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An Investigation of the Development and Expression of Sensitization to the Locomotor Activating Effects of Amphetamine and Morphine

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ABSTRACT

An Investigation of the Development and Expression of Sensitization to the Locomotor Activating Effects of Amphetamine and Morphine

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Concordia University, 1987

The development and expression of behavioral sensitization to the locomotor activating effects of amphetamine and morphine was investigated in six experiments.

Experiments 1 (amphetamine) and 2 (morphine) tested the DA autoreceptor subsensitivity hypothesis of sensitization. Animals were pretreated with D-1 or D-2 DA receptor antagonists prior to each of five exposures to the drug. It was reasoned that the D-2 antagonists would prevent the desensitization of DA autoreceptors and, thereby, prevent the development of sensitization. Results provided no support for this hypothesis. In Experiment 1, none, and in Experiment 2, only one of the three D-2 antagonists tested attenuated the development of sensitization. Curiously, the D-2 antagonist, pimozide, blocked the development of sensitization to morphine but not to amphetamine. Conversely, the D-1 antagonist, SCH-23390, blocked the development of sensitization to amphetamine but not to morphine. Although difficult to interpret, these latter findings suggest that the mechanisms underlying the development of sensitization to amphetamine and morphine are different.

In Experiment 3 (amphetamine) and 4 (morphine), the relation between conditioning and sensitization was explored. It was found that the expression of sensitization to amphetamine and morphine

can come under strong stimulus control. This control could be reduced by extinction training. This procedure, however, did not eliminate evidence for sensitization. These findings suggest that, although conditioning factors can exercise strong control over its expression, evidence for sensitization can be seen in their absence.

The final two experiments were conducted to investigate whether conditioning and/or sensitization would develop in animals exposed to intracranial amphetamine injections into either the ventral tegmental area (VTA, Experiment 5) or the nucleus accumbens (NAC, Experiment 6). Results suggest that the neuroanatomical site critical for the development of sensitization to amphetamine is the VTA and not the NAC. No evidence for conditioned activity or for stimulus control of sensitization was found when amphetamine infusions into either site were paired with a specific environment.

The implications of these findings for hypotheses that changes in mesolimbic DA neurons underlie behavioral sensitization to stimulant and opiate drugs and for current ideas about the role played by environmental stimuli in the expression of conditioned and sensitized drug effects are discussed.

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Since the late 1960's, there has developed an increasing interest in the phenomenon of behavioral sensitization of the locomotor activating effects of psychomotor stimulant drugs. Sensitization has been considered important for its possible relation to the progressive phenomenology of affective illnesses (e.g., Post, Rubinow and Ballenger, 1984) and to the changing effectiveness of drugs of abuse (see Iversen, 1983; Vezina, Kalivas and Stewart, 1987; Stewart and Vezina, submitted). Many attempts have been made to elucidate the biological basis of the changing effectiveness of these drugs on those neural systems thought to mediate their locomotor activating effects (e.g., Robinson and Becker, 1986).

Although behavioral sensitization to the psychomotor stimulants, and particularly to amphetamine, has been the most thoroughly investigated, recent studies have turned to similar effects following the repeated administration of the opiate drug, morphine, and of opioid peptides. These studies have led to the gradual emergence of the view that opiate and stimulant drugs act via a common neural system important in the development of behavioral sensitization (see Kalivas, 1985a). This thesis constitutes an attempt to explore similarities and dissimilarities between the development of sensitization to the locomotor activating effects of amphetamine and morphine in the rat, with a view to further elucidating the requirements of a biological system necessary to mediate these effects. In this introductory section, the acute effects of amphetamine and morphine on locomotor activity as well as the neural substrates

thought to mediate these effects will be discussed. The sensitization of these effects will then be characterized, and hypotheses proposed to account for this sensitization will be reviewed.

Characterization of the Locomotor Activating Effects of Amphetamine and Morphine

Amphetamine and morphine are drugs with multiple central nervous system actions, including effects on locomotion, eating, body temperature and pain sensation, and a host of actions on peripheral tissue (Mansky, 1978; Moore, 1978). Moreover, the effects of these drugs on locomotor activity are complex.

Amphetamine. In the rat, low doses of amphetamine, ranging roughly from 0.3 to 1.5 mg/kg injected systemically, produce an increase in forward locomotion persisting for approximately forty minutes to two hours. At higher doses, amphetamine elicits a multiphasic response pattern that consists of early and late phases of forward locomotion and an intermediate phase of focused stereotypy during which locomotion is absent. In this latter phase, animals typically engage in the repetitive performance of species-typical behaviors such as sniffing, nose poking, head and limb movements and oral behaviors (licking, biting and gnawing) expressed over a small area of the testing environment (Randrup and Munkvad, 1967; Segal, 1975a; Kelly, 1977; Fray, Sahakian, Robbins, Koob and Iversen, 1980; Rebec and Bashore, 1984).

Amphetamine is thought to act primarily on catecholamine [dopamine (DA) and norepinephrine (NE)] neurons. It has been suggested to promote the release of the transmitter into the

synapse, to block the inactivation of the released transmitter through inhibition of reuptake into the nerve terminal thereby prolonging its synaptic activity, and perhaps also to increase the availability of releasable transmitter through inhibition of the degradative enzyme monoamine oxidase (see Kuczenski, 1983). Not surprisingly, early views suggested that both DA and NE were important in the expression of amphetamine-induced locomotor activity (e.g., Taylor and Snyder, 1971). More recent pharmacological, biochemical and 6-hydroxydopamine (6-OHDA) lesion studies now suggest, however, a primary role for DA neurons, and, in particular, those neurons of the ascending mesolimbic DA system with cell bodies in the ventral tegmental area (VTA, the A10 nucleus) and terminals in the nucleus accumbens (NAC) and olfactory tubercle (OT; for reviews, see Kelly, 1977; Ungerstedt, 1979; Groves and Tepper, 1983). Thus, amphetamine-induced locomotor activity has been found to be antagonized when catecholamine synthesis is inhibited by alpha-methyl-p-tyrosine (Weissman, Koe and Tenen, 1966), but not when the conversion of NE from DA is prevented by DA-beta-hydroxylase inhibitors (Thornburg and Moore, 1973; although, cf, Archer, Fredriksson, Jonsson, Lewander, Mohammed, Ross and Soderberg, 1986; Mohammed, Danysz, Ogren and Archer, 1986), by DA, but not NE receptor blockade (Maj, Sowinska, Kapturkiewicz and Sarnek, 1972; Rolinski and Scheel-Kruger, 1973; Pijnenburg, Honig and Van Rossum, 1975) and by large though selective 6-OHDA lesions of DA cells in the substantia nigra, but not by similar lesions of the dorsal or ventral noradrenergic pathways (Creese and Iversen,

1975). Evidence specifically implicating the mesolimbic DA system in the mediation of amphetamine's locomotor activating effects comes from numerous studies. Pijnenburg et al. (1975), for example, found that injections of the DA receptor antagonist haloperidol into the NAC antagonized the locomotor activity induced by systemic injections of amphetamine. Conversely, amphetamine injected into the NAC (Pijnenburg, Honig, Van Der Heyden and Van Rossum, 1976; Staton and Solomon, 1984; Carr and White, 1987) and the OT (Pijnenburg et al., 1976) elicits increased locomotion which is blocked by systemic injections of haloperidol (Pijnenburg et al., 1976). Similar findings were obtained with injections of DA and apomorphine, a DA agonist, into these sites (Pijnenburg et al., 1976). In other studies, 6-OHDA lesions of the NAC, but not of the caudate nucleus (Creese and Iversen, 1974; Kelly, Sevoir and Iversen, 1975; Kelly and Iversen, 1976; Koob, Stinus and LeMoal, 1981) nor of the frontal cortex (Joyce, Stinus and Iversen, 1983), were found to attenuate amphetamine-induced locomotion. Interestingly, some reports have suggested that amphetamine exerts its effects on locomotion via a simultaneous ("mass") action at several DA terminal fields (e.g., ACC, OT and the anteroventral caudate nucleus) since lesions at all of these sites combined (via anterolateral hypothalamic 6-OHDA injections) were found to produce a more profound blockade of amphetamine locomotion than separate lesions at individual terminal regions (Fink and Smith, 1979, 1980; see also Winn and Robbins, 1985; Kafetzopoulos, 1986). Recent anatomical investigations have established that the ascending mesolimbic DA system projects to the ventromedial part of the neostriatum as

well, and injection of the DA receptor antagonist cis-flupenthixol into this site, but not into other striatal sites, has been found to attenuate exploratory locomotor activity in rats (see Ahlenius, Hillegaart, Thorell, Magnusson and Fowler, 1987).

Morphine. Like amphetamine, morphine has also been found to have dose-dependent effects on locomotor activity. In the rat, low doses of morphine (e.g., 1.0 to 5.0 mg/kg, injected systemically) produce an increase in locomotor activity persisting for two to three hours while higher doses produce an initial decrease that is followed by an increase (Babbini and Davis, 1972; Vasko and Domino, 1978).

Although several nuclei in the rat brain have been studied (e.g., substantia nigra, Iwamoto and Way, 1977; globus pallidus, Joyce, Koob, Strecker, Iversen and Bloom, 1981; nucleus raphe pontis, Broekkamp, LePichon and Lloyd, 1984; and other sites, Tseng, Wei, Loh and Li, 1980), the search for the substrate of morphine's locomotor activating effect has centered on the VTA and the NAC. Both of these sites have been found to have high concentrations of enkephalinergic terminals (Sar, Stumpf, Miller, Chang and Ciatrecasas, 1978; Wamsley, Young and Kuhar, 1980) and receptors (Hong, Yang, Fratta and Costa, 1977; Sar et al., 1978; Goodman, Snyder, Kuhar and Young, 1980).

Numerous studies have now demonstrated that the microinjection of a number of opiate substances into the VTA produces an increase in locomotor activity that is blocked or reversed by the opiate receptor blocker naloxone (Broekkamp,

Phillips and Cools, 1979; Joyce and Iversen, 1979; Kelley, Stinus and Iversen, 1980; Stinus, Koob, Ling, Bloom and LeMoal, 1980; Joyce et al., 1981; Vezina and Stewart, 1984; Kalivas, Taylor and Miller, 1985). Several lines of evidence suggest that this locomotor activating effect of opiates in the VTA may also be mediated by the ascending mesolimbic DA system. The cells of origin of this system (the A10 DA nucleus) are located in the VTA and both systemic and iontophoretic administrations of morphine produce an increase in the firing frequency of a subpopulation of these cells (Ostrowski, Hatfield and Caggiula, 1982; Gysling and Wang, 1983; Mathews and German, 1984). Injection of enkephalin into the VTA causes a dose-dependent increase in DA turnover in the NAC, a major terminal region of the A10 DA cells. Furthermore, simultaneous administrations of enkephalin into the VTA and DA into the NAC at doses subthreshold for either substance alone have been shown to produce a significant increase in locomotion (Kalivas, Widerlov, Stanley, Breese and Prange, 1983). Finally, destruction of the mesolimbic DA system by 6-OHDA, or blockade of dopamine receptors by systemic or intra-NAC injections of neuroleptics both interfere with the locomotor activating effects of intra-VTA morphine or other opioids (Joyce and Iversen, 1979; Kelley et al., 1980; Stinus et al., 1980; Joyce et al., 1981; Kalivas et al., 1983; Vezina and Stewart, 1984).

Microinjection of morphine (Pert and Sivit, 1977) and enkephalins (Havemann, Winkler and Kuschinsky, 1983; Kalivas et al., 1983; Pert and Sivit, 1977) into the NAC have also been shown to produce increased locomotor activity that can be blocked

by naloxone. However, unlike the motor activity induced by intra-VTA opiate injections, this effect is not blocked by systemic (Pert and Sivit, 1977) or intra-NAC (Kalivas et al., 1983) injections of neuroleptics or by destruction of the mesolimbic DA system by 6-OHDA (Kalivas et al., 1983). Furthermore, intra-NAC administration of enkephalin has been found to have no effect on DA turnover in the NAC (Kalivas et al., 1983; cf, Pollard, Llorens, Bonnet, Constentin and Schwartz, 1977; Biggio, Casu, Corda, DiBello and Gessa, 1978).

Characterization of the Behavioral Sensitization of the Locomotor Activating Effects of Amphetamine and Morphine

The term "behavioral sensitization," also known as reverse tolerance, behavioral augmentation, and behavioral facilitation, refers to the progressive enhancement of different drug-induced behaviors produced by the repeated intermittent administration of a drug. Numerous studies have now demonstrated that such repeated exposure to either amphetamine or morphine induces sensitization to the locomotor activating effects of these drugs.

Amphetamine. In the case of amphetamine, the sensitization of several behaviors has been reported, including locomotor activity (Tilson and Rech, 1973; Segal and Mandell, 1974; Short and Shuster, 1976; Browne and Segal, 1977; Bailey and Jackson, 1978; Hirabayashi and Alam, 1981; Leith and Kuczenski, 1981, 1982), rotational behavior (Echols, 1977, 1979; Robinson, Becker and Presty, 1982; Robinson, 1984), stereotypy (Segal and Mandell, 1974; Browne and Segal, 1977; Nelson and Ellison, 1978; Leith and Kuczenski, 1981, 1982; Kolta, Shreve, De Souza and Uretsky, 1985)

and others (for reviews, see Post, 1981; Robinson and Becker, 1986). Sensitization of the effects of amphetamine has been reported following a single preexposure injection although more extensive sensitization is generally seen following repeated injections (e.g., Robinson, 1984). In either case, behavioral sensitization is a long-term effect having been reported to persist for months. Robinson (1984), for example, has reported that a single injection of a low dose of amphetamine enhances rotational behavior induced by a second injection for up to 12 weeks. It should be noted that such behavioral sensitization is obtained with a regimen of intermittent drug injections and differs from the changes in behavior obtained with a regimen of continuous drug administrations (e.g., as with a subcutaneously implanted osmotic pump). These latter changes in behavior have been associated with degenerative brain damage reflective of the neurotoxic effects of maintained high brain levels of amphetamine (see Robinson and Becker, 1986). Moreover, some have reported that more temporally spaced injections are more efficacious than injections given more frequently. For example, Hirabayashi and Alam (1981) reported that injections given three to four or seven days apart resulted in greater sensitization of locomotor activity than injections given daily (see also Nelson and Ellison, 1978; Post, 1980; Robinson, 1984; Kolta et al., 1985).

In the studies cited above, the repeated administration of low doses of amphetamine resulted in the sensitization of locomotor activity, whereas the repeated administration of high doses resulted in the sensitization of both locomotor activity

and stereotypy. In the latter case, the enhanced locomotion appears in the late phase, after the stereotypy has subsided. The sensitization of stereotypy has been characterized as the emergence of more intense stereotyped behavior, reduced time to the onset of stereotypy following the injection and the elicitation of stereotypy by doses lower than would usually elicit stereotyped behavior (Segal and Mandell, 1974; Leith and Kuczenski, 1981, 1982). Given the obviously opposing nature of locomotor activity and stereotypy (e.g., see Joyce and Iversen, 1984), this latter finding, indicative of a leftward shift in the amphetamine dose-response curve, is important. Indeed, Segal and Mandell (1974) have suggested that with repeated administrations of amphetamine, the progressive emergence of stereotypy may compete with the expression of the sensitization of locomotor activity even when systemic doses as low as 1.0 mg/kg are used. They found that animals repeatedly administered amphetamine at a low dose of 0.5 mg/kg (i.p.) showed a progressive increase in locomotor activity throughout the experiment (no stereotypy appeared in these animals). Other animals, repeatedly administered a dose of 1.0 mg/kg (i.p.), showed a progressive increase in locomotion up to a point, however, after which no further increases were obtained (some stereotypy did appear in these animals). Such findings have led some (e.g., Rebec and Bashore, 1984) to recommend the simultaneous measuring of several indices of behavior (e.g., locomotor activity and stereotyped behaviors) in order to adequately monitor the changes that occur when amphetamine is administered repeatedly. Alternatively (or in addition), the sensitization of locomotor activity obtained with

low doses can be demonstrated by administering even lower (stereotypy-free) doses at test, a procedure concordant with the progressive shift to the left in the amphetamine dose-response curve as behavioral sensitization develops (see above).

Considerable evidence suggests that changes in the functioning of central DA neurons, themselves, underlie the behavioral sensitization to the effects of amphetamine. Much of this evidence has been reviewed recently by Robinson and Becker (1986) who, at the same time, concluded that there was little evidence for other types of changes often suggested as possible explanations for behavioral sensitization (e.g., peripheral drug dispositional factors, see also Rebec and Segal, 1979; postsynaptic DA receptor supersensitivity, see also Alloway and Rebec, 1984). Thus, it has been reported that the behavioral sensitization induced by repeated intermittent injections of amphetamine is accompanied by a long-term enhancement of amphetamine-stimulated DA release from striatal tissue in vitro (Robinson and Becker, 1982; Robinson et al., 1982). Using an in vitro striatal slice preparation, Kolta et al. (1985) subsequently reported that the amphetamine-stimulated release of DA was enhanced 15 and 30 days after pretreatment with amphetamine, but not after three days, findings which paralleled the time course of the development of the enhanced or sensitized behavioral response (stereotypy) to amphetamine. Peris and Zahniser (1987) also found increased amphetamine-induced release of tritium-labelled DA in vitro from striatal slices of animals which had previously received a single injection of cocaine (a

psychomotor stimulant that, like amphetamine, blocks DA reuptake but, unlike amphetamine, does not appear to promote DA release, see Kuczenski, 1983). In other studies, striatal levels of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were assayed following a systemic injection of amphetamine as indices of the changing effectiveness of amphetamine to induce DA utilization/release. DOPAC and HVA levels are found to be lowered by acute injections of amphetamine. This reduction is thought to result from blockade by amphetamine of DA reuptake into the neuron, where it is normally degraded by intracellular monoamine oxidase into DOPAC that is, in turn, subsequently degraded by extracellular catechol-O-methyltransferase into HVA (see Kuczenski, 1983). Thus, Kuczenski and Leith (1981) found that an injection of amphetamine decreased DOPAC and HVA levels to a greater extent in animals sensitized to amphetamine than in control animals. Similarly, Kalivas, DuMars and Skinner (submitted) found that the decrease in DOPAC and HVA in the striatum and the NAC induced by an acute injection of cocaine was greater in rats that had been pretreated with three daily cocaine injections 14 days earlier. This enhanced decrease of DA metabolites was thus interpreted to reflect a greater release of DA into the extracellular space which, combined with blockade of DA reuptake, would further decrease the intracellular DA available for degradation (Kuczenski and Leith, 1981; Kalivas et al., submitted). Interestingly, Nishikawa, Mataga, Takashima and Toru (1983) reported that an injection of methamphetamine increased DOPAC levels in the striatum and mesolimbic terminal fields (NAC, OT and a part of the septum combined) in

methamphetamine-pretreated compared to saline-pretreated animals, a finding that could also indicate enhanced DA release if it is assumed that methamphetamine is not as potent a DA reuptake blocker as d-amphetamine (used by Kuczenski and Leith, 1981) and cocaine (see Robinson and Becker, 1986). It should be noted that in all of the above studies, no differences in resting or baseline levels of DA release or DA metabolites were found between amphetamine-pretreated and saline-pretreated animals. This suggests, therefore, that it is not a change in the steady-state level of transmitter utilization/release by DA neurons that underlies the behavioral changes observed in sensitized animals. Rather, the important underlying neuronal change would appear to be in the way these neurons respond to pharmacological and environmental (see below) challenges. A notable exception to this lack of effect of sensitization on steady-state or resting levels of DA neuron function was reported recently by Robinson and Camp (1987). They found that female rats sensitized to amphetamine showed higher resting levels of DOPAC than saline-pretreated controls in the striatum and NAC (remember, animals were not injected with amphetamine prior to sacrifice so that there was therefore no blockade of DA reuptake). It was initially suggested by them that such increases in baseline DA function might be detected more readily in females since, although behavioral sensitization is obtained with both female and male animals, females (and castrated males) have been found to show greater sensitization to amphetamine than intact males (which, incidentally, also suggests the possible modulation of the

develoment of sensitization by a testicular hormone in males, Robinson et al., 1982; Robinson, 1984). These authors observed as well, however, that amphetamine-sensitized females did not show higher baseline levels of locomotor activity than untreated females, prompting them to suggest an alternative explanation of their neurochemical findings. They speculated that the increased DOPAC levels might not in fact reflect resting state, but rather a rapid and exaggerated response to a "stress challenge" by sensitized females prior to decapitation. It is known that amphetamine-sensitized animals show an increased neurochemical response to a stress challenge (Antelman and Chiodo, 1983; see below). Given the above sex difference in the development of sensitization to amphetamine, this response would be expected to be more pronounced in females (Robinson and Camp, 1987).

In addition to an enhanced cocaine-induced decrease in striatal and NAC (terminal field) DOPAC and HVA levels in animals sensitized to cocaine, Kalivas et al. (submitted) have also found changes in DA function at the cell body level of DA neurons. They compared the effects of an acute injection of cocaine in cocaine- and saline-pretreated animals. In saline-pretreated animals, VTA levels of DOPAC and HVA were decreased following the injection of cocaine as was DA synthesis [indexed by the accumulation of 3,4-dihydroxyphenylalanine (DOPA), a precursor of DA, after inhibition of the DA synthesizing enzyme DOPA-decarboxylase]. The latter finding was considered, as was the decrease in DA metabolite levels (see above), to be due to the increase in the concentration of DA outside the cell body brought about by reuptake blockade; DA synthesis would thus be expected to be

indirectly inhibited by the inhibitory effect of the increased stimulation of DA somatodendritic autoreceptors by DA on DA cell firing (see Roth, 1984). In sensitized animals, however, the changes in DA functioning at the cell body level did not parallel those found at terminal sites. Kalivas et al. (submitted) report that the cocaine-induced decrease in VTA levels of DA metabolites found in saline-pretreated animals was "significantly blunted" for up to an hour post injection in cocaine-sensitized animals rather than being enhanced as in terminal areas. That is, levels of DOPAC and HVA in cocaine-sensitized animals did not differ from those of saline-pretreated animals nor did they differ from baseline levels in cocaine-pretreated animals. Concordant with this result was the additional finding that the decline in DOPA accumulation seen after an acute injection in saline-pretreated animals was reversed in cocaine-pretreated animals. It was thus suggested that these findings reflected either a decrease in the cell body release of DA and a corresponding decrease in autoreceptor stimulation by extracellular DA, a decrease in the number and/or affinity of somatodendritic DA autoreceptors or a decrease in the capacity of these autoreceptors to inhibit synthesis. Direct support for the first possibility was subsequently obtained by Kalivas and Duffy (submitted, a). They measured the in vitro release of DA from ventromedial mesencephalic tissue slices (which included the nucleus interfascicularis, the VTA and the medial substantia nigra) induced by either amphetamine or depolarization (by increasing the superfusate potassium concentrations). The release of DA was

less in animals pretreated with cocaine than in those pretreated with saline. Again, as was found in the DA terminal fields, there were no alterations in resting or baseline levels of DA release or of DOPA accumulation. There was, however, a decrease in VTA baseline levels of DA metabolites, a finding that is without explanation (Kalivas, personal communication; see also Robinson and Camp, 1987, p. 825).

Morphine. There have been numerous reports of sensitization to the locomotor activating effects of morphine. Generally, it has been found that the repeated injection of high systemic doses results in the development of tolerance to the initial depressant effect and a progressive enhancement of the excitatory effect of morphine on locomotor activity (Kumar, Mitchell and Stolerman, 1971; Babbini and Davis, 1972; Oka, Nozaki and Hosoya, 1972; Nakamura, Ishii and Shimizu, 1978; Vasko and Domino, 1978; Brady and Holtzman, 1981a, b). This behavioral sensitization has been shown to be long-term, persisting for as long as eight months after the last injection (Babbini, Gaiardi and Bartoletti, 1975). Interestingly, low doses of morphine, which elicit only increases in locomotor activity, have been reported not to produce sensitization when administered repeatedly (Babbini and Davis, 1972). This finding, together with those obtained with higher doses, led some initially to view the development of sensitization obtained with high doses as the progressive unmasking of the excitatory effects of morphine on locomotor activity as tolerance developed to its depressant actions (e.g., Babbini and Davis, 1972; see Seevers and Deneau, 1963). Subsequent reports, however, showing that tolerance to the

depressant effects of morphine diminishes quickly after drug-injections are terminated (within at least 20 days), but that sensitization to the excitatory effects remains for several months, suggested that sensitization occurred independently of tolerance (Babbini et al., 1975; Bartoletti, Gaiardi, Gubellini, Bacchi and Babbini, 1983). Furthermore, the finding by Bartoletti et al. (1983) that a dose of 1.25 mg/kg (i.p.), that did not elicit increased locomotor activity in naive animals, would do so in animals pretreated with repeated injections of a higher dose of morphine, indicated a shift to the left of the morphine dose-response curve for increased locomotor activity. Such findings led to the more recent view that the development of tolerance to the depressant effects and sensitization to the excitatory effects of morphine on locomotor activity are mediated via actions on separate neuronal systems and that the net effect of an injection represents the algebraic sum of drug action on these two systems (Vasko and Domino, 1978; Bartoletti et al., 1983; Schnur and Rajgoza, 1986; cf, Hinson and Siegel, 1983). Of course, such a view does not account for the lack of development of sensitization with low doses reported by Babbini and Davis (1972). This finding remains puzzling and in need of an explanation. It may be, for example, that even though the development of sensitization and tolerance may represent changes occurring in two separate neuronal systems, each of these systems may influence the changes occurring in the other, especially when morphine is administered systemically (e.g., see Hand and Franklin, 1985). Nonetheless, in agreement with the above view

are the findings reviewed earlier that injection of opiates into the VTA produces increased locomotor activity in the absence of depressant effects, and, furthermore, that the repeated microinjection of enkephalin (Kalivas et al., 1985) or morphine (Joyce and Iversen, 1979; Vezina and Stewart, 1984) into this site results in a progressive enhancement or sensitization of this locomotor activity. The evidence, also reviewed earlier, that the locomotor activating effect of opiates in the VTA is mediated by the ascending mesolimbic DA system, and that changes in this system may underly the behavioral sensitization found with repeated injections of amphetamine, suggests that similar changes in this system may underly the behavioral sensitization found with repeated injections of morphine. As in the case of amphetamine, no evidence has been found for the involvement of peripheral drug dispositional factors (Kalivas and Duffy, 1987), as well as postsynaptic DA receptor supersensitivity (Kalivas, 1985b) in the development of behavioral sensitization with morphine.

In support of the view that changes in the ascending mesolimbic DA system similar to those found with amphetamine may underlie the behavioral sensitization found with repeated injections of morphine, it has been found that sensitization of the hyperactivity induced by repeated enkephalin injections into the VTA is associated with an enhanced or sensitized metabolism (as indicated by an enhanced increase in the DOPAC/DA ratio; remember, there is no reuptake blockade) of DA in the NAC in response to the drug (Kalivas 1985b). In another study, Kalivas and Duffy (1987) found that the behavioral sensitization obtained

following repeated systemic injections of morphine was accompanied by an enhanced utilization (as estimated by depletion of DA after inhibition of the DA synthesizing enzyme tyrosine hydroxylase with alpha-methyl-p-tyrosine) and synthesis (as indexed by accumulation of DOPA following inhibition of DOPA-decarboxylase) of DA in the NAC. Surprisingly, the increase in DA metabolism (i.e., DOPAC/DA ratio) in the ACC found after injection of morphine to saline-pretreated animals was actually less in morphine-pretreated animals. Note that this finding does not agree with that reported by Kalivas (1985b) in animals pretreated with intra-VTA enkephalin. No explanation was given for this anomolous finding (Kalivas and Duffy, 1987, p. 211). In addition to the enhancement of DA function found in the terminal region (NAC) of mesolimbic DA neurons in animals sensitized to morphine, Kalivas and Duffy (1987) also found changes at the cell body level similar to those found with cocaine above (Kalivas et al., submitted). That is, the increase in DA synthesis (DOPA accumulation) and metabolism (DOPAC/DA ratio) found in the VTA after a morphine injection in saline-pretreated animals was diminished in animals pretreated with morphine and showing a sensitized behavioral response to morphine. Morphine sensitized animals also demonstrated a decrease in DA utilization (depletion of DA after alpha-methyl-p-tyrosine) in the VTA. Furthermore, Kalivas and Duffy (submitted, a) have also demonstrated that the in vitro release of DA from mesencephalic tissue slices induced by potassium was reduced in morphine-pretreated compared to saline-pretreated animals, findings similar to those obtained in

cocaine pretreated animals. They considered these findings to reflect enhanced DA function (utilization/release) in the NAC and a corresponding decrease in DA function at the DA cell body level in animals sensitized to morphine, an interpretation similar to that given to the findings obtained with amphetamine and cocaine. Again, it is important to note that in none of the above studies was a change found in steady-state or resting level of DA function indicating, once again, that the important change underlying behavioral sensitization appears to be in the way DA neurons respond to pharmacological and environmental (see below) challenges.

Additional evidence that sensitization to the locomotor activating effects of morphine involves the mesolimbic DA system comes from studies in which the DA-dependent and the DA-independent effects of morphine and other neuropeptides on locomotor activity have been compared. For example, it has been reported that the DA-dependent increases in locomotor activity produced by intra-VTA injections of neurotensin (Kalivas and Taylor, 1985), but not the DA-independent increases induced by intra-VTA injections of substance P (Kalivas, 1985a), showed the development of sensitization when these injections were administered repeatedly. Similarly, Vezina et al. (1987) found that the DA-dependent increases in locomotor activity produced by intra-VTA injections of morphine or the mu opioid receptor agonist DAGO, but not the DA-independent increases produced by intra-NAC injections of these substances, showed the development of sensitization with repeated injections. Concordant with this finding, Kalivas and Duffy (1987) found that sensitization to the

locomotor activating effects of systemic morphine can be prevented by pretreatment with intra-VTA, but not intra-NAC, injections of the opiate receptor blocker naltrexone methobromide (cf, Amalric and Koob, 1985).

Some data that may be difficult to reconcile with the findings reviewed thus far were reported by Bunney, Massar and Pert (1984). They found, for example, that electrolytic lesions made in either the VTA or the NAC attenuate, but do not completely block, the development of sensitization that occurs with repeated systemic injections of morphine. Because DA cells of origin (VTA lesion) or DA terminals (NAC lesion) and the intrinsic enkephalin targets at either site would be among the neural processes destroyed by such lesions, they suggested that opiates can act at sites other than the VTA and the NAC to bring about the sensitization of locomotor activity. One such site may be the globus pallidus; enkephalin microinjections into this site have been shown to produce an increase in locomotor activity that is blocked by naloxone but not by DA receptor blockade (Joyce et al., 1981). It remains to be determined whether repeated opiate injections into this site would result in the development of sensitization. A likely alternative explanation of the results of Bunney et al. (1984), however, may be that, in addition to the sensitization of the hyperactivity induced directly by the action of morphine in the VTA, tolerance to the depressant actions of morphine contributes to the emergence or unmasking of the higher activity levels seen after repeated systemic injections (Seevers and Deneau, 1963; Schnur and Raigoza, 1986; see above). For

example, the repeated administration of opiates into the nucleus raphe pontis , a site implicated in the mediation of their depressant effects (Broekkamp et al., 1984), may result in the development of tolerance to these effects.

Cross-sensitization. The above findings, indicating that similar changes in the functioning of neurons in the ascending mesolimbic DA system underly the development of sensitization to the locomotor activating effects of both amphetamine and morphine, suggest that cross-sensitization between the effects of these two drugs should occur. This possibility was investigated by Stewart and Vezina (1987) who found that the increased locomotor activity induced by systemic or intra-VTA injections of morphine in saline-pretreated animals was indeed enhanced or sensitized in animals pretreated with amphetamine. Conversely, animals pretreated with morphine have also been found to show sensitized levels of locomotor activity induced by a subsequent injection of amphetamine when compared to saline-pretreated controls (Vezina, Giovino, Wise and Stewart, in preparation). Similar findings have been reported by Kalivas (1985b) in animals pretreated with intra-VTA enkephalin and tested with a systemic injection of amphetamine (see also Smee and Overstreet, 1976; Wiechman, Wood and Spratto, 1981). Numerous studies have also demonstrated cross-sensitization between the effects of amphetamine or morphine and environmental challenges such as stress. It has been shown that, in several ways, the effects of acute electric foot-shock and tail-pinch stress, for example, on DA function at the terminal (Thierry, Tassin, Blanc and Glowinski, 1976; Fadda, Argiolas, Melis, Tissari, Onali and

Gessa, 1978; D'Angio, Serrano, Rivy and Scatton, 1987; for a review, see Dunn and Kramarcy, 1984) and cell body level (Deutch, Tam and Roth, 1985) of mesencephalic DA neurons resemble the acute effects of amphetamine and morphine on these neurons. Thus, it has been reported that amphetamine-induced locomotor activity (Herman, Stinus and Le Moal, 1984), stereotypy (MacLennan and Maier, 1983) and rotational behavior (Robinson, Angus and Becker, 1985; Carlson, Glick and Hinds, 1987) are enhanced in animals preexposed to inescapable foot-shock stress compared to animals not previously stressed. Similar findings have been reported for tail-pinch (Antelman, Eichler, Black and Kocan, 1980) and other stressors as well as for electrical self-stimulation in mesolimbicocortical pathways (Eichler and Antelman, 1979; see also Predy and Kokkinidis, 1984; for a review, see Antelman and Chiodo, 1983). In agreement with these behavioral findings, it has been shown that preexposure to stress (saline injection and associated handling) enhances the amphetamine-induced release of DA in striatum in vitro (Wilcox, Robinson and Becker, 1986) and pretreatment with amphetamine enhances the increase in DA metabolism in mesencephalic DA neuron terminals in animals given an electric foot-shock test (Robinson, Becker, Young, Akil and Castaneda, 1987). Similarly, Kalivas and Duffy (submitted, b) report that DA function is enhanced in the NAC and decreased in the VTA whether animals are pretreated with cocaine and tested with electric foot-shock stress or vice versa. As with amphetamine, Leyton and Stewart (1987) have shown that rats preexposed to inescapable foot-shock were more active than no-

shock controls when subsequently tested with a systemic injection of morphine. Similarly, Kalivas, Richardson-Carlson and Van Orden (1986) found that animals showed sensitized levels of locomotor activity whether they were pretreated with intra-VTA enkephalin and tested with electric foot-shock stress or vice versa. These enhanced levels of locomotor activity were paralleled by sensitized levels of DA metabolism in the NAC (Kalivas et al., 1986; Kalivas and Duffy, 1987). Interestingly, Kalivas and Abhold (1987) reported that pretreatment with intra-VTA injections of naltrexone methobromide during preexposure to foot-shock stress blocked the cross-sensitization to the effects on locomotor activity and NAC DA metabolism of a subsequent intra-VTA injection of enkephalin, suggesting that electric foot-shock stress, and possibly other stressors, enhances the release of enkephalin into the VTA, and that this released enkephalin activates mesolimbic DA neurons.

In summary, the results reviewed in this section, although not without anomalies, support the notion that a change in the functioning of mesolimbic DA neurons underlies the sensitization to the locomotor activating effects of amphetamine and morphine. More specifically, these findings strongly support the view that this behavioral sensitization is mediated by increased terminal and decreased cell body release of DA by neurons in this system in response to pharmacological and environmental challenge. Although the more immediate and intuitive correlate of behavioral sensitization may appear to be enhanced release of DA from terminals in response to challenge, it should be noted that decreased DA release at the cell body level is also compatible

with a sensitized neuronal system. The release of DA from the cell body, and the resulting stimulation of somadendritic autoreceptors by this released DA, has been shown to have an inhibitory effect on DA cell firing (Wang, 1981) with the resulting attenuation in release of DA from terminals (see Roth, 1984). The finding that cell body release of DA is decreased in sensitized animals suggests, therefore, that the autoreceptor-mediated inhibition of cell firing should also be diminished in these animals. These DA cells would then more easily be fired by excitatory pharmacological and environmental challenges and subsequently release more DA at terminals. Given these consequences of decreased cell body release in sensitized animals, it has been suggested that it is this change in DA function that is critical to the development of behavioral sensitization and that increased terminal release of DA is a result of this change (Kalivas and Duffy, submitted, a). Certainly, the biochemical data suggesting that the challenge-induced utilization/release of DA from terminals is increased in sensitized animals is concordant with this view. Alternatively, in vitro data also indicate that amphetamine-induced release of DA from terminal field tissue is enhanced in amphetamine sensitized animals (Robinson and Becker, 1982; Robinson et al., 1982; Kolta et al., 1985). Because this terminal field tissue does not include the DA cell bodies of origin, these data suggest that changes occur at the cell terminal level independent from those at the cell body. In contrast, Kalivas and Duffy (submitted, a) have reported that the in vitro potassium-induced

release of DA from NAC and striatal tissue is not enhanced in cocaine or morphine-pretreated animals, although some of these data have been inconsistent (cf, Duffy and Kalivas, 1987). It remains to be determined, therefore, whether one or both of these changes in DA neuron function, at the cell body or terminal, is critical for the behavioral sensitization obtained with repeated injections of amphetamine and morphine.

Neural Basis of Sensitized Dopaminergic Function

It should be stated at the onset that the basis of the changes in DA function described above and thought to underlie behavioral sensitization to the locomotor activating effects of amphetamine and morphine is unknown. Nonetheless, several hypotheses have been proposed.

One hypothesis is that DA autoreceptor subsensitivity provides the neural basis of sensitized DA function (Martres, Costentin, Baudry, Marcais, Protais and Schwartz, 1977; Muller and Seeman, 1979; Anteman and Chiodo, 1981). DA autoreceptors are thought to mediate the modulatory effect of DA itself on the functioning of the DA neuron. The stimulation by DA or DA agonists of neuron terminal autoreceptors (Anden, Grabowska-Anden and Liljenberg, 1983a,b) is considered to inhibit the synthesis and release of DA, while the stimulation of somadendritic autoreceptors (Wang, 1981) is considered to inhibit DA cell firing (see Roth, 1984). According to this view, therefore, the repeated release of DA, induced by pharmacological and environmental stimuli, and the consequential repeated stimulation of these autoreceptors by the released DA would lead to their

down-regulation. DA would exert less of an inhibitory influence on DA cell function if DA autoreceptors were subsensitive and, as a result, neural and pharmacological events that led to DA release would show enhanced effectiveness. It should be noted that the term "subsensitivity" refers only to the decreased effectiveness of the agonist (in this case, DA) to produce its usual effects on target cell activity via the receptor and does not address the nature of the change in the receptor (e.g., change in affinity or number) or other cellular changes that might influence how the receptor-agonist complex affects cell function.

Initial support for this hypothesis was reported by Muller and Seeman (1979) who found that repeated amphetamine administrations produced a decrease in labelled apomorphine binding in striatum. They attributed this decreased binding to subsensitive autoreceptors (although, the significance of these findings has been questioned, see Antelman and Chiodo, 1983, p. 286; White and Wang, 1984, p. 290). More recent support for DA autoreceptor subsensitivity following repeated exposure to amphetamine has come from electrophysiological studies. White and Wang (1984), for example, found that pretreatment with amphetamine reduced the ability of amphetamine or apomorphine to suppress the firing of VTA, A10, DA neurons (when injected intravenously at doses that selectively stimulate DA autoreceptors, Skirboll, Grace and Bunney, 1979). The inhibitory effect of microiontophoretically applied DA was reduced likewise. Amphetamine pretreatment also increased the number of

spontaneously active A10 cells as well as their firing rate, further indicating a decrease in the ability of autoreceptor activation by DA to tonically inhibit these DA cells. Similar findings were obtained by Kamata and Rebec (1984a) in the A10 DA cells as well as in the substantia nigra DA cells (Antelman and Chiodo, 1981; Kamata and Rebec, 1983, 1984b). Interestingly, Antelman and Chiodo (1981) found that increasing the interval between the last amphetamine injection and test reduced even more the suppressant effect of apomorphine on DA cell firing as well as on locomotor activity (Antelman and Chiodo, 1983), a finding that is reminiscent of those reviewed earlier suggesting that more temporally spaced injections are more efficacious in producing behavioral sensitization than injections given more frequently.

In other studies, the effect of preexposure to amphetamine on the subsequent ability of low doses of apomorphine to lower both DA metabolite levels and locomotor activity (effects thought to be due to the selective stimulation of DA autoreceptors, see Strombom, 1976) was evaluated. Watanabe (1985) reported, for example, that pretreatment with amphetamine reduced the ability of apomorphine to lower DOPAC levels in the ACC and OT, but not in the striatum and frontal cortex. A similar lack of change in striatum has also been reported by others (Conway and Uretsky, 1982; Kuczenski, Leith and Applegate, 1983; but see Masuda, Murai, Saito, Kohori and Itoh, 1987). In agreement with the findings of Antelman and Chiodo (1983), Watanabe and Taniguchi (1986) also found that pretreatment with amphetamine attenuated the suppressant effect of apomorphine on locomotor activity (see

also Davis, Sant and Ellison, 1985; Masuda et al., 1987; but cf, Conway and Uretsky, 1982; Riffee and Wilcox, 1985). Given the findings, reviewed earlier, indicating that cross-sensitization occurs between the locomotor activating effects of amphetamine and some stressors, it is interesting that pretreatment with electroconvulsive shock stress has also been found to reduce the ability of apomorphine to inhibit substantia nigra DA cell firing (Chiodo and Antelman, 1980) as well as to decrease locomotor activity (Serra, Argiolas, Fadda, Melis and Gessa, 1981). A similar reduction in the ability of apomorphine to lower locomotor activity has been reported following prolonged immobilization stress (Cancela and Molina, 1986). In addition to these studies that lend support to the view that DA autoreceptor subsensitivity could underly sensitization following exposure to psychomotor stimulants, it has been suggested that DA autoreceptor subsensitivity could be involved in sensitization of the effects of opiates as well (Kalivas, 1985a). According to this view, pretreatment with any pharmacological or environmental stimulus that results in the repeated release of DA from mesolimbic DA neurons would be expected to lead to DA autoreceptor subsensitivity and to the subsequent enhanced effectiveness of the stimulus to release DA.

As Robinson and Becker (1986) point out, however, the autoreceptor subsensitivity hypothesis is not without problems. The main difficulty is that concurrent DA autoreceptor subsensitivity would not seem to account for the fact that sensitization to the locomotor activating and DA releasing

effects of amphetamine occurs after a single pretreatment injection of amphetamine (Browne and Segal, 1977; Antelman et al., 1980; Robinson et al., 1982), in that such a pretreatment does not alter the ability of apomorphine to inhibit DA cell firing in the substantia nigra (Antelman and Chiodo, 1981) or the VTA (White and Wang, 1984). Another phenomenon that is problematic for this explanation of sensitization is that animals remain sensitized to the locomotor activating and DA releasing effects of amphetamine for weeks to months after the last pretreatment injection (Robinson et al., 1982; Robinson, 1984; Kolta et al., 1985). And, although Antelman and Chiodo (1981) did report that the ability of apomorphine to inhibit substantia nigra DA cell firing remained decreased for at least 11 days after pretreatment, this interval does not approach those reported above. Moreover, White and Wang (1984) found that only eight days after pretreatment, the decrease in the inhibitory effect of apomorphine was either partially (50%) or completely reversed (depending on the pretreatment dose regimen) compared to the decrease in apomorphine's effect in animals tested one day after pretreatment. These latter findings would indicate, therefore, that pretreatment dose regimens known to produce long-term sensitization do not seem to produce parallel long-term changes in DA autoreceptor sensitivity.

Robinson and Becker (1986) make the further argument that DA autoreceptor subsensitivity is probably not involved in amphetamine sensitization inasmuch as these receptors are thought to regulate depolarization-induced, calcium-dependent release of DA (Fischer and Cho, 1979; Langer and Arbilla, 1983), whereas

amphetamine has been shown to release DA via a calcium-independent process unmodulated by DA autoreceptors (Kamal, Arbilla and Langer, 1981). A change in DA autoreceptor sensitivity should, therefore, not result in a change in amphetamine-induced DA release and, conversely, enhanced amphetamine-induced release should not be affected by the lack or presence of changes in DA autoreceptors (Robinson and Becker, 1986). It should be noted, however, that this argument would not apply to changes in morphine-induced release of DA, since morphine is known to increase the firing of mesencephalic DA cells, and would, thus, promote the release of DA via a depolarization-induced, calcium-dependent process regulated by DA neuron terminal, and, indirectly, cell body, autoreceptors. Consistent with the argument of Robinson and Becker, however, are those findings indicating no change in the ability of apomorphine to inhibit saline-induced (Conway and Uretsky, 1982) or amphetamine-induced (Riffee and Wilcox, 1985) locomotor activity after pretreatment with amphetamine. It was also found, in the latter study, that pretreatment with apomorphine enhanced the locomotor activating effects of a subsequent injection of amphetamine. Riffee and Wilcox (1985) suggested that this cross-sensitization between apomorphine and amphetamine could not be due to autoreceptor subsensitivity since it was also found that this pretreatment, like pretreatment with amphetamine, did not lower the subsequent ability of apomorphine to inhibit locomotor activity (cf, Masuda et al., 1987). Surprisingly, Riffee, Wanek and Wilcox (1987) also found that the development of

sensitization to the locomotor activating effects of amphetamine is blocked by concomitant injections of apomorphine (15 minutes after the amphetamine injection) during preexposure. Thus, while these results may be difficult to interpret, they clearly suggest that sensitization to the effects of amphetamine is not due to autoreceptor subsensitivity.

The data reviewed so far in this section, indicating equivocal support for the view that DA autoreceptor subsensitivity is the basis of sensitized DA function, do not rule out a role for DA autoreceptors in the development of behavioral sensitization to the effects of amphetamine and morphine. Robinson and Becker (1986), for example, suggest the possibility that there may be a cascade of cellular changes that leads to the enduring behavioral and neurochemical signs of sensitization, and that momentary changes in DA autoreceptors may represent but one stage in this process. This would mean that observed changes in DA autoreceptor sensitivity, for example, would not necessarily need to parallel the time course of the behavioral and neurochemical indices of sensitization. Support for the idea that DA autoreceptors are necessary for behavioral sensitization to occur was reported by Fujiwara, Kazahaya, Nakashima, Sato and Otsuki (1987) who found that the ability of repeated injections of amphetamine to produce sensitization corresponded to the establishment, in the brains of rat pups, of functionally mature DA autoreceptors. Of course, the nature of the contribution, other than subsensitivity, of DA autoreceptors to sensitized DA function would need to be determined.

Alternatively, or possibly in addition to DA autoreceptor-

mediated events, changes at other DA receptor sites may play a role in the sensitization of DA function. DA receptors are generally classified as D-1 and D-2 receptors. DA stimulates adenylate cyclase activity at D-1 receptors and has either no effect or an inhibitory effect on adenylate cyclase activity at D-2 receptors. D-1 receptors are generally considered to be located postsynaptic to DA neuron terminals while D-2 receptors are located post- and presynaptically (the "autoreceptors" on DA neuron terminals and cell bodies; for a review, see Stoof and Kebabian, 1984). Thus, Barnett, Segal and Kuczenski (1987) reported that pretreatment with amphetamine produced a desensitization of striatal postsynaptic D-1 DA receptors (as indexed by reduced adenylate cyclase activity in response to an amphetamine challenge). No change in adenylate cyclase activity was found in response to a saline challenge in amphetamine-pretreated animals. These investigators proposed that the change in adenylate cyclase activity may reflect a compensatory response by D-1 DA receptors to enhanced synaptic levels of DA (in sensitized animals). The resulting decrease in efficacy of DA at these receptors would then result in a predominance of postsynaptic D-2 DA receptor activation and a corresponding induction of stereotyped behaviors (but see Molloy, O'Boyle, Pugh and Waddington, 1986). Alternatively, desensitization of striatal postsynaptic D-1 DA receptors may result in a decrease in the inhibition of substantia nigra DA cell firing via a striato-nigral feedback pathway, similar to that reported to occur after postsynaptic DA receptor blockade (see Chiodo and Bunney, 1984).

Such a pathway has been suggested to mediate the increased firing of a subpopulation of substantia nigra DA cells induced by systemic injections of the selective D-1 DA receptor antagonist SCH-23390 (Carlson, Bergstrom and Walters, 1986; see also Mereu, Collu, Ongini, Biggio and Gessa, 1985; Onali, Mereu, Olianas, Bunse, Rossetti and Gessa, 1985; Carlson, Bergstrom, Weick and Walters, 1987). A similar inhibitory NAC-VTA feedback pathway has been proposed by Wang (1981). In addition, it has been suggested that this pathway may mediate, in part, the inhibition of VTA DA cell firing produced by cocaine, as well as the increased firing of some VTA DA cells produced by SCH-23390, and the ability of SCH-23390 to reverse the D-2 DA receptor agonist-induced inhibition of these cells (Einhorn and White, 1986; Wachtel and White, 1986). Given these results, it is possible that postsynaptic DA receptor desensitization (D-1 and possibly D-2) could provide a mechanism by which DA neuronal activity is enhanced. This possibility would need to be tested and the relevant population of postsynaptic DA receptors and the NAC cells of origin of such a feedback pathway identified.

Robinson and Becker (1986) suggest other possible bases for sensitized dopaminergic function such as an increase in the size of the intracellular pool of readily releasable DA (with a possible concomitant decline in the size of the storage pool) or presynaptic facilitation of DA release by afferents that would hyperpolarize DA terminals (but see Groves, Fenster, Tepper, Nakamura and Young, 1981, who propose that DA autoreceptor stimulation-induced hyperpolarization of neuron terminals is associated with inhibition of DA release). Recent studies have

demonstrated that pharmacological (Romo, Cheramy, Godeheu and Glowinski, 1986a,b,; Cheramy, Romo, Godeheu, Baruch and Glowinski, 1986) or electrical (Kilpatrick and Phillipson, 1986; Kilpatrick, Jones, Johnson, Cornwall and Phillipson, 1986; Kilpatrick, Jones, Pycock, Riches and Phillipson, 1986) stimulation of some thalamic nuclei induces the release of DA from striatal DA neuron terminals. These effects appear to involve a thalamo-cortico-striatal neuronal loop and to be due to the presynaptic facilitation of DA release by cortico-striatal glutaminergic neurons. It would be interesting to determine whether the potentially facilitatory effects of this neuronal loop are affected by repeated preexposure to amphetamine or morphine and, if so, how. Indeed, the possibility that presynaptic facilitation of DA release by cortico-striatal glutaminergic neurons may play a role in sensitized dopaminergic function is quite intriguing especially when one considers the importance of thalamic and cortical pathways in the relay of sensory information. The existence of a thalamo-cortico-striatal neuronal loop would provide at least the rudiments of a way in which sensory stimuli, and possibly conditioning, could contribute to sensitization.

In conclusion, it remains to be seen whether and, if so, how DA autoreceptor-mediated events influence the development of sensitized DA function. At mesolimbic DA cell terminals, DA autoreceptor subsensitivity or other, as of yet unknown, DA autoreceptor-mediated events could participate in the enhancement of DA released by amphetamine and morphine. At the cell body

level, it is difficult to see how somatodendritic autoreceptor subsensitivity could be coupled to the decrease in cell body release of DA reported to occur in amphetamine and morphine sensitized animals (see above). Interestingly, however, both of these changes (decreased DA release and autoreceptor subsensitivity) occurring, perhaps independently, at the cell body would render the neuron more vulnerable to excitatory pharmacological and environmental stimuli.

Alternatively, or in addition to events mediated by DA autoreceptors, changes at other sites may contribute to sensitized DA function. These include increases in the size of the intracellular pool of readily releasible DA, desensitization of postsynaptic DA receptors and changes in the effectiveness, or possibly, the recruitment of presynaptic facilitation of DA release by thalamo-cortico-striatal neuronal loops.

Conditioning and Behavioral Sensitization

As seen in the preceding sections, experiments investigating behavioral sensitization usually involve the repeated administration of a drug in the presence of a common set of environmental stimuli. Such a procedure also provides a situation in which animals can learn the contingent relation between such stimuli and the drug-produced stimulus, a situation in which conditioning can occur. In the terminology of Pavlovian conditioning, the initially "neutral" environmental stimuli that are paired repeatedly with a drug act as the conditioned stimulus (CS), and the drug-produced stimuli act as the unconditioned stimulus (US; see Mackintosh, 1974, ch 2). As an association is

formed between CS and US, the CS comes to be able to elicit, in the absence of the US, effects similar to those originally produced by the US.

It is well established that many drug effects can become conditioned or elicited by CS's previously paired with a drug (for reviews, see Pickens and Dougherty, 1971; Lynch, Stein and Fertziger, 1976; Eikelboom and Stewart, 1982; Stewart and Eikelboom, 1987). In addition, many studies have now demonstrated that the locomotor activating effects of amphetamine (e.g., Tilson and Rech, 1973; Schiff, 1982; Beninger and Hahn, 1983; Carey, 1986) and morphine (e.g., Kamat, Dutta and Pradhan, 1974; Mucha, Volkovskis and Kalant, 1981; Vezina and Stewart, 1984,1987) can come to be elicited by cues (the CS; usually the stimuli provided by the activity measuring device or environment) previously paired with the repeated administration of these drugs. Given these results and the fact that the same procedures are often used in sensitization experiments as are used in conditioning experiments, it has, not surprisingly, been proposed that conditioning may account for the development of behavioral sensitization (Tilson and Rech, 1973; Hinson and Poulos, 1981; Mansfield, Wenger, Benedict, Halter and Woods, 1981; Moller, Nowak and Kuschinsky, 1987). According to this view, effects produced by the CS and the drug US combine (are "additive", Tilson and Rech, 1973; or, act "in a synergistic way", Moller et al., 1987) so that an apparently greater effect is produced when the two are presented together, as in a test for sensitization. Behavioral sensitization would be expected, therefore, to be observed only in the presence of those cues previously paired

with the drug and would not be expected to be observed in their absence. Several reports have indicated support for this view. Thus, environment-specific behavioral sensitization (i.e., sensitization that is observed only when animals are tested in the environment previously associated with the drug) has been reported with amphetamine (Tilson and Rech, 1973), morphine (Mansfield et al., 1981; Vezina and Stewart, 1984), cocaine (Hinson and Poulos, 1981; Post, Lockfeld, Squillace and Contel, 1981) and apomorphine (Moller et al., 1987; see also Nowak, Moller and Kuschinsky, 1987). Such environment-specificity in the demonstration of sensitization to the locomotor activating effects of amphetamine and morphine has also been demonstrated in cross-sensitization studies, either in animals trained with amphetamine and tested with morphine (Stewart and Vezina, 1987) or vice versa (Vezina et al., in preparation; see also Stewart, 1981).

Not unexpectedly, one reaction to the view that conditioning may account for behavioral sensitization has been to attempt to demonstrate that conditioning factors are <u>not</u> involved in the development of behavioral sensitization. Some of the arguments levelled in support of this opposing view have been rather weak and/or unfounded, however. For example, Robinson and Becker (1986) argued that conditioning could not account for sensitization since the latter could be produced with a single drug injection while conditioning requires the repeated pairing of the CS and the drug US. Furthermore, according to these authors, sensitization develops with the passage of time, whereas

conditioning decrements with the passage of time. It should be apparent, however, that the single trial conditioning of both the aversive (e.g. conditioned taste aversion; for a review, see Goudie, 1987) and the appetitive (e.g., conditioned place preference; see Mucha, Van der Kooy, O'Shaughnessy and Bucenicks, 1982; Bozarth and Wise, 1983; Bardo and Neisewander, 1986) effects of psychoactive drugs is a well established phenomenon. It is equally well established that conditioning is well retained over time unless extinction training intervenes (i.e., pairing of the CS with the absence of the US; Kimble, 1961, p. 281; Mackintosh, 1974, ch. 8; e.g., see Hinson and Poulos, 1981).

One strategy, however, which has been more successful in providing support for the view that conditioning does not account for or explain behavioral sensitization has been to attempt to demonstrate that sensitization can be obtained in situations where the opportunity for conditioning to occur is slight or nonexistent. There is some evidence that perhaps it can. For example, Segal and his colleagues conducted experiments with amphetamine using procedures designed to minimize the contribution of conditioning factors (Segal and Mandell, 1974; Segal, 1975b; Browne and Segal, 1977). This was done by housing animals continuously in the testing chambers throughout the experiment proper as well as during a pre-experiment adaptation period of at least two weeks (during which animals were injected daily with saline). These procedures would reduce the likelihood of the animal forming an association between the amphetamine US and the testing chamber CS inasmuch as this CS would be paired not only with the drug but also with its absence. Furthermore,

the ability of cues that may have otherwise been predictive of the amphetamine US (e.g., the experimenter-injection ritual complex) to predict the occurrence of the US was diminished during the adaptation period. Not surprisingly, these investigators reported no evidence for conditioned increases in activity (as measured on a saline test) when these procedures were used. Nonetheless, animals did show a progressive increase in amphetamine-induced behaviors when injected repeatedly with amphetamine. Segal and his colleagues argued from these data that behavioral sensitization with amphetamine can occur in the absence of conditioned activity and that conditioning is not necessary for, and, therefore, cannot account for behavioral sensitization.

In several of the studies reviewed above, in which sensitization to the locomotor activating effects of amphetamine and morphine was demonstrated, animals had been pretreated with multiple injections of the drug before ever being exposed to the testing environment. In these experiments, therefore, no testing-environment-CS/drug-US pairings were made prior to the test for sensitization, precluding the formation of an association between these two stimuli. In spite of this, however, significant behavioral sensitization was demonstrated, suggesting, again, that conditioning is not necessary for behavioral sensitization to occur. This conclusion must be tempered, however, by the possibility that, in these experiments, the experimenter-injection ritual cues could have provided a CS predictive of the drug US; note that these cues always preceded the drug US and, in

these experiments, the drug US only. Such cues have been shown to be able to exercise strong control over behavior (e.g., see Dafters and Bach, 1985). Interestingly, a closer look at the data of Segal and his colleagues reveals that, even in their experiments, the injection ritual cues may have formed a predictive association with amphetamine. In order to rule out a role for conditioning, these experimenters tested their animals with saline and compared the activity of amphetamine animals (totalled over three hours) to their predrug baseline activity. A comparison of the activity of these animals to those of a saline control group over the same period on the previous day (these animals were either not given a saline test or their scores were not reported) reveals that, in the first 90 minutes after the saline injection, they were more active than the control group animals. Thus, conditioning, although perhaps relatively weak, may have occurred (compare saline control, day 36, figure 2, to amphetamine groups, day 37, figure 3, Segal, 1975b). These findings would indicate, therefore, that a role for conditioning in the development of behavioral sensitization could not be precluded.

In another experiment, said to demonstrate behavioral sensitization in the absence of conditioning, animals in one group were given injections of amphetamine, once a week on three occasions, immediately after being exposed to rotation testing boxes (Robinson, 1984; see also Kalivas et al., 1985). On a subsequent test with amphetamine in the testing boxes, these animals showed levels of amphetamine-induced rotation that were intermediate between the lower levels of saline control animals,

that received amphetamine for the first time, and the higher levels of the conditioning group animals that had previously received amphetamine paired with the testing boxes. Although these results were said to demonstrate that conditioning (i.e., pairing of the test box CS with the drug US) is not necessary for the development of behavioral sensitization, it should be clear that the testing boxes were predictive of amphetamine for the animals that received the drug immediately after being removed from them (especially when one considers that this event occurred only once a week). Thus, contrary to the conclusion of Robinson (1984), conditioning factors may have been involved in the demonstration of behavioral sensitization in this experiment as well. In one amphetamine group, the testing boxes could have provided a contingent CS (i.e., paired with the occurrence of the drug) and in the other amphetamine group these same boxes could have provided a predictive CS (i.e., reliably occurring before the drug). Interestingly, the contingent CS was associated with higher levels of amphetamine-induced rotation than the predictive CS, suggesting the possibility that the two may not influence the demonstration of conditioned effects in the same way.

To the extent that experimental procedures can minimize the contribution of conditioning, but not prevent the development of behavioral sensitization, it can be said that conditioning does not account for sensitization. However, as was seen above, experiments that have adopted this strategy (purposely or otherwise) have produced equivocal results mainly due to the fact that not all potential CS's (e.g., injection-ritual cues) were

(could be?) controlled for. Rather than attempting to prevent conditioning, therefore, perhaps another strategy might be to investigate whether behavioral sensitization is affected by procedures known to affect established conditioned effects. Hinson and Poulos (1981), for example, investigated the effect of extinction (i.e., repeatedly exposing animals to the CS in the absence of the drug US) on already established environment-specific sensitization to cocaine. They reasoned that if conditioning is a central factor in behavioral sensitization, then a procedure known to diminish it (extinction) should diminish behavioral sensitization as well. And, indeed, their findings in large part supported their hypothesis. Nonetheless, although extinction did attenuate sensitization to the behavioral effects of cocaine, it did not completely abolish it. These results suggest that, although conditioning factors may contribute to the manifestation of behavioral sensitization, they do not completely explain its development.

One of the difficulties in trying to interpret much of the data reviewed in this section is that the nature of the relation between conditioning and behavioral sensitization is not understood. Thus, although some of these data suggest that behavioral sensitization can be demonstrated in the absence of conditioned changes, it is very clear that behavioral sensitization can come under strong stimulus control. What needs to be explained, therefore, is how this occurs. For example, while Tilson and Rech (1973) suggested that behavioral effects elicited by the CS and the drug US are additive and that their sum produces the sensitized response to the drug, more recent

studies have demonstrated that environment-specific sensitization is more than the sum of conditioned and initial unconditioned effects (Post et al., 1981; Vezina and Stewart, 1984). Further, to label the interaction between conditioning and the unconditioned drug effect as synergistic (Moller et al., 1987) or even multiplicative (Stewart and Eikelboom, 1987) only restates the need to determine the nature of the interaction between the two.

The Present Experiments.

The experiments reported in this thesis form part of ongoing studies aimed at elucidating the development and expression of sensitization to the locomotor activating effects of amphetamine and morphine. Three main areas were investigated in the present experiments: the role played by DA autoreceptors in behavioral sensitization to amphetamine and morphine, the role of conditioning in the manifestation of behavioral sensitization to these two drugs, and the neuroanatomical site critical for the development of conditioning and behavioral sensitization to amphetamine.

The dependent variable in all of the experiments reported was locomotor activity, measurement of which often includes both horizontal locomotion and vertical activity counts (rearing) pooled together to reflect "total" activity. In preliminary studies conducted in this laboratory, however, it was found that horizontal locomotion and rearing were not always affected in the same way by experimental treatments intended to either increase or decrease activity. These two behaviors are therefore reported

separately below.

In Experiments 1 (with amphetamine) and 2 (with morphine), the effect of pretreatment with D-1 and D-2 DA receptor antagonists on the development of behavioral sensitization was investigated.

In experiments 3 (amphetamine) and 4 (morphine), the relation between conditioning and sensitization was explored. These experiments investigated what effect extinction, a procedure known to diminish observable conditioned effects, would have on established environment-specific sensitization.

In the final two experiments, an attempt was made to determine whether conditioning and/or sensitization of the locomotor activating effects of amphetamine would develop in animals exposed to intracranial injections into either the VTA (Experiment 5) or the NAC (Experiment 6).

EXPERIMENT 1

The stimulation of DA autoreceptors by the DA released repeatedly by either pharmacological agents or environmental stimuli has been suggested by some to lead to their down-regulation. Subsensitivity of DA autoreceptors has, in turn, been proposed to play a role in the development of behavioral sensitization to amphetamine. According to this view, subsensitive DA autoreceptors could be directly responsible for sensitization, preventing released DA from having its usual autoinhibitory effects on DA neuron function. Alternatively, changes in DA autoreceptor sensitivity might indirectly contribute to sensitization by being one of several cellular changes brought about by the repeated administration of amphetamine and necessary for sensitization to occur (see Introduction).

Experiment 1 was designed to test the notion that changes in the sensitivity of DA autoreceptors are involved, either directly or indirectly, in the development of sensitization to the locomotor activating effects of amphetamine. This was done by attempting to prevent down-regulation of DA autoreceptors, during repeated enhanced amphetamine-induced DA release, by pretreating animals with selective D-2 DA receptor antagonists. DA autoreceptors are considered to be of the D-2 type. It was reasoned that if DA were prevented from repeatedly stimulating DA autoreceptors, these would not become subsensitive and, if this change were indeed involved in the development of behavioral

sensitization, then sensitization should not occur.

Three selective D-2 DA receptor antagonists were tested: sulpiride (O'Connor and Brown, 1982), pimozide (Seeman, Watanabe, Grigoriadis, Tedesco, George, Svensson, Nilsson and Neumeyer, 1985) and Ro22-2586 (Molloy et al., 1986). For purposes of comparison and to investigate the possibility that changes in D-1 DA receptors might also be involved in the development of behavioral sensitization (see Introduction), the selective D-1 DA receptor antagonist SCH-23390 (Hyttel, 1983) was tested as well (for a review of the binding characteristics of these and other ligands for DA receptors, see Seeman and Grigoriadis, 1987).

Methods

Subjects

Ninety-three male Wistar rats (Charles River Canada Inc.), weighing 250-300 g on arrival, were housed individually in stainless steel cages (18 X 24 X 18 cm) located in a 12 h light/12 hour dark reverse cycle room. Food and water were continuously available in this room. Each animal was handled and weighed daily during the first week after arrival. Experimental testing began the following week. Animals were always tested during their dark cycle.

Apparatus

A bank of 12 activity boxes was used to measure locomotor activity. Each box (20 X 41 X 25 cm) was constructed of white pressed wood (rear and two side walls), a wire screen ceiling, a Plexiglas front hinged door, and a tubular stainless steel floor. Two photocells, positioned 3.5 cm above the floor and spaced

evenly along the longitudinal axis of each box, estimated horizontal locomotion. Two other photocells, positioned on the side walls 16.5 cm above the floor and 5 cm from the front and back walls, estimated rearing. The activity boxes were kept in a room lighted only by the dim red-light afforded by the photocell lights and with white noise (75 dB) continuously present. They were connected via an electrical interface to an Apple IIe computer situated in an adjacent room. Photocells were sampled for beam interruptions at approximately 6 Hz.

Drugs

All drugs were administered systemically at doses based on previous reports. Sulpiride (25 mg/kg, i.p.; Sigma Chemical Company, St. Louis, MO) was dissolved in 5% acetic acid (1/8 of the vehicle volume) and diluted with distilled water. Pimozide (0.5 mg/kg, i.p.; Janssen Pharmaceuticals, Beerse, Belgium) was dissolved in a 3% solution of heated tartaric acid. Ro22-2586 (0.2 mg/kg, s.c.; Hoffmann-La Roche Ltd, Etobicoke, Ont.) and SCH-23390 (0.04 and 0.2 mg/kg, s.c.; Schering Corporation, Bloomfield, NJ) were dissolved in distilled water. d-amphetamine sulphate (0.5 and 1.0 mg/kg, i.p.; Smith, Kline and French, I.A.C., Montreal, Que.) was dissolved in 0.9% NaCl solution. The control saline injections (i.p.) consisted of the 0.9% NaCl solution. All injections were made in a 1.0 ml/kg volume.

Design and Procedure

The experiment involved two phases: training, and testing for sensitization.

The training phase consisted of five 3-day blocks. In each

block, animals were tested on the first day and left undisturbed in their home cages on the other two. On test days, animals were first weighed and injected with one of the DA receptor antagonists or saline and returned to their home cages. After the appropriate interval, animals were removed from their home cages and carried, in groups of 12, to the testing room where they were injected with amphetamine (1.0 mg/kg, i.p.) or saline and placed immediately into the activity boxes. After a period of two hours, during which horizontal locomotion and rearing were measured and recorded at 10 minute intervals, animals were returned to their home cages. Animals were therefore randomly assigned to one of 12 groups depending on what pretreatment injection they received and whether they received amphetamine or saline in the activity boxes. These groups were:

1) SAL-AMP	(n=9)	saline-amphetamine
2) SUL-AMP	(n=10)	sulpiride-amphetamine
3) PIM-AMP	(n=10)	pimozide-amphetamine
4) Ro-AMP	(n=9)	Ro22-2586-amphetamine
5) SCH(.04)-AMP	(n=10)	SCH-23390 (.04 mg/kg)-amphetamine
6) SCH(.2)-AMP	(n=9)	SCH-23390 (0.2 mg/kg)-amphetamine
7) SAL-SAL	(n=12)	saline-saline
8) SUL-SAL	(n=5)	sulpiride-saline
9) PIM-SAL	(n=5)	pimozide-saline
10) Ro-SAL	(n=4)	Ro22-2586-saline
11) SCH(.04)-SAL	(n=5)	SCH-23390 (.04 mg/kg)-saline
12) SCH(.2)-SAL	(n=5)	SCH-23390 (0.2 mg/kg)-saline

Pimozide was injected four hours, sulpiride one hour and the remaining pretreatment injections, including saline, one half

hour prior to the activity box injection.

Animals were tested for sensitization on the day following the training phase (i.e., day 16). On this day, all animals were first administered their respective drug vehicle injections (no antagonists were administered) and following the appropriate interval, were carried to the testing room, administered 0.5 mg/kg amphetamine (i.p.) and tested in the activity boxes for two hours.

The data were analyzed by between-within analyses of variance (ANOVA). Analysis of simple main effects and post hoc Scheffé comparisons were made according to Kirk (1968).

Results

Training.

Figure 1 shows the mean horizontal activity counts obtained in Hours 1 and 2 of the five training days for each of the 12 groups. To better illustrate the data, results from the groups pretreated with the D-1 DA receptor antagonist are shown in Figure 1A, and those from the groups pretreated with the D-2 DA receptor antagonists are shown in Figure 1B. To ease the making of comparisons, results from the two groups not pretreated with antagonists (SAL-AMP and SAL-SAL) are shown in both A and B.

As can be seen, Group SAL-AMP showed levels of activity that were consistently higher than those of Group SAL-SAL and these increased moderately over days in the first hour. The D-1 DA receptor antagonist SCH-23390 completely blocked this effect of amphetamine in a dose-dependent manner for the duration of the

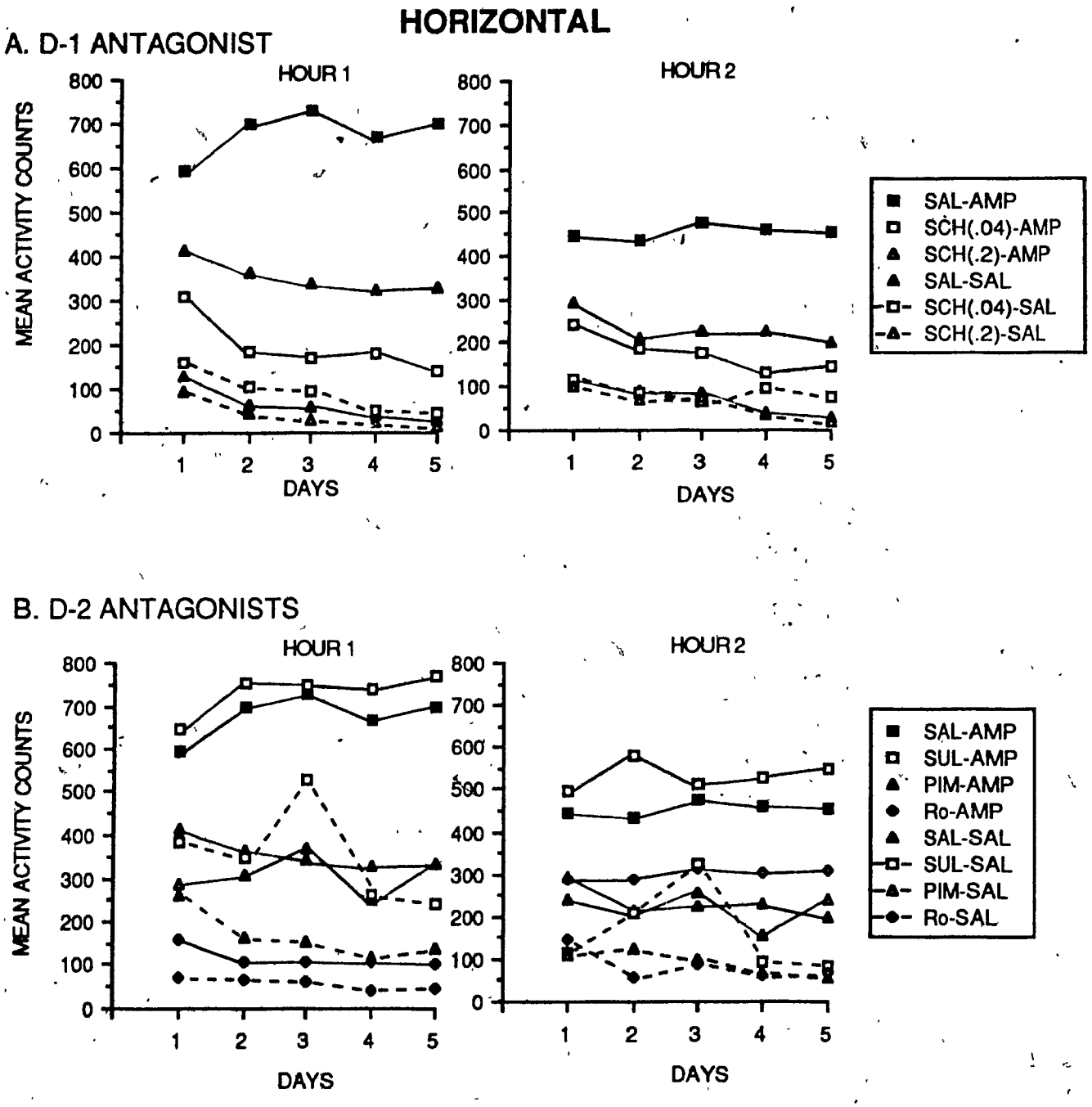


Figure 1. TRAINING. Mean horizontal activity counts obtained in Hours 1 and 2 of the five training days for each of the 12 groups in Experiment 1. A. Groups pretreated with the D-1 DA receptor antagonist. B. Groups pretreated with the D-2 DA receptor antagonists. Counts for the two groups not pretreated with antagonists are illustrated in both A and B.

training phase. The D-2 DA receptor antagonists, pimozide and RO22-2586 also completely blocked the locomotor activating effect of amphetamine for the duration of the training phase. Unlike these antagonists, however, sulpiride actually slightly enhanced amphetamine's effect on locomotor activity. Animals pretreated with the antagonists and administered saline in the activity boxes (antagonist alone controls) generally showed activity levels lower than those of Group SAL-SAL. The one exception was Group SUL-SAL whose activity levels were relatively more variable but generally similar to those of Group SAL-SAL in Hour 1 and lower in Hour 2.

Four ANOVA's were conducted on the data illustrated in Figure 1, one separate ANOVA for each hour in each antagonist category (see Table 1 for the source tables). The use of the data for groups SAL-AMP and SAL-SAL in the analysis of results from both antagonist categories was compensated for by lowering the acceptable level of significance to 0.04 from 0.05, a reduction proportional to the 20% contribution of these two groups to the total N for this experiment.

All four ANOVA's revealed significant effects of groups. Post hoc comparisons revealed that, in both Hour 1 and Hour 2, Group SAL-AMP was significantly more active than Group SAL-SAL and all groups pretreated with SCH-23390 ($p's<0.01$), and that these latter groups were significantly less active than Group SAL-SAL in Hour 1 ($p's<0.01$). Group SCH(.04)-AMP was significantly more active than Group SCH(.2)-AMP in the first hour only ($p<0.01$).

Comparisons made with groups pretreated with the D-2 antagonists showed that, although Group SUL-AMP was slightly more

Figure 1A, Hour 1.

Source	df	SS	MS	F	p
Groups	5	12148551.7	2429710.3	146.9	0.001
Error	44	914490.4	20783.9		
Days	4	172972.9	43243.2	9.2	0.001
G X D	20	283827.2	14191.4	3.0	0.001
Error	176	831202.7	4722.7		

Figure 1A, Hour 2.

Source	df	SS	MS	F	p
Groups	5	4498665.4	899733.1	38.0	0.001
Error	44	1042465.0	23692.4		
Days	4	135853.4	33963.3	9.8	0.001
G X D	20	82998.8	4149.9	1.2	0.258
Error	176	607906.6	3454.0		

Figure 1B, Hour 1.

Source	df	SS	MS	F	p
Groups	7	16767824.2	2395403.5	46.1	0.001
Error	56	2911673.1	51994.2		
Days	4	105766.8	26441.7	2.601	0.037
G X D	28	568596.0	20307.0	1.997	0.003
Error	224	2277558.7	10167.7		

Figure 1B, Hour 2.

Source	df	SS	MS	F	p
Groups	7	6737846.6	962549.5	24.2	0.001
Error	56	2230389.0	39828.4		
Days	4	56213.0	14053.2	2.7	0.031
G X D	28	369374.3	13191.9	2.54	0.001
Error	224	1163216.3	5192.9		

Table 1. Source tables for analyses of variance conducted on the horizontal activity data illustrated in Figure 1.

active than group SAL-AMP, this difference was not significant in either hour. However, these groups together were significantly more active than all groups pretreated with the other D-2 DA receptor antagonists in both hours ($p's<0.01$). Groups Ro-AMP and Ro-SAL were significantly less active than Group SAL-SAL in Hour 1 ($p's<0.01$), but did not differ significantly from this group in Hour 2. Groups PIM-AMP and PIM-SAL did not differ significantly from Group SAL-SAL in either hour. In Hour 1, the simple main effect of days was significant for Group SAL-AMP ($p<0.01$) and approached significance for Group SUL-AMP, reflecting the moderate increase over days in the activity levels of these two groups. This effect was highly significant in both hours for Group SUL-SAL ($p's<0.01$) but, in this case, reflected the relatively variable levels of activity shown by this group over days.

Figure 2 shows the mean rearing counts obtained in Hours 1 and 2 of the five training days for each of the 12 groups. These are illustrated and were statistically analyzed in the same way as the horizontal activity counts above (see Table 2 for the ANOVA source tables).

As can be seen, the relation between groups on this measure closely resembles that seen with horizontal locomotor activity. There were, however, some notable differences. For example, although Group SAL-AMP showed higher rearing levels than Group SAL-SAL, this difference did not achieve statistical significance in Hour 1 and only approached significance in Hour 2 ($p<0.05$). Further, the simple main effect of days was not significant for

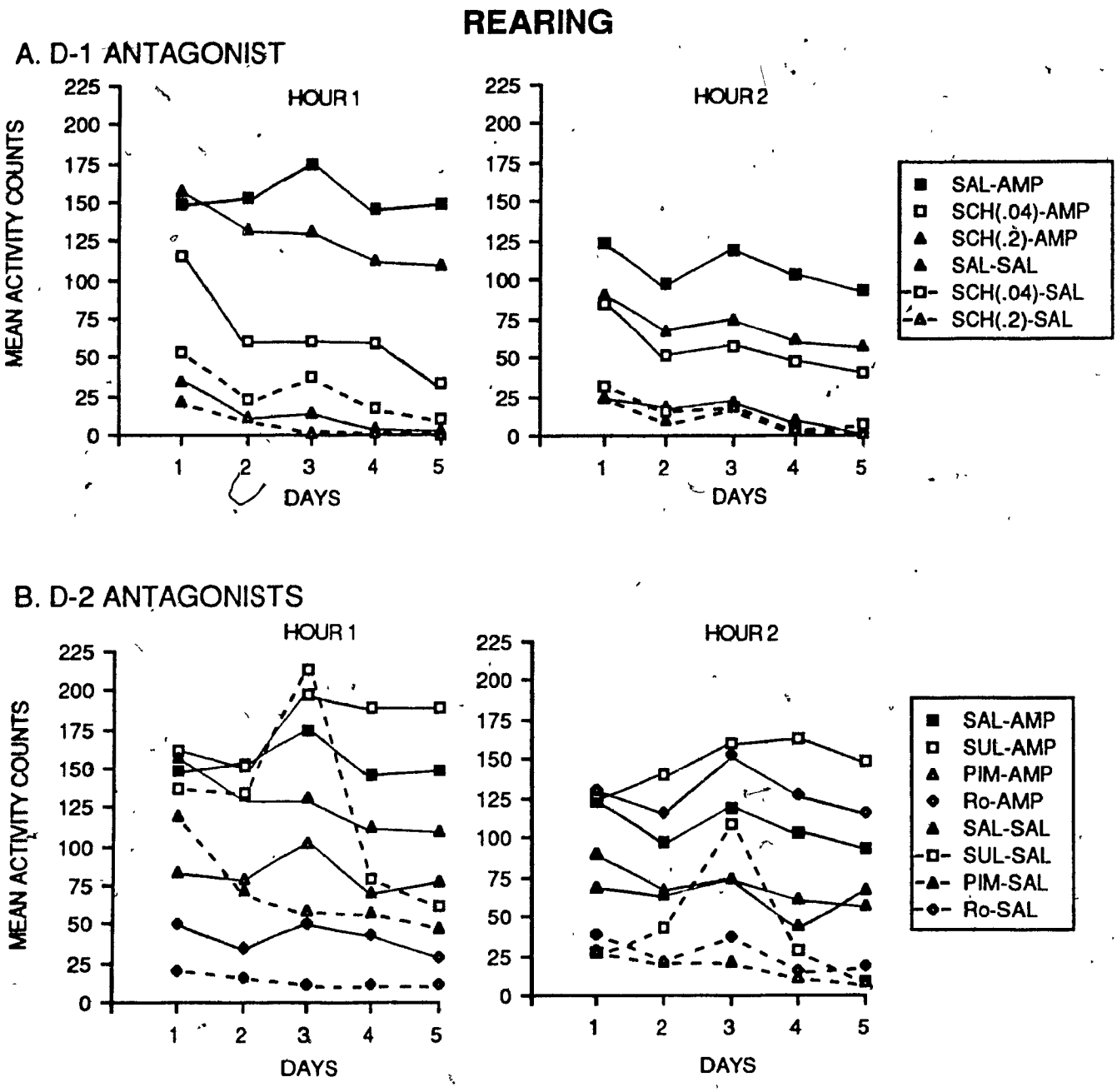


Figure 2. TRAINING. Mean rearing counts obtained in Hours 1 and 2 of the five training days for each of the 12 groups in Experiment 1. A. Groups pretreated with the D-1 DA receptor antagonist. B. Groups pretreated with the D-2 DA receptor antagonists. Counts for the two groups not pretreated with antagonists are illustrated in both A and B.

Figure 2A, Hour 1.

Source	df	SS	MS	F	p
Groups	5	793867.4	158773.5	29.9	0.001
Error	44	233363.4	5303.7		
Days	4	46622.4	11655.6	21.7	0.001
G X D	20	25925.3	1296.3	2.4	0.001
Error	176	94621.1	537.6		

Figure 2A, Hour 2.

Source	df	SS	MS	F	p
Groups	5	290448.7	58089.7	20.7	0.001
Error	44	123427.0	2805.2		
Days	4	27532.8	6883.2	13.7	0.001
G X D	20	3601.6	180.1	0.4	0.995
Error	176	88462.8	502.6		

Figure 2B, Hour 1.

Source	df	SS	MS	F	p
Groups	7	813316.1	116188.0	13.1	0.001
Error	56	494827.8	8836.2		
Days	4	37571.3	9392.8	7.4	0.001
G X D	28	97544.4	3483.7	2.7	0.001
Error	224	284530	1270.2		

Figure 2B, Hour 2.

Source	df	SS	MS	F	p
Groups	7	565232.7	80747.5	8.4	0.001
Error	56	539256.4	9629.6		
Days	4	25272.0	6318.0	7.3	0.001
G X D	28	43665.9	1559.5	1.8	0.010
Error	224	193637.7	864.5		

Table 2. Source tables for analyses of variance conducted on the rearing data illustrated in Figure 2.

Group SAL-AMP in either hour. Groups SAL-AMP and SUL-AMP together exhibited significantly more rearing in Hour 1 than all groups pretreated with the other D-2 antagonists ($p's<0.01$). In Hour 2, however, Groups SAL-AMP, SUL-AMP and Ro-AMP did not differ significantly from each other, but together displayed significantly higher levels of rearing than Group SAL-SAL ($p<0.04$). The simple main effect of days was significant for Group SUL-AMP in both hours ($p's<0.04$) and, as was found with horizontal locomotion, continued to be highly significant for Group SUL-SAL in both hours ($p's<0.01$).

Test for Sensitization.

The mean horizontal activity counts for all groups obtained on this test, in which all animals were pretreated with their respective vehicles and administered 0.5 mg/kg (i.p.) amphetamine in the activity boxes, are shown in Figures 3 and 4. As was done in the training phase, these data are illustrated separately for the two antagonist categories. Similarly, the data illustrated in each figure were statistically analyzed with separate ANOVA's. The acceptable level of significance remained, therefore, at 0.04. Results shown in each figure were further subdivided according to those groups administered amphetamine (A. antagonist + amphetamine) and those administered saline (B. antagonist alone controls) in the activity boxes during training. This was done for illustration only; the data for these groups were combined for statistical analysis.

As can be seen in Figure 3, Group SAL-AMP was more active than all other groups at the beginning of the test session, demonstrating sensitization to the locomotor activating effects

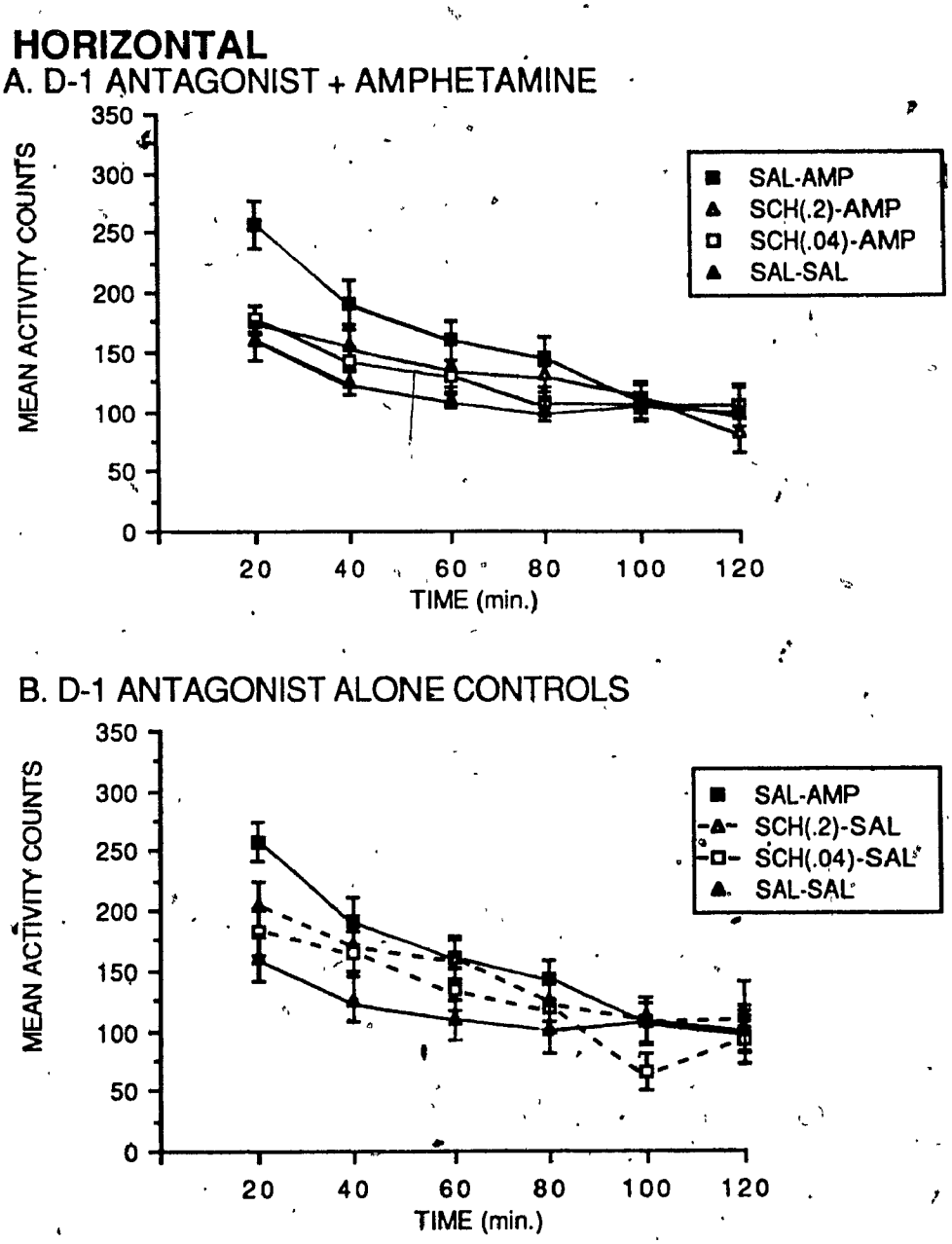


Figure 3. TEST FOR SENSITIZATION. Mean horizontal activity counts (±1 S.E.M.) obtained on the test for sensitization for Groups SAL-AMP, SAL-SAL and those pretreated with the D-1 DA receptor antagonist during the training phase. A, Groups administered amphetamine in the activity boxes during training. B. Groups administered saline in the activity boxes during training. Counts for Groups SAL-AMP and SAL-SAL are illustrated in both A and B.

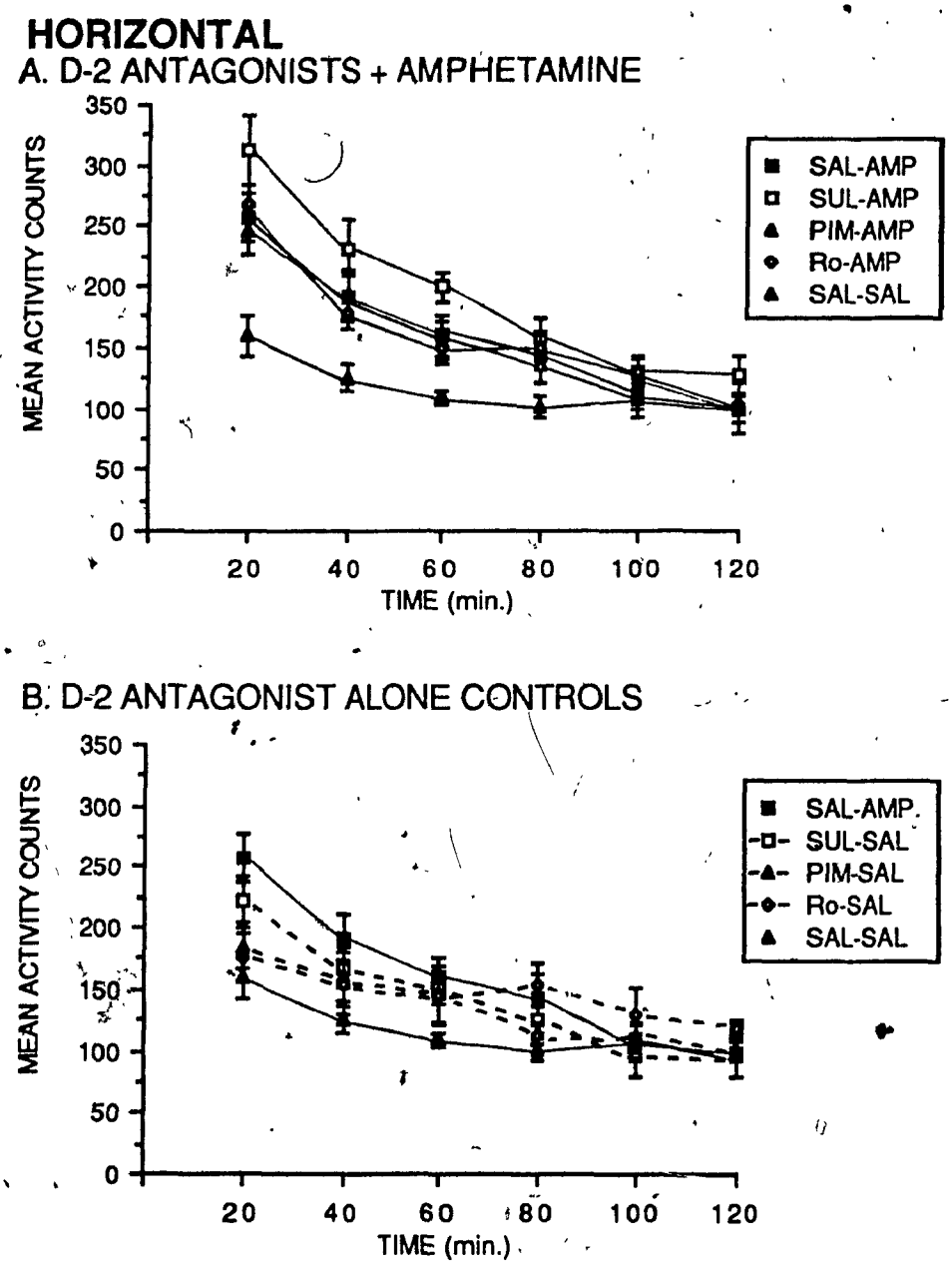


Figure 4. TEST FOR SENSITIZATION. Mean horizontal activity counts (±1 S.E.M.) obtained on the test for sensitization for Groups SAL-AMP, SAL-SAL and those pretreated with the D-2 DA receptor antagonists during the training phase. A. Groups administered amphetamine in the activity boxes during training. B. Groups administered saline in the activity boxes during training. Counts for Groups SAL-AMP and SAL-SAL are illustrated in both A and B.

of amphetamine. This difference between groups diminished as the session progressed. Interestingly, pretreatment with the D-1 DA receptor antagonist during training appeared to block the development of the sensitized levels of activity shown by Group SAL-AMP; both Groups SCH(.2)-AMP and SCH(.04)-AMP showed levels of activity similar to those of Group SAL-SAL. The ANOVA conducted on these data revealed that the groups effect was not significant. The groups x days interaction, however, was significant [$F(25,220)=2.86$, $p<0.001$]. Post hoc comparisons revealed that Group SAL-AMP was significantly more active than Group SAL-SAL during the initial 40 minutes ($p<0.01$) and significantly more active than Groups SCH(.2)-AMP and SCH(.04)-AMP during the initial 20 minutes of the test session. Although Group SAL-AMP was more active than Groups SCH(.2)-SAL and SCH(.04)-SAL in the first 20 minutes of the session, this difference did not achieve statistical significance.

Figure 4 shows the mean horizontal activity counts obtained on the test for those groups pretreated with the D-2 DA receptor antagonists during training. It is clear that none of these antagonists administered during training interfered with the development of sensitization to the locomotor activating effects of amphetamine. Pretreatment with sulpiride during training actually appeared to enhance the development of sensitization as expressed in horizontal activity on this test. The ANOVA conducted on these data revealed a significant effect of groups [$F(7,56)=3.41$, $p<0.004$] and a significant groups x days interaction [$F(35,280)=3.82$, $p<0.001$]. Post hoc comparisons

revealed that Groups SAL-AMP, SUL-AMP, PIM-AMP and Ro-AMP did not differ significantly from each other but that, together, they differed significantly from Group SAL-SAL ($p<.04$). The antagonist alone control groups showed activity levels intermediate to those of Group SAL-AMP and SAL-SAL, but none of these groups differed significantly from each other.

Figures 5 and 6 show the corresponding mean rearing counts obtained on this test for all 12 groups. As can be seen in Figure 5, repeated administration of amphetamine during training did not result in enhanced amphetamine-induced rearing on this test relative to Group SAL-SAL. And, although it might appear from Figure 5B that slightly enhanced levels of rearing were shown by groups pretreated with the D-1 DA receptor antagonist alone during training, these differences were not significant. The ANOVA conducted on these data revealed only a significant effect of time.

In Figure 6, it can be seen that two of the groups pretreated with D-2 DA receptor antagonists during training, one in combination with amphetamine (Group SUL-AMP) and one with the antagonist alone (Group PIM-SAL) showed higher levels of rearing than the other groups on the test for sensitization. The ANOVA conducted on these data revealed a significant groups x days interaction [$F(35,280)=2.18$, $p<0.001$] and post hoc comparisons revealed that Group SUL-AMP and PIM-SAL showed significantly more rearing than Group SAL-SAL in the first 20 minutes of the test session ($p<0.02$ and $p<0.01$, respectively). The remaining groups did not differ significantly from each other.

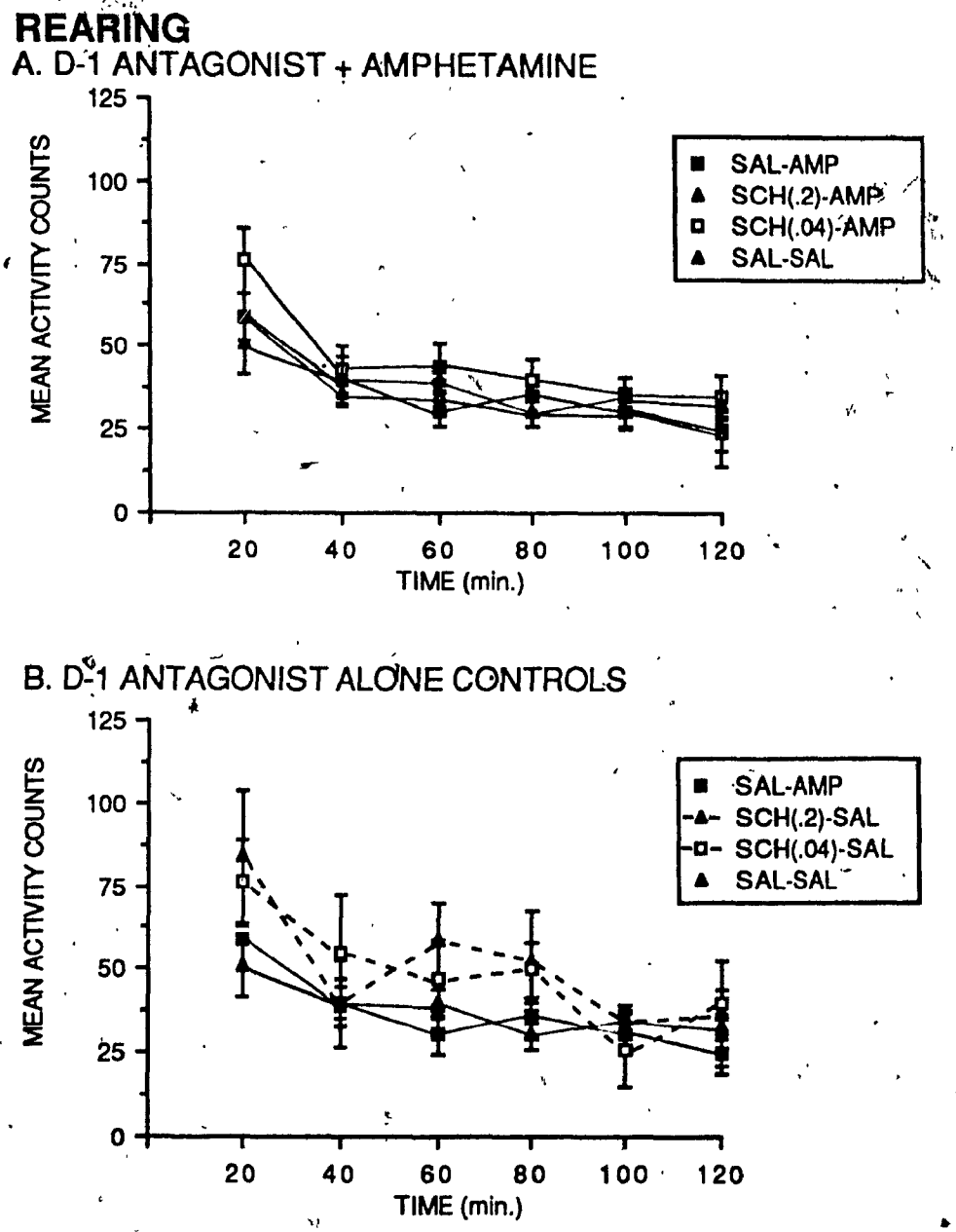


Figure 5. TEST FOR SENSITIZATION. Mean rearing counts (±1 S.E.M.) obtained on the test for sensitization for Groups SAL-AMP, SAL-SAL and those pretreated with the D-1 DA receptor antagonist during the training phase. A. Groups administered amphetamine in the activity boxes during training. B. Groups administered saline in the activity boxes during training. Counts for Groups SAL-AMP and SAL-SAL are illustrated in both A and B.

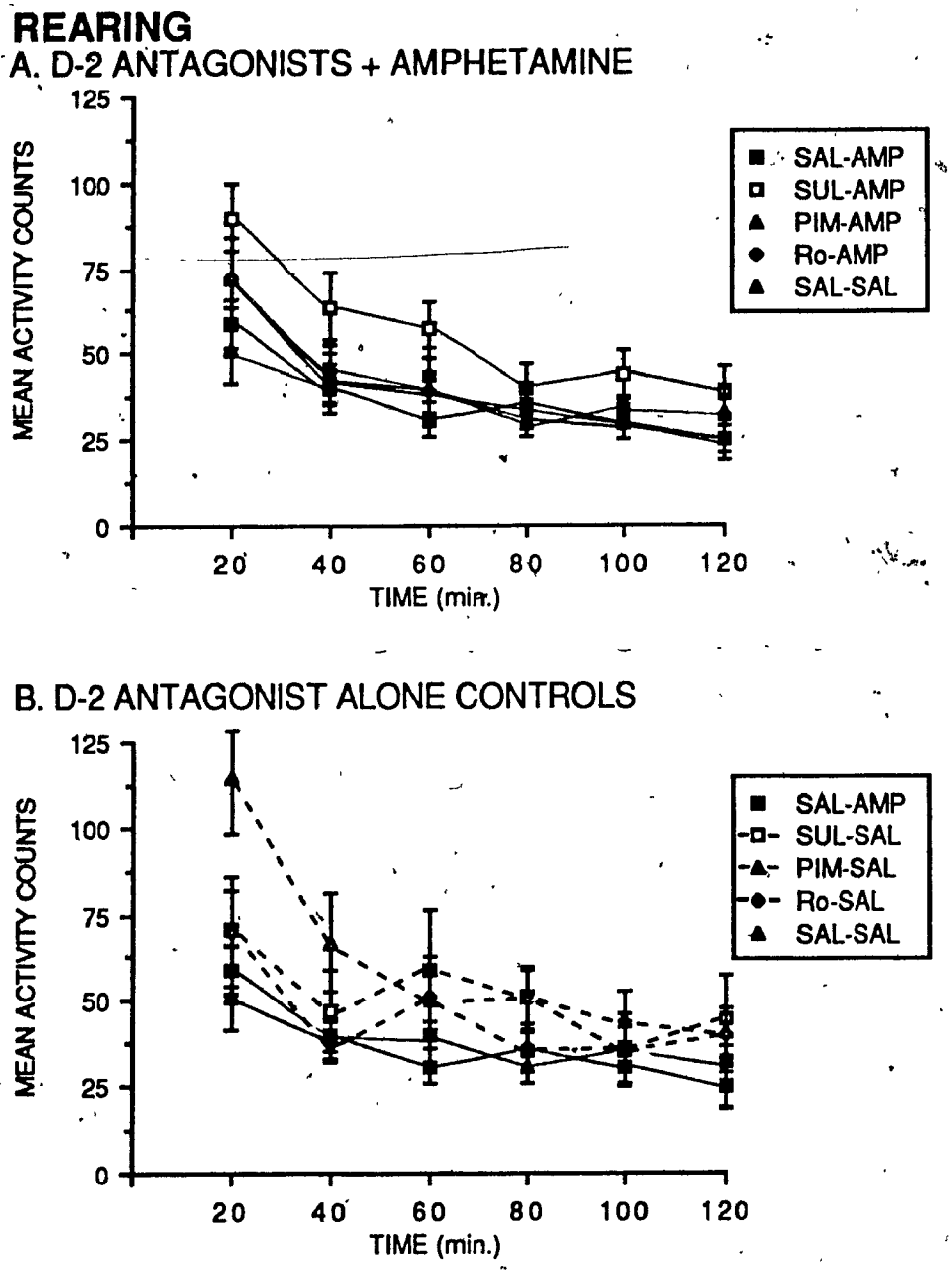


Figure 6. TEST FOR SENSITIZATION. Mean rearing counts (±1 S.E.M.) obtained on the test for sensitization for Groups SAL-AMP, SAL-SAL and those pretreated with the D-2 DA receptor antagonists during the training phase. A. Groups administered amphetamine in the activity boxes during training. B. Groups administered saline in the activity boxes during training. Counts for Groups SAL-AMP and SAL-SAL are illustrated in both A and B.

Discussion

The results of this experiment clearly do not support the view that subsensitive D-2 DA autoreceptors are involved, either directly or indirectly, in the development of sensitization to the locomotor activating effects of amphetamine. In spite of the fact that two of the three D-2 DA receptor antagonists blocked the acute effects of amphetamine during training, none of them attenuated the development of sensitization as expressed by the enhanced levels of horizontal locomotor activity in Group SAL-AMP on the test day (see Figure 4A).

Interestingly, pretreatment during training with the D-1 DA receptor antagonist, SCH-23390, did significantly attenuate the development of sensitization of amphetamine-induced horizontal locomotion (see Figure 3A), a finding suggesting a role for D-1 DA receptors rather than D-2 DA autoreceptors. This finding was unexpected when originally obtained, but is potentially of great interest. There was little in the literature that would have suggested a role for D-1 DA receptor activation in the development of behavioral sensitization to amphetamine. There are now, however, a few possible leads. For example, it was recently reported that; in animals with unilateral 6-OHDA lesions of the medial forebrain bundle, prior exposure to either a D-1 or D-2 agonist enhanced the contraversive circling induced by a subsequent injection of the D-1 agonist (Morelli, Fenu and Di Chiara, 1987; Morelli and Di Chiara, submitted; see also Parenti, Flauto, Parati, Vescovi and Groppetti, 1986). Although, as observed by these investigators, such findings may be difficult to reconcile with reports of the sensitization of the DA

releasing effects of amphetamine (see Introduction), they nonetheless do suggest a potential, although still unknown, role for D-1 DA receptors in the development of behavioral sensitization. As suggested in the Introduction, one possibility may be that increased amphetamine-induced levels of DA leads to desensitization of postsynaptic D-1 DA receptors which results, via a feedback pathway, in a decrease in the inhibition of mesencephalic DA cells. Pretreatment with SCH-23390 during training would prevent the desensitization of these receptors and thus prevent the development of sensitization. D-1 DA receptors in the substantia nigra may also be involved. These D-1 DA receptors have been shown to be located presynaptically on striato-nigral GABAergic afferent terminals, and it has been suggested that DA released from substantia nigra DA cell dendrites could modulate, via these receptors, the inhibition of nigral cells by GABA (Matthews and German, 1986; Porceddu, Giorgi, Ongini, Mele and Biggio, 1986). It is possible that such D-1 DA receptor mediated modulation of GABAergic inhibition plays a role in the development of behavioral sensitization to amphetamine. Finally, SCH-23390 has been shown to interact potently with brain serotonin receptors (Bischoff, Heinrich, Sonntag and Krauss, 1986). Although there is little evidence implicating serotonin in behavioral sensitization to amphetamine (see Robinson and Becker, 1986), there is one report indicating enhanced ventricular release of serotonin in amphetamine sensitized animals (Sparber and Tilson, 1972; cf, Segal, 1977). Thus, although the exact nature of D-1 DA receptor involvement

(or the mode of action of SCH-23390) in the development of behavioral sensitization to amphetamine remains unknown, several potential mechanisms exist and need to be investigated.

Both pimozide and Ro22-2586 effectively blocked the acute amphetamine-induced increases in horizontal locomotion, indicating that these antagonists blocked postsynaptic as well as presynaptic D-2 DA receptors. Sulpiride, on the other hand, did not block amphetamine's acute effect on horizontal locomotion but, rather, enhanced it slightly (although not significantly, see Figure 1B). Moreover, sulpiride significantly enhanced amphetamine's acute effect on rearing during training. These effects of sulpiride may be taken to indicate its preferential activation of presynaptic DA autoreceptors at the dose tested in this experiment. Sulpiride and other DA autoreceptor antagonists have been reported, at appropriate doses, to produce increases in locomotor activity (Costall, Domeney and Naylor, 1983; Svensson, Hjorth, Clark, Carlsson, Wikstrom, Andersson, Sanchez, Johansson, Arvidsson, Hacksell and Nilsson, 1986). The highly variable horizontal activity and rearing levels demonstrated when sulpiride was administered alone during training (Group SUL-SAL), however, are difficult to interpret in this way. It may be that, at this dose of sulpiride, the concurrent induction of release of DA by amphetamine is necessary for any enhancement of locomotor activity to become evident; given alone, there may be sufficient blockade of postsynaptic receptors to antagonize any presynaptic effects.

It is well known that chronic exposure to DA receptor antagonists renders animals supersensitive to subsequent

challenge injections of direct and indirect DA agonists. This effect has been interpreted to be due to the development of DA receptor supersensitivity and/or to other unspecified factors (e.g., Seeger, Thal and Gardner, 1982; Dewey and Fibiger, 1983; Meller, Bohmaker, Goldstein, Schweitzer and Friedhoff, 1985; Vogelsang and Piercey, 1985; Hess, Albers, Le and Creese, 1986; Vaccheri, Dall'Olio, Gandolfi, Roncada and Montanaro, 1987). Although the drug regimens used in such studies (e.g., two injections per day for 40 days) are usually much more severe than the regimen used in the present experiment (one injection every third day repeated five times), the possible effect of repeated exposure to DA receptor antagonists on amphetamine-induced test levels of activity was assessed nonetheless. As was seen in Figures 3B and 4B, on the amphetamine test day, all D-1 and D-2 antagonist alone control groups showed levels of horizontal activity that were initially intermediate to those of Groups SAL-AMP and SAL-SAL, although they did not differ significantly from either. The finding that both D-1 and D-2 antagonist alone control groups responded in the same direction on the test day, would appear to rule out supersensitivity of DA receptors as an explanation of the differential effect of D-1 and D-2 receptor blockade on the development of behavioral sensitization to amphetamine. The D-1 antagonist clearly blocked the development of sensitization (an effect that would be opposite in direction to the expected effect of supersensitivity) whereas the D-2 antagonists did not. Further, the initial test levels of horizontal activity of the D-2 antagonists + amphetamine groups

differed significantly from those of Group SAL-SAL whereas those of the D-2 antagonist alone control groups did not. One of the D-2 antagonist alone control groups did show significantly higher test levels of rearing (Group PIM-SAL, see Figure 6B). However, these are difficult to interpret since Group PIM-AMP did not show elevated rearing levels on the test.

Finally, two aspects of the rearing data obtained in this experiment were surprising. Although amphetamine has been shown to induce significant increases in rearing in this (e.g., see Experiment 3) and other laboratories (e.g., Russell, Giordano and Sanberg, 1987), the difference in rearing levels during training between Groups SAL-AMP and SAL-SAL in the present experiment was unusually small. This may have contributed to the finding that rearing did not show sensitization as assessed on the test day (see Figure 5). Having found a similar lack of sensitization of rearing, Mazurski and Beninger (1987) have suggested that rearing can be dissociated from horizontal activity by the fact that it does not show sensitization following repeated exposure to amphetamine. No explanation was offered, however, as to why rearing might not show the development of sensitization. Further, such a dissociation has not always been found in this laboratory (see Experiments 3 and 5).

EXPERIMENT 2

In this experiment, the involvement, either direct or indirect, of DA autoreceptor subsensitivity in the development of sensitization to the locomotor activating effects of intra-VTA morphine was investigated. This was done, as in Experiment 1, by pretreating animals with either D-1 or D-2 DA receptor antagonists prior to repeated administrations of morphine to the VTA. The four DA receptor antagonists tested in Experiment 1 were tested in the present experiment.

Methods

Subjects

Forty male Wistar rats, weighing 250-300 g on arrival, were used. The supplier and housing conditions were as specified in Experiment 1. Four to 12 days after arrival, during which time they were handled daily, animals were anaesthetized with sodium pentobarbital (55 mg/kg, i.p.; Somnotol, M.T.C. Pharmaceuticals Ltd., Mississauga, Ont.) and stereotaxically implanted with chronic bilateral guide cannulae (22 gauge, Plastic Products Co.) aimed at the VTA and positioned one mm above the final injection site. The VTA coordinates were: A/P -3.6, L $\pm$0.6 and D/V -8.9 trom skull. The incisor bar was placed 5.0 mm above the interaural line (Pellegrino, Pellegrino and Cushman, 1979). To avoid puncturing the cerebral aqueduct, guide cannulae were angled at 16 degrees to the vertical. Following surgery, 28 gauge Plastic Products obturators were inserted in the guide cannulae

(these protruded one mm beyond the guide cannulae tips) and the animals returned to their home cages for a minimum 10 day recovery period. During this period, animals were handled every other day. Home cages were now fitted with a flat bottom aluminum floor covered with beta chip and wire screen covering the front of the cage. These precautionary measures prevented animals from dislodging their implants. Bedding was replaced on days when animals were removed for testing.

Following the experiment, all animals were perfused transcardially with saline and a 10% formalin solution under deep anaesthesia. Brains were stored in a 10% formalin solution for at least 5 days. Histological verification of cannula tip placements was subsequently made on 40 μm thionin stained coronal sections. This examination revealed injector cannula tip placements that were either too caudal or too ventral (cannula tracks exited ventrally) in six animals. The data from these animals were, therefore, dropped from the experiment. In all remaining animals, both injector cannula tips were located in the VTA. Bilateral injector cannula tip placements are illustrated in Appendix, Figure A.

Design and Procedure

Like Experiment 1, this experiment involved a training phase and a test for sensitization.

The training phase consisted of five 2-day blocks. In each block, animals were tested on the first day and left undisturbed in their home cages on the second. On test days, animals were first weighed and injected with one of the DA receptor antagonists or saline and returned to their home cages. After the

appropriate interval, animals were removed from their home cages and carried, in groups of eight, to the testing room where they were administered morphine. Simultaneous bilateral microinjections into the VTA were made in the unrestrained rat. Morphine sulphate (BDH Chemicals, Toronto, Ont.) was dissolved in sterile 0.9% saline and administered at a dose of 5 µg/side. Microinjections were made in a volume of 0.5 µl/side over 45 seconds with slightly modified 28 gauge Plastic Products injector cannulae inserted to a depth of one mm below the guide cannulae tips. The injector cannulae were connected via PE-20 tubing to one µl syringes (Hamilton, Reno, NV). Seventy-five seconds after injection, the injector cannulae were removed, the obturators replaced and animals immediately placed in activity boxes. After a period of two hours, animals were returned to their home cages. Animals were randomly assigned to one of five groups depending on what pretreatment injection they received. These groups were:

1) SAL-MOR	(n=8)	saline-morphine
2) SUL-MOR	(n=6)	sulpiride-morphine
3) PIM-MOR	(n=7)	pimozide-morphine
4) Ro-MOR	(n=7)	Ro22-2586-morphine
5) SCH-MOR	(n=6)	SCH-23390-morphine

Animals were tested for sensitization on the day following the training phase (i.e., day 11). On this day, all animals were first administered their respective drug vehicle injections (no antagonists were administered) and, following the appropriate interval, were carried to the testing room, administered morphine (5 µg/side) into the VTA and tested in the activity boxes for two

hours.

The preparation, dose, route and time of administration of the DA receptor antagonists as well as the activity boxes used to test animals were as specified in Experiment 1. However, only the 0.2 mg/kg dose of SCH-23390 was tested in this experiment.

The data were analyzed by between-within ANOVA's followed by tests of simple main effects and post hoc Scheffé comparisons made according to Kirk (1968).

Results

Training.

Due to unexpected technical difficulties, the data for Day 5 of the training phase were lost, leaving only the data for Days 1-4 for analysis.

Figure 7 shows the mean horizontal activity counts obtained during training for each of the five groups. It can be seen that, in both Hours 1 and 2, Groups SAL-MOR and SUL-MOR showed activity levels that were higher than the remaining groups and that these increased progressively over days. ANOVA's conducted on the Hour 1 and Hour 2 data revealed significant groups effects [$F(4,29)=5.23$ and 3.93, $p<0.003$ and 0.011, respectively] and significant groups x days interactions [$F(12,87)=2.12$ and 2.49, $p<0.023$ and 0.008, respectively]. Post hoc comparisons confirmed that, in both hours, Groups SAL-MOR and SUL-MOR together were significantly more active than the remaining groups (p's<0.03) which did not differ significantly from each other. The simple main effect of days was significant for both Groups SAL-MOR ($p<0.01$) and SUL-MOR ($p<0.01$) in both hours, reflecting the

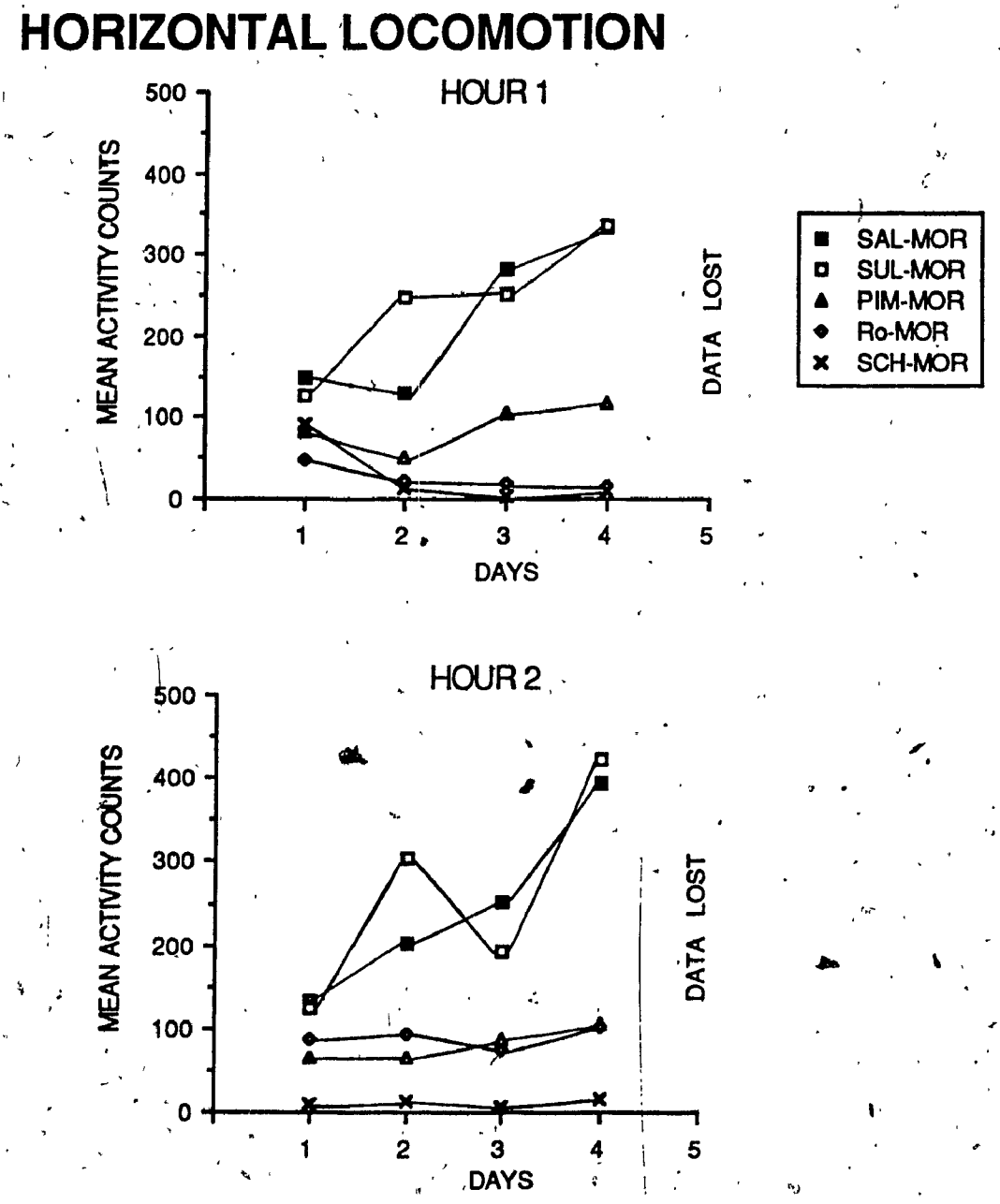


Figure 7. TRAINING. Mean horizontal activity counts obtained in Hours 1 and 2 of the five training days for each of the five groups in Experiment 2.

progressive increase over days in the activity levels of these two groups.

Figure 8 shows the corresponding mean rearing counts obtained on the first four days of training for each of the five groups. It can be seen that all groups, with the exception of Group SAL-MOR, showed relatively low levels of rearing throughout training. The ANOVA's conducted on the Hour 1 and the Hour 2 data revealed a significant effect of groups in Hour 1 [$F(4,29)=3.47$, $p<0.020$]. Group SAL-MOR reared significantly more than all other groups in this hour ($p's<0.05$).

Test for Sensitization

Figure 9 shows the mean horizontal activity (A) and rearing (B) counts obtained on the test day, in which all animals were pretreated with their respective vehicles and given an intra-VTA morphine injection in the activity boxes.

As can be seen in Figure 9A, Groups SUL-MOR, Ro-MOR and SCH-MOR all showed, in Hours 1 and 2, horizontal activity levels similar to the sensitized levels of Group SAL-MOR. In contrast, Group PIM-MOR showed lower levels of activity barely higher than those obtained after pretreatment with pimozide during training. Surprisingly, however, the effect of groups was not statistically significant. A closer examination of the data revealed that the variance in Group SUL-MOR was unusually high, perhaps reflecting problems of absorption and central action of sulpiride (S. Nakajima, personal communication). A second ANOVA was conducted, therefore, without the data for Group SUL-MOR and did reveal a significant effect of groups [$F(3,24)=3.35$, $p<0.036$]. Post hoc

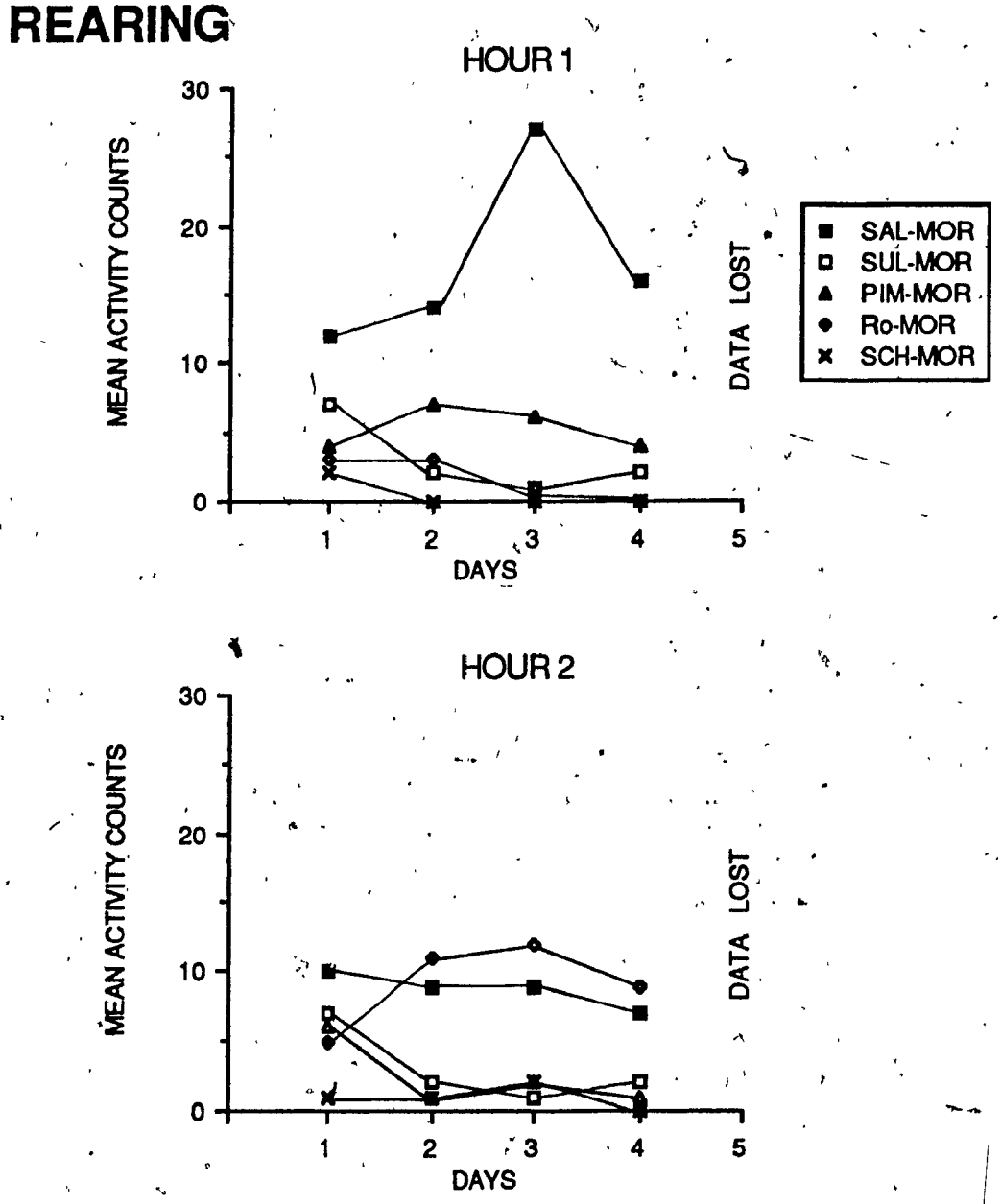


Figure 8. TRAINING. Mean rearing counts obtained in Hours 1 and 2 of the five training days for each of the five groups in Experiment 2.

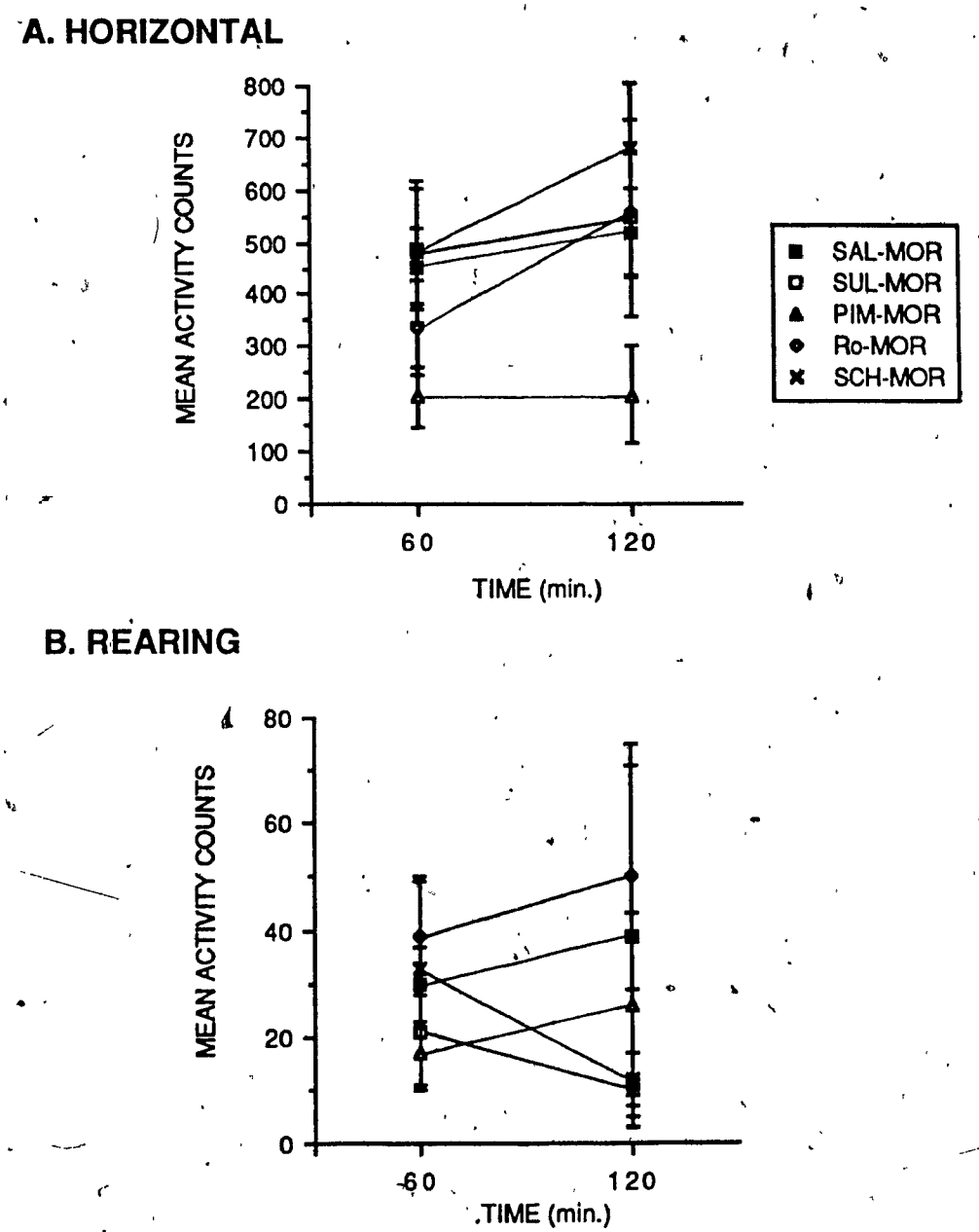


Figure 9. TEST FOR SENSITIZATION. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the test for sensitization for each of the five groups in Experiment 2.

comparisons revealed that Groups SAL-MOR, Ro-MOR and SCH-MOR did not differ significantly from one another but that, together, they were significantly more active than Group PIM-MOR ($p<0.05$).

As can be seen in Figure 9B, rearing levels on the test remained comparatively low and were characterized by high variances. The ANOVA conducted on these data revealed no significant effects.

Discussion

The present experiment revealed marked differences in the ability of three D-2 DA receptor antagonists to block both the acute effect of intra-VTA morphine on horizontal activity and the development of sensitization to this effect with repeated injections of morphine. Pimozide and Ro22-2586 both blocked the acute effect, whereas sulpiride had no observable effect. Pimozide effectively blocked the development of sensitization to the locomotor activating effects of intra-VTA morphine, whereas neither Ro22-2586 nor sulpiride interfered. The D-1 DA receptor antagonist, SCH-23390, had no effect on the development of behavioral sensitization to intra-VTA morphine even though it completely blocked the acute effect of morphine.

Two aspects of the results are surprising. The first is the differential effect of the three D-2 DA receptor antagonists on the development of behavioral sensitization to intra-VTA morphine. The second is that pimozide blocked the development of sensitization to intra-VTA morphine and SCH-23390 had no effect, whereas, in Experiment 1 with amphetamine, the opposite result was found.

As was discussed in relation to amphetamine in Experiment 1, the differential effect of these D-2 DA receptor antagonists during training might reflect postsynaptic as well as presynaptic DA autoreceptor blockade by pimozide and Ro22-2586 and preferential blockade of DA autoreceptors by sulpiride at the dose tested. There appears, however, to be sufficient discrepancy between the action of various D-2 DA receptor antagonists to suggest that there are factors operating that may be quite unrelated to the effect of these compounds on D-2 DA receptors. The finding that pimozide blocked the development of behavioral sensitization to intra-VTA morphine replicates the earlier identical finding by Vezina and Stewart (1984). The finding that Ro22-2586 and sulpiride were without effect is similar to the finding by Kalivas (1985a) that haloperidol, also a D-2 DA receptor antagonist, did not block the development of behavioral sensitization to intra-VTA enkephalin. Thierry, Le Douarin, Penit, Ferron and Glowinski (1986) also found that different D-2 DA receptor antagonists differed in their ability to block the inhibitory influence of DA neurons on the electrical activity of medial prefrontal cortex neurons (e.g., sulpiride was effective while haloperidol was ineffective at all doses tested). Given the fact that DA receptor antagonists are of divergent chemical structures and interact differentially with other neurotransmitter systems (Kebabian and Calne, 1979; see also Christensen, Arnt, Hyttel, Larsen and Svendsen, 1984), it may be possible that some of the above discrepancies are due to effects of D-2 DA receptor antagonists other than their effects on DA

receptors.

In the present experiment, sulpiride neither blocked the acute effect of intra-VTA morphine on horizontal locomotor activity nor did it affect the development of sensitization. These results were marked, however, with a high degree of variance. In order to confirm these findings, a subsequent experiment was undertaken to test the effects of two doses of sulpiride (10 and 50 mg/kg, i.p.) on the development of behavioral sensitization to intra-VTA morphine. Preliminary findings indicate that the lower dose enhanced, and the higher dose blocked, the acute effect of intra-VTA morphine on locomotor activity. Neither dose, however, prevented the development of behavioral sensitization. These findings obtained with sulpiride and Ro22-2586 would appear to indicate, as was the case for amphetamine in Experiment 1, that autoreceptor subsensitivity is not involved in the development of sensitization to the locomotor activating effects of intra-VTA morphine. No explanation can as yet be provided for the findings obtained with pimozide in the present experiment and in the previous study with intra-VTA morphine (Vezina and Stewart, 1984).

As stated above, the findings obtained with pimozide and SCH-23390 in the present experiment are in contrast to those obtained with amphetamine in Experiment 1. Even if the exact mode of action of these two compounds may not be known, these results suggest, nonetheless, that the mechanisms underlying the development of behavioral sensitization to amphetamine and morphine may differ even though these ultimately produce similar changes in the activity of mesencephalic DA neurons (see

Introduction). Inasmuch as the postsynaptic consequences of behavioral sensitization to morphine are the same as those to amphetamine (i.e., increased quantities of released DA in response to challenge), the present results, indicating a lack of effect of SCH-23390, may make it difficult to entertain the desensitization of postsynaptic D-1 DA receptors (and the consequential reduction in the inhibition of mesencephalic DA cells) as a mechanism for sensitization to amphetamine (see Experiment 1). Alternatively, sensitization to intra-VTA morphine may be less dependent on a role for NAC-VTA feedback fibers in the reduction of inhibition of DA cells in the VTA. Kalivas, Duffy, Dilts and Abhold (in press), for example, have proposed that endogenous enkephalin releases VTA DA neurons from tonic GABAergic inhibition by inhibiting GABA interneurons intrinsic to the VTA. Although this is not the event critical for the development of sensitization proposed by these authors, it is intriguing in light of reports showing that conditioned morphine-induced effects are blocked by opiate receptor blockade (Drawbaugh and Lal, 1974; Lal, Miksic and Smith, 1976; Neisewander and Bardo, submitted).

Finally, even though Group SAL-MOR showed levels of rearing that were significantly higher than those of the other groups in Hour 1 of training, this group did not show development of sensitization on this measure. Kalivas et al.(1985) similarly found that sensitization developed to the horizontal activity but not to the rearing induced by repeated intra-VTA injections of enkephalin at a dose that initially elicited comparable levels of

each behavior (Kalivas et al., 1983). Such results, together with those of the present experiment, suggest that sensitization does not develop to the rearing induced by intra-VTA opiate injections. Why this would be so is not immediately clear.

EXPERIMENT 3

The present experiment was conducted in an attempt to elucidate the relation between conditioning and behavioral sensitization to amphetamine. As discussed in the Introduction, there have been several demonstrations that the behavioral sensitization to amphetamine and other psychoactive drugs can come under strong stimulus control; that is, the sensitization is apparent in the environment where the drug was repeatedly administered, but not in another environment. These findings have suggested to some investigators that conditioning might be responsible for the development of behavioral sensitization to these drugs, and that conditioned activity might be serving to augment the pharmacological effects of the drug.

Inasmuch as there is evidence that behavioral sensitization can come under strong stimulus control when the conditions for its development meet the requirements for the development of conditioning, this and the following experiment were conducted to attempt to elucidate the relation between conditioning and behavioral sensitization to amphetamine (the present experiment) and to morphine (Experiment 4).

In the present experiment, the effect of extinction training on previously established environment-specific sensitization of the locomotor activating effects of amphetamine was investigated. It was hypothesized that if conditioned activity could explain, or were in some sense responsible for sensitization, then a procedure known to cause its decrement, extinction, should also

cause the decrement of sensitization. On the other hand, if conditioned activity were not able to account for sensitization, then extinction should have no effect on sensitization itself but might be seen to have an effect on the stimulus control of its manifestation; that is, following extinction, behavioral sensitization to amphetamine might still remain, but no longer be specific to the CS environment.

Methods

Subjects

Subjects were 35 male Wistar rats, weighing 275–325 g on arrival. The supplier, food and water availability, and the home cages used to house the animals, were as specified in Experiment 1. The animals were kept in a 12 h light/12 h dark animal colony room. During the first week after arrival, animals were handled daily. On the latter five days of this week, animals were also weighed and given saline injections (1.0 ml/kg, i.p.) in the colony room in an effort to habituate them to the injection procedure. Experimental testing began the following week and always took place in animals' light cycle.

Design and Procedure

This experiment involved three phases: conditioning, extinction and testing. The testing phase consisted of three tests. Test 1 (saline test for conditioning) was given during the conditioning phase. Test 2 (given after the conditioning phase) and Test 3 (given after the extinction phase) were tests for environment-specific sensitization to amphetamine.

Conditioning. In this phase of the experiment, animals in one

group (Group COND, conditioning, n=8) were administered amphetamine in a distinctive environment (the CS: the activity boxes in the testing room described in Experiment 1) and saline in their home cages. Animals in another group (Group PSEUDO, pseudoconditioning, n=8) were administered saline in the activity boxes and amphetamine in their home cages. Animals in a third group (Group CTL, saline control, n=8) were administered saline in both environments. Conditioning consisted of six 3-day blocks. Animals received their activity box injections on the first day, their home cage injections on the second and were left undisturbed in their home cages on the third. Thus, on the first day of each block, animals were carried, in groups of 11 or 12, to the testing room, given their respective injections, tested in the activity boxes for two hours and returned to their home cages. On the second day of each block, animals were injected in the colony room and returned immediately to their home cages. During conditioning, amphetamine was dissolved in saline and administered i.p. in a dose of 1.0 mg/kg in a 1.0 ml/kg volume; saline was injected in the same volume by the same route.

Test 1: Saline Test for Conditioning. Test 1 was given on the first day of a 3-day block imbedded between blocks four and five of conditioning. All animals were administered saline and tested in the activity boxes for two hours. Because this test constituted an extinction trial for Group COND, Group PSEUDO was also given an extinction trial on the day after when all animals were given saline injections in their home cages. On the following day, animals were left undisturbed in their home cages.

Conditioning resumed on the next with block five of conditioning.

Test 2: Amphetamine Test for Environment-Specific Sensitization. Test 2 was given on the day following the conditioning phase. On this test, all animals were administered amphetamine (0.5 mg/kg, i.p.) and tested in the activity boxes for two hours.

Extinction. The extinction phase began two days following Test 2. This phase, consisting of six 3-day blocks, was identical to the conditioning phase except that saline was substituted for all amphetamine injections.

Test 3: Amphetamine Test for Environment-Specific Sensitization. Test 3 was given on the day following the extinction phase and was identical to Test 2.

In order to confirm that the effects of extinction training on conditioning and behavioral sensitization were simply not due to the passage of time, two additional groups were tested: Group COND (PTC), conditioning passage-of-time control (n=6), and Group PSEUDO (PTC), pseudoconditioning passage-of-time control (n=5). These groups were treated identically to Groups COND and PSEUDO, respectively, but were not given extinction training. During this phase, these animals were left undisturbed in their home cages. It was expected that, with no extinction training intervening, environment-specific sensitization would still be evident in these two groups on Test 3.

The conditioning phase data were analyzed with 1-between 2-within ANOVA's with groups as the between factor and hours and days as the within factors. Data for the first and last day of extinction and the test days were analyzed with 1-between 1-

within ANOVA's. Tests of simple main effects and post hoc Scheffé comparisons were made according to Kirk (1968).

Results

Conditioning.

Figure 10 shows the mean horizontal activity counts obtained in Hours 1 and 2 of the six conditioning days for Groups COND, PSEUDO and CTL. It can be seen that, in both Hours 1 and 2, Group COND, which received amphetamine paired with the activity boxes, showed activity levels which were clearly much higher than those of the other two groups. These increased whereas the activity levels of Groups PSEUDO and CTL decreased slightly over days. The ANOVA conducted on these data revealed significant effects of groups [$F(2,21)=39.59$, $p<0.001$] and hours [$F(1,21)=253.86$, $p<0.001$] and significant groups x days [$F(10,105)=4.02$, $p<0.001$] and groups x hours x days [$F(10,105)=2.09$, $p<0.031$] interactions. Post hoc comparisons confirmed that, in Hours 1 and 2, Group COND was significantly more active than both other groups ($p's<0.01$) which did not differ from each other. As indicated by the significant hours effect, all groups were significantly less active in Hour 2 compared to Hour 1 ($p's<0.01$). The simple main effect of days was significant in Hours 1 and 2 for Group COND ($p's<0.01$), reflecting the slight increase in activity over days by this group, and only in Hour 1 ($p's<0.01$) for the remaining groups, reflecting the slight decrease over days in this hour by these groups.

Figure 11 shows the corresponding mean rearing counts

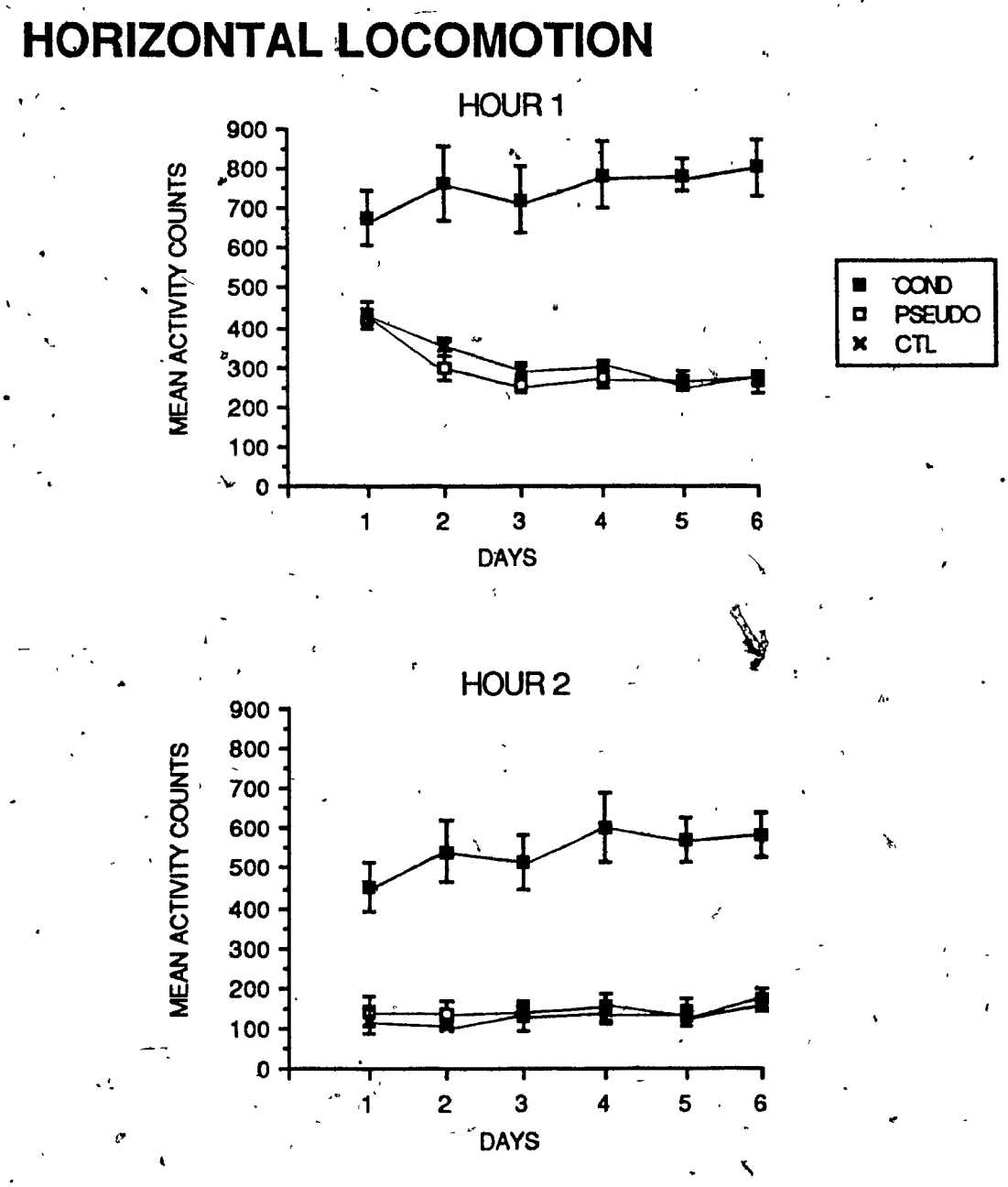


Figure 10. CONDITIONING. Mean horizontal activity counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six conditioning days for Groups COND, PSEUDO and CTL in Experiment 3.

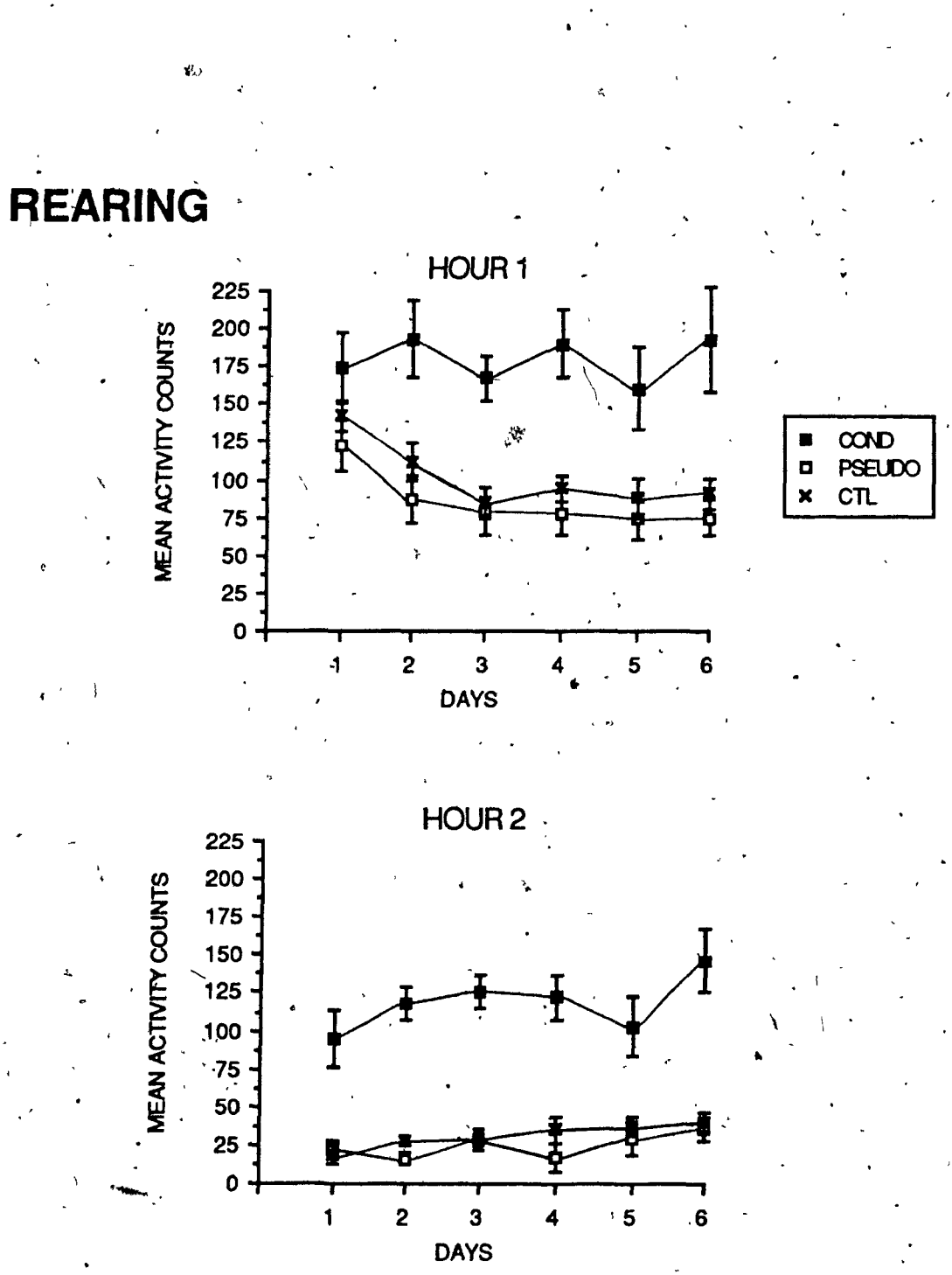


Figure 11. CONDITIONING. Mean rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six conditioning days for Groups COND, PSEUDO and CTL in Experiment 3.

obtained on the six conditioning days for these groups. It can be seen that the relation between groups obtained on this measure is similar to that seen for horizontal activity. The ANOVA conducted on these data revealed significant effects of groups [$F(2,21)=19.71$, $p<0.001$] and hours [$F(1,21)=98.16$, $p<0.001$] and a significant groups x days interaction [$F(10,105)=2.09$, $p<0.031$]. Post hoc comparisons confirmed that, in both hours, Group COND reared significantly more than the other two groups ($p's<0.01$) which did not differ significantly from each other. All groups showed significanty less rearing in Hour 2 compared to Hour 1 ($p's<0.01$). Finally, the significant groups x days interaction mostly reflects the fact that Group PSEUDO and CTL showed declining levels of rearing over days while Group COND showed variable levels of rearing from one day to the next.

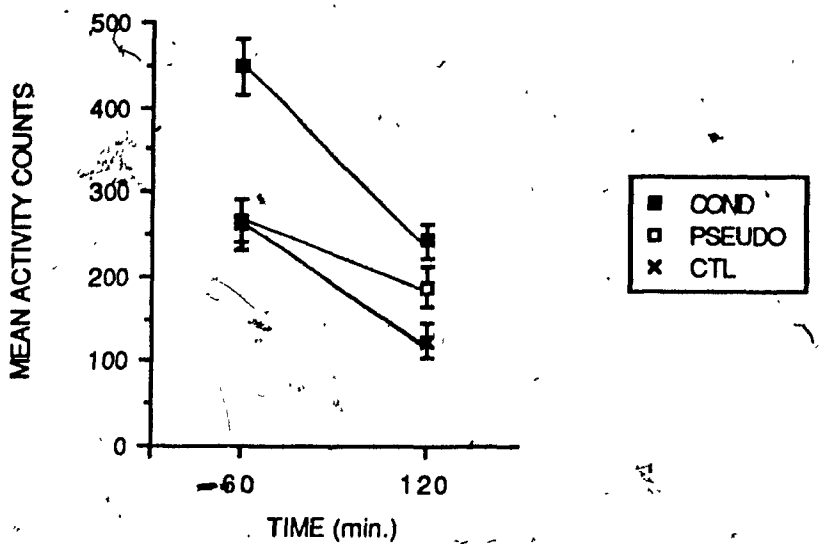
Test 1: Saline Test for Conditioning.

Figure 12 shows the group mean horizontal activity and rearing counts obtained on the test for conditioning, in which all animals were tested in the activity boxes after receiving a saline injection.

It can be seen in Figure 12A that, even in the absence of amphetamine, Group COND continued to show horizontal activity levels that were considerably higher than those of the other two groups, a finding again demonstrating that the locomotor activating effects of amphetamine could come to be elicited by a CS (i.e., the activity box) that had been paired with the drug. The ANOVA conducted on these day indicated that the groups effect [$F(2,21)=12.672$, $p<0.001$] was significant as well as the groups x time interaction [$F(2,21)=5.369$, $p<0.013$]. Post hoc comparisons

SALINE TEST

A. HORIZONTAL



B. REARING

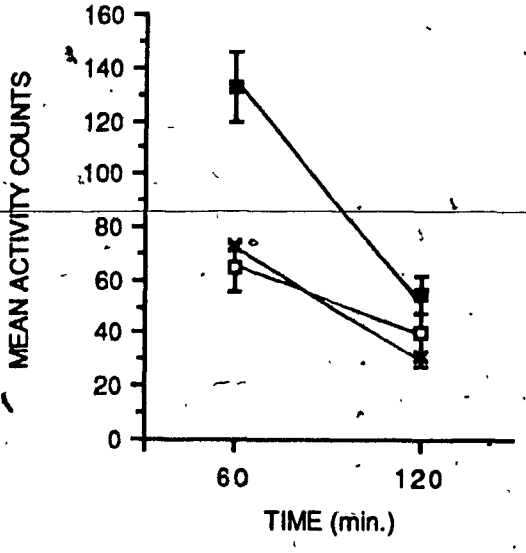


Figure 12. TEST 1. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the saline test for conditioning for Groups COND, PSEUDO and CTL in Experiment 3.

revealed that Group COND was significantly more active than Group CTL in Hours 1 and 2. The significant difference between Groups COND and PSEUDO in Hour 1 ($p<0.01$) was no longer significant in Hour 2. Groups PSEUDO and CTL did not differ significantly from each other.

As can be seen in Figure 12B, parallel results were obtained with rearing. The ANOVA revealed a significant effect of groups [$F(2,21)=7.29$, $p<0.004$] and a significant groups x time interaction [$F(2,21)=10.41$, $p<0.001$]. Post hoc comparisons confirmed that, in Hour 1, Group COND was significantly more active than both other groups ($p<0.01$) which did not differ from each other. The three groups did not differ significantly from one another in Hour 2.

Test 2: Amphetamine Test for Environment-Specific Sensitization.

Figure 13 shows the group mean horizontal activity and rearing counts obtained on the first (pre-extinction) test for environment-specific sensitization, in which all animals were tested in the activity boxes after an injection of 0.5 mg/kg amphetamine.

It can be seen in Figure 13A that, even though all animals had received an injection of amphetamine, Group COND continued to show higher levels of horizontal activity than the other groups. This finding again demonstrates that sensitization to the locomotor activating effects of amphetamine can come under strong stimulus control. Group PSEUDO, which had received an equal number of amphetamine injections as Group COND, but not paired with the activity boxes, showed activity levels similar to those

AMPHETAMINE TEST

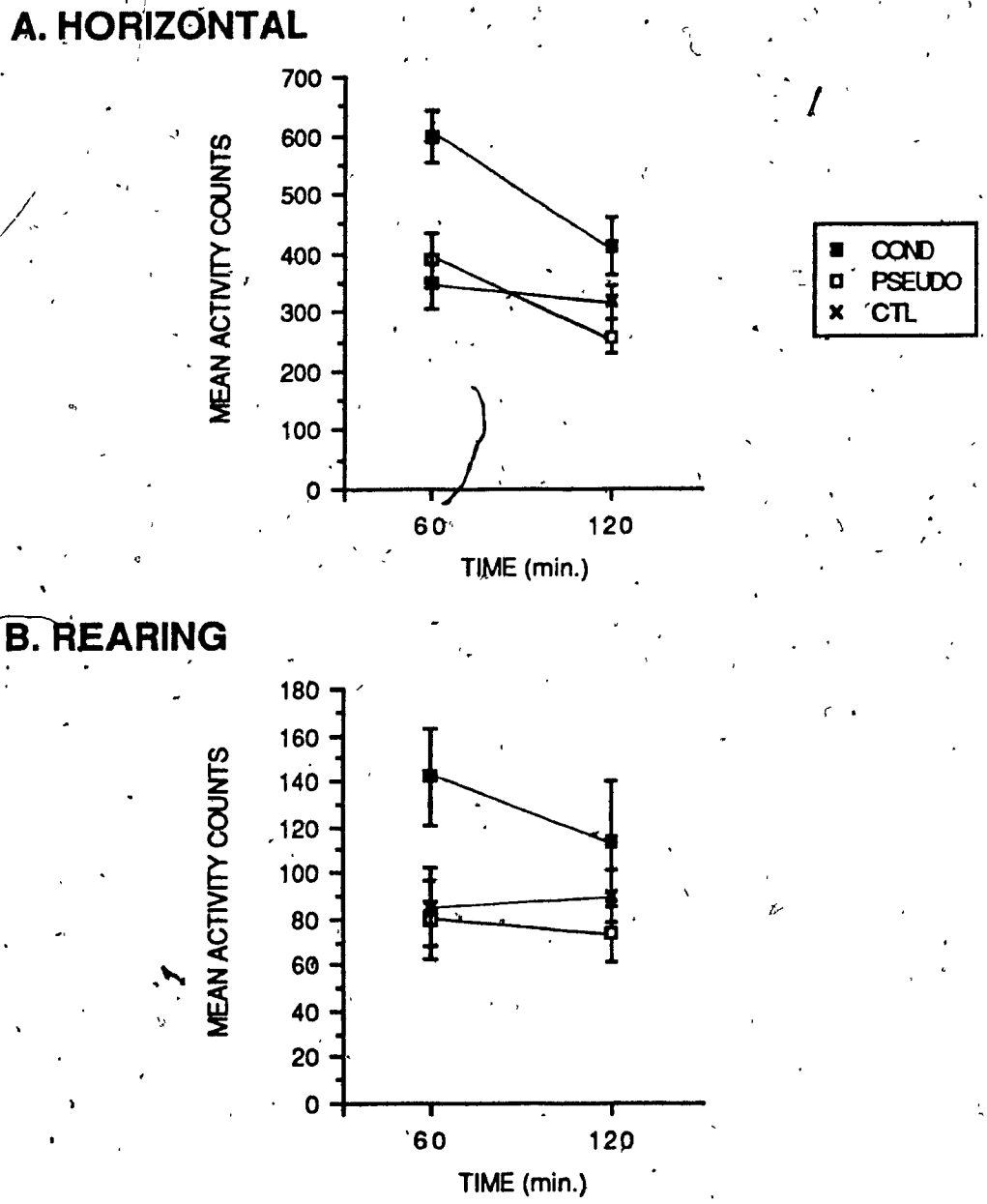


Figure 13. TEST 2. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the first (pre-extinction) amphetamine test for environment-specific sensitization for Groups COND, PSEUDO and CTL in Experiment 3.

of Group CTL which, on this test, received amphetamine for the first time. The ANOVA indicated a significant effects of groups [$F(2,21)=7.89$, $p<0.003$] and a significant groups x time interaction [$F(2,21)=5.243$, $p<0.014$]. Post hoc comparisons showed that Group COND was significantly more active than both groups in Hour 1 (p's<0.01) and significantly more active than Group PSEUDO in Hour 2 ($p<0.05$). Groups PSEUDO and CTL did not differ significantly from each other.

This relation between groups was paralleled in the rearing measure (Figure 13B), although the ANOVA revealed no significant effects. The probability value associated with the groups effect was $p<0.07$, however, indicating that the difference between Group COND and the other two groups approached statistical significance.

Extinction.

Figure 14 shows the mean horizontal activity counts obtained in Hours 1 and 2 of the six extinction days for Groups COND, PSEUDO and CTL. Differences between groups indicative of conditioning are still evident in Hour 1 of the first day of extinction. These differences, however, quickly diminished so that, by the last day of extinction, no differences between groups were apparent. The ANOVA's conducted on the data from the first and last days of extinction confirmed these observations. A significant groups x time interaction was found on the first day [$F(2,21)=4.82$, $p<0.018$] and post hoc comparisons indicated that, in Hour 1, Group COND was significantly more active than the remaining two groups combined ($p<0.05$). Only the time effect was significant on the last day of extinction.

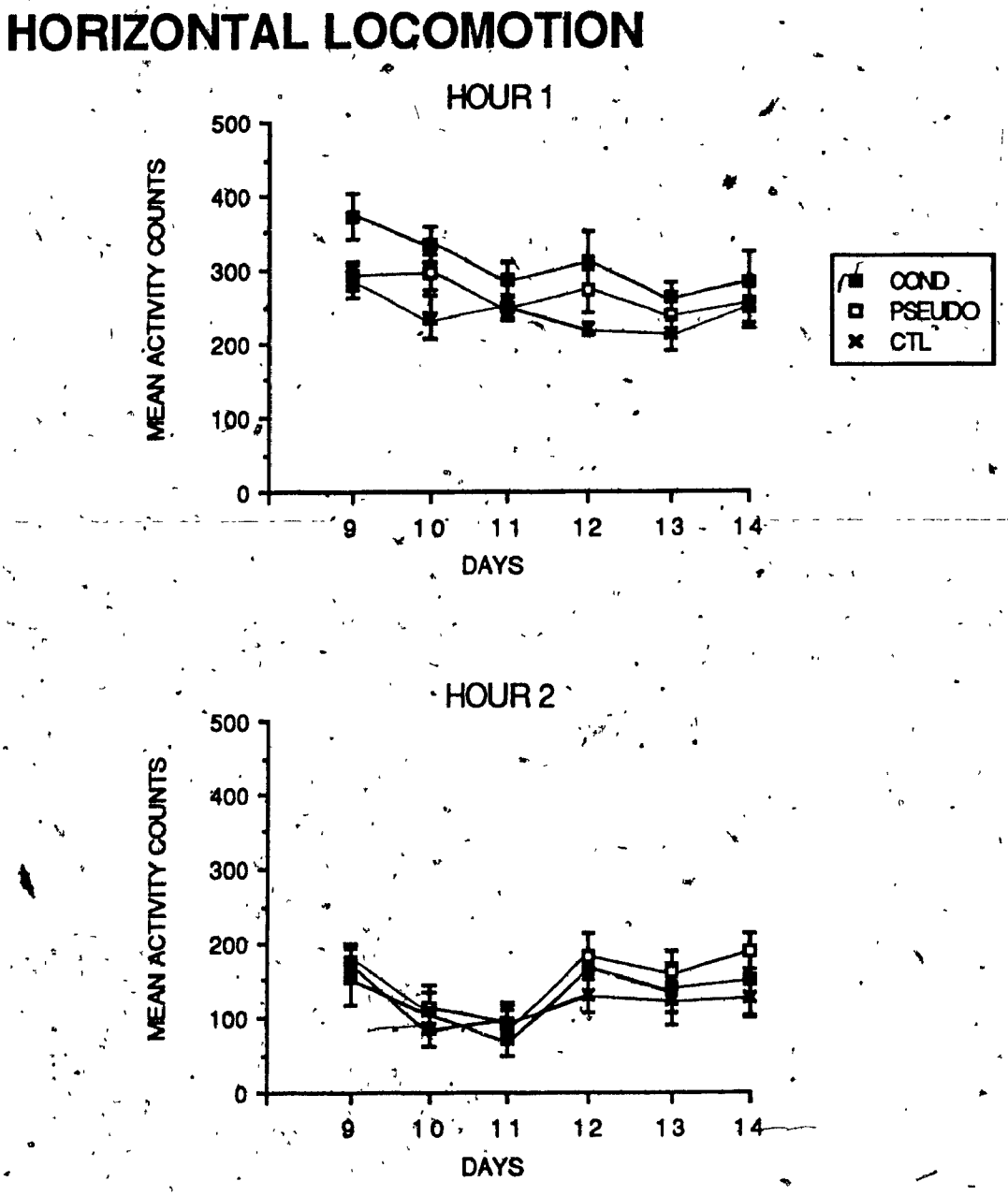


Figure 14. EXTINCTION. Mean horizontal activity counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six extinction days for Groups COND, PSEUDO and CTL in Experiment 3.

The relation between groups on the six days of extinction was similar for rearing (Figure 15). On the first day, a significant groups x time interaction was found [$F(2,21)=4.43$, $p<0.024$] and post hoc comparisons indicated that, in Hour 1, the difference between Groups COND and the other two groups approached statistical significance ($p<0.08$). Again, only the time effect was significant on the last day of extinction.

Test 3: Amphetamine Test for Environment-Specific Sensitization.

Figure 16 shows the group mean horizontal activity and rearing counts obtained on the second (post-extinction) test for environment-specific sensitization, in which all animals were tested in the activity boxes after receiving a 0.5 mg/kg injection of amphetamine.

As can be seen in Figure 16A, Group COND continued to show sensitized levels of horizontal activity relative to Group CTL, suggesting that extinction training had no effect on the previously established behavioral senstization to amphetamine. Importantly, Group PSEUDO now showed sensitized levels of horizontal activity similar to those of Group COND, suggesting that extinction training reduced the control previously exercised by environmental stimuli on the manifestation of behavioral sensitization. The ANOVA conducted on these data confirmed these observations. Although the groups effect was not significant, the groups x time interaction was [$F(2,21)=3.52$, $p<0.048$]. Post hoc comparisons confirmed that Groups COND and PSEUDO did not differ significantly from each other, but that, together, they were significantly more active than Group CTL in Hour 1. The groups did not differ significantly in Hour 2.

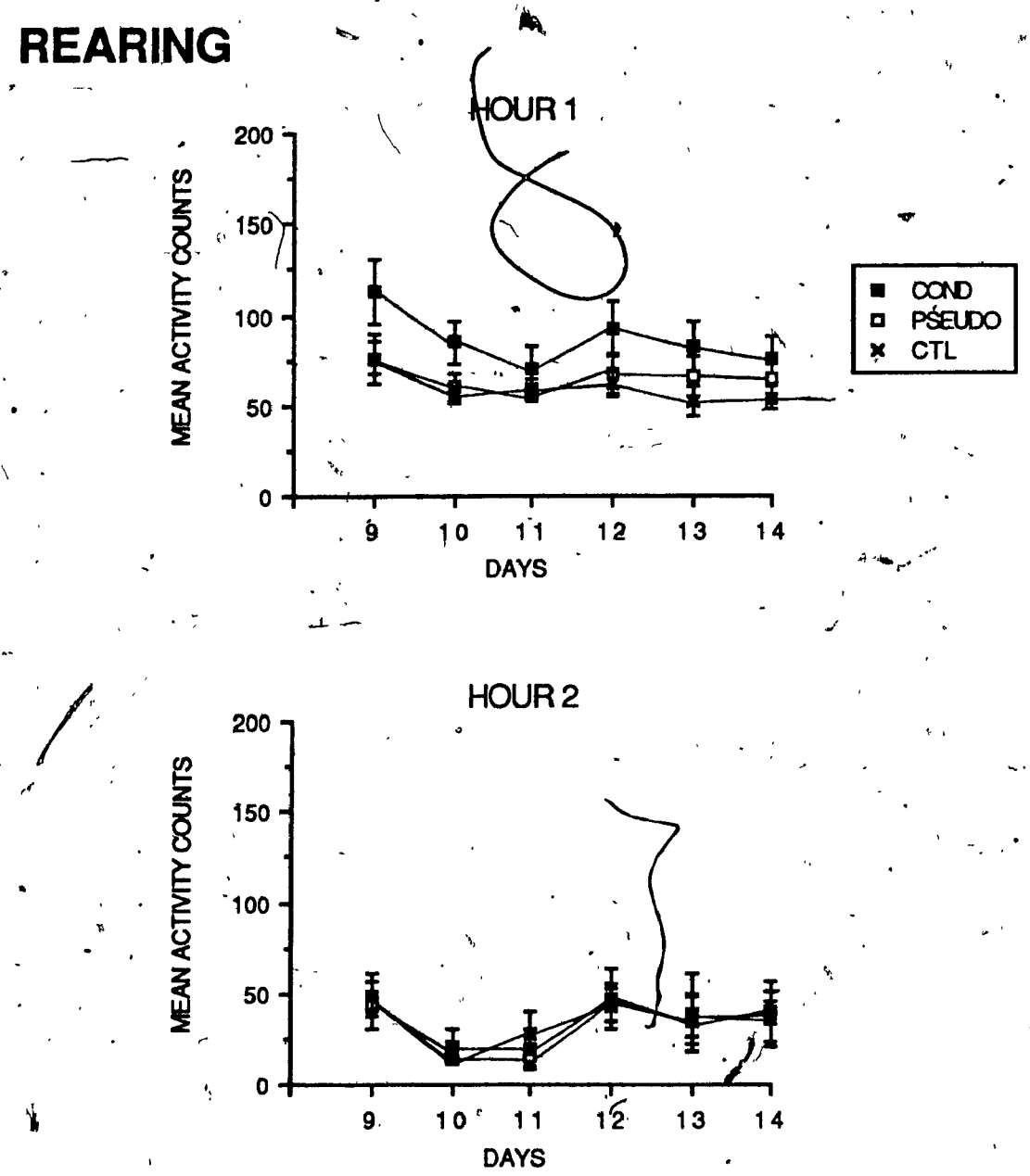


Figure 15. EXTINCTION. Mean rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six extinction days for Groups COND, PSEUDO and CTL in Experiment 3.

AMPHETAMINE TEST

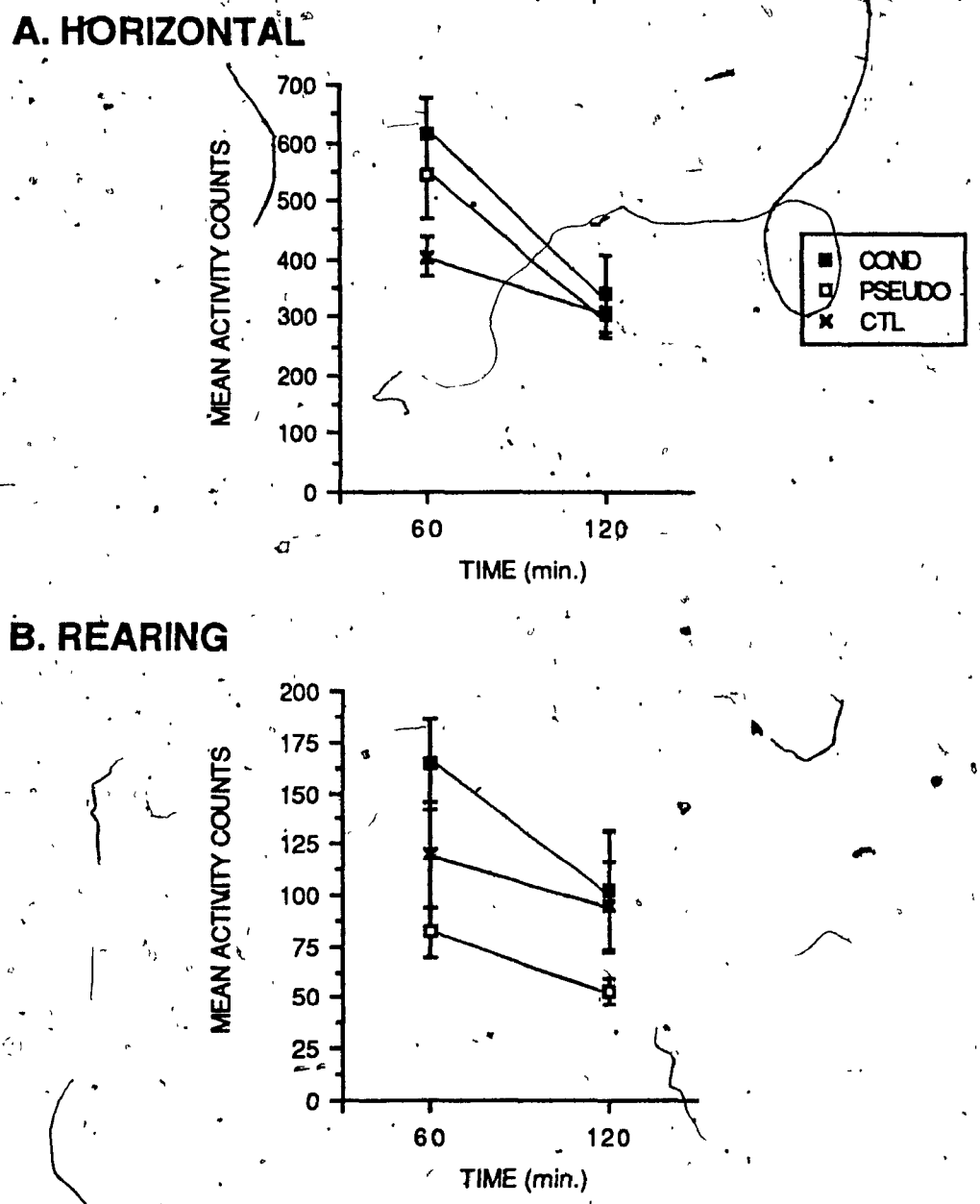


Figure 16. TEST 3. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the second (post-extinction) amphetamine test for environment-specific sensitization for Groups COND, PSEUDO and CTL in Experiment 3.

Curiously, extinction training had little effect on the relation between the rearing levels exhibited by Groups COND and PSEUDO (Figure 16B). The ANOVA revealed that the probability associated with the groups effect was $p<0.07$, indicating that, again, the difference in rearing levels between Groups COND and PSEUDO approached statistical significance. This was surprising especially in light of the fact that conditioning was no longer evident by the last day of extinction. No explanation can be given for this finding.

Passage-Of-Time Controls.

Figure 17 shows the mean horizontal activity and rearing counts obtained on the six conditioning days for Groups COND (PTC) and PSEUDO (PTC). As expected, these two groups showed patterns of horizontal activity and rearing that matched those of Groups COND and PSEUDO during conditioning (see Figures 10 and 11). The ANOVA's conducted on these data confirmed that Group COND (PTC) showed significantly higher levels of horizontal activity [$F(1,9)=52.04$, $p<0.001$] and rearing [$F(1,9)=31.39$, $p<0.001$], than Group PSEUDO (PTC).

Similarly, on Tests 1 and 2, the passage-of-time control groups showed the same patterns of results as those obtained by Groups COND and PSEUDO on these tests. Thus, on the saline test for conditioning (Test 1, Figure 18A), Group COND (PTC) showed significantly more horizontal activity [$F(1,9)=9.28$, $p<0.014$] and rearing [$F(1,9)=9.67$, $p<0.013$] than Group PSEUDO (PTC), indicating that conditioning had occurred. On the first amphetamine test for environment-specific sensitization (Test 2,

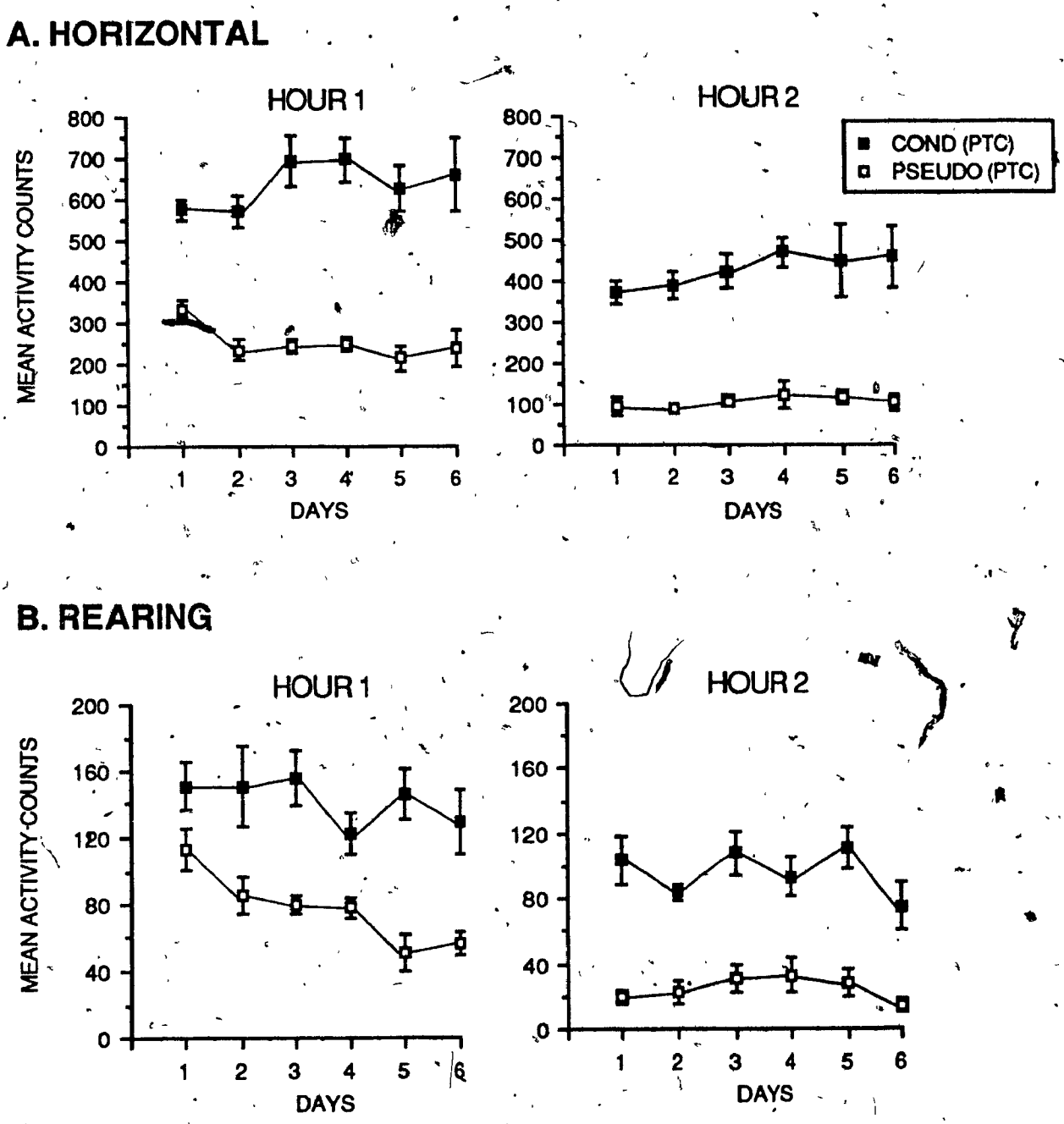


Figure 17. PASSAGE-OF-TIME CONTROLS: CONDITIONING. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six conditioning days for Groups COND(PTC) and PSEUDO(PTC) in Experiment 3.

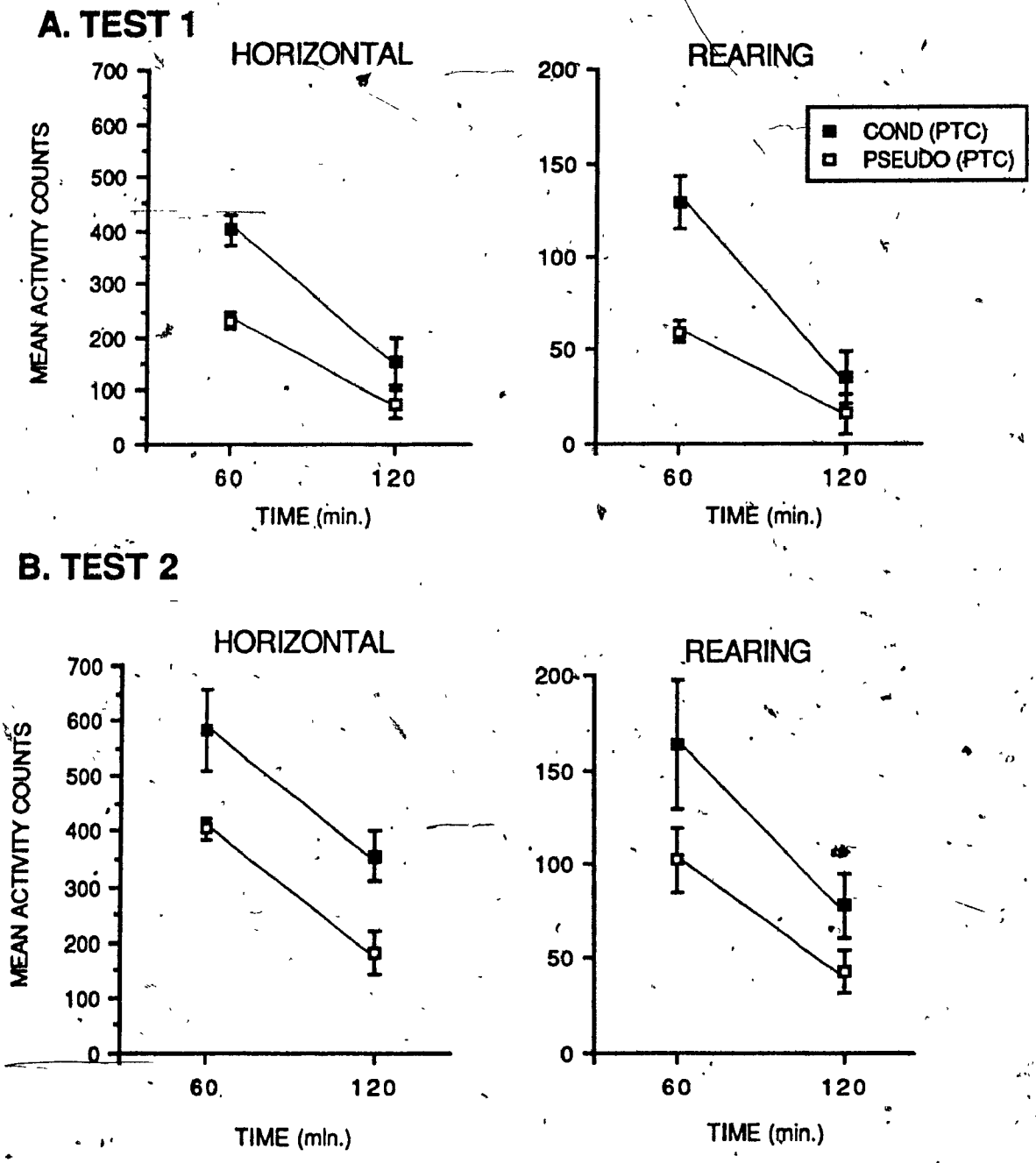


Figure 18. PASSAGE-OF-TIME CONTROLS: TESTS 1 AND 2. Mean horizontal activity and rearing counts (±1 S.E.M.) obtained on (A) Test 1, the saline test for conditioning, and (B) Test 2, the first amphetamine test for environment-specific sensitization, for Groups COND(PTC) and PSEUDO(PTC) in Experiment 3.

Figure 18B), Group COND (PTC) showed significantly higher levels of horizontal activity than Group PSEUDO (PTC) [$F(1,9)=8.29$, $p<0.018$], indicating that sensitization to amphetamine had come under stimulus control. However, like their counterparts in Figure 13B, although Group COND (PTC) showed higher rearing levels than Group PSEUDO (PTC) on this test, this difference did not achieve statistical significance.

Figure 19 shows the mean horizontal activity and rearing counts obtained for the passage-of-time control groups on the final amphetamine test for environment-specific sensitization. Remember that these two groups were left undisturbed in their home cages for the duration of the extinction phase and so had not been exposed to the activity boxes since Test 2 (i.e., 19 days). It can be seen that, although both groups showed similar levels of horizontal activity in the initial 30 minutes and second hour of the test, the two groups clearly differed in the latter half of the first hour. Not surprisingly, the ANOVA conducted on the data from the entire two hour test indicated only a significant effect of time. Noting, however, that Groups COND and PSEUDO differed significantly on this test only in Hour 1 (see Figure 16A), the data for the two passage-of-time control groups on the first 60 minutes of this test were analyzed separately. This analysis produced a significant groups x time interaction [$F(5,45)=2.81$, $p<0.026$], indicating that Group PSEUDO (PTC) showed levels of horizontal activity that declined significantly more over time than those of Group COND (PTC). A similar analysis of the data for Groups COND and PSEUDO revealed no significant differences between these two groups. It may be

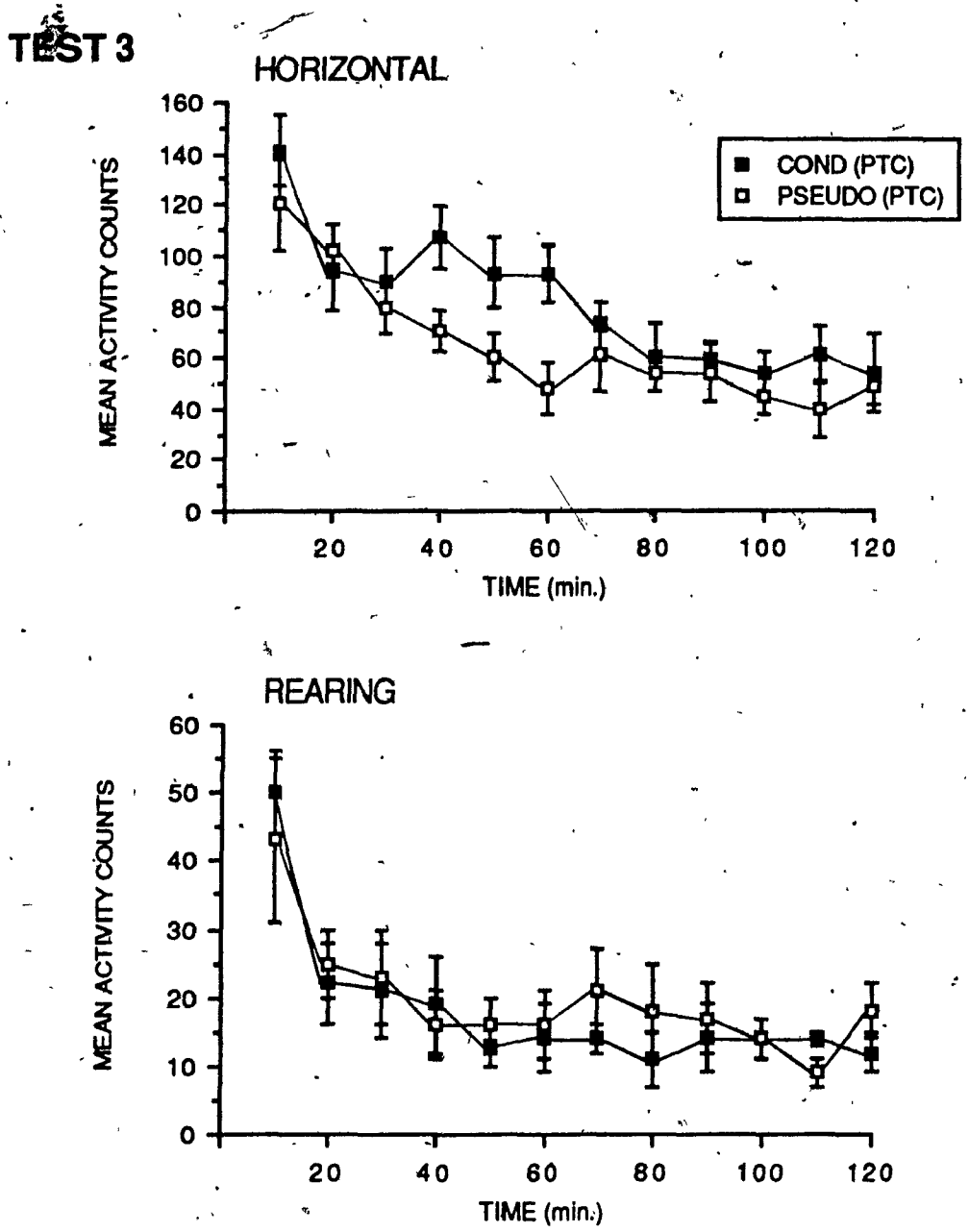


Figure 19. PASSAGE-OF-TIME CONTROLS: TEST 3. Mean (A) horizontal and (B) rearing counts (±1 S.E.M.) obtained on the second amphetamine test for environment-specific sensitization for Groups COND(PTC) and PSEUDO(PTC) in Experiment 3.

that exposure to the activity boxes and the experimental procedures after an extended absence produced heightened levels of activity in the initial segment of the test and that these interfered with the demonstration of the full extent of the difference between Groups COND (PTC) and PSEUDO (PTC). Consitent with this possibility, Hinson and Poulos (1981), who found greater differnces between two similar passage-of-time control groups, habituated their animals to the testing box for 15 minutes prior to injection and test.

Finally, it is clear that Groups COND (PTC) and PSEUDO (PTC), at no time during this test, showed different levels of rearing. The ANOVA conducted on these data indicated only a significant effect of time.

Discussion

The results of the present experiment confirmed the findings of previous studies (see Introduction) showing that the locomotor activating effects of amphetamine can come to be elicited by a CS paired with amphetamine (conditioning, Figure 12) and that the behavioral sensitization to amphetamine can come under strong stimulus control (environment-specific sensitization, Figure 13). More importantly, however, it was also found that extinction training, which reduced the ability of the CS to elicit conditioned activity and to control the expression of behavioral sensitization to amphetamine, did not cause sensitization to be eliminated (Figure 16A). Two aspects of these findings are important for an understanding of the relation between

conditioning and sensitization. First, these findings confirm that the expression of behavioral sensitization can come under strong stimulus control and that this control is subject to procedures that affect conditioning phenomena. Second, they support the view that sensitization of drug-induced behaviors can be observed when conditioned drug effects are absent. Thus, although conditioning factors may not cause or explain sensitization, the demonstration that they are able to control the manifestation of behavioral sensitization to the extent of completely preventing its expression (Figure 13 and other reports, see Introducton) illustrates dramatically that they cannot be treated as trivial or artifactual effects that get in the way of true pharmacological effects. Rather, it would seem that to gain an understanding of how environmental stimuli achieve such control may provide some insights into the basis of sensitization itself. At the very least, any biological system proposed to account for sensitization must have as one of its requirements the provision of mechanisms whereby such stimulus control could occur.

As discussed in the Introduction, the relation between conditioning and sensitization has been variously described as involving additive, multiplicative or synergistic interactions between CS's and drug US's. These views would seem to stem largely from the widely held view that the conditioning of drug effects reflects the acquisition by the CS of the ability to elicit, in the absence of the US, effects similar to those originally produced by the drug US (for a review, see Stewart and Eikelboom, 1987). The finding, in the present experiment, of

conditioned activity on the saline test (Figure 12) is consistent with this view. The findings from the tests for sensitization given before and after extinction, however, suggest that an association also developed between the CS and the absence of the drug, leading to conditioned inhibitory processes.

The conditioning procedure used in the present experiment (and most other drug conditioning experiments) involved training an animal to discriminate between a CS paired with the drug US (the CS+) and a CS paired with the absence of the US (the CS-). Thus, for Group COND, the activity box was the CS+ and the home cage stimulus complex, the CS-; for Group PSEUDO, the activity box was the CS- and the home cage, the CS+. In addition to promoting the development of an excitatory association between the CS+ and the US, as described above, such a procedure can impart the CS- with inhibitory properties. On tests when the CS- is presented alone, as when Group PSEUDO was tested for conditioning on Test 1 (Figure 12), there is often little or no evidence of inhibition. Techniques, such as presenting the CS- together with an effective CS+ and observing a reduction in the magnitude of the response elicited by this stimulus, have been developed, however, for demonstrating conditioned inhibition (see Rescorla and Solomon, 1967; Rescorla, 1969; Wagner and Rescorla, 1972).

Interestingly, the tests for environment-specific sensitization in the present experiment may have provided such a situation in which inhibition by the CS- could be observed. On these tests, the US, itself, was presented to Group PSEUDO rather

than a CS+ as described above. The finding that Group PSEUDO showed reduced levels of activity compared to Group COND and was no different from Group CTL on the first amphetamine test for sensitization (Test 2, Figure 13A) might suggest that the activity box cues (the CS- for Group PSEUDO) inhibited this group's sensitized response to the amphetamine US. This interpretation is supported by the results of the second amphetamine test for sensitization (Test 3, Figure 16A) given after extinction training, when the differentiation between CS+ and CS- should have been diminished. The fact that the activity scores of Group PSEUDO now approached those of Group COND and were higher than those of Group CTL suggests that the activity box CS- was exerting reduced inhibition of the sensitized response to the amphetamine US in Group PSEUDO.

As described in the Introduction, Hinson and Poulos (1981) conducted an experiment with cocaine very similar to the present experiment with amphetamine. They found, unlike the findings reported here, that extinction training attenuated the magnitude of the sensitized response to cocaine, although it did not completely abolish it. Unfortunately, they did not test animals in the presence of the CS- after extinction so that the contribution of this stimulus to the manifestation of the environmental specificity of the behavioral sensitization could not be assessed in their experiment.

The results of the present experiment suggest, therefore, that the relation between conditioning and sensitization may involve more than an interaction between the CS+ and the drug US. Rather, the stimulus critical for controlling the expression of

sensitization may be the CS-. Interestingly, Rescorla (1985) has suggested that such inhibitory (and facilitatory) stimuli may act to control a threshold for activation of the central representation of the US by excitatory stimuli. The present results suggest that a CS- may modulate the effectiveness of the US, itself, on the central nervous system.

EXPERIMENT 4

In this experiment, the effect of extinction training on previously established environment-specific sensitization to the locomotor activating effects of morphine was investigated in order to compare the nature of stimulus control of sensitization to morphine to that seen with amphetamine. The design and procedures used, therefore, essentially duplicated those used in Experiment 3.

Methods

Subjects

Thirty-six male Wistar rats, weighing 250-290 g on arrival were used. The supplier, all housing conditions and pre-experiment handling and habituation to the injection procedure were as specified in Experiment 3.

Design and Procedure

The design of this experiment and the procedures followed were identical to those of Experiment 3. The experiment had three phases: conditioning, extinction and testing. Test 1, the test for conditioning using saline, was given during the conditioning phase. Tests 2 and 3, given after conditioning and after extinction, respectively, were tests for environment-specific sensitization to morphine.

Three groups were tested, group names refering to treatment during the conditioning phase:

Group COND (n=14) morphine-activity box/saline-home cage

Group PSEUDO (n=14) saline-activity box/morphine-home cage

Group CTL (n=8) saline-activity box/saline-home cage

Morphine sulphate was dissolved in saline and administered in a dose of 5.0 mg/kg during conditioning and 2.5 mg/kg on Tests 2 and 3. Morphine and saline injections were made i.p. in a 1.0 ml/kg volume.

The conditioning phase data were analyzed with 1-between 2-within ANOVA's with groups as the between factor and hours and days as the within factors. Data for the first and last days of extinction, the test days and the subgroup analysis were analyzed with 1-between 1-within ANOVA's. When only two means were compared in the subgroup analysis, the t-test for independent samples was used. Post hoc Scheffé comparisons were made according to Kirk (1968).

Results

Conditioning.

Figure 20 shows the mean horizontal activity counts obtained by the three groups in Hour 1 and Hour 2 of the six conditioning days. Group COND, which received morphine paired with the activity boxes, showed levels of horizontal activity that were generally higher than the two remaining groups in both hours. These remained relatively constant across days. Although Group CTL showed activity levels comparable to those of Group COND in Hour 1 of days one to three, these diminished to lower levels for the remainder of the conditioning phase. The ANOVA revealed significant effects of groups [$F(2,33)=30.55, p<0.001$] and hours

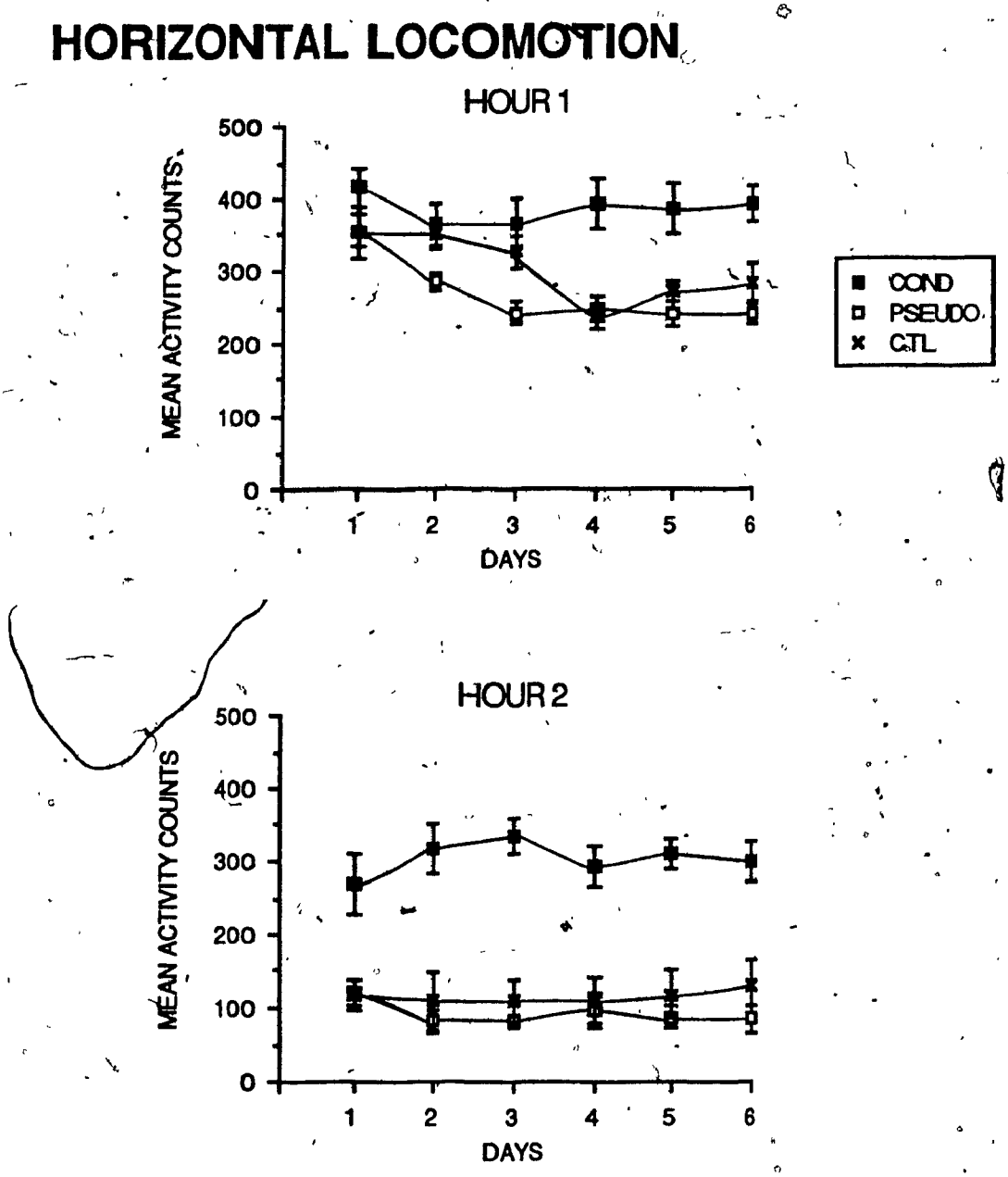


Figure 20. CONDITIONING. Mean horizontal activity counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six conditioning days for each of the three groups in Experiment 4.

[F(1,33)=328.46, p<0.001] and a significant groups x hours interaction [F(2,33)=16.42, p<0.001]. Post hoc comparisons confirmed that, in both Hours 1 and 2, Group COND was significantly more active overall than each of the other two groups (p's<0.01); these did not differ significantly from each other. All groups were significantly less active in Hour 2 than in Hour 1 (p's<0.01) although the decline in activity was considerably greater for Groups PSEUDO and CTL, accounting for the significant interaction.

The corresponding mean rearing counts obtained on the six days of conditioning for the three groups are shown in Figure 21. Group COND showed levels of rearing that were higher than those of the other two groups in Hour 2. In Hour 1, all groups showed comparable levels of rearing. There was no increase in rearing over days in either hour in Group COND. The ANOVA conducted on these data revealed significant effects of groups [F(2,33)=5.15, p<0.011] and hours [F(1,33)=268.36, p<0.001] and a significant groups x hours interaction [F(2,33)=35.86, p<0.001]. Post hoc comparisons confirmed that the groups did not differ significantly in Hour 1. In Hour 2, Group COND showed significantly more rearing than either of the other groups (p's<0.01); these did not differ significantly from each other. Group COND showed significantly less rearing in Hour 2 than in Hour 1 (p<0.05). This decline in rearing over hours was considerably greater for the two other groups (p's<0.01).

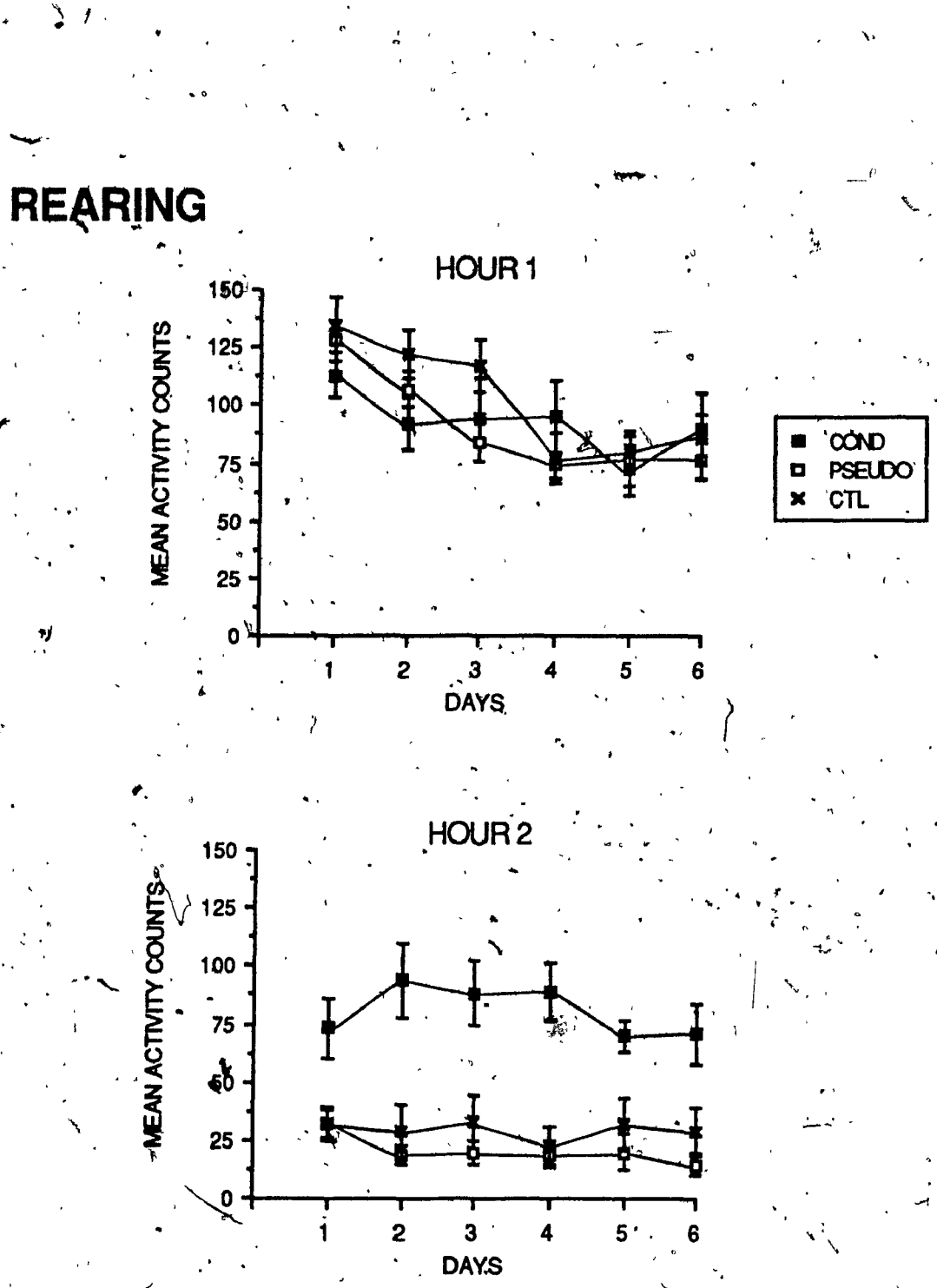


Figure 21. CONDITIONING. Mean rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six conditioning days for each of the three groups in Experiment 4.

Test 1: Saline Test for Conditioning.

Figure 22 shows the group mean horizontal activity and rearing counts obtained on the test for conditioning, in which all animals were tested in the activity boxes after receiving a saline injection.

It can be seen in Figure 22A that, even in the absence of morphine, Group COND continued to show levels of horizontal activity that were higher than those of the other two groups, indicating that conditioning had occurred. The ANOVA indicated a significant groups effect [$F(2,33)=13.79$, $p<0.001$] and post hoc comparisons confirmed that Group COND was significantly more active than the other two groups combined ($p<0.01$). These latter two groups did not differ from each other.

Parallel results were obtained with rearing (Figure 22B). A significant groups effect was found [$F(2,33)=4.27$, $p<0.022$] and post hoc comparisons confirmed that Group COND showed rearing levels that were significantly higher than the other two groups combined ($p<0.05$). Again, these latter two groups did not differ significantly from each other.

Test 2: Morphine Test for Environment-Specific Sensitization.

Figure 23 shows the group mean horizontal activity and rearing counts obtained on the first (pre-extinction) test for environment-specific sensitization, in which all animals were tested in the activity boxes after receiving an injection of 2.5 mg/kg morphine.

As seen in Figure 23A, Group COND showed higher levels of horizontal activity than the other two groups even though all animals had received an injection of morphine on this test. This

SALINE TEST

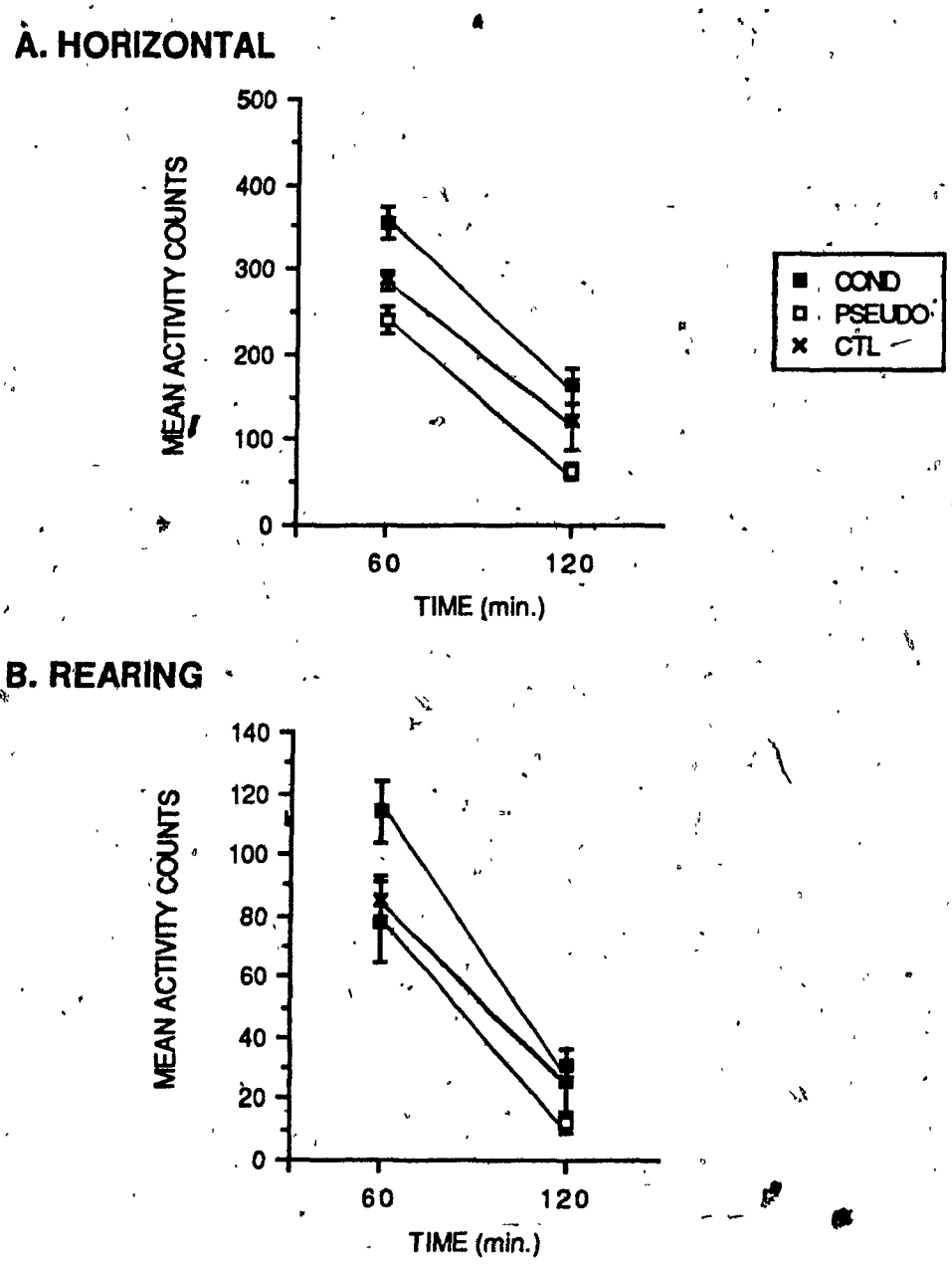


Figure 22. TEST 1. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the saline test for conditioning for each of the three groups in Experiment 4.

MORPHINE TEST

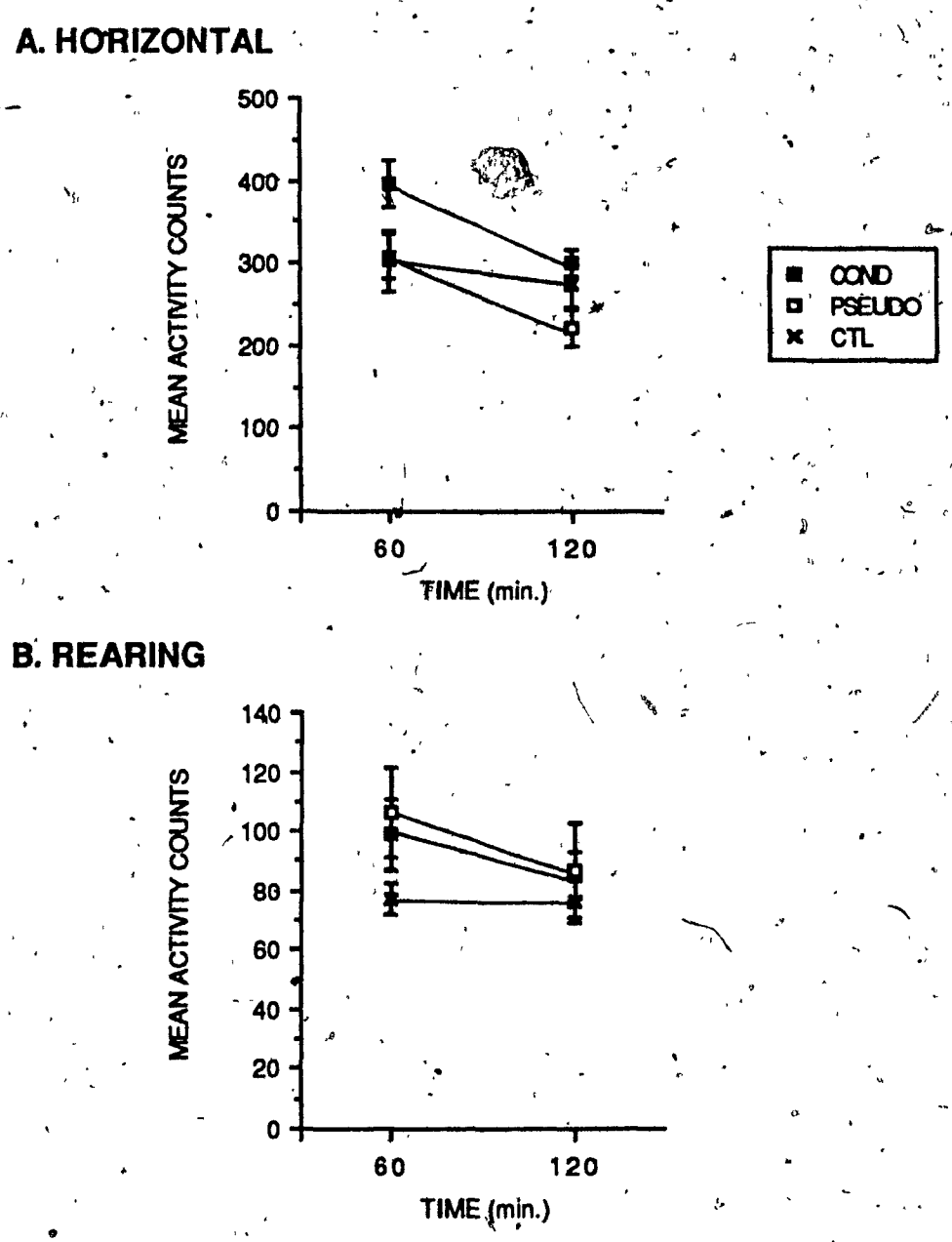


Figure 23. TEST 2. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the first (pre-extinction) morphine test for environment-specific sensitization for each of the three groups in Experiment 4.

finding with morphine, like that with amphetamine in Experiment 3, demonstrates that sensitization to the locomotor activating effects of the drug can come under stimulus control. Group PSEUDO, which had received an equal number of morphine injections as Group COND, but not paired with the activity boxes, showed activity levels similar, or slightly lower, than those of Group CTL which, on this test, received morphine for the first time. The ANOVA conducted on these data confirmed these observations. A significant groups effect was found [$F(2,33)=3.71$, $p<0.035$] and post hoc comparisons confirmed that Group COND was significantly more active than Groups PSEUDO and CTL combined ($p<0.05$). These latter two groups did not differ significantly from each other.

Unlike with horizontal activity, both Groups COND and PSEUDO showed rearing levels that were somewhat higher than those of Group CTL on this test (Figure 23B). Only the effect of time, however, was found to be significant.

Extinction.

Figure 24 shows the mean horizontal activity counts obtained in Hours 1 and 2 of the six extinction days for the three groups. Evidence of conditioned locomotor activity is still apparent on the first day of extinction after which differences between groups diminish. ANOVA's conducted on the data from the first and last days of extinction confirmed these observations. A significant effect of groups was found on the first day [$F(2,33)=6.34$, $p<0.004$] and post hoc comparisons confirmed that Group COND was significantly more active than the other two groups combined ($p<0.01$). Only the time effect was significant on

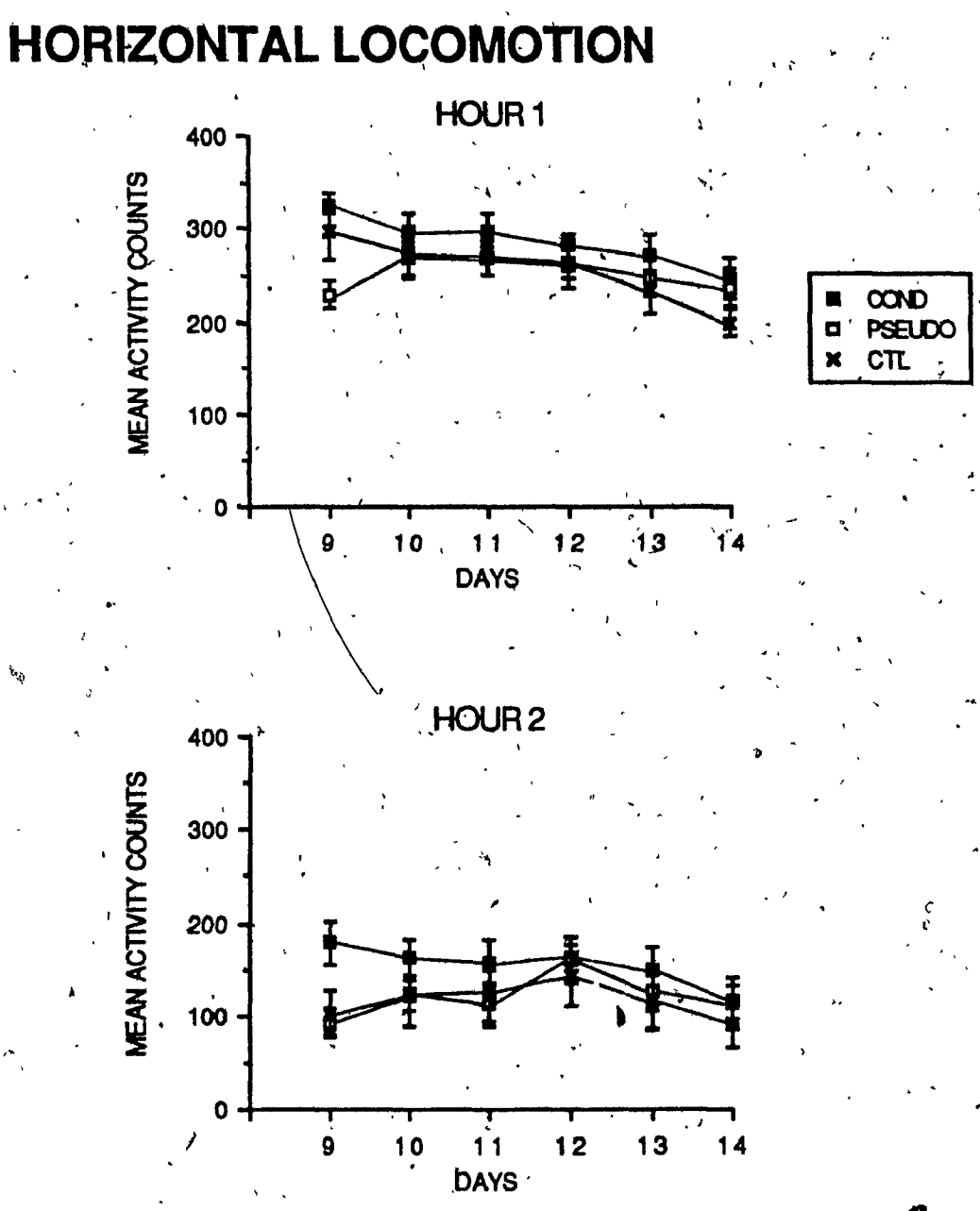


Figure 24. EXTINCTION. Mean horizontal activity counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six extinction days for each of the three groups in Experiment 4.

the last day of extinction.

Similarly, Group COND showed slighty more rearing than the other two groups on the first day of extinction (Figure 25). This difference between groups, however, did not achieve statistical significance. Groups did not differ significantly on the remaining days of extinction, although these were punctuated with occasional increases in variability. Only the time effect was significant on the last day of extinction.

Test 3: Morphine Test for Environment-Specific Sensitization.

Figure 26 shows the group mean horizontal activity and rearing counts obtained on the second (post-extinction) test for environment-specific sensitization, in which all animals were tested in the activity boxes after receiving a 2.5 mg/kg injection of morphine.

Although Group COND continued to show somewhat higher levels of horizontal activity than the other two groups, the difference between groups was diminished from that seen on Test 2 (Figure 26A). The ANOVA conducted on these data confirmed that all three groups did not differ significantly from one another. Only a significant effect of time was found.

Group PSEUDO showed somewhat higher levels of rearing than the other two groups, but these were accompanied by considerable variance (Figure 26B). The ANOVA conducted on these data revealed no significant effects, indicating that the three groups did not differ significantly from one another on this measure.

Subgroup Analysis.

In Experiment 3, it was found that, although extinction training eliminated the control of the CS's on the manifestation

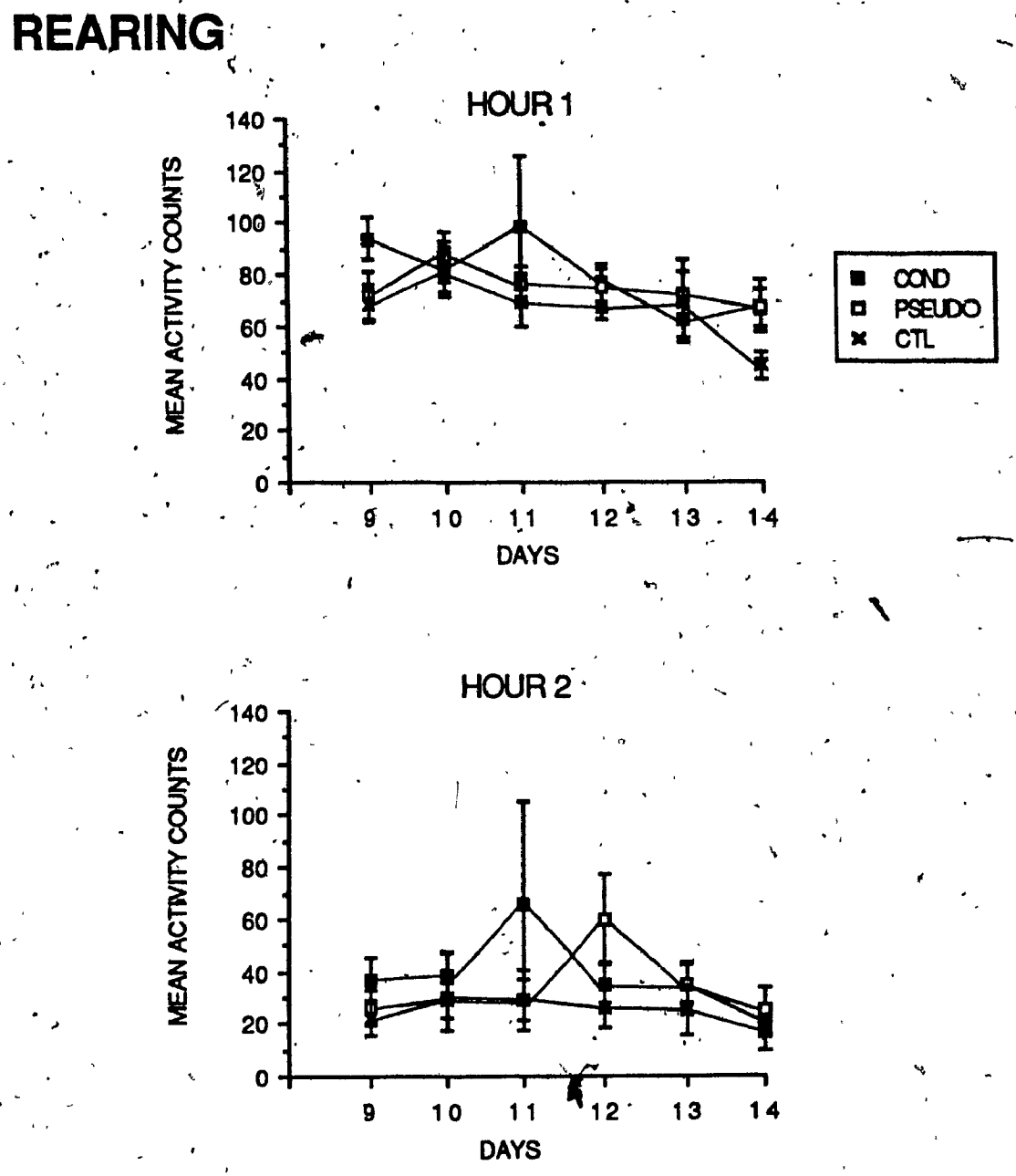


Figure 25. EXTINCTION. Mean rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the six extinction days for each of the three groups in Experiment 4.

MORPHINE TEST

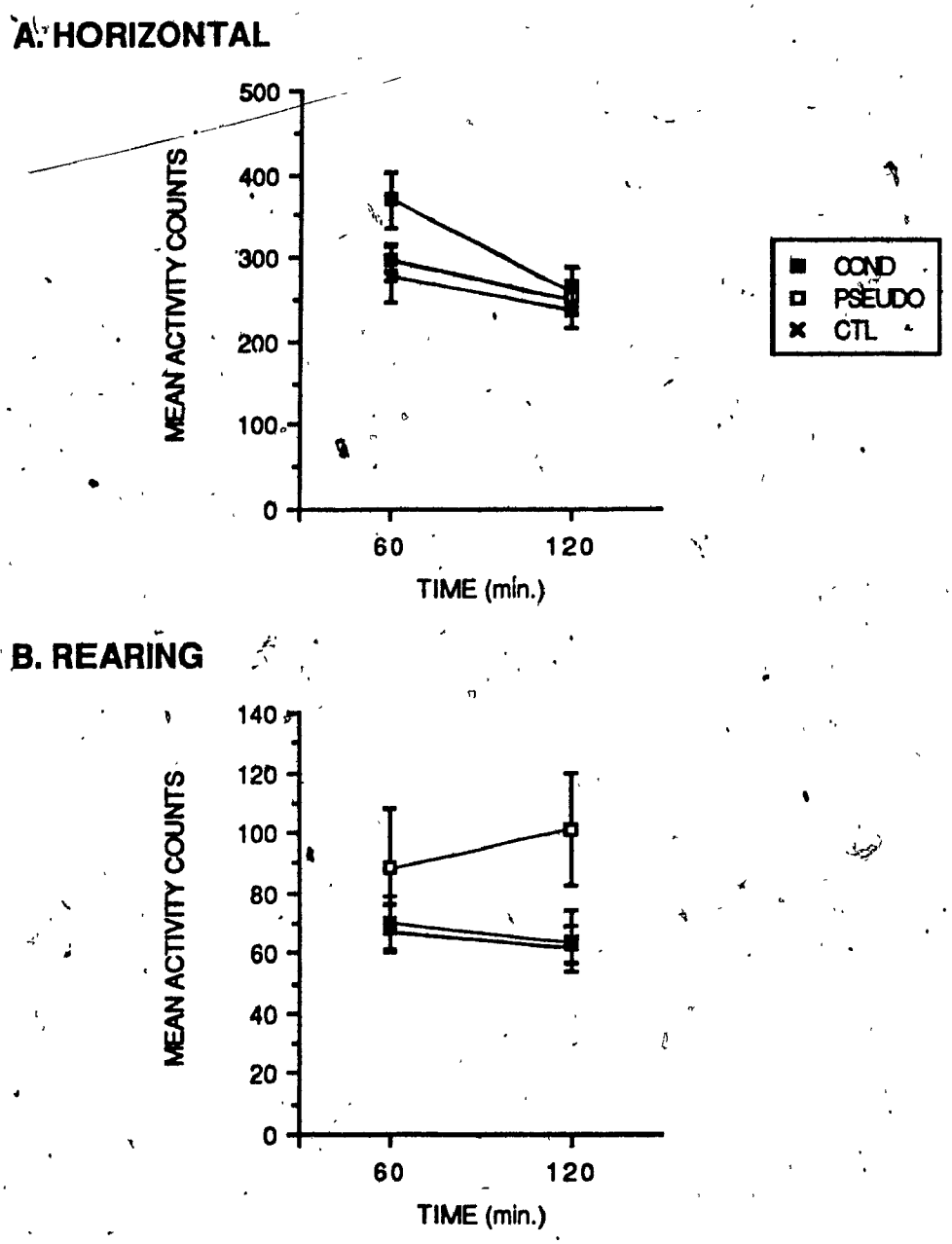


Figure 26. TEST 3. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the second (post-extinction) morphine test for environment-specific sensitization for each of the three groups in Experiment 4.

of behavioral sensitization, it did not affect sensitization itself. That is, behavioral sensitization to amphetamine was still present after extinction training, although it was now apparent in the presence of both CS+ and CS- (see Figure 16A). It was suggested that extinction training may have reduced the ability of the CS- to inhibit the sensitized response to amphetamine in Group PSEUDO.

It was, therefore, surprising to find, in the present experiment, that Groups COND and PSEUDO did not show levels of horizontal activity that were significantly higher than those of Group CTL on the final morphine test for sensitization (Figure 26A). However, upon closer examination of the data from the first morphine test for sensitization (Test 2, Figure 23A), it was found that there was considerable overlap between individual scores in Groups COND and PSEUDO, suggesting that not all animals in these groups showed environment-specific sensitization on this test. Each of these two groups, therefore, was divided into two subgroups based on the relation of animals' total horizontal activity scores on Test 2 to the median score obtained for each group on this test. Thus, Group COND was subdivided into Group COND-Hl (animals showing the highest activity scores, n=7) and Group COND-LO (animals showing the lowest activity scores, n=7). Likewise, Group PSEUDO was subdivided into Groups PSEUDO-Hl (n=7) and PSEUDO-LO (n=7).

Figure 27A shows the mean total horizontal activity scores obtained on Tests 2 and 3 for these subgroups and Group CTL. It can be seen that Group COND-HI was considerably more active than

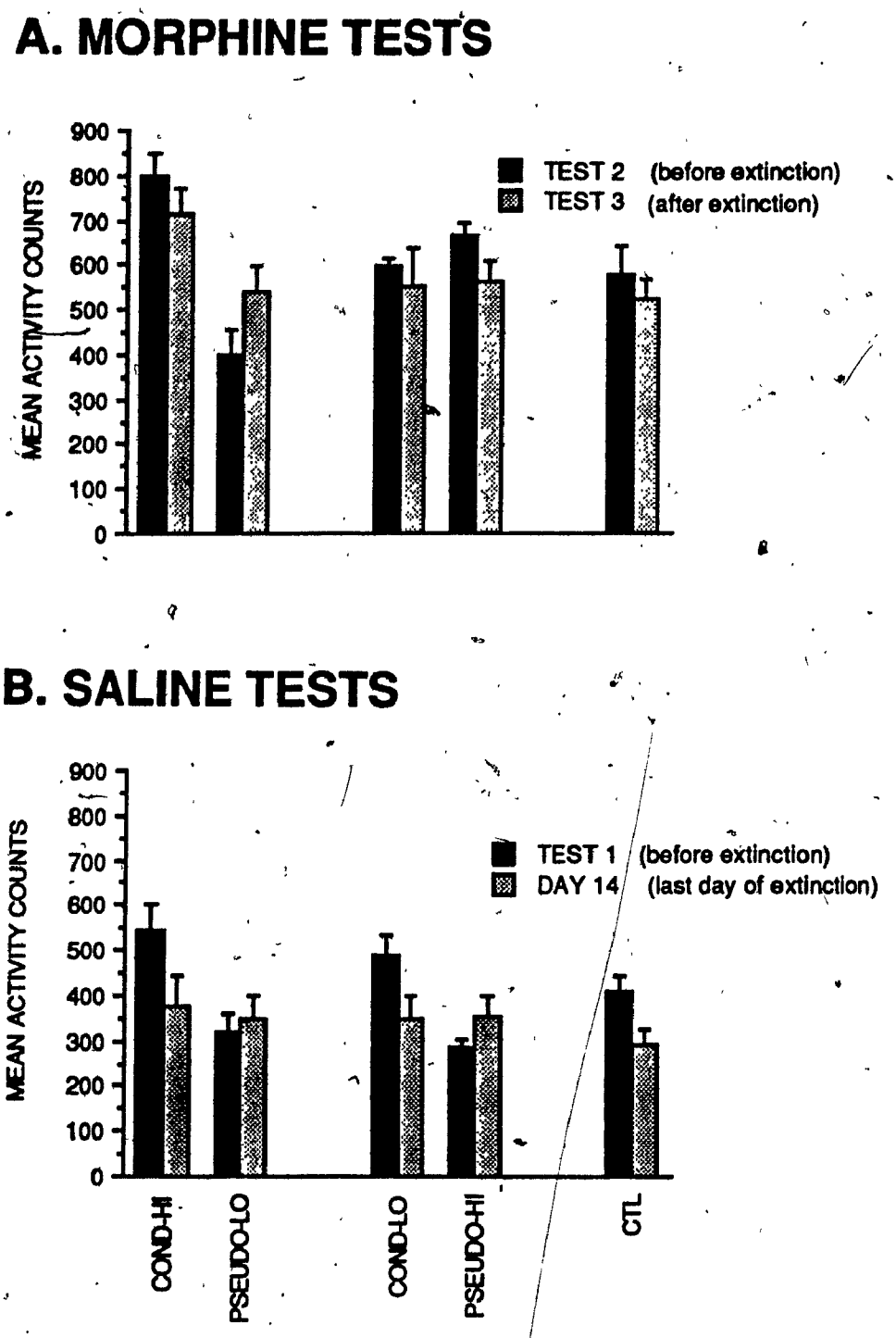


Figure 27. SUBGROUP ANALYSIS. Mean horizontal activity counts (+1 S.E.M.) obtained on (A) morphine Tests 2 and 3 and (B) the saline test (Test 1) and the last day of extinction for the Group COND and PSEUDO subgroups and Group CTL. Groups COND and PSEUDO were subdivided into subgroups showing the highest (HI) and lowest (LO) activity on Test 2.

Groups PSEUDO-LO and CTL on Test 2, providing evidence for environment-specific sensitization to morphine. On the other hand, Groups COND-LO and PSEUDO-HI showed similar levels of activity on this test. Further, these were similar to those of Group CTL.

Interestingly, although all the other groups showed small declines in activity levels from Test 2 to Test 3, Group PSEUDO-LO showed an increase, suggesting that extinction training may have reduced the inhibitory effects of the CS- on this group. An ANOVA conducted on the data from Groups COND-HI and PSEUDO-LO and another conducted on the data from Groups COND-LO and PSEUDO-HI confirmed these observations. Only the first ANOVA revealed significant effects: a significant effect of groups [$F(1,12)=21.59$, $p<0.001$] and a significant groups x test interaction [$F(1,12)=5.87$, $p<0.03$]. Post hoc comparisons confirmed that Group COND-HI was significantly more active than Group PSEUDO-LO on both tests ($p's<0.01$). Further, Group PSEUDO-LO was significantly more active on Test 3 than on Test 2 ($p<0.05$). The activity levels of Group COND-HI on Tests 2 and 3 did not differ significantly. T-tests showed that Group COND-HI was significantly more active than Group CTL on both tests [$t(13)=2.73$ and 2.65, $p's<0.02$, two-tailed]. The same tests showed that Group COND-LO did not differ significantly from Group CTL, indicating that this group did not show sensitization to morphine.

Figure 27B shows the mean total horizontal activity scores obtained by these subgroups on Test 1, the saline test for conditioning, and on the last day of extinction (also a saline

day). It can be seen that both Groups COND-HI and -LO and PSEUDO-LO and -HI showed differences indicative of conditioning on Test 1, even though only Groups COND-HI and PSEUDO-LO showed evidence for environment-specific sensitization. Further, it can be seen that, by the last day of extinction, these conditioned effects were no longer evident. The ANOVA conducted on the data from Groups COND-HI and PSEUDO-LO and the other conducted on the data from Groups COND-LO and PSEUDO-HI both showed significant groups x test interactions [$F(1,12)=12.96$ and 20.92, $p<0.003$ and 0.001]. Post hoc comparisons confirmed that both COND subgroups were significantly more active than their respective PSEUDO subgroups on Test 1 ($p's<0.01$) but not on the last day of extinction.

Discussion

As was found with amphetamine in Experiment 3, the results of the present experiment confirmed the findings of previous studies (see Introduction) showing that the locomotor activating effects of morphine can come to be elicited by a CS paired with morphine (conditioning, Figure 22) and that the behavioral sensitization to morphine can come under stimulus control (environment-specific sensitization, Figure 23A).

Unlike the results of Experiment 3, however, extinction training was found to reduce both conditioning and sensitization to morphine (Figure 26A). This was a surprising finding and may be misleading. Indeed, when Groups COND and PSEUDO were divided into subgroups showing the highest and lowest horizontal activity on the first morphine test for sensitization, it was found that

not all animals showed environment-specific sensitization on this test (Test 2, Figure 27A). For example, Groups COND-LO and PSEUDO-HI showed similar levels of activity on this test. Further, the activity in Group COND-LO was similar to that of Group CTL, suggesting that the animals in Group COND-LO did not become sensitized to morphine. When these animals were tested again with morphine after extinction (Test 3, Figure 27A), their behavior was similar to that on Test 2; Groups COND-LO and PSEUDO-HI still showed similar levels of activity and those of Group COND-LO remained similar to those of Group CTL.

On the other hand, Group COND-HI showed activity levels that were clearly higher than Groups CTL and PSEUDO-LO, providing evidence for environment-specific sensitization to morphine (Test 2, Figure 27A). More importantly, extinction training did not significantly reduce the sensitized activity levels of Group COND-HI but did significantly increase those of Group PSEUDO-LO (Test 3, Figure 27A). These findings are similar to those obtained with amphetamine in Experiment 3 and suggest that, in animals showing environment-specific sensitization to morphine, extinction training does not eliminate behavioral sensitization, but does reduce the control of CS's on its manifestation.

Although Group PSEUDO-LO was significantly more active on Test 3 than on Test 2, it remained less active than Group Cond-HI on this test. This would indicate that the activity box CS- may still have been exerting some inhibition on the sensitized response to morphine in this group. Similar differences, although not as great, were seen with amphetamine (see Figure 16A). Thus, although six extinction sessions may have been sufficient to

eliminate the ability of the CS+ to elicit increased activity (e.g., see Figure 27B), more may have been required to completely eliminate the inhibitory properties of the CS-. This is not an unreasonable expectation, for, although extinction of the CS+ would require only that this stimulus lose its predictive-associative relation to the US, extinction of the CS- would require the more complex process of diminishing the differential value of the CS- and the CS+ (see Wagner and Rescorla, 1972).

There is no clear explanation for the finding that not all animals showed development of sensitization to morphine, or evidence of inhibition by the CS-. That is, Group COND-LO did not show activity levels that were higher than those of Group CTL on either Test 2 or 3. In addition, Group PSEUDO-HI did not show the inhibition of activity shown by Group PSEUDO-LO on Test 2 nor the increase in activity shown by this group on Test 3. These differences between groups were not due to differences in basal levels of activity since, as can be seen in Figure 27B, all COND and PSEUDO subgroups showed similar levels of activity on the last day of extinction. Further, the dose of morphine used during training (5mg/kg) was sufficient to elicit unconditioned locomotor activity and to produce conditioned activity in both pairs of Group COND and PSEUDO subgroups (Figure 27B). This dose may have been only a threshold dose for producing sensitization, however. Babbini and Davis (1972) showed, for example, that high doses of morphine (over 5 mg/kg) but not low doses (5mg/kg and lower) produced sensitization when administered repeatedly. Although their finding is also in need of an explanation (see

Introduction), it may account for why not all Group COND animals showed sensitization in the present experiment.

The finding that Group PSEUDO-HI did not show any evidence of inhibition by the CS- on Test 2, is more difficult to explain. For example, it can be argued that the fact that these animals showed lower activity levels than Group COND-HI on Test 2 is evidence of inhibition by the CS-. However, if this were so, it would be difficult to explain why the activity levels of Group PSEUDO-HI did not increase after extinction on Test 3. It can also be argued that perhaps these animals did not become sensitized to morphine (like animals in Group COND-LO) and that CS- inhibition would not develop in the absence of sensitization. However, a comparison of the activity levels of Groups COND-LO, PSEUDO-HI, and CTL on the last day of extinction to the activity levels of these groups on Test 2 (Figure 27) reveals that the test dose of morphine used (2.5 mg/kg) elicited considerable increases in activity in all of these groups and that these would be sufficient to reveal CS- inhibition (e.g., Group PSEUDO-LO).

EXPERIMENT 5

As discussed in the Introduction, in vitro studies of DA release and biochemical studies of DA neuron function in the whole animal have implicated changes in DA function at the cell body and the terminals of mesencephalic DA neurons in the mediation of behavioral sensitization following repeated systemic injections of amphetamine. And, although the changes in DA function found at either site (increased DA release at terminals and decreased DA release at the cell body to an amphetamine challenge) are compatible with a sensitized neuronal system, it has not yet been determined whether one, or both, of these changes is critical.

An interesting, but much less used approach to the question of which site might be critical to the development and manifestation of sensitized responses to amphetamine, has been to investigate the effect of repeated administrations of amphetamine into particular brain sites. Such an approach has been highly successful at elucidating the site of action critical for the development of behavioral sensitization to opiates (e.g., Vezina et al., 1987). Two studies have applied this strategy to the study of amphetamine sensitization. In one, Dougherty and Ellinwood (1981) reported that repeated intra-NAC administrations of amphetamine did not produce behavioral sensitization, suggesting that amphetamine action at DA neuron terminals (at least in the NAC) is not critical for the development of sensitization. In the other study, Kalivas and Weber (submitted)

found that two daily administrations of amphetamine into the VTA, but, as reported by Dougherty and Ellinwood (1981), not into the NAC, produced sensitized responses to subsequent systemic injections of amphetamine, cocaine and morphine, suggesting that amphetamine action at A10 DA cell bodies is critical for the development of behavioral sensitization.

It was the purpose of this experiment to attempt to replicate some of these findings with intra-VTA amphetamine, but, more importantly, to determine whether conditioned activity would develop with repeated administrations of amphetamine to this site and, therefore, whether the behavioral sensitization obtained would come under stimulus control (i.e., environment-specific sensitization). The prediction was made that it would not. Intuitively, it appeared that under these conditions, where amphetamine would be acting to release DA locally but where the mesolimbic DA neurons and their interconnections were not activated, the conditions for the formation of associations would not be obtained. In the Kalivas and Weber study, the clearest evidence for behavioral sensitization had been obtained when animals were subsequently tested with a systemic injection of morphine. Animals in the present study, therefore, were trained with intra-VTA amphetamine and tested for environment-specific sensitization with morphine and later for conditioning with saline.

Methods

Subjects

Thirty male Wistar rats, weighing 250-300 g on arrival, were used. Four to seven days after arrival, these were stereotaxically implanted with chronic bilateral guide cannulae aimed at the VTA. The animal supplier, housing conditions, handling, surgery, perfusion and histology were as specified in Experiment 2.

Upon histological verification after the experiment, it was found that injector cannula tip placements were too ventral (cannula tracks exited ventrally) in three animals. The data from these animals were, therefore, dropped from the experiment. In all remaining animals, both injector cannula tips were located in the VTA. Bilateral injector cannula tip placements are illustrated in Appendix, Figure B.

Design and Procedure

This experiment consisted of a conditioning phase followed by two tests, the first for environment-specific sensitization with morphine, and the second, for conditioned locomotor activity with saline.

The conditioning phase consisted of four 3-day blocks. On the first day of each block, animals were carried to the testing room, in groups of seven or eight, administered an intra-VTA injection of amphetamine or saline and tested in the activity boxes for two hours. On the second day, animals were given intra-VTA injections of amphetamine or saline in the colony room and immediately returned to their home cages. Animals were left undisturbed in their home cages on the third day.

Animals were randomly assigned to one of three groups depending on what injections they received on the first and second days of each block of conditioning:

Group COND (n=9) amphetamine-activity box/saline home cage

Group PSEUDO (n=9) saline-activity box/amphetamine-home cage

Group CTL (n=9) saline-activity box/saline-home cage

D-amphetamine sulphate was dissolved in saline and administered in a dose of 2.5 µg/side in a volume of 0.5 µl/side. Saline injections were made in the same volume. Microinjection procedures were as specified in Experiment 2. The testing room and the activity boxes are described in Experiment 1.

Test 1. Animals were tested for environment-specific sensitization on the day following the conditioning phase (i.e., day 13). All animals were given a systemic injection of morphine and tested in the activity boxes for two hours. Morphine sulphate was dissolved in saline and administered i.p. at a dose of 1.0 mg/kg in a 1.0 ml/kg volume.

Test 2. Three days following Test 1, animals were tested for conditioning. All animals were given a saline injection (1 ml/kg, i.p.) and tested in the activity boxes for two hours.

The conditioning phase data were analyzed by 1-between 2-within ANOVA's with groups as the between factor and hours and days as the within factors. The test data were analyzed by 1-between 1-within ANOVA's. Post hoc Scheffé comparisons were made according to Kirk (1968).

Results

Conditioning.

Figure 28 shows the mean horizontal activity counts obtained during conditioning for each of the three groups. It can be seen that, overall, intra-VTA injections of amphetamine had little effect on the behavior of Group COND relative to the two other groups. Group CTL showed somewhat lower levels of activity than Groups COND and PSEUDO in Hour 2 of the first two days. However, by Day 4, all groups showed very similar levels of activity. The effects of days [$F(3,72)=13.85$, $p<0.001$] and hours [$F(1,24)=84.56$, $p<0.001$] were significant, reflecting the decline in activity over hours and days by all groups.

Figure 29 shows the corresponding rearing counts obtained during conditioning for each of the three groups. Again, it can be seen that, overall, intra-VTA injections of amphetamine had little effect on the behavior of Group COND relative to the two other groups. Group PSEUDO did show somewhat more rearing in Hour 1 of the first two days of conditioning. By Day 4, all groups showed identical levels of rearing. The ANOVA revealed significant effects of days [$F(3,72)=33.52$, $p<0.001$] and hours [$F(1,24)=96.10$, $p<0.001$], again reflecting the decline in this behavior over hours and days in all groups.

Test 1: Morphine Test for Environment-Specific Sensitization

The results of this test, in which all animals were tested in the activity boxes after receiving an injection of 1.0 mg/kg morphine, are shown in Figure 30.

Groups COND and PSEUDO clearly showed horizontal activity levels that were higher than those of Group CTL, demonstrating

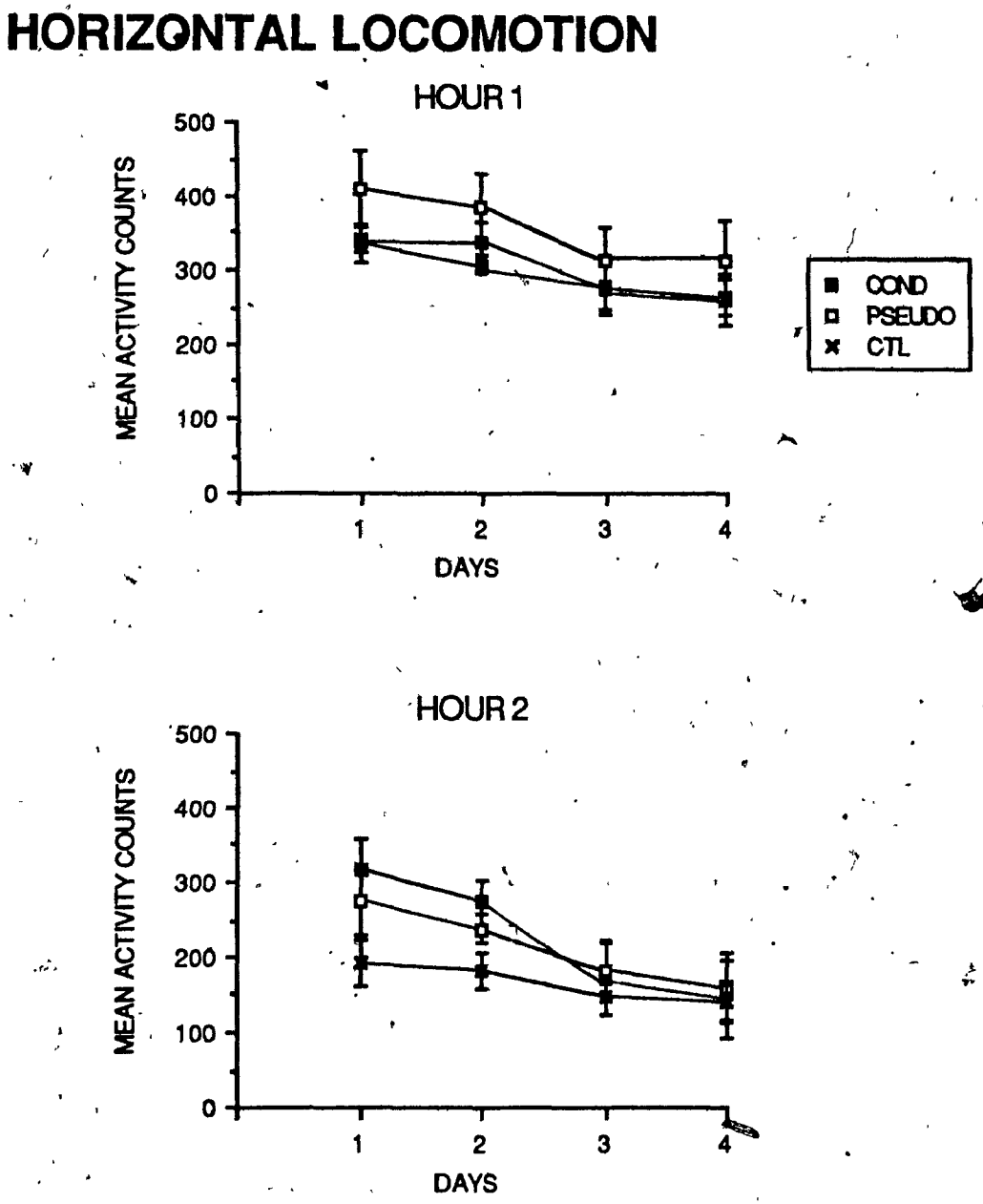


Figure 28. CONDITIONING. Mean horizontal activity counts (±1 S.E.M.) obtained in Hours 1 and 2 of the four conditioning days for each of the three groups in Experiment 5.

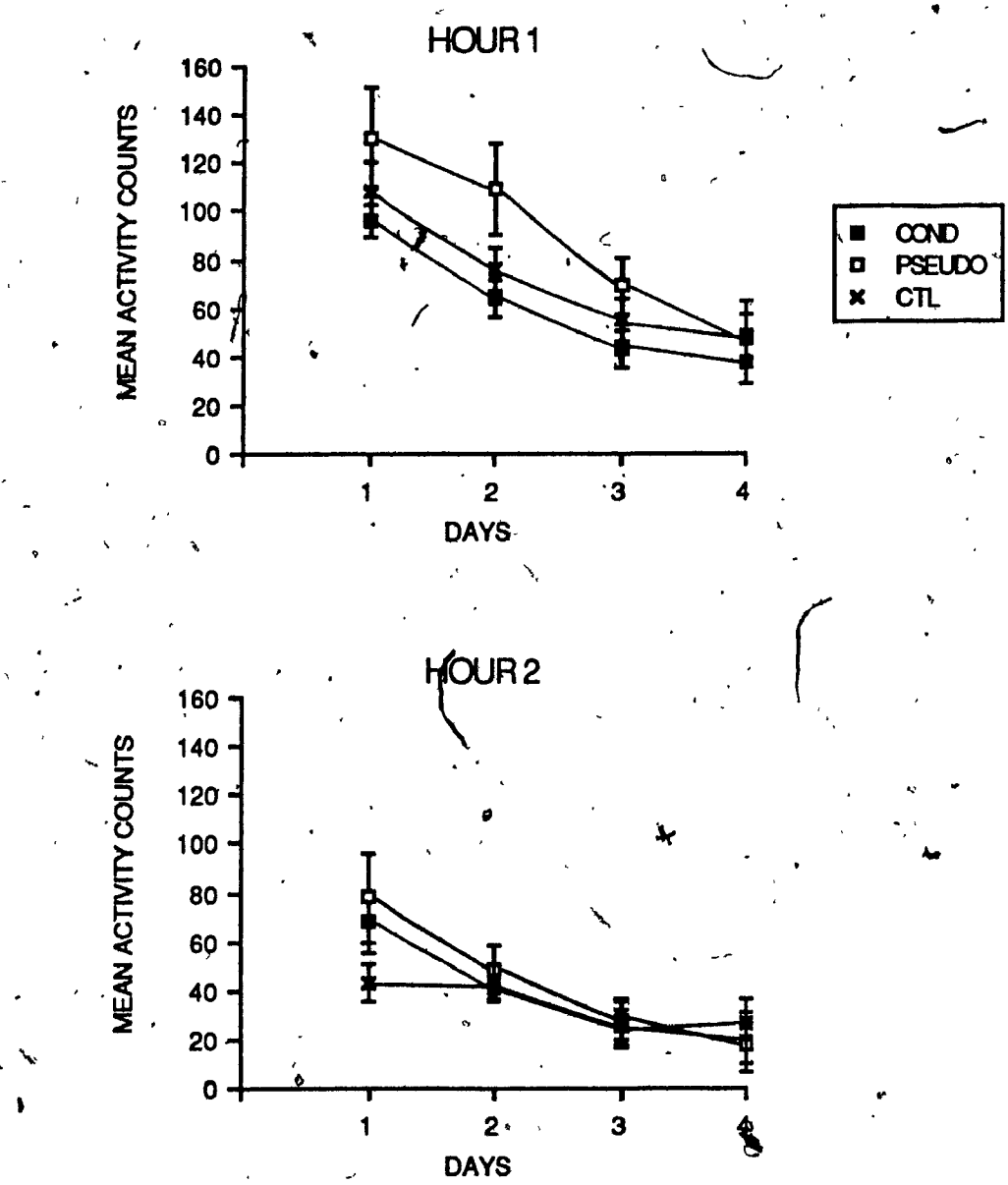


Figure 29. CONDITIONING. Mean rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the four conditioning days for each of the three groups in Experiment 5.

MORPHINE TEST

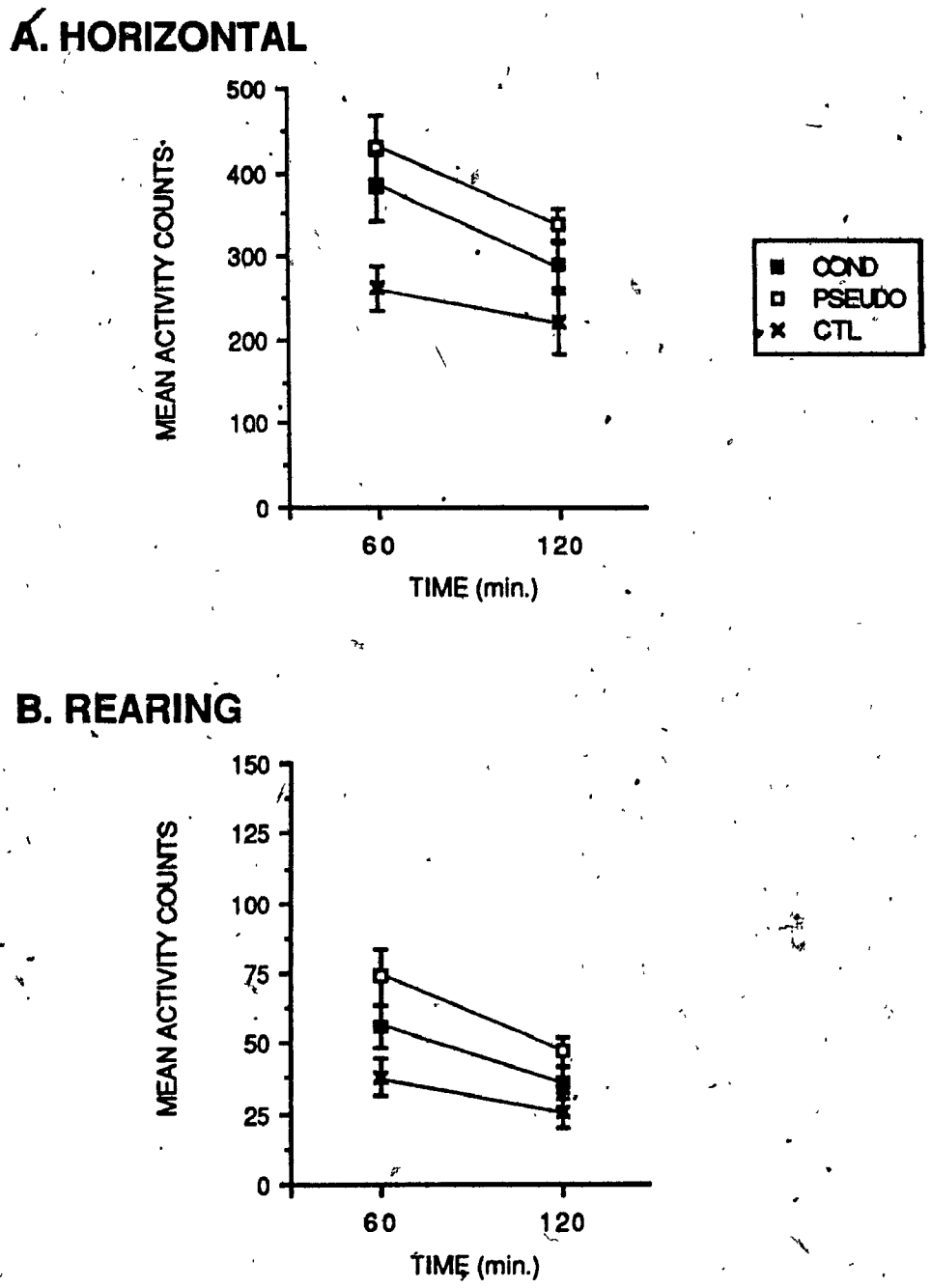


Figure 30. TEST 1. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the morphine test for environment-specific sensitization for each of the three groups in Experiment 5.

that pretreatment with intra-VTA amphetamine produced a sensitized response to morphine (Figure 30A). Because both Groups COND and PSEUDO showed the sensitized response, these findings do not show any evidence for the control of the manifestation of behavioral sensitization by the CS's. That is, sensitization was not found to be environment-specific. The ANOVA confirmed these observations. A significant effect of groups was found [$F(2,24)=6.84$, $p<0.005$] and post hoc comparisons showed that both Groups COND and PSEUDO were significantly more active than Group CTL in Hour 1 (p's<0.05 and 0.01, respectively). Only Group PSEUDO differed significantly from Group CTL in Hour 2 ($p<0.05$). Groups COND and PSEUDO did not differ significantly from each other.

Similar results were obtained with rearing (Figure 30B). A significant effect of groups was found [$F(2,24)=5.29$, $p<0.013$] and post hoc comparisons confirmed that Groups COND and PSEUDO did not differ significantly from each other but, combined, showed significantly more rearing than Group CTL ($p<0.05$).

Test 2: Saline Test for Conditioning.

Figure 31 shows the group mean horizontal activity and rearing counts obtained on the test for conditioning, in which all animals were tested after receiving an injection of saline. It can be seen that in neither measure were group differences apparent, suggesting that conditioning did not accrue with repeated pairings of the CS and intra-VTA amphetamine administrations. The ANOVA conducted on the horizontal activity data and that conducted on the rearing data both indicated only a

SALINE TEST

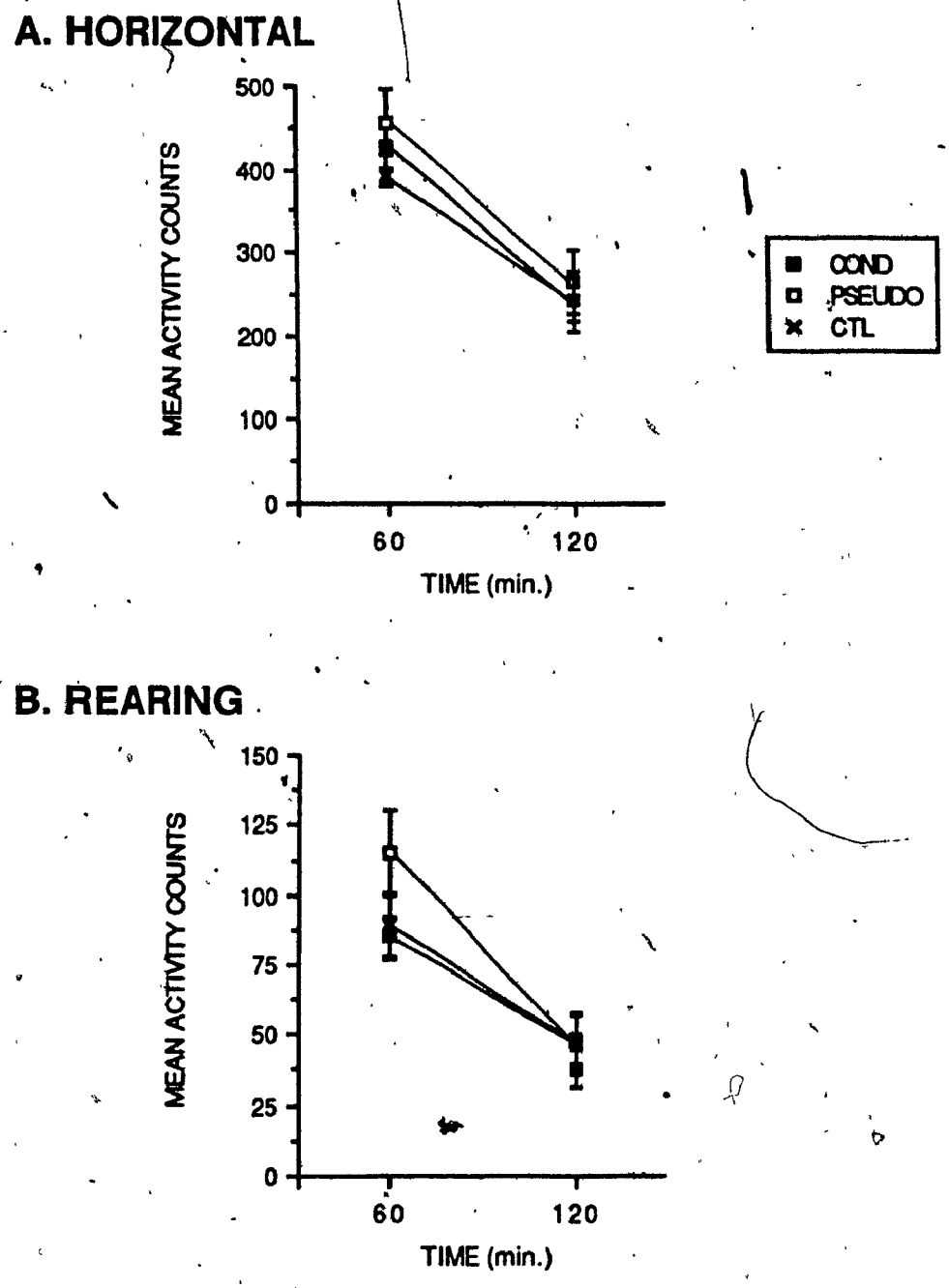


Figure 31. TEST 2. Mean (A) horizontal activity and (B) rearing counts (±1 S.E.M.) obtained on the saline test for conditioning for each of the three groups in Experiment 5.

significant effect of time.

Discussion

The results of the present experiment, with intra-VTA amphetamine, replicate those reported by Kalivas and Weber (submitted). These findings, together with those indicating that repeated intra-NAC injections of amphetamine do not produce behavioral sensitization (Dougherty and Ellinwood, 1981; Kalivas and Weber, submitted; Experiment 6), support the view that the site of amphetamine action critical for the development of behavioral sensitization to amphetamine is the VTA. As reviewed in the Introduction, the repeated administration of amphetamine into the VTA may produce a progressive decrease in the DA released from the A10 DA cell bodies by amphetamine (Kalivas and Duffy, submitted, a; Kalivas and Weber, submitted). This would result in decreased autoreceptor-mediated inhibition of these cells and make them more easily excited by pharmacological and environmental stimuli. The mechanism by which the repeated administration of amphetamine (or other drugs) might bring about such changes in the cell body release of DA remains to be determined.

Interestingly, conditioning did not develop in the present experiment. Thus, while amphetamine action in the VTA is sufficient to produce sensitization, it does not appear to be sufficient to produce conditioning, providing further evidence that conditioning cannot account for sensitization. Given that intra-VTA infusions of amphetamine would not be expected to stimulate DA release from mesolimbic DA neuron terminals, this

finding is consistent with others suggesting that the pairing of a CS with the postsynaptic consequences of drug-US-induced DA release is necessary for conditioning to occur (Spyraki, Fibiger and Phillips, 1982; Beninger and Hahn, 1983; Vezina and Stewart, 1984).

EXPERIMENT 6

In Experiments 3 and 4, it was found that behavioral sensitization could still be observed after the effects of stimulus control had been removed by extinction. These findings were interpreted to suggest that behavioral sensitization and conditioning do not arise from the same set of neurochemical events, but that the latter could modulate the expression of the former. The results of Experiment 5 extended this interpretation by suggesting that the site of amphetamine action responsible for the development of sensitization, but not conditioning, was the VTA, the site of the A10 DA cell bodies.

The present experiment investigated whether amphetamine action in the NAC might be responsible for the development of conditioned control of behavioral sensitization. This possibility was suggested by findings indicating that repeated administration of amphetamine into the NAC did not produce behavioral sensitization (Dougherty and Ellinwood, 1981; Kalivas and Weber, submitted), but that, if these were paired with a CS, place preference conditioning could be obtained (Carr and White, 1983, 1986). In the present experiment, therefore, animals were repeatedly administered amphetamine into the NAC and tested for conditioned locomotor activity and environment-specific behavioral sensitization to morphine and amphetamine.

Methods

Subjects

Twenty-four male Wistar rats, weighing 250-300 g on arrival, were used. Four to six days after arrival, these were stereotaxically implanted with chronic bilateral guide cannulae aimed at the NAC and positioned one mm above the final injection site. The NAC coordinates were: A/P +3.4, L ±1.5, and D/V -7.5 from skull (Pellegrino et al., 1979). The guide cannulae were angled at 10 degrees to the vertical. The remaining surgical details as well as the animal supplier, housing conditions, handling, perfusion and histology were as specified in Experiment 2.

Upon histological verification after the experiment, it was found that injector cannula tip placements fell outside the NAC in three animals. The data from these animals were, therefore, dropped from the experiment. In all remaining animals, both injector cannula tips were located in the NAC. Bilateral injector cannula tip placements are illustrated in Appendix, Figure C.

Design and Procedure

This experiment involved two phases: conditioning and testing. The testing phase consisted of three tests. Tests 1 (given during conditioning) and 2 (given after conditioning) were saline tests for conditioned locomotor activity. Tests 3 and 4 were tests for environment-specific sensitization to morphine and amphetamine, respectively.

The conditioning phase consisted of five 3-day blocks. On the first day of each block, animals were carried to the testing room, in groups of eight, administered an intra-NAC injection of

amphetamine or saline and tested in the activity boxes for two hours. On the second day, animals were given intra-NAC injections of amphetamine or saline in the colony room and immediately returned to their home cages. Animals were left undisturbed in their home cages on the third day.

Animals were randomly assigned to one of three groups depending on what injections they received on the first and second days of each block of conditioning:

Group COND (n=7) amphetamine-activity box/saline-home cage
Group PSEUDO (n=7) saline-activity box/amphetamine-home cage
Group CTL (n=7) saline-activity box/saline-home cage

D-amphetamine sulphate was dissolved in saline and administered in a dose of 2.5 µg/side in a volume of 0.5 µl/side. Saline injections were made in the same volume. Microinjection procedures were as specified in Experiment 2. The testing room and the activity boxes are described in Experiment 1.

Test 1: Saline Test for Conditioning. Test 1 was given on the first day of a 3-day block imbedded between blocks three and four of conditioning. All animals were administered an intra-NAC saline injection and tested in the activity boxes for two hours. Likewise, animals were given an intra-NAC saline injection in their home cages on the second day. On the third day, animals were left undisturbed in their home cages. Conditioning resumed on the next day with block four of conditioning.

Test 2: Saline Test for Conditioning. Test 2 was given on the day following the conditioning phase. All animals were given an i.p. injection of saline and tested in the activity boxes for two

hours. Saline, as well as all the subsequent injections were made in a 1 ml/kg volume.

Test 3: Morphine Test for Environment-Specific Sensitization. This test was given two days following Test 2. All animals were given an injection of 1.0 mg/kg morphine (i.p.) and tested in the activity boxes for two hours. Morphine sulphate was dissolved in saline.

Test 4: Amphetamine Test for Environment-Specific Sensitization. This test was given two days following Test 3. All animals were injected with 0.5 mg/kg amphetamine (i.p.) and tested in the activity boxes for two hours. Ampetamine sulphate was dissolved in saline.

The conditioning data were analyzed by 1-between 2-within ANOVA's with groups as the between factor and hours and days as the within factors. The test data were analyzed by 1-between 1-within ANOVA's. Tests for simple main effects and post hoc Scheffe comparisons were made according to Kirk (1968).

Results

Figure 32 shows the mean horizontal activity counts obtained on the five conditioning days for each of the three groups. As expected, intra-NAC injections of amphetamine produced sizable increases in activity in Group COND compared to the other two groups. These increased levels of activity were apparent only in the first hour and decreased somewhat over days but remained higher than those of the other two groups throughout conditioning. The ANOVA conducted on these data revealed significant effects of groups [$F(2,18)=10.22$, $p<0.001$], hours

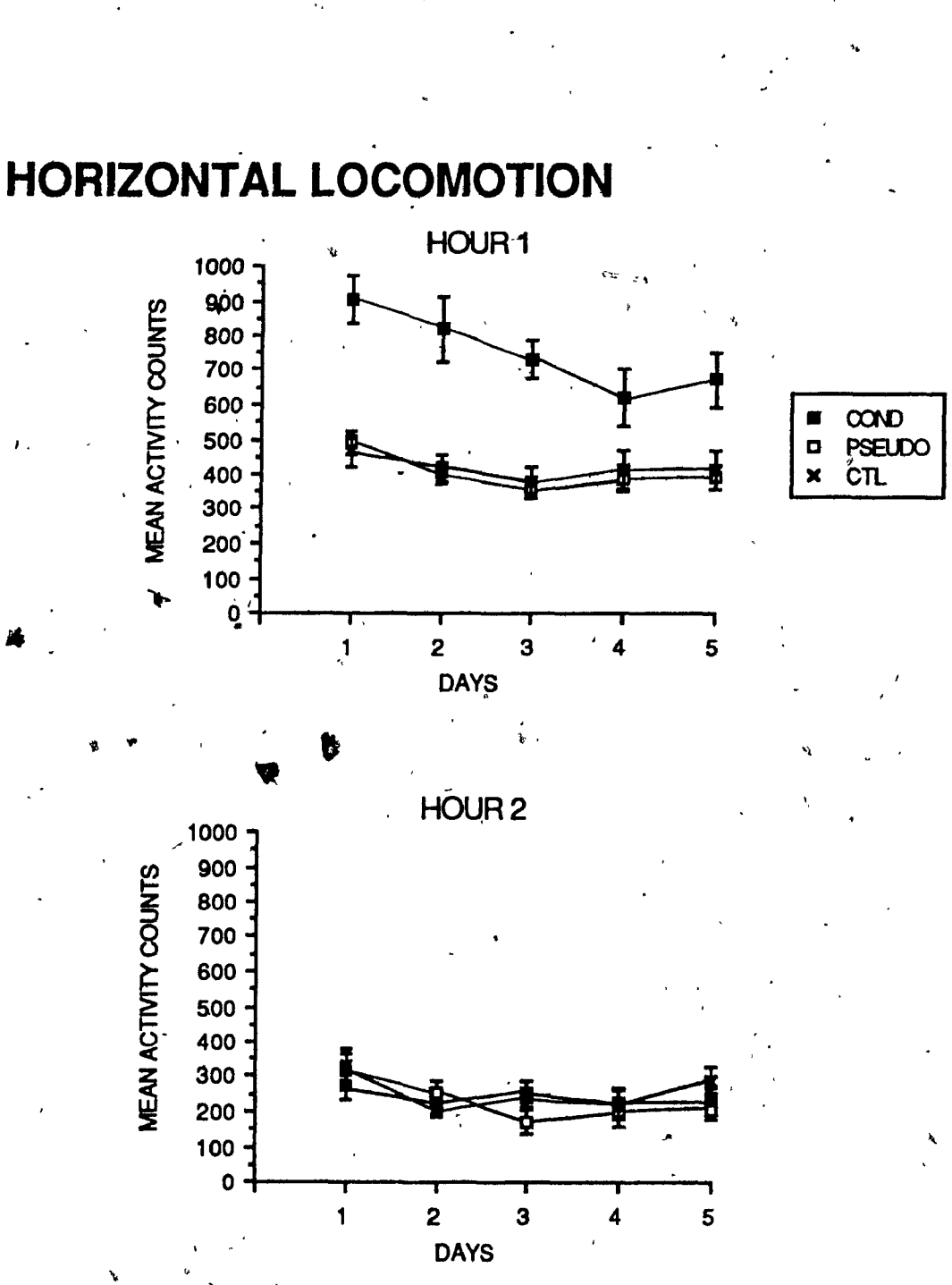


Figure 32. CONDITIONING. Mean horizontal activity counts (±1 S.E.M.) obtained in Hours 1 and 2 of the five conditioning days for each of the three groups in Experiment 6.

[F(1,18)=128.80, p<0.001], days [F(4,72)=10.16, p<0.001] and significant groups x hours [F(2,18)=20.90, p<0.001] and groups x hours x days [F(8,72)=2.96, p<0.006] interactions. Post hoc comparisons confirmed the above observations. Group COND was significantly more active than both other groups in Hour 1 (p's<0.01) but not in Hour 2. Groups PSEUDO and CTL did not differ significantly from each other. All groups were significantly less active in Hour 2 compared to Hour 1, although this decline was more considerable for Group COND (p<0.01) than the other two groups (p's<0.05). The simple main effect of days was significant in Hour 1 for Group COND (p<0.01) and barely significant for Groups PSEUDO and CTL (p's<0.05).

Similar results were obtained with rearing (Figure 33). Again, significant effects of groups [F(2,18)=9.19, p<0.002], hours [F(1,18)=71.95, p<0.001], days [F(4,72)=19.33, p<0.001] and significant groups x hours [F(2,18)=18.47, p<0.001] and groups x hours x days [F(8,72)=3.60, p<0.001] interactions were found. Post hoc comparisons confirmed that Group COND showed significantly higher levels of rearing than both other groups in Hour 1 (p's<0.01) but not in Hour 2. Groups PSEUDO and CTL did not differ significantly from each other. Only Group COND reared significantly more in Hour 1 than in Hour 2 (p<0.01). The simple main effect of days in Hour 1 was significant for all groups (p's<0.01). However, the decline in rearing levels over days was much more considerable for Group COND, accounting for the significant groups x hours x days interaction.

REARING

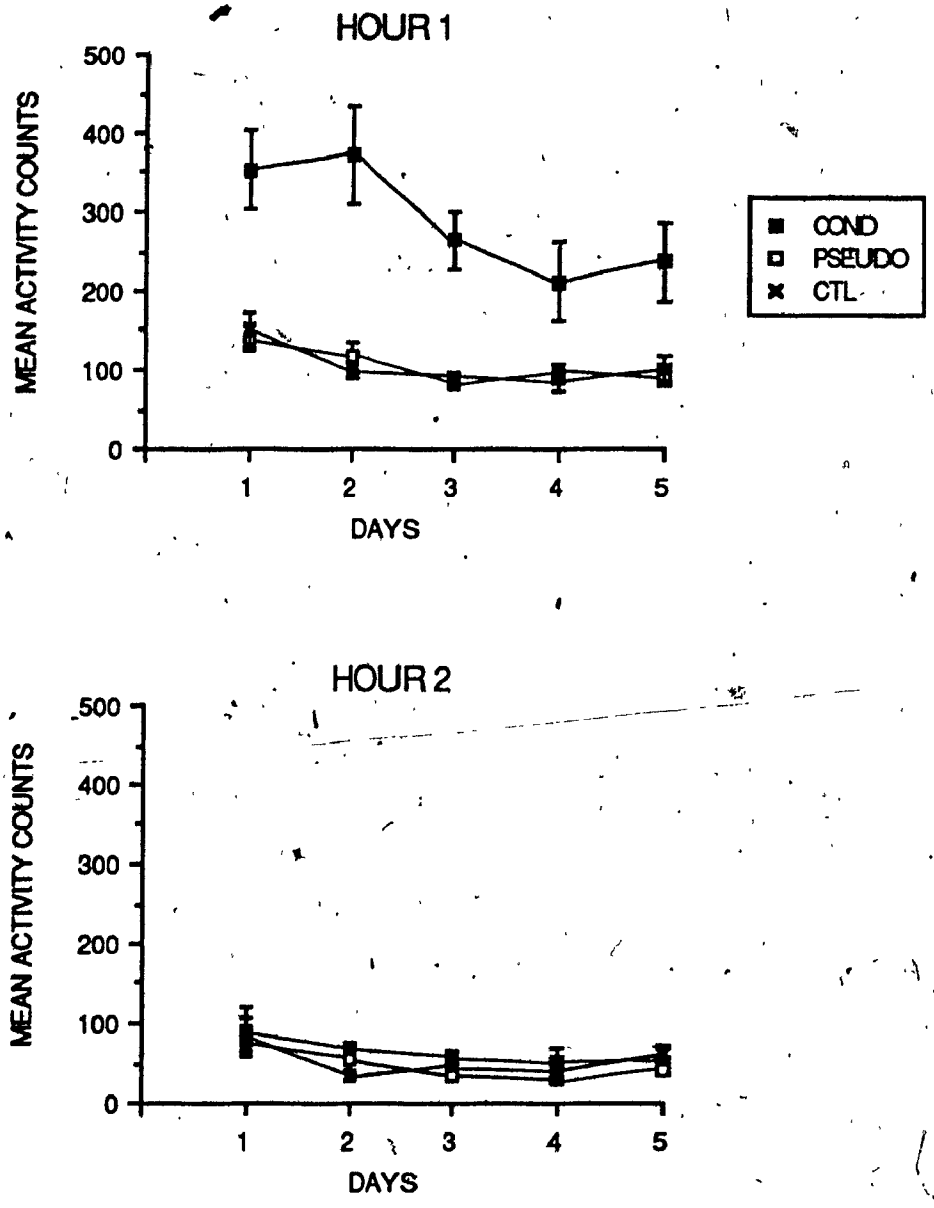


Figure 33. CONDITIONING. Mean rearing counts (±1 S.E.M.) obtained in Hours 1 and 2 of the five conditioning days for each of the three groups in Experiment 6.

Test 1: Saline Test for Conditioning.

The results of this test, given during conditioning and in which all animals were tested after receiving an intra-NAC injection of saline, are shown in Figure 34. It is clear that no differences between groups indicative of conditioning are present. The ANOVA's conducted on the horizontal activity and rearing data indicated only significant effects of time.

Test 2: Saline Test for Conditioning.

Figure 35 shows the results of the second test for conditioning, given after the conditioning phase and in which all animals were tested after receiving an i.p. injection of saline. Again, no differences between groups indicative of conditioning are apparent. Only the time effect was significant in both measures.

Test 3: Morphine Test for Environment-Specific Sensitizaton.

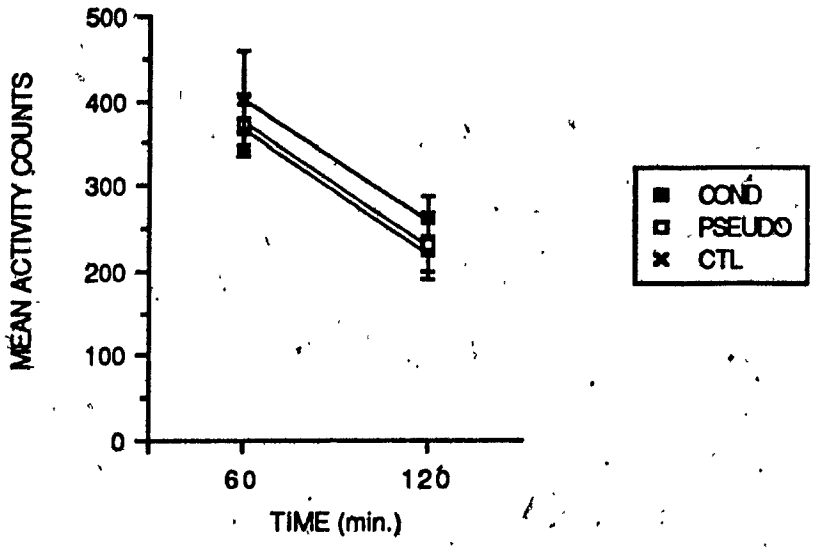
The results of this test, in which all animals were tested after receiving an i.p. injection of morphine, are shown in Figure 36. As can be seen, there is no evidence for sensitization in either measure. Not surprisingly, given the results of Tests 1 and 2, there is also no evidence for environmental specificity of activity. Both ANOVA's again revealed only significant effects of time.

Test 4: Amphetamine Test for Environment-Specific Sensitization.

Figure 37 shows the results of the final test, in which all animals were tested after receiving an i.p. injection of amphetamine. Again, no differences between groups indicative of sensitization or environmental specificity can be seen. Group COND did show somewhat higher levels of rearing than the other

SALINE TEST

A. HORIZONTAL



B. REARING

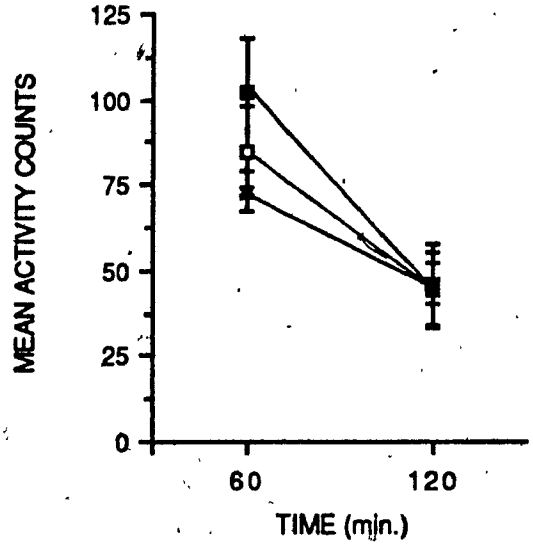
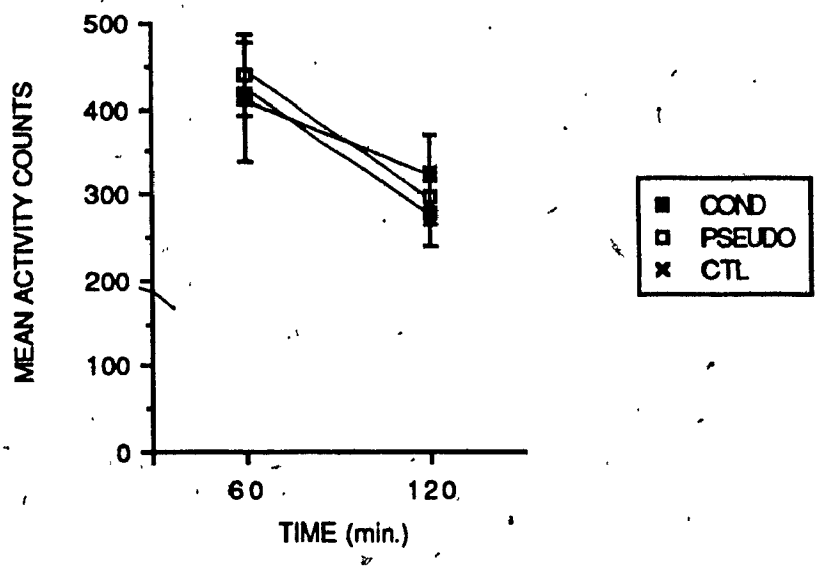


Figure 34. TEST 1. Mean (A) horizontal activity and (B) rearing counts obtained on the first saline test for conditioning for each of the three groups in Experiment 6.

SALINE TEST

A. HORIZONTAL



B. REARING

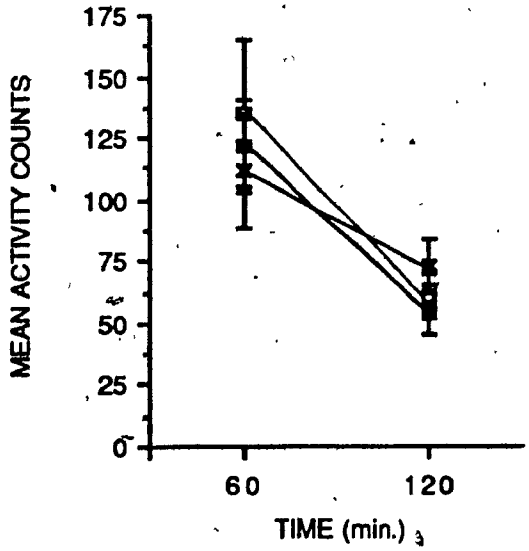


Figure 35. TEST 2. Mean (A) horizontal and (B) rearing counts obtained on the second saline test for conditioning for each of the three groups in Experiment 6.

MORPHINE TEST

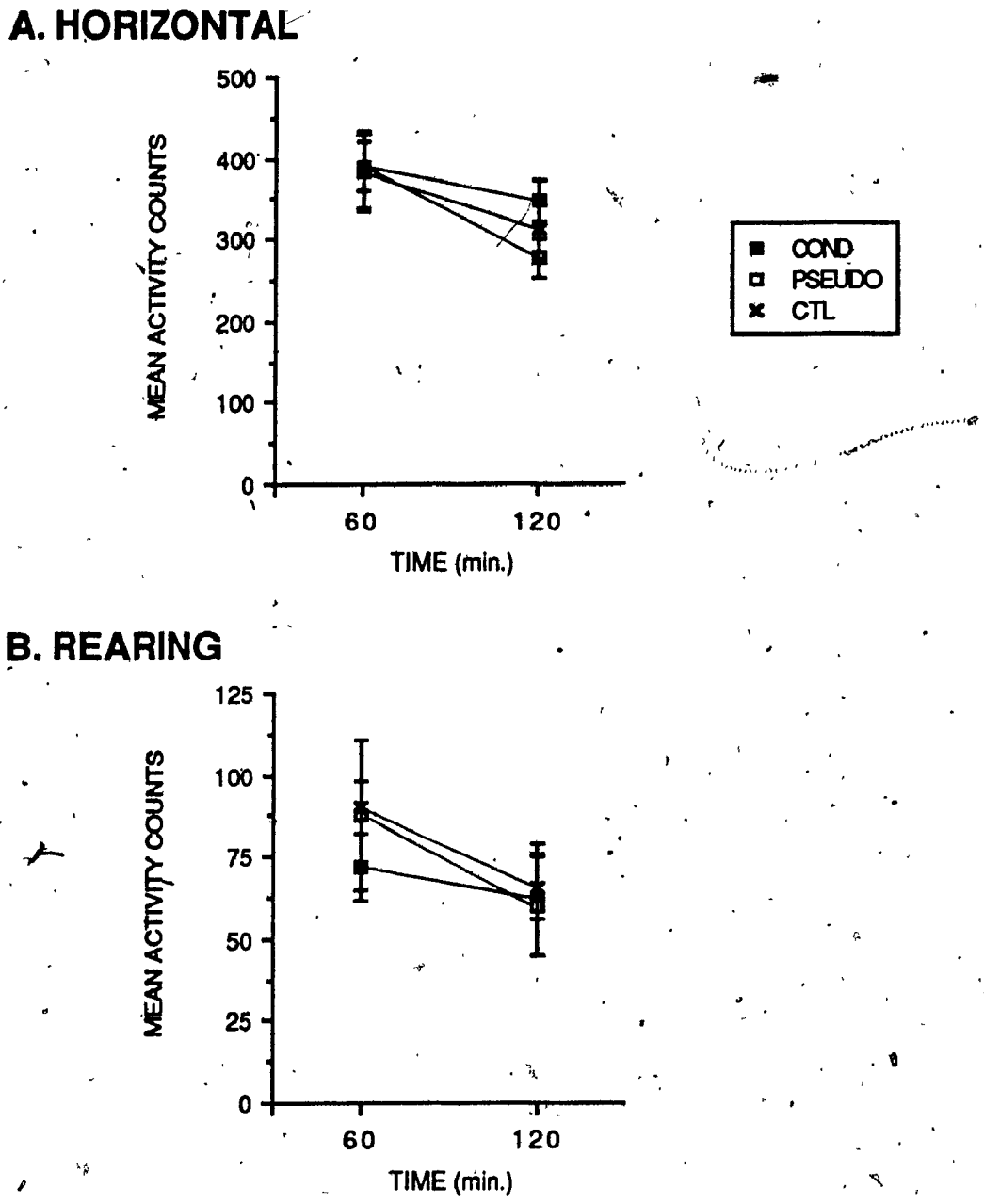


Figure 36. TEST 3. Mean (A) horizontal and (B) rearing counts obtained on the morphine test for environment-specific sensitization for each of the three groups in Experiment 6.

AMPHETAMINE TEST

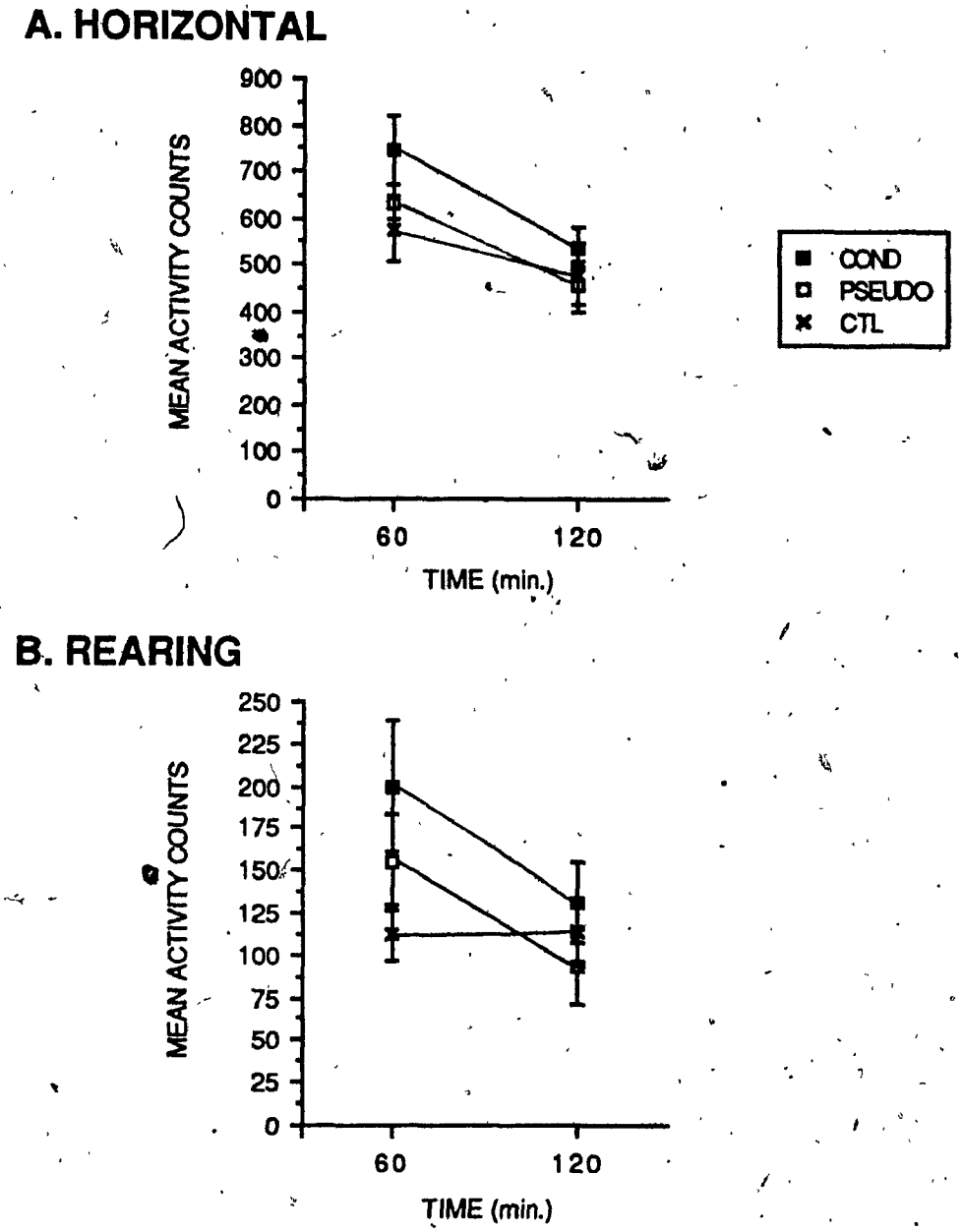


Figure 37. TEST 4. Mean (A) horizontal and (B) rearing counts obtained on the amphetamine test for environment-specific sensitization for each of the three groups in Experiment 6.

two groups, but scores were highly variable. Again, both ANOVA's yeilded only significant effects of time.

Discussion

The results of the present experiment, showing that repeated injections of amphetamine into the NAC did not produce behavioral sensitization, confirmed the similar findings of Dougherty and Ellinwood (1981) and Kalivas and Weber (submitted). These findings, together with those reviewed and obtained in Experiment 5, suggest that the site of amphetamine action critical for the development of behavioral sensitization is the VTA and not the NAC.

Such a conclusion might appear to ignore the findings obtained from in vitro DA release experiments. As reviewed in the Introduction, these studies suggest that changes at DA neuron terminals (increased DA release in response to superfused amphetamine), independent of cell body changes, underlie behavioral sensitization. What the results of the present experiment and those cited above do make clear, however, is that amphetamine action in the NAC is not responsible for the development of these changes at DA neuron terminals. These may be brought about by the consequences of amphetamine action at other sites. Indeed, it would be interesting to determine whether pretreatment with intra-VTA amphetamine would result in sensitized amphetamine-induced levels of DA release from NAC tissue, in vitro.

The surprising finding in the present experiment was that no

evidence for conditioning was obtained in any of the tests despite the fact that the intra-NAC injections of amphetamine produced considerable amounts of locomotor activity during training. This finding, suggesting that the repeated amphetamine-induced release of DA in the NAC is not sufficient to produce conditioning, would appear to be at odds with the finding that it is capable of producing conditioned place preference (Carr and White, 1983, 1986). There is no clear explanation for this discrepancy. It may be that conditioned activity is dependent on a different substrate from conditioned place preference, a possibility not without precedent (see Kucharski, Johanson and Hall, 1986; Durivage and Miliaressis, 1987). Thus, while intra-NAC injections may be sufficient to produce conditioned place preference, the recruitment of additional systems may be necessary for the conditioning of locomotor activity. The finding, for example, that both conditioned activity (Vezina and Stewart, 1984) and conditioned place preference (Phillips and LePiane, 1980) are produced by repeated intra-VTA injections of morphine may reflect the action of released DA at several mesolimbic DA neuron terminals.

This suggestion, that the development of conditioned place preference and conditioned locomotion may involve different or overlapping mechanisms, is not necessarily inconsistent with the view that both conditioned effects reflect the rewarding properties of drugs (Bindra, 1968; Iversen, 1983; Stewart, deWit and Eikelboom, 1984; Stewart and Eikelboom, 1987). Rather, it is consistent with the different but overlapping classes of behavior seen in each effect: approach and the maintenance of sensory

contact in conditioned place preference and approach and behavioral excitation in conditioned locomotion (see Vezina and Stewart, 1987).

GENERAL DISCUSSION

The aim of the experiments reported in this thesis was to investigate some of the processes that have been proposed to account for the development and expression of sensitization to the locomotor activating effects of amphetamine and morphine. Three main areas were explored: the role played by DA autoreceptors in behavioral sensitization to amphetamine and morphine, the role of conditioning in the manifestation of behavioral sensitization to these two drugs, and the neuroanatomical site critical for the development of conditioning and behavioral sensitization to amphetamine.

No support was found for the view that subsensitive D-2 DA autoreceptors are involved, either directly or indirectly, in the development of sensitization to the locomotor activating effects of amphetamine and morphine. In Experiment 1, it was found that none of the D-2 DA receptor antagonists tested attenuated the development of behavioral sensitization to amphetamine. In Experiment 2, two of these three D-2 antagonists were without effect on the development of behavioral sensitization to intra-VTA morphine. Curiously, the D-2 DA antagonist, pimozide, blocked the development of sensitization to intra-VTA morphine although it had no effect on the development of sensitization to amphetamine. Conversely, the D-1 DA receptor antagonist, SCH-23390, blocked the development of sensitization to amphetamine but was without effect on the development of sensitization to intra-VTA morphine.

In Experiments 3 and 4, it was found that the expression of behavioral sensitization to amphetamine and morphine can come under strong stimulus control and that this control is reduced by extinction training. This procedure, however, did not cause sensitization to be eliminated. Thus, while conditioning factors can play an important role in the expression of behavioral sensitization to pyschoactive drugs, sensitization can still be observed when evidence for conditioned drug effects is absent.

The neuroanatomical site critical for the development of behavioral sensitization to amphetamine appears to be the VTA and not the NAC. In Experiment 5, it was found that pretreatment with intra-VTA injections of amphetamine sensitized animals to subsequent systemic injections of morphine, whereas, Experiment 6 showed that pretreatment with intra-NAC injections was without effect. No evidence of either environment-specific sensitization or conditioned stimulus control of activity was found when amphetamine injections to either the VTA or the NAC were paired with a specific environment.

Dopamine Receptors and Behavioral Sensitization

The main purpose of Experiments 1 and 2 was to test the autoreceptor subsensitivity hypothesis of behavioral sensitization. The results obtained in these experiments clearly do not support this hypothesis nor do they support the possibility that DA autoreceptor changes may be indirectly involved in the development of sensitization. The results obtained with SCH-23390 and pimozide were surprising, however, and in need of explanation.

The finding, in Experiment 1, that SCH-23390 blocked the

development of sensitization to amphetamine, together with the finding of Barnett et al. (1987) that preexposure to amphetamine produces desensitization of D-1 DA receptors postsynaptic to DA cell terminals, would seem to suggest that subsensitive postsynaptic D-1 DA receptors may be responsible for behavioral sensitization by reducing the inhibition of mesencephalic DA cells via feedback pathways (see Introduction). This possibility appears unlikely, however, in view of the finding, in Experiment 2, that SCH-23390 had no effect on the development of sensitization to intra-VTA morphine. Further, the finding, in Experiment 5, that intra-VTA injections of amphetamine could produce behavioral sensitization is also difficult to interpret in terms of concurrently developing postsynaptic D-1 DA receptor desensitization, since these injections would not be expected to produce increased release of DA from DA neuron terminals. Finally, in Experiment 6, it was found that intra-NAC injections of amphetamine, which do produce increased release of DA from DA neuron terminals and would thus provide the conditions for postsynaptic D-1 DA receptor desensitization, did not produce behavioral sensitization.

D-1 DA receptors have also been shown to be located presynaptically on GABAergic afferent terminals in the substantia nigra, and it has been suggested that DA released from mesencephalic DA cell dendrites could modulate, via these receptors, the inhibition of nigral cells by GABA (Matthews and German, 1986; Porceddu et al., 1986). How these D-1 DA receptors might be involved in the development of behavioral sensitization

to amphetamine and, thus, how SCH-23390 might act at this site to prevent the development of sensitization remains, however, an open question. Moreover, the possibility that it is D-1 DA receptors that are involved is brought into question by the lack of effect of SCH-23390 on the development of sensitization to intra-VTA morphine.

Finally, the finding that pimozide blocked the development of behavioral sensitization to intra-VTA morphine is difficult to interpret in terms of its blockade of D-2 DA receptors since the other two D-2 DA receptor antagonists tested were without effect on the development of sensitization to intra-VTA morphine. This suggests that some other action of pimozide, unrelated to its effect on D-2 DA receptors, may be responsible for the findings obtained.

Whatever the exact mode of action of these two compounds, their differential effect on the development of behavioral sensitization to amphetamine and morphine suggests that the mechanisms underlying the development of sensitization to these two drugs differ, even though these ultimately produce similar changes in the activity of mesencephalic DA neurons. It has been suggested, for example, that the decreased release of DA from mesencephalic DA cell bodies in response to pharmacological challenge is the enduring change in DA neuron function that is critical for the expression of behavioral sensitization to amphetamine and morphine (Kalivas and Duffy, submitted, a). Whether, and if so, how, SCH-23390 and pimozide (and other DA receptor antagonists) might influence the development of this change in the release of DA from mesencephalic DA cell bodies

remains to be determined.

Conditioning and Behavioral Sensitization

As reviewed in the Introduction, much of the literature concerned with the relation between conditioning and sensitization has, unfortunately, been concerned with demonstrating whether conditioning does or does not account for behavioral sensitization (e.g., Robinson and Becker, 1986). The results of Experiments 3 and 4 of the present thesis suggest that a more fruitful approach to gaining an understanding of environment-specific control of the expression of sensitization might be to study the relation between these two phenomena.

Two aspects of the results of these two experiments are important in this regard. First, the expression of behavioral sensitization can come under strong stimulus control and this control is subject to procedures that affect conditioning phenomena (i.e., extinction). Second, the sensitization of drug-induced behaviors can be observed when evidence for conditioned drug effects is absent. Further evidence that conditioning cannot account for sensitization was obtained in Experiment 5 where it was found that amphetamine action in the VTA is sufficient to produce sensitization but not conditioning.

Although conditioning may not explain or cause the development of sensitization, the demonstration that it is able to control the manifestation of behavioral sensitization to the extent of completely preventing its expression illustrates dramatically that the relation between the two is not trivial. There are several reasons why an understanding of the relation

between these two phenomena would be beneficial.

First, it would seem that to gain an understanding of how environmental stimuli achieve such strong control over the expression of sensitization may provide some insights into the basis of sensitization itself. At the very least, any mechanism proposed to account for sensitization must have as one of its requirements the provision whereby such stimulus control could occur.

Second, there is an extensive literature indicating an important, although not completely understood, role for environmental stimuli in the expression of the acute effects of pyschoactive drugs (for a review, see Wise and Bozarth, in press). What effect might prior sensitization to a drug have on how an animal interacts with such stimuli especially if previous drug exposures have been paired with these stimuli?

Third, it has been suggested that the rewarding properties of psychoactive drugs may also show sensitization (Gaiardi, Bartoletti, Gubellini, Bacchi and Babbini, 1986; Mansky, 1978). This possibility, together with the demonstration that environmental stimuli can exert strong control over the expression of sensitization (i.e., environment-specific sensitization), could have important implications for the role of unconditioned and conditioned drug effects in the self-administration of drugs (see Stewart et al., 1984).

As discussed in the Introduction, the conditioning of drug effects is widely believed to reflect the acquisition by the CS+ of the ability to elicit, in the absence of the US, effects similar to those originally produced by the drug US (see Stewart

and Eikelboom, 1987). It should also be clear from the Introduction, however, that the relation of the CS+ to behavioral sensitization is not well understood. If the additivity of CS+ elicited conditioned effect and initial unconditioned drug effect is incapable of accounting for behavioral sensitization, then what might be the role of the CS+ in sensitization? One possibility may be that it is directive. For example, it has been shown that water deprived rats, administered the stimulant pipradol (Robbins, 1976) or intra-NAC injections of amphetamine (Taylor and Robbins, 1984, 1986), will press a lever that produces a light previously associated with water (but not an inactive lever) more often than rats administered a vehicle injection. These investigators concluded that motivationally significant stimuli (CS's) can direct the manifestation of the behavioral effects of a drug. Interestingly, Robbins (1976) suggested that the lever directed responses of rats injected with pipradol were part of a stereotyped pattern of behavior induced by the drug, and, vice versa, that stereotyped behavior might even arise from the persistent directing of behavior to the lever stimuli.

If the drug experience were the event associated with a CS (as in drug conditioning and sensitization experiments), then it might be expected that the drug would come to potentiate the directing effects of this very stimulus. Beck, Chow and Cooper (1986), for example, found that rats administered a high dose of amphetamine engaged in stereotyped behaviors directed at those stimuli afforded by the testing apparatus. When new stimuli were

subsequently added to the testing apparatus, these rats did not respond to them, but persevered in stereotyped behavior directed at the initial stimuli. Although these data are based on a single drug administration, they support, together with the findings of Robbins and his colleagues, the possibility that the CS+ may have the effect of directing sensitized responding. The results of Experiments 3 and 4, showing that, although extinction training reduced the ability of the CS+ to elicit conditioned activity, it did not significantly reduce behavioral sensitization, suggest that the CS+ does not contribute to the augmented responding seen in sensitization. It would be interesting to determine, therefore, whether the presence or absence of the opportunity for conditioning during the development of behavioral sensitization would produce any differences in the manner in which animals subsequently manipulate environmental stimuli on a test for sensitization.

As outlined in Experiments 3 and 4, the expression of behavioral sensitization appears to be inhibited by a CS that has been paired repeatedly with the non-occurence of the drug (CS-). Extinction of the CS-, as was found in these experiments, would reduce the inhibition exerted on the sensitized response to the drug, making it more visible in the presence of the CS-. Such results suggest that a CS- may be able to modulate the "pharmacological" effectiveness of the drug itself (i.e., modulate the US properties of the drug). If this were the case, then one could imagine that the effectiveness of the inhibitory properties of the CS- would be determined by the potency of the US. If, for example, a high dose of a drug were administered on a

test for sensitization, it might be that the inhibitory properties of the CS- would be insufficient to overcome the direct US action of the drug. Preliminary experiments with amphetamine conducted in this laboratory have found, for example, that when a 1.0 mg/kg training dose was used to test animals for environment-specific sensitization, variable results were obtained: in some experiments, environment-specific sensitization was observed, in others, not. If, in fact, the test US overwhelms the influence of the CS-, it might explain some of the reported failures to obtain environment-specific sensitization even when conditioning procedures have been followed. In the present experiments, to avoid this potential interference between the effects of CS's and drug action in the manifestation of environment-specific sensitization, the test dose used was half that used in training. The potential relationship between training dose, test dose, and the manifestation of environment-specific sensitization obviously needs to be explored more fully.

Finally, it is tempting to speculate about the neural substrates that might mediate these directing and inhibitory effects of CS+ and CS-. For example, it has been suggested that the neuronal associations necessary for the conditioning of dopaminergic activity occur "beyond the dopaminergic neurons" (Moller et al., 1987). The thalamo-cortico-striatal neuronal loop described separately by both Glowinski and colleagues and Phillipson and colleagues (see Introduction) is particularly attractive in this regard. The activation of this loop has been shown to be capable of facilitating and reducing the release of

DA from DA neuron terminals in the caudate nucleus (see Cheramy et al., 1986). Further, neurons in different nuclei in this loop have been implicated in the mediation of learning of different contingencies in operant conditioning (Sakurai and Hirano, 1983). Although all the elements of a biological system that would provide for the differential appetitive and inhibitory effects of CS+ and CS- on dopaminergic activity are evidently not known, the above findings suggest that the neuronal processes capable of mediating these effects exist. Furthermore, conditioned changes in dopaminergic activity have been reported following CS-drug US pairings. For example, increases in DA turnover have been reported to be elicited by CS's previously paired with morphine (Perez-Cruet, 1976) and amphetamine (Schiff, 1982). And, increases in the single-unit activity of mesencephalic DA cells have been found to be elicited by a CS previously paired with a gustatory US (Miller, Sanghera and German, 1981). It would be interesting to determine what relation thalamo-cortico-striatal fibers might have to these conditioned effects.

Rearing

In preliminary studies conducted in this laboratory, it was found that horizontal activity and rearing were not always affected in the same way by experimental manipulations intended to either increase or decrease locomotor activity. The results obtained in the present experiments confirmed these observations and support the recommendation that the two behaviors be measured separately, especially when the effect of repeated drug infusions is the object of study.

In Experiments 3 and 4 (but not 1), systemic injections of

amphetamine and morphine both produced increased levels of rearing as well as increased horizontal activity. Furthermore, both produced conditioned rearing, results similar to those obtained with horizontal activity. However, on the tests for sensitization, results obtained with rearing diverged from those obtained with horizontal activity. For example, in the experiment with morphine, no evidence for sensitization of rearing was found in either drug group. On the other hand, although amphetamine produced increases in rearing that paralleled the environment-specific sensitization of horizontal activity, it was found that extinction training had no effect on the environment-specificity of the sensitized rearing levels seen in the pre-extinction test.

Finally, while intra-VTA morphine did not produce sensitization of rearing in Experiment 2 (see also Kalivas et al., 1985), in Experiment 5, a systemic injection of morphine produced sensitized levels of rearing in animals pretreated with intra-VTA amphetamine (see also Kalivas and Weber, submitted).

There is no clear explanation for these findings. Although some have suggested that drug-induced horizontal activity and rearing are dissociable (Itoh, Murai, Yoshida, Masuda, Saito and Chen, 1987; Mazurski and Beninger, 1987), no basis for such a dissociation was proposed.

REFERENCES

Ahlenius, S., Hillegaart, V., Thorell, G., Magnusson, O. and Fowler, C.J. Suppression of exploratory locomotor activity and increase in dopamine turnover following the local application of cis-flupenthixol into limbic projection areas of the rat striatum. Brain Research, 1987, 402, 131-138.

Alloway, K.D. and Rebec, G.V. Apomorphine-induced inhibition of neostriatal activity is enhanced by lesions induced by 6-hydroxydopamine but not by long-term administration of amphetamine. Neuropharmacology, 1984, 23, 1033-1039.

Amalric, M. and Koob, G.F. Low doses of methylnaloxonium in the nucleus accumbens antagonize hyperactivity induced by heroin in the rat. Pharmacology Biochemistry & Behavior, 1985, 23, 411-415.

Anden, N.-E., Grabowska-Anden, M. and Liljenberg, B. On the presence of autoreceptors on dopamine neurons in different brain regions. Journal of Neural Transmission, 1983, 57, 129-137.(a)

Anden, N.-E., Grabowska-Anden, M. and Liljenberg, B. Demonstration of autoreceptors on dopamine neurons in different brain regions of rats treated with gammabutyrolactone. Journal of Neural Transmission, 1983, 58, 143-152.(b)

Antelman, S.M. and Chiodo, L.A. Dopamine autoreceptor subsensitivity: A mechanism common to the treatment of depression and the induction of amphetamine psychosis? Biological Psychiatry, 1981, 16, 717-727.

Antelman, S.M. and Chiodo, L.A. Amphetamine as a stressor. In I. Creese (Ed.), Stimulants: Neurochemical, behavioral, and clinical perspectives. New York: Raven Press, 1983.

Antelman, S.M., Eichler, A.J., Black, C.A. and Kocan, D. Interchangeability of stress and amphetamine in sensitization. Science, 1980, 207, 329-331.

Archer, T., Fredriksson, A., Jonsson, G., Lewander, T., Mohammed, A.K., Ross, S.B. and Soderberg, U. Central noradrenaline depletion antagonizes aspects of d-amphetamine-induced hyperactivity in the rat. Psychopharmacology, 1986, 88, 141-146.

Babbini, M. and Davis, W.M. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. British Journal of Pharmacology, 1972, 46, 213-224.

Babbini, M., Gaiardi, M. and Bartoletti, M. Persistence of chronic morphine effects upon activity in rats 8 months after ceasing the treatment. Neuropharmacology, 1975, 14, 611-614.

Bailey, R.C. and Jackson, D.M. A pharmacological study of changes in central nervous system receptor responsiveness after long-term dexamphetamine and apomorphine administration. Psychopharmacology, 1978, 56, 317-326.

Bardo, M.T. and Neisewander, J.L. Single-trial conditioned place preference using intavenous morphine. Pharmacology Biochemistry & Behavior, 1986, 25, 1101-1105.

Barnett, J.V., Segal, D.S. and Kuczenski, R. Repeated amphetamine pretreatment alters the responsiveness of striated dopamine-stimulated adenylate cyclase to amphetamine-induced desensitization. The Journal of Pharmacology and Experimental Therapeutics, 1987, 242, 40-47.

Bartoletti, M., Gaiardi, M., Gubellini, G, Bacchi, A. and Babbini, M. Long-term sensitization to the excitatory effects of morphine. Neuropharmacology, 1983, 22, 1193-1196.

Beck, C.H.M., Chow, H.L. and Cooper, S.J. Initial environment influences amphetamine-induced stereotypy: Subsequently environment change has little effect. Behavioral and Neural Biology, 1986, 46, 383-397.

Beninger, R.J. and Hahn, B.L. Pimozide blocks establishment but not expression of amphetamine-produced environment-specific conditioning. Science, 1983, 220, 1304-1306.

Biggio, G., Casu, M., Corda, M.G., Di Bello, C. and Gessa, G.L. Stimulation of dopamine synthesis in caudate nucleus by intrastriatal enkephalins and antagonism by naloxone. Science, 1978, 200, 552-554.

Bindra, D. Neuropsychological interpretation of the effects of drive and incentive-motivation on general activity and instrumental behavior. Psychological Review, 1968, 75, 1-22.

Bischoff, S., Heinrich, M., Sonntag, J.M. and Krauss, J. The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT2) receptors. European Journal of Pharmacology, 1986, 129, 367-370.

Bozarth, M.A. and Wise, R.A. Dissociation of the rewarding and physical dependence-producing properties of morphine. In L.S. Harris (Ed.), Problems of drug dependence, 1981. Rockville, Maryland: National Institute on Drug Abuse, 1983.

Brady, L.S. and Holtzman, S.G. Locomotor activity in morphine-dependent and post-dependent rats. Pharmacology Biochemistry and Behavior, 1981, 14, 361-370.(a)

Brady, L.S. and Holtzman, S.G. Effects of intraventricular morphine and enkephalins on locomotor activity in non-dependent, morphine-dependent and post-dependent rats. The Journal of Pharmacology and Experimental Therapeutics, 1981, 218, 613-620.(b)

Broekkamp, C.L.E., Phillips, A.G. and Cools, A.T. Stimulant effects of enkephalin injection into the dopaminergic A10 areas. Nature (London), 1979, 278, 560-562.

Broekkamp, C.L., LePichon, M. and Lloyd, K.G. Akinesia after local[illegible]pplied morphine near the nucleus raphe pontis of the ra[illegible]euroscience Letters, 1984, 50, 313-318.

Browne, R.G. and Segal, D.S. Metabolic and experiential factors in the behavioral response to repeated amphetamine. Pharmacology Biochemistry & Behavior, 1977, 6, 545-552.

Bunney, W.C., Massari, V.J. and Pert, A. Chronic morphine-induced hyperactivity in rats is altered by nucleus accumbens and ventral tegmental lesions. Psychopharmacology, 1984, 82, 318-321.

Cancela, L.M. and Molina, V.A. Reduced apomorphine-induced sedation following chronic stress. European Journal of Pharmacology, 1986, 134, 117-119.

Carey, R.J. Conditioned rotational behavior in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Brain Research, 1986, 365, 379-382.

Carlson, J.H., Bergstrom, D.A. and Walters, J.R. Neurophysiological evidence that D-1 dopamine receptor blockade attenuates postsynaptic but not autoreceptor-mediated effects of dopamine agonists. European Journal of Pharmacology, 1986, 123, 237-251.

Carlson, J.H., Bergstrom, D.A., Weick, B.G. and Walters, J.R. Neurophysiological investigation of effects of the D-1 agonist SKA 38393 on tonic activity of substantia nigra dopamine neurons. Synapse, 1987, 1, 411-416.

Carlson, J.N., Glick, S.D. and Hinds, P.A. Changes in d-amphetamine elicited rotational behavior in rats exposed to uncontrollable footshock stress. Pharmacology Biochemistry & Behavior, 1987, 26, 17-21.

Carr, G.D. and White, N.W. Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. Life Sciences, 1983, 33, 2551-2557.

Carr, G.D. and White, N.W. Anatomical disassociation of amphetamine's rewarding and aversive effects: An intracranial microinjection study. Psychopharmacology, 1986, 89, 340-346.

Carr, G.D. and White, N.M. Effects of systemic and intracranial amphetamine injections on behavior in the open field: A detailed analysis. Pharmacology Biochemistry & Behavior, 1987, 27, 113-122.

Cheramy, A., Romo, R., Godeheu, G., Baruch, P. and Glowsinski, J. In vivo presynaptic control of dopamine release in the cat caudate nucleus--II. Facilitatory or inhibitory influence of l-glutamate. Neuroscience, 1986, 19, 1081-1090.

Chiodo, L.A. and Antelman, S.M. Electroconvulsive shock: Progessive dopamine autoreceptor subsensitivity independent of repeated treatment. Science, 1980, 210, 799-801.

Chiodo, L.A. and Bunney, B.S. Effects of dopamine antagonists on midbrain dopamine cell activity. In A. Dahlstrom and E. Usdin (Eds.), Catecholamines: Neuropharmacology and central nervous system - Theoretical aspects. New York: Alan R. Liss, 1984.

Christensen, A.V., Arnt, J., Hyttel, J., Larsen, J.-J. and Svendsen, O. Pharmacological effects of a specific dopamine D-1 antagonist SCH 23390 in comparison with neuroleptics. Life Sciences, 1984, 34, 1529-1540.

Conway, P.G. and Uretsky, N.J. Role of striatal dopaminergic receptors in amphetamine-induced behavioral facilitation. The Journal of Pharmacology and Experimental Therapeutics, 1982, 221, 650-655.

Costall, B., Domeney, A.M. and Naylor, R.J. Stimulation of rat spontaneous locomotion by low doses of haloperidol and (-)-sulpiride: Importance of animal selection and measurement technique. European Journal of Pharmacology, 1983, 90, 307-314.

Creese, I. and Iversen, S.D. The role of forebrain dopamine systems in amphetamine induced stereotyped behavior in the rat. Psychopharmacologia, 1974, 39, 345-357.

Creese, I. and Iversen, S.D. The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Research, 1975, 83, 419-436.

Dafters, R. and Bach, L. Absence of environment-specificity in morphine tolerance acquired in non-distinctive environments: habituation or stimulus overshadowing? Psychopharmacology, 1985, 87, 101-106.

D'Angio, M., Serrano, A., Rivy, J.P. and Scatton, B. Tail-pinch stress increases extracellular DOPAC levels (as measured by in vivo voltammetry) in the rat nucleus accumbens but not frontal cortex: antagonism by diazepam and zolpidem. Brain Research, 1987, 409, 169-174.

Davis, R.E., Sant, W.W. and Ellison, G. Continous low-level apomorphine administration induces motor abnormalities and hallucinogen-like behaviors. Psychopharmacology, 1985, 85, 1-7.

Deutch, A.Y., Tam, S.-Y. and Roth, R.H. Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. Brain Research, 1985, 333, 143-146.

Dewey, K.J. and Fibiger, H.C. The effects of dose and duration of chronic pimozide administration on dopamine receptor supersensitivity. Naunyn-Schmiedeberg's Archives of Pharmacology, 1983, 322, 261-270.

Dougherty, G.G., Jr. and Ellinwood, E.H., Jr. Chronic d-amphetamine in nucleus accumbens: Lack of tolerance or reverse tolerance of locomotor activity. Life Sciences, 1981, 28, 2295-2298.

Drawbaugh, R. and Lal, H. Reversal by narcotic antagonist of a narcotic action elicited by a conditioned stimulus. Nature, 1974, 247, 65-67.

Duffy, P. and Kalivas, P.W. Inhibition of endogenous dopamine release from the ventromedial mesencephalon after daily cocaine or morphine. Abstract presented at The Conference on the Mesocorticolimbic Dopamine System. The New York Academy of Sciences, Miami, 1987.

Dunn, A.J. and Kramarey, N.R. Neurochemical responses in stress: Relationships between the hypothalmic-pituitary-adrenal and catecholamine systems. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), Handbook of psychopharmacology. New York: Plenum Press, 1984.

Durivage, A. and Miliaressis, E. Anatomical dissociation of the substrates of medial forebrain bundle self-stimulation and exploration. Behavioral Neuroscience, 1987, 101, 57-61.

Echols, S.D. Circling of mice bearing unilateral striated lesions: Development of increased response to d-amphetamine. Life Sciences, 1977, 21, 563-568.

Echols, S.D. Reverse tolerance to amphetamine of mice bearing unilateral striated lesions: Effect upon the circling response to apomorphine. Life Sciences, 1979, 24, 691-696.

Eichler, A.J. and Antelman, S.M. Sensitization to amphetamine and stress may involve nucleus accumbens and medial frontal cortex. Brain Research, 1979, 176, 412-416.

Eikelboom, R. and Stewart, J. The conditioning of drug-induced physiological responses. Psychological Review, 1982, 89, 507-528.

Einhorn, L.C. and White, F.J. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Studies in the ventral tegmental area. Society for Neuroscience Abstracts, 1986, 12, 1516.

Fadda, F, Argiolas, A., Melis, M.R., Tissari, A.M., Onali, P.L. and Gessa, G.L. Stress-induced increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the cerebral cortex and in n. accumbens: Reversal by diazepam. Life Sciences, 1978, 23, 2219-2224.

Fink, J.S. and Smith, G.P. Abnormal pattern of amphetamine locomotion after 6-OHDA lesion of anteromedial caudate. Pharmacology Biochemistry & Behavior, 1979, 11, 22-30.

Fink, J.S. and Smith, G.P. Relationships between selective denervation of dopamine terminal fields in the anterior forebrain and behavioral responses to amphetamine and ampomorphine. Brain Research, 1980, 201, 107-127.

Fischer, J.F. and Cho, A.K. Chemical release of dopamine from striatal homogenates: Evidence for an exchange diffusion model. The Journal of Pharmacology and Experimental Therapeutics, 1979, 208, 203-209.

Fray, P.J., Sahakian, B.J., Robbins, T.W., Koob, G.F. and Iversen, S.D. An observational method for quantifying the behavioural effects of dopamine agonists: Contrasting effects of d-Amphetamine and apomorphine. Psychopharmacology, 1980, 69, 253-259.

Fujiwara, Y., Kazahaya, Y., Nakashima, M., Sato, M. and Otsuki, S. Behavioral sensitization to methamphetamine in the rat: An ontogenic study. Psychopharmacology, 1987, 91, 316-319.

Gaiardi, M., Bartoletti, M., Gubellini, C., Bacchi, A. and Babbini, M. Sensitivity to the narcotic cue in non-dependent, morphine-dependent and post-dependent rats. Neuropharmacology, 1986, 25, 119-123.

Goodman, R.R., Snyder, S.H., Kuhar, M.J. and Young, W.S., III. Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. Proceedings of the National Academy of Science, 1980, 77, 119-127.

Goudie, A.J. Aversive stimulus properties of drugs: The conditioned taste aversion paradignm. In A.J. Grenshaw and C.T. Dourish (Eds.), Experimental approach in psychopharmacology. Crescent Manor, NJ: Humana Press, 1987.

Groves, P.M., Fenster, G.A., Tepper, J.M., Nakamura, S. and Young, S.J. Changes in dopaminergic terminal excitability induced by amphetamine and haloperidol. Brain Research, 1981, 221, 425-431.

Groves, P.M. and Tepper, J.M. Neuronal mechanisms of action of amphetamine. In I. Creese (Ed.), Stimulants: Neurochemical, behavioral and clinical perspectives. New York: Raven Press, 1983.

Gysling, K. and Wang, R.Y. Morphine-induced activation of A10 dopamine neurons in the rat. Brain Research, 1983, 277, 119-127.

Hand, T.H. and Franklin, K.B.J. Lesions of ventral tegmental dopamine neurons delay the development of tolerance to morphine catalepsy. Neuroscience Letters, 1985, 55, 367-370.

Havemann, U., Winkler, M. and Kuschinsky, K. The effects of D-ala2,D-Leu5-enkephalin injections into the nucleus accumbens on the motility of rats. Life Sciences, 1983, 33, 627-630.

Herman, J.-P., Stinus, L. and Le Moal, M. Repeated stress increases locomotor response to amphetamine. Psychopharmacology, 1984, 84, 431-435.

Hess, E.J., Albers, L.J., Le, H. and Creese, I. Effects of chronic SCH23390 treatment on the biochemical and behavioral properties of D1 and D2 dopamine receptors: Potentiated behavioral responses to a D2 dopamine agonist after selective D1 dopamine receptor upregulation. The Journal of Pharmacology and Experimental Therapeutics, 1986, 238, 846-854.

Hinson, R.E. and Poulos, C.X. Sensitization to the behavioral effects of cocaine: Modification by pavlovian conditioning. Pharmacology Biochemistry and Behavior, 1981, 15, 559-562.

Hinson, R.E. and Siegel, S. Anticipatory hyperexcitability and tolerance to the narcotizing effect of morphine in the rat. Behavioral Neuroscience, 1983, 97, 759-767.

Hirabayashi, M. and Alam, M.R. Enhancing effect of methamphetamine on ambulatory activity produced by repeated administration in mice. Pharmacology Biochemistry & Behavior, 1981, 15, 925-932.

Hong, J.S., Yang, H.Y.T., Fratta, W. and Costa,E. Determination of methionine enkephalin in discrete regions of rat brain. Brain Research, 1977, 134, 383-386.

Hyttel, J. SCH 23390 - The first selective dopamine D-1 antagonist. European Journal of Pharmacology, 1983, 91, 153-154.

Itoh, T., Murai, S., Yoshida, H., Masuda, Y., Saito, H. and Chen, C.H. Effects of methamphetamine and morphine on the vertical and horizontal motor activities in mice. Pharmacology Biochemistry & Behavior, 1987, 27, 193-197.

Iversen, S.D. Brain endorphins and reward function: Some thoughts and speculations. In J.E. Smith and J.D. Lane (Eds.), The neurobiology of opiate reward processes. Amsterdam: Elsevier/North Holland Biomedical Press, 1983.

Iwamoto, E.T. and Way, E.L. Circling behavior and stereotypy induced by intranigral opiate microinjections. The Journal of Pharmacology and Experimental Therapeutics, 1977, 203, 347-359.

Joyce, E.M. and Iversen, S.D. The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neuroscience Letters, 1979, 14, 207-212.

Joyce, E.M. and Iversen, S.D. Dissociable effects of 6-OHDA-induced lesions of neostriatum on anorexia, locomotor activity and sterotypy: The role of behavioural competition. Psychopharmacology, 1984, 83, 363-366.

Joyce, E.M., Koob, G.F., Strecker, R. Iversen, S.D. and Bloom, F.E. The behavioral effects of enkephalin analogues injected into the ventral tegmental area and globus pallidus. Brain Research, 1981, 221, 359-370.

Joyce, E.M., Stinus, L. and Iversen, S.D. Effect of injections of 6-OHDA into either nucleus accumbens septi or frontal cortex on spontaneous and drug-induced activity. Neurophamacology, 1983, 22, 1141-1145.

Kafetzopoulos, E. Effects of amphetamine and apomorphine on locomotor activity after kainic acid lesion of the nucleus accumbens septi in the rat. Psychopharmacology, 1986, 88, 271-274.

Kalivas, P.W. Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon. Neuroscience and Biobehavioral Reviews, 1985, 9, 573-587.(a)

Kalivas, P.W. Sensitization to repeated enkephalin administration into the ventral tegmental area of the rat.II.Involvement of the mesolimbic dopamine system. The Journal of Pharmacology and Experimental Therapeutics, 1985, 235, 544-550.(b)

Kalivas, P.W. and Abhold, R. Enkephalin release into the ventral tegmental area in response to stress: Modulation of mesocorticolimbic dopamine. Brain Research, 1987, 414, 339-348.

Kalivas, P.W. and Duffy, P. Sensitization to repeated morphine injection in the rat: Possible involvement of A10 dopamine neurons. The Journal of Pharmacology and Experimental Therapeutics, 1987, 241, 204-212.

Kalivas, P.W. and Duffy, P. Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release, submitted.(a)

Kalivas, P.W. and Duffy, P. Sensitization of the mesocorticolimbic dopamine system: III. Interaction between cocaine and stress, submitted.(b)

Kalivas, P.W., Duffy, P., Dilts, R. and Abhold, R. Enkephalin modulation of A10 dopamine neurons: A role in dopamine sensitization. Annals of the New York Academy of Sciences, in press.

Kalivas, P.W., DuMars, L.E. and Skinner, C. Sensitization of the mesocorticolimbic dopamine system: I. Effects of daily cocaine, submitted.

Kalivas, P.W., Richardson-Carlson, R. and Van Orden, G. Cross-sensitization between foot shock stress and enkephalin-induced motor activity. Biological Psychiatry, 1986, 21, 939-950.

Kalivas, P.W. and Taylor, S. Behavioral and neurochemical effect of daily injection with neurotensin into the ventral tegmental area. Brain Research, 1985, 358, 70-76.

Kalivas, P.W., Taylor, S. and Miller, J.S. Sensitization to repeated enkephalin administration into the ventral tegmental area of the rat. I. Behavioral characterization. The Journal of Pharmacology and Experimental Therapeutics, 1985, 235, 537-543.

Kalivas, P.W. and Weber, B. Amphetamine injection onto the dopamine perikarya sensitizes rats to peripheral amphetamine, cocaine and morphine, submitted.

Kalivas, P.W., Widerlov, E., Stanley, D., Breese, G. and Prange, A.J. Jr. Enkephalin action on the mesolimbic dopamine system: A dopamine-dependent and a dopamine-independent increase in locomotor activity. The Journal of Pharmacology and Experimental Therapeutics, 1983, 227, 229-237.

Kamal, L.A., Arbilla, S. and Langer, S.Z. Presynaptic modulation of the release of dopamine from the rabbit caudate nucleus: Differences between electrical stimulation, amphetamine and tyramine. The Journal of Pharmacology and Experimental Therapeutics, 1981, 216, 592-598.

Kamat, K.A., Dutta, S.N. and Pradhan, S.N. Conditioning of morphine-induced enhancement of motor activity. Research Communications in Chemical Pathology and Pharmacology, 1974, 7, 367-373.

Kamata, K. and Rebec, G.V. Dopaminergic and neostriatal neurons: Dose-dependent changes in sensitivity to amphetamine following long-term treatment. Neuropharmacology, 1983, 22, 1377-1382.

Kamata, K. and Rebec, G.V. Long-term amphetamine treatment attenuates or reverses the depression of neuronal activity produced by dopamine agonists in the ventral tegmental area. Life Sciences, 1984, 34, 2419-2427.(a)

Kamata, K. and Rebec, G.V. Nigral dopminergic neurons: Differential sensitivity to apomorphine following long-term treatment with low and high doses of amphetamine. Brain Research, 1984, 321, 147-150.(b)

Kebabian, J.W. and Calne, D.B. Multiple receptors for dopamine. Nature, 1979, 277, 93-96.

Kelley, A.H., Stinus, L. and Iversen, S.D. Interactions between D-Ala-Met-enkephalin, A10 dopaminergic neurons, and spontaneous behavior in the rat. Behavioral Brain Research, 1980, 1, 3-24.

Kelly, P.H. Drug-Induced Motor Behavior. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), Handbook of psychopharmacology, Vol. 8. New York: Plenum Press, 1977.

Kelly, P.H. and Iversen, S.D. Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. European Journal of Pharmacology, 1976, 40, 45-56.

Kelly, P.H., Seviour, P.W. and Iversen, S.D. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research, 1975, 94, 507-523.

Kilpatrick, I.C., Jones, M.W., Johnson, B.J., Cornwall, J. and Phillipson, O.T. Thalamic control of dopaminergic functions in the caudate-putamen of the rat--II. Studies using ibotenic acid injection of the parafascicular-intralaminar nuclei. Neuroscience, 1986, 19, 979-990.

Kilpatrick I.C., Jones, M.W., Pycock, C.J., Riches, I. and Phillipson, O.T. Thalamic control of dopaminergic functions in the caudate-putamen of the rat--II. The effects of lesions in the parafascicular-intralaminar nuclei on D2 dopamine receptors and high affinity dopamine uptake. Neuroscience, 1986, 19, 991-1005.

Kilpatrick, I.C. and Phillipson, O.T., Thalamic control of dopaminergic functions in the caudate-putamen of the rat--I. The influence of electrical stimulation of the parafascicular nucleus on dopamine utilization. Neuroscience, 1986, 19, 965-978.

Kimble, G. Conditioning and learning. New York: Appleton-Century-Crofts, 1961.

Kirk, R.E. Experimental design: Procedures for the behavioral sciences. Belmont, CA: Brooks/Cole Publishing Company, 1968.

Kolta, M.G., Shreve, P. De Souza, V. and Uretsky, N.J. Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. Neuropharmacology, 1985, 24, 823-829.

Koob, G.F., Stinus, L. and LeMoal, M. Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. Behavioral Brain Research, 1981, 3, 341-359.

Kucharski, D., Johanson, I.B. and Hall, W.G. Unilateral olfactory conditioning in 6-day-old rat pups. Behavioral and Neural Biology, 1986, 46, 472-490.

Kuczenski, R. Biochemical actions of amphetamine and other stimulants. In I. Creese (Ed.), Stimulants: Neurochemical, behavioral and clinical perspectives. New York: Raven Press, 1983.

Kuczenski, R. and Leith, N.J. Chronic amphetamine: Is dopamine a link in or mediator of the development of tolerance and reverse tolerance? Pharmacology Biochemistry and Behavior, 1981, 15, 405-413.

Kuczenski, R., Leith, N.J. and Applegate, C.D. Striatal dopamine metabolism in response to apomorphine: The effects of repeated amphetamine pretreatment. Brain Research, 1983, 258, 333-337.

Kumar, R., Mitchell, E. and Stolerman, I.P. Disturbed patterns of behaviour in morphine tolerant and abstinent rats. British Journal of Pharmacology, 1971, 42, 473-484.

Lal, H., Miksic, S. and Smith, N. Naloxone antagonism of conditioned hyperthermia: An evidence for release of endogenous opioid. Life Sciences, 1976, 18, 971-975.

Langer, S.Z. and Arbilla, S. Presynaptic regulation of neurotransmitter release: Physiological and pharmacological implications. In R. Caputto and C. Ajmone Marsan (Eds.), Neural transmission, learning and memory. New York: Raven Press, 1983.

Leith, N.J. and Kuczenski, R. Chronic amphetamine: Tolerance and reverse tolerance reflect different behavioral actions of the drug. Pharmacology Biochemistry & Behavior, 1981, 15, 399-404.

Leith, N.J. and Kuczenski, R. Two dissociable components of behavioral sensitization following repeated amphetamine administration. Psychopharmacology, 1982, 76, 310-315.

Leyton, M. and Stewart, J. Locomotor activity shows progressive increases following repeated exposure to a stressor similar to those observed following repeated exposure to morphine. Society for Neuroscience Abstracts, 1987, 13, 920.

Lynch, J.J., Stein, E.A. and Fertziger, A.P. An analysis of 70 years of morphine classical conditioning: Implications for clinical treatment of narcotic addiction. The Journal of Nervous and Mental Disease, 1976, 163, 47-58.

Mackintosh, N.J. The psychology of animal learning. New York: Academic Press, 1974.

MacLennan, A.J. and Maier, S.F. Coping and the stress-induced potentiation of stimulant stereotypy in the rat. Science, 1983, 219, 1091-1092.

Maj, J., Sowinska, H., Kapturkiewicz, Z. and Sarnek, J. The effect of L-dopa and (+)-amphetamine on the locomotor activity after pimozide and phenoxybenzamine. Journal of Pharmacy and Pharmacology, 1972, 24, 412-413.

Mansfield, J.G., Wenger, J.R., Benedict, R.S., Halter, J.B. and Woods, S.C. Sensitization to the hyperthermic and catecholamine-releasing effects of morphine. Life Sciences, 1981, 29, 1697-1704.

Mansky, P.A. Opiates: Human psychopharmacology. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), Handbook of psychopharmacology, Vol 12. New York: Plenum Press, 1978.

Martres, M.P., Costentin, J., Baudry, M., Marcaise, H., Protais, P. and Schwartz, J.D. Long-term changes in the sensitivity of pre- and postsynaptic dopamine receptors in mouse striatum evidenced by behavioural and biochemical studies. Brain Research, 1977, 136, 319-337.

Masuda, Y., Murai, S., Saito, H., Kohori, I. and Itoh, T. Repeated low dose apomorphine induced subsensitivity of presynaptic dopamine receptors. Pharmacology Biochemistry & Behavior, 1987, 28, 35-37.

Mathews, R. and German, D. Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience, 1984, 11, 617-628.

Mathews, R.T. and German, D.C. Evidence for a functional role of dopamine type-1 (D-1) receptors in the substantia nigra of rats. European Journal of Pharmacology, 1986, 120, 87-93.

Mazurski, E.J. and Beninger, R.J. Environment-specific conditioning and sensitization with (+)-amphetamine. Pharmacology Biochemistry and Behavior, 1987, 27, 61-65.

Meller, E., Bohmaker, K., Goldstein, M., Schweitzer, J.W. and Friedhoff, A.J. Chronic haloperidol does not alter agonist affinity for dopamine receptors in vitro. European Journal of Pharmacology, 1985, 109, 389-394.

Mereu, G., Collu, M., Ongini, E., Biggio, G. and Gessa, G.L. SCH 23390, A selective dopamine D1 antagonist, activates dopamine neurons but fails to prevent their inhibition by apomorphine. European Journal of Pharmacology, 1985, 111, 393-396.

Miller, J.D., Sanghera, M.K. and German, D.C. Mesencephalic dopaminergic unit activity in the behaviorally conditioned rat. Life Sciences, 1981, 29, 1255-1263.

Mohammed, A.K., Danysz, W., Ogren, S.O. and Archer, T. Central noradrenaline depletion attenuates amphetamine-induced locomotor behavior. Neuroscience Letters, 1986, 64, 139-144.

Moller, H.-G., Nowak, K. and Kuschinsky, K. Studies on interactions between conditioned and unconditioned behavioural responses to apomorphine in rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 1987, 335, 673-679.

Molloy, A.G., O'Boyle, K.M., Pugh, M.T. and Waddington, J.L. Locomotor behaviors in response to new selective D-1 and D-2 dopamine receptor agonists, and the influence of selective antagonists. Pharmacology Biochemistry and Behavior, 1986, 25, 249-253.

Moore, K.E. Amphetamines: Biochemical and behavioral actions in animals. In L.L. Iversen, S.D. Iversen and S.D. Snyder (Eds.), Handbook of psychopharmacology, Vol 11. New York: Plenum Press, 1978.

Morelli, M. and Di Chiara, G. Agonist-induced homologous and heterologous sensitization to D-1 and D-2 dependent contraversive turning, submitted.

Morelli, M. Fenu, S. and Di Chiara, G. Behavioral expression of D-1 receptor supersensitivity depends on previous stimulation of D-2 receptors. Life Sciences, 1987, 40, 245-251.

Mucha, R.F., Van der Kooy, D., O'Shaughnessy, M. and Bucenieks, P. Drug reinforcement studied by the use of place conditioning in rat. Brain Research, 1982, 243, 91-105.

Mucha, R.F., Volkovskis, C. and Kalant, H. Conditioned increases in locomotor activity produced with morphine as an unconditioned stimulus, and the relation of conditioning to acute morphine effect and tolerance. Journal of Comparative and Physiological Psychology, 1981, 95, 351-362.

Muller, P. and Seeman, P. Presynaptic subsensitivity as a possible basis for sensitization by long-term dopamine mimetics. European Journal of Pharmacology, 1979, 55, 149-157.

Nakamura, H., Ishii, K. and Shimizu, M. Some altered responses in rats formerly dependent on morphine. Psychopharmacology, 1978, 56, 269-277.

Neisewander, J.L. and Bardo, M.T. Expression of morphine-conditioned hyperactivity is attenuated by naloxone and pimozide, submitted.

Nelson, L.R. and Ellison, G. Enhanced stereotypies after repeated injections but not continuous amphetamines. Neuropharmacology, 1978, 17, 1081-1084.

Nishikawa, T., Mataga, N., Takashima, M. and Toru, M. Behavioral sensitization and relative hyperresponsiveness of striatal and limbic dopaminergic neurons after repeated methamphetamine treatment. European Journal of Pharmacology, 1983, 88, 195-203.

Nowak, K., Moller, H.-G. and Kuschinsky, K. Conditioning of behavioral signs produced by nomifensine and by B-HT 920 in rats. Psychopharmacology, 1987, 93, 182-187.

O'Connor, S.E. and Brown, R.A. The pharmacology of sulpiride - a dopamine receptor antagonist. General Pharmacology, 1982, 13, 185-193.

Oka, T, Nozaki, M. and Hosoya, E. The effect of cholinergic antagonists on increases of spontaneous locomotor activity and body weight induced by the administration of morphine to tolerant rats. Psychopharmacologia, 1972, 23, 231-237.

Onali, P., Mereu, G., Olianas, M.C., Bunse, B., Rossetti, Z. and Gessa, G.L. SCH23390, a selective D-1 dopamine receptors blocker, enhances the firing rate of nigral dopaminergic neurons but fails to activate striatal tyrosine hydroxylase. Brain Research, 1985, 340, 1-7.

Ostrowski, N.L., Hatfield, C.B. and Caggiula, A.R. The effects of low doses of morphine on the activity of dopamine-containing cells and on behavior. Life Sciences, 1982, 31, 2347-2350.

Parenti, M., Flauto, C., Parati, E., Vescovi, A. and Groppetti, A. Differential effect of repeated treatment with l-dopa on dopamine-D1 or -D2 receptors. Neuropharmacology, 1986, 25, 331-334.

Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J. A stereotaxic atlas of the rat brain. New York: Plenum Press, 1979.

Perez-Cruet, J. Conditioning of striatal dopamine metabolism with methadone, morphine, or bulbocapnine as an unconditioned stimulus. Pavlovian Journal of Biological Science, 1976, 11, 237-250.

Peris, J. and Zahniser, N.R. One injection of cocaine produces a long-lasting increase in (3H)-dopamine release. Pharmacology Biochemistry & Behavior, 1987, 27, 533-535.

Pert, A. and Sivit, C. Neuroanatomical focus for morphine and enkephalin-induced hypermotility. Nature (London), 1977, 265, 645-647.

Phillips, A.G. and LePiane, F.G. Reinforcing effects of morphine microinjected into the ventral tegmental area. Pharmacology Biochemistry and Behavior, 1980, 12, 965-968.

Pickens, R. and Dougherty, J.A. Conditioning and the activity effects of drugs. In T. Thompson and R. Pickens (Eds.), Stimulus properties of drugs. New York: Appleton-Century-Crofts, 1971.

Pijnenburg, A.J.J., Honig, W.M.M., Van Der Heyden, J.A.M. and Van Rossum, J.M. Effects of chemical stimulation on the mesolimbic dopamine system upon locomotor activity. European Journal of Pharmacology, 1976, 35, 45-58.

Pijnenburg, A.J.J., Honig, W.M.M. and Van Rossum, J.M. Inhibition of d-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacologia (Berl.), 1975, 41, 87-95.

Pollard, H., Llorens, C., Bonnet, J.J., Costentin and Schwartz, J.D. Opiate receptors on mesolimbic dopaminergic neurons. Neuroscience Letters, 1977, 7, 295-299.

Porceddu, M.L., Giorgi, O., Ongini, E., Mele, S. and Biggio, G. 3H-SCH 23390 binding sites in the rat substantia nigra: Evidence for a presynaptic localization and innervation by dopamine. Life Sciences, 1986, 39, 321-328.

Post, R.M. Intermittent versus continuous stimulation: Effect of time interval on the development of sensitization or tolerance. Life Sciences, 1980, 26, 1275-1282.

Post, R.M. Central stimulants: Clinical and experimental evidence on tolerance and sensitization. In Y. Israel, F.B. Glaser, H. Kalant, R.E. Popham, W. Schmidt and R.G. Smart (Eds.), Research advances in alcohol and drug problems, Vol. 6. New York: Plenum Publishing Corporation, 1981.

Post, R.M., Lockfeld, A., Squillace, K.M. and Contel, N.R. Drug-environment interaction: Context dependency of cocaine-induced behavioral sensitization. Life Sciences, 1981, 28, 755-760.

Post, R.M., Rubinow, D.R. and Ballenger, J.C. Conditioning, sensitization, and kindling: Implications for the course of affective illness. In R.M. Post and J.C. Ballenger (Eds.), Neurobiology of mood disorders. Frontiers of clinical neuroscience. Baltimore: Williams and Wilkins, 1984.

Predy, P.A. and Kokkinidis, L. Sensitization to the effects of repeated amphetamine administration on intracranial self-stimulation: Evidence for changes in reward processes. Behavioural Brain Research, 1984, 13, 251-259.

Randrup, A. and Munkvad, I. Stereotyped activities produced by amphetamine in several animal species and man. Psychopharmacologia (Berl.), 1967, 11, 300-310.

Rebec, G.V. and Bashore, T.R. Critical issues in assessing the behavioral effects of amphetamine. Neuroscience & Biobehavioral Reviews, 1984, 8, 153-159.

Rebec, G.V. and Segal, D.S. Enhanced responsiveness to intraventricular infusion of amphetamine following its repeated systemic administration. Psychopharmacology, 1979, 62, 101-102.

Rescorla, R.A. Pavlovian conditioned inhibition. Psychological Bulletin, 1969, 72, 77-94.

Rescorla, R.A. Conditioned inhibition and facilitation. In R.R. Miller and N.E. Spear (Eds.), Information processing in animals: Conditioned inhibition. Hillsdale, NJ: Lawrence Erlbaum, 1985.

Rescorla, R.A. and Solomon, R.L. Two-process learning theory. Psychological Review, 1967, 74, 151-182.

Riffee, W.H., Wanek, E. and Wilcox, R.E. Prevention of amphetamine-induced behavioral hypersensitivity by concomitant treatment with microgram doses of apomorphine. European Journal of Pharmacology, 1987, 135, 255-258.

Riffee, W.H. and Wilcox, R.E. Effects of multiple pretreatment with apomorphine and amphetamine on amphetamine-induced locomotor activity and its inhibition by apomorphine. Psychopharmacology, 1985, 85, 97-101.

Robbins, T.W. Relationship between reward-enhancing and stereotypical effects of psychomotor stimulant drugs. Nature, 1976, 264, 57-59.

Robinson, T.E. Behavioral sensitization: Characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology, 1984, 84, 466-475.

Robinson, T.E., Angus, A.L. and Becker, J.B. Sensitization to stress: The enduring effects of prior stress on amphetamine-induced rotational behavior. Life Sciences, 1985, 37, 1039-1042.

Robinson, T.E. and Becker, J.B. Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striated tissue in vitro. European Journal of Pharmacology, 1982, 85, 253-254.

Robinson, T.E. and Becker, J.B. Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Research Reviews, 1986, 11, 157-198.

Robinson, T.E., Becker, J.B. and Presty, S.K. Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: Sex differences. Brain Research, 1982, 253, 231-241.

Robinson, T.E., Becker, J.B., Young, E.A., Akil, H. and Castaneda E. The effects of footshock stress on regional brain dopamine metabolism and pituitary b-endorphin release in rats previously sensitized to amphetamine. Neuropharmacology, 1987, 26, 679-691.

Robinson, T.E. and Camp, D.M. Long-lasting effects of escalating doses of d-amphetamine on brain monoamines, amphetamine-induced stereotyped behavior and spontaneous nocturnal locomotion. Pharmacology Biochemistry & Behavior, 1987, 26, 821-827.

Rolinski, Z. and Scheel-Kruger, J. The Effect of dopamine and noradrenaline antagonists on amphetamine induced locomotor activity in mice and rats. Acta Pharmacologica (Kbh.), 1973, 33, 385-399.

Romo, R., Cheramy, A., Godeheu, G. and Glowinski, J. In vivo presynaptic control of dopamine release in the cat caudate nucleus--I. Opposite changes in neuronal activity and release evoked from thalamic motor nuclei. Neuroscience, 1986, 19, 1067-1079.(a)

Romo, R., Cheramy, A. Godeheu, G. and Glowinski, J. In vivo presynaptic control of dopamine release in the cat caudate nucleus--II. Further evidence for the implication of corticostriatal glutamatergic neurons. Neuroscience, 1986, 19, 1091-1099.(b)

Roth, R.H. CNS dopamine autoreceptors: Distribution, pharmacology, and function. Annals of the New York Academy of Sciences, 1984, 430, 27-53.

Russell, K.H., Giordano, M. and Sanberg, P.R. Amphetamine-induced on- and off-wall rearing in adult laboratory rats. Pharmacology Biochemistry and Behavior, 1987, 26, 7-10.

Sakurai, Y. and Hirano, T. Multiple unit response in reward areas during operant conditioning reinforced by lateral hypothalamic stimulation in the rat. Behavioral Brain Research, 1983, 8, 33-48.

Sar, M., Stumpf, W.E., Miller, R.J., Chang, K.-J. and Ciatrecasas, P. Immunohistochemical localization of enkephalin in rat brain and spinal cord. Journal of Comparative Neurology, 1978, 182, 17-38.

Schiff, S.R. Conditioned dopaminergic activity. Biological Psychiatry, 1982, 17, 135-154.

Schnur, P. and Raigoza, V.P. Effects of naloxone on morphine induced sedation and hyperactivity in the hamster. Pharmacology, Biochemistry & Behavior, 1986, 24, 849-854.

Seeger, T.F., Thai, L. and Gardner, E.L. Behavioral and biochemical aspects of neuroleptic-induced dopaminergic supersensitivity: Studies with chronic clozapine and haloperidol. Psychopharmacology, 1982, 76, 182-187.

Seeman, P. and Grigoriadis, D. Dopamine receptors in brain and periphery. Neurochemistry International, 1987, 10, 1-25.

Seeman, P., Watanabe, M., Grigoriadis, D., Tedesco, J.L., George, S.R., Svensson, U., Nilsson, J.L.G. and Neumeyer, J.L. Dopamine D2 receptor binding sites for agonists. Molecular Pharmacology, 1985, 28, 391-399.

Seevers, M.G. and Deneau, G.A. Physiological aspects of tolerance and physical dependence. In W.S. Root and F.G. Hofmann (Eds.), Physiological pharmacology, Vol. 1. New York: Academic Press, 1963.

Segal, D.S. Behavioral characterization of d- and l-amphetamine: Neurochemical implications. Science, 1975, 190, 475-477.(a)

Segal, D.S. Behavioral and neurochemical correlates of repeated d-amphetamine administration. In A.J. Mandell (Ed.), Neurobiological Mechanisms of Adaptation and Behavior. New York: Raven Press, 1975.(b)

Segal, D.S. Differential effects of serotonin depletion on amphetamine-induced locomotion and stereotypy. In E.H. Ellinwood and M.M. Kilbey (Eds.), Cocaine and other stimulants. New York: Plenum, 1977.

Segal, D.S. and Mandell, A.J. Long-term administration of d-amphetamine: Progressive augmentation of motor activity and stereotypy. Pharmacology Biochemistry and Behavior, 1974, 2, 249-255.

Serra, G., Argiolas, A., Fadda, F., Melis, M.R. and Gessa, G.L. Repeated electroconvulsive shock prevents the sedative effect of small doses of apomorphine. Psychopharmacology, 1981, 73, 194-196.

Short, P.H. and Shuster, L. Changes in brain norepinephrine associated with sensitization to d-amphetamine. Psychopharmacology, 1976, 48, 59-67.

Skirboll, L.R., Grace, A.A. and Bunney, B.S. Dopamine auto- and postsynaptic receptors: Electrophysiological evidence for differential sensitivity to dopamine agonists. Science, 1979, 206, 80-82.

Smee, M.L. and Overstreet, D.H. Alterations in the effects of dopamine agonists and antagonists on general activity in rats following chronic morphine treatment. Psychopharmacology, 1976, 49, 125-130.

Sparber, S.B. and Tilson, H.A. The releasability of central norepinephrine and serotonin by peripherally administered d-amphetamine before and after tolerance. Life Sciences, 1972, 11, 1059-1067.

Spyraki, C., Fibiger, H.C. and Phillips, A.G. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Research, 1982, 253, 185-193.

Staton, D.M. and Solomon, P.R. Microinjections of d-amphetamine into the nucleus accumbens and caudate-putamen differentially affect stereotypy and locomotion in the rat. Physiological Psychology, 1984, 12 (2), 159-162.

Stewart, J. Changes in sensitivity to apomorphine following repeated morphine injections are specifically related to the place where morphine is experienced. Society for Neuroscience Abstracts, 1981, 7, 49.

Stewart, J., deWit, H. and Eikelboom, R. The role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychological Review, 1984, 91, 251-268.

Stewart, J. and Eikelboom, R. Conditioned drug effects. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), Handbook of psychopharmacology, Vol 19. New York: Plenum Press, 1987.

Stewart, J. and Vezina, P. Environment-specific enhancement of the hyperactivity induced by systemic or intra-VTA morphine injections in rats preexposed to amphetamine. Psychobiology, 1987, 15, 144-153.

Stewart, J. and Vezina, P. A comparison of the effects of intra-accumbens injections of amphetamine and morphine on reinstatement of heroin intravenous self-administration behavior, submitted.

Stinus, L., Koob, G.F., Ling, N., Bloom, F.E. and LeMoal, M. Locomotor activation induced by infusion of endorphins into the ventral tegmental area: Evidence for opiate-dopamine interactions. Proceedings of the National Academy of Science, U.S.A., 1980, 77, 2323-2327.

Stoof, J.C. and Kebabian, M.W. Two dopamine receptors: Biochemistry, physiology and pharmacology. Life Sciences, 1984, 35, 2281-2296.

Strombom, U. Catecholamine receptor agonists: Effects on motor activity and rate of tyrosine hydroxylation in mouse brain. Naunyn-Schmiedeberg's Archives of Pharmacology, 1976, 292, 167-176.

Svensson, K., Hjorth, S., Clark, D., Carlsson, A., Wikstrom, H., Andersson, B., Sanchez, D., Johansson, A.M., Arvidsson, L.-E., Hacksell, U. and Nilsson, J.L.G. (+)-UH232 and (+)-UH242: Novel stereoselective dopamine receptor antagonists with preferential action on autoreceptors. Journal of Neural Transmission, 1986, 65, 1-27.

Taylor, J.R. and Robbins, T.W. Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacology, 1984, 84, 405-412.

Taylor, J.R. and Robbins, T.W. 6-hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacology, 1986, 90, 390-397.

Taylor, K.M. and Snyder, S. Differential effects of d- and l-amphetamine on behavior and on catecholamine disposition in dopamine and norepinephrine containing neurons of the rat brain. Brain Research, 1971, 28, 295-309.

Thierry, A.M., Le Douarin, C., Penit, J. Ferron, A. and Glowinski, J. Variation in the ability of neuroleptics to block the inhibitory influence of dopaminergic neurons on the activity of cells in the rat prefrontal cortex. Brain Research Bulletin, 1986, 16, 155-160.

Thierry, A.M., Tassin, J.P., Blanc, G. and Glowinski, J. Selective activation of the mesocortical DA system by stress. Nature, 1976, 263, 242-243.

Thornburg, J.E. and Moore, K.E. The relative importance of dopaminergic and noradrenergic neuronal systems for the locomotor stimulation induced by amphetamine and other drugs. Neuropharmacology, 1973, 12, 853-866.

Tilson, H.A. and Rech, R.H. Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity. Pharmacology Biochemistry and Behavior, 1973, 1, 149-153.

Tseng, L.F., Wei, E.T., Loh, H.H. and Li, C.H. Beta-endorphin central sites of analgesia, catalepsy and body temperature changes in rats. The Journal of Pharmacology and Experimental Therapeutics, 1980, 214, 328-332.

Ungerstedt, U. Central dopamine mechanisms and unconditioned behaviour. In A.S. Horn, J. Korf and B.H.C. Westerink (eds.), The Neurobiology of Dopamine. New York: Academic Press, 1979, pp. 577-596.

Vaccheri, A., Dall'Olio, R., Gandolfi, O., Roncada, P. and Montanaro, N. Enhanced stereotyped response to apomorphine after chronic D-1 blockade with SCH 23390. Psychopharmacology, 1987, 91, 394-396.

Vasco, M.R. and Domino, E.F. Tolerance development to the biphasis effects of morphine on locomotor activity and brain acetylcholine in the rat. The Journal of Pharmacology and Experimental Therapeutics, 1978, 207, 848-858.

Vezina, P., Giovino, A.A., Wise, R.A. and Stewart, J. Environment-specific cross-sensitization between the locomotor activating effects of morphine and amphetamine, in preparation.

Vezina, P., Kalivas, P.W. and Stewart, J. Sensitization occurs to the locomotor effects of morphine and the specific mu opioid receptor agonist, DAGO, administered repeatedly to the ventral tegmental area but not to the nucleus accumbens. Brain Research, 1987, 417, 51-58.

Vezina, P. and Stewart, J. Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacology Biochemistry and Behavior, 1984, 20, 925-934.

Vezina, P. and Stewart, J. Conditioned locomotion and place preference elicited by tactile cues paired exclusively with morphine in an open field. Psychopharmacology, 1987, 91, 375-380.

Vogelsang, G.D. and Piercey, M.F. The supersensitivity of dopaminergic neurons to apomorphine in rats following chronic haloperidol. European Journal of Pharmacology, 1985, 110, 267-269.

Wachtel, S.R. and White, F.J. Interactions between D-1 and D-2 receptors within the rat mesoaccumbens dopamine system: Electrophysiological studies. Society for Neuroscience Abstracts, 1986, 12, 1516.

Wagner, A.R. and Rescorla, R.A. Inhibition in pavlovian conditioning: Application of a theory. In R.A. Boakes and M.S. Halliday (Eds.), Inhibition and learning. New York: Academic Press, 1972.

Wamsley, J.K., Young, W.S. and Kuhar, M.J. Immuno-histochemical localization of enkephalin in rat forebrain. Brain Research, 1980, 20, 925-934.

Wang, R.Y. Dopaminergic neurons in the rat ventral tegmental area.II.Evidence for autoregulation. Brain Research Reviews, 1981, 3, 141-151.

Watanabe, H. Subchronic methamphetamine treatment selectively attenuates apomorphine-induced decrease in 3,4-dihydroxyphenylacetic acid level in mesolimbic dopaminergic regions. Life Sciences, 1985, 37, 2319-2325.

Watanabe, H. and Taniguchi, M. Effect of subchronic treatment with methamphetamine on apomorphine-induced changes in locomotor activity in mice. Japanese Journal of Pharmacology, 1986, 40, 185-187.

Weissman, A., Koe, B.K. and Tenen, S.S. Antiamphetamine effects following inhibition of tyrosine hydroxylase. The Journal of Pharmacology and Experimental Therapeutics, 1966, 151, 339-352.

White, F.J. and Wang, R.Y. Electrophysiological evidence for A10 dopamine autoreceptor subsensitivity following chronic d-amphetamine treatment. Brain Research, 1984, 309, 283-292.

Wiechman, B.E., Wood, T.E. and Spratto, G.R. Locomotor activity in morphine-treated rats: Effects of and comparisons between cocaine, procaine, and lidocaine. Pharmacology Biochemistry & Behavior, 1981, 15, 425-433.

Wilcox, R.A., Robinson, T.E. and Becker, J.B. Enduring enhancement in amphetamine-stimulated striatal dopamine release in vitro produced by prior exposure to amphetamine or stress in vivo. European Journal of Pharmacology, 1986, 124, 375-376.

Wise, R.A. and Bozarth, M.A. A psychomotor stimulant theory of addiction, in press.

Winn, P. and Robbins, T.W. Comparative effects of infusions of 6-hydroxydopamine into nucleus accumbens and anterolateral hypothalamus on the response to dopamine agonists, body weight, locomotor activity and measures of exploration in the rat. Neurophamacology, 1985, 24, 25-31.

APPENDIX

Figure A. Bilateral injector cannula tip placements in the VTA for the 34 animals included in the data analysis of Experiment 2. The coronal sections are from the atlas of Pellegrino et al. (1979). Numbers to the left indicate mm from bregma. The different symbols indicate group affiliation:

■ , SAL-MOR (n=8)

□ , SUL-MOR (n=6)

△ , PIM-MOR (n=7)

◇ , Ro-MOR (n=7)

○ , SCH-MOR (n=6).

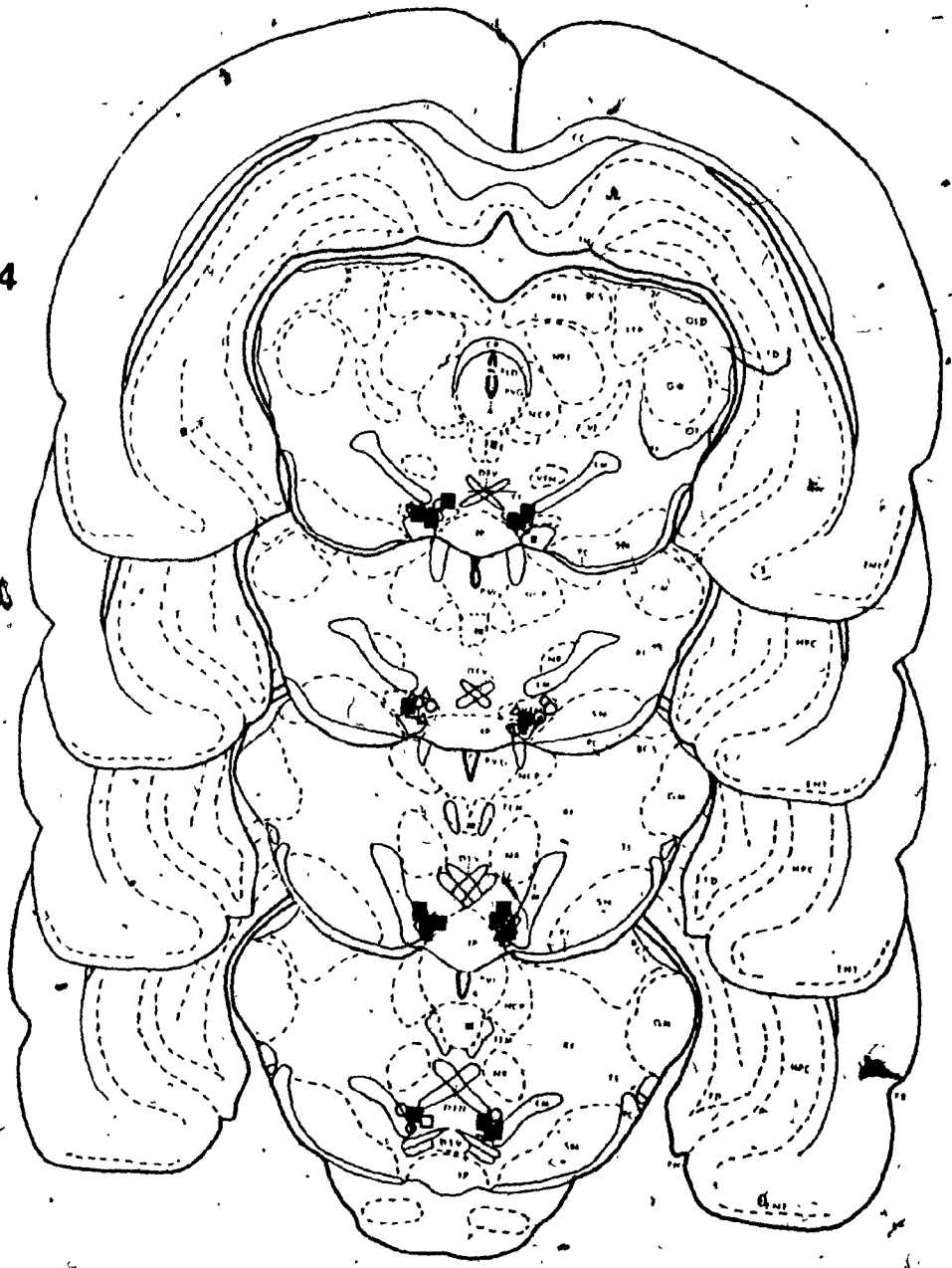

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Figure B. Bilateral injector cannula tip placements in the VTA for the 27 animals included in the data analysis of Experiment 5. The coronal sections are from the atlas of Pellegrino et al. (1979). Numbers to the left indicate mm from bregma. The different symbols indicate group affiliation:

■ , COND (n=9)

□ , PSEUDO (n=9)

○ , CTL (n=9).

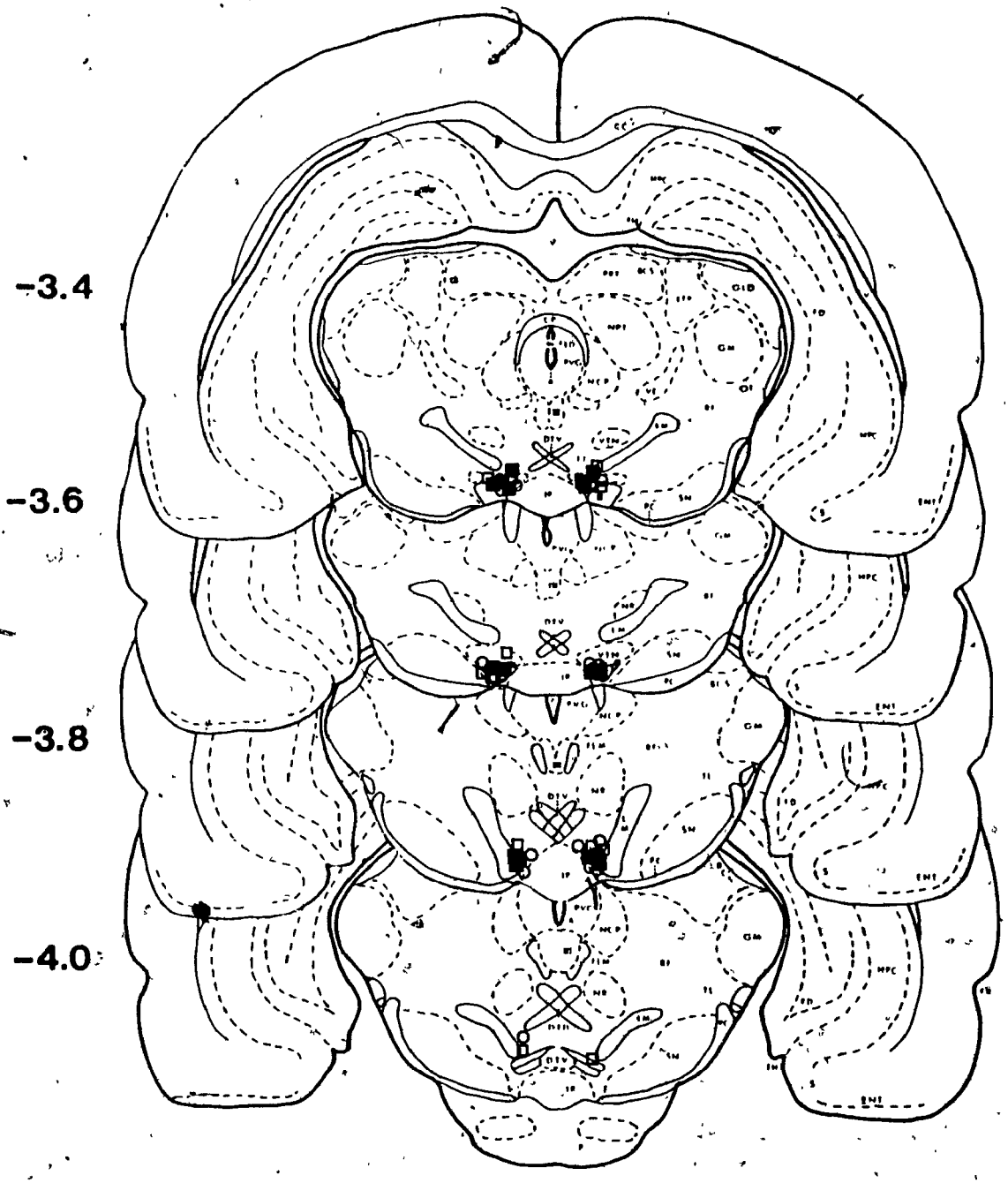
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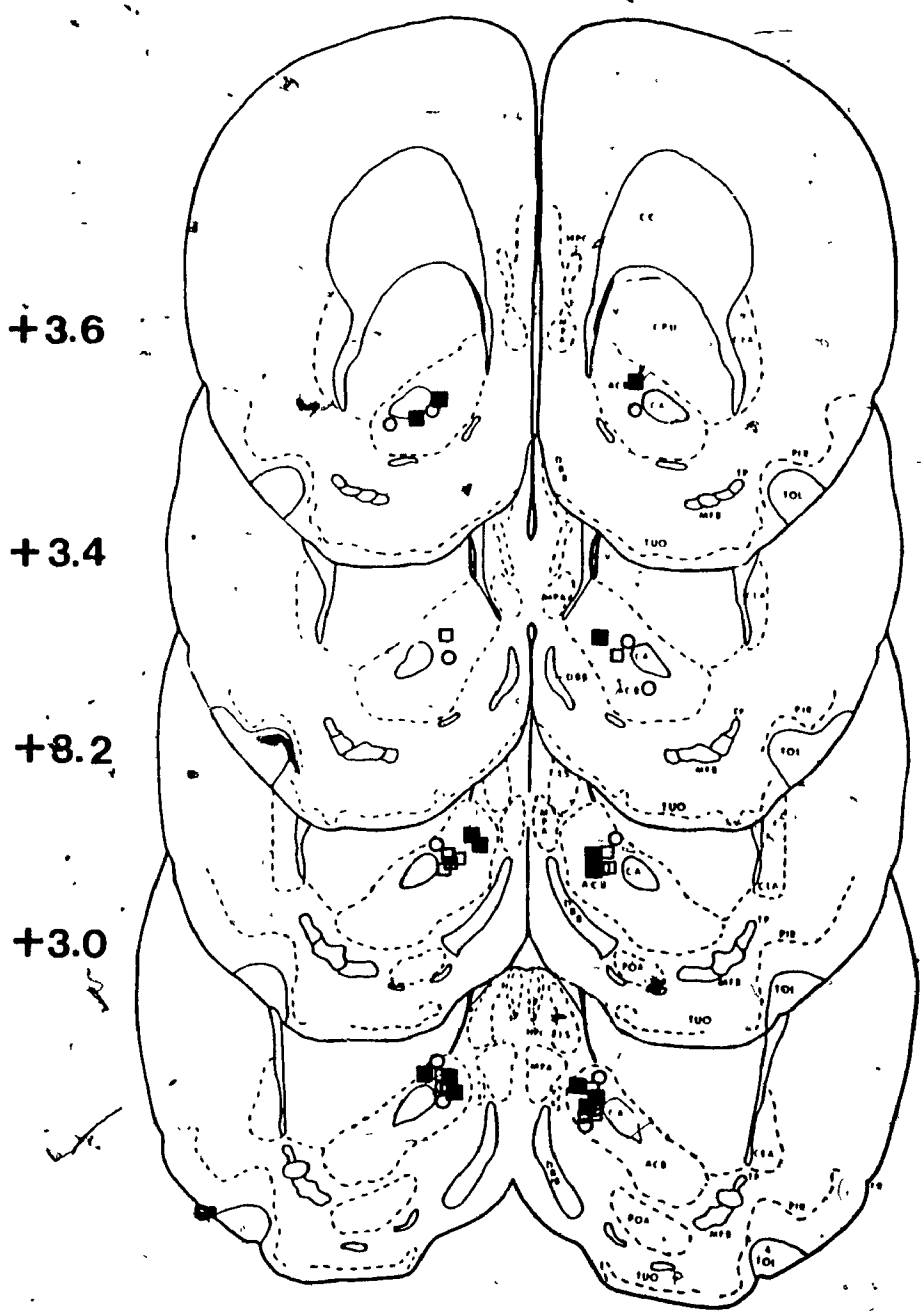
Figure C. Bilateral injector cannula tip placements in the NAC for the 21 animals included in the data analysis of Experiment 6. The coronal sections are from the atlas of Pellegrino et al. (1979). Numbers to the left indicate mm from bregma. The different symbols indicate group affiliation:

■ , COND (n=7)

□ , PSEUDO (n=7)

○ , CTL (n=7).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