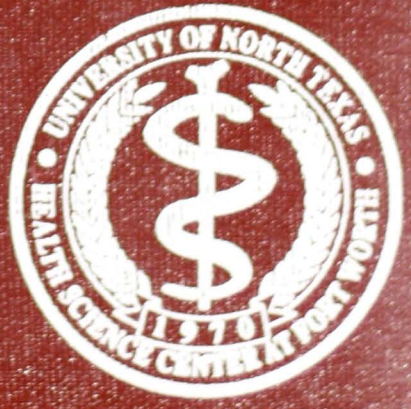
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Odom, Linda Ann, SENSITIZATION TO COCAINE: BEHAVIORAL AND GENETIC CHARACTERIZATION. Doctor of Philosophy (Pharmacology). April 1998, 141 pages, 2 tables, 23 figures, 89 references.

Conditioned associations between environmental context and cocaine effects may play a significant role in acquisition and maintenance of cocaine dependence. Conditioning may also contribute significantly to cocaine sensitization, a leftward shift in the cocaine dose-response curve that is attributable to cocaine pre-exposure. Both studies examined the sensitization of cocaine's behavioral effects after one or four prior exposures to cocaine in two distinct environments, allowing evaluation of the acquisition and magnitude of sensitization to cocaine and the contribution of conditioning to sensitization. An extinction component was added to the second study to allow determination of persistence of context-dependent sensitization in C57BL/6 and DBA/2 mice. The purpose of the first study was to fully characterize the quantity and quality of the sensitized behavioral response to cocaine in Swiss Webster mice and to determine parameters for sensitization in the second study. Results of this study indicated that pairing cocaine to the testing environment resulted in a leftward shift of the dose-response curves for both horizontal and stereotypy measures and a concurrent decrease in maximal effect of cocaine on horizontal distance and an increase in maximal effect of cocaine on stereotypy. The multivariate behavior profile indicated that the sensitized response to cocaine was best observed in response to 1 to 5 mg/kg cocaine, and that the conditioned response elicited by

saline following cocaine pre-exposure closely resembled the 10 mg/kg acute cocaine response. The overall purpose of the second study was to determine if genetic differences in various aspects of such conditioned associations could contribute to individual differences in cocaine dependence. It was determined that, although DBA/2 mice had a faster rate of acquisition of context-dependent sensitization to cocaine than C57BL/6 mice, the multivariate behavior profile of the conditioned response of C57BL/6 mice resembled the behavior observed with a higher dose of acute cocaine and had greater magnitude and greater persistence than that of DBA/2 mice, which may explain in part the susceptibility of the C57BL/6 mice to cocaine dependence.

SENSITIZATION TO COCAINE:

BEHAVIORAL AND GENETIC

CHARACTERIZATION

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SENSITIZATION TO COCAINE:

BEHAVIORAL AND GENETIC CHARACTERIZATION

DISSERTATION

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas Health Science Center at Fort Worth

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Linda Ann Odom, B.A.

Fort Worth, Texas

April 1998

Acknowledgments

I would like to express my sincere gratitude to Dr. Michael Forster of the Department of Pharmacology at the University of North Texas Health Science Center at Fort Worth for his long hours spent working on this project with me, his generous financial support of the project, and his great sense of humor about everything. I would also like to thank him for providing me with an excellent, reliable and dedicated technician, Samantha Wild, to do the work my handicap prevented me from completing. My appreciation is also extended to Samantha for her long hours of work.

I also thank Dr. Michael Gatch for his help with the statistical problems presented in this research. His patience in helping me was invaluable. I would like to thank the remaining members of my committee, Drs. Glenn Dillon, Harbans Lal, Scott Stoll, Cleatus Wallis and Judy Wilson, for their critical review and constructive comments concerning my research proposal and manuscripts.

I must conclude by thanking my mother, Gale Odom, who provided consistent encouragement and financial support, which made this entire effort possible. She believed in me when I did not, and for that, I will always be grateful. This dissertation is dedicated to her.

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CHAPTER I

INTRODUCTION

The Problem of Relapse in Cocaine Dependence

Cocaine addiction is a serious public health problem in the USA, and currently there is no effective treatment. As of 1991, twenty-one million Americans had used cocaine and estimates of the dependent population varied from one to three million people (figures cited in Noble et al, 1993 from a National Institute of Drug Abuse (NIDA[1]) 1991 report). Drug dependence is a chronic relapsing disorder and as such, relapse can be anticipated in the course of treatment, just as exacerbations are expected in the management of diabetes or heart disease (O'Brien et al, 1992a). Most often, relapses are blamed on obsessive drug cravings initiated by exposure to cocaine-associated environmental triggers (Childress et al, 1993; O'Brien et al, 1992b).

Craving has been defined in the United Nations International Drug Control Programme and the World Health Organization's 1992 report as "the desire to experience the effect(s) of a previously experienced psychoactive substance" (Markou et al, 1993). Robinson and Berridge (1993) postulate that craving occurs as internal and environmental cues, through repeated pairings with cocaine use, acquire the motivational properties of the drug itself. These cues can then

enhance the "central motivational state" of an organism, which in turn elicits exploratory and instrumental responses that can lead to drug-taking (Markou et al, 1993). Examples of neutral stimuli which can come to elicit cocaine craving in humans after repeated pairing with drug effects include the sight of a white, powdery substance, a pharmaceutical smell, passing by a drug-buying location, or seeing someone with whom the subject once used cocaine (O'Brien et al, 1993).

When skin conductance and a craving questionnaire were used to measure physiological reactions and craving in response to cocaine-related stimuli in humans, the group which had used cocaine in the past showed higher skin conductance scores and craving than the group with no cocaine history (Ehrman et al, 1992; Negrete and Emil, 1992). In addition, this reactivity was specific for cocaine-related cues since the same craving and physiological responses were not observed with opiate-related stimuli (Ehrman et al, 1992). These environmental triggers can precipitate a conditioned state of arousal similar to that obtained with cocaine use. In effect, these environmental cues can serve as a small dose of cocaine or a priming dose. Studies in animals and humans have shown that a priming dose will induce a conditioned "high," craving and drug-seeking behavior (Weissenborn et al, 1995; Jaffe et al, 1989; Stewart and deWit, 1987; Gerber and Stretch, 1975). Addicts report experiencing a cocaine taste at the back of the throat, a faint ringing in the ears, a feeling of excitement and sexual arousal,

and a hot or cold "rush" when environmental cues trigger their sensation of craving (Childress et al, 1993).

Currently, some cocaine abstinence programs employ extinction training to remove the conditioned responses humans have to certain environmental stimuli. This extinction training has shown increased abstinence rates, but is not perfect since not all triggers can be anticipated much less replicated safely in the rehabilitation program (O'Brien et al, 1992b). For example, in order to promote the ability of recovering cocaine addicts to stay abstinent even if they find themselves in the places in which they previously purchased cocaine, it was suggested that they be escorted to these places repeatedly to extinguish their conditioned craving through being exposed to the location without subsequent cocaine use. It was decided that this was too dangerous both for the recovering addicts and for the staff who would accompany them (O'Brien et al, 1992b).

The Role of the Mesolimbic Dopaminergic System in Drug Dependence

Incentive salience (wanting or craving) and reinforcement (liking or pleasure) are both thought to be controlled by the same part of the brain which controls locomotor behavior (forward motion or drug-seeking). That part of the brain is the mesolimbic dopaminergic system, which is composed in part by the ventral tegmental area's (VTA) dopaminergic projections to the nucleus accumbens (Robinson and Berridge, 1993; Kalivas and Duffy, 1990; Wise and Bozarth, 1987). Repeated, intermittent use of cocaine or other psychostimulant drugs

leads to an enhancement of drug effects, which is termed sensitization, while chronic use results in a decrease in drug effects at a given dose which is termed tolerance (c.f. Fontana et al, 1993; Emmett-Oglesby et al, 1993; Emmett-Oglesby and Lane, 1992; Reith et al, 1987). Sensitization of the mesolimbic dopaminergic system by repeated cocaine doses results in enhanced motor responses to cocaine and may promote the evolution of drug-wanting into obsessive drug-craving (Robinson and Berridge, 1993). There is also evidence that the reinforcing effects of cocaine become sensitized after repeated exposure to cocaine (Shippenberg and Heidbreder, 1995). Prior exposure to cocaine has been reported to enhance cocaine conditioned place preference, an experimental procedure which measures the reinforcing effects of cocaine by offering the animals a choice of two compartments, one of which has been paired with cocaine, and the other which has been paired with saline (Shippenberg and Heidbreder, 1995). After several pairings, the animals begin to associate the drug-paired compartment with the effects of the drug and show a preference for that compartment, and those that were pre-exposed to cocaine show a sensitization of cocaine conditioned place preference.

As mentioned previously, environmental stimuli present at the time of drug use become associated with the effects of the drug and, through repeated pairing with the drug, can acquire the ability to elicit craving (Robinson and Berridge, 1993). Craving for cocaine cannot be easily assessed in animal models (Markou et al, 1993). However, one could consider the context-dependent increases in

motor behavior observed after repeated cocaine exposure as a model of the conditioned aspect of drug-craving, recognizing that both sensitization and craving appear to be mediated by the mesolimbic dopaminergic system.

To simulate the human development of craving in response to environmental cues, the experiments contained herein paired cocaine with the environment of the testing chamber used to monitor behavior in the "paired" group of mice and with the home cage in the "unpaired" group. Presumably through Pavlovian conditioning the previously neutral stimulus of the testing chamber becomes paired with cocaine, an unconditioned stimulus (US), which elicits an unconditioned response (UR), such as euphoria, increased heart rate or altered motor behavior. After enough pairings, the previously neutral, now conditioned stimulus of the testing chamber can elicit a portion of the UR in the absence of the US, cocaine, and this is termed a conditioned response (Siegel, 1989; Kling, 1971).

The Cellular and Behavioral Effects of Cocaine

Cocaine is thought to have its euphoric effects by its competitive inhibition of the presynaptic uptake of dopamine, norepinephrine and serotonin by binding to the reuptake transporters of these neurotransmitters (Ritz et al, 1990). Competitive inhibition of the transporters leads to increased levels of neurotransmitters at the synapses and increased stimulation of the post-synaptic neurons (Cooper et al, 1996). A drug that exhibits similar blockade of the dopamine as

well as the norepinephrine reuptake transporters is bupropion, a commonly used antidepressant. Although bupropion has not been considered euphoric by humans (Rothman and Glowa, 1995), it was self-administered by baboons trained on cocaine and substituted for cocaine in a rat cocaine discrimination paradigm (Asher et al, 1995; Lamb and Griffiths, 1990). On the other hand, selective serotonin reuptake inhibitors, which have also been used as antidepressants, have been shown to reduce the subjective effects of cocaine in humans (Walsh and Cunningham, 1997). This would be in keeping with a recent theory that serotonin may contribute an aversive component to the subjective effects of cocaine. For example, destruction of the serotonergic neurons in the amygdala enhanced the motivation to acquire cocaine in a progressive ratio self-administration paradigm (Cunningham et al, 1996; Loh and Roberts, 1990). The amygdala has been shown to modulate the reinforcing, discriminative and hyperlocomotive effects of cocaine through D_1-dopamine receptor stimulation (Callahan et al, 1995).

Rothman and Glowa (1995) review studies pertaining to dopaminergically active drugs. In their review, they discuss other drugs which competitively inhibit dopamine reuptake transporters such as nomifensine, an antidepressant which was removed from the market for severe allergic reactions in some patients; mazindol which is an anti-obesity drug; and GBR12909 which has been tested in Europe as an antidepressant. According to the authors, GBR12909 was found to have sedative effects even though it was several hundred times more potent

than cocaine in inhibiting radiolabeled dopamine reuptake; mazindol was found to be dysphoric in humans; and nomifensine was found to be dissimilar to the subjective effects of amphetamine.

When low doses of cocaine act on the mesolimbic dopaminergic neurons of the ventral tegmental area (VTA), levels of dopamine increase post-synaptically at the neurons of the nucleus accumbens and increased locomotor activity occurs in animals (Kalivas and Duffy, 1990). At high doses of cocaine or repeated moderate doses of cocaine (Johanson and Fischman, 1989), inhibition of the dopamine reuptake transporters results in higher levels of dopamine in the synapse between the axons of the substantia nigra and the cell bodies of the neostriatum (the mesostriatal dopaminergic system), and this causes stereotypy (Wise and Bozarth, 1987), which refers to small, often abnormal, repetitive motions occurring in a restricted space such as sniffing, gnawing and head-bobbing in rodents, and compulsive foraging, akathisia and chorea in humans (Tolliver et al, 1994; Daras et al, 1994; Rosse et al, 1994).

The exact neural circuitry underlying the rewarding effects of cocaine has been debated vigorously since Wise and Bozarth's hypothesis in 1987. A recent review by Bardo (1998) proposes that cocaine has its rewarding actions by first stimulating the medial prefrontal cortex via activation of D_2 dopamine receptors. Glutamatergic projections from the prefrontal cortex to the nucleus accumbens stimulate what Bardo refers to as the accumbal-pallidal-pontine reward circuitry. Within the nucleus accumbens, D_1 and D_2 dopamine receptors, as well as glu-

tamatergic receptors, modulate cocaine reward. The glutamate receptors receive input primarily from the amygdala, which in turn is stimulated by collateral dopaminergic fibers from the ventral tegmental area. Much of Bardo's (1998) work summarized here echoes Kalivas, (1995).

The Phenomenon of Sensitization

It appears that the reinforcing effects of cocaine can become sensitized with prior exposure to psychostimulants. Animals pre-exposed to caffeine, nicotine, amphetamine or cocaine acquire cocaine self-administration faster and administer a lower dose of cocaine than those previously exposed to saline (Horger et al, 1991,1992 and 1990).

In addition, sensitization of the motor-stimulating effects of cocaine has been extensively studied in many laboratories (c.f. Shuster et al, 1977; Post, 1977; Stripling and Ellinwood, 1977; and Post and Rose, 1976). It has also been found that psychomotor stimulant drugs can cross-sensitize motor behavior. For example, Schenk and colleagues found that pre-exposure to caffeine and amphetamines, but not nicotine, resulted in sensitization of the motor stimulating effects of cocaine in rats (Schenk et al, 1989 and 1991). Regarding the sensitization of the motor response to cocaine after amphetamine pre-exposure, Schenk's findings were corroborated by Bonate et al (1997). With respect to the sensitization of cocaine's motor effects after caffeine pre-exposure, Misra et al (1986) showed similar results to Schenk's report in response to low

doses of caffeine (20 mg/kg) only. Schenk's caffeine findings were also supported by a study in her lab using a self-administration model (Worley et al, 1994). In that study rats which had acquired cocaine self-administration were put through extinction by turning off the cocaine infusion pumps, such that lever depressions continued to be recorded and resulted in a light stimulus that had previously been paired with drug infusion. Within 5 hr all rats had ceased to respond for a 60-min period. Then the rats were injected intraperitoneally (i.p.) with either saline, caffeine or cocaine. Extinction conditions remained active and lever presses were counted until the rats ceased responding for 60 min. Both caffeine and cocaine i.p. injections were able to reinstate lever pressing for, respectively, 8 hr and 6 hr. These findings emphasize the importance of conditioning in sensitization and cocaine dependence.

In addition, prior stressful events such as a tail-pinch are known to sensitize the response to amphetamine (Piazza et al, 1990). As reported by Piazza, stress alters the dopaminergic system such that dopamine neurons display an increase in metabolism and that behavioral reactivity is enhanced in response to psychostimulants. Along these same lines, exposure to a novel environment has been shown to enhance sensitization to cocaine and amphetamine (Badiani et al, 1995). A further finding in Badiani's study was that conditioned responses to contextual cues only developed when cocaine was given in the novel environment. This confirmed findings from a study by Hinson and Poulos (1981) which

indicated that sensitization to cocaine was enhanced by Pavlovian conditioning. Furthermore, they showed that conditioned sensitization was extinguishable.

Context-Dependent Sensitization

Sensitization usually occurs only in a context-dependent manner (c.f. Badiani et al, 1995; Fontana et al, 1993; Jackson and Nutt, 1993; Post et al, 1992). Animals that had been exposed to cocaine in the locomotor testing chamber showed sensitization of cocaine's behavioral effects while those exposed to cocaine in another environment did not. It has been shown through the use of the D_1-receptor antagonist SCH23390 and the D_2-receptor antagonist haloperidol that both D_1- and D_2-receptor stimulation were required to establish context-dependent sensitization of cocaine's motor effects, and D_2-receptor stimulation was required to elicit previously established sensitization (Mattingly et al, 1996; Fontana et al, 1993; Weiss et al, 1989). In addition to the striatum and the nucleus accumbens, the dopaminergic projections from the VTA also reach the prefrontal cortex as part of the mesocortical dopaminergic system and the amygdala as part of the mesolimbic dopaminergic system (Cooper et al, 1996). According to a study by Carey and Damianopoulos (1994), serotonin in the prefrontal cortex appears to be involved in context-dependent sensitization. Animals that had experienced cocaine previously in the locomotor testing chamber had higher levels of serotonin and its metabolite in their prefrontal cortices in response to a saline challenge in the testing chamber than animals which had pre-

viously received cocaine in another environment (Carey and Damianopoulos, 1994). The amygdala and the ventral striatum have been implicated in the pathway controlling instrumental behavior by conditioned reinforcers, and lesions of the basolateral amygdala can impair the acquisition of a new response through conditioned reinforcement (Altman et al, 1996). A final point emphasizing the importance of conditioning in the sensitization of cocaine's effects concerns the observation that Battleboro rats, a strain which is genetically deficient in vasopressin, a peptide important in learning, do not become sensitized to the effects of cocaine (Post et al, 1982). As mentioned above, the conditioned element of the craving phenomenon is a major contributor to relapse into drug-taking; thus, the study of conditioned cocaine responses may contribute to the treatment of drug addiction.

Locomotor Behavior and Sensitization

Locomotor behavior is studied not only because it is controlled by the same area of the brain which mediates craving (Robinson and Berridge, 1993), but also because it has been associated with the abuse potential and reinforcing effects of drugs as shown by Piazza et al (1989) who showed that those animals showing a higher locomotor response to a novel environment later acquired and maintained drug self-administration at lower doses than those animals that showed a lower response to the novel environment. It is an adequate paradigm for studying sensitization because it requires no previous exposure to the drug.

Self-administration and drug discrimination are inadequate for sensitization studies because the training involved exposes the animal to cocaine in such a way that sensitization may be obscured by the development of tolerance. Many studies have been done to determine the appropriate dose and dosing interval necessary to induce sensitization to cocaine in animals (Tolliver et al, 1994; Reith et al, 1987). Tolerance has been shown to develop in rats after 10 days of chronic cocaine (Emmett-Oglesby et al, 1993; Emmett-Oglesby and Lane, 1992). Reith et al (1987) showed that cocaine (25 mg/kg) given for 18 days intraperitoneally (i.p.) once a day versus chronic release of the same dose via minipump caused, respectively, sensitization and tolerance to the locomotor stimulating effects of cocaine. In order to establish sensitization in the following experiments, the 24-h dosing interval was employed.

Previous studies of sensitization using locomotor behavior were inadequate because they did not use full dose response curves when determining the presence or absence of sensitization (Koff et al, 1994; Fontana et al, 1993; Post et al, 1992). Furthermore, with the exception of Fontana et al (1993), they did not quantify the context-dependent aspect of sensitization. One laboratory attempted to measure the context-dependent aspect of sensitization in mice using full dose-response curves; however, their design was limited in that a direct measure of the effects of conditioning could not be determined (Tolliver and Carney, 1994; Tolliver et al, 1994). These researchers performed experiments using a paired group which was exposed to the testing chamber following an in-

jection of 32 mg/kg cocaine for six days while the "unpaired" group received the same treatment without exposure to the testing chamber until day seven when both groups were placed into the testing chamber after challenge injections with saline or cocaine (from 1 to 100 mg/kg). In this way, the unpaired group represented not only context-independent sensitization but also the novelty effect as the animals explored the testing chamber for the first time. The experiments herein differed from such studies by conducting a full dose-response curve on the challenge day to determine sensitization of cocaine-induced motor behavior. Additionally, the current experiments included an unpaired group that was exposed to the testing chamber as many times as the paired group. By using full dose-response curves, the effect of sensitization on the lower doses of cocaine could be determined. Doses of cocaine under 10 mg/kg proved to be more variable as the length of cocaine pre-exposure was changed. If the 40 mg/kg dose of cocaine had been used to induce sensitization in these mice, and then only that dose, or perhaps a 20 mg/kg dose, had been used to test for sensitization, then the observed effect would often have appeared to be tolerance (see chapters 2 and 3). By using full dose-response curves, the shift of the entire curve as a function of sensitization could be observed.

Genetic Variance in Cocaine Dependence

Craving induced by the presence of cocaine-associated environmental stimuli has been shown to occur in roughly 60% of the Caucasian cocaine ad-

dicts in rehabilitation programs (O'Brien et al, 1988). This implies a genetic variance in the strength of cocaine craving that may be related to an individual's ability to make conditioned associations between the environment and the effects of cocaine. To address this issue, an experiment was performed that tested the hypothesis that context-dependent sensitization of the behavioral effects of cocaine varies between genetically dissimilar strains of mice. If such differences in sensitization were causes of differential susceptibility to dependence then a strain that was more susceptible to drug dependence would be predicted to show stronger conditioning than a strain which is resistant to drug dependence.

Inbred mouse strains provide an invaluable tool to researchers, namely a reproducible genetically defined background. They are strains which have been brother-sister mated for enough generations that each individual in the strain is virtually identical to the others (McClearn, 1991). For this experiment, the strains chosen were C57BL/6, previously characterized as a drug-seeking strain, and DBA/2, characterized as a drug-avoiding strain. These strains have been shown to be different in their susceptibility to drug addiction in general, and to cocaine addiction specifically through many different experiments. For example, when oral morphine consumption was compared between C57BL/6J and DBA/2J mice, the C57BL/6 mice consumed 90% of their daily fluid intake from the morphine-saccharin bottle, while DBA/2J mice consumed only 13% (Phillips et al, 1991). The C57BL/6J mice also have higher alcohol acceptance than DBA/2J

mice when alcohol acceptance is measured by amount of alcohol consumed after a 24-h period of water deprivation (Plomin and McClearn, 1993). The reinforcing effects of cocaine and, by inference, its dependence liability can be measured through the self-administration paradigm wherein the animal presses a lever or pokes its nose through a specific hole in the chamber in order to receive an intravenous dose of a drug. One study showed that C57BL/6J mice initiated self-administration of cocaine, morphine, methamphetamine and pentobarbital, while DBA/2J mice self-administered all but cocaine (Carney et al, 1991). Other investigators, however, found that DBA/2J mice do indeed self-administer cocaine albeit at lower doses than C57BL/6J mice (Grahame and Cunningham, 1995; Rocha et al, 1996). In addition, Seale and Carney (1991) showed that cocaine conditioned place preference was stronger in C57BL/6 mice than it was in DBA/2 mice, indicating stronger rewarding effects of cocaine in C57BL/6 mice. Tolliver et al (1994) found that the paired group of the C57BL/6 mice displayed classically conditioned locomotor behavior in response to saline after six days of exposure to 32 mg/kg cocaine in the testing chamber. The paired group of the DBA/2 mice did not show conditioning in response to the same treatment. In this study neither strain showed sensitization of the locomotor effects of cocaine; however, in their previous study (Tolliver and Carney, 1994) the DBA/2 mice showed sensitization of the stereotypy effects of cocaine. As mentioned previously, this study did not include an unpaired group equivalent to that used in the experiment described herein; therefore, that experiment

should be repeated with modifications to more directly address the question of context-dependent sensitization.

The Hypotheses

The studies described herein sought to test two hypotheses. The first hypothesis was that specific variables of motor behavior would be more greatly effected by context-dependent sensitization than by context-independent sensitization, and that the character of the context-dependent response would be similar to that of the response to acute cocaine. The second hypothesis was that genetic differences in susceptibility to cocaine addiction and the development and persistence of context-dependent sensitization contribute to cocaine seeking behavior and, ultimately, to risk for development and persistence of cocaine dependence.

Endnote

Abbreviations used:

- ANOVA, analysis of variance
- dB, decibel
- i.p., intraperitoneal
- multivariate behavior measures:
  - AD, average distance
  - AS, average speed
  - HC, horizontal counts
  - HD, horizontal distance
  - HM, horizontal movements
  - HT, horizontal time
  - SC, stereotypy counts
  - SM, stereotypy movements
  - ST, stereotypy time
  - VC, vertical counts
  - VM, vertical motions
  - VT, vertical time
- NIDA, National Institute of Drug Abuse
- 6-OHDA, 6-hydroxydopamine
- PET, positron emission tomography
- QTL, quantitative trait loci
- RI, recombinant inbred
- UR, unconditioned response
- US, unconditioned stimulus
- VTA, ventral tegmental area

CHAPTER II

EXPERIMENT I

Multivariate Parametric Analysis of Context-Dependent and Context-Independent Sensitization to the Motor-Stimulating Effects of Cocaine in Swiss Webster Mice

Abstract

The purpose of this study was to test the hypotheses that variables of motor behavior would be more greatly affected by context-dependent sensitization than by context-independent sensitization, and that the character of the conditioned response would be similar to that of acute cocaine. Cocaine (40 mg/kg) was paired for one or four days to the testing chamber for the Paired group and to the home cage for the Unpaired group, while the Saline Control group received saline in both environments. Then each group was challenged with saline or cocaine (doses from 1 to 40 mg/kg). The paired and unpaired groups provided measurements of context-dependent and context-independent sensitization, respectively, relative to acute cocaine effects in the saline control group. Some context-dependent sensitization was evident after only one exposure to cocaine, but after four, the paired group showed significant differences from the other two groups on eleven of the twelve motor variables tested. Sen-

sitization was observed in the unpaired group on only three variables. The conditioned response of the paired group was similar in character to the acute response to 10 mg/kg cocaine. Our conclusion was that sensitization is mainly a context-dependent phenomenon, which is enhanced by multiple exposures to cocaine.

Introduction

Some of the earliest papers to investigate the enhancement of cocaine's stimulatory effects with repeated drug exposure, a phenomenon called "reverse tolerance" or sensitization, were published in the late 1970's (Shuster et al, 1977; Post, 1977; Stripling and Ellinwood, 1977; and Post and Rose, 1976). A great deal of the literature since then has focused on finding the areas of the brain that are responsible for the effects of cocaine and the sensitization phenomenon (cf. Wise and Bozarth, 1987; Robinson and Berridge, 1993; and Kalivas, 1995).

In addition to the neurochemistry behind sensitization, there have also been efforts to investigate the observation that sensitization to the behavioral effects of cocaine most frequently occurs in a context-dependent manner. For example, Hinson and Poulos (1981) found that cocaine's stimulatory effects on the locomotor and stereotypical behavior of rats were enhanced when the drug was administered in the presence of cues previously associated with cocaine administration than without those cues. Furthermore, they found that sensitiza-

tion to be extinguishable, when saline was presented in the presence of the cocaine-associated cues, thus indicating further the importance of context in sensitization to cocaine. Dr. Robert Post's laboratory at the National Institute of Mental Health has published several studies that have attempted to identify the neurological substrates responsible for context-dependent sensitization (Weiss et al, 1989; Fontana et al, 1993). However, none have examined the multivariate behavior profile of the motor behavior elicited by exposure to cocaine-related environmental cues. Zubrycki et al (1990) characterized the multivariate motor behavior induced by acute doses of cocaine in rats. Findings included increased rotational and ambulatory behavior with cocaine (20 and 30 mg/kg) over that stimulated by saline. Furthermore, the cocaine "activity print" that Zubrycki et al observed was dose-dependent and qualitatively different from the "activity print" of amphetamine in the same behavioral paradigm.

The present study attempted to characterize the multivariate behavior profile of both acute cocaine and repeated cocaine as well as the multivariate behavior profile of conditioned responses to cocaine-associated cues. Moreover, this study used mice in order to design a model that could later be manipulated genetically. Furthermore, this study employed full dose-response curves to reflect more completely the effects of sensitization to cocaine. The purpose of this study was to test the hypotheses that variables of motor behavior would be more greatly affected by context-dependent sensitization than by context-

independent sensitization, and that the character of the conditioned response would be similar to that of the response to low dose acute cocaine.

Methods

Subjects

Two-month-old, male Swiss Webster (Harlan Sprague-Dawley) mice were housed in groups of 3-5 for at least one week before the experiments began. At the start of the experiments, mice were housed singly on either side of divided clear polypropylene cages (7 x 11 x 5 in) with ad libitum access to food and water. The colony room was maintained at 23° ± 1°C at 50 ± 5% humidity, under a normal 12-h light/dark cycle beginning at 0600.

Apparatus

Motor behavior was measured using a Digiscan Animal Activity Monitoring System (Omnitech Electronics, Inc., Columbus, OH), which consisted of individual acrylic testing chambers (40.5 x 40.5 x 30.5 cm) surrounded by red-filtered horizontal and vertical activity sensors [Model RXYZCM(16)], and a Digiscan Analyzer [Model DCM(8)] for collection and initial sorting of motor variables (Forster and Lal, 1991). Two arrays of 16-infrared photocell beams were arranged to detect movements in the horizontal plane. An additional array of 16-photocell beams was located at a fixed height of 7.7 cm above the floor to detect vertical movement. The Digiscan equipment was housed in sound-attenuated

chambers equipped with a ventilation fan that provided 80-dB ambient noise. A 7.5-watt incandescent light above each chamber provided dim illumination via a rheostat set at 20% of full scale.

Description of motor behavior

A number of different components of motor behavior were measured in order to provide a comprehensive description of behavior for qualitative comparison of the cocaine-naive and cocaine-sensitized mice. The components were defined by variables falling into categories of ***horizontal motion*** (walking and running), ***vertical motion*** (climbing and rearing), and ***stereotypy*** (repeated motions occurring in a restricted amount of space such as head-bobbing, gnawing, grooming and scratching).

Horizontal motion. Variables of horizontal motion included Horizontal Counts (HC), which represented the total photocell interruptions within the apparatus during the session; Horizontal Distance (HD), a measure of the total horizontal displacement in cm; Horizontal Movements (HM), the number of times the mouse initiated motion in the horizontal plane; Average Distance (AD), which was the average distance per horizontal movement; Horizontal Time (HT); the total duration of horizontal motions; Average Speed (AS), which equaled HD/HT.

Vertical motion. These measures included Vertical Counts (VC), the total vertical photocell interruptions within a session; number of Vertical Movements

(VM), the number of times the mouse initiated motion in the vertical plane; and Vertical Time (VT), the total duration of vertical motion.

Stereotypy. Stereotypy measures included Stereotypy Counts (SC), the number of times that one photocell or a group of photocells were broken repeatedly; the number of Stereotypy movements, or bouts of stereotypy (SM); and Stereotypy Time (ST), the total duration of each bout of stereotypy. It should be noted that the Digiscan equipment may not in fact be measuring what is classically known as stereotypy. It merely counts the single, repeated interruptions of the photocell beams. No direct human observation of the animals' behavior was made.

Procedure

Acute cocaine. Initially, a dose-response study was performed in cocaine-naïve mice, to determine doses to be used in subsequent sensitization studies and to allow characterization of the multivariate behavior profile of cocaine-induced behavior. Mice received intraperitoneal (i.p.) injections of saline or cocaine (5, 10, 20 or 40 mg/kg) immediately prior to placement in the Digiscan testing chamber for 30 min.

Sensitization. A dose of 40 mg/kg was selected for pairings in the sensitization experiment, to ensure maximum duration of effect and for consistency with previous literature (Weiss et al, 1989; Koff et al, 1994; Hirabayashi et al, 1991). Mice were exposed to the following pairing conditions for either one or

four days. Each animal received two i.p. injections daily of either cocaine (40 mg/kg) or saline (0.9 %). The first injection was given immediately prior to placement in the Digiscan testing chamber for 30 min. The second injection was given 2 h after the animals returned to their home cages in the animal facility. As seen in Table 1, mice were divided into three groups: 1) Saline Control, which received saline for both injections; 2) Unpaired, which received saline for the first injection and cocaine for the second; and 3) Paired, which received cocaine prior to placement in the testing chamber and saline in the home cage. On the day following the last pairing session, mice from each pairing condition were divided into seven groups and were challenged with either saline or doses of cocaine ranging from 1 to 40 mg/kg. Sensitization was defined as an increase in motor response to a saline injection and/or a leftward shift in the dose-response curve for cocaine-induced motor behavior that was attributable to cocaine pre-exposure. Context-dependent sensitization was inferred by a difference in sensitization between the paired and unpaired groups of mice.

Statistics

Statistical analyses were performed using SYSTAT version 5.0 (Wilkinson, 1990). Perusal of the acute cocaine time-response curves for the twelve motor variables tested showed peak effects of cocaine in the first 15-min; therefore, the following analyses were performed using an average of the first three 5-min intervals for each variable.

Repeated measures ANOVAs using Group as the between subjects factor and Pairing Session as the within groups factor were performed on the data from the four-pairing condition to show the changes of the measured parameters as a function of time. A three-way ANOVA using Number of Pairings, Group, and Dose as between subjects factors was performed on the data from the challenge day. Fisher's LSD comparisons were performed. The response of the paired group to saline on the challenge day was compared to the cocaine response of the paired group during the last pairing session to calculate a percentage of the cocaine response. This percentage was used to quantify the strength of conditioning after one pairing session and after four sessions, and a t-test was performed to determine if there was a significant difference between the two. For comparisons of acute cocaine responses to context-dependent sensitization responses, values were calculated for the acute cocaine effect by dividing the value for each subject receiving a particular dose of acute cocaine by the average saline control value, multiplying the result by 100, and then subtracting 100. The context-dependent cocaine effect was obtained by dividing the unpaired group's average response to a particular dose of cocaine or saline into each subject of the paired group at the same dose, and again multiplying by 100 and then subtracting 100.

Results

Time-Response and Dose-Response Curves for Cocaine

On the left side of Figures 2-1 through 2-4, the motor variable data are plotted as a function of dose and time. Perusal of the data indicated that the maximum effects of cocaine on horizontal measures occurred within the first 15 min following the injection of cocaine. For stereotypy, the effects of the 5 and 10 mg/kg doses were maximal in the first 15 min, whereas the 20 and 40 mg/kg doses began to peak in the second 15 min of the session. It was felt that this was simply the effect of the blood levels of cocaine dropping closer to 10 mg/kg cocaine, which resulted in the maximal effect observed in the first 15 min. Rather than use different time intervals for analysis of different variables, it was decided that all subsequent statistical analyses would be performed on the mean of the means of the first three 5-min intervals. In this manner, the dose-response curves were plotted on the right side of figures 2-1 through 2-4.

In Figure 2-1, the time-response and dose-response curves for Horizontal Distance, Average Distance and Average Speed are plotted. Cocaine increased all three measures in a dose-dependent manner. Maximum effects of cocaine occurred in response to the 20 mg/kg dose of cocaine. It appears from these data that the mice are traveling somewhat faster, thus covering more distance with each movement. Figure 2-2 depicts the time-response and dose-response curves with respect to Horizontal Counts, Time and Movements. Doses of co-

caine greater than 10 mg/kg reduced the number of Horizontal Movements made by the animals indicating that they stopped less under the influence of cocaine and spent more time in horizontal behavior. The combination of slightly enhanced speed and more time spent in ambulation lead to greater distances being traveled.

Figure 2-3 displays the time-response and dose-response curves for Stereotypy Counts, Time and Movements. Maximum effects of cocaine on all three measures occurred in response to 10 mg/kg cocaine. Cocaine increased Stereotypy Counts in a dose-dependent manner. Time spent in Stereotypy and number of Stereotypy Movements in response to cocaine were not increased beyond levels induced by saline. This may indicate that the intensity of the stereotypical behavior was increased. As can be seen in Figure 2-4, Vertical Counts, Time and Movements were all reduced by the presence of cocaine in doses greater than 10 mg/kg. These data indicated that more time was being spent in horizontal activity than in vertical while stereotypy time changed little.

The Sensitization Experiment

Figures 2-5 through 2-8 represent the data from the pairing and challenge days for the twelve measures of cocaine-induced motor behavior. The left side of the figures shows the data from the one pairing condition while the right side shows the data from the four pairing condition. The left panel of each graph dis-

plays the data from the pairing days. The right panel of each graph shows the data from the challenge day.

In Figure 2-5, the calculated measures of Horizontal Distance, Average Distance and Average Speed are plotted. With regard to the data from challenge day, there was an upward shift of the lower portion of the dose-response curve for HD in the paired group, relative to the saline control group after only one pairing session. The maximal effect of cocaine on the HD, AD or AS variables of the paired group was not significantly different from that of the saline control group. After four pairings, there was a leftward shift in the dose-response curves for HD and AD in the paired group, relative to the saline control group. Relative to the unpaired group, the paired group displayed significantly greater response to almost all doses of cocaine and in response to saline. This increase likely reflected the conditioned element of sensitization. The maximal effects of cocaine on HD, AD and AS were decreased in the paired group relative to the saline control group. A related finding was that the behavior of the unpaired group was significantly depressed on the challenge day relative to the saline control group after four pairings with cocaine on the HD and AD measures. During the cocaine pairing sessions of the four pairings experiment, there was a decline in the horizontal distance traveled by the paired group beginning on the third day of cocaine pairing, and this was concurrent with an increase in stereotypy behavior (see Figure 2-7). Simultaneously, the unpaired group began to show a progressive decline in distance traveled in response to saline.

There was a significant difference between the unpaired and saline control groups with regard to horizontal distance traveled on days 3 ($F_{1,687} = 41.711$, $p<.001$) and 4 ($F_{1,687} = 70.982$, $p<.001$). A three-way ANOVA using Number of Pairings, Group and Dose as between subjects factors was performed on the data from the challenge days of both the one and four pairing conditions and revealed significant interactions between Number of Pairings X Group (HD: $F_{2,637} = 5.763$, $p<.003$; AD: $F_{2,637} = 3.669$, $p<.026$; AS: $F_{2,637} = 3.007$, $p<.050$), thus indicating that increased exposures to cocaine in the same environment strengthens context-dependent sensitization. Group X Dose interactions were also significant for two of these variables (HD: $F_{12,637} = 5.062$, $p<.001$; AS: $F_{12,637} = 3.226$, $p<.001$), thus indicating a difference between the groups relative to cocaine dose regardless of number of previous pairings of cocaine.

In Figure 2-6, the measures of Horizontal Counts, Time and Movements are plotted. With regard to the data from the challenge day, there was an enhancement of the paired group's response to saline on the HC, HT and HM measures relative to both the saline control and unpaired groups. Additionally, the lower portion of the paired group's dose-response curves for HC and HT shifted upward relative to the unpaired and saline control group after only one pairing with cocaine. Then after four pairings, there was a leftward shift in the dose-response curves of the paired group relative to the saline control group on HC and HT. The paired group's response to the saline challenge after four pairings with cocaine remained enhanced relative to the unpaired and saline

control groups on all measures except HC where the paired group was only different from the unpaired group. Unlike the distance measures, there was no reduction in maximum effect of cocaine on these measures in the paired group relative to the saline control group. As with the distance measures, the cocaine pairing sessions were marked by the decrease in the maximal response of the paired group to cocaine at the third and fourth pairing sessions, except on Horizontal Movements which increased as the pairings continued. Again, this could be accounted for by the increase in stereotypy counts and time. As above, the unpaired group showed a progressive decrease in horizontal behavior in response to saline after the second pairing with cocaine, and this reduction in behavior surpassed the habituation effect observed in the saline control group (F's ranging from 18.598 to 91.614, all p's<.001). A three-way ANOVA using Number of Pairings, Group and Dose as between subjects factors was performed on the data from the challenge days of both the one and four pairing conditions and revealed a significant interaction between Number of Pairings X Group X Dose ($F_{12,637} = 2.103$, $p<.015$) for HT, thus indicating that the number of pairings had an effect on the response to cocaine for each group and dose. All three measures showed a significant interaction of Group X Dose (HC: $F_{12,637} = 3.636$, $p<.001$;HT: $F_{12,637} = 3.534$, $p<.001$;and HM: $F_{12,637} = 5.876$, $p<.001$) and two measures showed that the interaction of Number of Pairings X Group was significant also (HC: $F_{2,637} = 5.587$, $p<.004$; HT: $F_{2,637} = 6.847$, $p<.001$).

In Figure 2-7, the measures of Stereotypy Counts, Time and Movements are plotted. As with the horizontal variables of cocaine-induced behavior on the cocaine challenge day, SC and ST showed an upward shift of the dose-response curves in the paired group relative to the saline control group after only one pairing with cocaine. The paired group's response to saline was also enhanced on SC, ST and SM measures after only one pairing with cocaine. After four pairings, there was both an upward and leftward shift in the dose-response curves for all three stereotypy measures in the paired group relative to the saline control and unpaired groups, and the paired group displayed significantly higher responses to saline after four pairings with cocaine on all three measures with respect to the response of the unpaired group. The maximal effects of cocaine on SC, ST and SM were increased in the paired group relative to the saline control and unpaired groups after four pairings with cocaine. As the paired group spent more time in stereotypy behavior and made more stereotypy motions, the overall SC measure was increased. There was also an increase in the maximal effect of cocaine on the unpaired group's stereotypy time after four pairing sessions, although this curve was not shifted leftward as much as that of the paired group. This finding may indicate context-independent sensitization; however, it is not supported by a concurrent increase in SC or SM. With respect to the cocaine pairing sessions, as mentioned above, there was an increase in the maximal effect of cocaine on SC and ST in the paired group relative to the other two groups. As with the horizontal measures, the unpaired group showed

a progressive decline in stereotypy measures in response to saline after the second cocaine pairing session, and this reduction in behavior surpassed the habituation effect observed in the saline control group (F's ranging from 4.149 to 65.212, all p's<.042). A three-way ANOVA using Number of Pairings, Group and Dose as between subjects factors was performed on the data from the challenge days of both the one and four pairing conditions and revealed a significant interaction between Number of Pairings X Group X Dose ($F_{12,637} = 3.334$, $p<.001$) for ST. For ST ($F_{12,637} = 2.660$, $p<.002$) and SM ($F_{12,637} = 2.433$, $p<.004$) the interaction of Group X Dose was significant. For SC, significant effects were found in Number of Pairings ($F_{1,637} = 23.911$, $p<.001$), Group ($F_{2,637} = 26.255$, $p<.001$) and Dose ($F_{6,637} = 28.175$, $p<.001$), but the only significant interaction was between Number of Pairings X Dose ($F_{6,637} = 5.368$, $p<.001$).

In Figure 2-8, the measures of Vertical Counts, Time and Movements are plotted. Data from the challenge days indicate that one pairing with cocaine did not alter the response of the groups in any significant way, as the variability of response is very high on the vertical measures. After four days of pairing, a pattern of suppression in the activity of both the paired and unpaired groups was observed on the VC and VT measures. The paired group was also suppressed on the VM measure after four pairings with cocaine. The suppression of vertical motion in the paired and unpaired groups in response to low doses of cocaine on the challenge day was equal to that induced by the higher doses of cocaine in the saline control group, and this finding indicates a leftward shift in the dose-

response curves of the paired and unpaired groups. With regard to the response to saline, there was an enhancement of activity by the paired group relative to the unpaired group on all three measures of vertical activity after both one and four pairings with cocaine. While higher doses of cocaine suppress vertical activity, the lower doses enhance it. As with the above measures, the unpaired group showed a progressive decrease in vertical measures in response to saline beginning on the second cocaine pairing session. As before this reduction in behavior surpassed the habituation effect observed in the saline control group (F's ranging from 15.120 to 88.395, all p's<.001). A three-way ANOVA using Number of Pairings, Group and Dose as between subjects factors was performed on the data from the challenge days of both the one and four pairing conditions and revealed significant interactions between Number of Pairings X Group (VC: $F_{2, 632} = 10.335$, $p<.001$; VT: $F_{2, 632} = 8.751$, $p<.001$; VM $F_{2, 632} = 7.345$, $p<.001$) and Group X Dose (VT: $F_{12, 632} = 1.795$, $p<.046$; VM: $F_{12, 632} = 1.274$, $p<.043$).

Strength and Character of the Context-Dependent Response

The response of the paired group to saline on the challenge day was compared to the paired group's response to 40 mg/kg cocaine the previous day. From the comparison that is detailed in Figure 2-9, it was evident that four pairings increased the conditioned response to cocaine on several of the horizontal variables, specifically horizontal distance, average distance and speed and hori-

zontal time. The one pairing condition resulted in conditioned horizontal movements that far surpassed that of the four pairing condition. A t-test confirmed the significance of the difference between the pairing conditions (all p's<.031) for each locomotor variable displayed. There was no difference between the pairing conditions with regard to any of the stereotypy measures. Vertical measures were not subjected to this calculation because large doses of cocaine suppress vertical behavior while the conditioned effects of cocaine involve enhancing it; therefore the calculations would result in values of 300-400% increases in behavior. It was felt that the effects of cocaine on the horizontal and stereotypy measures were of more import and would be minimized in a graph displaying the vertical measures.

Finally, comparisons of the multivariate behavior profile of acute cocaine and context-dependent sensitization can be seen in fig. 10. In the bottom panel, the conditioned behavior elicited by cocaine-associated cues was determined by administering saline to the paired and unpaired groups of mice. As described in the methods section, the values for the one and four pairing conditions were derived by dividing the average response to saline of the unpaired group into the response to saline by each subject in the paired group and then multiplying by 100 and subtracting 100. The conditioned response after one and four pairings were remarkably similar to one another, and most resembled the acute response to 10 mg/kg cocaine especially on the measures of HD, HC, HT, VM and SC. In comparison to the context-dependent sensitization observed in response to 1,

2.5, 5 and 10 mg/kg cocaine, the conditioned response to saline was higher in magnitude on almost all measures.

In the top four panels of fig. 10, the context-dependent sensitization after one and four pairings with cocaine was compared to the response to acute cocaine. Sensitization surpassed the acute response on most measures of horizontal activity and stereotypy with respect to the 1, 2.5 and 5 mg/kg doses of cocaine.

Discussion

This study attempted to test the hypotheses that specific variables of motor behavior would be more greatly affected by context-dependent sensitization than by context-independent sensitization, that the character of the conditioned response would be similar to that of the response to acute cocaine, and that multiple pairings with cocaine would produce stronger context-dependent sensitization than only one pairing. From the data it was concluded that 1) sensitization of the behavioral effects of cocaine was enhanced by multiple pairings with cocaine in specific environments, 2) context-dependent sensitization developed on eleven of the twelve motor variables that were tested, specifically all except average speed, while context-independent sensitization was observed on only stereotypy time, vertical counts and vertical time, and 3) the conditioned response to cocaine-associated cues occurred on all measures of motor behavior

except average distance and average speed and was similar in character to the acute response to 10 mg/kg cocaine.

The behavioral measures that seemed to respond most strongly to context-dependent sensitization were the horizontal and stereotypy variables. It was observed that initially the Swiss Webster mice showed more horizontal activity for longer times at the same relative speed, but as the mice were exposed repeatedly to cocaine, they displayed less horizontal activity at the same speed and more stereotypy activity. Vertical behavior was inhibited by high doses of cocaine and mildly stimulated by the lower doses of cocaine. Stewart and Badiani (1993) suggest that drugs have multiple effects such that tolerance can develop to some while sensitization develops to others. The one variable that did not become sensitized in the Swiss Webster experiment was average speed. Context-independent sensitization occurred on only three measures: vertical counts, vertical time and stereotypy time; and even on these measures, it did not approach the magnitude of context-dependent sensitization. Based on the results of this experiment, it was decided that the horizontal distance and stereotypy counts measures of behavior would be used for the experiment with the C57BL/6 and DBA/2 mice.

Endnote

1. Support for this research was provided by the NIDA contract N01DA-2-9305, and this work was part of a dissertation project by Linda A. Odom.

TABLE 2-1. Schedule of injections during the pairing phase of the experiments.

Group	Testing chamber	Home Cage
Paired	Cocaine (40 mg/kg)	Saline
Unpaired	Saline	Cocaine (40 mg/kg)
Saline Control	Saline	Saline

Fig. 2-1. Dose-response curves for cocaine-induced horizontal distance, average distance and average speed in Swiss Webster mice. The left panels plot the dose-response curve against time in 5-min intervals. Each line represents a separate dose of cocaine. The right panels depict the mean of the means of the first three 5-min time intervals. Values plotted ± SEM. Number of subjects was at least 15.

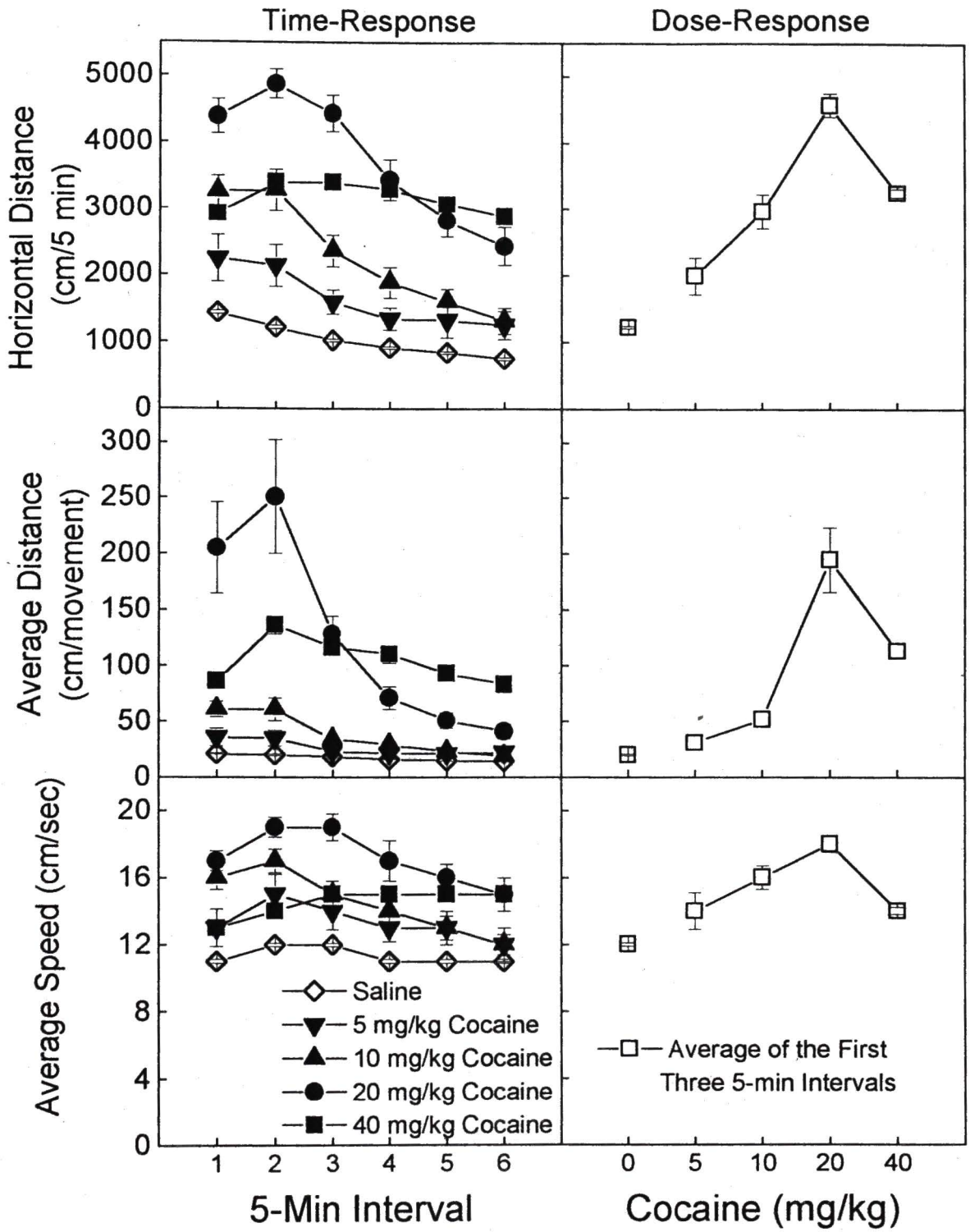

Time-Response
Dose-Response
Horizontal Distance (cm/5 min)
Average Distance (cm/movement)
Average Speed (cm/sec)
Saline
5 mg/kg Cocaine
10 mg/kg Cocaine
20 mg/kg Cocaine
40 mg/kg Cocaine
Average of the First Three 5-min Intervals
5-Min Interval
Cocaine (mg/kg)

Fig. 2-2. Dose-response curves for cocaine-induced horizontal counts, time and movements in Swiss Webster mice. The left panels plot the dose-response curve against time in 5-min intervals. Each line represents a separate dose of cocaine. The right panels depict the mean of the means of the first three 5-min time intervals. Values plotted ± SEM. Number of subjects was at least 15.

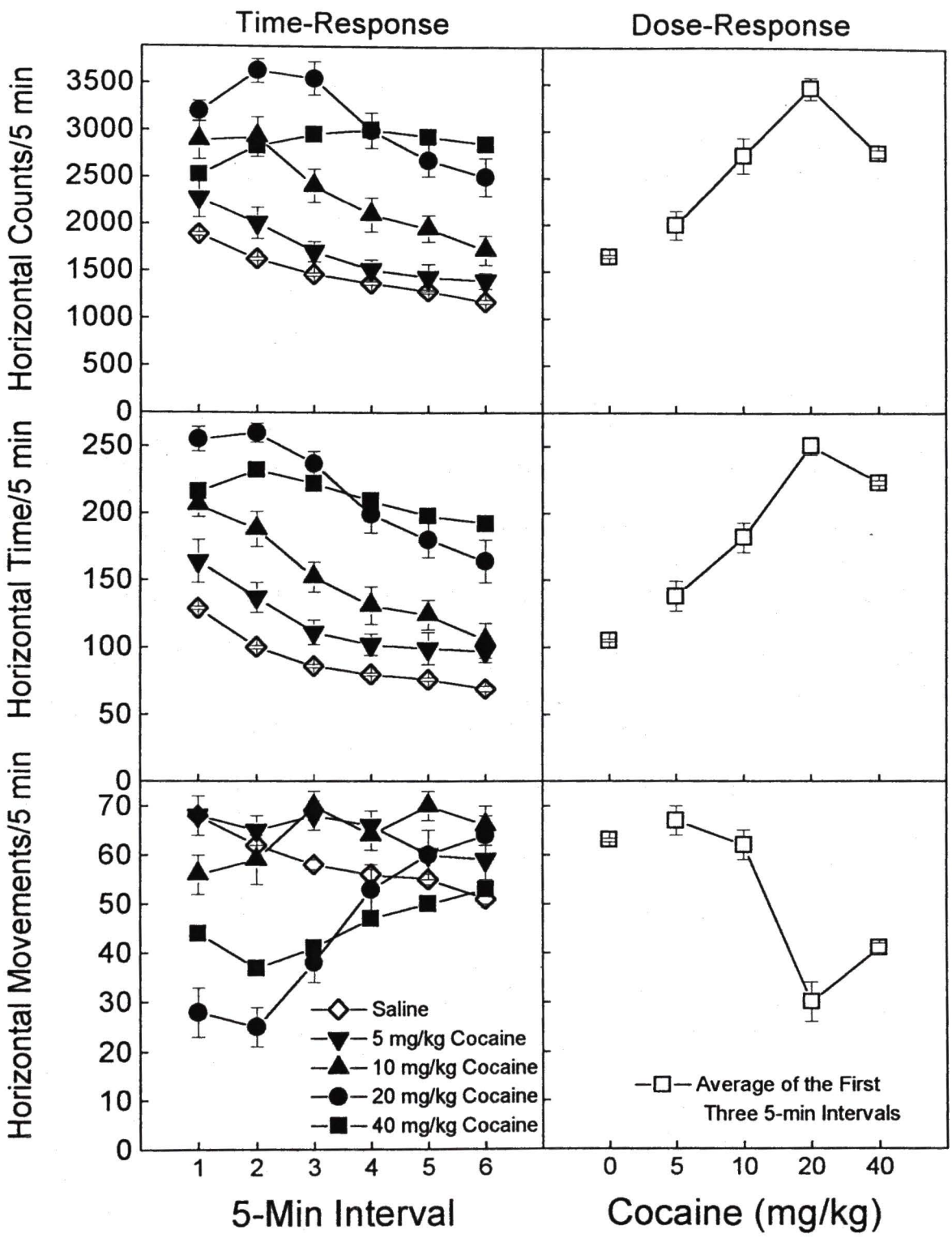

Time-Response
Dose-Response
Horizontal Counts/5 min
Horizontal Time/5 min
Horizontal Movements/5 min
3500
3000
2500
2000
1500
1000
500
0
250
200
150
100
50
0
70
60
50
40
30
20
10
0
Saline
5 mg/kg Cocaine
10 mg/kg Cocaine
20 mg/kg Cocaine
40 mg/kg Cocaine
Average of the First
Three 5-min Intervals
1
2
3
4
5
6
0
5
10
20
40
5-Min Interval
Cocaine (mg/kg)

Fig. 2-3. Dose-response curves for cocaine-induced stereotypy counts, time and movements in Swiss Webster mice. The left panels plot the dose-response curve against time in 5-min intervals. Each line represents a separate dose of cocaine. The right panels depict the mean of the means of the first three 5-min time intervals. Values plotted ± SEM. Number of subjects was at least 15.

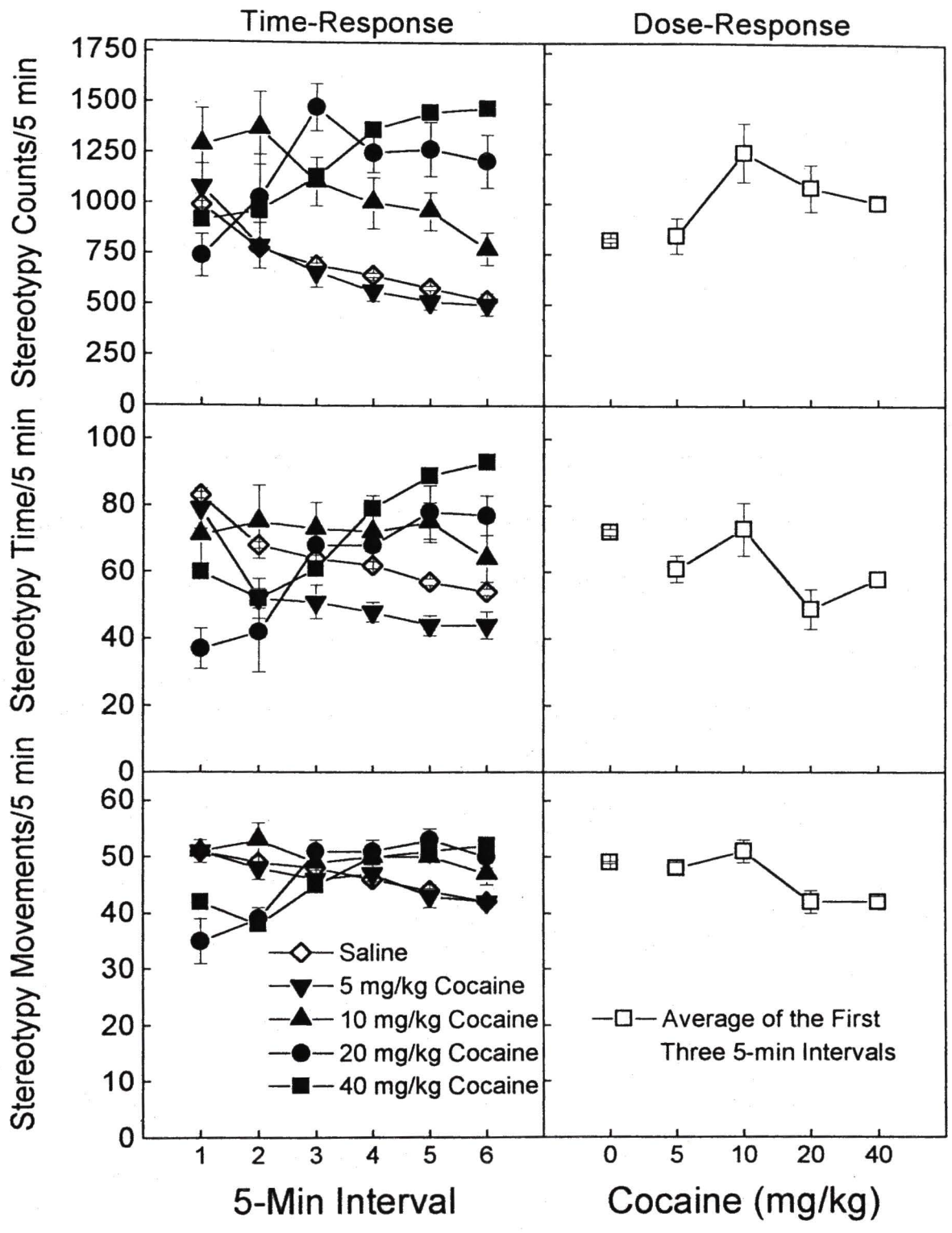

Time-Response
Dose-Response
Stereotypy Movements/5 min Stereotypy Time/5 min Stereotypy Counts/5 min
1750
1500
1250
1000
750
500
250
0
100
80
60
40
20
0
60
50
40
30
20
10
0
Saline
5 mg/kg Cocaine
10 mg/kg Cocaine
20 mg/kg Cocaine
40 mg/kg Cocaine
Average of the First Three 5-min Intervals
1
2
3
4
5
6
0
5
10
20
40
5-Min Interval
Cocaine (mg/kg)

Fig. 2-4. Dose-response curves for cocaine-induced vertical counts, time and movements in Swiss Webster mice. The left panels plot the dose-response curve against time in 5-min intervals. Each line represents a separate dose of cocaine. The right panels depict the mean of the means of the first three 5-min time intervals. Values plotted ± SEM. Number of subjects was at least 15.

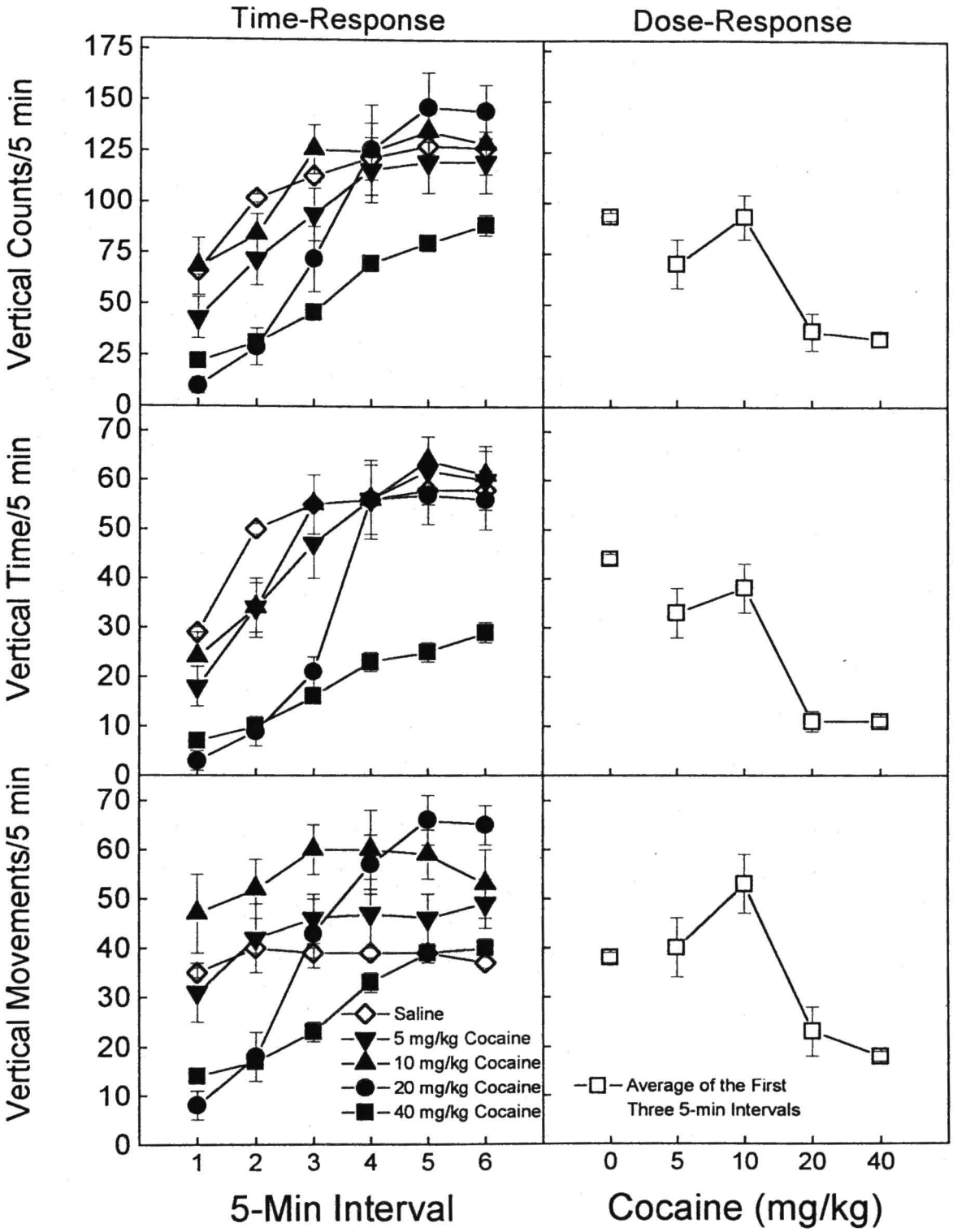

Time-Response
Dose-Response
Vertical Counts/5 min
Vertical Time/5 min
Vertical Movements/5 min
175
150
125
100
75
50
25
0
70
60
50
40
30
20
10
0
Saline
5 mg/kg Cocaine
10 mg/kg Cocaine
20 mg/kg Cocaine
40 mg/kg Cocaine
Average of the First
Three 5-min Intervals
1
2
3
4
5
6
0
5
10
20
40
5-Min Interval
Cocaine (mg/kg)

Fig. 2-5. Sensitization of cocaine-induced horizontal distance, average distance and average speed in Swiss Webster mice following one or four pairing sessions. The left half of the figure displays the data from the one pairing condition and the right side shows the four pairing condition. The left side of each panel depicts data from the pairing days and the right side depicts data from the challenge day. The X-axes indicate the days of pairing and the doses of cocaine given on the challenge day. Each group received two injections daily: one prior to placement in the testing chamber and one 2 h later in the home cage. The injection schedules are described in Table 1. Each value represents the mean of the means of the first three 5-min intervals of observation. Values plotted ± SEM. Number of subjects in each group is at least 15. Significant differences ($p<.05$) relative to the saline control group are marked with *, and those between the paired and unpaired groups are marked with ϕ.

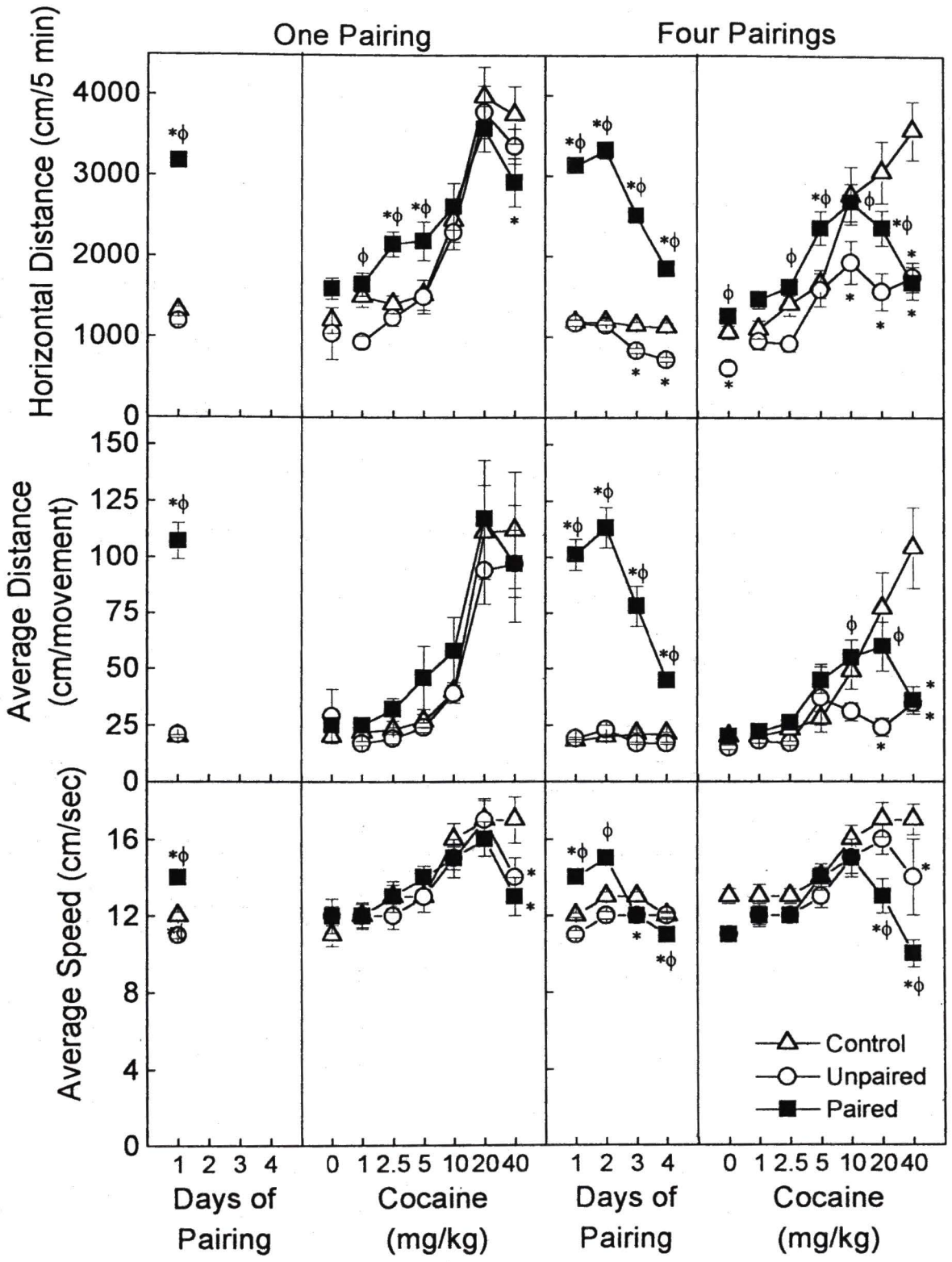

One Pairing
Four Pairings
Horizontal Distance (cm/5 min)
Average Distance (cm/movement)
Average Speed (cm/sec)
Control
Unpaired
Paired
Days of Pairing
Cocaine (mg/kg)
Days of Pairing
Cocaine (mg/kg)

Fig. 2-6. Sensitization of cocaine-induced horizontal counts, time and movements in Swiss Webster mice following one or four pairing sessions. The left half of the figure displays the data from the one pairing condition and the right side shows the four pairing condition. The left side of each panel depicts data from the pairing days and the right side depicts data from the challenge day. The X-axes indicate the days of pairing and the doses of cocaine given on the challenge day. Each group received two injections daily: one prior to placement in the testing chamber and one 2 h later in the home cage. The injection schedules are described in Table 1. Each value represents the mean of the means of the first three 5-min intervals of observation. Values plotted ± SEM. Number of subjects in each group is at least 15. Significant differences ($p<.05$) relative to the saline control group are marked with *, and those between the paired and unpaired groups are marked with ϕ.

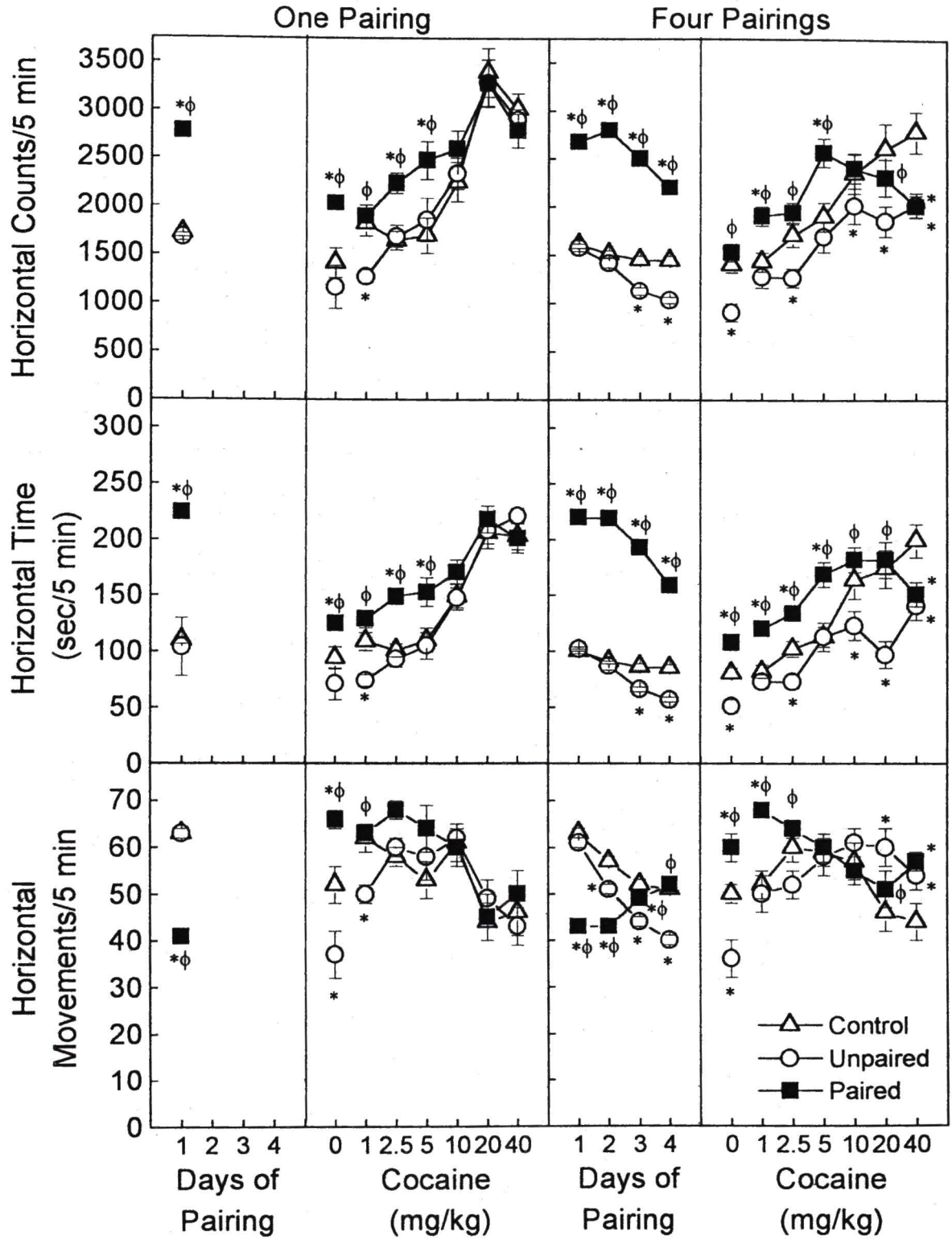

One Pairing
Four Pairings
Horizontal Counts/5 min
Horizontal Time (sec/5 min)
Horizontal Movements/5 min
Control
Unpaired
Paired
Days of Pairing
Cocaine (mg/kg)
Days of Pairing
Cocaine (mg/kg)

Fig. 2-7. Sensitization of cocaine-induced stereotypy counts, time and movements in Swiss Webster mice following one or four pairing sessions. The left half of the figure displays the data from the one pairing condition and the right side shows the four pairing condition. The left side of each panel depicts data from the pairing days and the right side depicts data from the challenge day. The X-axes indicate the days of pairing and the doses of cocaine given on the challenge day. Each group received two injections daily: one prior to placement in the testing chamber and one 2 h later in the home cage. The injection schedules are described in Table 1. Each value represents the mean of the means of the first three 5-min intervals of observation. Values plotted ± SEM. Number of subjects in each group is at least 15. Significant differences ($p<.05$) relative to the saline control group are marked with *, and those between the paired and unpaired groups are marked with ϕ.

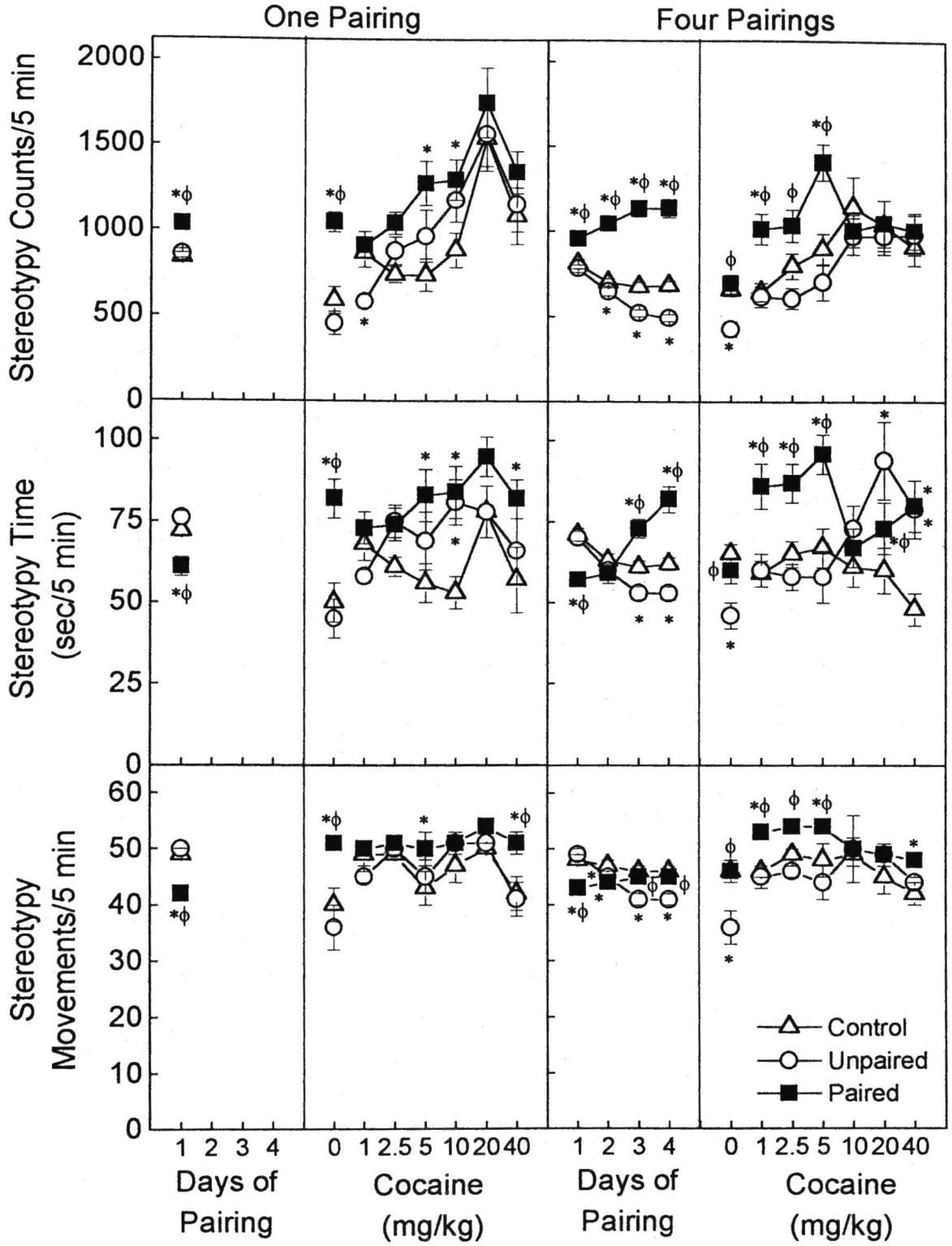

One Pairing
Four Pairings
Stereotypy Counts/5 min
2000
1500
1000
500
0
Stereotypy Time (sec/5 min)
100
75
50
25
0
Stereotypy Movements/5 min
60
50
40
30
20
10
0
Control
Unpaired
Paired
1 2 3 4
Days of Pairing
0 1 2.5 5 10 20 40
Cocaine (mg/kg)
1 2 3 4
Days of Pairing
0 1 2.5 5 10 20 40
Cocaine (mg/kg)

Fig. 2-8. Sensitization of cocaine-induced vertical counts, time and movements in Swiss Webster mice following one or four pairing sessions. The left half of the figure displays the data from the one pairing condition and the right side shows the four pairing condition. The left side of each panel depicts data from the pairing days and the right side depicts data from the challenge day. The X-axes indicate the days of pairing and the doses of cocaine given on the challenge day. Each group received two injections daily: one prior to placement in the testing chamber and one 2 h later in the home cage. The injection schedules are described in Table 1. Each value represents the mean of the means of the first three 5-min intervals of observation. Values plotted ± SEM. Number of subjects in each group is at least 15. Significant differences ($p<.05$) relative to the saline control group are marked with *, and those between the paired and unpaired groups are marked with ϕ.

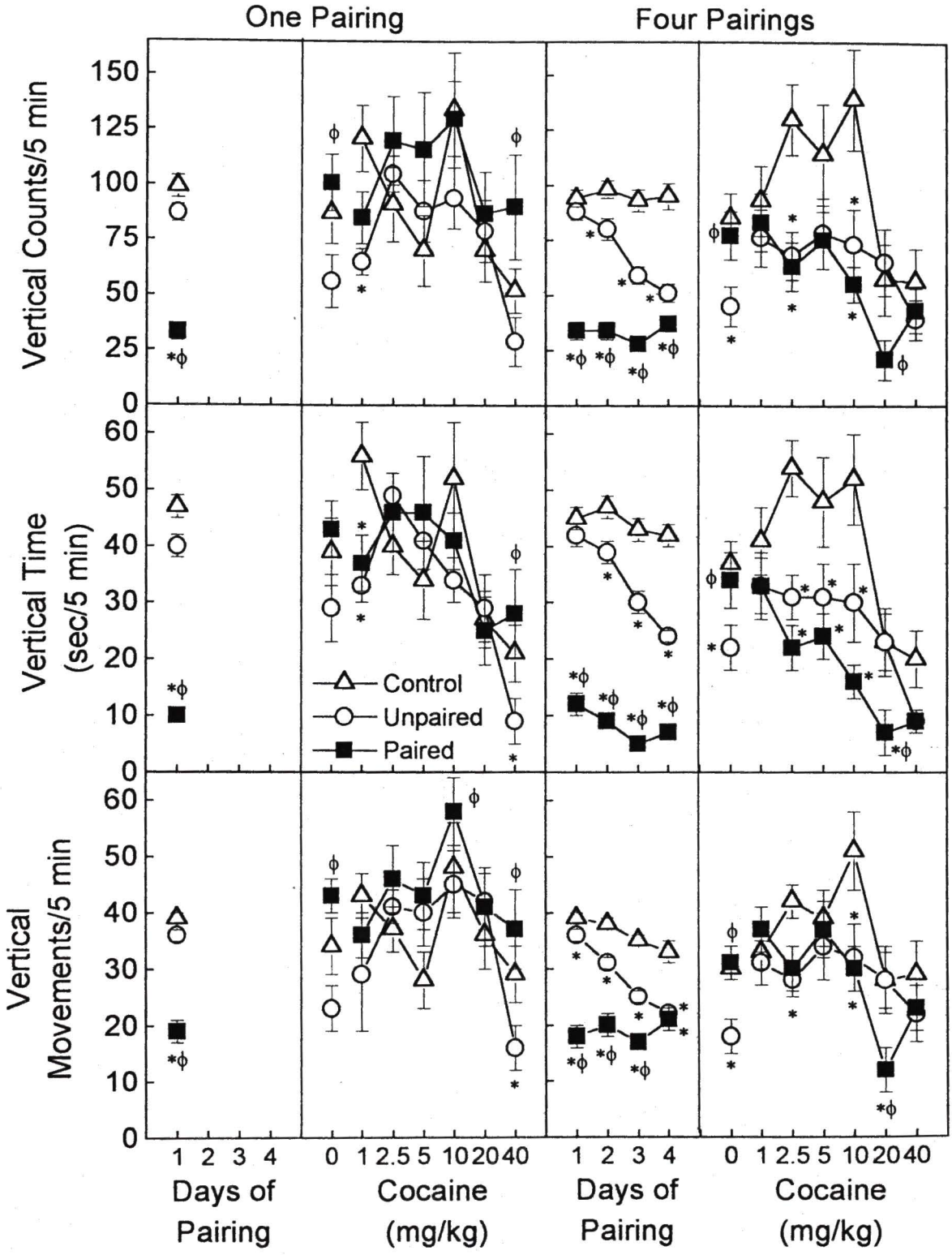

One Pairing
Four Pairings
Vertical Counts/5 min
Vertical Time (sec/5 min)
Vertical Movements/5 min
Control
Unpaired
Paired
Days of Pairing
Cocaine (mg/kg)
Days of Pairing
Cocaine (mg/kg)

Fig. 2-9. Magnitude of the conditioned response to cocaine after one or four pairings in Swiss Webster mice. The Y-axis represents the saline response of the paired group on challenge day / the cocaine response of the paired group on previous day * 100 in both pairing conditions. The X-axis indicates variables of motor behavior: Horizontal Distance (HD), Average Distance (AD) and Speed (AS); Horizontal Counts (HC), Time (HT) and Movements (HM), Stereotypy Counts (SC), Time (ST) and Movements (SM). Values plotted ± SEM. Number of subjects in each group was at least 19. Significant differences ($p<.05$) between the pairing conditions are marked with *.

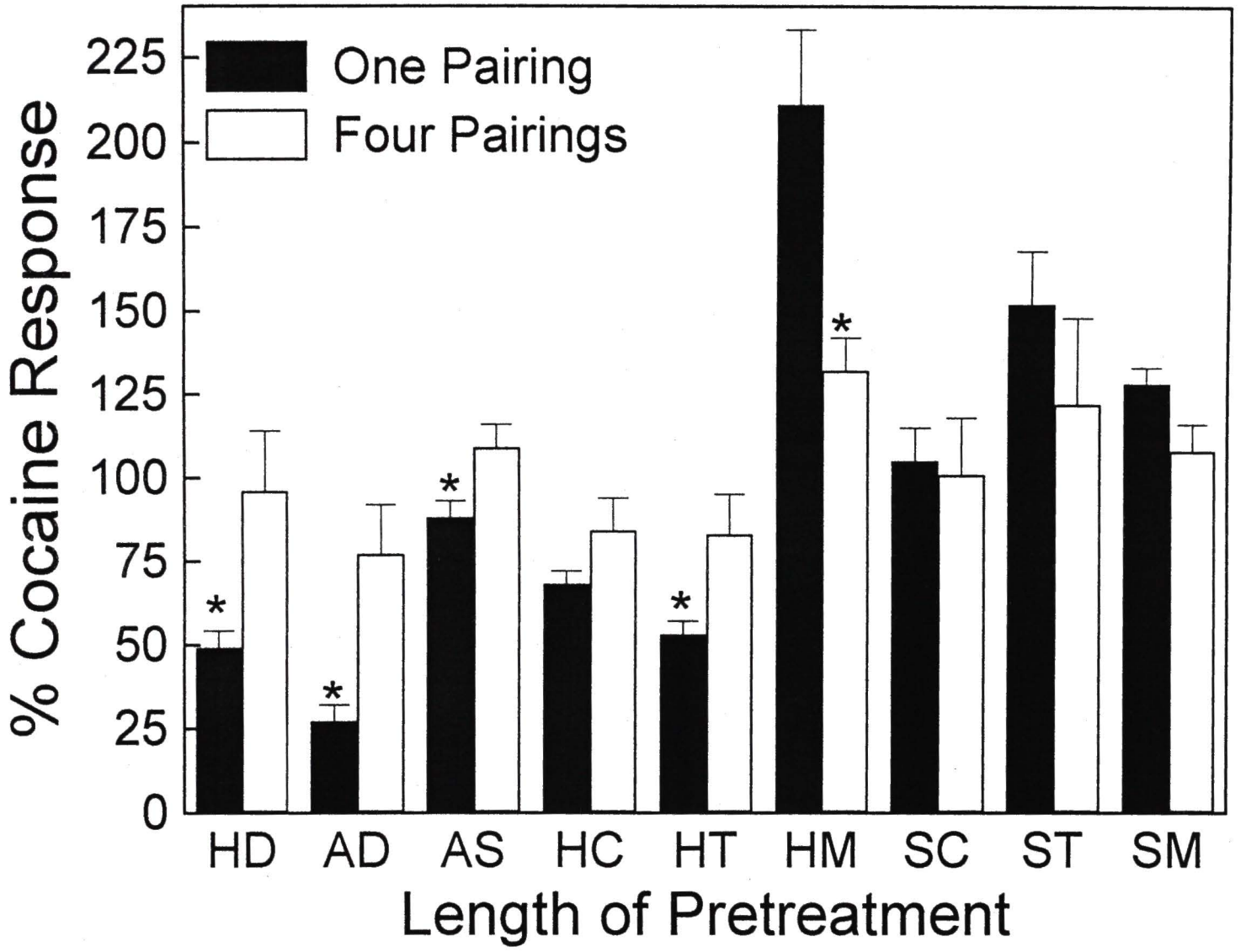
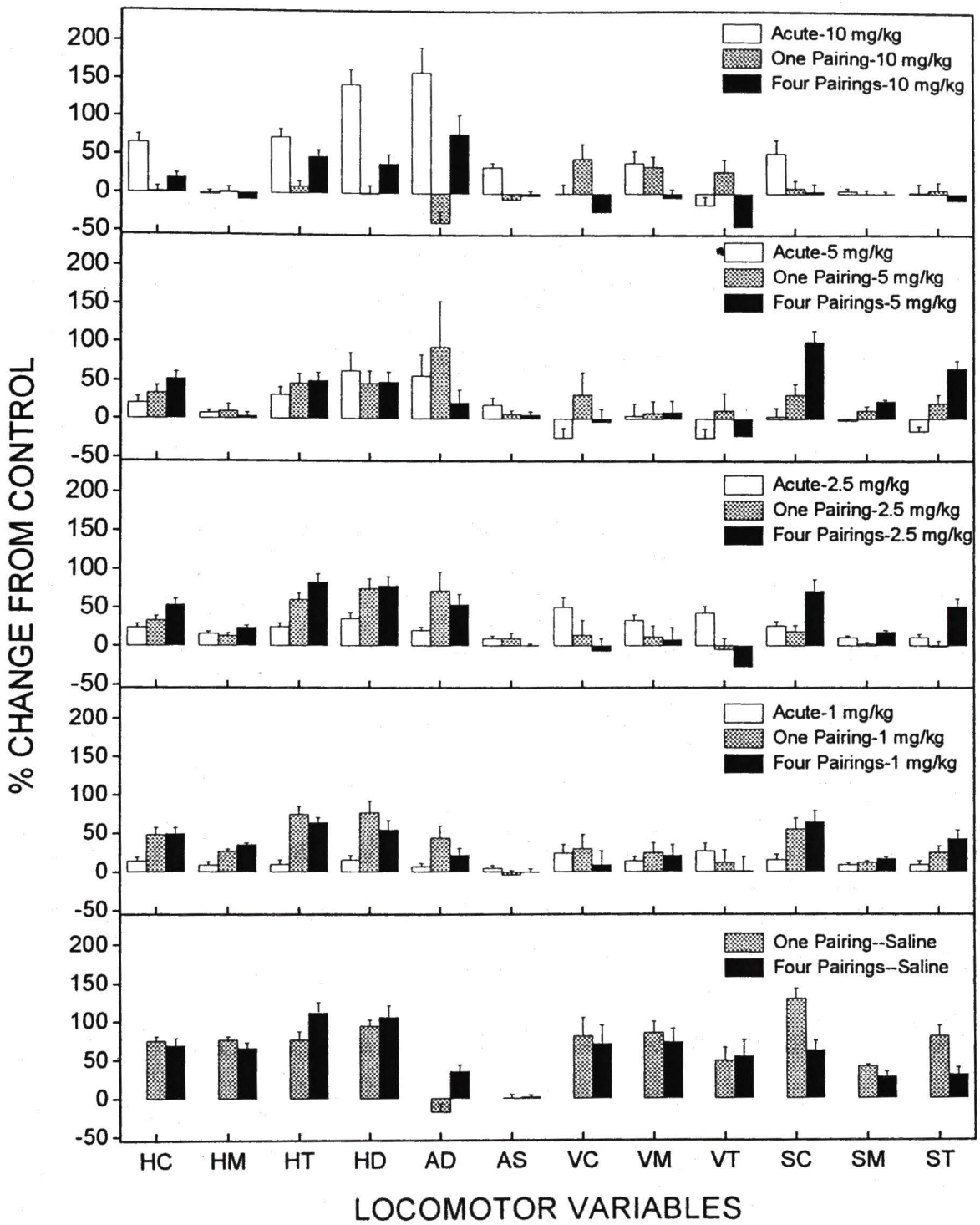

One Pairing
Four Pairings
% Cocaine Response
225
200
175
150
125
100
75
50
25
0
HD
AD
AS
HC
HT
HM
SC
ST
SM
Length of Pretreatment

Fig. 2-10. Comparison of the quality of the acute cocaine response in the saline control group with the context-dependent sensitization response following a single pairing with 40 mg/kg cocaine or four pairings with 40 mg/kg cocaine in Swiss Webster mice. Acute values = (Saline Control Group's response to each dose / mean of the Saline Control Group's response to saline * 100) – 100. Context-dependent sensitization values for both the one and four pairing conditions = (Paired Group's response to each dose / mean of the Unpaired Group's response to same dose * 100) – 100. Locomotor variables are Horizontal Counts (HC), Time (HT) and Movements (HM), Horizontal Distance (HD), Average Distance (AD) and Speed (AS), Vertical Counts (VC), Movements (VM)and Time (VT) and Stereotypy Counts (SC), Movements (SM)and Time (ST). Values plotted ± SEM. Number of subjects was at least 15 for each group.


% CHANGE FROM CONTROL
200
150
100
50
0
-50
Acute-10 mg/kg
One Pairing-10 mg/kg
Four Pairings-10 mg/kg
Acute-5 mg/kg
One Pairing-5 mg/kg
Four Pairings-5 mg/kg
Acute-2.5 mg/kg
One Pairing-2.5 mg/kg
Four Pairings-2.5 mg/kg
Acute-1 mg/kg
One Pairing-1 mg/kg
Four Pairings-1 mg/kg
One Pairing--Saline
Four Pairings--Saline
HC
HM
HT
HD
AD
AS
VC
VM
VT
SC
SM
ST
LOCOMOTOR VARIABLES

CHAPTER III

EXPERIMENT II

Context-Dependent Sensitization of Cocaine's Motor Stimulant Effects in C57BL/6 and DBA/2 Mice

Abstract

The hypothesis tested by this study was that genetic differences in the development and persistence of context-dependent sensitization contribute to cocaine seeking behavior and, ultimately, to risk for development and persistence of cocaine dependence. Sensitization in this study was defined as an increase in cocaine-induced motor behavior in response to repeated drug exposure. By pairing cocaine with different environments and for different lengths of time, measures of the onset and magnitude of context-dependent and context-independent sensitization were obtained for the inbred mouse strains, C57BL/6 and DBA/2. Then by giving saline in the environments associated with cocaine for different lengths of time, measurements of the resistance to extinction were obtained. C57BL/6 mice had delayed onset of context-dependent sensitization relative to the DBA/2 mice. However, once developed, the context-dependent sensitization of the C57BL/6 mice was of greater magnitude and more resistant to extinction than that of the DBA/2 mice. Furthermore, the response to saline by the C57BL/6 mice that received cocaine in the

testing chamber was qualitatively similar to a 10 mg/kg dose of cocaine, whereas that of the DBA/2 mice was similar to a 5 mg/kg dose of cocaine.

Introduction

The susceptibility to drug dependence of C57BL/6 and DBA/2 mice has been compared across many classes of abused drugs and via many different paradigms. For example, when oral morphine consumption of C57BL/6J mice was compared to that of DBA/2J mice, the C57BL/6 mice consumed 90% of their daily fluid intake from the morphine-saccharin bottle, whereas DBA/2J mice consumed only 13% (Phillips et al, 1991). The C57BL/6J mice also consumed more after a 24-h period of water deprivation than did DBA/2J mice (Plomin and McClearn, 1993). Another study showed that C57BL/6J mice initiated self-administration of cocaine, morphine, methamphetamine and pentobarbital, while DBA/2J mice self-administered all but cocaine (Carney et al, 1991); however, other investigators observed acquisition of cocaine self-administration in DBA/2J mice when doses lower than those used by C57BL/6J mice were given as the training dose (Grahame and Cunningham, 1995; Rocha et al, 1996). Further comparisons of these strains' susceptibility to cocaine abuse involved testing the conditioned place preference established after pairing 32 mg/kg cocaine with one chamber of a two-chambered compartment (Seale and Carney, 1991). The cocaine conditioned place preference was found to be greater in C57BL/6 than in DBA/2 mice. Thus, it appears that the C57BL/6 mice are a rela-

tively drug-seeking strain, while the DBA/2 mice are a relatively drug-avoiding strain.

The current study evaluated the context-dependent sensitization to the behavior-stimulating effects of cocaine in both C57BL/6 and DBA/2 mice. In this study, sensitization refers to the enhancement of cocaine's stimulatory effects with repeated drug exposure. Previous studies have shown that sensitization to the behavioral effects of cocaine most frequently occurs in a context-dependent manner (c.f. Badiani et al, 1995; Fontana et al, 1993; Jackson and Nutt, 1993; Post et al, 1992).. For example, Hinson and Poulos (1981) found that cocaine's stimulatory effects on the locomotor and stereotypical behavior of rats were enhanced when the drug was administered in the presence of cues previously associated with cocaine administration than without those cues. Furthermore, they found that sensitization to be extinguishable, when saline was presented in the presence of the cocaine-associated cues, thus indicating further the importance of context in sensitization to cocaine.

Conditioned aspects of sensitization are important to the overall study of drug dependence because environmental triggers can precipitate a conditioned state of arousal similar to that obtained with cocaine use. Human addicts report experiencing a cocaine taste at the back of the throat, a faint ringing in the ears, a feeling of excitement and sexual arousal, and a hot or cold "rush" when environmental cues trigger their sensation of craving (Childress et al, 1993). In effect, environmental cues can serve as a priming dose of cocaine, which can induce craving and drug-

seeking behavior (Weissenborn et al, 1995; Jaffe et al, 1989; Stewart and deWit, 1987). Currently, some cocaine abstinence programs employ extinction training to remove the conditioned responses humans have to certain environmental stimuli (O'Brien et al, 1992). This extinction training has shown increased abstinence rates.

Previous studies of sensitization using locomotor behavior failed to include full dose response curves when determining the presence or absence of sensitization (Koff et al, 1994; Fontana et al, 1993; Wolf et al, 1993; Post et al, 1992). Furthermore, with the exception of Fontana et al (1993), they did not quantify the context-dependent aspect of sensitization. Another group of researchers attempted to measure the context-dependent aspect of sensitization in mice (Tolliver and Carney, 1994; Tolliver et al, 1994). For six days, they gave a 32 mg/kg cocaine injection to a "paired" group immediately prior to being placed in an activity cage whereas an "unpaired" group was given the same injection schedule without exposure to the activity cage. On day seven, both groups were placed into the activity cage after challenge injections with saline or cocaine (from 1 to 100 mg/kg). In this way, the unpaired group represented not only context-independent sensitization but also the novelty effect as the animals explored the activity cage for the first time. Therefore, Tolliver et al (1994) were unable to detect genetic associations involved in the context-dependent sensitization to cocaine when they performed a quantitative trait loci (QTL) analysis on their data. The present study differed from such ex-

periments by conducting a full dose-response curve on the challenge day and by having an unpaired group that was equally exposed to the activity cage.

The hypothesis tested by this study was that genetic differences in the development and persistence of context-dependent sensitization contribute to cocaine-seeking behavior and, ultimately, to risk for development and persistence of cocaine dependence. If the hypothesis is correct, then genetically defined mice which differ in drug-seeking behavior should also show parallel differences in the rate of acquisition of context-dependent sensitization, the magnitude of such sensitization, or the persistence of context-dependent effects during extinction.

Methods

See Chapter II for description of apparatus and behavioral measures.

Animals

Two-month-old, male C57BL/6 and DBA/2 mice (Jackson Laboratories) were housed in groups of 4 to 5 for at least seven days before the experiments began. Mice were housed under same conditions as described in Chapter II.

Cocaine hydrochloride (National Institute of Drug Abuse (NIDA), Bethesda, MD) was dissolved in isotonic saline (0.9%) at concentrations of 0.5, 1.0, 2.0, 4.0 and 6.0 mg/ml and was injected intraperitoneally (i.p.) in a volume of 0.01 ml/g of body weight. The final cocaine HCl dosages were 5, 10, 20, 40 and 60 mg/kg.

Procedure

Cocaine-induced motor behavior was defined as an increase in the total distance traveled or the number of stereotypy counts as measured in a 30-min session using a Digiscan motor activity testing chamber attributable to the presence of cocaine. Sensitization was defined as an increase in motor response to a saline injection and/or a leftward shift in the dose-response curve for cocaine-induced motor behavior that was attributable to cocaine pre-exposure. Context-dependent sensitization was inferred by a difference in sensitization between groups of mice that had been pre-exposed to cocaine in the testing chamber versus those pre-exposed in their home cages. The rate of acquisition was estimated by comparing context-dependent sensitization in separate groups of mice receiving differing degrees of cocaine pre-exposure. The magnitude of context-dependent sensitization was evaluated by comparing the response to a challenge injection of saline in the group pre-exposed to cocaine in the testing chamber with their response to cocaine the previous day. The persistence of context-dependent sensitization was determined by comparing the context-dependent sensitization present after subjecting the groups to differing degrees of extinction.

A dose-response curve for cocaine was calculated by administering saline or cocaine (5, 10, 20, 40 and, for DBA/2 only, 60 mg/kg) i.p. in animals from each strain immediately prior to placement in the activity cage a 30 min session. The Digiscan apparatus recorded motor activity in 5-min intervals.

In order to test the rate of acquisition of context-dependent sensitization, groups of mice were exposed to either one or four days of cocaine and saline pairing. During the pairing days, each animal received two i.p. injections: 1) immediately prior to placement in the Digiscan activity cage for 30 min, and 2) in the home cage in the animal colony 2 h later. The contents of those injections varied for each group (see Table 1). Cocaine pre-exposure occurred in the activity cage for the Paired group and in the home cage for the Unpaired group. The Saline Control group received saline in each environment. On the day after the last pairing day, mice from each pairing condition were challenged with either saline or cocaine (5, 10, 20 or 40 mg/kg) via i.p. injection immediately prior to placement in the activity cage for 30 min. Data was collected in six 5-min intervals.

The maximum effect of cocaine on the horizontal distance and stereotypy counts measures in the C57BL/6 mice was in response to 20 mg/kg cocaine, according to the dose-response curves. Therefore, a preliminary experiment was performed in the C57BL/6 mice using 20 mg/kg cocaine as the pairing dose to determine whether or not this dose would increase their context-dependent sensitization after one pairing relative to pairing with the 40 mg/kg cocaine dose. It was concluded that context-dependent sensitization was optimized by pairing 40 mg/kg cocaine to the activity cage.

Resistance of context-dependent sensitization to extinction was tested by adding to the sensitization experiment one or four days wherein saline was given in place of all cocaine injections. On the day after the last extinction session, the mice

were challenged with either saline or cocaine (5 mg/kg) via i.p. injection immediately prior to placement in the activity cage for 30 min. The 5 mg/kg cocaine dose was chosen because of the strains' volatile sensitization response elicited by this dose.

Statistics

The statistical analyses were performed using SYSTAT version 5.0 (Wilkinson, 1990). Perusal of the acute cocaine time-response curves for the motor variables tested showed peak effects of cocaine in the first 15 min; therefore, the following analyses were performed using an average of the first three 5-min intervals for each variable.

For the dose-response data, a two-way ANOVA using Strain and Dose as the between subjects factors was performed. Also comparison of this data to the conditioned effect observed after a single pairing with 20 or 40 mg/kg cocaine was performed using a one-way ANOVA with Dose as the between subjects factor, followed by a Dunnett two-sided test to determine in each strain which acute cocaine dose the conditioned response was most similar to in character, as well as determining which pairing dose produced the greater sensitization in the C57BL/6 mice.

Two-way repeated measures ANOVAs were performed on the motor behavior data from the pairing and extinction days using Group as the within subjects factor and Pairing Day or Extinction Day as within groups factors. On the data from the challenge days, three-way ANOVAs using Number of Pairings or Extinctions, Group, and Dose as between subjects factors were performed. Fisher's LSD comparisons

were performed as well. In addition, four-way ANOVAs using Strain, Number of Pairings or Extinctions, Group, and Dose as between subjects factors were executed. To calculate a measure of the magnitude of context-dependent sensitization, a percentage of the cocaine effect from the final day of pairing was calculated by dividing the paired group's cocaine response into the paired group's saline response on the initial day of extinction. Two-way ANOVAs using Strain and Number of Pairings as between subjects factors were performed on that data.

Comparisons of the multivariate behavior profile of context-dependent cocaine sensitization versus that of acute cocaine were made using calculations of context-dependent and acute behavior. Context-dependent values were determined by dividing the average of the unpaired group's response to a particular dose of drug into the response by each subject of the paired group at that same dose, then multiplying by 100 and subtracting 100 to give a measure of percent change from control. In a similar fashion, acute values were calculated by dividing the average response to saline into the response to each dose of cocaine acutely, and again multiplying by 100 and then subtracting 100.

Results

Dose-Response Curves

Figures 3-1 and 3-2 represent the horizontal distance and stereotypy counts induced by various doses of cocaine in both strains plotted against the 5-min time intervals in which the data was collected. Perusal of this data indicated that the

peak effects of cocaine were generally observed within the first 15 min; therefore, an average of the first three intervals was used in the following analyses and graphs.

Figure 3-3 shows the dose-response curves for cocaine-induced horizontal distance and stereotypy in both strains. Significant differences in horizontal distance were found between the strains in response to the 10, 20 and 40 mg/kg doses using a two-way ANOVA (Strain: $F_{1, 142} = 58.873$, $p<.001$; Dose: $F_{4, 142} = 150.863$, $p<.001$; Strain X Dose: $F_{4, 142} = 23.197$, $p<.001$). Also, significant differences in baseline and 5 mg/kg cocaine-induced stereotypy were determined by using a two-way ANOVA (Strain: $F_{1, 142} = 8.073$, $p<.005$; Dose: $F_{4, 142} = 4.751$, $p<.001$; Strain X Dose: $F_{4, 142} = 3.492$). The DBA/2 mice were given 60 mg/kg cocaine to determine whether or not the dose-response curve had peaked with the response to 40 mg/kg cocaine.

Determination of the Cocaine Dose for Pairing

A single pairing preliminary study using 20 and 40 mg/kg cocaine as the pairing dose in C57BL/6 mice showed that the 20 mg/kg dose was slightly better at inducing context-dependent sensitization of the horizontal distance-enhancing effects of cocaine (see Fig. 4). There was no improvement in the context-dependent sensitization of stereotypy by using the 20 mg/kg dose instead of the 40 mg/kg dose. However, comparisons of the multivariate behavior profiles of acute low doses of cocaine with the conditioned response developed after a single pairing

with 20 or 40 mg/kg cocaine (see Fig. 5) showed that there was little difference between the conditioned responses developed by the two pairing doses of cocaine. It was felt that administering the same dose of cocaine to both strains would ease interpretation of the data; therefore, the pairing dose was chosen to be 40 mg/kg cocaine for the remaining experiments.

For comparison with the C57BL/6 mice, the multivariate behavior profile of the acute and conditioned responses to cocaine in DBA/2 mice are provided in Fig. 6. The conditioned responses of DBA/2 mice are more like the 5 mg/kg than the 10 mg/kg cocaine dose. As shown in Fig. 5, the conditioned response in C57BL/6 mice developed after one pairing with 40 mg/kg cocaine resembled the acute response to 10 mg/kg cocaine. Also of note in Fig. 6 is the large difference between the acute responses to 5 and 10 mg/kg cocaine in the DBA/2 mice. In the C57BL/6 mice, there was no significant difference between the responses to the two doses of cocaine.

Sensitization of Cocaine's Effect on Horizontal Distance

The effects of cocaine on horizontal distance in both strains after the one and four pairings with cocaine can be seen in Fig. 7. As determined by a two-way ANOVA performed on the data from the one pairing condition using Group and Dose as between subjects factors, there was no significant difference between the groups of C57BL/6 mice in response to the cocaine challenge doses. However, because the standard error differs between the saline dose and the cocaine doses, a

one-way ANOVA using Group as the between subjects factor was performed using the horizontal distance data from only the saline dose, and it showed a significant difference between the paired group and the other two groups (Group: $F_{2, 21} = 6.167$, $p<.008$). After four pairings with cocaine, there was a leftward shift as well as a reduction in the maximal effect of cocaine in the C57BL/6 paired group's dose-response curve for horizontal distance relative to the saline control group and an upward shift of the curve relative to the unpaired group. Using a two-way ANOVA on the horizontal distance data from the C57BL/6 four pairing condition with Group and Dose as between subjects factors, significant differences between the groups were found (Group: $F_{2,101} = 16.804$, $p<.001$; Dose: $F_{4,101} = 20.433$, $p<.001$; Group X Dose: $F_{8,101} = 5.892$, $p<.001$). As with the one pairing condition, a one-way ANOVA using Group as the between subjects factor and using the horizontal distance data from only the saline dose showed a significant difference between the paired group and the other two groups (Group: $F_{2, 20} = 21.490$, $p<.001$). Also of note was the decline in the C57BL/6 paired group's horizontal distance response to cocaine with each successive cocaine pairing on days 1-4. On the day following the last pairing session, the paired group's response to the saline challenge resulted in an increase in horizontal distance traveled that was very similar to the previous day's response to 40 mg/kg cocaine. In addition, after the third pairing session with cocaine, the C57BL/6 unpaired group showed a downward shift in their horizontal distance response to saline that surpassed the habituation observed in the saline control group.

In contrast to the C57BL/6 mice, the dose-response curve for horizontal distance in the DBA/2 paired group was shifted upward relative to the other two groups after only one pairing with cocaine. A two-way ANOVA confirmed the significant differences between the groups in response to the 5 and 10 mg/kg doses of cocaine (Group: $F_{2,105} = 10.201$, $p<.001$; Dose: $F_{4,105} = 67.221$, $p<.001$; Group X Dose: $F_{8,105} = 2.561$, $p<.014$). Again, because the standard error differs between the saline dose and the cocaine doses, a one-way ANOVA using Group as the between subjects factor was performed using the horizontal distance data from only the saline dose, and it showed a significant difference between the paired group and the other two groups (Group: $F_{2, 18} = 8.202$, $p<.003$). After four pairings with cocaine, the upward shift of the dose-response curve for horizontal distance in the DBA/2 paired group was not as pronounced as after only one pairing. A one-way ANOVA using Group as the between subjects factor and using the horizontal distance data from only the saline dose showed a significant difference between the Paired group and the other two groups (Group: $F_{2, 18} = 8.202$, $p<.003$). A two-way ANOVA that was performed on the horizontal distance data from the four pairing condition using Group and Dose as the between subjects factors did not show a significant difference between the groups overall (Group: $F_{2, 105} = 2.782$, $p<.066$), but there was a significant difference between the paired and other two groups in response to 5 mg/kg cocaine. The DBA/2 mice did not show a decline in their horizontal distance response to cocaine until the fourth pairing session. Unlike the C57BL/6 mice, the DBA/2 paired group's horizontal distance response to a saline challenge was only a

fraction of the previous day's response to cocaine. Furthermore, the DBA/2 unpaired group did not display the decreased horizontal distance response to saline that was observed in the C57BL/6 mice during the pairing sessions.

The overall effects of Strain and Number of Pairings on horizontal distance were significant as determined by a four-way ANOVA using Strain, Number of Pairings, Group and Dose as between subjects factors (Strain: $F_{1, 415} = 123.130$, $p<.001$; Number of Pairings: $F_{1,415} = 22.294$, $p<.001$; Group: $F_{2,415} = 19.057$, $p<.001$; Dose; $F_{4,415} = 131.319$, $p<.001$; Strain X Dose: $F_{4, 415} = 13.764$, $p<.001$; Group X Dose: $F_{8,415} = 4.449$, $p<.001$; Strain X Number of Pairings X Group: $F_{2,415} = 4.128$, $p<.017$).

Sensitization of Cocaine's Effect on Stereotypy Counts

The effects of cocaine on stereotypy counts in both strains after the one and four pairings with cocaine can be seen in Fig. 8. As determined by a two-way ANOVA performed on the stereotypy data from the one pairing condition using Group and Dose as between subjects factors, there was no significant difference between the groups of C57BL/6 mice in response to the cocaine challenge doses. However, a one-way ANOVA using Group as the between subjects factor was performed using the stereotypy data from only the saline dose, and it showed a significant difference between the paired group and the other two groups (Group: $F_{2, 21} = 5.064$, $p<.016$). After four pairings with cocaine, there was an upward shift as well as an increase in the maximal stereotypy effect of cocaine in the C57BL/6 paired group's dose-response curve relative to the saline control group and an upward and

leftward shift of the curve relative to the unpaired group. Using a two-way ANOVA on the stereotypy data from the C57BL/6 four pairing condition with Group and Dose as between subjects factors, significant differences between the groups were found (Group: $F_{2,101} = 10.611$, $p<.001$; Dose: $F_{4,101} = 6.494$, $p<.001$; Group X Dose: $F_{8,101} = 2.542$, $p<.015$). Furthermore, there was no decline in the C57BL/6 paired group's stereotypy response to cocaine during the pairing sessions. On the day following the last pairing session, the paired group's response to the saline challenge resulted in an increase in stereotypy counts that was very similar to the previous day's response to 40 mg/kg cocaine. In addition, after the third pairing session with cocaine, the C57BL/6 unpaired group showed a downward shift in their stereotypy response to saline that surpassed the habituation observed in the saline control group.

In contrast to the C57BL/6 mice, the dose-response curve for stereotypy in the DBA/2 paired group was shifted upward relative to the other two groups after only one pairing with cocaine. There was also an upward shift of the dose-response curve for stereotypy in the DBA/2 unpaired group relative to the saline control group, which indicates context-independent sensitization. A two-way ANOVA confirmed the significant differences between the groups (Group: $F_{2,105} = 11.509$, $p<.001$; Dose: $F_{4,105} = 6.752$, $p<.001$). Again, because the standard error differs between the saline dose and the cocaine doses, a one-way ANOVA using Group as the between subjects factor was performed using the stereotypy data from only the saline dose, and it showed a significant difference between the paired group and

the other two groups (Group: $F_{2, 18} = 10.335$, $p<.001$). After four pairings with cocaine, the upward shift of the dose-response curve for stereotypy in the DBA/2 paired group relative to the unpaired group was not as pronounced as after only one pairing. This is mainly due to the increased upward shift of the dose-response curve of the DBA/2 unpaired group relative to the saline control group. A two-way ANOVA that was performed on the stereotypy data from the four pairing condition using Group and Dose as the between subjects factors showed a significant difference between the groups (Group: $F_{2, 105} = 21.807$, $p<.001$; Dose: $F_{4, 105} = 24.617$, $p<.001$; Group X Dose: $F_{8, 105} = 2.924$, $p<.005$). During the pairing sessions, there was no decline in the saline-induced activity of the DBA/2 unpaired group on the stereotypy measure. There was an increase in the DBA/2 paired group's stereotypy response during the second pairing session, and this was followed on days three and four by a slight decline in the stereotypy response to 40 mg/kg cocaine. On following day, the stereotypy response of the DBA/2 paired group to saline was approximately half of the response to cocaine from the previous day.

The overall effects of Strain and Number of Pairings on stereotypy were not significant as determined by a four-way ANOVA using Strain, Number of Pairings, Group and Dose as between subjects factors; however, several interactions between the factors were significant (Group: $F_{2,415} = 29.183$, $p<.001$; Dose; $F_{4,415} = 24.369$, $p<.001$; Strain X Group: $F_{2,415} = 8.444$, $p<.001$; Strain X Dose: $F_{4, 415} = 9.951$, $p<.001$; Number of Pairings X Dose: $F_{4,415} = 2.893$, $p<.022$; Group X Dose: $F_{8,415} = 3.057$, $p<.002$; Strain X Number of Pairings X Group: $F_{2,415} = 4.954$, $p<.007$).

The Effect of Extinction on Horizontal Distance

Figure 3-9 shows the horizontal distance results from the one pairing and one or four extinction sessions condition. T-tests performed separately on the saline and cocaine challenge data for horizontal distance from both strains showed that the paired and unpaired groups were significantly different after one extinction (C57BL/6: saline, $p<.013$; cocaine, $p<.010$. DBA/2: saline, $p<.008$; cocaine, $p<.020$), but not after four extinction sessions. The C57BL/6 paired group showed a significant increase in horizontal distance relative to the unpaired group on the second day of the extinction sessions as confirmed by a repeated measures ANOVA using Group as the between subjects factor and Extinction as the within groups factor (on day 2 of the extinction sessions, Group: $F_{1, 90} = 9.228$, $p<.003$). By the second extinction day, the horizontal distance response by the DBA/2 paired and unpaired groups were not significantly different.

The horizontal distance effects of four pairings with cocaine and one or four extinction sessions can be seen in Fig. 10. The reaction of the paired and unpaired groups to four pairing sessions greatly resembled the previously described sensitization experiment. In addition, the responses to saline on the first extinction day were similar in magnitude to the saline response in the sensitization experiment. After four pairings and one extinction session, the paired groups of both strains evidenced increased horizontal distance responses to the saline and cocaine challenges relative to their unpaired groups. T-tests performed separately on the saline and cocaine challenge data for horizontal distance from both strains showed that

the paired and unpaired groups were significantly different after one extinction (C57BL/6: saline, $p<.001$; cocaine, $p<.001$. DBA/2: saline, $p<.002$; cocaine, $p<.008$). After four extinction sessions, the DBA/2 groups showed a significant difference in horizontal distance traveled only in response to the cocaine challenge ($p<.039$), whereas the C57BL/6 mice displayed a significant difference between the groups in response to saline ($p<.009$). Also of note is the downward trend in saline-induced activity of the C57BL/6 unpaired group during the cocaine pairing days and the upward trend in saline-induced activity of the C57BL/6 unpaired group throughout the days of extinction.

The Effects of Extinction on Stereotypy Counts

The stereotypy effects of cocaine following one pairing and one or four extinction sessions can be seen in Fig. 11. After one pairing and one extinction session, the C57BL/6 paired group showed increased stereotypy in response to cocaine, but not to saline, relative to the unpaired group. T-tests performed separately on the data from the saline and cocaine challenges confirmed the significant differences between the groups ($p<.016$). After four extinction sessions, there was no difference between the response of the C57BL/6 groups to saline or cocaine. The paired and unpaired groups of the DBA/2 mice showed no significant difference after one or four extinction sessions. It is important to point out, however, that the C57BL/6 paired group showed significant differences from the unpaired group up to and including the fourth day of the extinction sessions as confirmed by a repeated

measures ANOVA using Group as the between subjects factor and Extinction as the within groups factor (on day 4, Group: $F_{1, 90} = 5.842$, $p<.018$). The DBA/2 paired group only showed a significant difference from the unpaired group through day 2 of the extinction sessions.

The stereotypy effects of cocaine following four pairings with cocaine and one or four extinction sessions can be seen in Fig. 12. After one extinction session, both strains showed significant differences between their paired and unpaired groups in response to both the saline and cocaine challenges, and this was confirmed by t-tests (C57BL/6: saline, $p<.009$; cocaine, $p<.004$. DBA/2: saline, $p<.001$; cocaine, $p<.006$). Both strains showed significant differences between the paired and unpaired groups with regard to stereotypy behavior during all four extinction sessions. This was confirmed by a repeated measures ANOVA using Group as the between subjects factor and Extinction as the within groups factor. However, on the day after the last extinction session, only the C57BL/6 mice showed any difference between groups, and that was only in response to cocaine, (t-test, $p<.017$.)

Magnitude of Conditioned Response

In Figure 3-13 the data from both the one and four extinction experiments were combined to allow a comparison of the magnitude of the saline response on the first extinction session with the magnitude of the cocaine response on the last pairing session. A two-way ANOVA performed on the horizontal distance and stereotypy count data using Strain and Number of Pairings as between subjects

factors confirmed the significance of the differences reported in Fig. 13 (Horizontal distance: Strain: $F_{1, 142} = 76.971$, $p<.001$; Pairing: $F_{1, 142} = 6.248$, $p<.014$. Stereotypy counts: Strain: $F_{1, 142} = 8.465$, $p<.004$; Pairing: $F_{1, 142} = 25.557$, $p<.001$).

Endnotes

1. Partial support was provided by the NIDA contract N01DA-2-9305. This work was part of a dissertation project by Linda A. Odom.

Table 3-1. Schedule of injections during pairing phase of the experiments.

Group	Testing chamber	Home Cage
Paired	Cocaine (40 mg/kg)	Saline
Unpaired	Saline	Cocaine (40 mg/kg)
Saline Control	Saline	Saline

Fig. 3-1. Time-response curve for cocaine-induced horizontal distance and stereotypy counts in C57BL/6. Horizontal distance and stereotypy counts in the C57BL/6 mice following injections of saline or cocaine (from 5 to 40 mg/kg) are plotted against the 5-min time intervals in which the data was collected. Each line is a separate dose. Values are plotted ± SEM. Number of subjects in each group was eight.

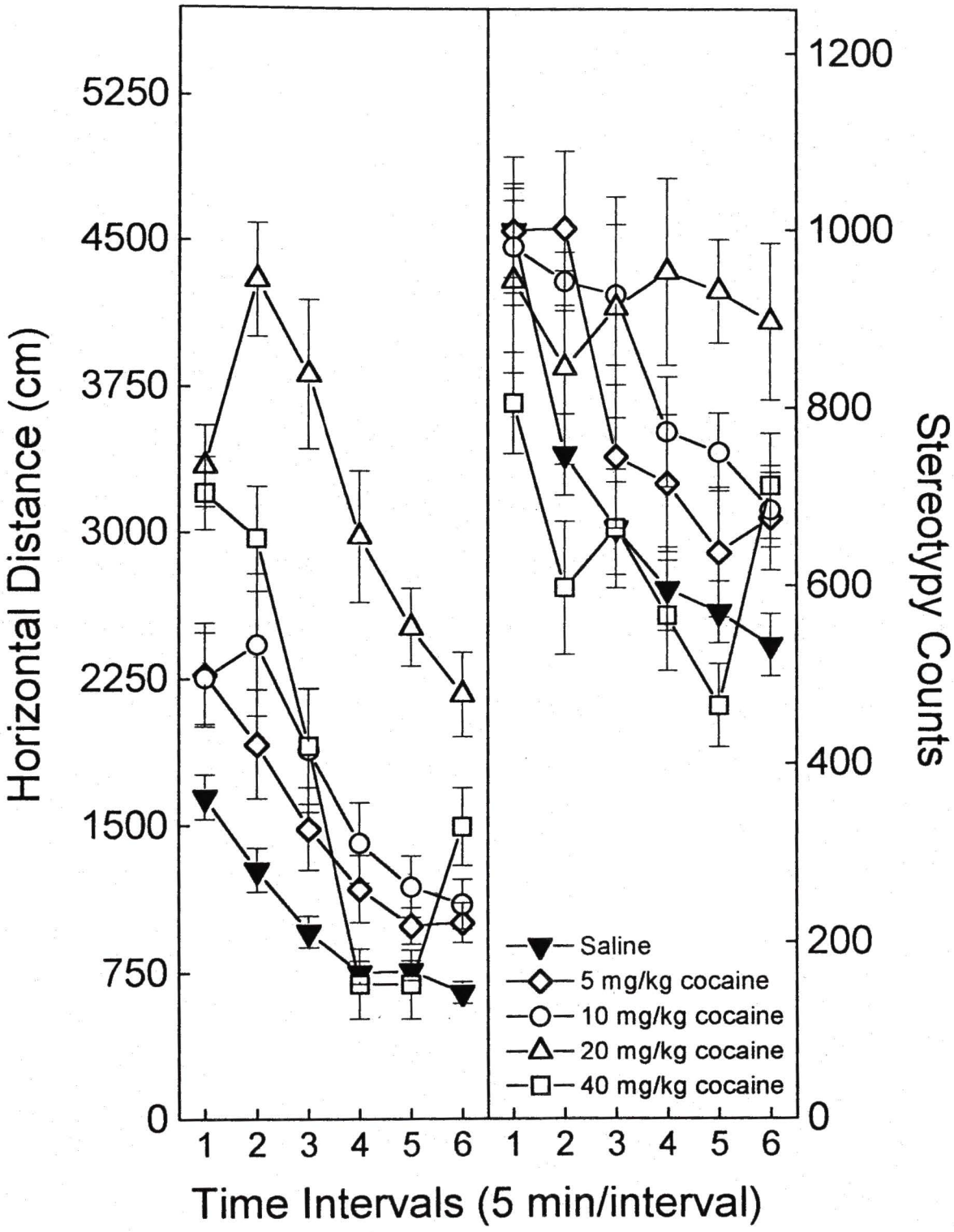

Horizontal Distance (cm)
5250
4500
3750
3000
2250
1500
750
0
1200
1000
800
600
400
200
0
Stereotypy Counts
1 2 3 4 5 6 1 2 3 4 5 6
Time Intervals (5 min/interval)
Saline
5 mg/kg cocaine
10 mg/kg cocaine
20 mg/kg cocaine
40 mg/kg cocaine

Fig. 3-2. Time-response curve for cocaine-induced horizontal distance and stereotypy counts in DBA/2 mice. Horizontal distance and stereotypy counts in the DBA/2 mice following injections of saline or cocaine (from 5 to 40 mg/kg) are plotted against the 5-min time intervals in which the data was collected. Each line is a separate dose. Values are plotted ± SEM. The number of subjects in each group was eight.

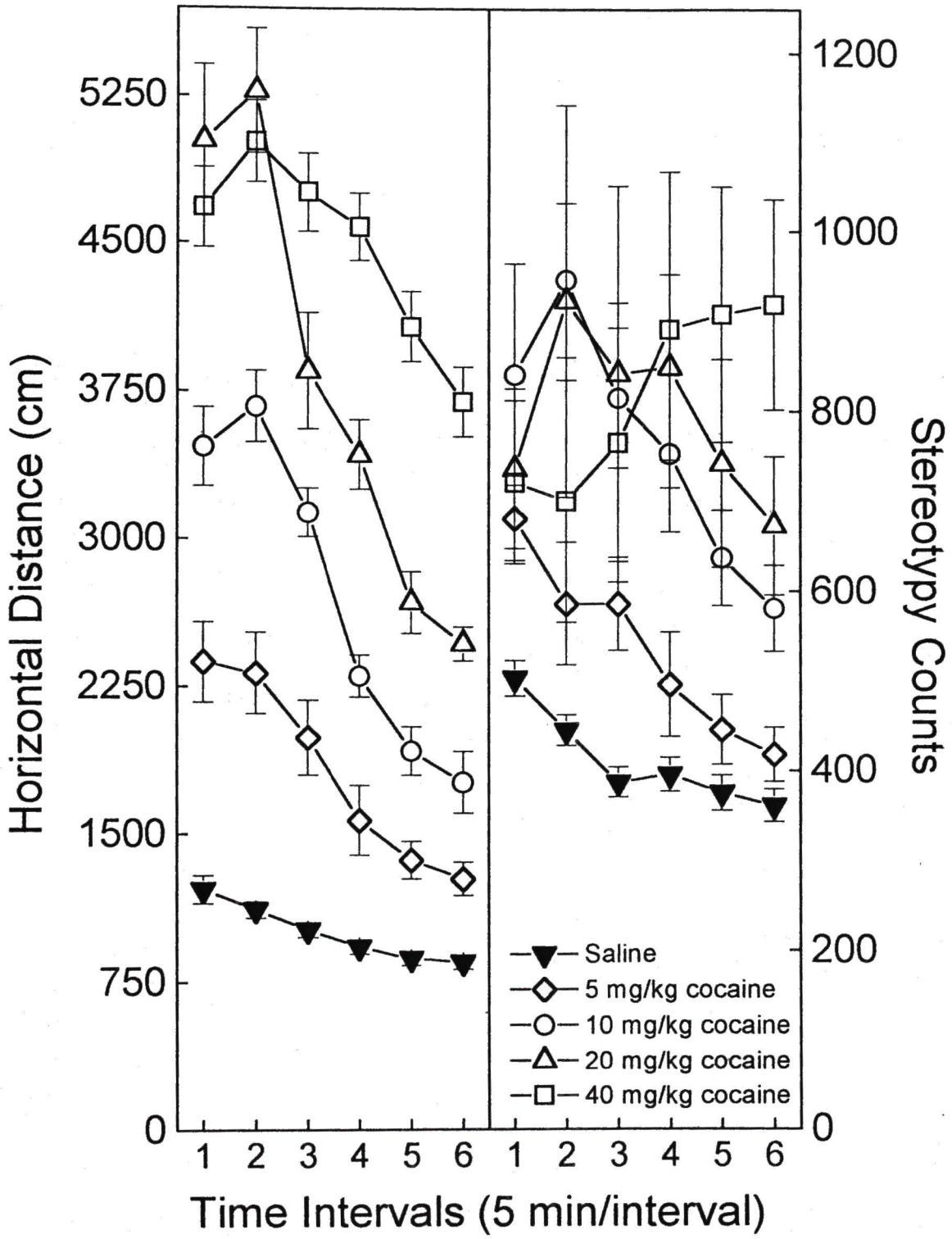

Horizontal Distance (cm)
5250
4500
3750
3000
2250
1500
750
0
1200
1000
800
600
400
200
0
Stereotypy Counts
1 2 3 4 5 6 1 2 3 4 5 6
Time Intervals (5 min/interval)
Saline
5 mg/kg cocaine
10 mg/kg cocaine
20 mg/kg cocaine
40 mg/kg cocaine

Fig. 3-3. Dose-response curve for cocaine-induced horizontal distance and stereotypy counts in C57BL/6 and DBA/2 mice. The top panel depicts the horizontal distance traveled in cm following injection with the indicated doses of cocaine. The bottom panel plots the stereotypy behavior in response to cocaine. Values are plotted ± SEM. Number of subjects in each group was eight. Significant difference ($p<.05$) between strains in response to that dose are marked with *.

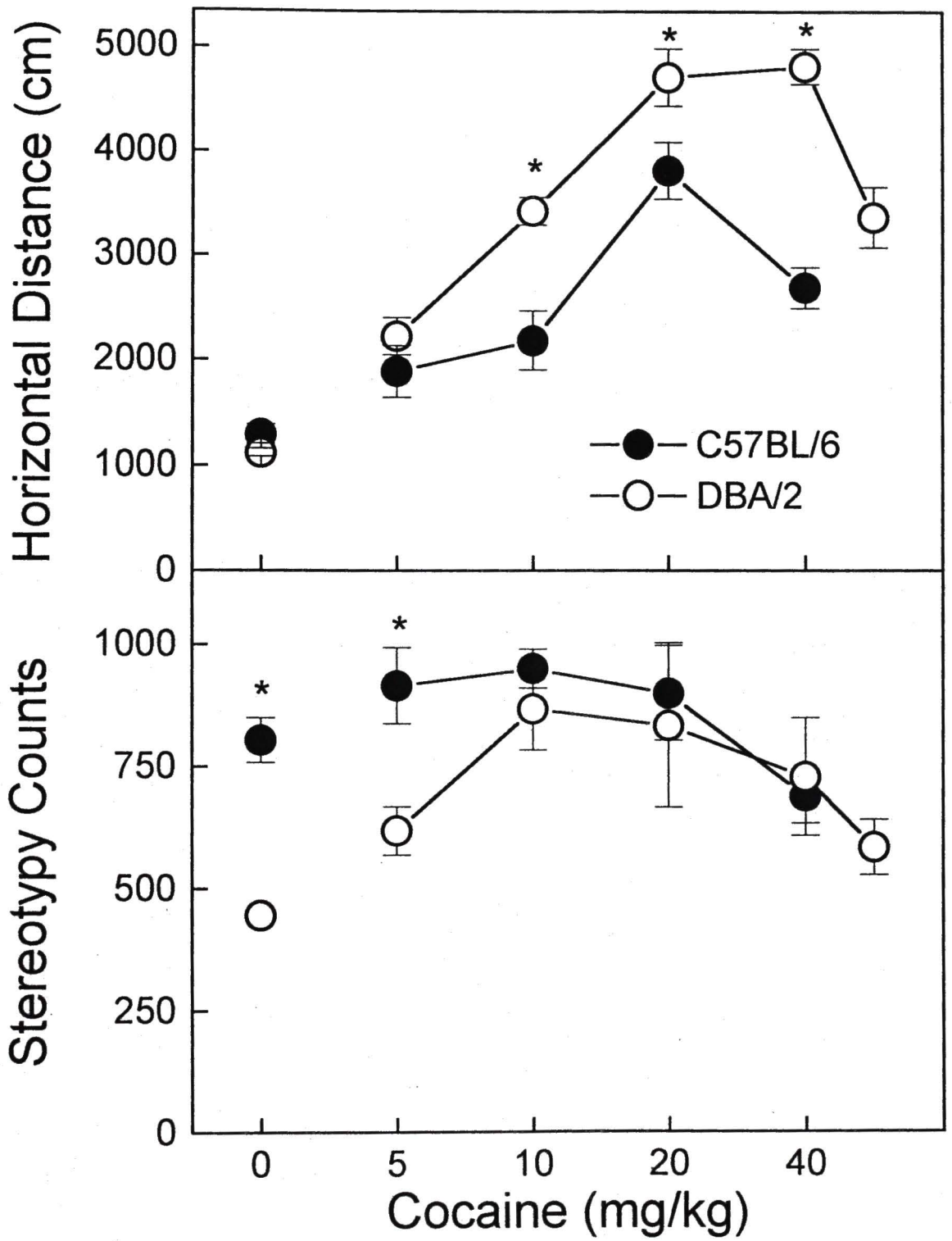

Horizontal Distance (cm)
5000
4000
3000
2000
1000
0
C57BL/6
DBA/2
Stereotypy Counts
1000
750
500
250
0
0
5
10
20
40
Cocaine (mg/kg)

Fig. 3-4. Comparison of horizontal distance and stereotypy counts of C57BL/6 mice after one pairing with either 20 or 40 mg/kg cocaine. P20 and U20 are the paired and unpaired groups of the 20 mg/kg pairing experiment, and P40 and U40 are the paired and unpaired groups of the 40 mg/kg pairing experiment. Values are plotted ± SEM. Number of subjects in each group was eight. Significant differences ($p<.05$) between the pairing experiments are indicated by *, and between the paired and unpaired groups by ϕ.

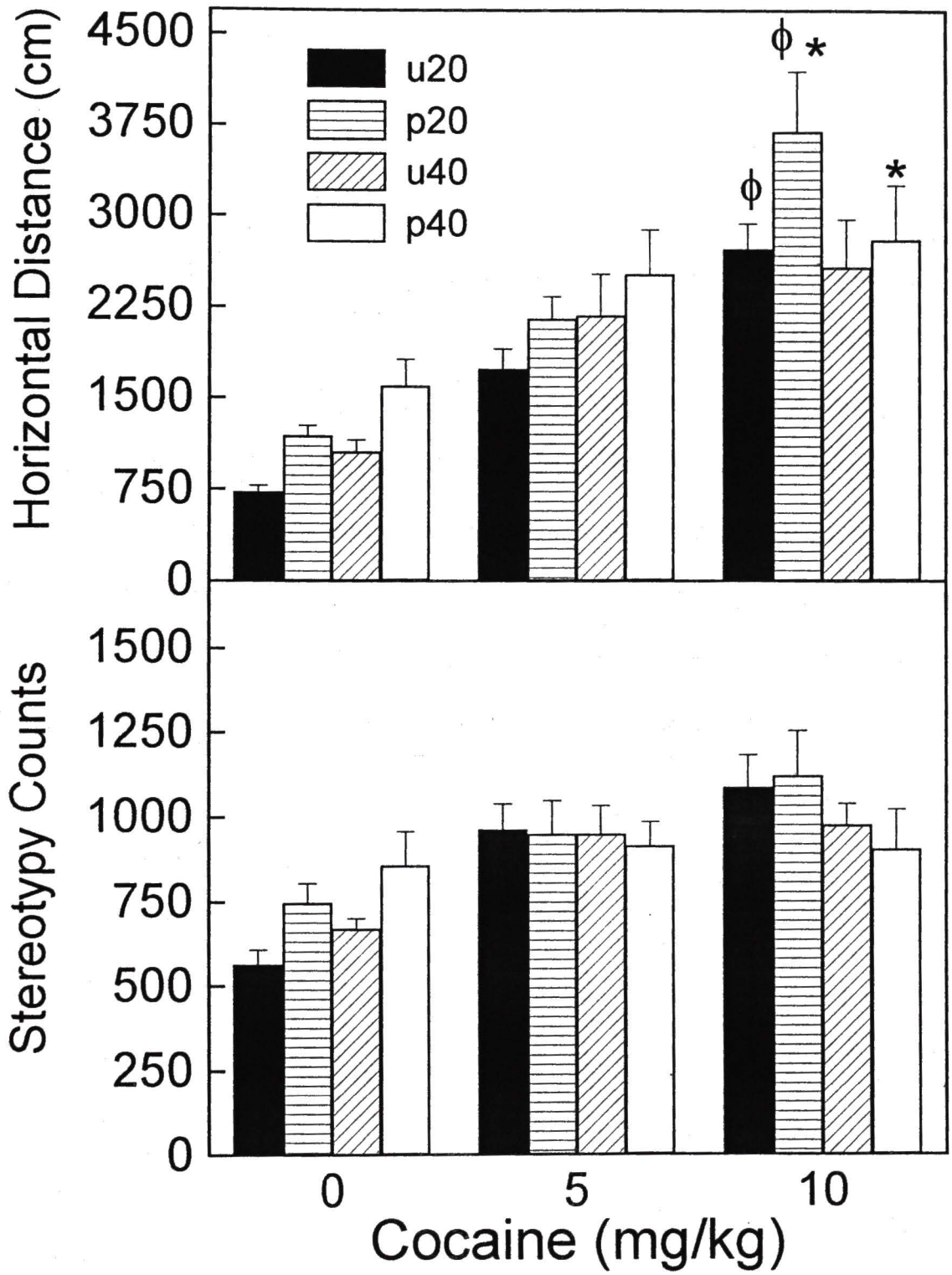

Horizontal Distance (cm)
4500
3750
3000
2250
1500
750
0
u20
p20
u40
p40
ϕ
ϕ *
*
Stereotypy Counts
1500
1250
1000
750
500
250
0
0
5
10
Cocaine (mg/kg)

Fig. 3-5. Comparison of the multivariate profiles of the behavior induced acutely by 5 and 10 mg/kg cocaine with the behavior of the conditioned response following a single pairing with 20 or 40 mg/kg cocaine in C57BL/6 mice. Acute values = Acute response to each dose / mean of the acute response to saline * 100 - 100. Conditioned values = paired group's response to saline / mean of the unpaired group's response to saline * 100 - 100. Variables of motor behavior are Horizontal Counts (HC), Distance (HD), Movements (HM), and Time (HT); Average Distance (AD) and Speed (AS); Stereotypy Counts (SC), Stereotypy Movements (SM) and Stereotypy Time (ST); and Vertical Counts (VC), Movements (VM) and Time (VT). Values are plotted ± SEM. Number of subjects was eight for each group. Significant differences ($p<.05$) from the acute groups are indicated by * for the 5 mg/kg dose and ϕ for the 10 mg/kg dose.

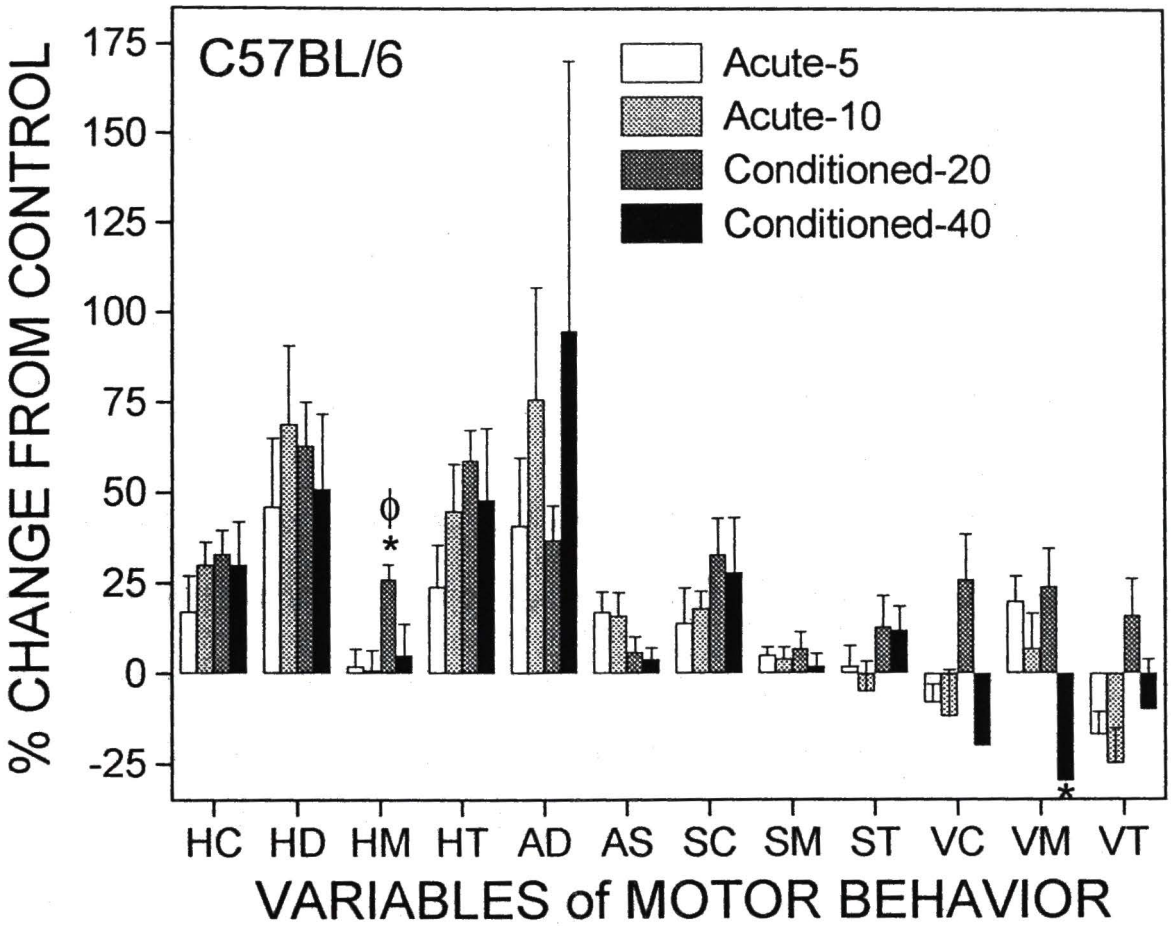

C57BL/6
Acute-5
Acute-10
Conditioned-20
Conditioned-40
% CHANGE FROM CONTROL
175
150
125
100
75
50
25
0
-25
ϕ
*
*
HC HD HM HT AD AS SC SM ST VC VM VT
VARIABLES of MOTOR BEHAVIOR

Fig. 3-6. Comparison of the multivariate profiles of the acute response to 5 and 10 mg/kg cocaine with the profiles of the conditioned response following a single pairing with 40 mg/kg cocaine in DBA/2 mice. Acute values = Acute response to each dose / mean of the acute response to saline * 100 - 100. Conditioned values = paired group's response to saline / mean of the unpaired group's response to saline * 100 - 100. Variables of motor behavior are Horizontal Counts (HC), Distance (HD), Movements (HM), and Time (HT); Average Distance (AD) and Speed (AS); Stereotypy Counts (SC), Stereotypy Movements (SM) and Stereotypy Time (ST); and Vertical Counts (VC), Movements (VM) and Time (VT). Values are plotted ± SEM. Number of subjects was eight for each group. Significant differences ($p<.05$) from the acute groups are indicated by * for the 5 mg/kg dose and ϕ for the 10 mg/kg dose.

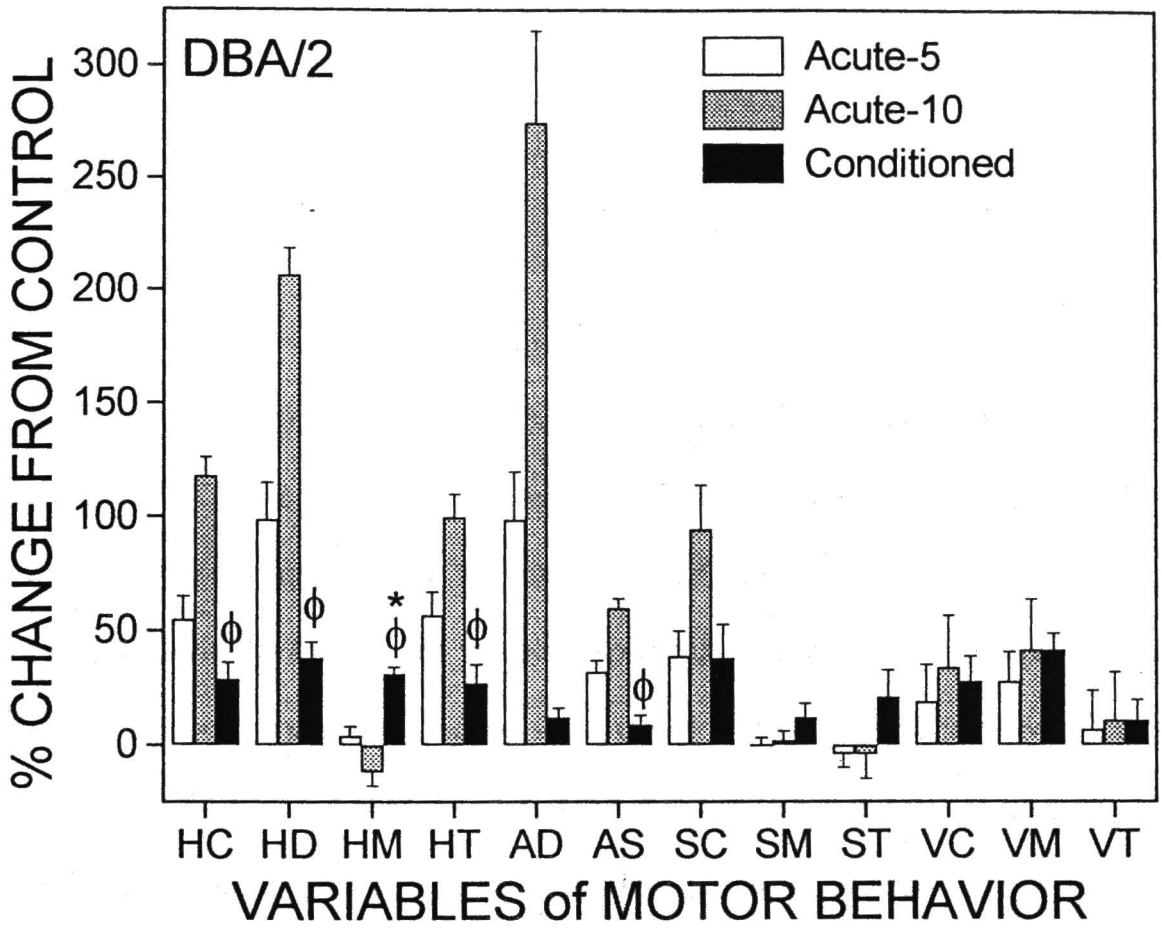

DBA/2
Acute-5
Acute-10
Conditioned
% CHANGE FROM CONTROL
300
250
200
150
100
50
0
HC HD HM HT AD AS SC SM ST VC VM VT
VARIABLES of MOTOR BEHAVIOR

Fig. 3-7. Context-dependent sensitization of cocaine-induced horizontal distance in C57BL/6 and DBA/2 mice after one and four pairings with cocaine. Data from C57BL/6 mice are presented in the top two panels, and data from the DBA/2 mice are displayed in the bottom two panels. Horizontal distance in cm is plotted against the days of pairing on the left side of each panel. On the right side of each panel, horizontal distance is plotted against the challenge doses of cocaine given the day after the last pairing session. Values are plotted ± SEM. Number of subjects in each group was at least 40 during pairing, and at least seven at all doses on the challenge day. Significant differences ($p<.05$) relative to the Saline Control group are marked with *, and those between the paired and unpaired groups are marked with ϕ.

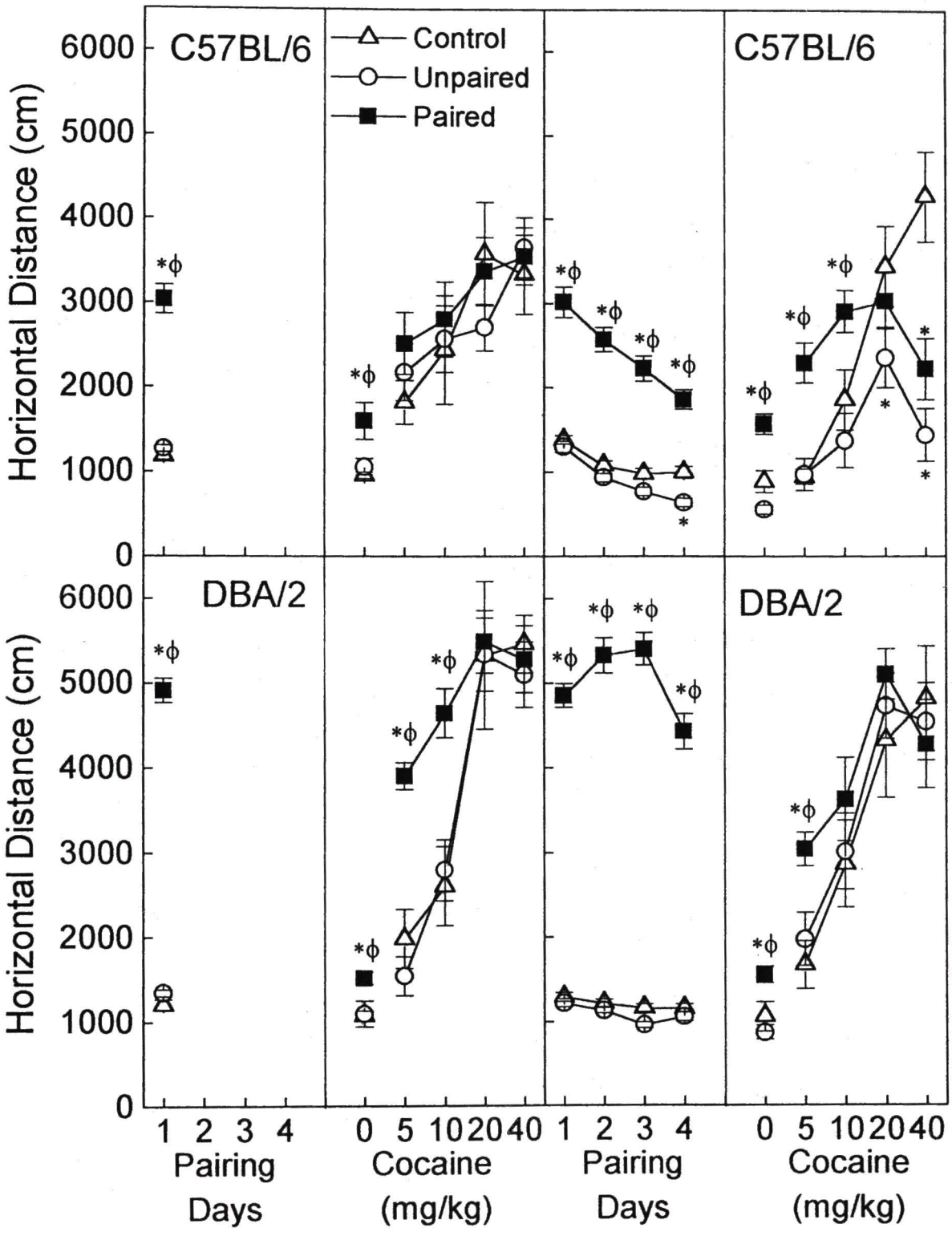

C57BL/6
Control
Unpaired
Paired
C57BL/6
DBA/2
DBA/2
Horizontal Distance (cm)
Horizontal Distance (cm)
0
1000
2000
3000
4000
5000
6000
1 2 3 4
Pairing Days
0 5 10 20 40
Cocaine (mg/kg)
1 2 3 4
Pairing Days
0 5 10 20 40
Cocaine (mg/kg)

Fig. 3-8. Context-dependent sensitization of cocaine-induced stereotypy counts in C57BL/6 and DBA/2 mice following one and four pairings with cocaine. Data from C57BL/6 mice are presented in the top two panels, and data from the DBA/2 mice are displayed in the bottom two panels. Stereotypy counts are plotted against the days of pairing on the left side of each panel. On the right side of each panel, stereotypy counts are plotted against the challenge doses of cocaine given the day after the last pairing session. Values are plotted ± SEM. Number of subjects in each group was at least 40 during pairing and at least seven at all doses on the challenge day. Significant differences ($p<.05$) relative to the Saline Control group are marked with *, and those between the paired and unpaired groups are marked with ϕ.

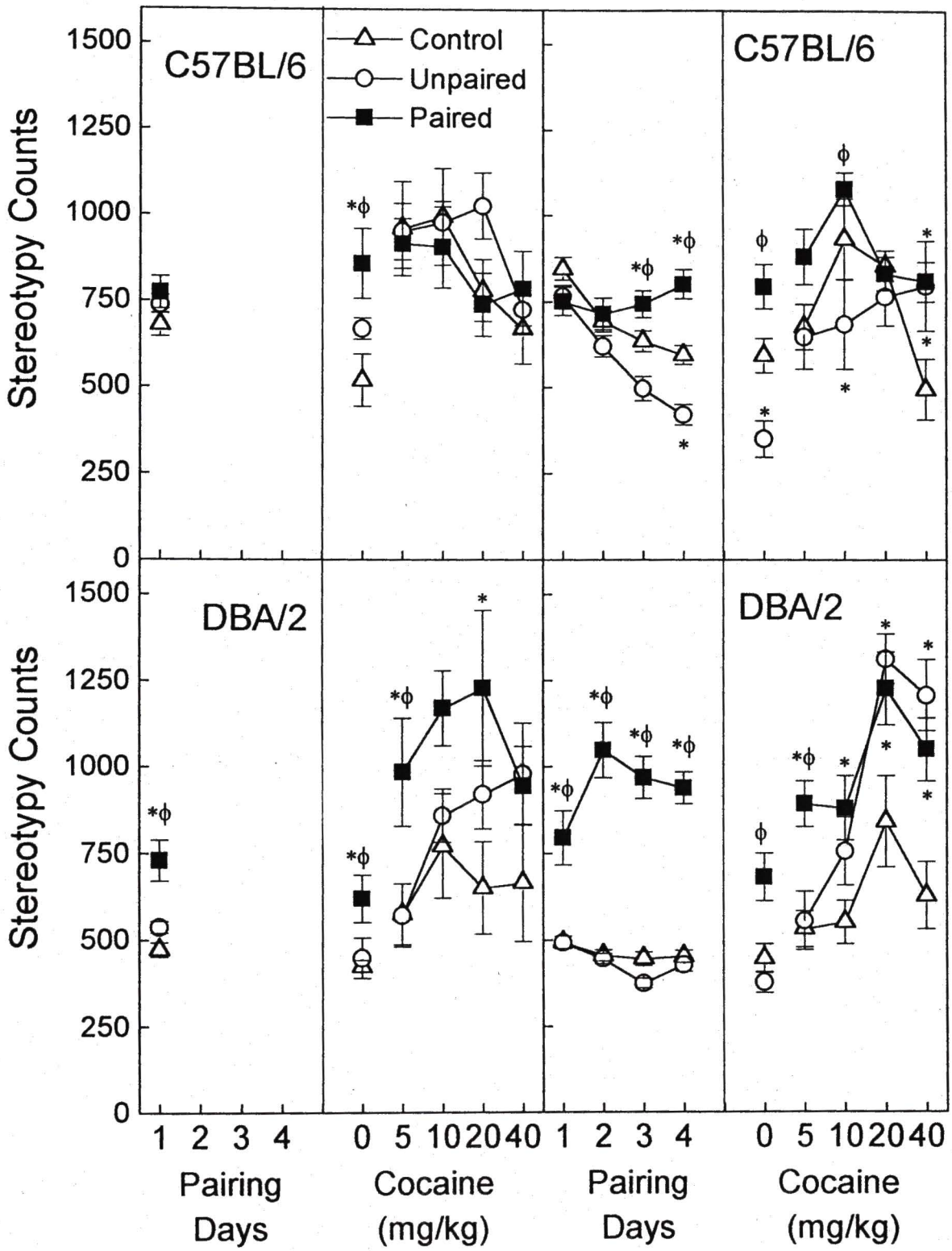

C57BL/6
Control
Unpaired
Paired
C57BL/6
DBA/2
DBA/2
Stereotypy Counts
Stereotypy Counts
1500
1250
1000
750
500
250
0
1 2 3 4
0 5 10 20 40
1 2 3 4
0 5 10 20 40
Pairing Days
Cocaine (mg/kg)
Pairing Days
Cocaine (mg/kg)

Fig. 3-9. Extinction of context-dependent sensitization of cocaine-induced horizontal distance in C57BL/6 and DBA/2 mice after one pairing with cocaine. Horizontal distance is plotted against the days of pairing on the left side of each panel, days of extinction in the middle of each panel and the challenge doses of cocaine on the right side of each panel. Values are plotted ± SEM. Number of subjects in each group was at least 16 during pairing and extinction and at least eight at all doses on the challenge day. Significant differences ($p<.05$) from the unpaired group are marked with *.

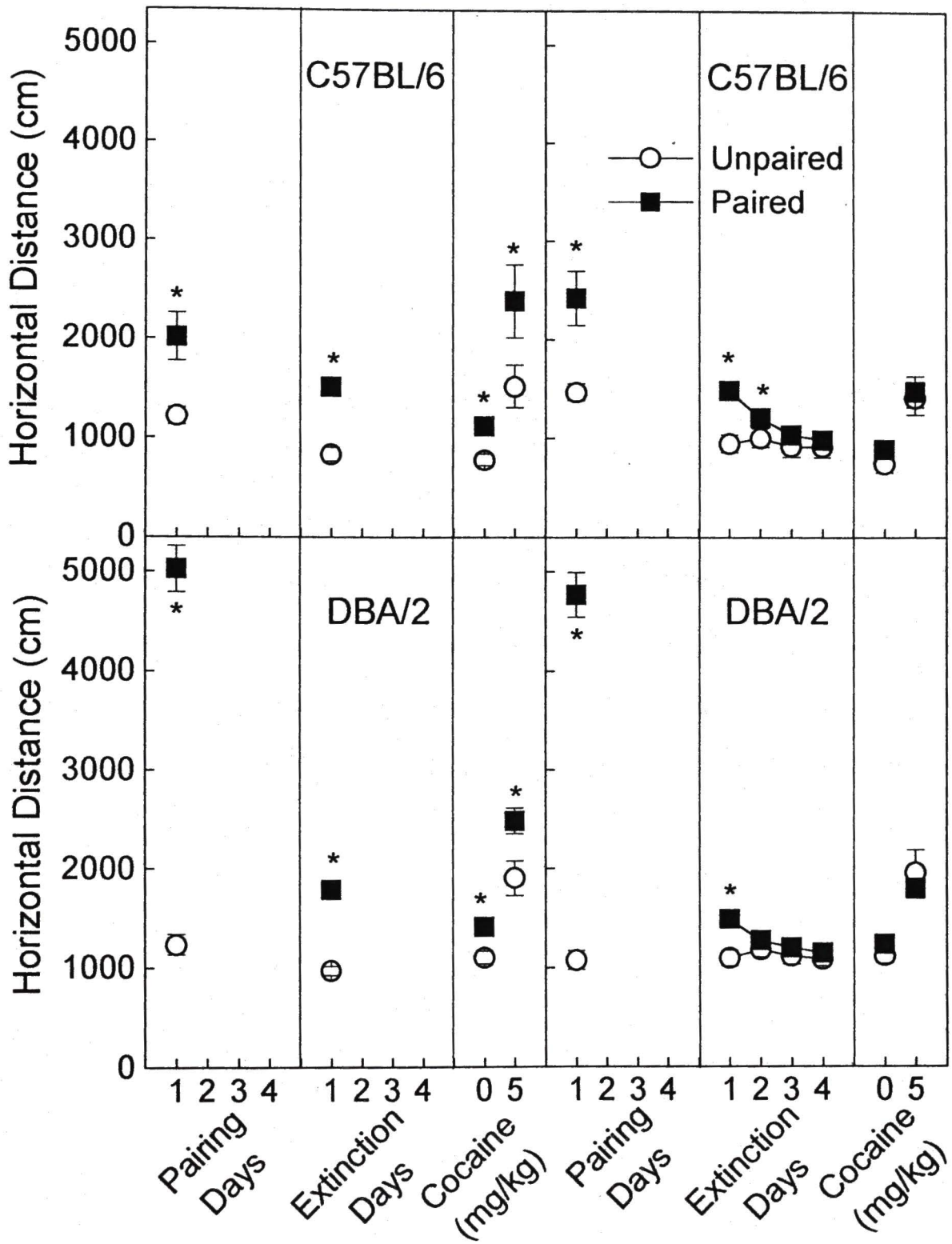

C57BL/6
C57BL/6
DBA/2
DBA/2
Unpaired
Paired
Horizontal Distance (cm)
Horizontal Distance (cm)
0
1000
2000
3000
4000
5000
1 2 3 4
0 5
Pairing Days
Extinction Days
Cocaine (mg/kg)

Fig. 3-10. Extinction of context-dependent sensitization of cocaine-induced horizontal distance in C57BL/6 and DBA/2 mice after four pairings with cocaine. Horizontal distance is plotted against the days of pairing on the left side of each panel, days of extinction in the middle of each panel and the challenge doses of cocaine on the right side of each panel. Values are plotted ± SEM. Number of subjects in each group was at least 16 during pairing and extinction and at least eight at all doses on the challenge day. Significant differences ($p<.05$) from the unpaired group are marked with *.

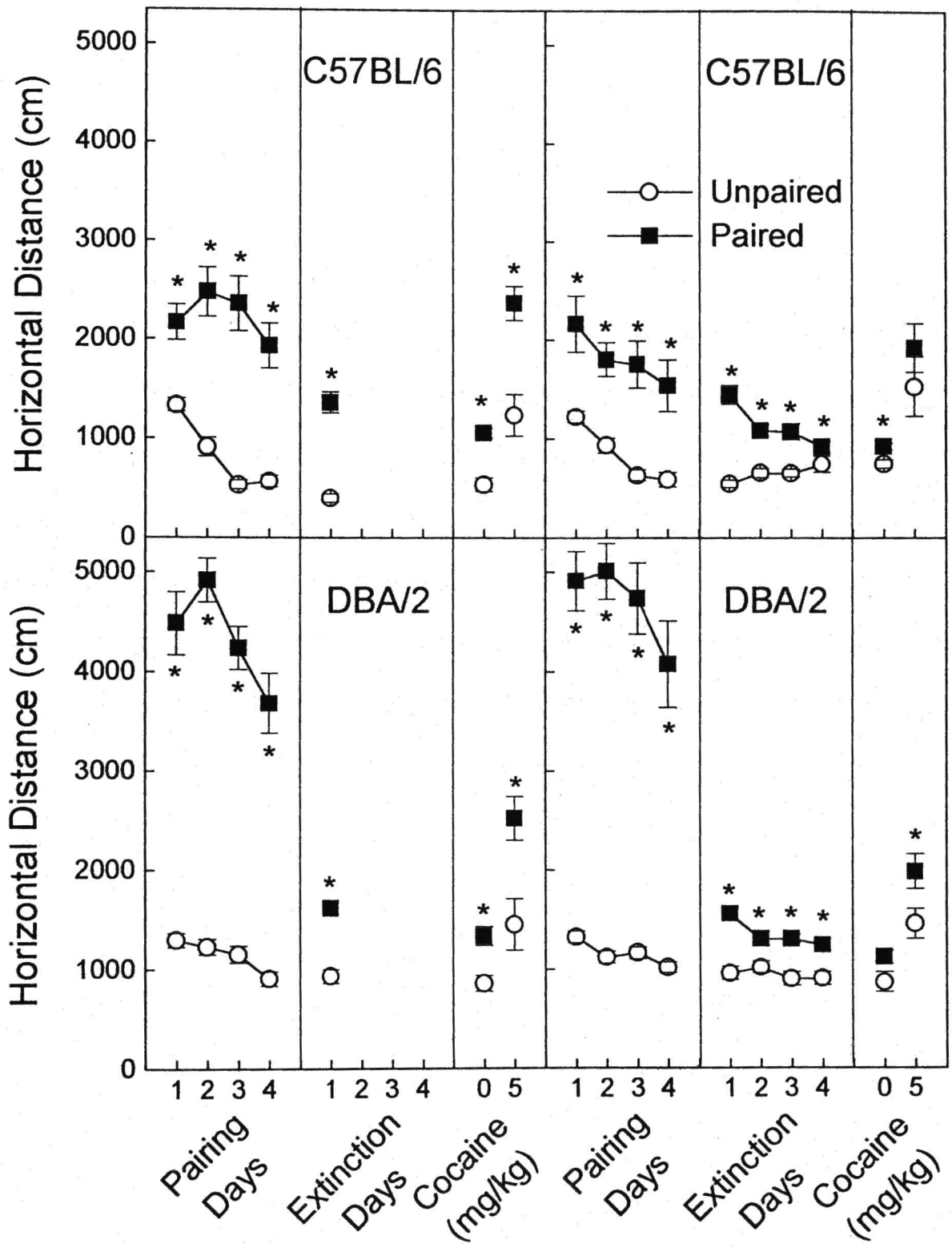

Horizontal Distance (cm)
5000
4000
3000
2000
1000
0
C57BL/6
C57BL/6
Unpaired
Paired
DBA/2
DBA/2
1 2 3 4
1 2 3 4
0 5
1 2 3 4
1 2 3 4
0 5
Pairing Days
Extinction Days
Cocaine (mg/kg)
Pairing Days
Extinction Days
Cocaine (mg/kg)

Fig. 3-11. Extinction of conditioned responses to cocaine-induced stereotypy counts in C57BL/6 and DBA/2 mice after one pairing with cocaine. Stereotypy counts are plotted against the days of pairing on the left side of each panel, days of extinction in the middle of each panel and the challenge doses of cocaine on the right side of each panel. Values are plotted ± SEM. Number of subjects in each group was at least 16 during pairing and extinction and at least eight at all doses on the challenge day. Significant differences ($p<.05$) from the unpaired group are marked with *.

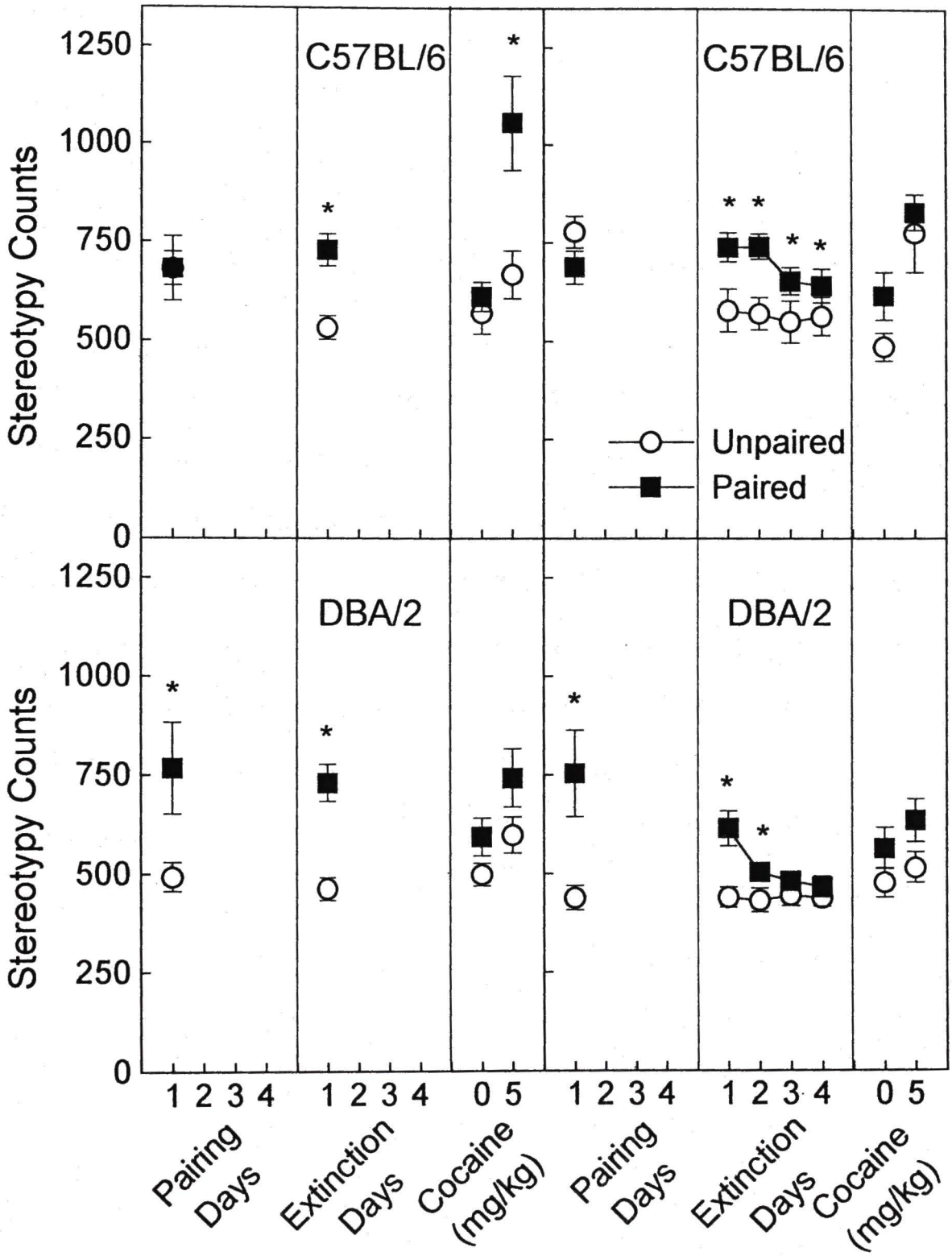

C57BL/6
C57BL/6
DBA/2
DBA/2
Stereotypy Counts
Stereotypy Counts
1250
1000
750
500
250
0
Unpaired
Paired
1 2 3 4
0 5
Pairing Days
Extinction Days
Cocaine (mg/kg)

Fig. 3-12. Extinction of conditioned responses to cocaine-induced stereotypy counts in C57BL/6 and DBA/2 mice after four pairings with cocaine. Stereotypy counts are plotted against the days of pairing on the left side of each panel, days of extinction in the middle of each panel and the challenge doses of cocaine on the right side of each panel. Values are plotted ± SEM. Number of subjects in each group was at least 16 during pairing and extinction and at least eight at all doses on the challenge day. Significant differences ($p<.05$) from the unpaired group are marked with *.

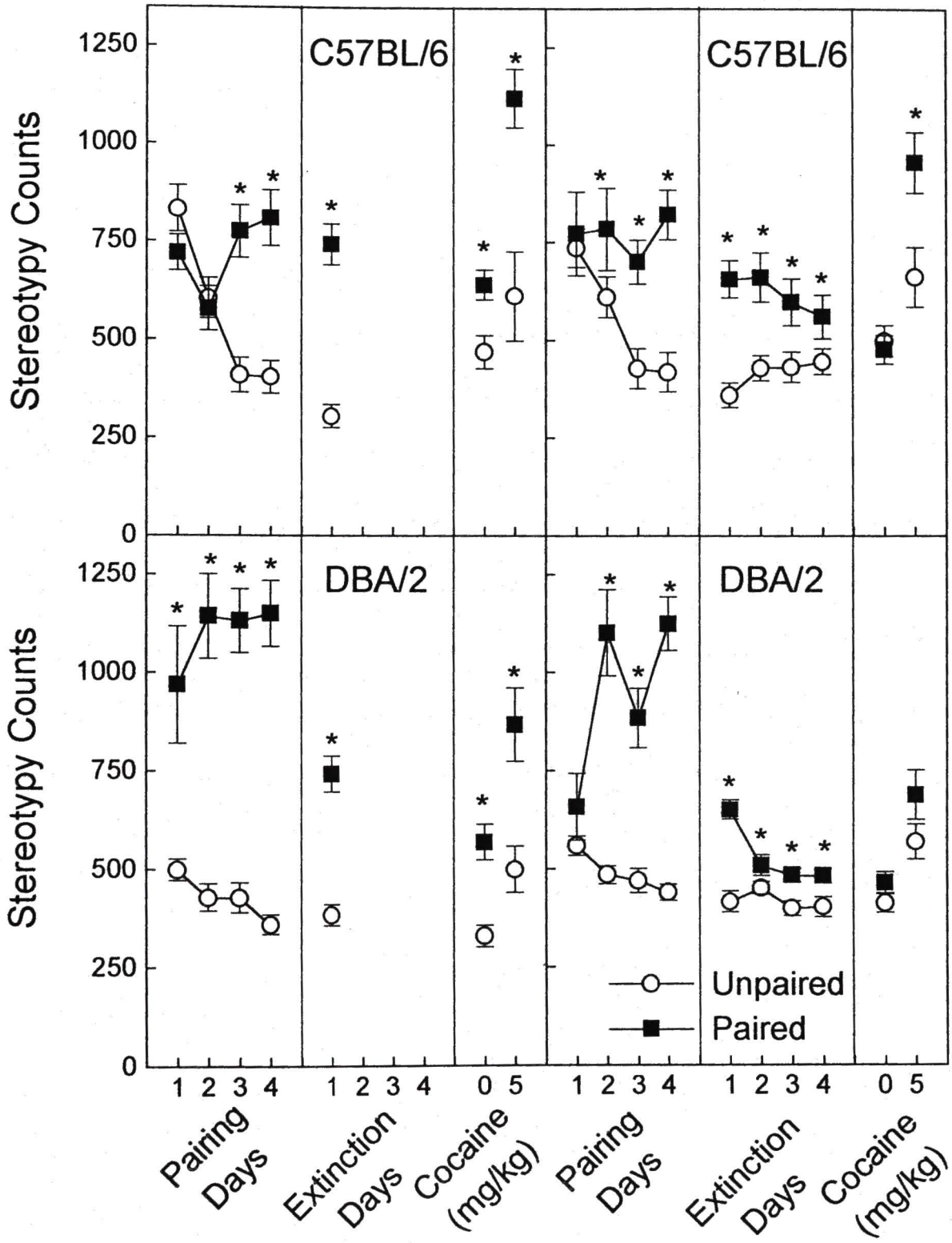
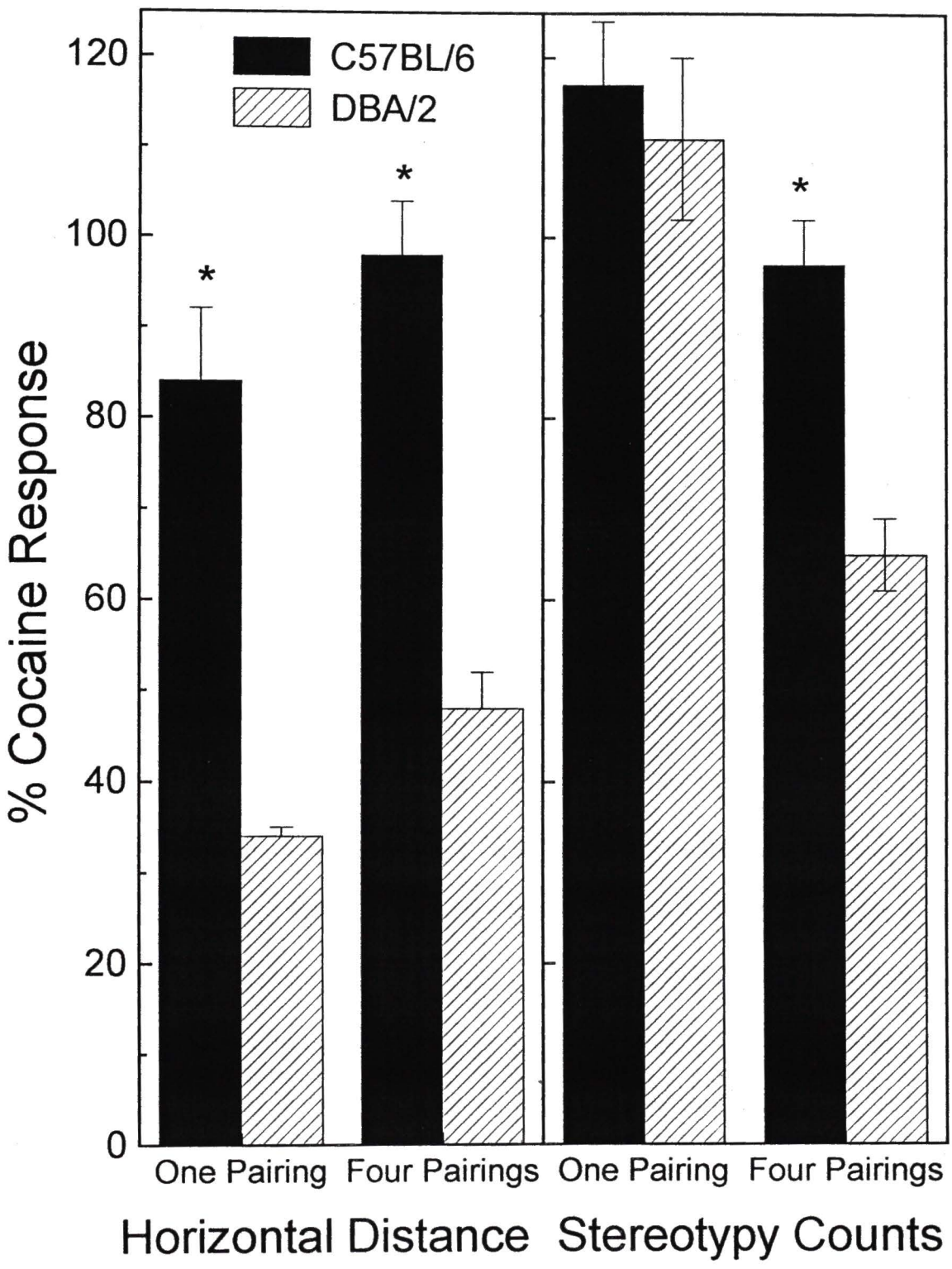

Stereotypy Counts
1250
1000
750
500
250
0
C57BL/6
C57BL/6
DBA/2
DBA/2
Unpaired
Paired
1 2 3 4
0 5
Pairing Days
Extinction Days
Cocaine (mg/kg)

Fig. 3-13. Magnitude of the conditioned response to cocaine after one or four pairings in both C57BL/6 and DBA/2 mice. The left panel depicts the conditioned horizontal distance response, and the right panel shows the conditioned stereotypy response. The X-axes indicate the number of pairings with cocaine. Percent cocaine response is plotted on the Y-axes and is calculated by (the activity level of the Paired group on the first day of extinction) / (the activity level on the last day of pairing) * 100. Values are plotted ± SEM. Number of subjects in each group was at least 32. Significant differences ($p<.05$) between strains are marked with *.


C57BL/6
DBA/2
% Cocaine Response
120
100
80
60
40
20
0
*
*
*
One Pairing
Four Pairings
One Pairing
Four Pairings
Horizontal Distance
Stereotypy Counts

CHAPTER IV

General Discussion

These experiments sought to quantify the contribution of context-dependent sensitization to the overall phenomenon of the sensitization of cocaine-induced motor behavior and to establish the existence of genetic variance in that context-dependent sensitization.

Experiment 1

Effect of Acute Cocaine

The first study found large increases in total and average distance traveled; horizontal counts, movements and time; and stereotypy counts and time in response of the Swiss Webster mice to 10 mg/kg acute cocaine, while Zubrycki et al (1990) found only mild increases in horizontal counts and movements in response to that dose in rats. The findings in Zubrycki et al with regard to the 20 mg/kg dose were similar to those found in this study only on the horizontal measures. While they showed increases in vertical and stereotypy behavior above control levels, this study did not (see figures 2-3 and 2-4). There are significant methodological differences between the present study and Zubrycki et al (1990). First, Zubrycki averaged data over a 60 min session, whereas this study

used the first 15 min of a 30 min session. Second, Zubrycki habituated the animals for 3 h, while this study did not habituate the animals. Finally, a different species was used for the two experiments. As mentioned in the results section, vertical and stereotypy behavior in the current study peaked in the second 15-min interval of the 30-min observation period. This may account for the difference in the results of the two studies.

The Swiss Webster mice responded to increasing doses of acute cocaine with increasing levels of horizontal behavior, peaking at 20 mg/kg cocaine. The relative reduction in response to 40 mg/kg cocaine was probably secondary to drug effects that were incompatible with continued increases in locomotor behavior. These could have included cocaine's known anesthetic, seizure or cardiac effects (Boghdadi and Henning, 1997; Koppel et al, 1996; Szabo et al, 1995).

Conditioned Response to Cocaine

In classical conditioning, a neutral stimulus comes to be a conditioned stimulus when paired repeatedly to the unconditioned stimulus (Kling, 1971). After enough pairings, which generally numbers around 50, the conditioned stimulus alone can elicit some portion of the unconditioned response. The neutral stimulus is not supposed to elicit a response that is similar to the unconditioned response. In these studies, the neutral stimulus of the testing chamber does, in fact, elicit a locomotor response as the animal explores the environment

without the pairing of it to the unconditioned stimulus of the cocaine. The unconditioned response with cocaine is an enhancement of that behavior and the addition of stereotypy behavior. In addition, only one pairing with cocaine is needed for the neutral stimulus to elicit the conditioned response seen. It is possible, therefore, that the conditioning observed in these experiments is not Pavlovian in nature. Furthermore, the exposure of both the paired and unpaired groups to the neutral stimulus of the testing environment was to control for the possibility of pseudoconditioning occurring. None was seen.

The first study found that the conditioned response to saline after one or four exposures to 40 mg/kg cocaine resembled low dose cocaine, specifically the 10 mg/kg dose. This was the expected result since conditioned responses generally match the unconditioned responses in direction, but have less magnitude (Kling, 1971). An unexpected finding was the conditioned increases in vertical and stereotypy behavior beyond the stimulation observed with 10 mg/kg acute cocaine. Those measures more closely resemble the responses to 1 and 2.5 mg/kg cocaine. Again, it should be mentioned that the Digiscan apparatus measures "stereotypy" as a repeated interruption of a single photocell beam, and this likely reflects a behavior that is distinct from what is classically considered stereotypy. As early as 1979, it was thought that stereotypy and locomotion were controlled by separate neurological systems, and studies since then have appeared to corroborate this finding (Bhattacharyya and Pradhan, 1979; cf. Wise and Bozarth, 1987 and Tolliver et al, 1994).

Another possible explanation is that these conditioning data are inflated due to the comparison of the paired group's response to that of the unpaired group which was displaying a consistent decline in activity in response to saline during the pairing sessions. This same confound was present in a study by Carey and Gui (1997) in which they noted that the conditioned response they saw in the paired group was not due to an increase above pretreatment levels of activity, but rather was observed to be increased because of comparison to the reduced activity of their unpaired group. However, if this were the case, then it would be expected that the conditioned responses observed after one pairing with cocaine would be smaller than those observed after four pairings with cocaine, and this was not the case; therefore it is most likely that the locomotor and stereotypy behaviors are controlled by separate neuronal mechanisms which respond differently to sensitization and conditioning.

Decline in the Behavior of the Unpaired Group

As mentioned above, a progressive decline in the response of the unpaired group to saline was observed in the Swiss Webster mice over the four days of cocaine pairing. A modest decline was observed in the saline control group as they became habituated to the environment over the pairing sessions, but the unpaired group's decrease in behavior was much greater than that of the saline control group. The session-related decline of the unpaired group was global in that it involved horizontal, vertical and stereotypy measurements except

average speed and average distance. One explanation for this could be that the unpaired group had a conditioned inhibitory response to the saline injection in the testing chamber. For this to occur, the unpaired group would have to perceive no difference between the injection in the testing chamber and the injection in the home cage such that no difference in expectations were created. The environments were in fact quite different. The testing chamber was housed inside a darkened wood box and lacked bedding while the home cages afforded the mice with views of the neighboring cages in a well-lit room as well as having bedding and being smaller. However, if no difference was perceived, then the saline injection in the testing chamber elicited the same compensatory responses that the cocaine injection had come to elicit, such that activity was inhibited to compensate for the hyperlocomotive response to cocaine. Conditioned inhibitory responses have been described by Siegel (1989) and Stewart and Badiani (1993), and involve physiological responses to addictive drugs which counteract various effects of the drugs, such as hyperthermia to protect from the hypothermic effect of alcohol in rats repeatedly exposed to alcohol injections (Siegel, 1989), and the hyperalgesic response following multiple exposures to morphine and its analgesic effects.

Another explanation for the session-related decline in the behavior of the Swiss Webster unpaired group could be that the unpaired group was in a state of cocaine withdrawal when tested, since it had been 22 h since their last injection of cocaine. Recent studies of the brain in human cocaine addicts have

shown that dysphoria and depression follow the euphoria response to snorting, smoking or injecting cocaine (Hurd and Herkenham, 1993). For example, the neurochemistry of the neostriatum in human subjects who died with a history of cocaine use and with cocaine or cocaine metabolites present in the bloodstream was compared with subjects that had no history of cocaine use and no plasma cocaine or plasma cocaine metabolites at death (Hurd and Herkenham, 1993). It was found that the cocaine users had increased dynorphin mRNA and κ opiate receptor levels along with decreased enkephalin mRNA and μ opiate receptor levels. This combination of neurochemistry in the neostriatum of cocaine users indicated that they suffered from dysphoria, which may have led to acute withdrawal symptoms, relapse and/or drug craving (Hurd and Herkenham, 1993). Furthermore, using positron emission tomography (PET) scans in living human subjects, Volkow et al (1993) determined that cocaine users recruited from the detoxification unit of a medical center had fewer D_2-dopamine receptors than non-cocaine users. This receptor availability correlated negatively with Beck Depression Inventory scores, again indicating that the cocaine addicts were experiencing dysphoria in this drug-free state. Therefore, it is possible that the unpaired group was exhibiting decreased activity secondary to a state of withdrawal and dysphoria.

Effect of Context-Dependent Sensitization

As one can see in Figure 2-10, after one or four exposures to cocaine at 40 mg/kg in the testing chamber, the context-dependent sensitization then elicited by only 1 mg/kg cocaine was significantly greater than that of the acute response to 1 mg/kg cocaine. This type of sensitization was seen also at the 2.5 and 5 mg/kg challenge doses. The unpaired group showed context-independent sensitization on three measures: stereotypy time and vertical counts and time. The paired group showed context-dependent sensitization on 11 of the 12 measures, with average speed being unaffected by sensitization.

Regarding the conditioning aspect of sensitization, several previous studies have addressed this issue in rats and mice. Jackson and Nutt (1993) showed as did the current study that one pre-exposure to cocaine results in sensitization of the locomotor effects of cocaine in mice. This study involved injecting the mice in a novel environment similar to the current study's testing chamber. This study did not employ an unpaired group, nor did it challenge the paired mice with multiple doses of cocaine. They did use a group injected in the home cage on day 1, but not exposed to the testing chamber until day 2 during testing, thus confounding the results with the novelty effect. However, with one exposure to 40 mg/kg cocaine on day 1, their paired group displayed activity levels on day 2 in response to 10 mg/kg cocaine equal to that of the previous day. There was also a conditioned response to saline on day 2 after prior exposure to cocaine 40 mg/kg cocaine on day 1 that surpassed the response ob-

served in the group given saline both days. This experiment was repeated in groups that where habituated for 40 min in the testing chamber prior to injection with cocaine or saline. These groups did not display as strong conditioning or sensitization as the unhabituated mice mentioned above.

Two other studies showed that the novel environment results in greater sensitization of cocaine effects in rats. Hinson and Poulos (1981) injected two separate groups of mice with 13 cocaine (first at 30 mg/kg and remaining 12 at 40 mg/kg) and 13 saline injections given alternately every other day. For their paired group, cocaine injections were given in a distinctive chamber, while saline injections were given in the home cages. No habituation was used in the novel chamber. The second group received saline in both environments. Afterwards, all animals were challenged with 30 mg/kg cocaine. Half of each group received the challenge dose in the cocaine-associated chamber and the other half in the home cage. They were videotaped for 3 min at 10 min intervals for 50 min and scored by observers blind to their testing conditions for increased locomotor activity and stereotyped behaviors. The cocaine-pretreated rats spent 99% of the time in cocaine-related behaviors when challenged in the novel environment, and 68% of the time when tested in the home cage. The saline-pretreated rats spent 30% of their time in cocaine-related behaviors regardless of the environment in which they were tested. The conditioned responses to cocaine-associated cues and context-dependent sensitization of the behavioral effects of cocaine were also observed in a study by Badiani et al (1995), which showed

that pretreating and challenging with cocaine in the novel environment resulted in greater sensitization to the behavioral effects of cocaine than those pretreated in the home cages and tested in the novel environment. In addition, conditioned behavior was observed in their paired group in response to saline when challenged in the novel chamber. Thus, although this type of work has been done before, none have used the type of unpaired group the present study employed nor did they test the cocaine sensitization with more than one dose of cocaine, which limited their interpretation of the results as much as if the current study had used only 10 or 40 mg/kg cocaine challenges.

Connection to the Craving Phenomenon

Context-dependent sensitization of the locomotor-stimulating effects of cocaine may be related to the phenomenon of craving. Incentive salience (wanting or craving) and reinforcement (liking or pleasure) are both thought to be controlled by the same part of the brain which controls locomotor behavior (forward motion or drug-seeking), namely the mesolimbic dopaminergic system (Robinson and Berridge, 1993; Kalivas and Duffy, 1990; Wise and Bozarth, 1987). As the mesolimbic dopaminergic system is sensitized by repeated exposure to cocaine, there is evidence that the motor-stimulatory effects of cocaine are enhanced and that drug wanting evolves into obsessive drug craving (Robinson and Berridge, 1993).

The context-dependent sensitization of motor behavior following repeated cocaine exposure could provide information about the conditioned aspect of drug craving since both motor behavior and drug craving appear to be mediated by the mesolimbic dopaminergic system. The first experimental design may shed some light on the conditioned aspects of cocaine craving. The findings of the present study predict that those who use cocaine repeatedly in the presence of specific environmental or internal cues will likely experience a conditioned response when they are subjected to those cues again, and this can lead to strong feelings of craving or even to a conditioned "high" (Childress et al, 1993). Thus, the present study provided a solid model for examining the conditioned response to cocaine-associated cues and context-dependent sensitization of cocaine's effects, and this model was then manipulated further to facilitate the study of cocaine dependence by examining the possibility of genetic variance in context-dependent sensitization to the effects of cocaine.

Experiment 2

As with the Swiss Webster mice, it was found that the C57BL/6 and DBA/2 inbred strains would develop sensitization after pre-exposure to cocaine in the novel testing environment. This sensitization was completely context-dependent in the C57BL/6 mice and mostly context-dependent in the DBA/2 mice. Conditioning was seen in both strains to the effects of cocaine on horizontal and stereotypy behavior.

Variance in Response to Acute Cocaine

The C57BL/6 and DBA/2 mice differed in their response to acute cocaine with respect to both horizontal stimulation and stereotypy behavior. For horizontal measures, the dose-response curve of the DBA/2 mice was shifted upward (see figure 3-3). For stereotypy, the dose-response curve of the C57BL/6 began with a baseline and 5 mg/kg cocaine response much higher than that of the DBA/2, but then the two strains were indistinguishable in their stereotypy response to acute cocaine (see also figure 3-3). Again, it must be mentioned that the stereotypy measure in the current experiment actually refers to the repeated interruption of a single photocell beam and may not reflect classical stereotypy behavior on the part of the mice.

Variance in Strength of Conditioning

Three lines of evidence indicated that the C57BL/6 mice are more strongly conditioned to the behavioral effects of cocaine than are the DBA/2 mice. The first was that the conditioned multivariate behavior profile of the C57BL/6 mice more closely resembled their behavioral response to 10 mg/kg acute cocaine while that of the DBA/2 mice resembled a response to 5 mg/kg cocaine (figures 3-5 and 3-6). Second, after only one prior exposure to cocaine, the conditioned response of the C57BL/6 mice on both horizontal distance and stereotypy measures persisted longer through the extinction sessions than did that of the DBA/2 mice (figures 3-9 and 3-11). The last line of evidence con-

cerned the magnitude of the conditioned response as compared to the cocaine response. Figure 3-13 clearly indicated that the C57BL/6 mice had stronger conditioning after one and four pairings with cocaine than did the DBA/2 mice.

One criticism of the data could come from the dose-response curve for acute cocaine in which the DBA/2 mice showed a pronounced increase in locomotor stimulation over that of the C57BL/6 mice. Given that large hyperlocomotive response, their conditioned response appeared relatively small when compared to the C57BL/6 conditioned response. However, normalization of these results was attempted by dividing through by the response of the unpaired group of each strain (see Figures 3-5 and 3-6). It is also possible that the conditioned response reaches a maximal level that is comparable between the strains as can be seen in the response to saline by the both strains after one or four pairings with cocaine (see Figures 3-7 and 3-8). If this is the case, then the comparison made in Figure 3-13 is biased.

Variance in Sensitization

The DBA/2 mice were remarkable in two aspects. First was the emergence of context-independent sensitization of the stereotypy response to cocaine after both one and four pairings with cocaine (figure 3-8). Second was the rapid onset of the DBA/2's context-dependent sensitization relative to that of the C57BL/6 mice on both the horizontal and stereotypy measures (figures 3-7 and 3-8). However, it is of note that this sensitization was not long-lived with respect

to the horizontal distance measure following four pairings with cocaine except for the 5 mg/kg dose (figure 3-7). Last, the DBA mice showed no decline in the behavior of their unpaired group over the course of the cocaine pairing sessions (figures 3-7 through 3-12). If indeed context-dependent sensitization is enhanced by the presence of a novel environment, then the lack of decline in the DBA/2 response to saline may indicate that the DBA/2 mice did not habituate to the environment, and thus continued to experience it as novel. In this case, only the one pairing experiment would have subjected the DBA/2 and C57BL/6 mice to an equivalent amount of novelty. After one pairing, the sensitization effect in the DBA/2 remained strong. After four pairings with cocaine in the testing chamber, the sensitization was relatively attenuated, seeming to contradict the theory that novelty is required for sensitization to be developed.

Variance Between the Strains on Related Measures Found in Literature

C57BL/6 and DBA/2 mice are known to differ at approximately 37% of their genome (Seale and Carney, 1991). They are also known to differ specifically in restriction endonuclease mapping in the area of the D2-, D3-, and D4-dopamine receptors (Scott et al, 1995). Several cocaine self-administration studies have shown that C57BL/6 mice self-administer more cocaine, faster, with more resistance to extinction than the DBA/2 mice do (Carney et al, 1991; Grahame and Cunningham, 1995; Rocha et al, 1996). Several studies of the behavioral profile induced by cocaine sensitization have shown results similar to

those of the present study. Using direct observation, stereotypy was measured in both C57BL/6 and DBA/2 mice following one, four and seven days of daily injections with either saline or 32 mg/kg cocaine in a study by Tolliver and Carney (1994). As with the current study, DBA/2 mice were found to show no significant increase in stereotypy behavior after only one exposure to cocaine, but after four pairings, both context-dependent and context-independent sensitization of the stereotypy response were observed. However, as mentioned previously, the "unpaired" group of the Tolliver and Carney (1994) study was not exposed to the testing chamber at all during the pairing sessions, and thus the response during testing may have also contained an element of novelty. Unlike the present study, no stereotypy was observed in the C57BL/6 mice. Part of this difference may be due to the measurement of stereotypy via direct human observation versus Digiscan computer observation. Studies in the BXD recombinant inbred mice derived from the C57BL/6 and DBA/2 strains of mice suggest that stereotypy and locomotion may be controlled by separate groups of genes (Tolliver et al, 1994). It appears from that study that stereotypy was associated with chromosomes 1, 6 and 12 of the DBA/2 mouse genome, while horizontal motion was associated with chromosome 9 of the DBA/2 mouse genome and chromosome 17 of the C57BL/6 mouse genome.

The stronger context-dependent sensitization exhibited by the C57BL/6 mice in the current study was also shown in a study by Elmer et al (1996). Using horizontal activity as the behavioral measure, Elmer et al (1996) exposed

C57BL/6 and DBA/2 mice to either one, two or three days of pairing followed by a challenge session. In their study the pairing doses chosen represented the ED_{50}, so for C57BL/6 mice it was 5.8 mg/kg and for DBA/2 mice it was 10 mg/kg, while the challenge doses were the ED_{25} for the respective strains. Under all three pairing conditions, the context-dependent sensitization of the C57BL/6 mice exceeded that of the DBA/2 mice.

Tolliver et al (1994) looked at both horizontal activity and stereotypy measures between not only the C57BL/6 and DBA/2 mice but also between their 26 recombinant inbred BXD strains. Unlike the current study they did not see any sensitization of horizontal activity measures on either the C57BL/6 or DBA/2 strains, but this may have been because their data reflected an average of behavior over a 60-min period rather than the first 15-min as the current study used. The same study also looked at the effect of context on sensitization, but as mentioned earlier, their "unpaired" group did not have exposures to the testing chamber during the cocaine pairing sessions; thus, the novelty effect may also be represented in their data. Neither the paired group of the DBA/2 or C57BL/6 strains displayed context-dependent sensitization that surpassed the response of the saline control group to cocaine when Tolliver et al exposed the mice to 6 pairing sessions with 32 mg/kg cocaine and a challenge injection of 32 mg/kg cocaine the following day. However, the C57BL/6 paired group did show a conditioned response to saline on the challenge day while the DBA/2 paired group did not (Tolliver et al, 1994). It is also of note that this same study found

no difference in the brain cocaine levels between strains after either acute or repeated cocaine administration.

Comparison of the Results of Both Experiments

These experiments sought to quantify the contribution of context-dependent sensitization to the overall phenomenon of the sensitization of cocaine-induced motor behavior and to establish the existence of genetic variance in that context-dependent sensitization. The expected results were achieved in that context-dependent sensitization of cocaine's behavioral effects was shown to be a significant component of sensitization in three different strains of mice: Swiss Webster, C57BL/6 and DBA/2 mice. Only Swiss Webster and DBA/2 mice showed any significant context-independent sensitization. Furthermore, the existence of genetic variance was established in that the drug-seeking C57BL/6 mouse strain displayed stronger context-dependent sensitization and conditioning of cocaine's behavioral effects than did the drug-avoiding DBA/2 strain.

The dissociation of context-dependent and context-independent sensitization of the motor effects of cocaine was established in this mouse model as had not been done before by pairing 40 mg/kg cocaine to differing environments, while exposing all animals to both environments an equal number of times and by testing the sensitization by using full dose-response curves. After establishing that dissociation of the context-dependent and context-independent aspects

of sensitization of cocaine-induced motor behavior was possible, it was determined as had been noted in prior studies (Badiani et al, 1995; Carey and Damianopoulos, 1994; Fontana et al, 1993; Jackson and Nutt, 1993; Post et al, 1992; Hinson and Poulos, 1981) that sensitization was almost exclusively context-dependent and that many of cocaine's behavioral effects could be conditioned.

It was determined that the C57BL/6 mice possessed stronger conditioning to the behavioral effects of cocaine than did the DBA/2 mice because 1) the C57BL/6 mice had a cocaine-conditioned behavior profile which was similar to a higher dose of cocaine than that of the DBA/2 mice, 2) the C57BL/6 cocaine-conditioned behavior was more resistant to extinction than that of the DBA/2 mice, and 3) the magnitude of the C57BL/6 cocaine-conditioned response was larger than that of the DBA/2 mice. Therefore, it was concluded that genetic differences between the strains regarding their context-dependent sensitization of the behavioral effects of cocaine may account for the differences observed in their drug-seeking behavior demonstrated in other paradigms (Rocha et al, 1996; Grahame and Cunningham, 1995; Carney et al, 1991; and Seale and Carney, 1991).

An additional finding in the unpaired groups of both the Swiss Webster and the C57BL/6 mice was a progressive decline in their saline-induced behavior over the four days of cocaine pairing. A slight decline was evident in the saline control groups of both strains as they became habituated to the environment, but the unpaired groups' decrease in behavior was much greater than that

of the saline control groups. This decline was global in that it effected all horizontal, vertical and stereotypy measurements except average speed and distance. One explanation for this could be that the unpaired mice were in a state of cocaine withdrawal, and experiencing depression and dysphoria. The fact that the unpaired group of the DBA/2 mice did not exhibit this decline in saline-induced behavior following cocaine pairing sessions indicates further reason for their resistance to cocaine dependence, since they may lack the incentive generated by the dysphoria of withdrawal to seek the drug again. Since dysphoria induced by a withdrawal state has been associated with relapse to cocaine use in humans (Johanson and Fischman, 1989), this may add further information toward the quest for the genetic basis of the C57BL/6's increases susceptibility to cocaine dependence.

Application of this Research to Isolation of Genes Important in Cocaine Abuse

As was done by Tolliver et al (1994) regarding locomotion and stereotypy, the phenotypes of magnitude of conditioning and decline in saline-induced behavior following repeated cocaine exposure could be determined in the BXD RI strains using the experimental model herein described in order to identify regions of the genome which contribute to the development of conditioned responses to cocaine and to dysphoria. Ultimately this may lead to a better understanding of the conditioning and dysphoria phenomena and perhaps to ways of manipulating them pharmacologically to the benefit of cocaine addicts attempting

abstinence. As with most drug-induced behavioral responses, there are likely several genetic loci which all contribute a small portion to the overall effect of a drug. These loci are called quantitative trait loci (QTL) (Belknap and Crabbe; 1992; Copeland et al; 1993). In order to identify the QTL responsible for these phenotypes, one would have to repeat the above study in the 26 BXD strains and then perform a QTL analysis, which is a procedure by which the variance in known markers on the chromosomes of the mice are compared with the variance in phenotype such that certain markers are identified which are significantly associated with the phenotype. Within the vicinity of these markers, theoretically, one would find the QTL responsible for context-dependent sensitization of the behavioral effects of cocaine (Gora-Maslak, 1991). Depending on what types of genes are involved, it may be possible to pharmacologically intervene in context-dependent sensitization of the cocaine-induced motor behavior or at least to screen for genes predicting susceptibility to cocaine dependence in humans.

In 1991, Gora-Maslak et al performed a QTL analysis on data from a 1985 experiment on amphetamine-induced core temperature changes by Seale. It was found that the Lamb-2 locus was significantly associated with the phenotypic variation, ($r = 0.96$). The DBA/2J allele of Lamb-2 was associated with large changes in core-temperature while the C57BL/6J was associated with small changes. Lamb-2 is a marker in a gene which codes for the B2 subunit of the extracellular matrix protein, laminin. Laminin may be involved in changing the blood-brain barrier permeability to amphetamine. This association was not

one that would have occurred intuitively to most investigators, unlike the D2-dopamine receptor involvement in four alcohol-associated and two methamphetamine-associated traits (Crabbe et al, 1994). Herein lies the rationale for the use of QTL analysis: associations that are not intuitively obvious may be uncovered. Therefore, unanticipated genes may prove to be important in conditioning, and it may be possible to manipulate them to the advantage of the recovering cocaine addict. The studies herein presented lay the foundation for such an investigation.

CHAPTER V

DISSERTATION REFERENCES

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