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EFFECTS OF CHRONIC NALTREXONE PRETREATMENT ON AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY IN THE OPEN-FIELD: DIFFERENTIAL RESPONSE WITH TWO DIFFERENT POPULATIONS OF WISTAR RATS AND NOISE STRESS

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A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montréal, Québec, Canada

September 1981

ABSTRACT

Effects of chronic naltrexone pretreatment on amphetamine-induced locomotor activity in the open-field: Differential response with two different populations of Wistar rats and noise stress

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The relationship between opiate receptor and the dopamine system was examined using the opiate supersensitivity phenomenon in two different populations of Wistar rats, and in the presence or absence of white noise. The animals were chronically treated with naltrexone for 8 days to induce opiate receptor proliferation. After a 2-day rest period, animals were tested with amphetamine for locomotor activity in the open-field in the presence or absence of white noise. The animals were similarly tested on Day 7 and Day 14 after the termination of the naltrexone pretreatment. In Experiment 1, chronic naltrexone treatment significantly attenuated amphetamine-induced locomotor activity in new colony Wistar rats at a dose of 1 mg/kg of amphetamine, but not at doses of 0.5 or 2.0 mg/kg and in the absence

of white noise. In Experiments 2 and 3, the chronic naltrexone pretreatment significantly decreases the locomotor activity of 1 mg/kg of amphetamine in old colony Wistar rats in the presence of white noise, but not in the no-noise condition. Finally, Experiment 4 showed that under the white noise condition, there was no effect of chronic naltrexone pretreatment on the amphetamine's effect on locomotion in the new colony rats. The attenuating effect of chronic naltrexone pretreatment on amphetamine-induced locomotor activity seen under two sets of conditions suggests that opiate receptor has an inhibitory role on dopamine transmission. These experiments also suggest that a very complex interaction exists between the opiate receptor, the dopamine system, the organism's predispositions, and environmental stimuli.

ACKNOWLEDGMENTS

I would like to express my appreciation and gratitude to my thesis adviser, Dr. Zalman Amit for his support, interest, and encouragement throughout the course of these studies.

I am deeply grateful to Dr. Richard Blair for his participation in the supervision of this research and his criticisms on the original draft of this manuscript.

Sincere thanks are also extended to Larry Holmes for his help in the conduct of the research and to Elizabeth Chau for her excellent typing of the thesis.

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Morphine produces a wide variety of pharmacological effects. For example, morphine is well known for its analgesic properties (Herz, Albus, Metys, Schubert, & Teschemacher, 1970; Irwin, Houde, Bennett, Hendershot, & Seevers, 1951; Yaksh & Rudy, 1976, 1978), its effects on thermoregulation (Clark & Clark, 1980) and respiratory functions (Florez, McCarthy, & Borison, 1968). In addition, morphine and opiates in general affect locomotor activity (Babbini & Davis, 1972; Oka & Hosoya, 1976; Sloan, Brooks, Eisenman, & Martin, 1962; Vasko & Domino, 1978), electrical self-stimulation (Adams, Lorenz, & Mitchell, 1972; Esposito, McLean, & Kornetsky, 1979; Wauquier, Niemegeers, & Lal, 1974), memory (Staubli & Huston, 1980).

It is generally accepted that most, if not all, of the effects of the opiates are mediated by the recently-discovered opiate receptor. The binding of opiates to the opiate receptor appears to be the first step in a series of physiochemical reactions involved in the manifestation of the pharmacological actions of the opiates. The discovery of these opiate receptors in the central nervous system has now prompted research interest concerning the opiate receptor's physiological functions, and the mechanisms of its interaction with other neurotransmitter

systems. The present thesis addressed the question of the possible interaction between the dopamine system and the opiate receptor and the nature of the relationship. A short review of the studies on the distribution of opiate receptors and endogenous ligands in relation to the dopamine system will thus be presented. Following that, electrophysiological, biochemical, and behavioral studies will be reviewed in terms of their contribution in the understanding of the relationship between the dopamine system and the opiate receptor.

Opiate receptor and its ligands in relation to dopamine pathways.

The opiate receptor is conceptualized as a three-dimensional structure in the cell membrane to which active levo-isomers of opiates bind in a stereospecific way. Following the discovery of the opiate receptor (Pert & Snyder, 1973; Simon, Hiller, & Edelman, 1973; Terenius, 1973), regional autoradiographic binding studies have revealed very high densities of opiate receptors in the amygdaloid complex and the striatum (Atweh & Kuhar, 1977; Kuhar, Pert, & Snyder, 1973; Pert, Kuhar, & Snyder, 1975, 1976). The existence of such receptors in the central nervous system has led to speculation about, and subsequently to

the identification of endogenous morphine-like substances (Cox, Goldstein, & Li, 1976; Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975). Using radioimmunoassay techniques, it has also been shown that the neostriatum contains substantial quantities of morphine-like pentopeptides, methionine- and leucine-enkephalins (Miller, Chang, Cooper, & Cuatrecasas, 1978; Simantov, Kuhar, Pasternak, & Snyder, 1976; Yang, Hong, & Costa, 1977). Interestingly, both the neostriatum and the amygdala also contain high densities of dopamine receptors (Hollts, Czlonkowski, & Herz, 1977; Laduron & Leysen, 1977) and are very rich in dopamine-containing terminals (Ungerstedt, 1971b). Further, combined histofluorescence-immunocytochemical studies have uncovered a distinct overlap between distribution of enkephalin immunofluorescence, opiate receptors, and dopamine cell bodies and terminals in areas such as the substantia nigra, ventral tegmentum, striatum, and the nucleus accumbens (Johannsson, Hökfelt, Elde, Schulzberg, & Terenius, 1978; Uhl, Goodman, Kuhar, & Snyder, 1978). In addition, lesioning of the dopamine presynaptic terminals in the terminal areas of the dopamine pathways with 6-hydroxydopamine, a chemical sympathectomy agent, is accompanied by a 20 - 30% decrease in opiate receptor

binding sites (Pollard, Llorens-Cortes, & Schwartz, 1977). Such findings have suggested that some of the opiate receptors are localized on the terminals of the dopamine neurons. Additional studies have demonstrated that the interruption of the nigrostriatal bundle by hemisection at the hypothalamic level is followed by a decrease in opiate receptors, but no change in methionine- or leucine-enkephalin levels was observed (Pollard, Llorens, Schwartz, Gros, & Dray, 1978). In the same study, a 6-hydroxydopamine-induced lesion of dopamine neurons in the substantia nigra caused a 30% decrease in opiate binding sites suggesting that the dopamine cell bodies and/or dendrites also contain opiate receptors. One should note that the substantia nigra also contains substantial amounts of enkephalins as shown by radioimmunoassay and immunocytochemistry (Elde, Hökfelt, Johannsson, & Terenius, 1976; Hong, Yang, Fratta, & Costa, 1977). More extensive studies have found an enhancement in the number of binding sites of radiolabelled domperidone, a dopamine agonist, following a six-day chronic morphine treatment (de la Baume, Patey, Marçais, Protais, Costentin, & Schwartz, 1979). Since this morphine effect is similar to the effects of dopamine blocking agents on dopamine receptor binding, an issue that emerges from

these studies is whether the opiate receptor and the dopamine receptor are in fact the same receptor. To rule out this possibility, it has been demonstrated that opiates do not bind to the dopamine receptors as neuroleptics do and vice versa (Leysen, Tollenaere, Koch, & Laduron, 1977). Thus, it seems very likely that there exists a functional relationship between the opiate receptor and the dopamine system.

Electrophysiological Studies.

Another body of evidence for the functional relationship between the opiate receptor and the dopamine system comes from electrophysiological studies. It was originally shown that electromyographic activity induced by morphine administration can be reversed by either l-dopa or apomorphine (Wand, Kuschinsky, & Sontag, 1978). This finding is in agreement with the notion that the opiate receptor has an inhibitory effect on dopamine neurotransmission. On the other hand, Zieglgänsberger, Siggins, French, & Bloom (1978) have pointed out that the most frequently observed response of single unit activity to phoretic application of opiate agonists is a naloxone-reversible depression of firing rate. More specifically, it is known that the striatal neurons are depressed by

opioid and opiate agonists applied microiontophoretically (Nicoll, Siggins, Ling, Bloom, & Guillemin, 1977; Frederickson & Norris, 1976; Lee, Wong, & Chan, 1977; Finnerty & Chan, 1981). Similar inhibitory effects on neuronal activities in the striatum are found with dopamine (Siggins, Hoffer, Bloom, & Ungerstedt, 1976). Assuming that the opiates are acting on the presynaptic dopamine terminals in these regions, these studies would suggest a facilitatory role of opiate receptor on the dopamine system. Additional evidence for this facilitatory role of the opiate receptor has been obtained in the caudate nucleus where morphine inhibitory effect on neuronal activities was prevented by the dopamine receptor blockers, haloperidol and pimozide (Lee et al., 1977).

Contrary to this conclusion, Bradley and Gayton (1976) failed to observe any effect of the dopamine antagonist, α-flupenthixol on morphine inhibitory action on caudate neurons even though the same drug successfully blocked the dopamine inhibitory effect. In addition, the same investigators also found that morphine reduced the dopamine inhibitory effect in some neurons. The interpretation of these early studies become controversial in view of the fact that electrical stimulation of the substantia nigra most often excites rather than

depresses striatal neurons suggesting that dopamine may itself act as an excitatory neurotransmitter in the striatum. Furthermore, although the striatum contains a large number of dopamine neurons, these neurons represent only a small percentage of the total number of striatal neurons. Yet, in these studies, there is no indication about the nature of the neurons whose electrical activities were being monitored, hence making interpretation about the cellular site of the drugs actions is very difficult.

A technique developed by Bunney, Walters, Roth, & Aghajanian (1973) has made it possible to identify dopamine neurons based on their electrophysiological parameters. Using this technique, it was demonstrated that systemic injection of morphine increases the firing rates of dopamine-containing neurons in the pars compacta of the substantia nigra (Iwatsubo & Clouet, 1977; Nowycky, Walters, & Roth, 1978; Finnerty & Chan, 1981). These same nigral dopamine-containing neurons are also depressed by dopamine receptor agonists such as l-dopa, apomorphine, and d-amphetamine and activated by dopamine receptor blockers such as haloperidol (Bunney et al., 1973; Iwatsubo & Clouet, 1977). Furthermore Iwatsubo and Clouet (1977) also showed that l-dopa and apomorphine

both reversed the stimulation of firing rates induced by morphine. Based on these data, and also data from behavioral and biochemical studies, several investigators have proposed a negative striato-nigral feedback pathway that impinges on the dopamine cell bodies. Thus, it was shown that the depressant effects of d-amphetamine on dopamine-containing neurons in the substantia nigra was markedly attenuated after lesions of the striato-nigral pathway (Bunney & Aghajanian, 1976). Likewise, Kondo and Iwatsubo (1980) have reported diminished responses of nigral dopaminergic neurons to haloperidol and morphine in animals following kainic-lesions in the striatum. The similarity of morphine and dopamine antagonists actions on the nigral neurons supports the contention of an inhibitory input to dopamine transmission by the opiate receptor localized on the dopamine neuron terminals.

Other investigators have argued instead that direct activation of opiate receptors of morphine localized on the cell bodies causes a depressive effect on spontaneous neuronal activities in the caudate nucleus by increasing the dopamine output in the striatum (Lee et al., 1976). To support the latter hypothesis, Finnerty and Chan (1981) have recently shown that microinjection of

morphine directly into the substantia nigra zone compacta resulted in a naloxone-reversible depression of caudate activity. However, given the relatively low density of opiate receptors in the substantia nigra compared to the density in the neostriatum, the excitatory component of the opiate receptor on the dopamine transmission at the cell bodies might be of minor importance.

Biochemical Studies

Biochemical studies have also been carried out to investigate the actions of opiates on neurotransmitter turnover, axonal transport, release, and metabolism in the dopamine neuron. The first demonstration of morphine biochemical effect on catecholamines was reported by Vogt (1954) who found a marked decrease in brain catecholamine levels following a morphine injection in the cat. This finding might reflect a morphine action on either turnover or release or metabolism of any of the members of the catecholamine family. A more discriminative study was reported by Clouet and Ratner (1970) in which morphine administered one hour before sacrificing the animals caused an accumulation of ^{14}C-dopamine from ^{14}C-tyrosine. This increase in dopamine synthesis by morphine has been confirmed by other researchers (Costa,

Carenzi, Guidotti, & Revuelta, 1973; Gauchy, Agid, Glowinski, & Cheramy, 1973; Kuschinsky & Hornykiewicz, 1972; Westerink & Korf, 1975). The opiate effect on dopamine turnover has been observed to be dose-dependent as well as stereospecific (Gessa, Vargiu, Biggio, & Tagliomonte, 1973; Smith, Sheldon, Bednarczyk, & Villarreal, 1972) thus implicating a role for the opiate receptor in dopamine turnover. In addition, morphine and other opiates increase dopamine metabolism in brains and striata of rats (Gunne, Jonsson, & Fuxe, 1969; Kuschinsky & Hornykiewicz, 1972; McMillen, 1980; Sasame, Perez-Cruet, Di Chiara, Tagliamonte, Tagliamonte, & Gessa, 1972) and in brains and striata of mice (Fukui & Takagi, 1972; Kuschinsky & Hornykiewicz, 1974). Similar effects on the dopamine synthesis and metabolism have been demonstrated with methionine-enkephalin and the synthetic opioid D-ala-leu-enkephalinamide (Algeri, Calderini, Consaloziona, & Garattini, 1977; Biggio, Casu, Borda, Dibello, & Gessa, 1978). When one compares these effects of opiates and opioids to the well-established responses of dopamine agonists and antagonists on the dopamine neurons (Carlsson, 1975; Groves & Rebec, 1976; Puri, Reddy, & Lal, 1973; Westerink & Korf, 1975), one is very likely to conclude that opiates inhibit dopaminergic

neurotransmission, at least in the striatum. The fact that, unlike haloperidol, morphine does not inhibit the dopamine stimulation of adenylate cyclase activity in caudate synaptosomal preparations suggests that the opiates do not act on the postsynaptic dopamine receptor as haloperidol does (Carenzi, Cheney, Costa, Guidotti, & Racagni, 1975; Iwatsubo & Clouet, 1975). On the other hand, there is some evidence of a presynaptic action of the opiate receptor on the dopamine system. Both morphine and β-endorphin have been observed to inhibit the K^+-induced release of dopamine from striatal slices in vitro (Loh, Brase, Sampath-Khanna, Mar, Way, & Li, 1976).

Morphine also shows a strong inhibitory effect on dopamine-stimulated cyclic AMP formation in intact slices of rat striatum (Minneman, 1977). The potency of dopamine in stimulating the synthesis of cyclic AMP is unchanged in striatal homogenates of morphine-withdrawn rats while the K^+-induced release of dopamine is increased in similarly-treated rats (Bosse & Kuschinsky, 1976). This finding suggests that an adaptation to chronic morphine administration involves a presynaptic mechanism. Although the criterion of opiate antagonist-reversibility is not met in these studies, they nevertheless support the involvement of the opiate receptor in

the release of dopamine. Overall, it has been proposed that activation of the opiate receptor blocks the release of dopamine and thereby activates a negative feedback loop which in turn stimulates the dopamine cell bodies, and increases turnover and metabolism of dopamine. To show that opiates act on the same feedback loop as the dopamine agonists and antagonists, it was reported that haloperidol potentiated morphine stimulation of dopamine synthesis in the striatum while apomorphine blocked the morphine effect (Puri et al., 1973).

Biggio et al. (1978) have instead proposed a local feedback process that works through presynaptic dopamine autoreceptors. They observed that intrastriatal injections of D-ala-leu-enkephalinamide increased dopamine turnover even after destruction of striatal cell bodies with kainic acid and suggested that this opioid effect might be mediated by a decreased activation of presynaptic dopamine receptors. But data from another report by Andén and Grabowska-Andén (1978) do not support this contention. The interruption of dopamine nerve impulse flow following γ-butyrolactone, an agent that completely inhibits neuronal firing in dopamine cells, markedly increased the dopa accumulation in the corpus striatum. Apomorphine inhibits this effect presumably by stimula-

ting dopamine autoreceptors on nerve terminals. Morphine did not affect the γ-butyrolactone-induced increase in dopa accumulation nor did it alter the apomorphine-induced inhibition of the dopa accumulation in the corpus striatum indicating that morphine does not block dopamine autoreceptors.

Another alternative mechanism for opiate actions on the dopamine neuron was suggested by Celsen and Kuschinsky (1974). Because morphine and other opiates induce symptoms of decreased dopaminergic neurotransmission and show supra-additive effects on dopamine utilization with neuroleptics, these investigators argued that the opiates might induce a diversion of newly-synthesized dopamine from storage sites to sites of catabolism. Thus, a dopamine deficiency in the extragranular pool would cause disinhibition of tyrosine hydroxylation, resulting in an increased dopamine synthesis. Interestingly, McMillen (1979) had found that, unlike haloperidol, morphine together with amfonelic acid, an impulse-dependent dopamine-releasing agent as well as a reuptake dopamine blocker (Shore, McMillen, Miller, Sanghera, Kiser, & German, 1979), have additive effects on the increase in dopamine metabolite, dihydroxyphenylacetic acid in the striatum. This finding

argues against the idea that the potentiating effect on dopamine metabolism is a result of a compensatory mechanism mediated through dopamine synthesis and turnover, and it also suggests that the morphine effect on dopamine metabolism is not secondary to its inhibitory effect on dopamine release. Hence, it seems that morphine effects on synthesis, turnover, metabolism and release of dopamine are to a large degree independent of each other. This independence of opiates effects agrees to a certain extent with the distribution of opiate receptors both at the terminals and on cell bodies of the dopamine neurons. Lastly, it should be noted that chronic administration of neuroleptics causes an increase in methionine-enkephalin levels in the striatum and nucleus accumbens which is presumed to result from an increase in the biosynthesis of the opioid peptide (Hong, Yang, Gillin, & Costa, 1980). This advances the notion that the converse situation where the dopamine system can affect the endogenous opioid system probably also exists.

Behavioral Studies

The number of behavioral studies bearing on the dopamine-opiate receptor interrelationship has been

tremendously high in the past decade. A variety of behavioral measures have been used to demonstrate such interaction. Lal (1975) has reviewed the literature on the involvement of the dopamine system in morphine-withdrawal aggression and the jumping-response. He concluded that morphine-withdrawn animals are supersensitive to dopamine agonists and subsensitive to the antagonists presumably as a result of the inhibitory effect of acute morphine administration on dopamine transmission. Chronic treatment with morphine is believed to induce supersensitivity to dopamine agonists in terms of increasing the number of dopamine receptors. This supersensitivity effect develops as a compensatory reaction to the continuous inhibitory influence of the opiate receptor activated by the chronic morphine treatment, and is manifested in morphine-withdrawn rats. Among the other behaviors that have been implicated in the dopamine-opiate receptor interaction are catalepsy (Dunstan, Broekkamp, & Lloyd, 1981; Namba, Quock, & Malone, 1980), grooming (Green, Isaacson, Dunn, & Lanthorn, 1979), free-access food intake (Schulz, Wuster, & Herz, 1980), intracranial self-stimulation (Esposito, Perry, & Kornetsky, 1980; Seeger, Nazzaro, & Gardner, 1980), and apomorphine-induced stereotypies (Buckett, 1979; Carlson

& Almasi, 1978). However, considerable attention has been paid to the effects of opiates and drugs that affect dopaminergic activity alone, and in combination, on locomotor activity. The intensity of the research in this area is such that closer detailed examination is justified.

Rotational behavior. One measure of locomotor activity that has been widely used to test dopamine function is the turning behavior following unilateral lesion of the nigrostriatal neurons (Glick, Jerussi, & Fleisher, 1976; Ungerstedt, 1971a). In animals with such unilateral lesion of the dopamine nigrostriatal pathway, dopamine receptor agonists such as apomorphine produce contralateral rotation which is believed to be the result of the development of increased receptor sensitivity on the lesioned side. D-amphetamine, on the other hand, causes ipsilateral rotation which is due to its dopamine release action from the intact nigrostriatal nerve endings. Using such a model of dopamine function, Cowan, Dettmar, and Walter (1975b) showed a dose-dependent antagonistic effect of morphine on apomorphine-induced contralateral turning behavior in rats. Since morphine at such low doses does not bind to dopamine receptors, it is very likely that such an

effect is being mediated by opiate receptors localized on postsynaptic neurons, and is related to the inhibitory effect of morphine on the dopamine-stimulated cyclic AMP production in vitro (Minneman, 1977) which would neutralize the activating action of apomorphine on the dopamine receptor. Another possibility is that morphine which alone produces ipsilateral rotation in unilateral nigrostriatal-lesioned animals (Watanabe, Ikeda, & Watanabe, 1979), balances the tendency to rotate contralaterally as induced by the apomorphine. Such a proposition would be consistent with the facilitatory role of the opiate receptor on dopamine transmission. Additional evidence comes from a report showing that low doses of naloxone antagonized the d-amphetamine ipsilateral circling in the nigral-lesioned rats (Dettmar, Cowan, & Walter, 1978).

In contrast, an earlier study by the same investigators has revealed that morphine at 10 mg/kg but not at lower doses, significantly antagonized the amphetamine-induced circling behavior (Cowan, Dettmar, & Walter, 1975a). This latter finding has recently been replicated and, in addition the morphine effect was found to be dose-dependent and naloxone-reversible (Slater & Blundell, 1979; Slater, Blundell, & Crossman, 1979). These

data make a strong argument for the inhibitory role of opiate receptor on dopamine release. Finally, one has to take into consideration the fact that unilateral intranigral injection of morphine produces contralateral circling behavior (Iwamoto & Way, 1977; Pert, DeWald, Liao, & Sivit, 1979). Pretreatment with d-amphetamine or haloperidol potentiates or attenuates respectively this morphine-induced contralateral circling behavior (Kaakkola, 1980) suggesting that activation of opiate receptors on the dopamine cell bodies stimulates dopamine neuronal transmission. However, given the inconsistency of the results across studies and the likely involvement of other transmitters in the rotation behavior (Glick et al., 1976), and the fact that most studies do not satisfy the criteria for receptor specificity, the question concerning the directionality of the opiate receptor input to the dopamine transmission still remains confusing on this behavioral measure.

Locomotor activity in the open-field. One behavioral assay that has been extensively used in pharmacological studies is locomotor activity. A variety of techniques and procedures have been developed to measure locomotor activity, ranging from simple counts of the number of squares crossed by the animal in an open-

field, activity wheels, stabilimeters to sophisticated vistration-monitoring device in the floor of an open-field chamber to pick up selective movements of the animal. However, the most often used index of locomotor activity is photocell counts in the open-field. Although the open-field itself is a very complex test (Walsh & Cummins, 1978, 1976), the measure of activity or ambulation has repeatedly been shown to be a reliable (Broadhurst, 1960; Manosewitz, 1970; Weasner, Finger, & Read, 1960; Whimbey & Denenberg, 1967) and valid measure of emotional stability (Henderson, 1970; Royce, 1977; Whimbey & Denenberg, 1967). In his review, Royce (1977) identified one of the three lower-order constructs of emotional stability to be motor discharge, and the primary indices of the latter factor as being latency to move, activity, and penetration to the center of the field.

Amphetamine effects on locomotor activity are well established (Dews, 1958). It has generally been found that amphetamine disrupts normal locomotor patterns of ambulation above doses of 3 mg/kg (Randrup & Munkvad, 1970) or 5 mg/kg at which point stereotyped behaviors like biting, gnawing, sniffing, kicking, and small head movements start to emerge. Such observations evidently

warrant some attention to the problematic issue of external validity of photocell counts measure in drugged animals particularly when the drug is amphetamine. Nevertheless, Krsiak, Steinberg, & Stolerman (1970) have reported high correlations between observed walks and photocell counts when animals were administered doses of amphetamine ranging from 0.25 to 2 mg/kg. Another related problem of validity is whether changes on photocell counts in the open-field reflect actions on locomotor activity or exploration or both (Broadhurst, 1960; Christmas & Maxwell, 1970). With regard to amphetamine, the drug has consistently been shown to increase locomotor activity while inhibiting exploration (Cox & Tye, 1975; Robbins & Iversen, 1973). The neuroanatomical site of amphetamine-induced locomotor activity appears to be localized in the nucleus accumbens of the limbic system (Kelly & Iversen, 1976; Kelly, Sevious, & Iversen, 1975; Fink & Smith, 1980; Franklin & Robertson, in press). Furthermore, the primary neurotransmitter involved in this amphetamine effect seems to be dopamine (Hollister, Breese & Cooper, 1974; Pijnenburg, Hönig, & van Rossum, 1975; Pijnenbrug & van Rossum, 1973; Roberts, Zis, & Fibiger, 1975). The mechanism of amphetamine action seems to consist of a

simultaneous release of dopamine as well as an inhibition of its re-uptake from the synaptic cleft (Cooper, Bloom, & Roth, 1974; Groves & Rebec, 1974; Taylor & Snyder, 1971).

Opiates have received as much attention in terms of their effects on locomotor activity. In general, low doses of opiates produce a biphasic effect starting with an initial depressant effect followed by an excitatory effect (Babbini & Davis, 1972; Browne & Segal, 1980; Fog, 1970; Oka & Hosoya, 1976; Sloan et al., 1962). Both the depressive and excitatory effects of the opiates seem to be mediated by the opiate receptor (Holtzman, 1976; Oka & Hosoya, 1976). In parallel to amphetamine-induced locomotor activity, the nucleus accumbens has also been implicated in the morphine-induced changes in locomotor activity (Pert & Sivit, 1977). In contrast, enhanced naloxone-reversible motor activity was reported after administration of morphine and the synthetic opioid D-ala-enkephalinamide in the ventral tegmental area, a region which contains most of the cell bodies of the mesolimbic dopaminergic pathway (Broekkamp, Phillips, & Cools, 1979; Joyce & Iversen, 1979; Kelley, Stinus, & Iversen, 1980; Stinus, Koob, Ling, Bloom, & LeMoal, 1980). To explain these results, it was pro-

posed that opiates act through opiate receptors to stimulate A10 dopamine cell bodies by two different mechanisms. One is inhibition of a tonic inhibitory system afferent to the dopamine cells with the opiate receptor localized on the terminals of the presynaptic neurons. The other is inhibition of dendritic release of dopamine on the same afferent output with the opiate receptor localized on the dopamine cell bodies.

However, the first report on direct interaction between the opiate receptor and the dopamine system on locomotor activity showed that the activity produced by amphetamine is reduced by morphine (van Nueten, 1962). This finding has subsequently been successfully replicated by Fog (1970), and Yehuda, Zadina, Kastin, and Coy (1980). The situation becomes more complex, on the other hand, when one looks at studies using the opiate antagonists and/or different species other than the rat. For instance, Holtzman (1973) found that naloxone decreased amphetamine-induced locomotor activity in rats but not in mice even at doses as high as 100 mg/kg. And while Haber, Hatsukami, Berger, Barchas, and Akil (1978) reported no effect of naloxone on amphetamine locomotor activity in rats, Dettmar et al. (1978) showed an antagonistic effect of naloxone on the stimu-

lant activity of amphetamine in mice. In addition, electrolytic lesion and chemical blockade with haloperidol of the posterior nucleus accumbens completely abolished amphetamine hyperactivity but only reduced morphine-induced increase in activity (Teitelbaum, Giammatteo, & Mickley, 1979).

In summary, the set of behavioral studies mentioned above seems to favour the hypothesis that morphine and opiates or opioids through activation of the opiate receptors increase dopamine transmission at least in the mesolimbic dopaminergic pathway assuming that such an increase in dopamine neurotransmission leads to enhancement of motor activity (Pijnenburg & van Rossum, 1973). Such a conclusion is inconsistent with the idea of an inhibitory influence of the opiate receptor on the dopamine transmission discussed in the electrophysiological and biochemical studies sections. A reconciliation of these data could be made based on a biphasic opiate action on the dopamine neuron as proposed by Di Chiara, Vargiu, Proceddu, Longoni, Mulas, and Gessa (1977). These investigators suggested that opiates produce their depressant effects by an inhibitory action on the terminals of the dopamine system, whereas the excitatory effects seem to depend on an indirect

stimulatory action on the dopamine cell bodies. In agreement with the proposal, studies have shown that injection of opiates or opioids directly into the terminal areas of the dopamine nigrostriatal system (Dill & Costa, 1977; Dunstan, Broekkamp, & Lloyd, 1980; Koffer, Berney, & Hornykiewicz, 1978) or the dopamine mesolimbic system (Costall, Fortune, & Naylor, 1978; Dill & Costa, 1977) produced catalepsy, which might be considered as absence of locomotion in this context. It remains to be seen how well this mechanism of opiate action will stand up against future research.

The Present Investigation

Meanwhile, there are some important general factors to consider in all these studies. First, some species of animals like cats and mice show predominantly excitatory effects in response to opiates unlike rats that show both the depressive and the excitatory effects. Second, given that opiates have biphasic effects when administered systematically it is important to look at the time course of the opiate effect over an extended period of time. Third, there has been little concern about the possible interaction between stress and drug response in these studies. Consideration of these problems could potentially reconcile the conflicting data in this area of research. The object of the present thesis is to elaborate further on the interaction between the opiate receptor and the dopamine system at the behavioral level using the open-field locomotor activity measure. Recently, Amir, Blair, and Amit (1979) have demonstrated that chronic naltrexone pretreatment enhanced amphetamine locomotor activity. Using the same paradigm, a series of experiments will be conducted to investigate the interaction between chronic naltrexone pretreatment, amphetamine, kind of animals used, and stress of environmental stimuli.

Experiment 1 is designed firstly to study the effect of chronic naltrexone pretreatment on amphetamine action, in a new population of Wistar rats, and secondly, to find a dose-dependent effect of amphetamine on the locomotor activity measure. Experiment 2 will re-examine the interaction between chronic naltrexone pretreatment and amphetamine on the old population of Wistar rats. In Experiment 3, the same interaction will be investigated in the old colony rats but this time in the presence of a noise stressor. Finally, Experiment 4 will look at the interaction of the chronic naltrexone and amphetamine on new colony rats in the presence of the noise stressor.

EXPERIMENT 1

In rats chronic treatment with the opiate antagonist, naloxone results in supersensitivity to the analgesic actions of morphine on the tail-flick test (Tang & Collins, 1978). A similar supersensitivity effect was found to dopamine after chronic administration with dopamine antagonists (Burt, Creese, & Snyder, 1977). Consistent with Collier's (1965) third concept of receptor induction, these findings support the view that if a living organism is distorted by excess or deficiency of a chemical substance, induction of receptors in the direction lessening that distortion is to be expected. Just as the dopamine supersensitivity effect was associated with an increase in dopamine receptor sites with no change in binding affinity (Burt et al., 1977), Lahti and Collins (1978) similarly demonstrated that chronic naloxone treatment for four weeks in rats increased the ^{3}H-naloxone binding with no change in affinity constants.

This supersensitivity phenomenon provides a good tool for investigating the interactions between drugs and neurotransmitters mainly for two reasons. First, the supersensitivity effect usually lasts for several days, and even weeks (Lahti & Collins, 1978; Freidhoff,

1979) thus giving ample time for the drug to leave the system completely. One is then left with a drug-induced structural change that is physiologically functional and that can be studied very selectively without the complications of drugs interaction. Second, it is a laborious and arduous task trying to differentiate between the selective actions of drugs on motivational systems and their motor debilitation effects because some drugs like morphine are known to have general motor depressant properties. Interestingly, the supersensitivity phenomenon provides an attractive model because it basically shifts the dose-response curve to the left so that low doses of the drugs that do not usually produce changes in motor activity can possibly be investigated for their effects on the selected behavioral system. In the case of drugs that affect neurotransmitter systems, it is expected that the supersensitivity effect will be reflected in these systems too.

Following the above rationale, Amir et al. (1979) chronically pretreated rats with the opiate antagonist, naltrexone and found an enhancement of amphetamine-induced locomotor activity in the naltrexone-pretreated animals as compared to the activity of the saline-pretreated rats. Naltrexone itself is a long-lasting

opiate antagonist that is almost devoid of agonistic opiate properties (Blumberg & Ikeda, 1978; Pace, Parrish, Lieberman, Wong, & Blatnick, 1978). Like naloxone, it has also been used successfully to induce opiate supersensitivity in infant rats (Paul, Diaz, & Bailey, 1978). The enhanced response to amphetamine in naltrexone-pretreated animals as observed in Amir et al's (1979) study supports the contention that the dopamine neurotransmitter system is closely linked to the opioid system. The present experiment was directed to extend the data base of Amir et al's (1979) study by using three doses of amphetamine.

Method

Subjects. The subjects were 48 drug-naive Wistar rats of the new colony (see Appendix A; Canadian Breeding Laboratories Ltd.) weighing 200-250 g at the beginning of the experiment. Animals were housed individually in stainless steel cages in a temperature-regulated room (20° C) with a 12-hr day-night cycle (light on from 0800 to 2000 hr). Food (Purina Lab Chow) and water were made available ad libitum. The animals were handled for at least two days before the beginning of the experiment.

Apparatus. Four wooden chambers (45.7 cm x 45.7 cm

x 39.4 cm) were each illuminated by a 40-watt incandescent bulb placed 80 cm above the center of the floor of the chamber. Each chamber was equipped with four sets of light sources and photocells. These pairs of light sources and photocells were located 3.8 cm above the floor and arranged so that a pair of light beams crossed the other pair of light beams perpendicularly, dividing the activity chamber into nine equal squares. Locomotor activity, as measured by the number of times the rat crossed the light beams, was recorded automatically on counters connected to the four intersecting photocell beams.

Drugs and Injections. Naltrexone hydrochloride (Endo Laboratories Inc.) was dissolved in injectable saline solution to a concentration of 5 mg/ml (pH = 5.7). D-amphetamine sulphate (University Hospital Pharmacy) was also dissolved in injectable saline solution to 2 mg/ml (pH = 6.65), 1 mg/ml (pH = 6.5), and 0.5 mg/ml (pH = 6.35). Saline solution was used for control purposes. The naltrexone pretreatment injections were given subcutaneously in a volume of 2 ml/kg and the amphetamine treatment injections were administered intraperitoneally in a volume of 1 ml/kg.

Design and Procedure. Animals were randomly

assigned to the eight experimental groups defined by four levels of amphetamine doses, 0 (saline), 0.5, 1, or 2 mg/kg, and two levels of pretreatment, saline or 10 mg/kg of naltrexone (n = 6 for each group). After 2 to 3 days of adaptation to the animal room, the animals were pretreated with either saline or naltrexone (10 mg/kg, subcutaneously) daily for 8 days between 1200 hr and 1400 hr. Two days after the last injection, amphetamine was injected to the animals at different doses (saline, 0.5, 1, or 2 mg/kg, intraperitoneally). Fifteen minutes following the injection, the animals were placed individually in one corner of the activity chamber. Activity counts, as measured by the number of light beam interruptions, were noted after 30 minutes. The activity chambers were located in a sound-insulated room and testing always took place between 1100 hr and 1700 hr. The animals were retested 7 and 14 days after the termination of the naltrexone pretreatment using the same experimental procedure.

Results

Data for one animal in the saline-saline group were lost. The means and standard errors of the mean of the activity counts for different combinations of chronic drug pretreatment (naltrexone, N or saline, S)

and drug treatment (saline, S; 0.5 mg/kg, A1; 1 mg/kg, A2; or 2 mg/kg of amphetamine, A3) for the different trials are shown in Figures 1, 2, and 3 (see Appendix B, Table 1). A factorial analysis of variance of the data (see Appendix C, Table 1) indicates a significant increase in locomotor activity was produced by amphetamine, F (3, 39) = 12.16, $p < .001$, as well as a significant interaction between the naltrexone pretreatment and the amphetamine treatment, F (3, 39) = 3.26, $p < .05$. There was neither an overall change in activity over the three trials nor any Drug x Trial interactions.

Insert Figures 1, 2, & 3 about here

Post-hoc Tukey tests show that amphetamine increased activity at all doses used over the three trials ($p < .001$). Similar tests reveal a significant decrease in activity in the chronically naltrexone-pretreated animals when compared to the saline-pretreated animals at the 1 mg/kg dose of amphetamine ($p < .05$) but no significant change at the two other doses of amphetamine. The most profound attenuating effect of the chronic naltrexone pretreatment is seen on Day 14 after the termination of the pretreatment regimen (≈41% decrease).

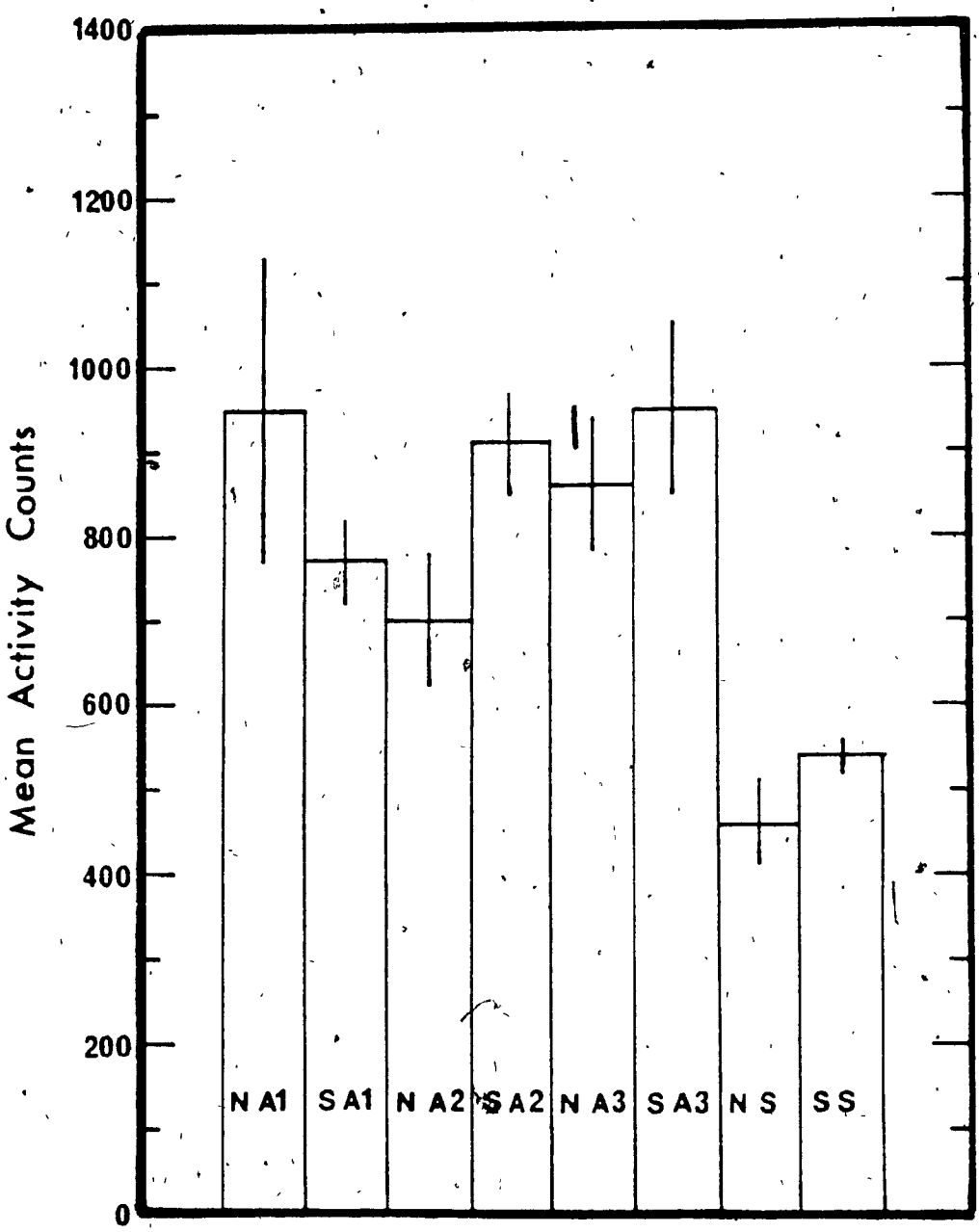

Figure 1. Means (±S.E.) of activity counts for new colony animals in different combinations of pretreatment (saline, S or naltrexone, N) and treatment (saline, S; 0.5, A1; 1, A2; or 2 mg/kg of amphetamine, A3) under no noise condition (testing session Day 2).

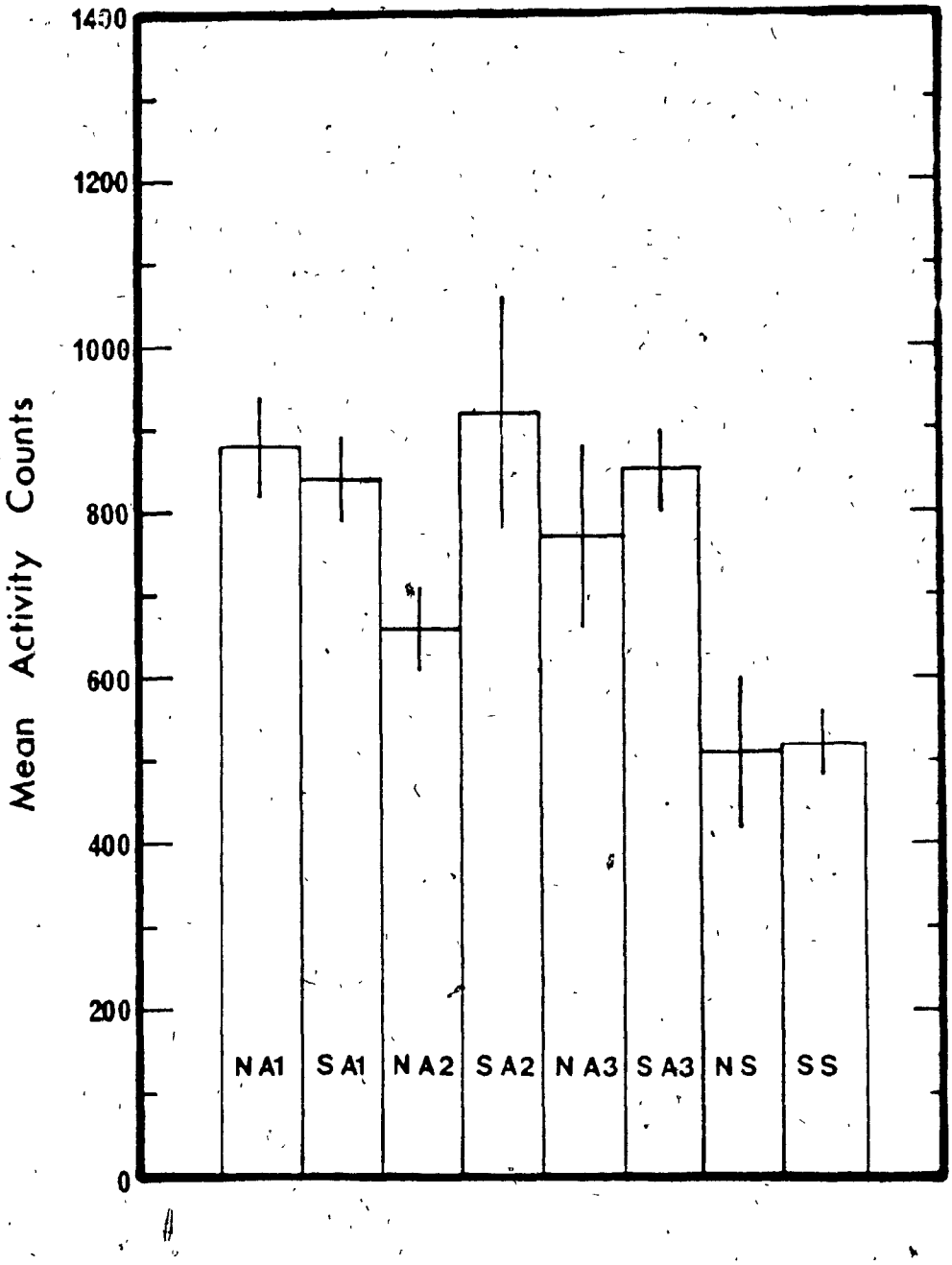

Figure 2. Means (±S.E.) of activity counts for new colony animals in different combinations of pretreatment (saline, S or naltrexone, N) and treatment (saline, S; 0.5, A1; 1, A2; or 2 mg/kg of amphetamine, A3) under no noise condition (testing session Day 7).

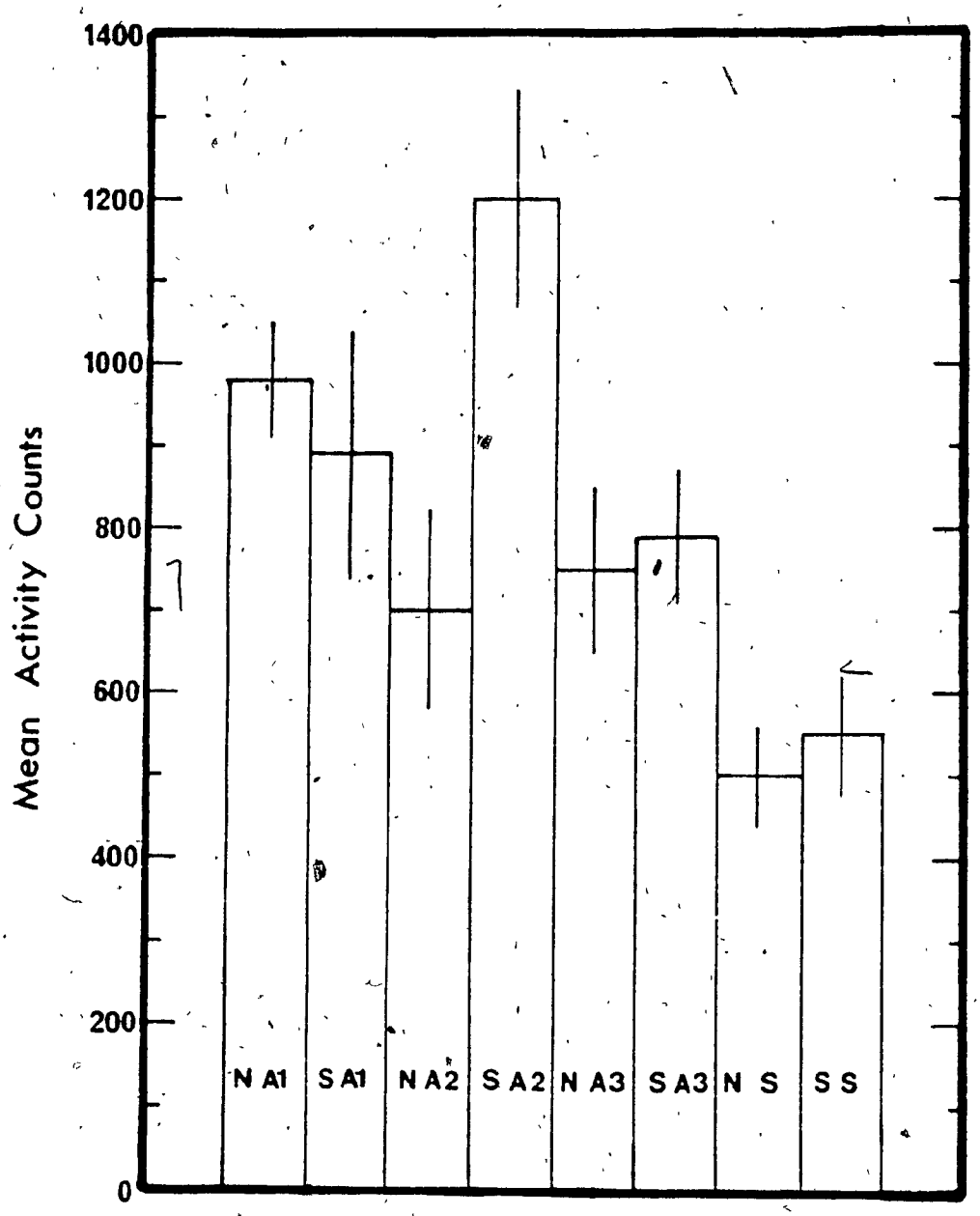

Figure 3. Means (±S.E.) of activity counts for new colony animals in different combinations of pretreatment (saline, S or naltrexone, N) and treatment (saline, S; 0.5, A1; 1, A2; or 2 mg/kg of amphetamine, A3) under no noise condition (testing session Day 14).

Discussion

The present findings are in contradiction to Amir et al's (1979) data. In their study, it was reported that the chronic naltrexone pretreatment enhances the stimulant action of 1 mg/kg dose of amphetamine on locomotor activity in the open-field. Using the identical experimental paradigm it is now found that the chronic naltrexone pretreatment instead attenuates the 1 mg/kg dose of amphetamine. The most pronounced attenuating effect of the naltrexone treatment is seen on the Day 14 trial session. In contrast, the potentiating effect of naltrexone in Amir et al's (1979) study was greatest on Day 7 trial session. Some other important points should be noted too. For instance, although Amir et al. reported a significant drug effect which included a dose of 1 mg/kg of apomorphine as one level of the drug treatment, there were no statistical reports of the effects of amphetamine alone over the three trial sessions. In the present experiment, there were significant increases in activity produced by all three doses of amphetamine at all trials. From the graph in Amir et al's (1979) study, it seems that the amphetamine-induced hyperactivity was observed only on Day 2 trial session. The average activity counts for the amphetamine

alone at the 1 mg/kg dose ranged from 919 to 1200 in contrast to the values of 250 to 450 in a similar group in Amir et al's study. But the values for the saline control animals differ in a similar way. An average activity counts of 536 over the three trials was found in the present experiment while Amir et al (1979) observed a lower average of 260 activity counts for similar control animals. In both studies, amphetamine seems to increase the activity of the animals significantly. Hence, the high activity counts in the amphetamine animals at the 1 mg/kg dose are not artifactual and do not account for the opposite results to Amir et al's (1979) findings. Instead, one would tend to think that the tolerance to the amphetamine effect on the last two trials might have contributed to the large difference between the saline-pretreated and naltrexone-pretreated animals in the early study. Yet, it certainly does not explain the difference in activity between these two groups on the first trial. In the present case, no such tolerance to amphetamine developed in terms of the activity of the animals in the open-field.

Also one can note that even though the motor activity level was higher in naltrexone-pretreated animals than in the saline-pretreated animals at the

0.5 mg/kg dose of amphetamine, the difference was not significant at any trial session. Unlike the observation made by Amir et al. (1979), there was no habituation to the open-field chamber in the saline control animals. Given all these differences in findings between the two studies, one would tend to conclude that either there were some differences in the experimental procedures or that the sample of animals in the two studies did not belong to the same population of animals.

EXPERIMENT 2

One possible explanation for the discrepancy between the data in Experiment 1 and those of Amir et al. (1979) is that the animals used might have been different in some respects. Recently, the Canadian Breeding Farm and Laboratories Ltd. which is the main supplier of laboratory rats in Quebec, has been providing our laboratories with a new kind of animals that are referred to as "new colony animals" ; on the other hand, the animals that have been used earlier are called "old colony animals". The differences in terms of rearing conditions or treatment procedures between the two kinds of animals are summarized in Appendix A.

It is known now that the animals used in the Amir et al. (1979) study were from the old colony (Amir, Note 1). This difference might explain partly the performance of the saline control animals in the open-field. The data gathered so far in our laboratory suggest that the new colony animals when compared to the old colony animals are more hyperactive, more difficult to handle, very susceptible to infection, and do not consistently gain weight. When tested on some of the laboratory behavioral tests, they are less susceptible to the morphine conditioned taste-aversion effect, and

drink less ethanol in ethanol preference studies (Hunt, Note 2; Socaransky, Note 3). It is conceivable; by extension of the above mentioned differences that the new colony animals from Experiment 1 reacted differently to drugs in a stressful situation like the open-field. To elucidate this question, Experiment 2 was conducted on old colony animals using the same experimental paradigm, and only the 1 mg/kg dose of amphetamine.

Method

Subjects. The subjects were 24 male Wistar rats of the old colony (see Appendix A; Canadian Breeding Laboratories Ltd.) weighing 200-250 g at the beginning of the experiment. Animals were housed individually in stainless steel cages in a temperature-regulated room (21° C) with a 12-hr day-night cycle (light on from 0800 hr to 2000 hr). Food (Purina Lab Chow) and water were made available ad lib. The animals were handled for at least two days before the beginning of the experiment.

Apparatus. Four wooden locomotor chambers as described in Experiment 1 were used.

Drugs and Injections. Similarly as in Experiment 1, naltrexone hydrochloride was dissolved in injectable saline solution to a concentration of 5 mg/ml (pH = 5.7).

D-amphetamine sulphate was dissolved in injectable saline solution to 1 mg/ml (pH = 6.5). Saline solution was used for control purposes. The naltrexone daily pretreatment injections were given subcutaneously in a volume of 2 ml/kg and the amphetamine treatment injections were administered intraperitoneally in a volume of 1 ml/kg.

Design and Procedure. Animals were randomly assigned to the four experimental groups defined by two levels of amphetamine doses, 0 (saline) or 1 mg/kg of amphetamine, and two levels of pretreatment, saline or 10 mg/kg of naltrexone (n = 6 for each group). After 2 to 3 days of adaptation to the animal room, the animals were pretreated with either saline or naltrexone (10 mg/kg, subcutaneously) daily for 8 days between 1200 hr and 1400 hr. Two days after the last injection, the animals were injected with either saline or amphetamine (1 mg/kg) intraperitoneally. Fifteen minutes following the injection, the animals were placed individually in one corner of the activity chamber. Activity counts as measured by the number of light beam interruptions were noted after 30 minutes. The activity chambers were located in a sound-insulated room and testing always took place between 1100 hr and 1700 hr.

The animals were retested 7 and 14 days after the termination of the naltrexone pretreatment using the same experimental procedures.

Results

Figure 4 graphically presents the mean activity counts and standard error of the mean for the old colony animals under different combinations of pretreatment (naltrexone or saline) and treatment (saline or 1 mg/kg of amphetamine) for the three trial sessions (see Appendix, B, Table 2). A factorial analysis of variance of the data (see Appendix C., Table 2) shows a main effect of amphetamine, F (1, 20) = 72.65, p < .001, a Naltrexone x Trial interaction, F (2, 40) = 3.37, p < .05, but no Naltrexone x Amphetamine interaction.

Insert Figure 4 about here

Post-hoc Tukey tests show that the 1 mg/kg of amphetamine significantly increases activity in both saline-pretreated and naltrexone-pretreated animals over the three trial sessions (p < .001). Chronic naltrexone pretreatment decreases overall activity at Day 14 after the termination of the pretreatment as

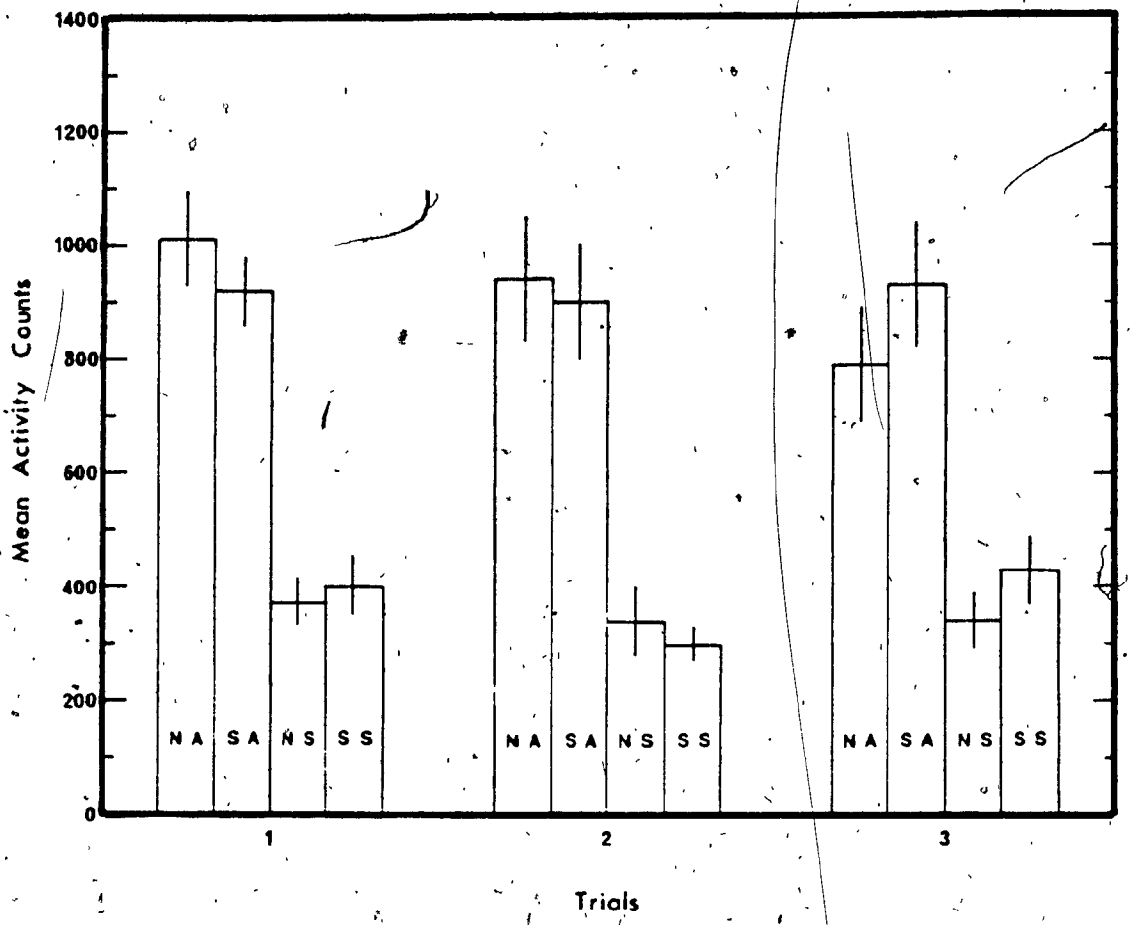

Figure 4. Means (±S.E.) of activity counts for old colony animals in different combinations of pretreatment (saline, S or naltrexone, N) and treatment (saline, S or 1 mg/kg of amphetamine, A) under no noise condition.

shown by the simple main effect of naltrexone at Trial 3, $F(1, 40) = 5.98$, $p < .05$.

Discussion

The data in this second experiment fail to show any interaction between the chronic naltrexone pretreatment and amphetamine treatment on the animal locomotor activity in the open-field. The naltrexone-pretreated animals showed more motor activity than the saline-pretreated animals when challenged with 1 mg/kg dose of amphetamine for the first two trials but the differences were not statistically significant. Here again, the saline control animals displayed more motor activity than the same control group animals in Amir et al.'s (1979) work, and there was no habituation to the open-field chamber either. The animals that were treated with naltrexone alone had average activity counts lower than the saline control animals at the first and last trial sessions, but the decrease in motor activity by naltrexone was only statistically significant at the last trial session. Instead, Amir et al. (1979) have found an overall decrease in activity over all three trials. Finally, as an index of internal validity, one can note that the 1 mg/kg dose of amphetamine about doubled the activity of the amphetamine-

treated animals when compared to the activity of the saline-treated animals as similarly reported in Experiment 1. Again, as observed in Experiment 1, there was no tolerance to the amphetamine effect across trials. To conclude, the difference in the kind of animals used does not seem to explain the discrepancy in findings between Amir et al's (1979) study and Experiment 1. The failure to show any interaction between the naltrexone pretreatment and the amphetamine effect in this second experiment suggests that there might be some environmental conditions under which the interactive effect can be triggered and observed.

EXPERIMENT 3

The failure to replicate Amir et al's (1979) finding does not seem to be the result of the differences between the new and old colony animals alone. Even though the data in Experiment 2 are less discrepant from those in Amir et al's (1979) study in that the saline control displayed less activity than similar control animals in Experiment 1, and there were statistically nonsignificant increases in amphetamine hyperactivity in naltrexone-pretreated animals relative to the activity of the saline-pretreated animals.

Another possible factor might have affected the results in the two earlier experiments. White noise to mask the background noise during the open-field testing is routinely used by some researchers as part of the experimental procedure. This practice is sometimes taken for granted and omitted in the published description of the procedure. Since such was the case in Amir et al's (1979) study, it might explain the low level of activity in the saline control animals as it has been previously observed (Cox & Lee, 1975; Cunha & Masur, 1978; Hall, 1936) and might equally shed some light on the exact nature of the chronic naltrexone and amphetamine interaction. In this third experiment,

using the same paradigm except that white noise was piped in the testing room throughout the testing period, the interaction between chronic naltrexone and amphetamine on locomotor activity was again studied in the old colony animals.

Method

Subjects. The subjects were 24 male Wistar rats of the old colony (Canadian Breeding Laboratories Ltd.) weighing 200-250 g at the beginning of the experiment. The animals were housed under similar room conditions and treated similarly as in the previous two experiments.

Apparatus. The same four wooden locomotor activity chambers as described in Experiment 1 were used. In addition, a noise generator Grason-Stadler Model 901 B (West Concord, Massachusetts) was used to provide white noise.

Drugs and Injections. Similarly as in Experiment 1, the daily pretreatment injections of naltrexone hydrochloride in saline solution (5 mg/ml) were given subcutaneously in a volume of 2 ml/kg. Amphetamine sulphate which was also in saline solution (1 mg/ml) was administered intraperitoneally in a volume of 1 ml/kg. As usual saline solution was used for control

purposes.

Design and Procedure. As in Experiment 2, animals were randomly assigned to the four experimental groups defined by two levels of amphetamine doses, 0 (saline) or 1 mg/kg of amphetamine, and two levels of pretreatment, saline or 10 mg/kg of naltrexone (n = 6 for each) group. Using the same experimental procedure, the animals were chronically pretreated with either naltrexone or saline for 8 days. Two days after the last day of the naltrexone pretreatment, animals were tested for locomotor activity in the activity chambers 15 min following an injection of either saline or amphetamine. In addition to the previous experiments, white noise was introduced into the testing room through a loudspeaker connected to the noise generator. The noise level in each chamber was maintained at 70 db throughout testing. Similar testing was carried out at Day 7 and Day 14 after the termination of the naltrexone regimen.

Results

An illustration of the mean activity counts and standard error of the mean for the old colony animals under noise condition for different combinations of drug pretreatment (saline or naltrexone) and drug treatment (saline or 1 mg/kg of amphetamine) at Days

2, 7, and 14 after termination of drug pretreatment is given in Figure 5 (see Appendix B, Table 3). A 2 x 2 x 3 analysis of variance of the data (see Appendix C, Table 3) reveals a significant main effect of amphetamine, F (1, 20) = 72.39, $p < .001$ and a significant interaction between naltrexone and amphetamine, F (1, 20) = 4.50, $p < .05$. There were no significant interactions between trials and drug treatment or drug pretreatment.

Insert Figure 5 about here

Amphetamine increased activity counts in animals independent of the pretreatment condition. However, post-hoc Tukey tests show that the chronic naltrexone pretreatment significantly attenuated the amphetamine-induced locomotor activity over the three trial sessions, $p < .05$. But naltrexone alone did not seem to have any significant effect on the locomotor activity of the animals under the present experimental conditions.

Discussion

As observed in Experiment 1, the data in Experiment 3 once again showed that chronic naltrexone pretreatment attenuates rather than enhances locomotor activity. In the present case, old colony animals were studied under

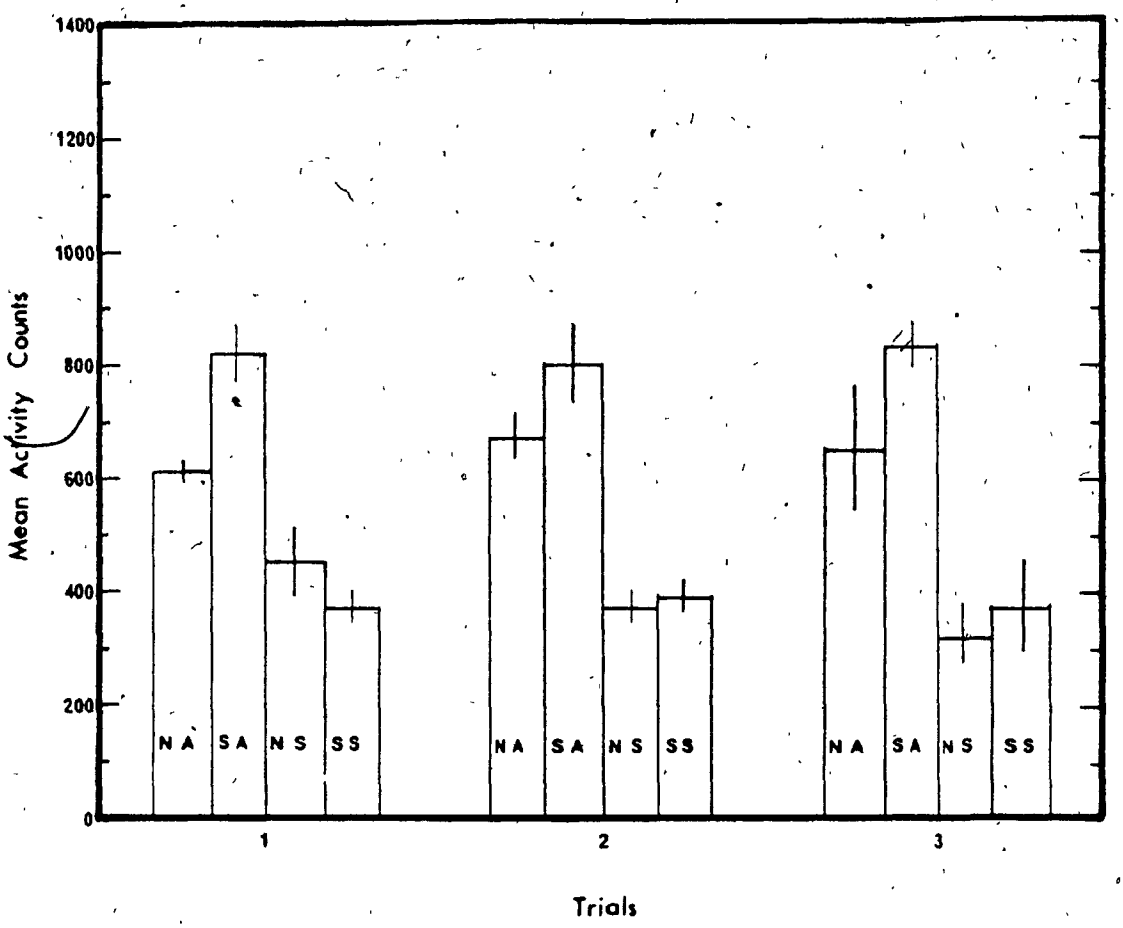

Figure 5. Means (±S.E.) of activity counts for old colony animals in different combinations of pretreatment (saline, S or naltrexone, N) and treatment (saline, S or 1 mg/kg of amphetamine, A) under noise condition.

white noise condition while in Experiment 1 the animals were from the new colony and were tested under no noise condition. These findings agree with the observation that the new colony animals are more hyperactive and more susceptible to handling stress. In this respect they behaved similarly to old colony animals under noise stress when they were themselves not subjected to such stress during testing.

Here also one can observe some consistent patterns in the findings with the first two experiments of this investigation. As noted in both Experiments 1 and 2, there was again no tolerance to the effect of amphetamine on locomotor activity over the three trials. Even though the saline control animals showed a lower activity level in the presence of white noise they exhibited no differential response to the amphetamine in this particular stressful situation. Naltrexone pretreatment alone also did not affect the locomotor activity of the animals at any trial sessions. Except at the last trial in Experiment 2, chronic naltrexone pretreatment in general does not seem to alter the locomotor activity of animals significantly under this experimental condition. Although an explanation for the depressive effect of chronic naltrexone pretreatment in the second experi-

ment is yet to be found, it appears that the effect of the naltrexone treatment is very sensitive to environmental conditions. White noise seems to elicit the manifestation of the naltrexone pretreatment effect on amphetamine-induced locomotor activity in old colony animals. In new colony animals, the interaction effect between the opiate antagonist treatment and the amphetamine effect was observed in the absence of white noise.

EXPERIMENT 4

Twice in this series of experiments the data revealed that chronic naltrexone pretreatment decreases the amphetamine locomotor activity in the open-field. These findings do not agree with Amir et al's (1979) results which suggested an opposite effect of chronic naltrexone on the amphetamine-induced locomotor activity. Neither the kind of animals used nor the noise stress condition seem to account for the discrepancy in these findings. But some consistent patterns in the data in the first three experiments of the present research provide a good measure of their internal validity.

In old colony animals, white noise appears to unmask or precipitate the interaction between chronic naltrexone and amphetamine actions. For the new colony animals, the interactive effect was evident even in the absence of the white noise. It would be of interest to know what would be the effect of white noise on the chronic naltrexone-amphetamine interaction in the new colony animals. Based on the previous experiments, one can speculate two likely possibilities. One is that the noise factor would show greater effect of the naltrexone treatment in attenuating the amphetamine hyperactivity. In such a case, the noise stressor

would be acting in a similar manner in the new colony animals as it did in the old colony animals, and that is making the animals more susceptible to the combined actions of naltrexone pretreatment and the amphetamine challenge. A second possibility is that the additional stressor might cause excessive arousal in these otherwise hyperactive new colony animals. This excessive arousal could in one way or another suppress the interaction effects. Experiment 4 was therefore conducted to answer this last question.

Method

Subjects. The subjects were 24 drug-naive male Wistar rats of the new colony (Canadian Breeding Laboratories Ltd.) weighing 200-250 g at the start of the experiment. Animals were housed and treated in a similar way as in the previous experiments.

Apparatus. As in Experiment 3, the apparatus consists of the same set of four wooden locomotor activity chambers together with the noise generator which provides white noise into the testing room through a loudspeaker.

Drugs and Injections. The drug concentrations (naltrexone, 5 mg/ml; amphetamine, 1 mg/ml), the volume of injection (naltrexone, 2 ml/kg; amphetamine, 1 ml/kg),

and the route of administration (naltrexone, subcutaneously; amphetamine, intraperitoneally) were the same as in the previous experiments.

Design and Procedure. Random assignments of animals to the eight experimental groups was carried out as usual. In a similar fashion, animals were chronically pretreated with either naltrexone or saline daily for 8 days. They were then tested in the activity chambers following an injection of amphetamine or saline 15 min earlier at Days 2, 7, and 14 after the termination of the naltrexone regimen. White noise level was maintained at 70 db throughout the 30-min testing period as in Experiment 3.

Results

The means and standard errors of the mean for the different groups of animals for the three trial sessions are shown graphically in Figure 6 (see Appendix B, Table 4). An analysis of variance of the data (see Appendix C, Table 4) shows no main effect of naltrexone, no main interaction between naltrexone and amphetamine, but a main effect of amphetamine, F (1, 20) = 27.60, $p < .001$. Amphetamine at the 1 mg/kg dose consistently

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Insert Figure 6 about here

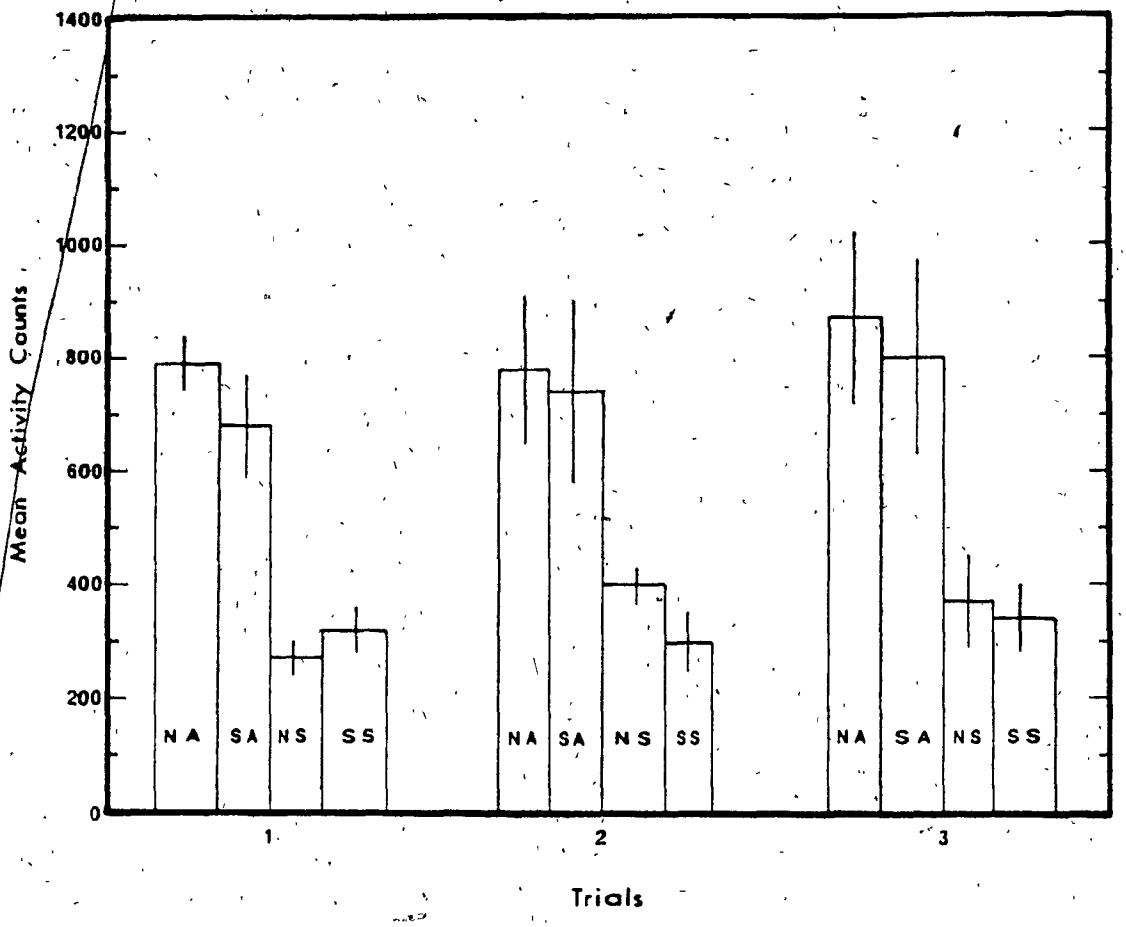

Figure 6. Means (±S.E.) of activity counts for new colony animals in different combinations of pretreatment (saline, S or naltrexone, N) and treatment (saline, S or 1 mg/kg of amphetamine, A) under noise condition.

increases activity counts in saline- and naltrexone-pretreated animals at all three trial sessions. There were no effects of trial or of any Trial x Drug treatment interaction.

Discussion

In this last experiment, following an injection of amphetamine, the naltrexone-pretreated animals exhibited a higher level of locomotor activity relative to the saline-pretreated animals. The increase in locomotor activity was not statistically significant at any trial sessions. When the data for the saline control group are compared to those obtained for the same group in Experiment 1, the noise stress seems to decrease locomotor activity in the new colony animals to a greater extent than in the old colony animals. This supports the idea that the new colony animals are more susceptible to stress including white noise stress. As repeatedly observed before, the animals showed no habituation to the open-field and no tolerance to the effect of amphetamine on locomotor activity. As observed in Experiment 4, chronic naltrexone pretreatment again did not affect locomotor activity of rats in the open-field at any trial sessions.

In contrast to the old colony animals, an inter-

action effect was found in new colony animals under no noise condition but not under noise condition. It thus appears that moderate stress in old colony animals under noise condition and in new colony animals under no noise condition precipitates the interaction. No interaction can be observed under low stress as in the old colony animals under no noise condition or under excessive stress as in the new colony animals under noise condition.

GENERAL RESULTS

To assess the contribution of the kind of animals and the noise condition to the variability of the activity measure, the data for all the experiments were pooled together (for balance, only data for the saline-saline, saline- 1 mg/kg of amphetamine, naltrexone-saline, and naltrexone- 1 mg/kg of amphetamine groups from Experiment 1 were used). An analysis of variance of the data (see Appendix C, Table 5) reveals that noise causes an overall decrease in activity counts, F (1, 79) = 10.78, $p < .05$ while amphetamine at the 1 mg/kg dose increases locomotor activity counts, F (1, 79) = 173.80, $p < .001$. There were significant Colony x Noise Condition x Naltrexone interaction, F (1, 79) = 5.70, $p < .05$; significant Colony x Noise Condition x Amphetamine interaction, F (1, 79) = 5.92, $p < .05$; and significant Colony x Noise Condition x Naltrexone x Amphetamine, F (1, 79) = 4.14, $p < .05$. These interactions are in agreement with the earlier analyses which have shown the attenuating effect of chronic naltrexone on amphetamine-induced locomotor activity in the new colony animals under no noise condition and in the old colony animals under noise condition only. In addition, tests for simple main effects

show that white noise significantly decreases the activity of naltrexone-amphetamine old colony animals at trials 1 and 2, F (1, 79) = 12.194, $p < .05$, and F (1, 79) = 5.589, $p < .05$, respectively. White noise equally attenuates the activity of saline-amphetamine new colony animals at the third trial, F (1, 79) = 11.653, $p < .05$.

Finally, there was also a significant Colony x Trial interaction, F (2, 158) = 3.78, $p < .05$. Figure 7 shows the means and standard errors of the mean for the two kinds of animals over the three trial sessions (see Appendix B, Table 5). A significant simple, simple main effect of colony at Trial 3, F (1, 237) = 4.39, $p < .05$ indicates that old colony animals exhibited lower levels of activity than the new colony animals at Day 14.

Insert Figure 7 about here

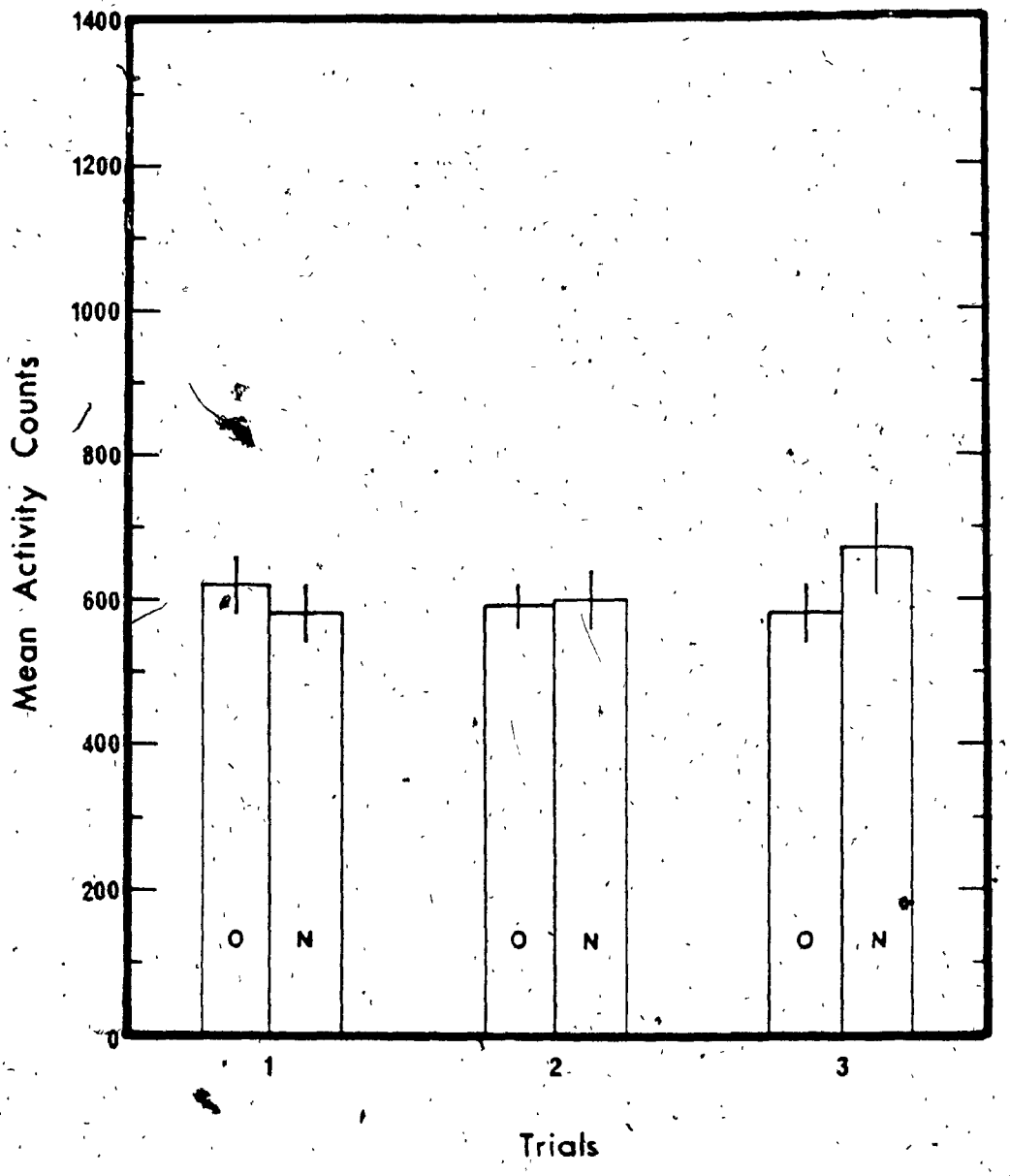

Figure 7. Means (±S.E.) of activity counts for old colony (O) and new colony (N) animals across the different experimental conditions at Trials 1, 2, and 3.

GENERAL DISCUSSION

The overall analysis confirmed the previous analyses in several ways. To recapitulate, one finds that amphetamine increases locomotor activity independently of pretreatment or noise conditions or kind of animals used. The amphetamine hyperactivity effect showed no tolerance when tested 5 and 7 days after the first test. The consistency of the amphetamine effect across all four experiments can be regarded as an index of cross-validation. Tolerance to the stimulatory effect of amphetamine on locomotion has been observed in cats (Jacobs, Heym, & Trulson, 1980) but the parameters of the experimental procedure differ largely from those in the present studies. In the above-mentioned study, the animals were administered a dose of 7.5 mg/kg of amphetamine twice daily and significant decrease in locomotor activity was seen on the fourth day of the chronic treatment. The animals in the present studies were allowed sufficient time to recuperate from the acute dose of amphetamine before the next session (5 and 7 days), and one, therefore, would not expect tolerance to develop to the amphetamine effect. Noise generally inhibits locomotor activity in the open-field (Cox & Lee, 1975; Cunha & Masur, 1978; Hall, 1936).

Although there was neither a significant main effect of noise nor a significant interaction between noise and the kind of animals used, the mean activity of the new colony animals was lower under the noise condition than that for the same kind of animals under no noise for all three trials. Significant attenuation of activity was only observed in naltrexone-amphetamine old colony animals at Trials 1 and 2, and in saline-amphetamine new colony animals at Trial 3. Next, it is noted that chronic naltrexone alone does not in general affect activity. Though previous studies have demonstrated that opiate antagonists reduce ambulation, it is important to note that the animals were expected to be devoid of naltrexone at all three test trials. To this end, two days were allowed between the last naltrexone injection and the first test session. Given that naltrexone has a half-life of 30 minutes in the rat (Berkowitz, Spector, & Lee, 1976), there should not be any significant amounts of the drug even on the first test day. Though it is very likely that the active metabolite naltrexol might play a significant role in the long-lasting effects of naltrexone, it too must have reached an ineffective level at the first test session even assuming a much longer half-life than naltrexone.

Yet, the supersensitivity effect of chronic naltrexone, if any, usually lasts for a long period of time (Lahti & Collins, 1978; Friedhoff, 1979). The data showed an attenuation of amphetamine-induced locomotor activity by the chronic naltrexone pretreatment under two specific sets of conditions. A significant reduction of the amphetamine activity was first observed in the new colony animals under no noise condition as a function of the naltrexone pretreatment, and a similar reduction was found in the old colony animals under noise condition. The noise condition differentially affects the responses of the animals to the drug manipulations. In the old colony animals, noise reduced amphetamine hyperactivity to a much larger degree in animals chronically-pretreated with naltrexone than in the saline-pretreated animals. On the other hand, in the new colony animals, noise seemed to increase amphetamine activity in animals with chronic naltrexone pretreatment and to decrease it in animals without the naltrexone pretreatment.

There are some possible explanations that account for these findings. First of all, it is now evident that the supersensitivity phenomenon of chronic treatment with opiate antagonists is due to the proliferation

of opiate receptors (Herz, Schulz, & Wüster, 1980). This increase in the number of opiate receptors could lead to enhanced inhibition of dopamine release by the action of endogenous opioids on these opiate receptors localized on dopamine terminals. Such an increased inhibition on dopamine release could account for the attenuating effect of the chronic naltrexone pretreatment on amphetamine-induced locomotor activity which is believed to be mediated mainly through the dopamine pathways (Hollister, Breese, & Cooper, 1974; Pijnenbrug, Honig, & van Rossum, 1975; Pijnenbrug & van Rossum, 1973; Roberts, Zis, & Fibiger, 1975). In addition, as it has been demonstrated with other stressors (Baizman, Cox, Osman, & Goldstein, 1979; Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale, & Bloom, 1977), noise may also activate the release of endogenous opioid peptides systems that would further inhibit the amphetamine effects as seen in the old colony animals. The effectiveness of those opiate receptors to inhibit the dopamine release seems to depend on a complex interaction between the organism predispositions and the stress of the environmental stimuli. As one would infer in old colony animals under noise condition and in new colony animals under no noise condition, moderate

stress level seems to optimize the interaction between the opiate receptor and the dopamine system. On the other hand, excessive stress level as in new colony animals under noise condition or low stress level as in old colony animals under no noise condition undermine this interaction. It is conceivable that, in the latter two situations, stress activates an additional population of opiate receptors localized on the dopamine cell bodies that instead stimulate dopamine transmission. Similar to this idea, Joyce and Iversen (1979) have proposed that the difference in sensitivities of opiate receptors according to their location could account for the biphasic effect of opiates on behavior.

Even though the present research does not answer specific questions concerning the possible mechanisms of the opiate-dopamine interaction, it clearly demonstrates a complex interplay between environmental conditions, kind of animals used, and drugs actions. Such conclusion evidently brings into question the reliability of previous studies in this area from different laboratories. In addition, as noted before, the supersensitivity phenomenon might likely be a more suitable model to examine drug actions on neurotransmitter systems essentially because it avoids the complication of drug

interactions. Finally, one observes that the behavioral component of the interaction between the opiate system and the dopamine system seen in the present investigation agrees with the majority of the physiological and biochemical studies. That is, since pretreatment with opiate antagonist that leads to proliferation of opiate receptors attenuates the behavioral effect of a dopamine reuptake blocker, it suggests that the activation of the opiate receptor inhibits dopamine transmission.

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APPENDIX A

Differences between the new colony and old colony animals

Differences between the new colony and the old colony animals.

The new colony animals were originally derived from the old colony animals in the following way. Fifteen pairs of old colony animals were randomly selected for breeding. The F_1 progenies were caesarean-delivered and reared under new conditions as are described below. A batch of these F_1 animals were used at Canadian Breeding Farm & Laboratories Ltd. to generate a total of 2000 animals which were then divided into four groups labelled A, B, C, and D, respectively. New colony animals were then obtained through cross-breeding animals from different combinations of those divisions (e.g., A vs. B, A vs. C, B vs. C, etc.). No cross-breeding within the same division was allowed. In contrast to the 1:1 mating ratio for the old colony animals, the new colony animals were bred in a ratio of 25 females to 5 males. The whole process described extended over a period of about 2 years. In addition, the rearing conditions differ for the two kinds of animals as follows:

1. Temperature, humidity, air pressure, and the purity of the air were more tightly controlled for the new colony rats than the old colony rats.
2. In addition to the RV and H-1 viruses that are

controlled for in the old colony animals, the new colony animals are also free from Sendai, SDA, and PVM.

3. New colony animals were housed 40 animals per cage ($2\frac{1}{2}$ x $1\frac{1}{2}$ ft). Subsequently after weanling, they are placed 20 animals per cage. All the cages for the new colony animals have grid floors whereas the old colony animals have contact-bedding floors and are housed 10 animals per cage and 1 or 2 per cage after weanling.
4. Old colony animals receive water from water bottles ($\frac{1}{2}$-litre size) and are provided with pasteurized food (Charles River Formula A). On the other hand, an automatic water system which delivers water through valves is available to the new colony animals. In addition, their pasteurized food is autoclaved.
5. Old colony rats receive more handling as a result of the more frequent changes of contact bedding and water bottles.

APPENDIX B

Tables for the means and standard errors of the mean of activity counts

Table 1

Mean activity counts for the new stock animals under no-noise condition

Group	n	Trial 1	2	3
saline-saline	5	535 (23)	524 (31)	549 (81)
naltrexone-saline	6	457 (48)	513 (86)	498 (59)
saline-amphetamine (0.5 mg/kg)	6	768 (54)	836 (53)	889 (151)
naltrexone-amphetamine (0.5 mg/kg)	6	952 (179)	884 (62)	982 (72)
saline-amphetamine (1 mg/kg)	6	970 (59)	919 (143)	1200 (129)
naltrexone-amphetamine (1 mg/kg)	6	699 (80)	656 (48)	704 (122)
saline-amphetamine (2 mg/kg)	6	953 (99)	848 (48)	793 (81)
naltrexone-amphetamine (2 mg/kg)	6	864 (83)	771 (111)	751 (102)

Note: Numbers in parentheses indicate the corresponding standard error of the mean.

Table 2

Mean activity counts for the old stock animals under no-noise condition

Group[a]	Trial 1	Trial 2	Trial 3
saline-saline	400 (51)	297 (35)	433 (64)
naltrexone-saline	371 (40)	338 (59)	337 (46)
saline-amphetamine (1 mg/kg)	923 (63)	898 (98)	934 (114)
naltrexone-amphetamine (1 mg/kg)	1012 (79)	942 (112)	787 (98)

Note: Numbers in parentheses indicate the corresponding standard error of the mean.

[a] $n = 6$ for each group.

Table 3

Mean activity counts for the old stock animals under noise condition

Group[a]	Trial 1	2	3
saline-saline	368 (30)	395 (27)	367 (83)
naltrexone-saline	449 (60)	375 (33)	316 (54)
saline-amphetamine (1 mg/kg)	817 (53)	801 (72)	827 (38)
naltrexone-amphetamine (1 mg/kg)	610 (12)	669 (39)	652 (117)

Note: Numbers in parentheses indicate the corresponding standard error of the mean.

[a] $n = 6$ for each group.

Table 4

Mean activity counts for the new stock animals under noise condition

Group[a]	Trial 1	2	3
saline-saline	317 (44)	304 (50)	340 (56)
naltrexone-saline	273 (34)	405 (28)	372 (82)
saline-amphetamine (1 mg/kg)	679 (94)	737 (162)	805 (168)
naltrexone-amphetamine (1 mg/kg)	790 (50)	783 (127)	871 (151)

Note: Numbers in parentheses indicate the corresponding standard error of the mean.

[a] $n = 6$ for each group.

Table 5

Mean activity counts for the new and old colony animals

Group	n	Trial 1	2	3
new stock[a]	47	582 (37)	605 (43)	667 (55)
old stock	48	619 (40)	589 (43)	582 (43)

Note: Numbers in parentheses indicate the corresponding standard error of the mean.

[a]From Experiment 1, only data for the saline-saline, naltrexone-saline, saline-amphetamine (1 mg/kg), and naltrexone-amphetamine (1 mg/kg) were used to compute the means in this table.

APPENDIX C

Summary Tables for the analyses of variance

Table 1

Analysis of Variance

Experiment 1

Source	df	MS	F
Naltrexone (N)	1	238840	2.789
Amphetamine (a)	3	1,041270	12.160*
N x A	3	278966	3.258†
Subjects within groups	39	85631	
Trials (T)	2	31883	0.927
N x T	2	17213	0.501
A x T	6	49565	1.442
N x A x T	6	23387	0.680
T x Subjects within groups	78	34383	

*$p < .001$

†$p < .05$

Table 2

Analysis of Variance

Experiment 2

Source	df	MS	F
Naltrexone (N)	1	4818	0.064
Amphetamine (A)	1	5,510650	72.648*
N x A	1	2509	0.033
Subjects within groups	20	75854	
Trials (T)	2	24919	1.690
N x T	2	49736	3.374†
A x T	2	27581	1.871
N x A x T	2	11366	0.771
T x Subjects within groups	40	14742	

*$p < .001$

†$p < .05$

Table 3

Analysis of Variance

Experiment 3

Source	df	MS	F
Naltrexone (N)	1	127176	4.155
Amphetamine (A)	1	2215510	72.386*
N x A	1	137638	4.497†
Subjects within groups	20	30607	0.207
Trials (T)	2	3193	
N x T	2	4024	0.261
A x T	2	13044	0.847
N x A x T	2	14504	0.942
T x Subjects within groups	40	15392	

*$p < .0001$

†$p < .05$

Table 4

Analysis of Variance

Experiment 4

Source	df	MS	F
Naltrexone (N)	1	49141	0.385
Amphetamine (A)	1	3520530	27.593*
N x A	1	9000	0.071
Subjects within groups	20	127588	
Trials (T)	2	40525	1.504
N x T	2	2538	0.094
A x T	2	8709	0.323
N x A x T	2	16701	0.620
T x Subjects within groups	40	26946	

*$p < .0001$

Table 5

Overall Analysis of Variance[a]

Source	df	MS	F
Animals (S)	1	33413	0.462
Environmental condition (E)	1	779175	10.783†
Naltrexone (N)	1	240445	3.328
Amphetamine (A)	1	12558400	173.797*
S x E	1	26112	0.361
S x N	1	4483	0.062
S x A	1	72042	0.997
E x N	1	126913	1.756
E x A	1	39938	0.553
N x A	1	162166	2.244
S x E x N	1	411744	5.698†
S x E x A	1	427525	5.917†
S x N x A	1	7019	0.097
E x N x A	1	16457	0.228
S x E x N x A	1	298944	4.137†
Subjects within groups	79	72259	
Trials (T)	2	21147	0.894
S x T	2	89475	3.782†
E x T	2	14493	0.613
N x T	2	57525	2.431
A x T	2	11650	0.493
S x E x T	2	2338	0.099
S x N x T	2	2786	0.118
S x A x T	2	36828	1.557
E x N x T	2	29028	1.227
E x A x T	2	5730	0.242
N x A x T	2	18491	0.782
S x E x N x T	2	1417	0.060
S x E x A x T	2	35627	1.506
S x N x A x T	2	20929	0.885
E x N x A x T	2	26054	1.101
S x E x N x A x T	2	13778	0.582
T x Subjects within groups	158	23659	

[a]From Experiment 1, only the data for naltrexone-saline, naltrexone amphetamine (1 mg/kg), saline-saline, saline-amphetamine (1 mg/kg) groups were used for the analysis of variance.

*$p < .0001$
†$p < .05$