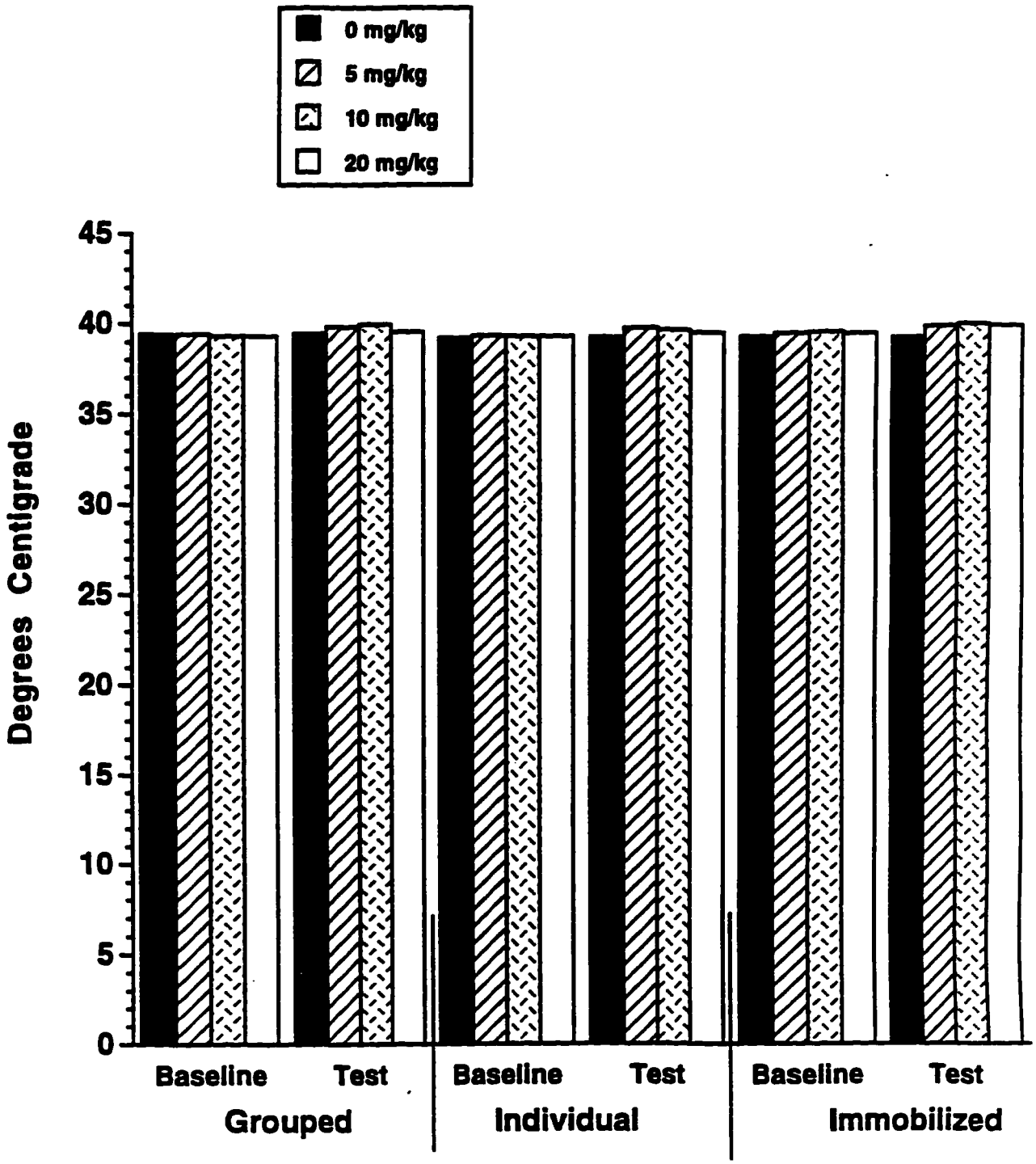


Figure 15. Experiment 1. Body temperature of F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

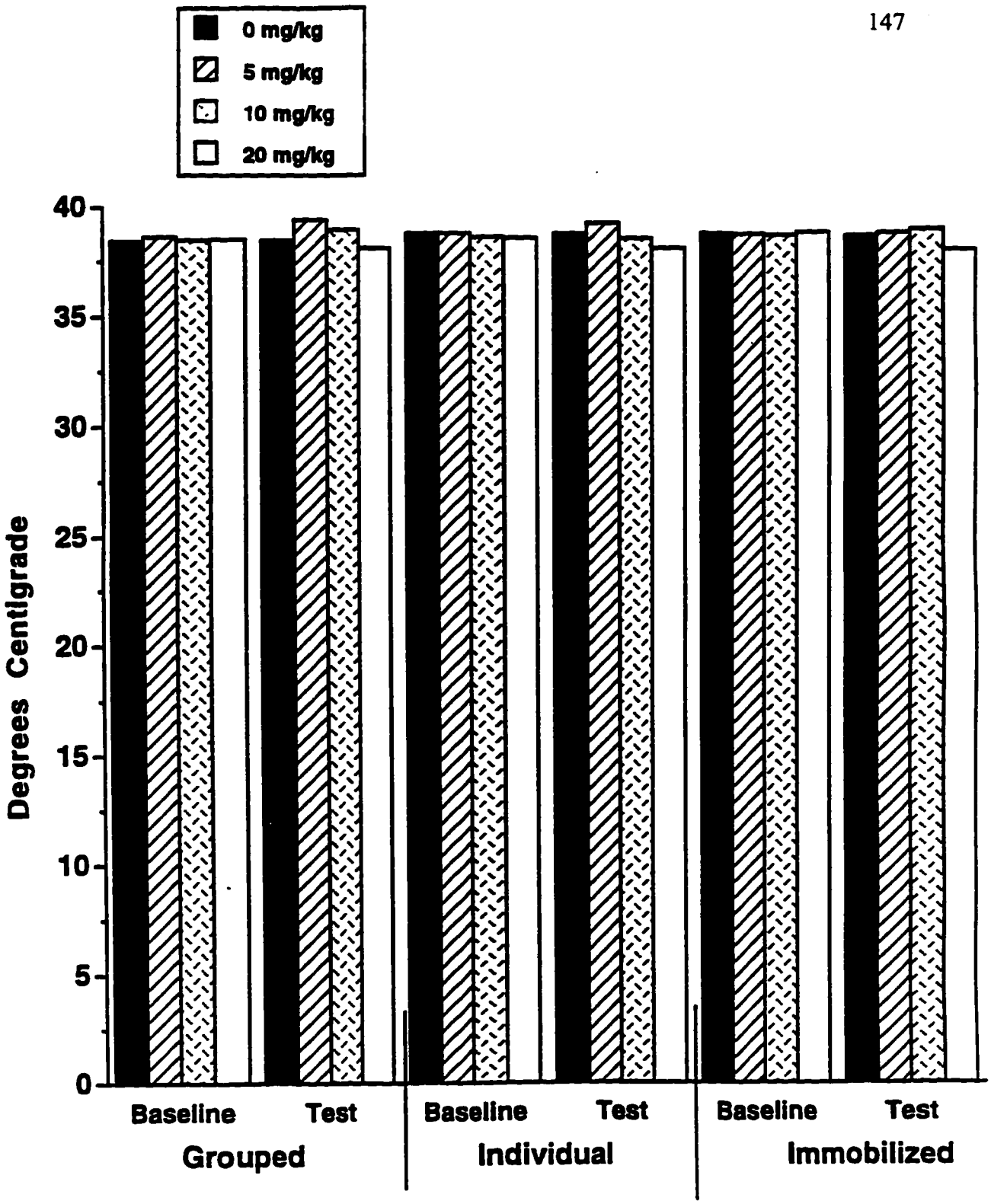


Figure 16. Experiment 1. Body temperature of Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

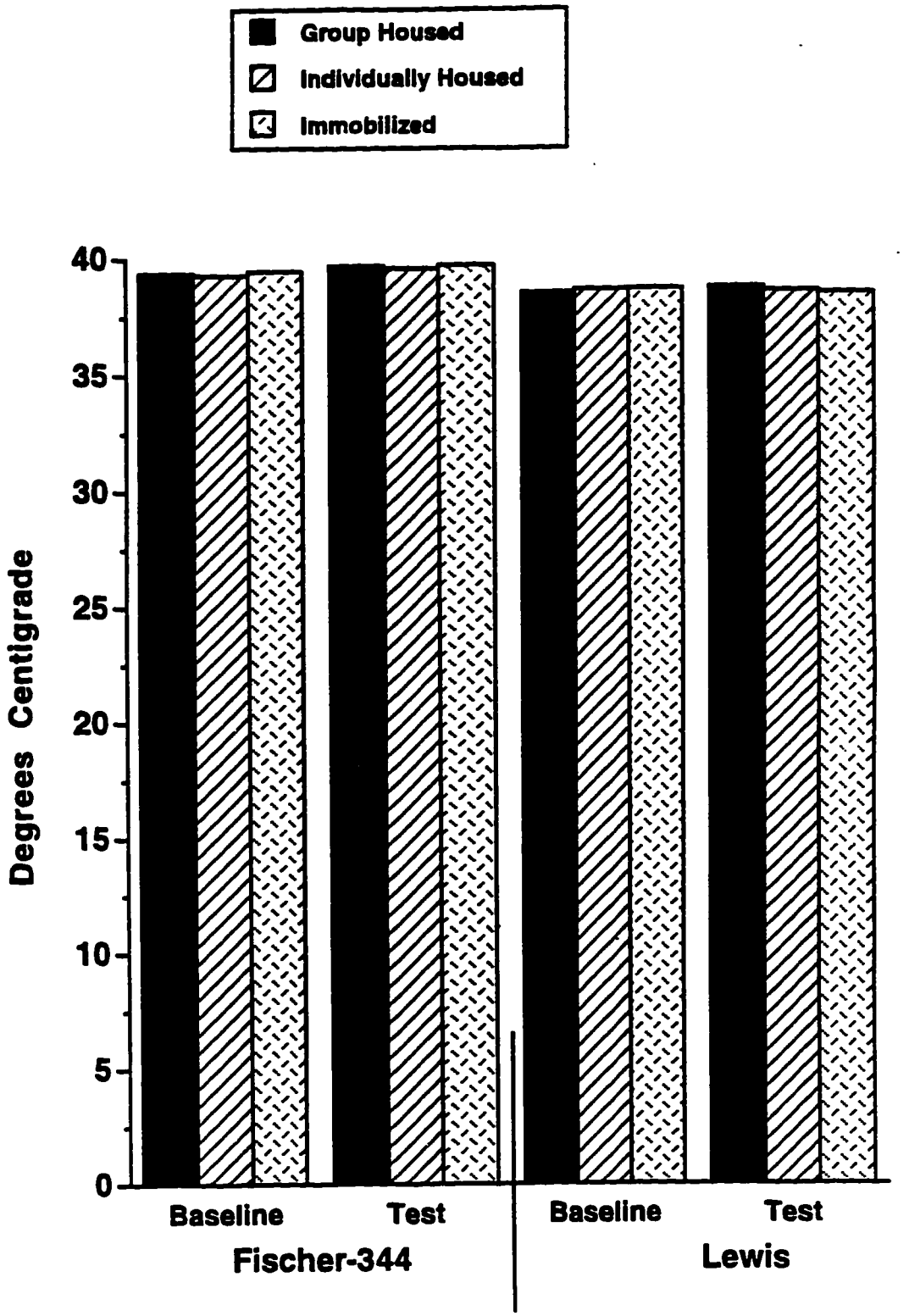
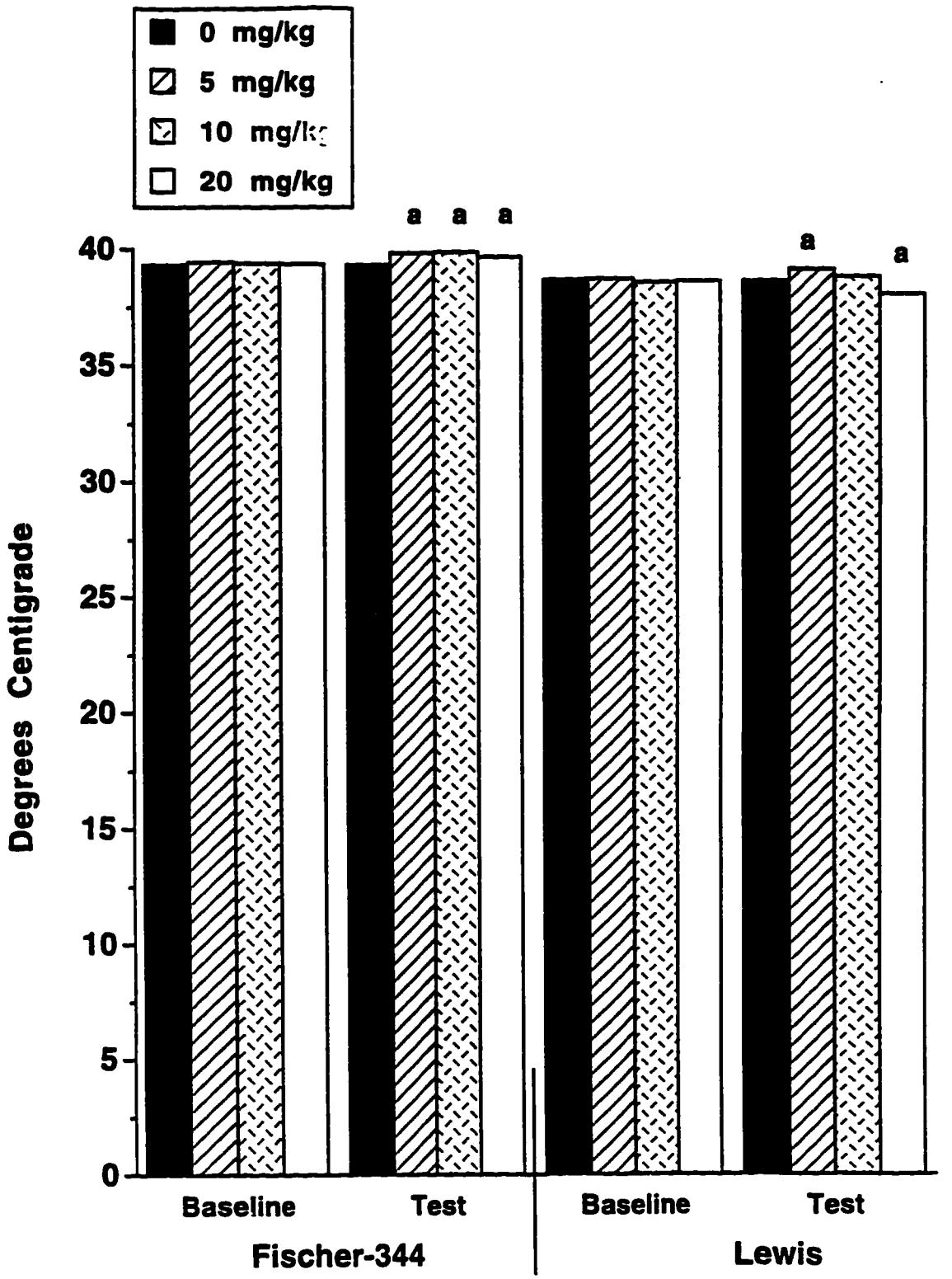


Figure 17. Experiment 1. Body temperature of F-344 and Lewis rats during baseline and testing separated by experimental conditions (means and standard errors).



a Significantly different from 0 mg/kg dose on test day for respective strain (Dunnett, $p<.05$).

Figure 18. Experiment 1. Body temperature for F-344 and Lewis rats during baseline and testing separated by drug dose (means and standard errors).

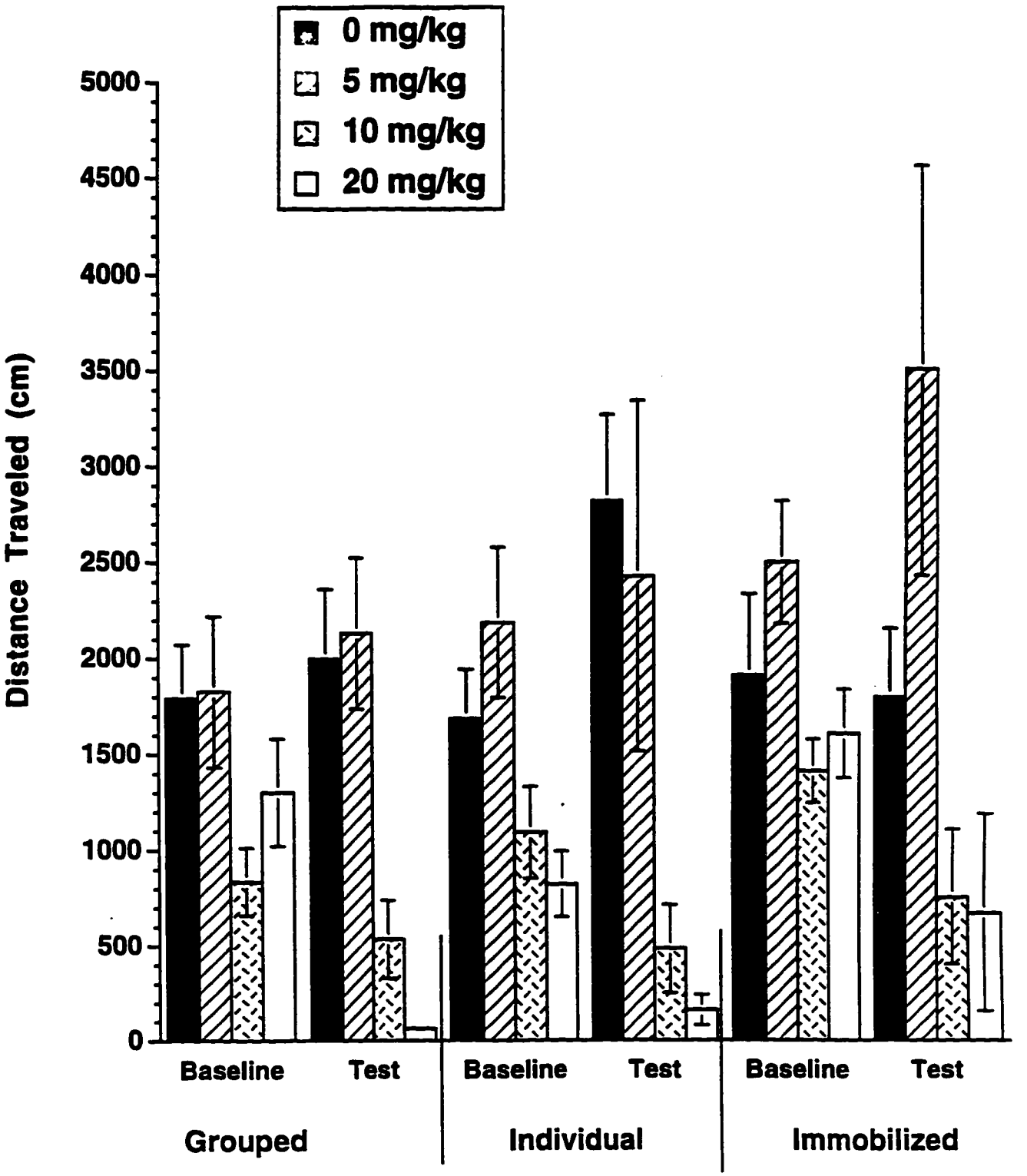


Figure 19. Experiment 1. Distance traveled by F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

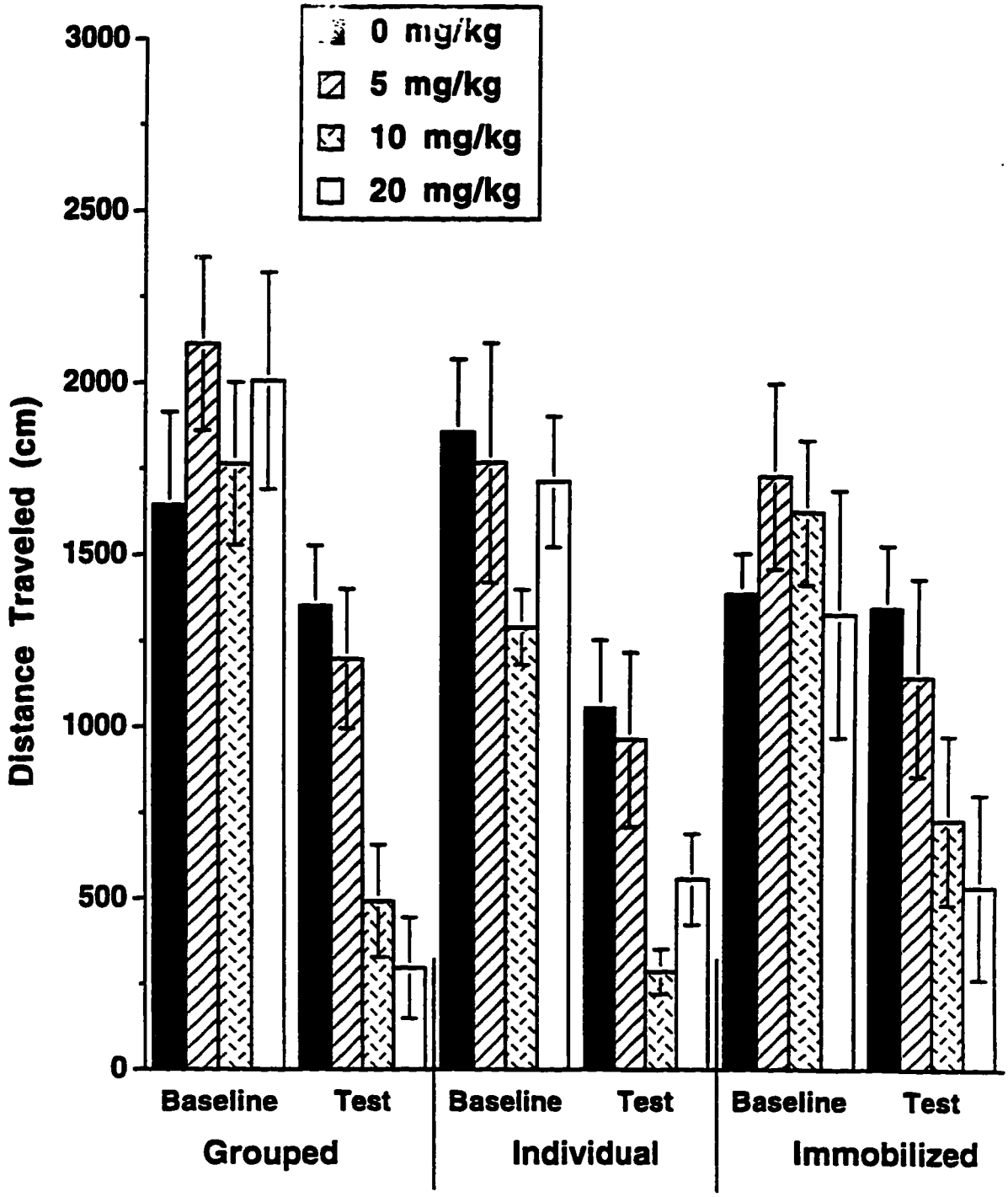
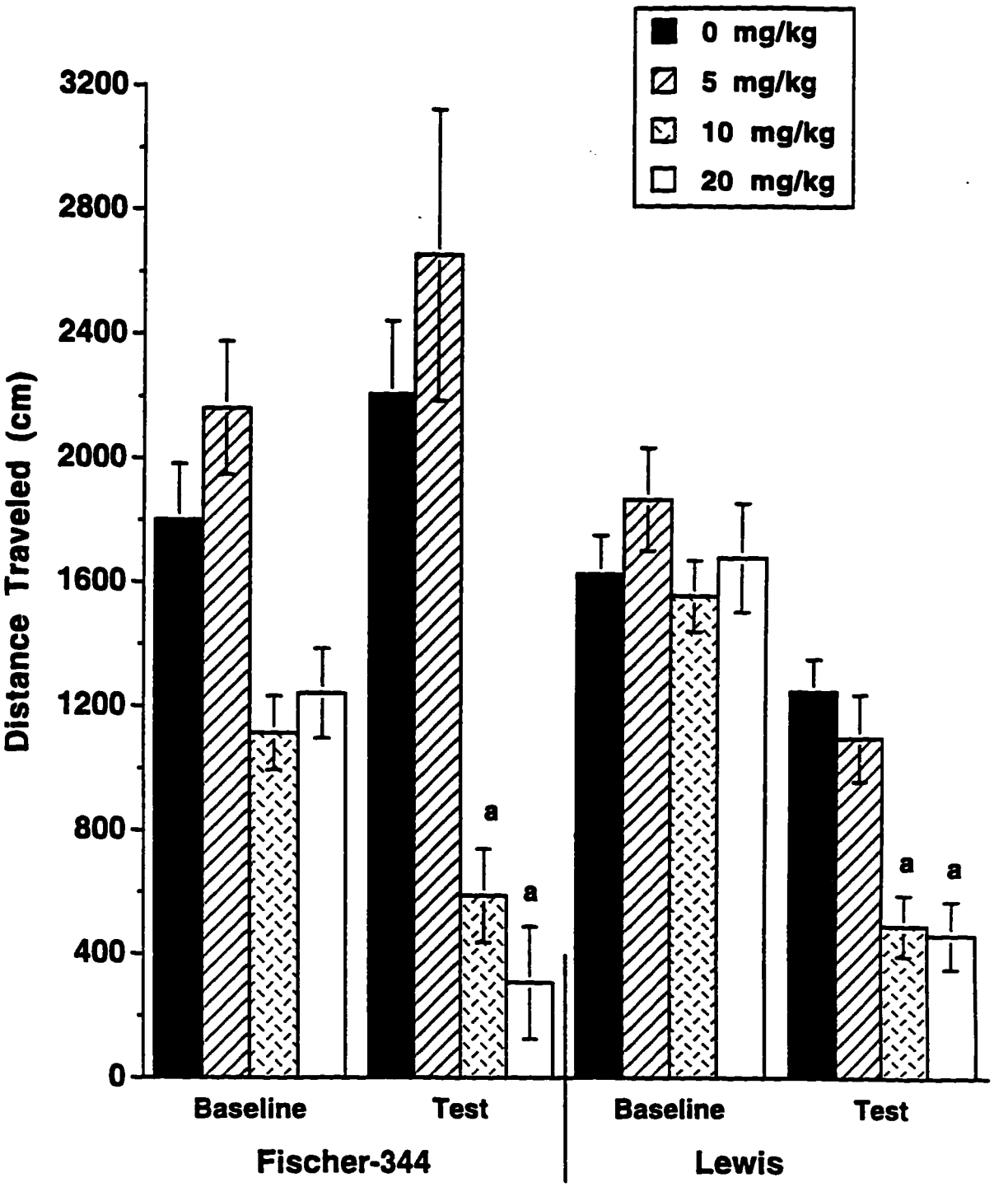


Figure 20. Experiment 1. Distance traveled by Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from 0 mg/kg dose on test day for respective strain (Dunnett, $p<.05$).

Figure 21. Experiment 1. Distance traveled by F-344 and Lewis rats during baseline and testing separated by drug dose (means and standard errors).

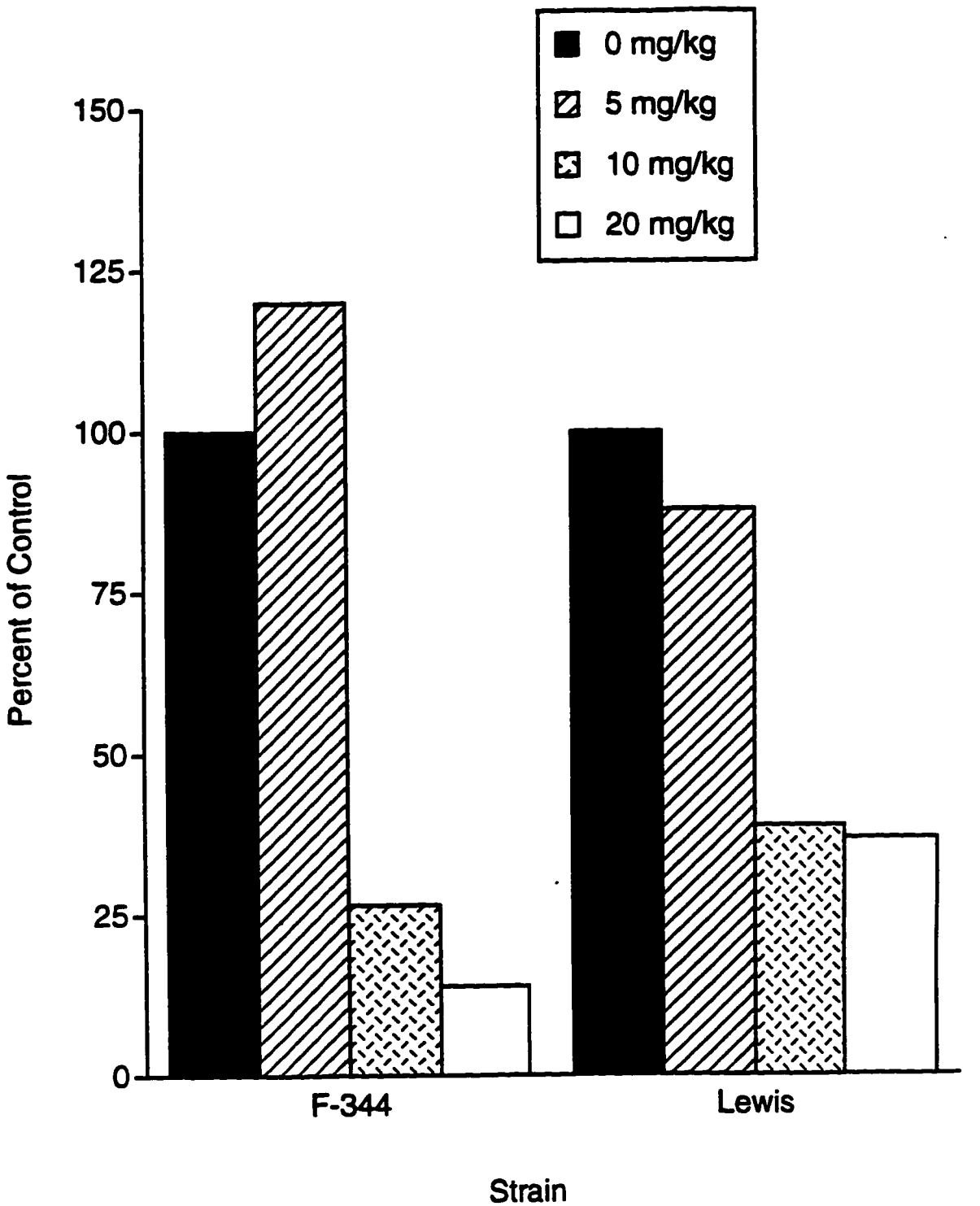


Figure 22. Experiment 1. Distance traveled presented as percent of control for F-344 and Lewis rats during testing separated by drug dose.

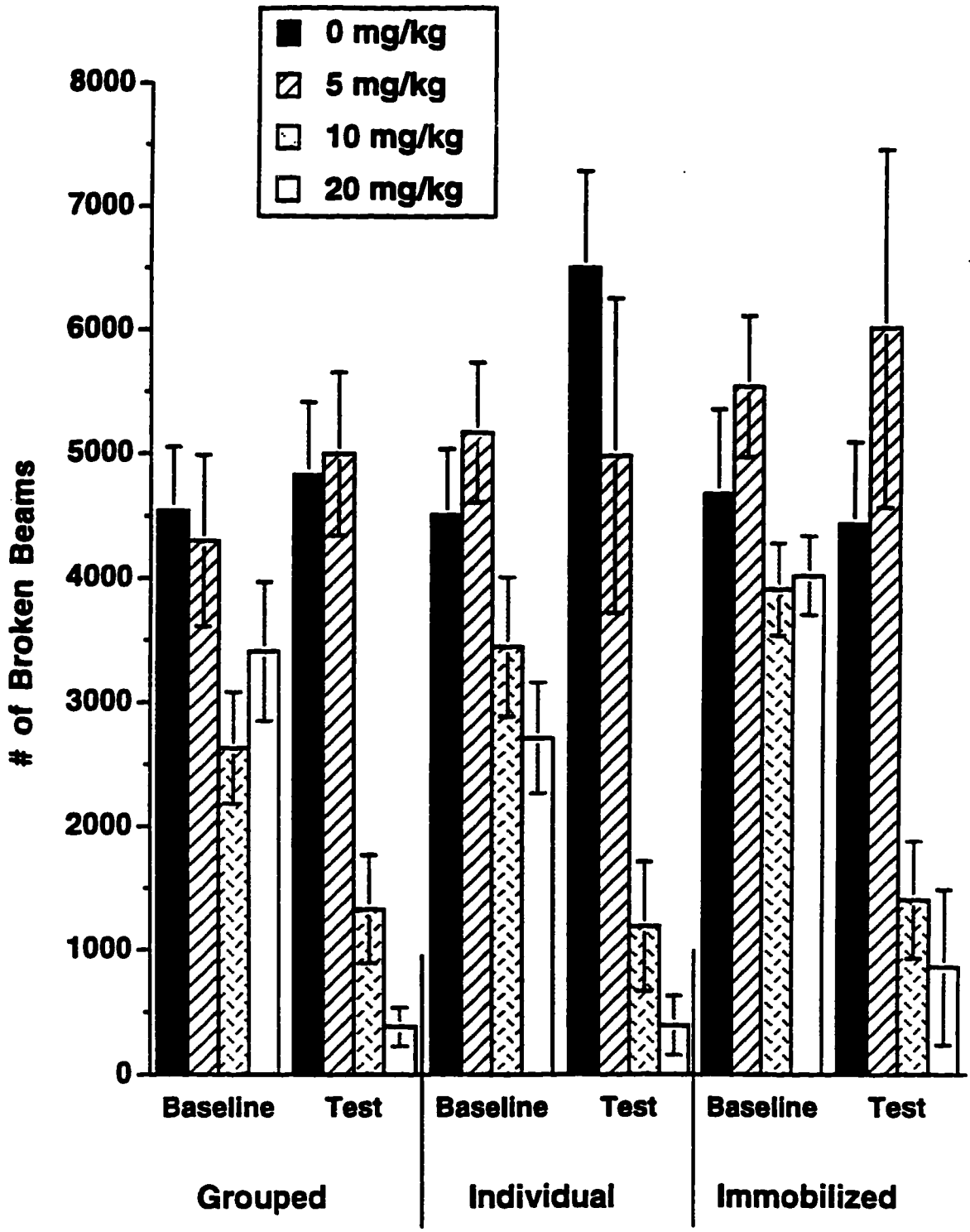


Figure 23. Experiment 1. Horizontal activity of F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

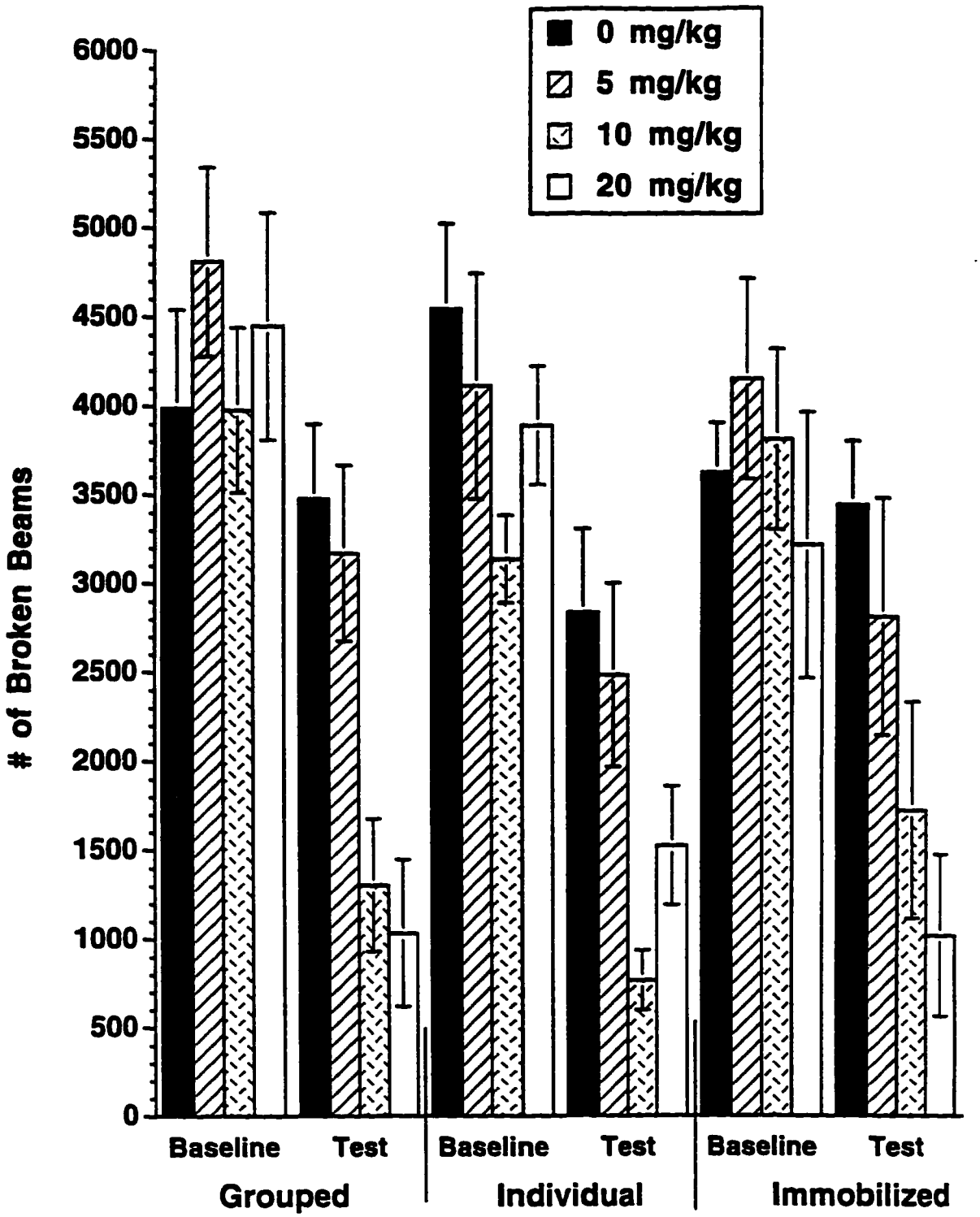
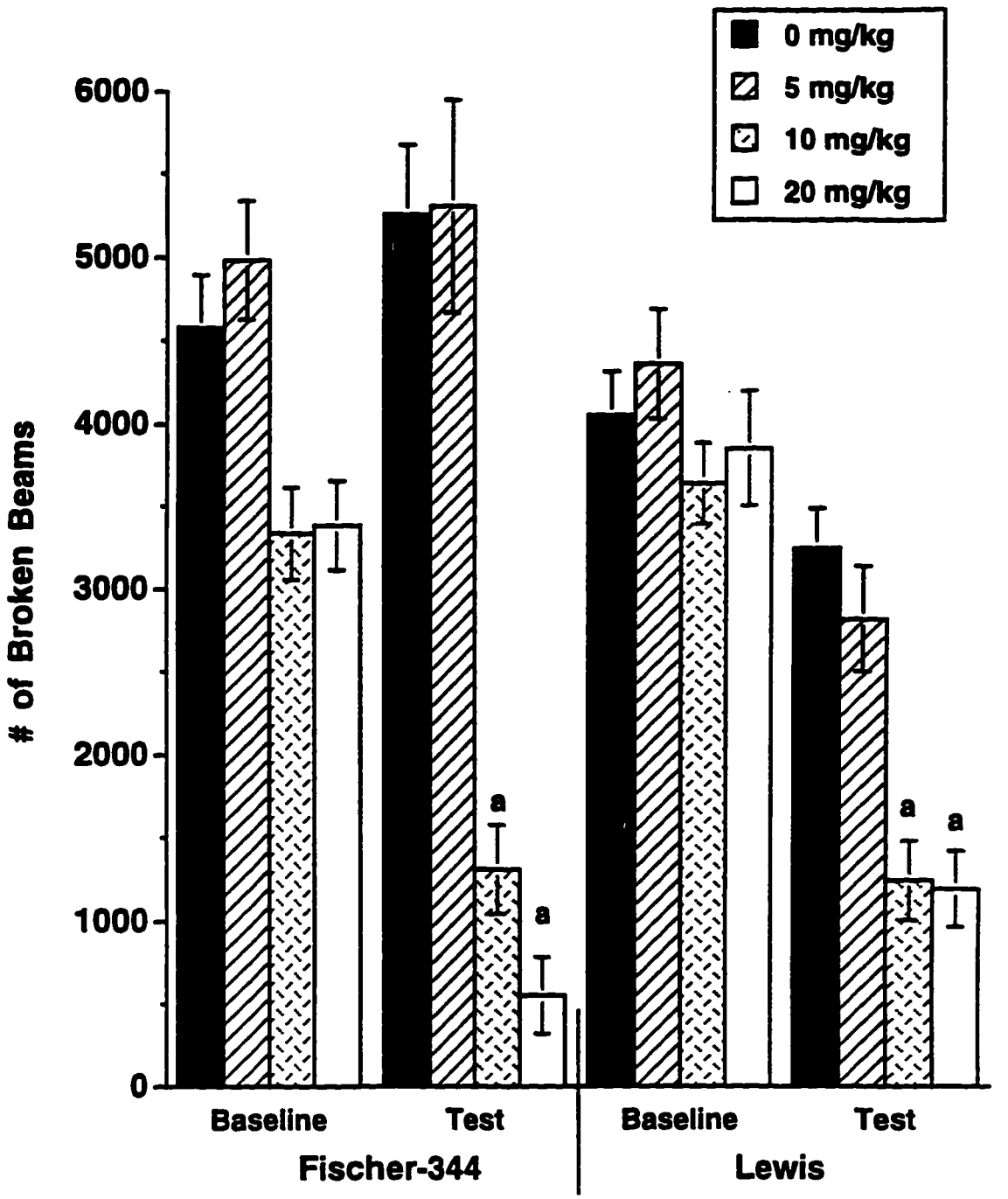


Figure 24. Experiment 1. Horizontal activity of Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from 0 mg/kg dose on test day for respective strain (Dunnett, P<.05).

Figure 25. Experiment 1. Horizontal activity of F-344 and Lewis rats during baseline and testing separated by drug dose (means and standard errors).

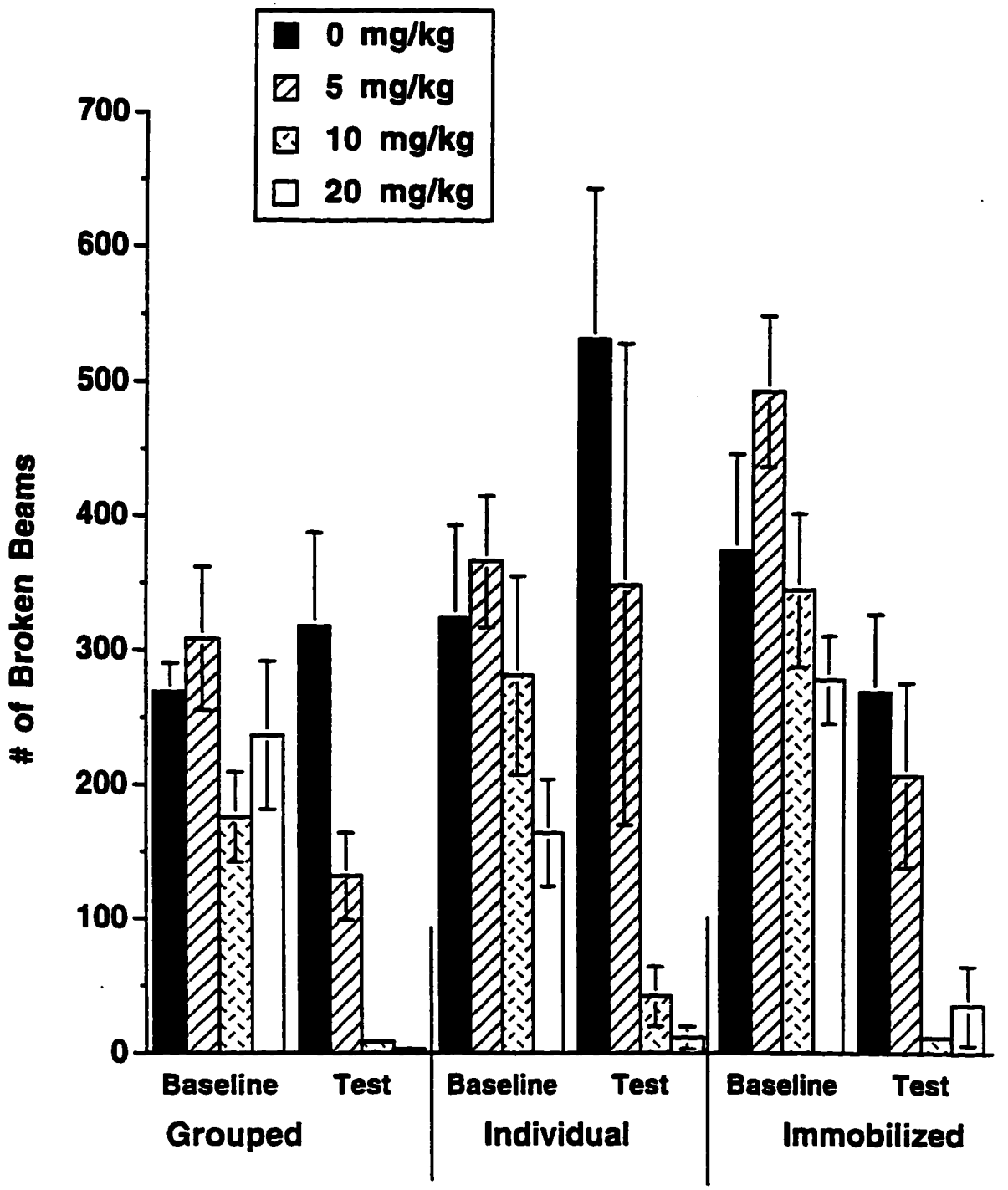


Figure 26. Experiment 1. Vertical activity of F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

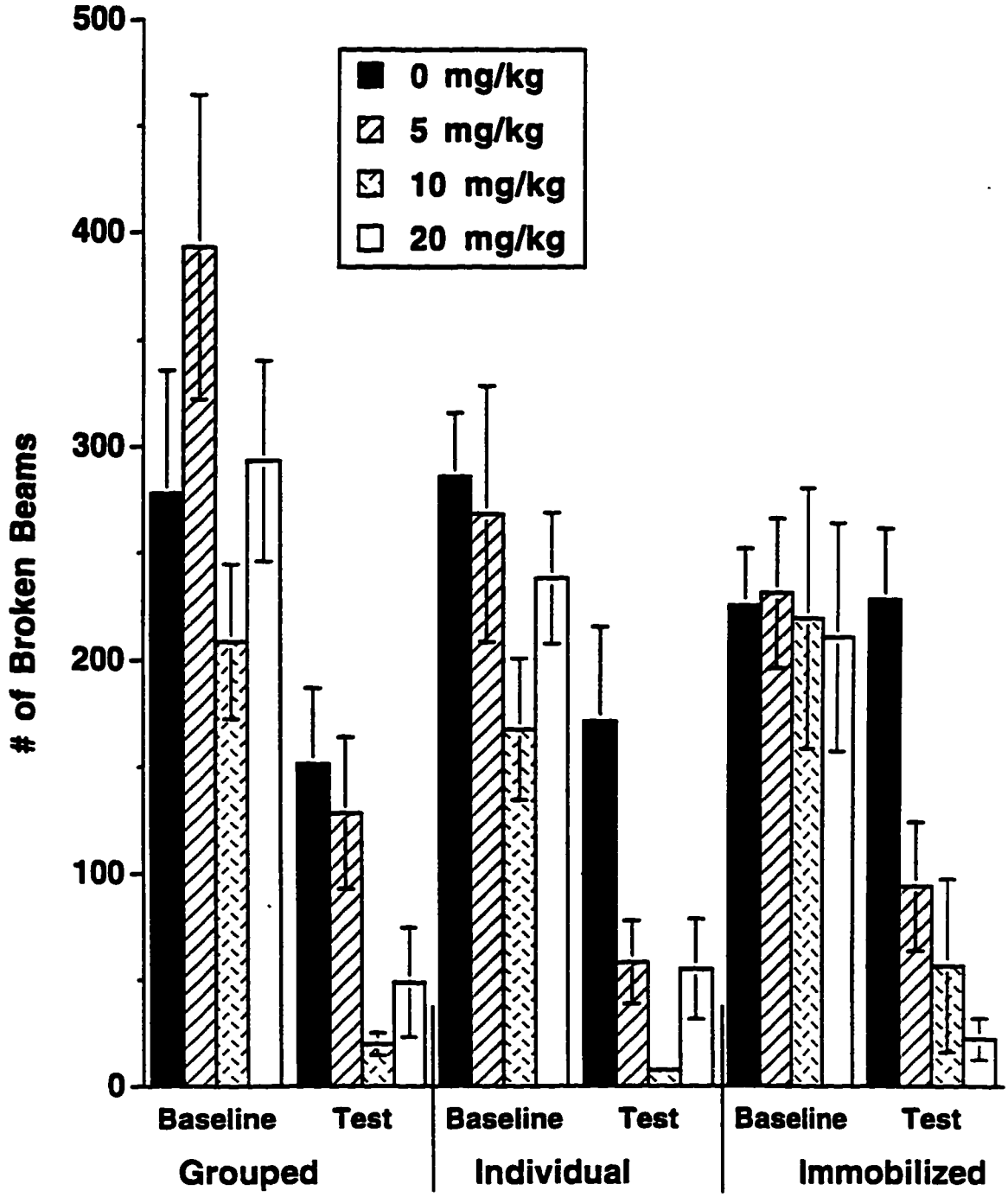


Figure 27. Experiment 1. Vertical activity of Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

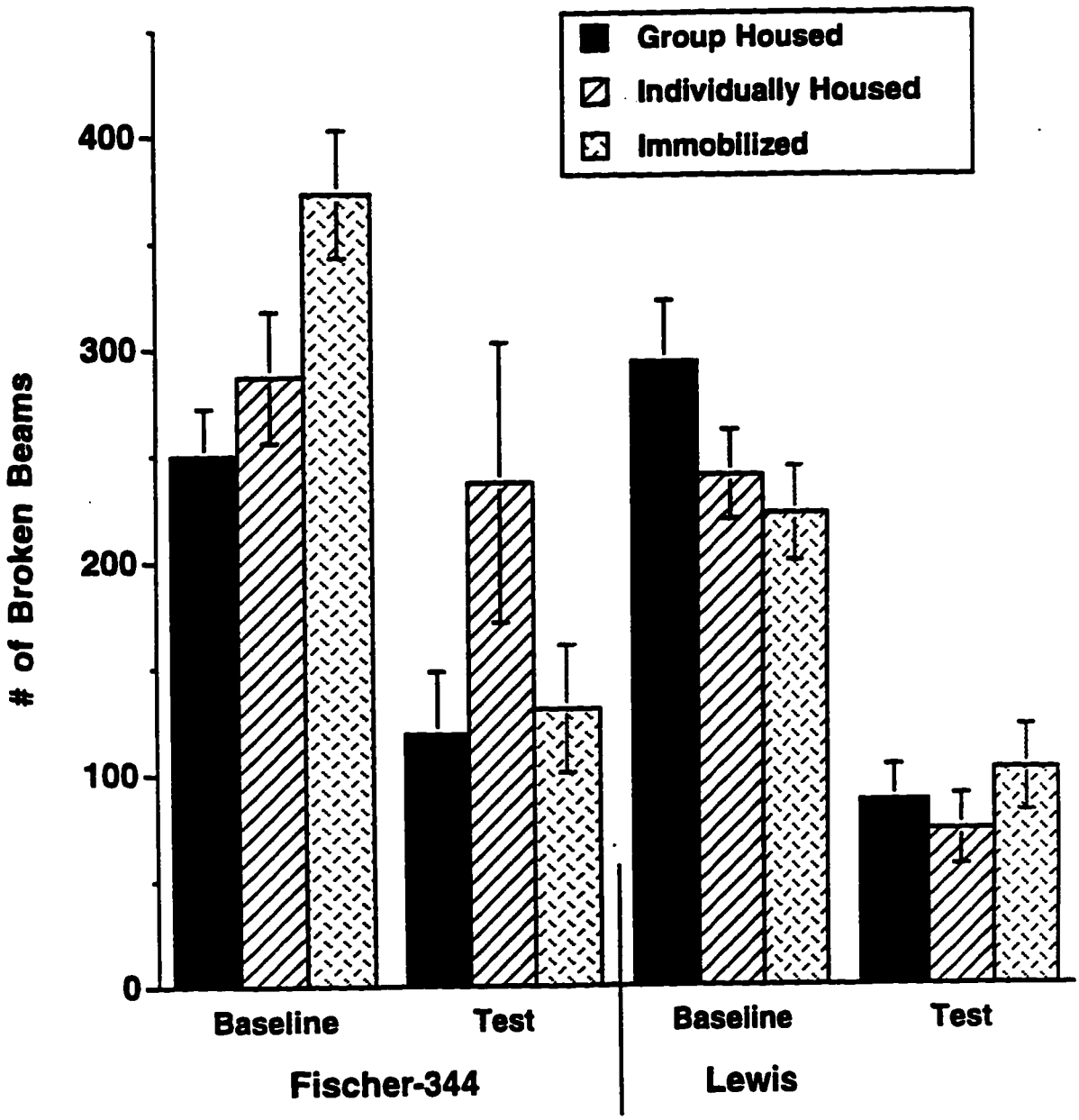
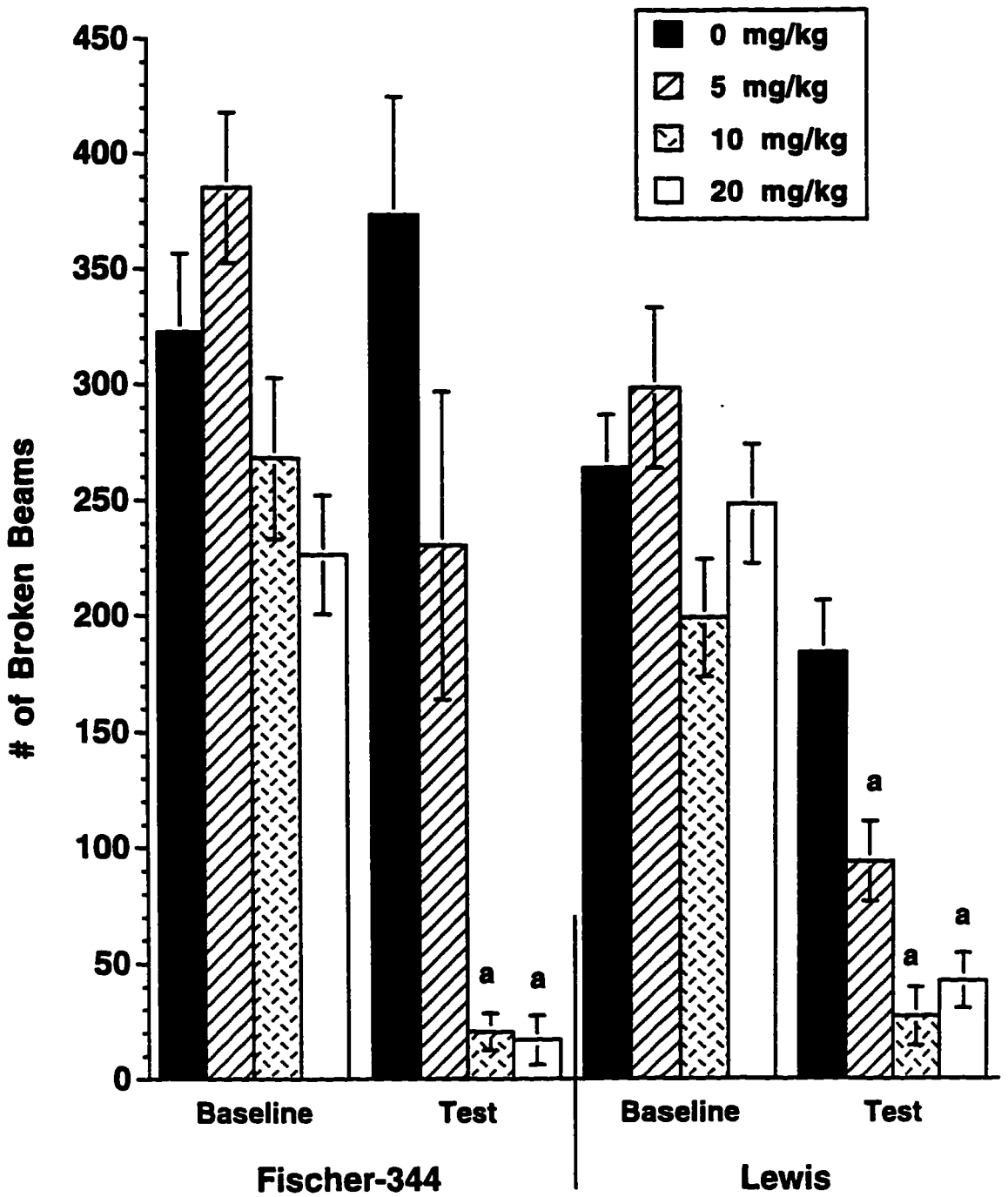
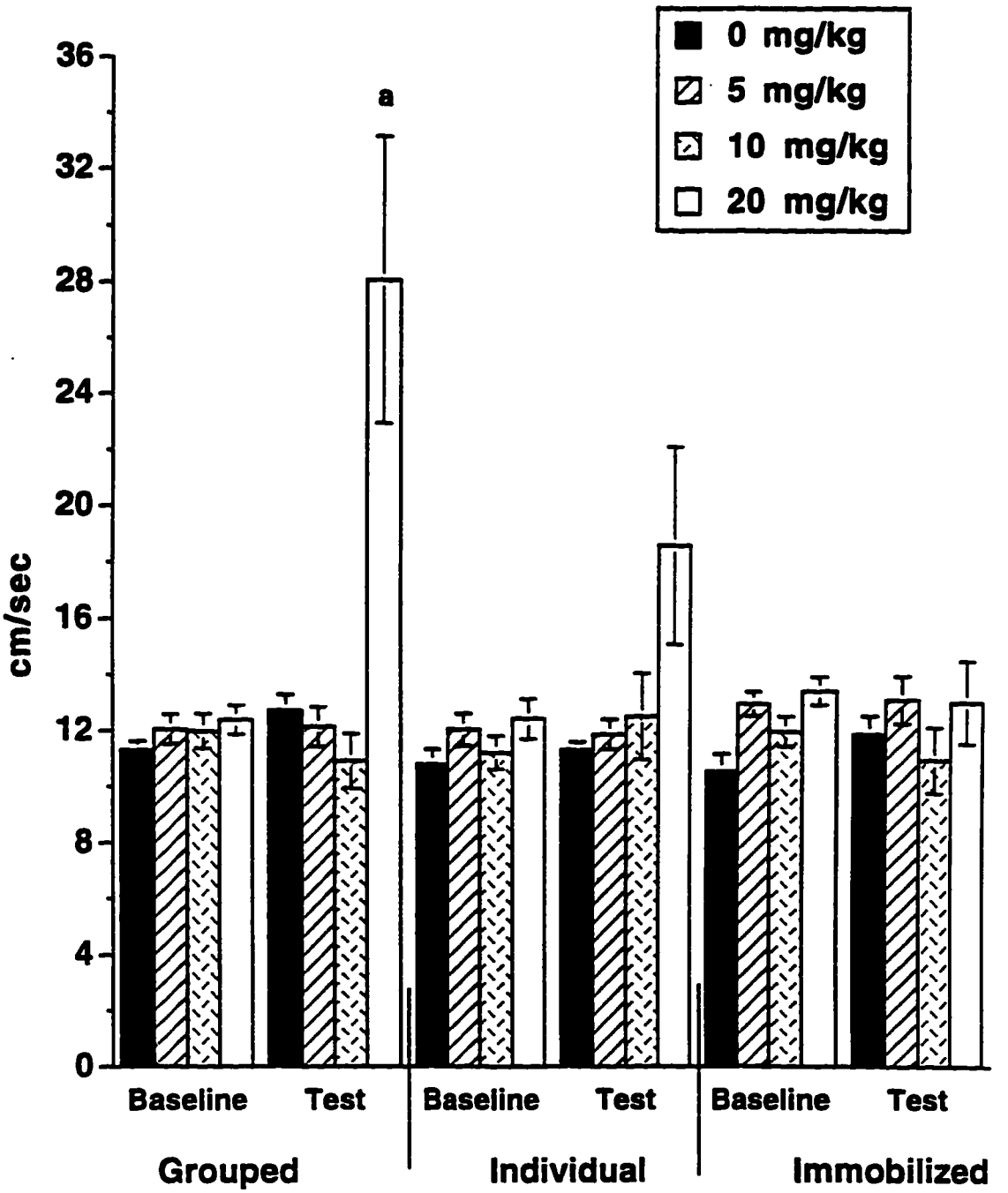


Figure 28. Experiment 1. Vertical activity of F-344 and Lewis rats during baseline and testing separated by experimental condition.



a Significantly different from 0 mg/kg dose group on test day for respective strain (Dunnett, $p<.05$).

Figure 29. Experiment 1. Vertical activity of F-344 and Lewis rats during baseline and testing separated by drug dose (means and standard errors).



a Significantly different from individually housed or immobilized rats given 20 mg/kg of morphine on test day (Tukey HSD, $p<.05$).

Figure 30. Experiment 1. Speed of F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

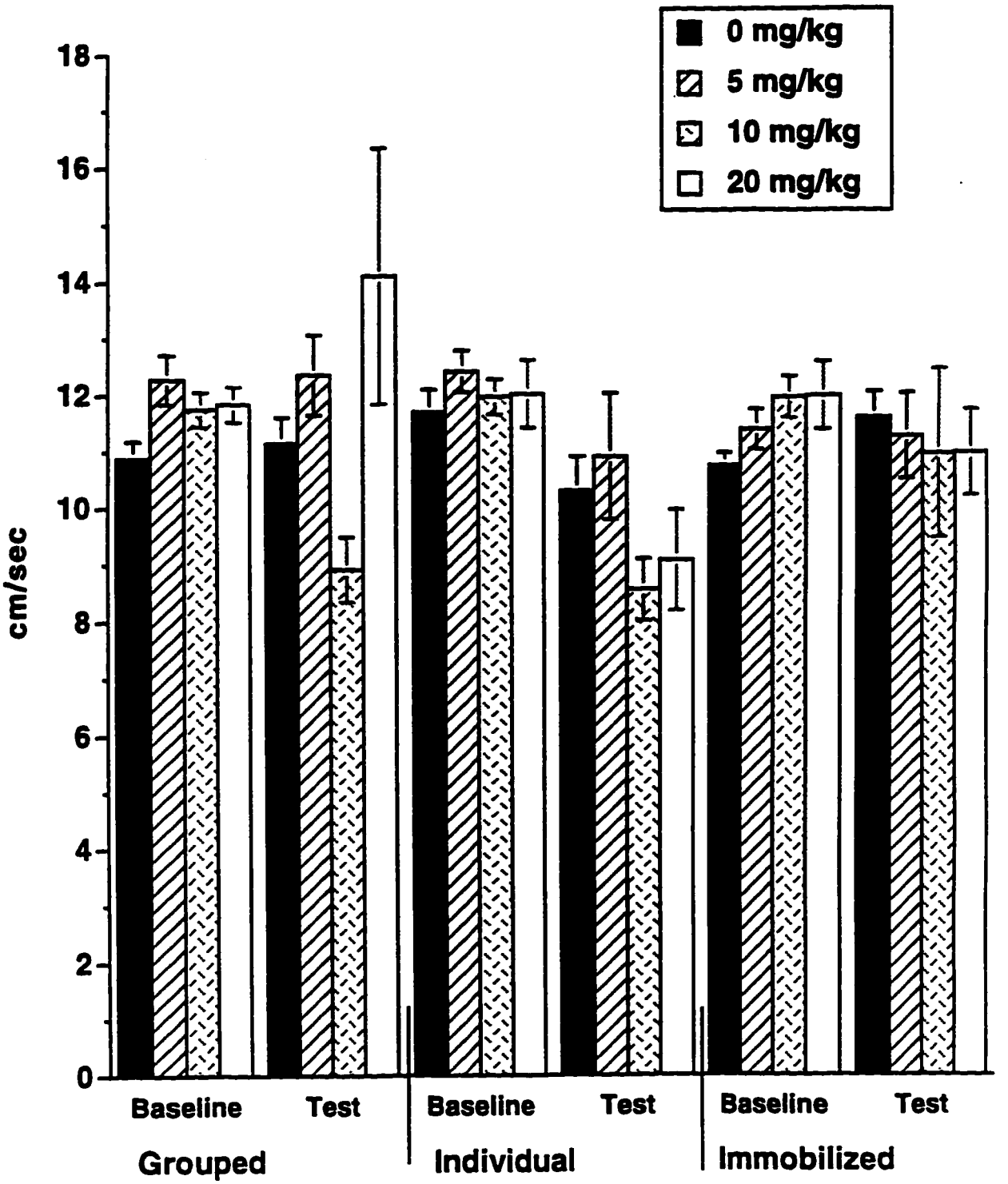
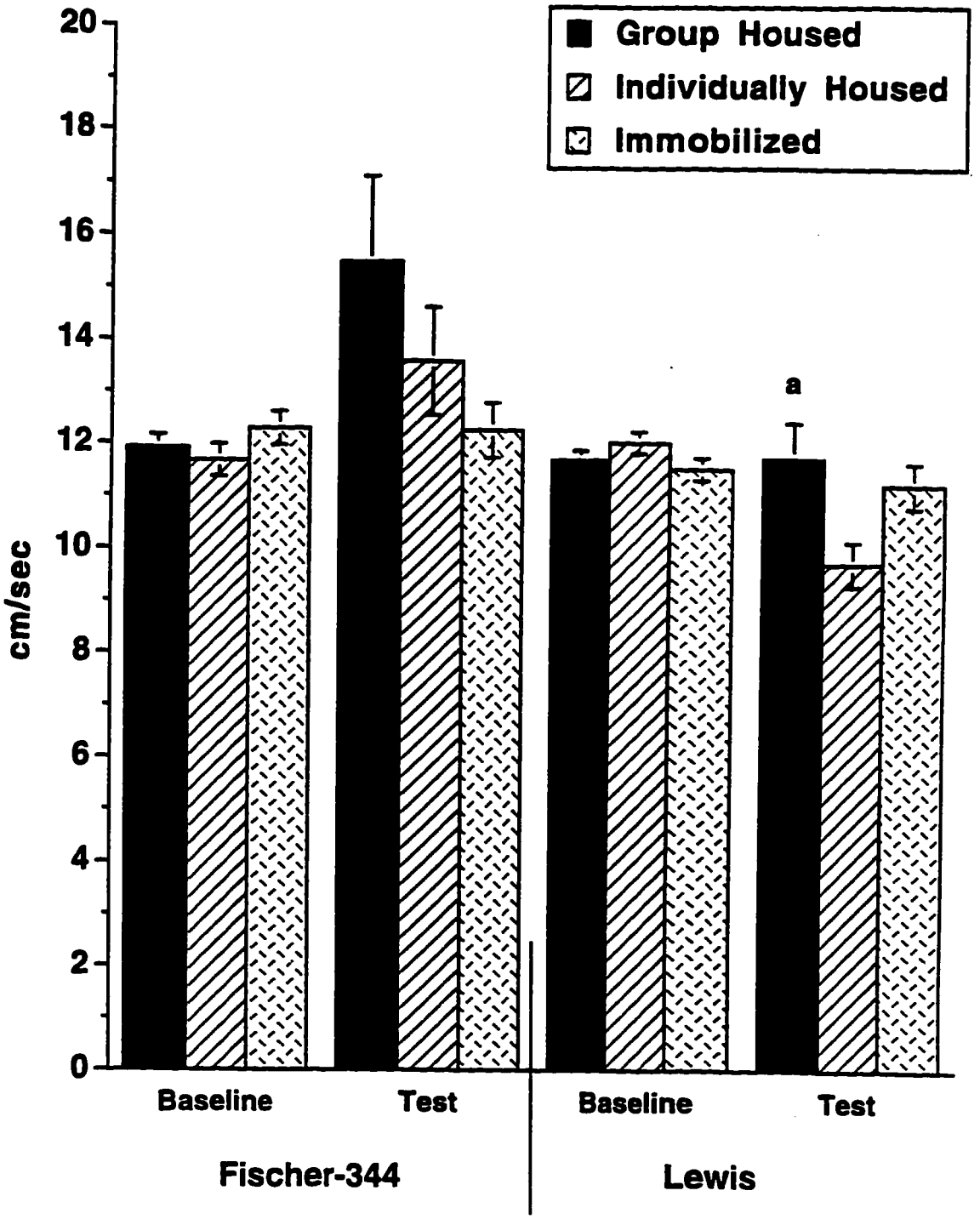
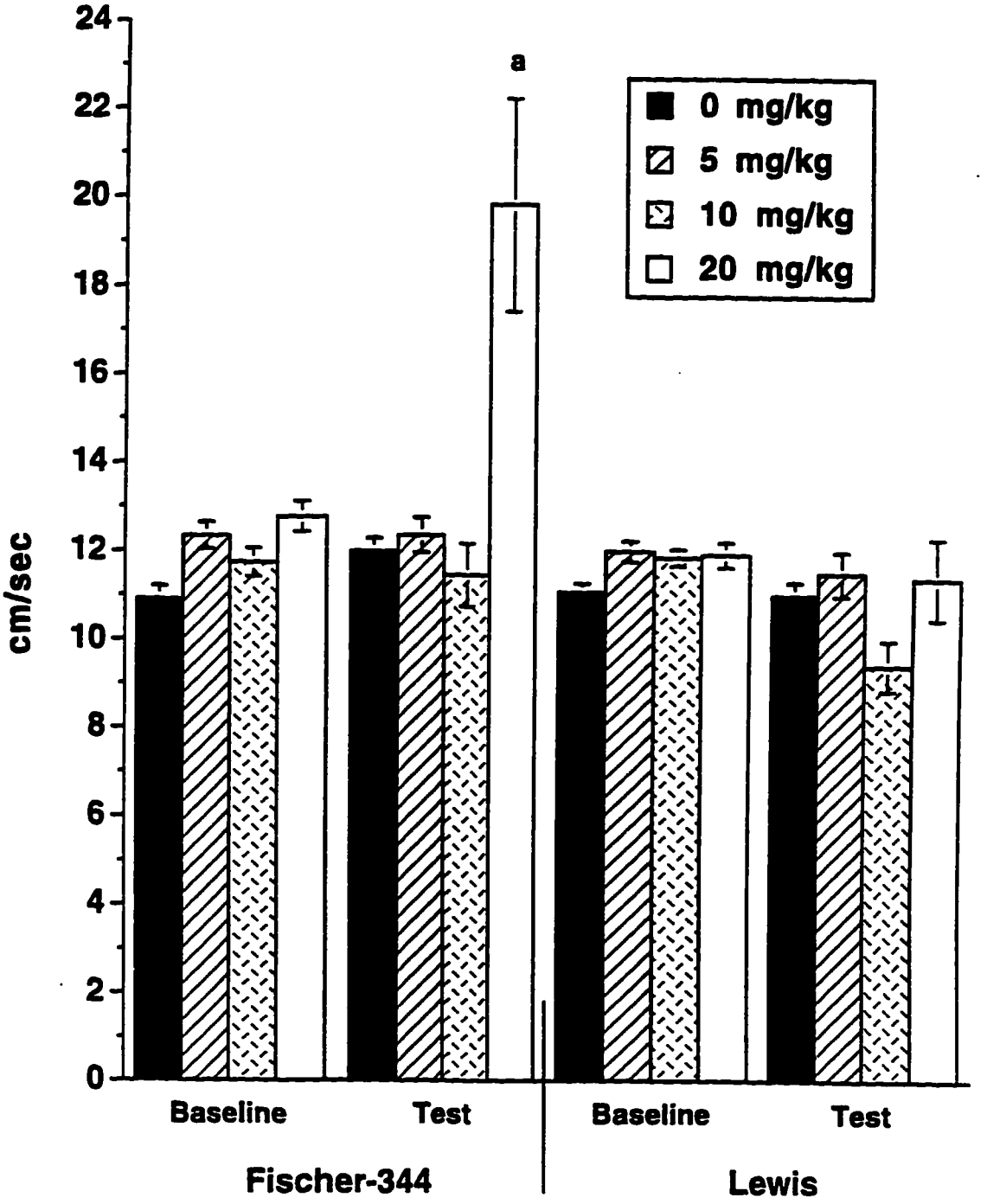


Figure 31. Experiment 1. Speed of Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).



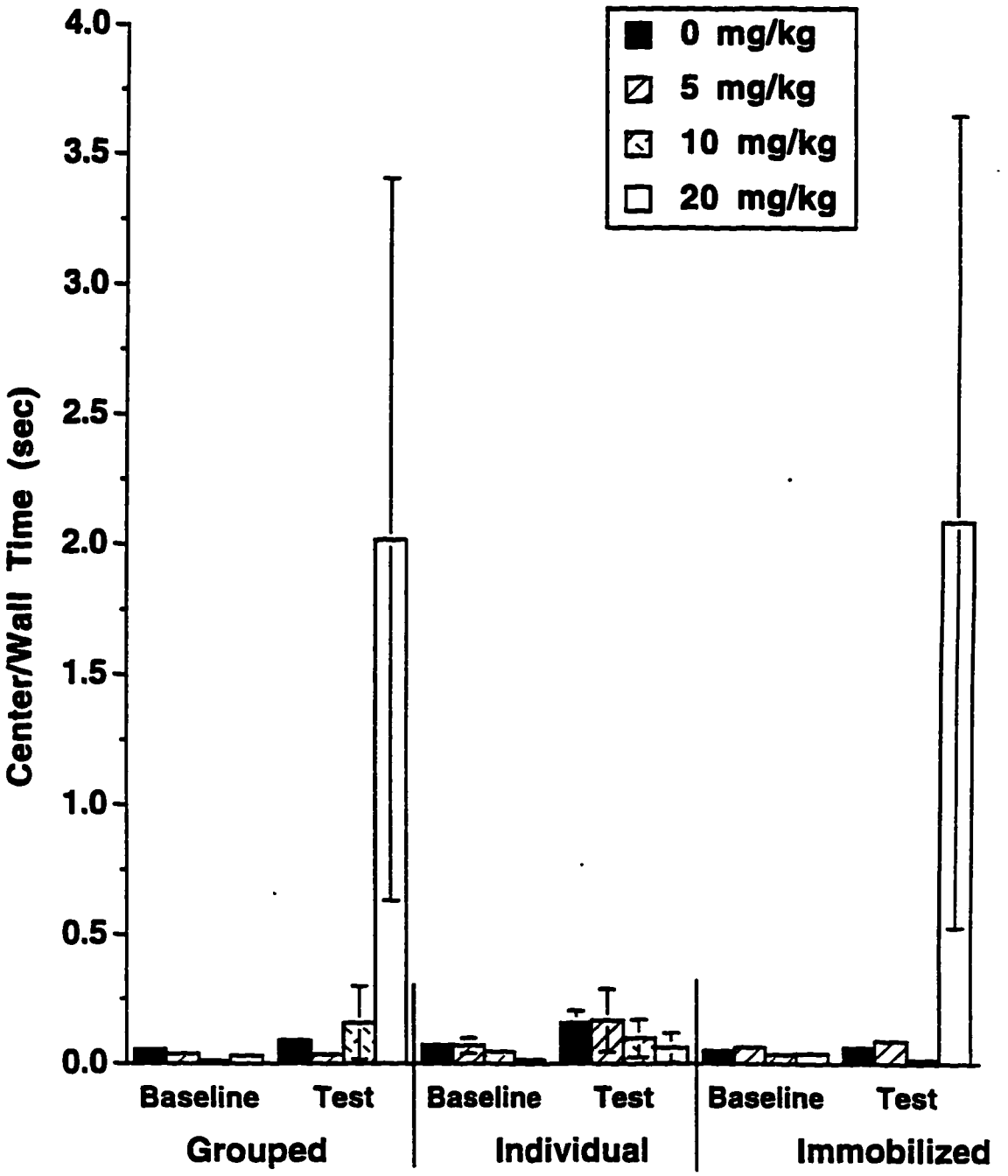
a Significantly different from individually housed Lewis rats on test day (Tukey HSD, $p<.05$).

Figure 32. Experiment 2. Speed of F-344 and Lewis rats during baseline and testing separated by experimental conditions (means and standard errors).



a Significantly different from F-344 rats given 0, 5, or 10 mg/kg morphine on test day (Tukey HSD, $p<.05$).

Figure 33. Experiment 1. Speed of F-344 and Lewis rats during baseline and testing separated by drug dose (means and standard errors).



Note: A higher number is indicative of more center time or less anxiety.

Figure 34. Experiment 1. Thigmotaxic behavior of F-344 rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

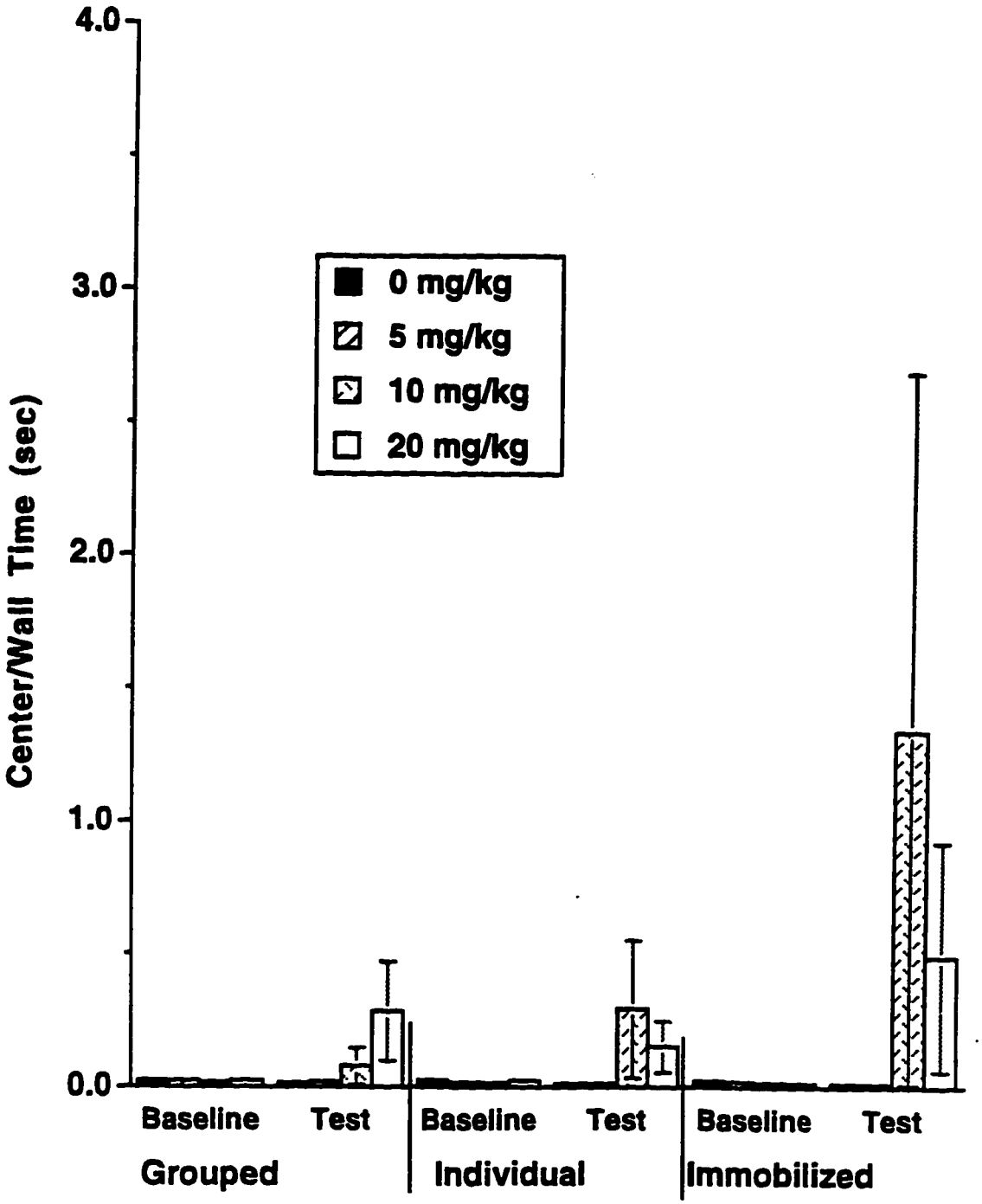


Figure 35. Experiment 1. Thigmotaxic behavior of Lewis rats during baseline and testing separated by drug dose within each experimental condition (means and standard errors).

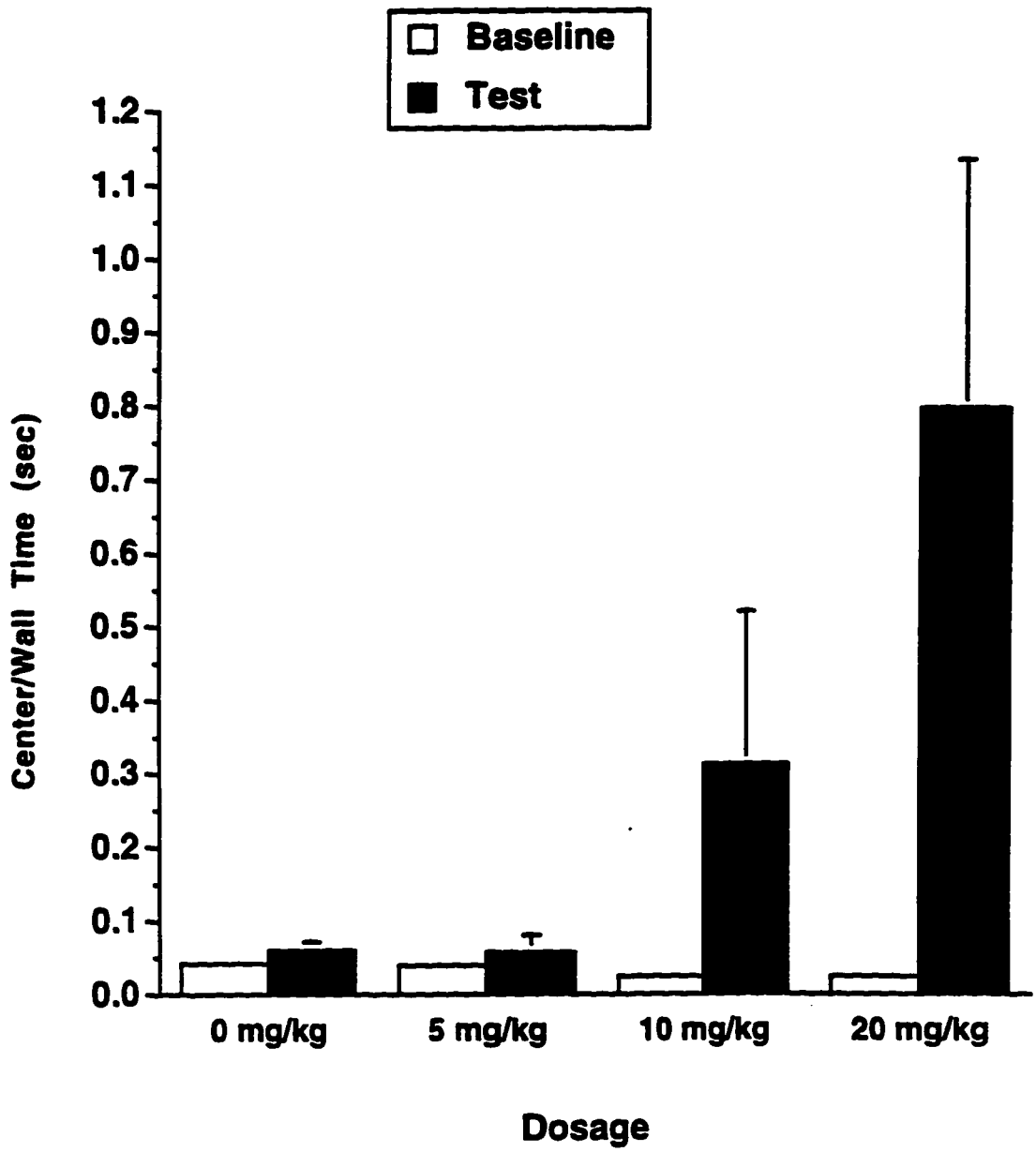


Figure 36. Experiment 1. Thigmotaxic behavior during baseline and testing separated by drug dose (means and standard errors).

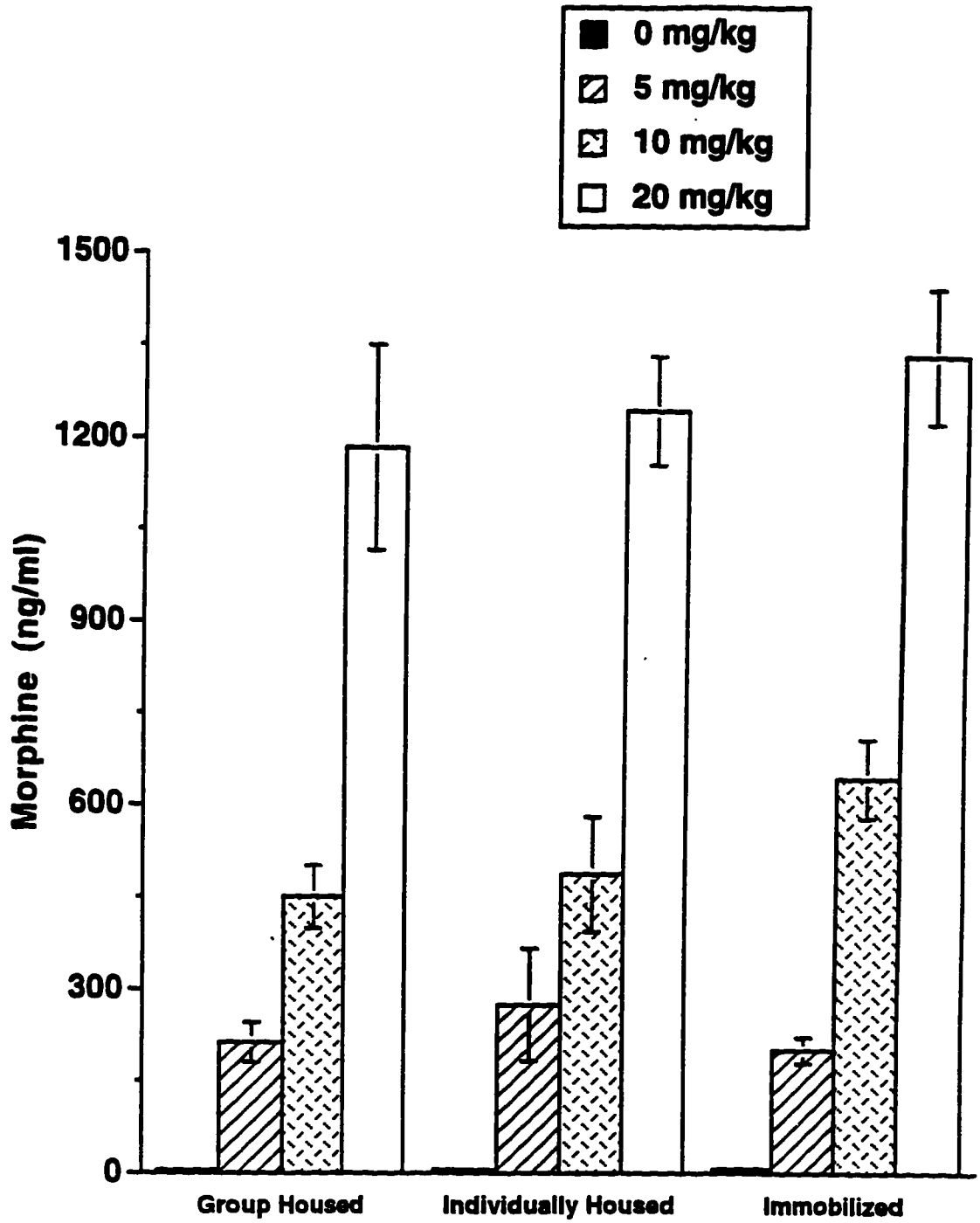


Figure 37. Experiment 1. Serum morphine levels of F-344 rats separated by drug dose within each experimental condition (means and standard errors).

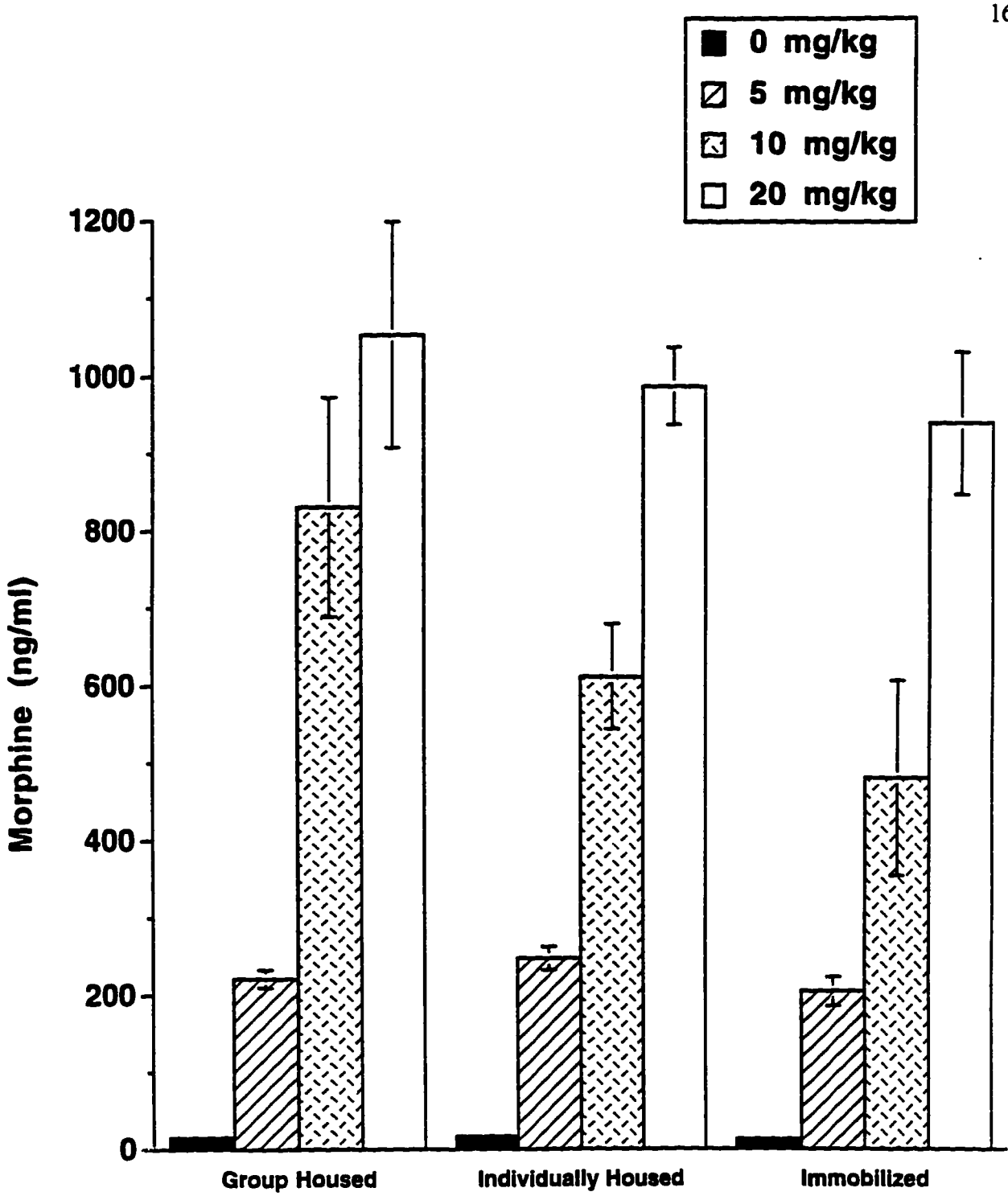


Figure 38. Experiment 1. Serum morphine levels of Lewis rats separated by drug dose within each experimental condition (means and standard errors).

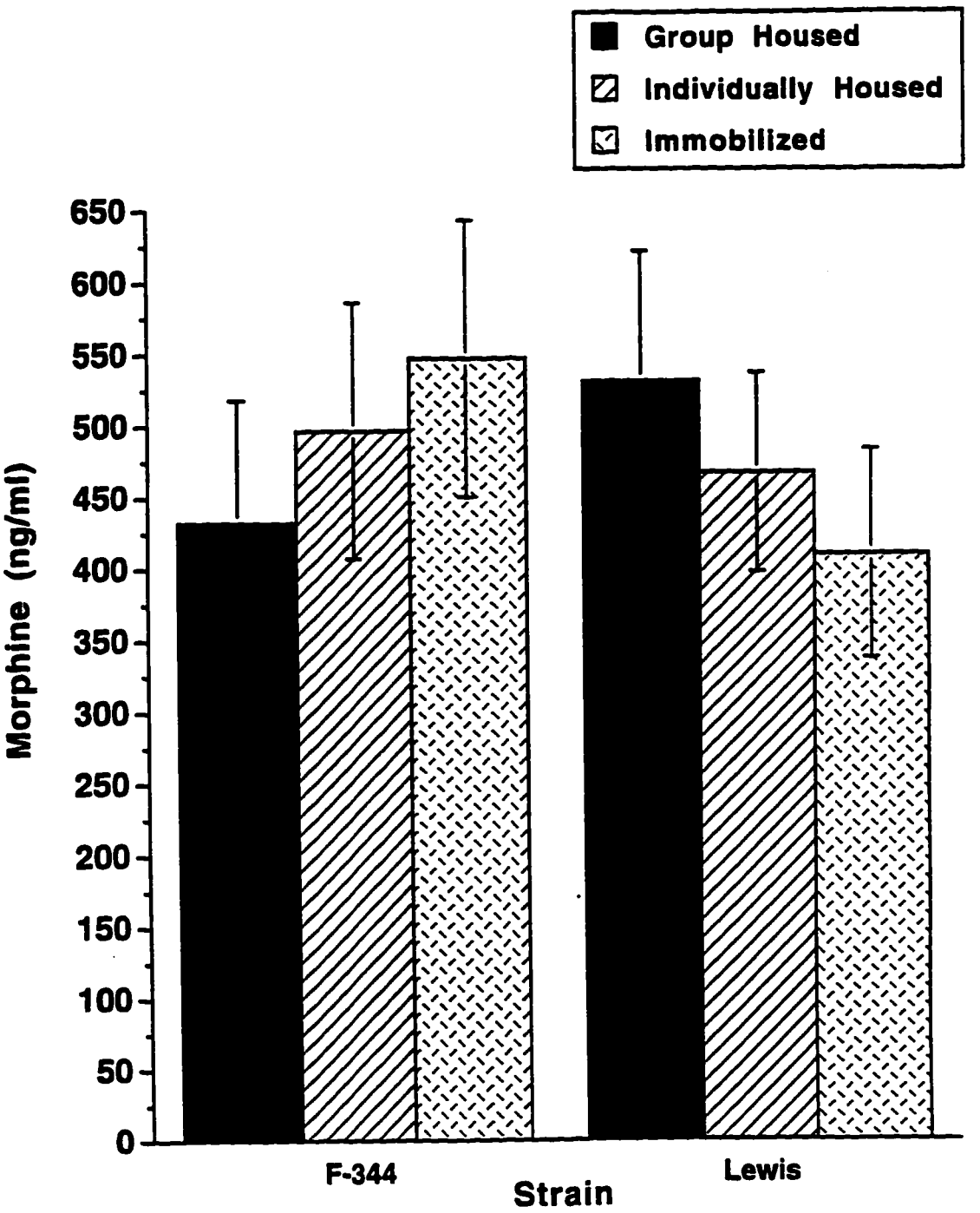
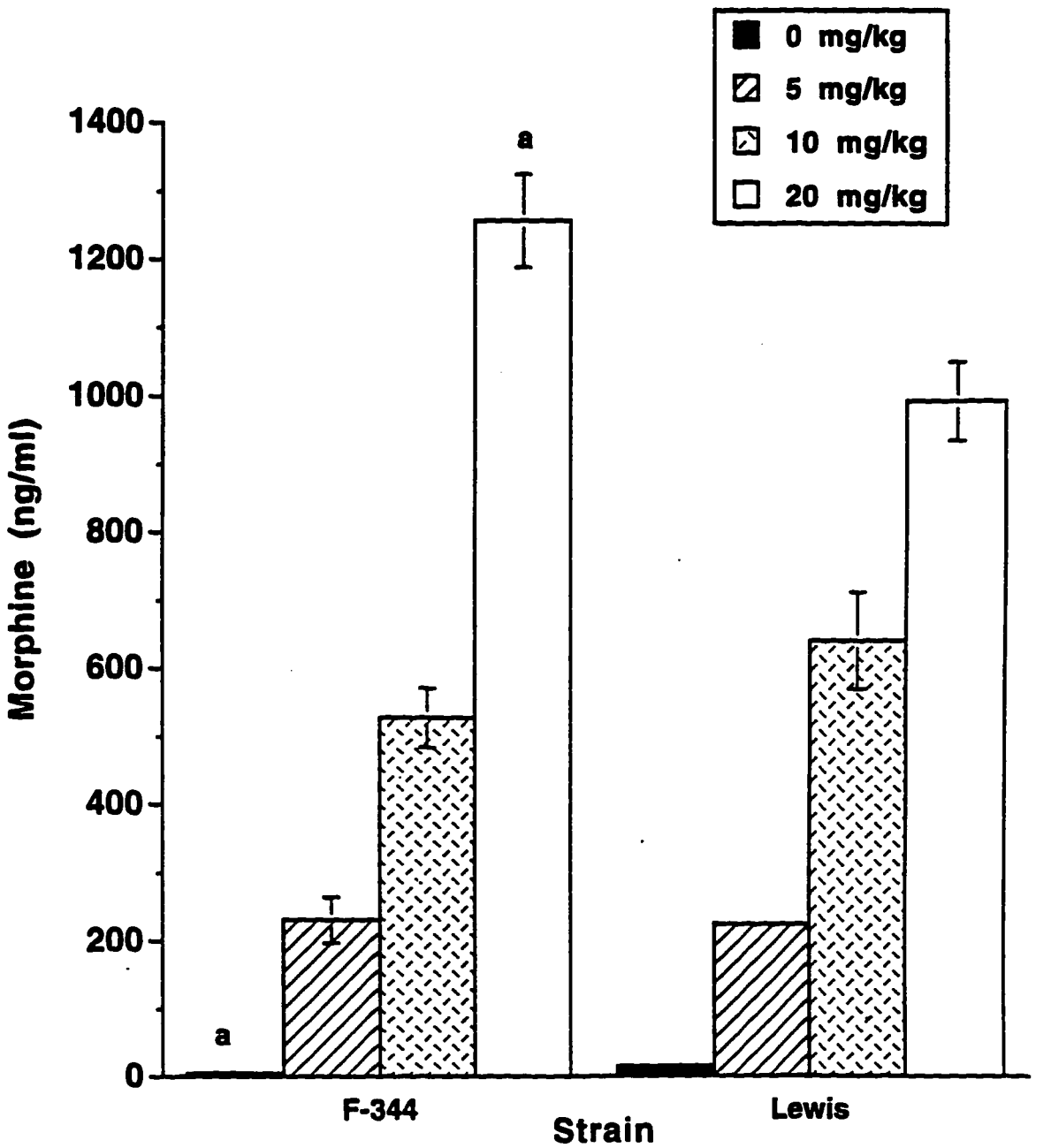


Figure 39. Experiment 1. Serum morphine levels of F-344 and Lewis rats separated by experimental condition (means and standard errors).



a Significantly different from Lewis rats at same drug dose (Tukey HSD, $p<.05$).

Figure 40. Experiment 1. Serum morphine levels of F-344 and Lewis rats separated by drug dose (means and standard errors).

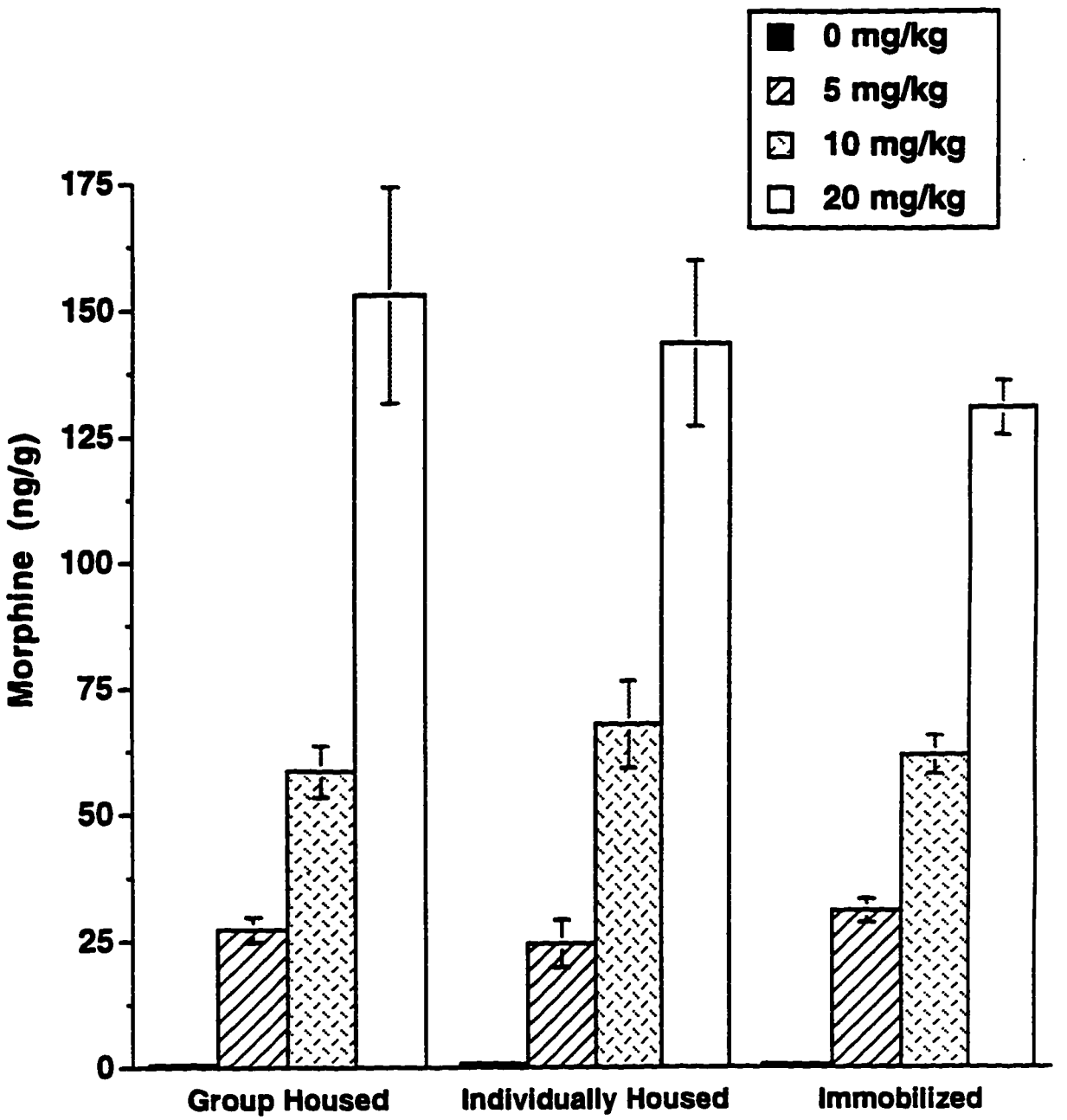
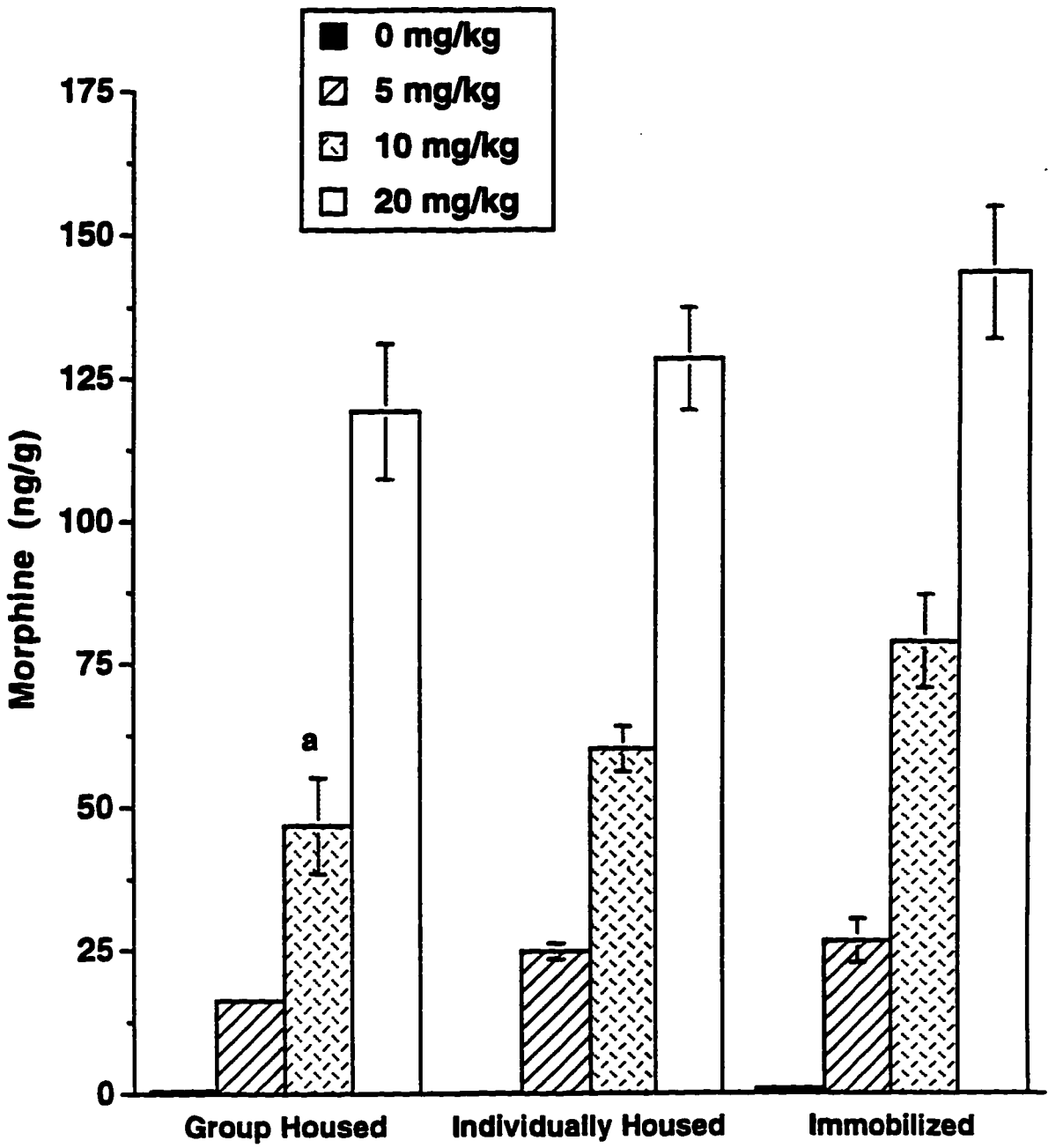


Figure 41. Experiment 1. Brain morphine levels of F-344 rats separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from immobilized rats receiving 10 mg/kg dose of morphine (Tukey HSD, $p<.05$).

Figure 42. Experiment 1. Brain morphine levels of Lewis rats separated by drug dose within each experimental condition (means and standard errors).

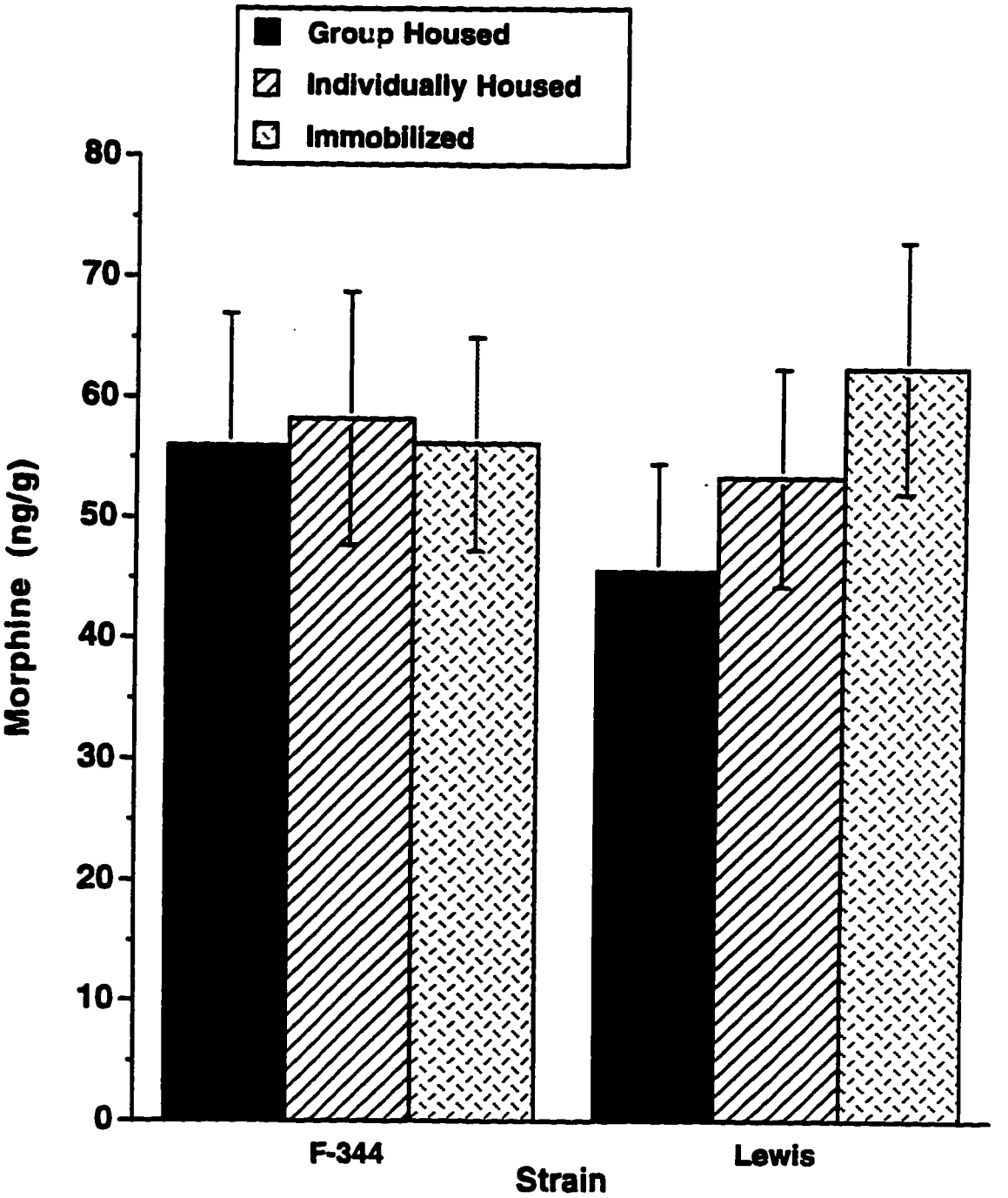
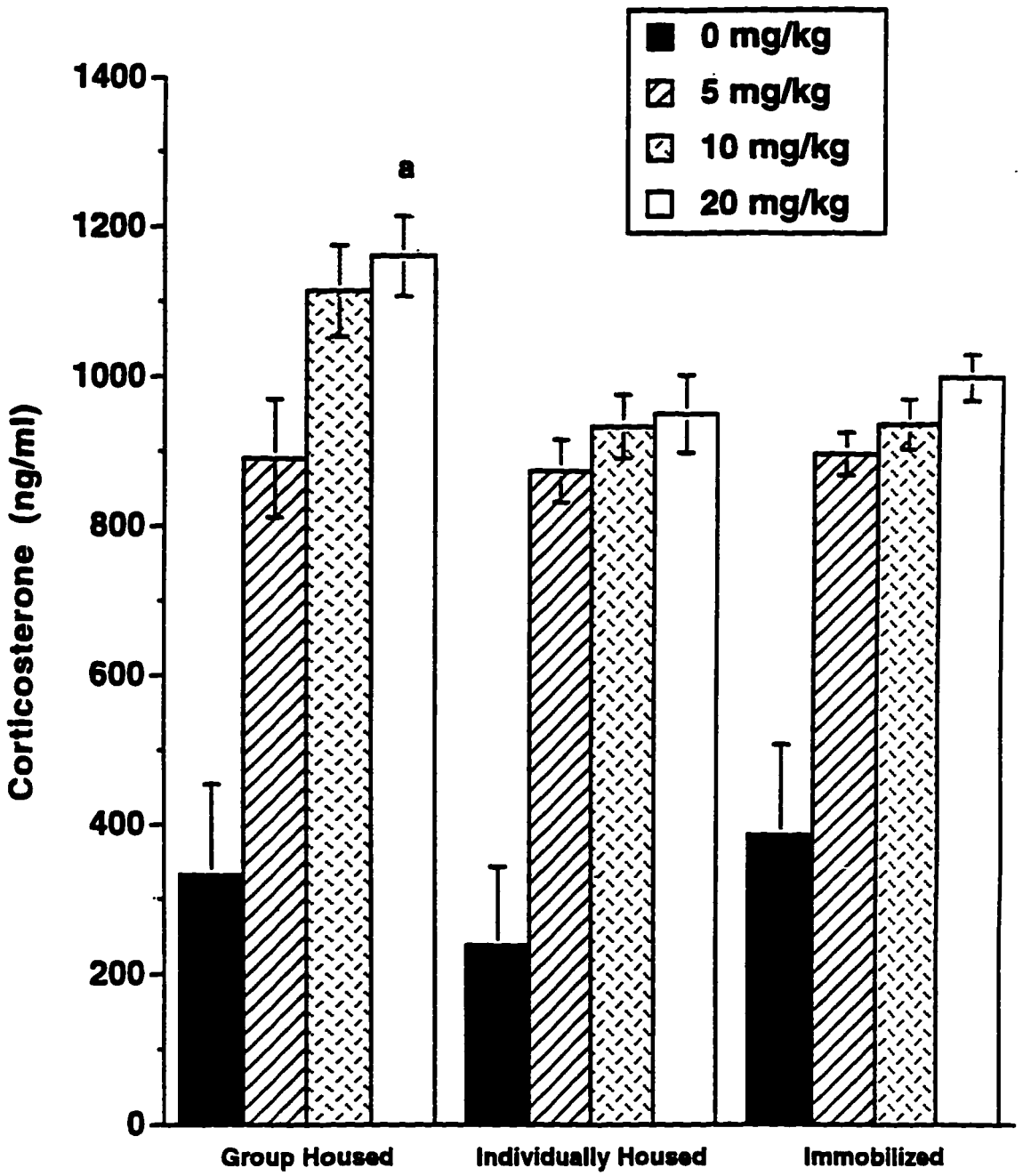
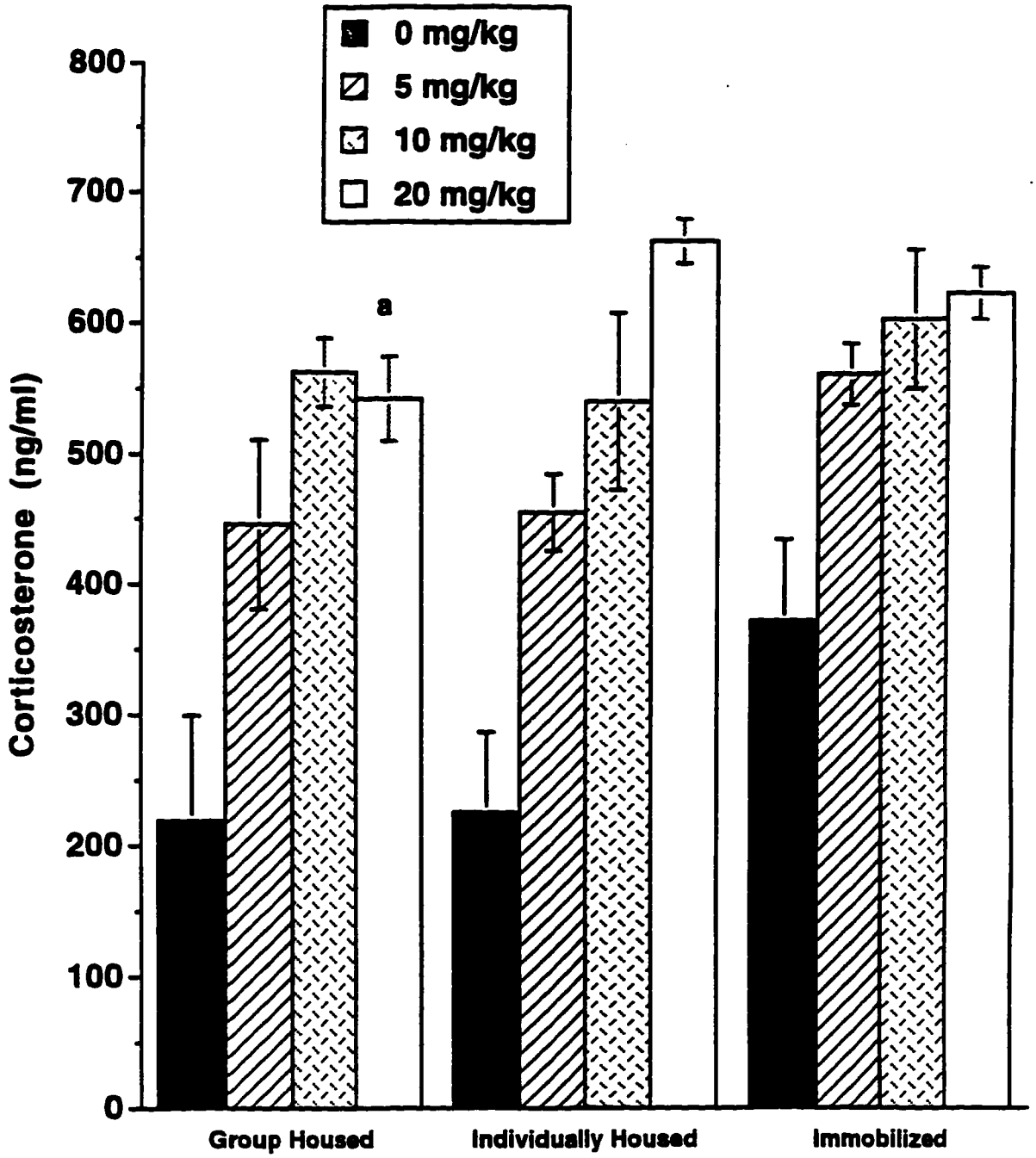


Figure 43. Experiment 1. Brain morphine levels of F-344 and Lewis rats separated by experimental condition (means and standard errors).



a Significantly different from individually housed rats receiving 20 mg/kg dose of morphine (Tukey HSD, $p<.05$).

Figure 44. Experiment 1. Corticosterone levels of F-344 rats separated by drug dose within each experimental condition (means and standard errors).



a Significantly different from individually housed rats receiving 20 mg/kg dose of morphine (Tukey HSD, $p<.05$).

Figure 45. Experiment 1. Corticosterone levels of Lewis rats separated by drug dose within each experimental condition (means and standard errors).

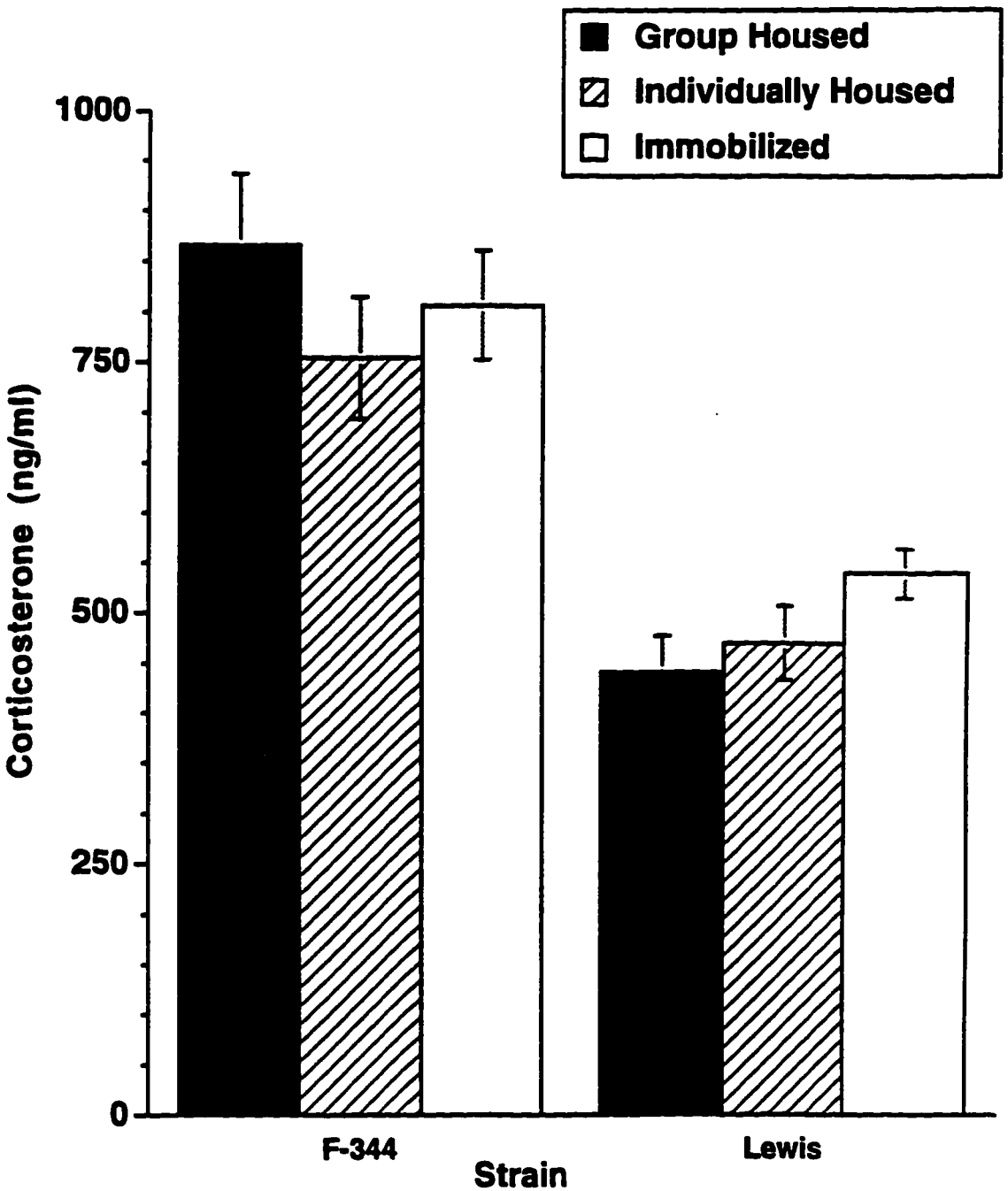
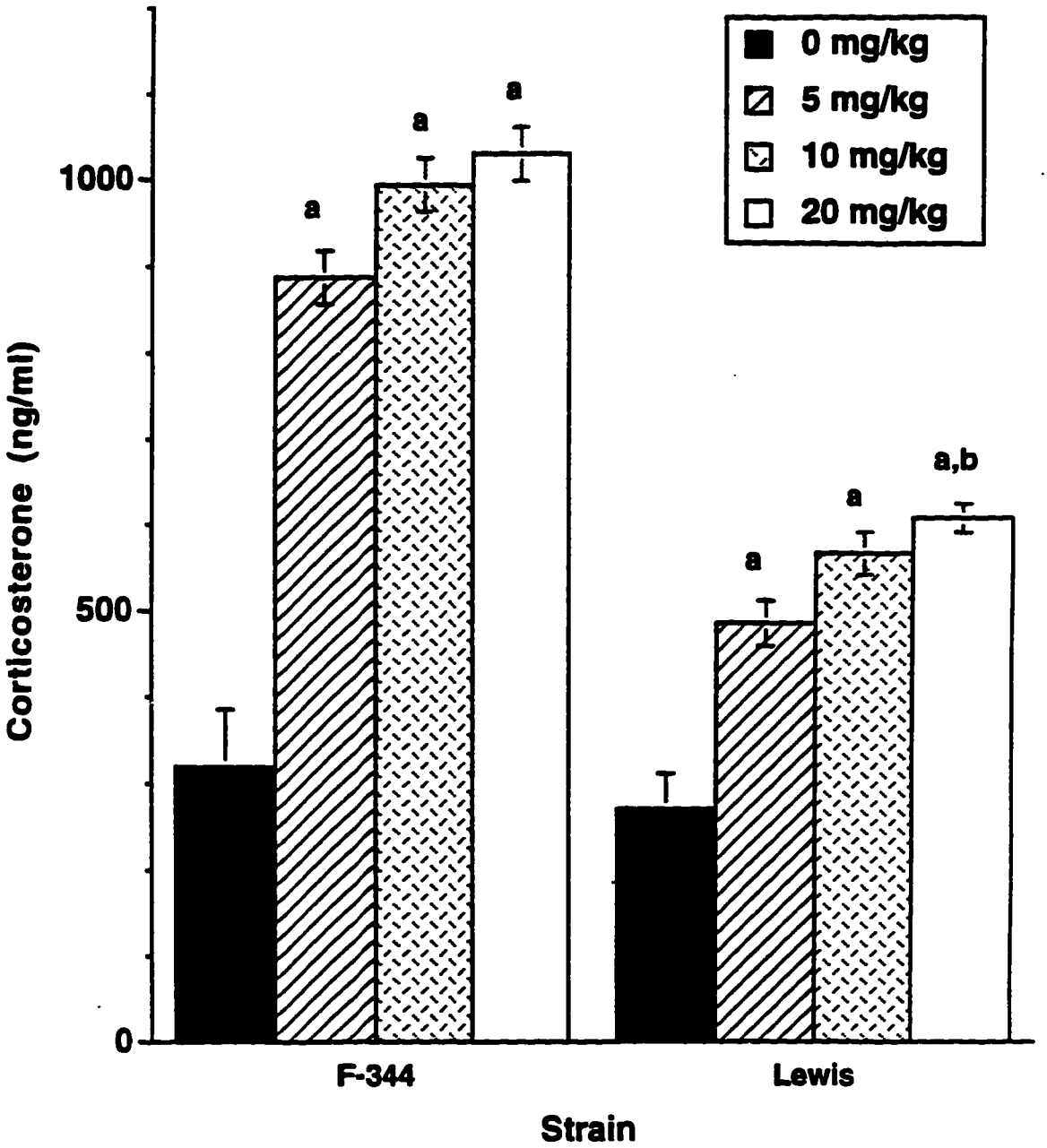


Figure 46. Experiment 1. Corticosterone levels of F-344 and Lewis rats separated by experimental conditions (means and standard errors).



a Significantly different from subjects receiving 0 mg/kg dose for respective strain (Dunnett, $p<.05$)
b Significantly different from Lewis rats receiving 5 mg/kg dose of morphine (Tukey HSD, $p<.05$).

Figure 47. Experiment 1. Corticosterone levels of F-344 and Lewis rats separated by drug dose (means and standard errors).

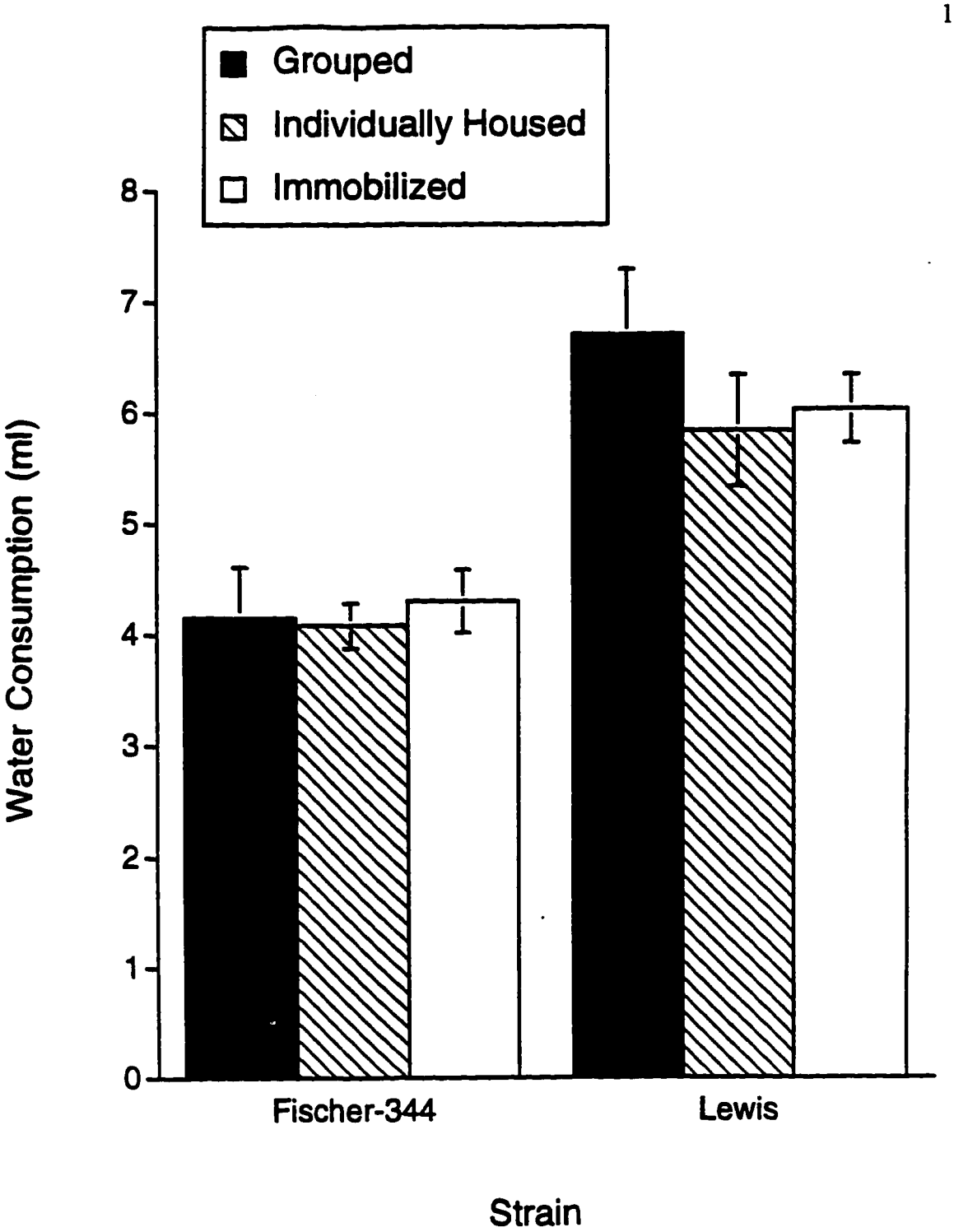
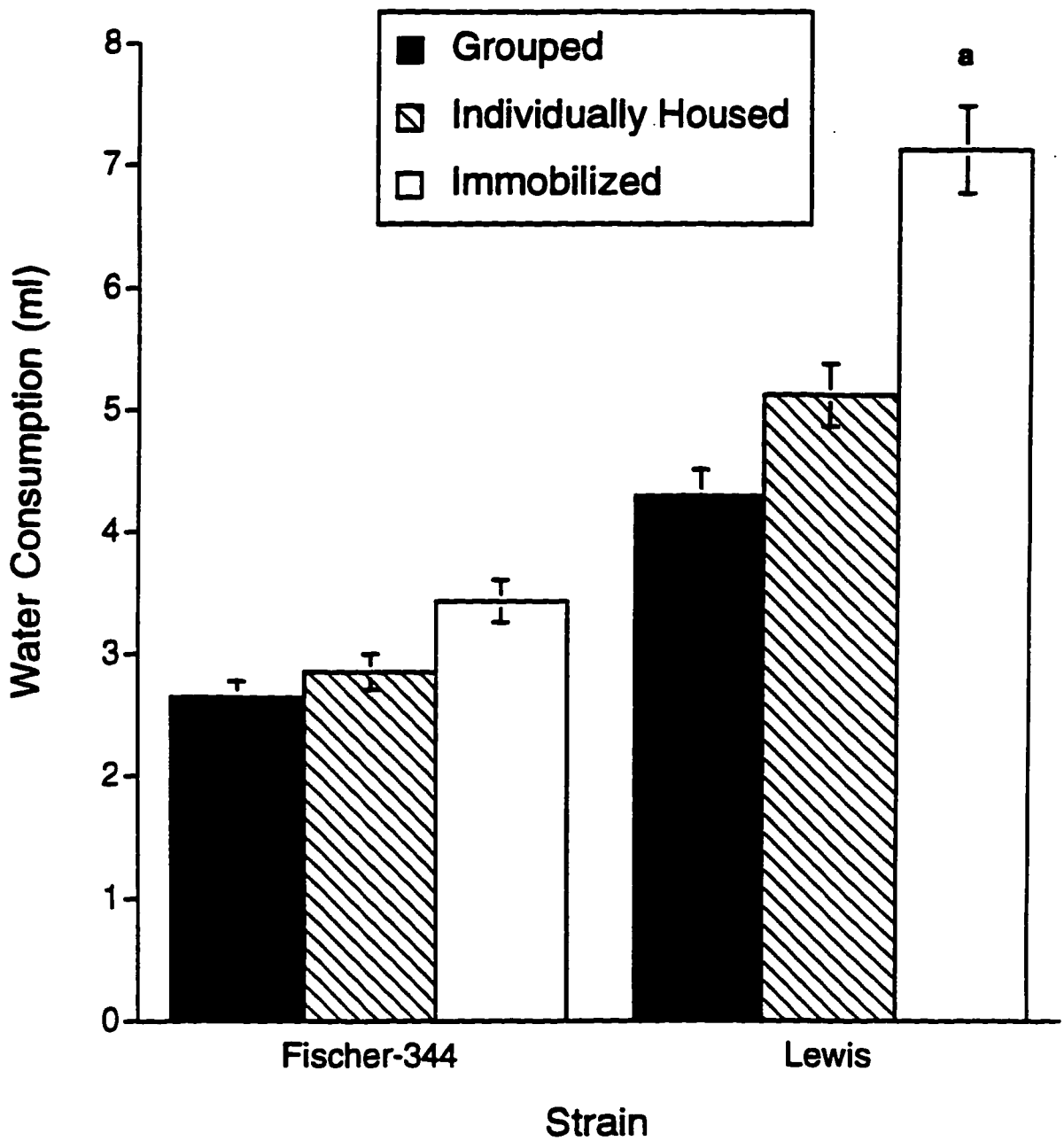
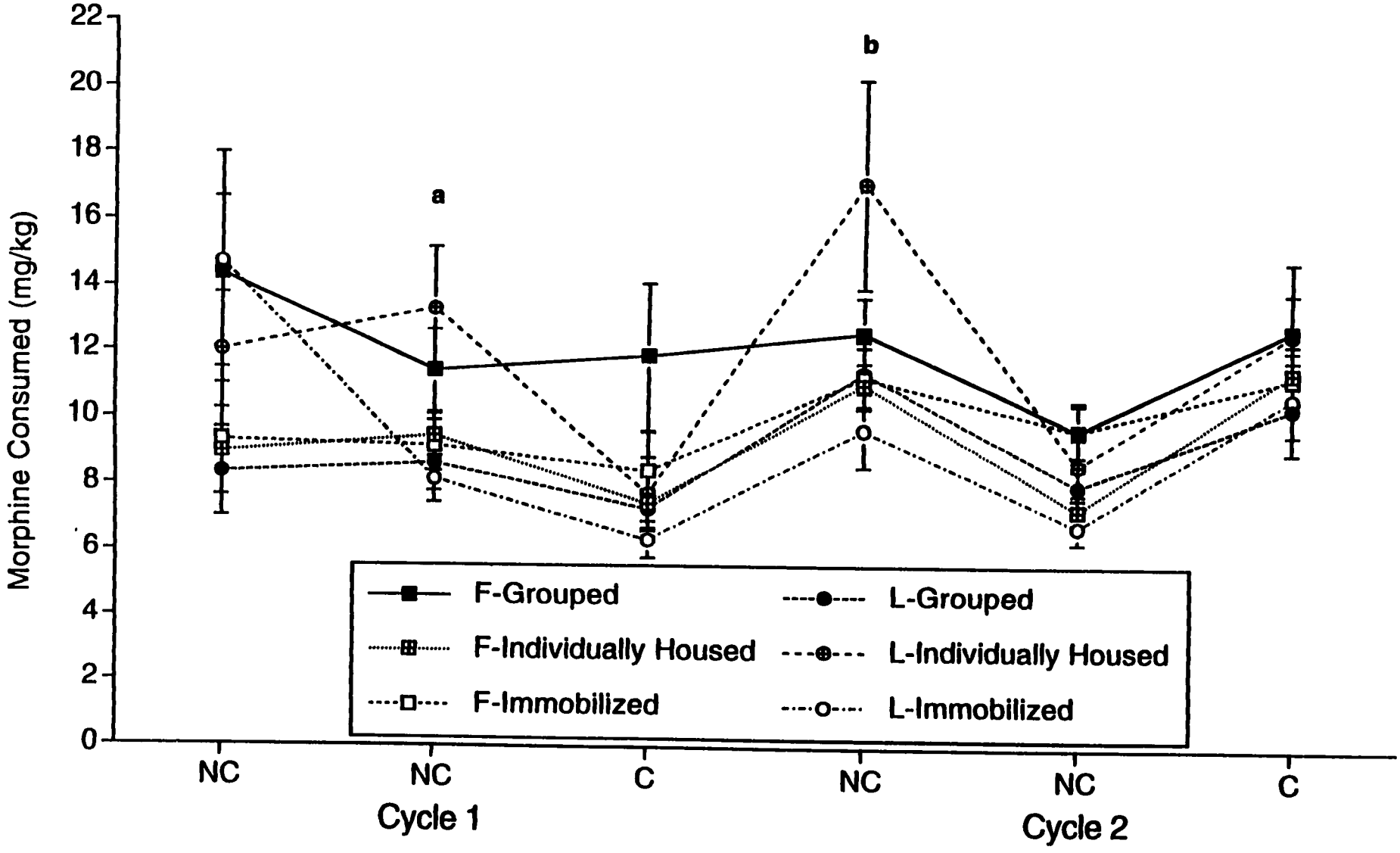


Figure 48. Experiment 2. Phase 1: Two day average of 6-hour water consumption during baseline for F-344 and Lewis rats prior to their assignment to respective treatment condition (means and standard errors).



a Significantly different from grouped or individually housed Lewis rats (Tukey HSD, $p<.05$).

Figure 49. Experiment 2. Phase II: Two day average of 6-hour water consumption during treatment for F-344 and Lewis rats (means and standard errors).



a IH Lewis > G Lewis; IH Lewis >IM Lewis (Tukey HSD, $p<.05$)
b IH Lewis >IM Lewis (Tukey HSD, $p<.05$)

Figure 50. Experiment 2. Phase III: Amount (mg/kg) of 0.5 mg/ml morphine concentration consumed by grouped, individually housed, and immobilized F-344 and Lewis rats across two 3-day cycles consisting of 2 no-choice days (NC) and 1 choice day (C) (means and standard errors).

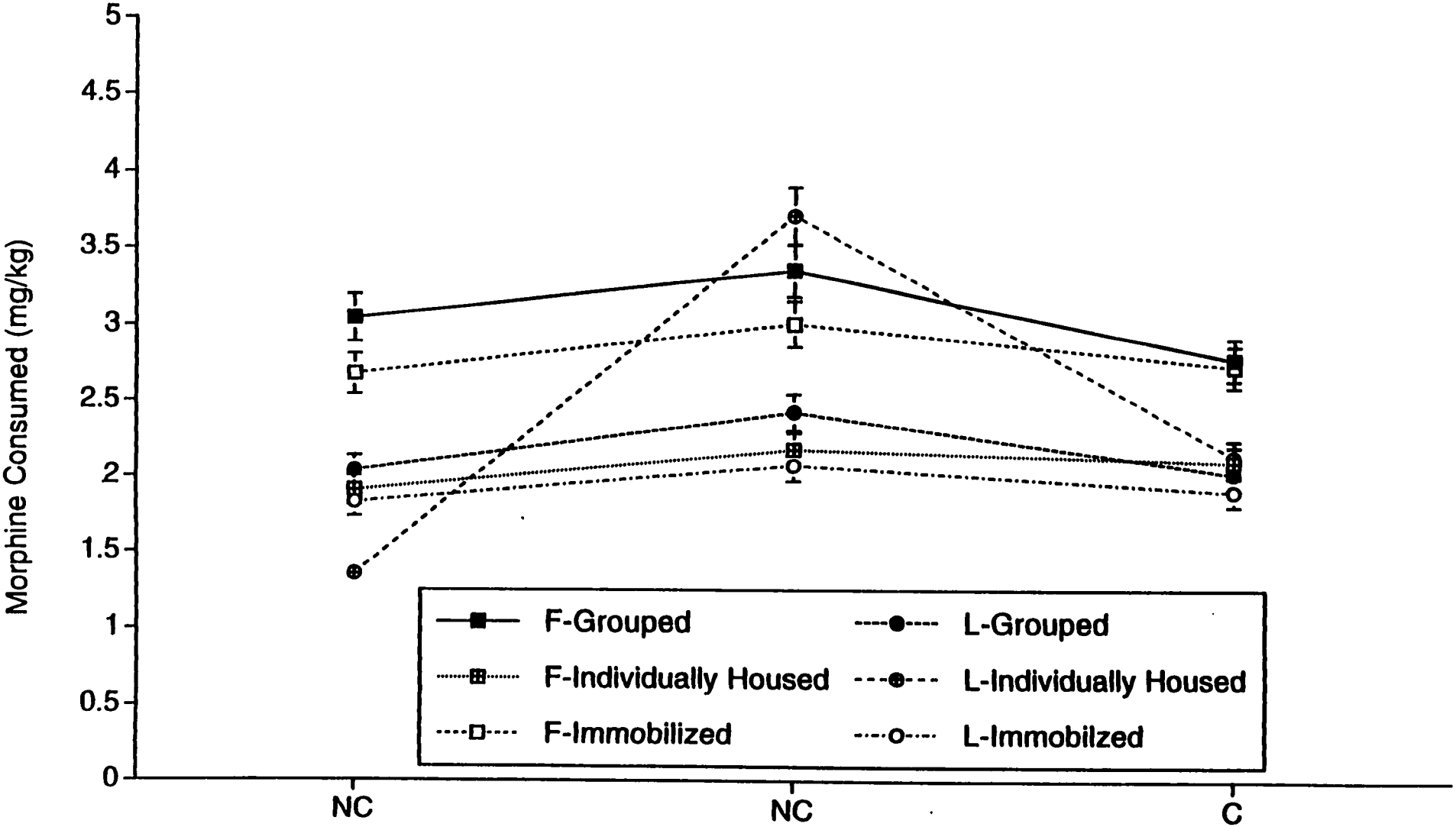


Figure 51. Experiment 2. Phase IV: Amount (mg/kg) of 0.25 mg/ml morphine concentration consumed by grouped, individually housed, and immobilized F-344 and Lewis rats across one 3-day cycle consisting of 2 no-choice (NC) and 1 choice (C) day (means and standard errors).

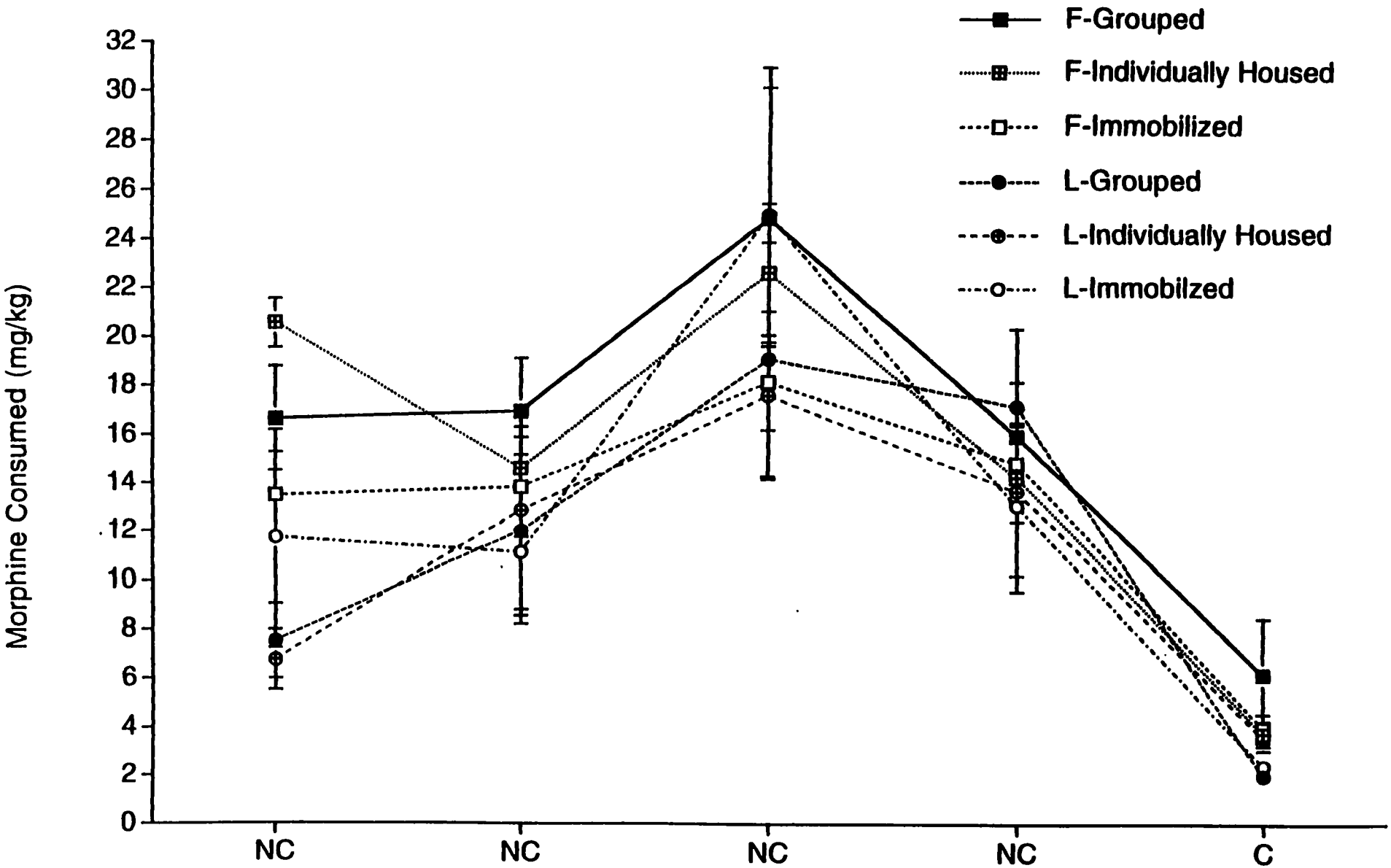
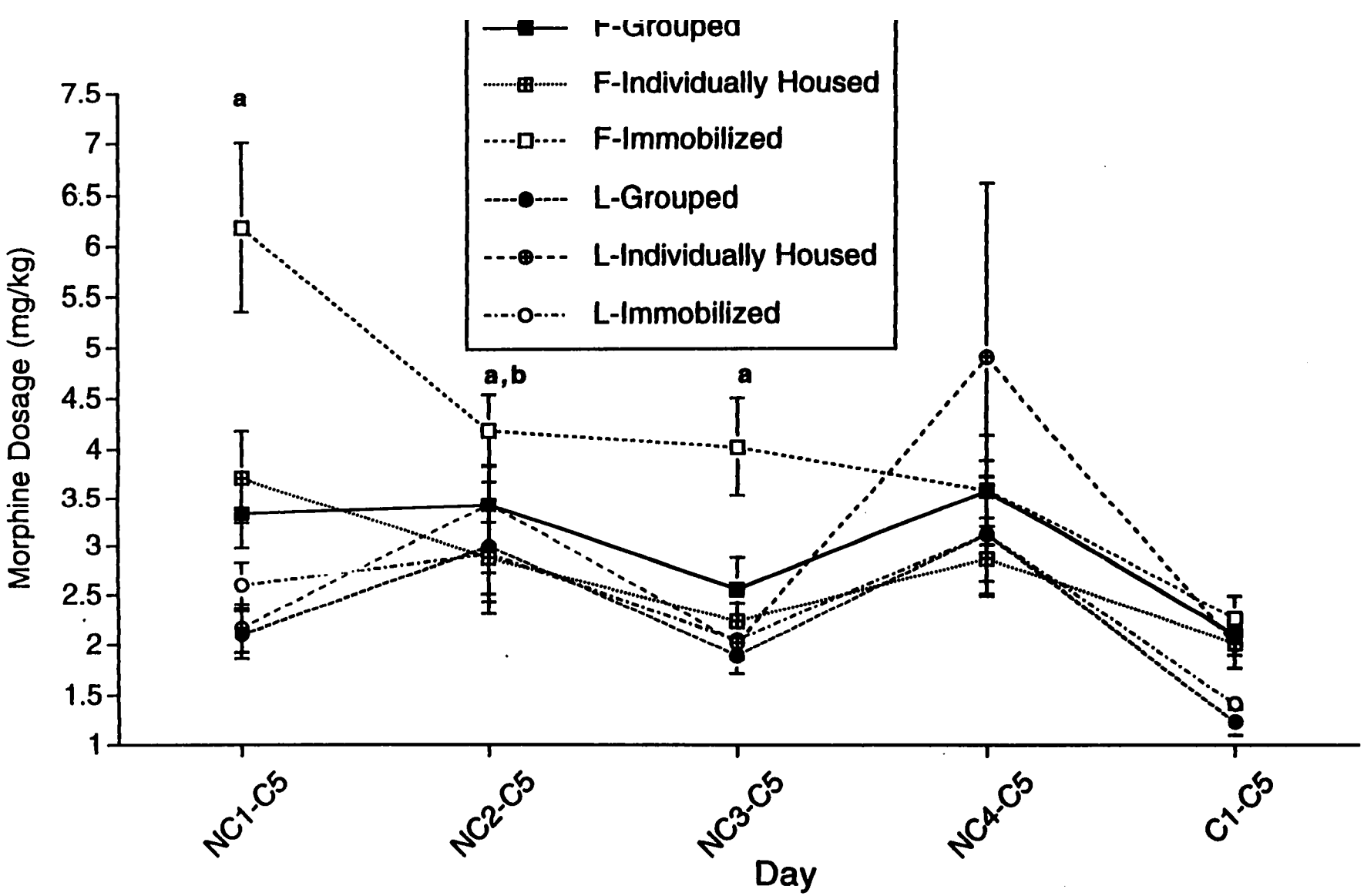


Figure 52. Experiment 2. Phase V: Amount (mg/kg) of 0.25 mg/ml morphine concentration consumed by grouped, individually housed, and immobilized F-344 and Lewis rats following 18-hours of water deprivation across one 5-day cycle consisting of 4 no-choice (NC) and 1 choice (C) day (means and standard errors).



a IM F-344 > GH F-344; IM F-344 > IN F-344 (Tukey HSD, $p<.05$)
b IM F-344 > IN F-344

Figure 53. Experiment 2. Phase VI: Amount (mg/kg) of 0.25 mg/ml morphine concnetration consumed by grouped, individually housed, and immobilized F-344 and Lewis rats across one 5-day cycle (means and standard errors).

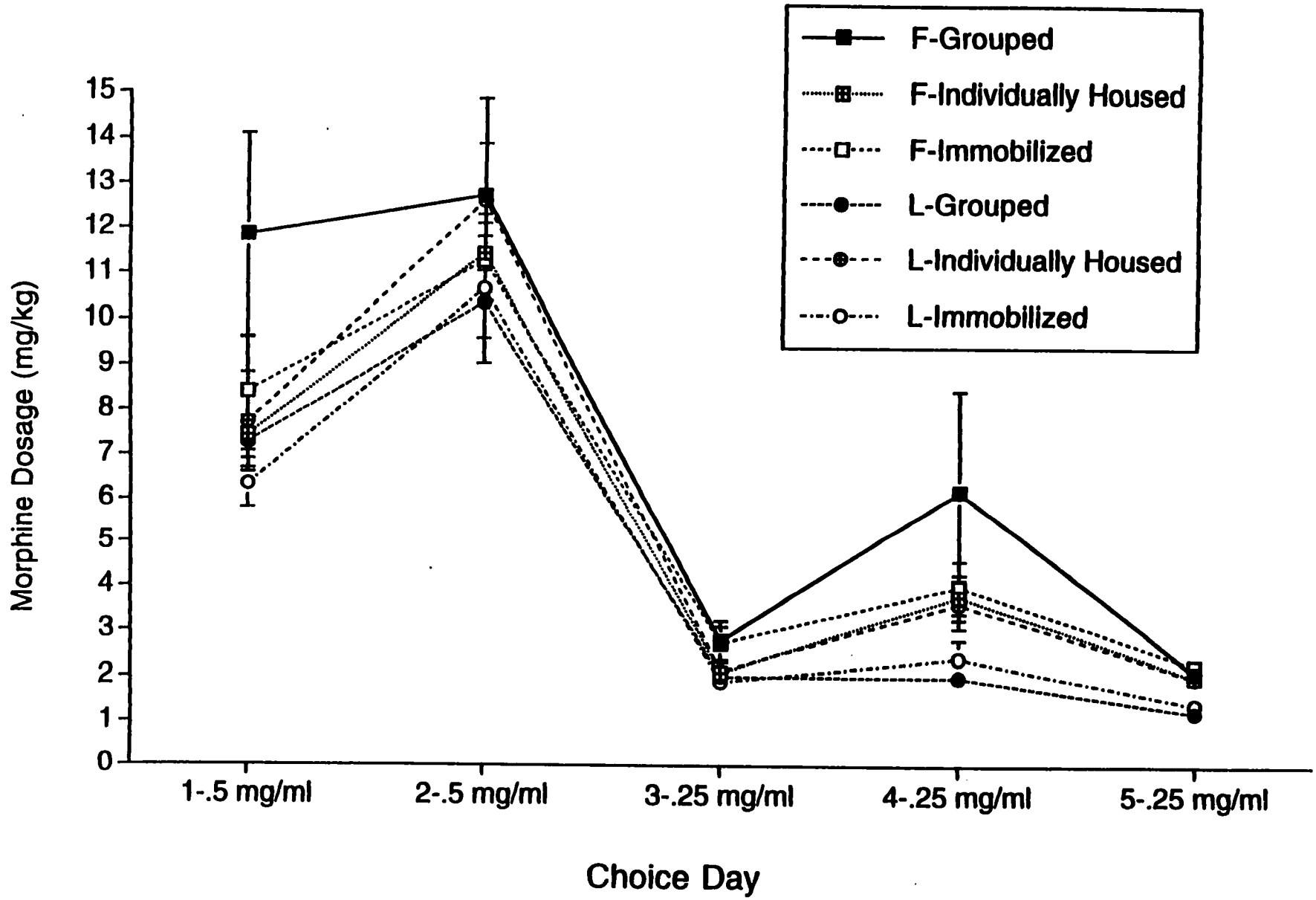
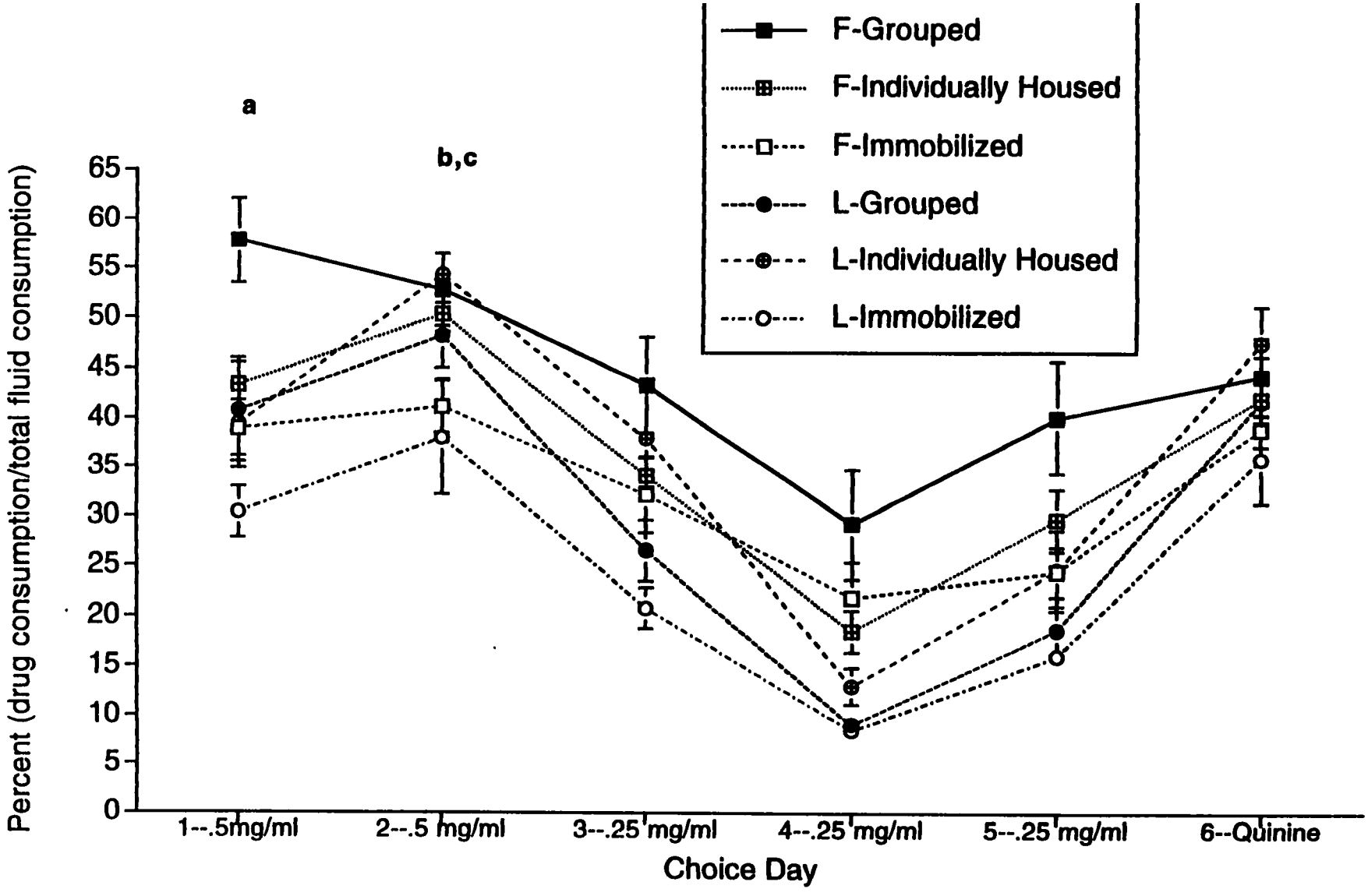


Figure 54. Experiment 2. Choice Days: Amount (mg/kg) of morphine consumed by grouped, individually housed, and immobilized F-344 and Lewis rats on choice days 1-5 at the specified drug concentration (means and standard errors).



a GH F-344 > IN F-344; GF-344 > IM (Tukey HSD, $p<.05$)
b GH F-344 > IM F-344 (Tukey HSD, $p<.05$)
c IN Lewis > IM Lewis

Figure 55. Experiment 2. Choice Days: Percent of morphine or quinine consumed by grouped, individually housed, and immobilized F-344 and Lewis rats on choice days 1-6 at the specified drug concnetration(means and standard errors).

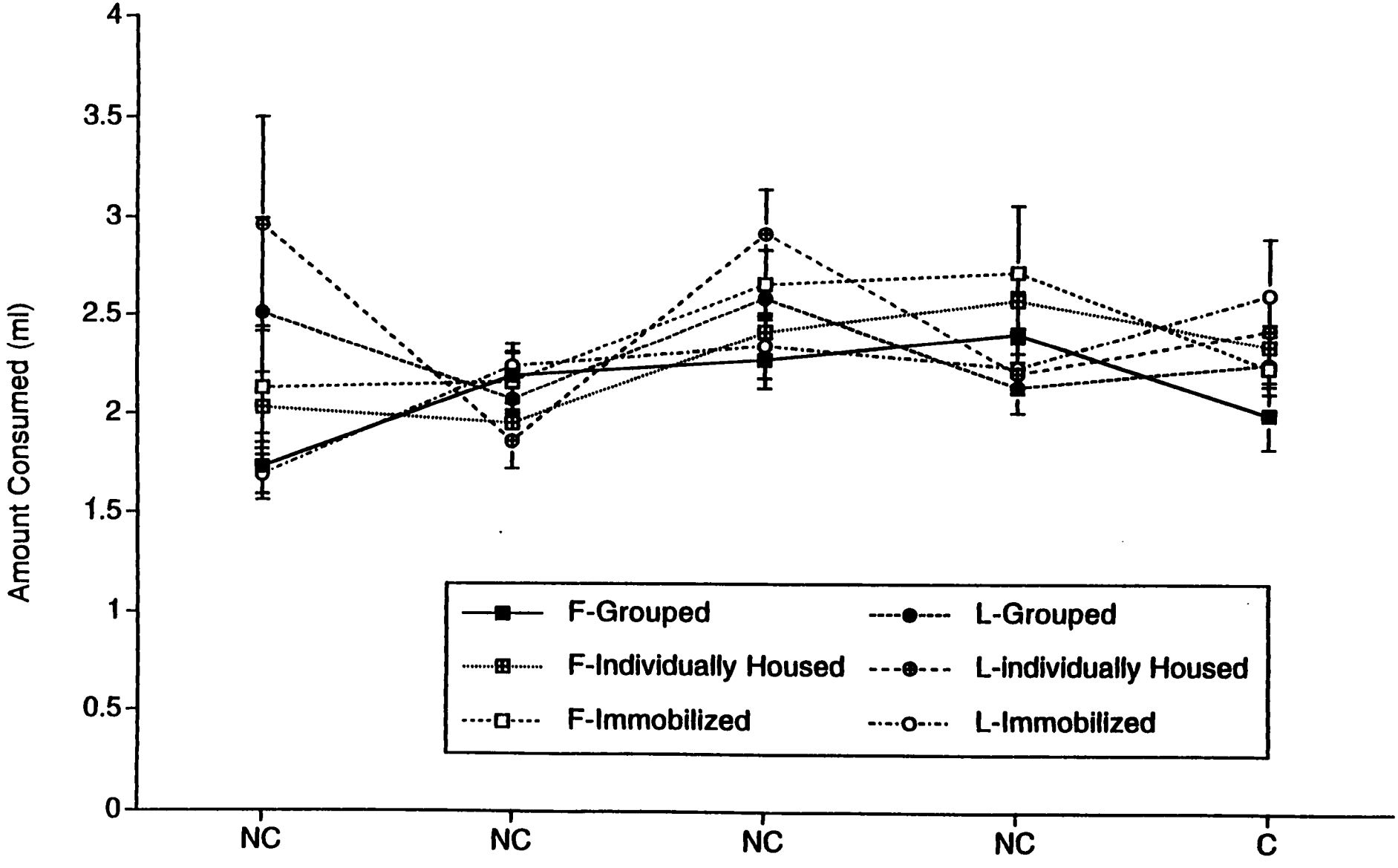
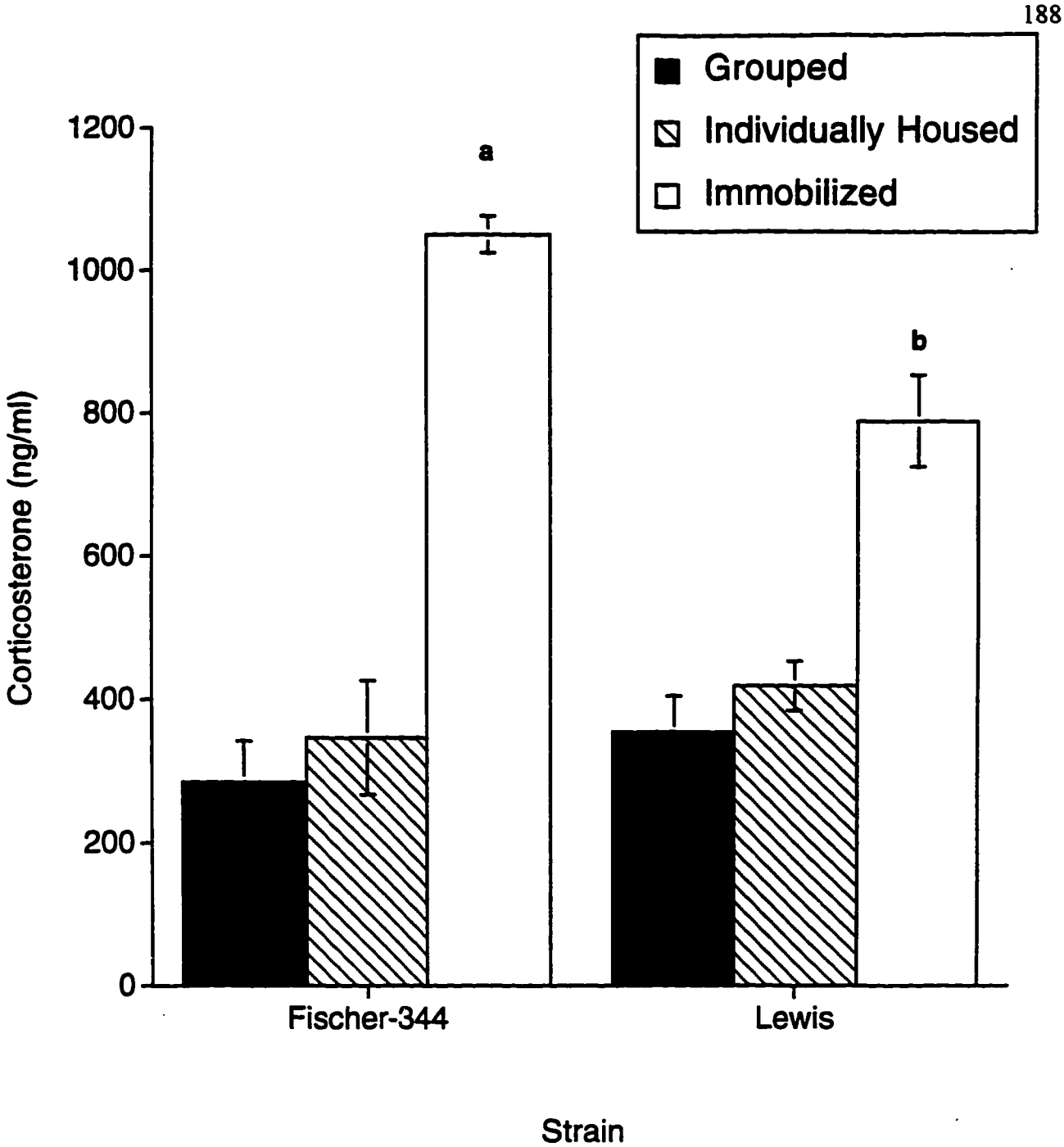


Figure 56. Experiment 2. Phase VI: Amount of quinine consumed by grouped, individually housed, and immobilized F-344 and Lewis rats across one 5-day cycle consisting of 4 no-choice (NC) and 1 choice (C) day (means and standard errors).



a IM F-344 > GH F-344; IM F-344 > IN F-344 (Tukey HSD, $p<.05$)
b IM Lewis > GH Lewis; IM Lewis > IN Lewis (Tukey HSD, $p<.05$)

Figure 57. Experiment 2. Corticosterone levels of F-344 and Lewis rats separated by experimental condition (means and standard errors).

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