THE ROLE OF CENTRAL NORADRENERGIC SYSTEMS IN MORPHINE TOLERANCE DEVELOPMENT.

by

PAMELA SUSAN KLONOFF

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Department of Psychiatry, Division of Neurological Sciences

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

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ABSTRACT

The role of noradrenaline (NA) in the behavioural and pharmacological effects of morphine was evaluated in rats. Animals received specific injections of 6-hydroxydopamine (6-OHDA) into the dorsal noradrenergic bundle (DB) resulting in selective depletion of telencephalic NA levels and increased levels of noradrenaline in the spinal cord and cerebellum. Employing changes in the hypoactive phase of morphine-induced locomotor activity as an index of tolerance development, it was observed that injection of 6-OHDA into the dorsal noradrenergic bundle resulted in a slower rate and a lesser degree of tolerance development to morphine. The effect of the DB-6-OHDA lesion on physical dependence was assessed by measuring naltrexone-induced withdrawal in lesioned and control animals who had received chronic morphine treatment. Results indicate that although NA is important in tolerance development, it does not mediate a dominant role in withdrawal, although behavioural evidence suggesting a secondary or modulatory role is presented. The interaction of amphetamine and morphine with the dopamine (DA) system was also assessed by studying the behavioural effects of amphetamine in animals following either acute or chronic morphine treatment. It was observed that amphetamine potentiated the spontaneous locomotor hyperactivity following both acute and chronic morphine treatment. The DB-6-OHDA lesion did not affect the locomotor potentiation of amphetamine in morphine pre-treated animals, and the hypothesis that another transmitter system mediates this effect, specifically DA, is discussed.

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GENERAL INTRODUCTION

The characterization of opiate receptors by binding and autoradiographic studies and the identification of opioid peptides as their endogenous ligands has aided in the understanding of the acute and chronic behavioural actions of morphine-like drugs.

Lesion studies indicate that opiate receptors are found on dopaminergic (DA) and noradrenergic (NA) nerve-endings and cell bodies (see review by Schwartz, 1979) and it has been suggested that the biochemical mechanisms underlying the psychopharmacological actions of morphine involve a direct interaction with the catecholamine systems.

Acute Studies: Interaction With the Dopamine System:

The striatum is one of the richest brain areas for enkephalin and opiate binding sites (Pollard, Llorens, Schwartz, Gros and Dray, 1978), and therefore much research has been aimed at determining the effects of opiates on dopaminergic neurons.

In rats, morphine administration initially elicits acute symptoms of decreased DA neurotransmission, i.e. catalepsy, hypokinesia and muscular rigidity. Following this, a compensatory increase in endogenous DA levels as a result of enhanced DA synthesis and utilization has been attributed to a feedback activation of biosynthesis (Gauchy, Agid, Glowinski and Cheramy, 1973; Fukui, Shiomi and Takagi, 1972; see review by Kuschinsky, 1976).

Strong evidence for this theory has been provided by Nowycky, Walters and Roth (1978), who plotted the time course of morphine's effect on DA metabolism. They reported that striatal DOPA synthesis rates were increased between 30-60 minutes after acute morphine administration and that striatal DOPAC (3,4 dihydroxyphenylacetic acid) levels were not significantly different from control levels 30 minutes following morphine injection, but were

doubled by 60 minutes post-injection.

Kuschinsky and Hornykiewicz (1972) found that acute injections of morphine induced catalepsy in rats and raised the homovanillic acid (HVA) concentration in the striatum. The morphine-induced catalepsy could be abolished with L-DOPA or apomorphine. These authors hypothesized that morphine influences DA metabolism presynaptically and that the increase in HVA is a consequence of a diversion of newly synthesized DA from storage sites to sites of catabolism. Increased breakdown of the newly formed DA results in a dearth of the amine at the receptor site.

Lal, Gianutsos and Puri (1975) compared the action of morphine with a neuroleptic, haloperidol, known to block DA receptors, on a series of behavioural measures (i.e. stereotypy, catalepsy, etc.). In these behavioural tests, both morphine and haloperidol resembled each other in their acute actions. However, the authors also reported that the morphine-induced increase in the firing rate of DA cells in the zona compacta of the substantia nigra can be further stimulated by haloperidol. In addition, anticholinergic drugs reverse many of haloperidol's actions, but not the morphine effects with the same true of naloxone. The authors therefore concluded that morphine blocks postsynaptic DA receptors, but that the action is indirect. Furthermore, behavioural evidence and biochemical evidence that endogenous opioid peptides and morphine produce markedly different response profiles than haloperidol has recently been presented (Weinberger, Arnsten and Segal, 1979).

Diamond and Borison (1978) have reported that following unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra, naloxone potentiated agents with postsynaptic dopaminergic actions (apomorphine, L-DOPA) and antagonized agents with presynaptic dopaminergic actions (d-amphetamine, phenylethylamine). The authors therefore proposed the following model for the ac-

tions of enkephalins in the nigrostriatal system. A presynaptic enkephalin interneuron facilitates nigrostriatal transmission and a postsynaptic enkephalinergic interneuron inhibits activation of dopamine receptors. Drugs that potentiate enkephalin mechanisms in the brain, potentiate presynaptic dopaminergic actions (i.e. increase DA synthesis) while antagonizing postsynaptic effects, while naloxone, potentiates postsynaptic but not presynaptic mechanisms.

Pollard et al (1978) explored the hypothesis for the presence of opiate receptors on DA neurons by assessing the effects of extensive degeneration of DA neurons. Effects of intranigral 6-OHDA lesions or hemitransections, intrastriatal administration of kainic acid and 6-OHDA lesions of the substantia nigra on opiate receptors, indicated that one-third of striatal opiate receptors are localized on dopaminergic neurons, while two-thirds are localized on neurons intrinsic to the striatum. Furthermore, opiate receptors might be present on DA cells in the substantia nigra although contrary evidence indicating that opiate receptors are not on nigral DA cell bodies, but may be on GABAergic or substance P afferents from the striatum has recently been reported (Reisine, Nagy, Beaumont, Fibiger and Yamamura, in press). Pollard et al go on to describe the model explaining the mechanisms underlying the acute action of morphine: presynaptic opiate receptors on DA neurons mediate presynaptic inhibition. This inhibition accounts for the symptoms of decreased dopaminergic transmission observed after acute administration. This primary effect triggers a second phase - a compensatory increase in DA synthesis, which is translated into symptoms of increased DA transmission (i.e. locomotor activity, jumping, stereotyped and aggressive behaviours).

Acute Studies: Interaction With the Noradrenergic System:

Opiate receptors have been located presynaptically on cortical and cerebellar noradrenergic nerve terminals originating from the locus coeru-

leus (LC) (Llorens, Martres, Baudry, Schwartz, 1978; Pert and Snyder, 1973).

Opiate admininstration results in inhibition of the spontaneous firing rate of NA cells in the locus coeruleus, and this effect is reversed by naloxone (Korf, Bunney and Aghajanian, 1974).

Herz, Teschemacher, Albus and Zeiglgansberger (1972) identified the medullary and pontine areas of the lower brain stem as primary sites of morphine drug action and have suggested involvement of such aminergic brain structures as the locus coeruleus and raphe nuclei. In addition, Atweh and Kuhar (1977b) have demonstrated high concentrations of opiate receptors in the locus coeruleus using both binding studies and autoradiography techniques.

Acute administration of morphine has been reported to increase the levels of MHPG-SO_4 (3 methoxy-4-hydroxyphenylglycol), a major metabolite of brain norepinephrine in the rat brain (Roffman, Reigle, Orsulak, Cassens and Schildkraut, 1979), while Watanabe (1971) reported a significant decrease in brain noradrenaline after an acute intraventricular injection of morphine.

Chronic Studies:

A major theory first proposed by Collier (1965) and Jaffe and Sharpless (1968), to explain the mechanisms underlying tolerance and physical dependence is that of "disuse hypersensitivity". According to this theory, opiate administration results in decreased catecholamine transmission, and prolonged presynaptic inhibition during long-term morphine administration results in a compensatory mechanism of increased responsiveness of the postsynaptic target cell to catecholamines. With morphine withdrawal, catecholamine release is restored and a rebound response of hypersensitive target cells results. Details of the disuse hypersensitivity theory are presented in Figure A (Schwartz, 1979).

Figure 1: Model for the effects of acute and chronic morphine treatment on noradrenergic transmission. (Schwartz, 1979).

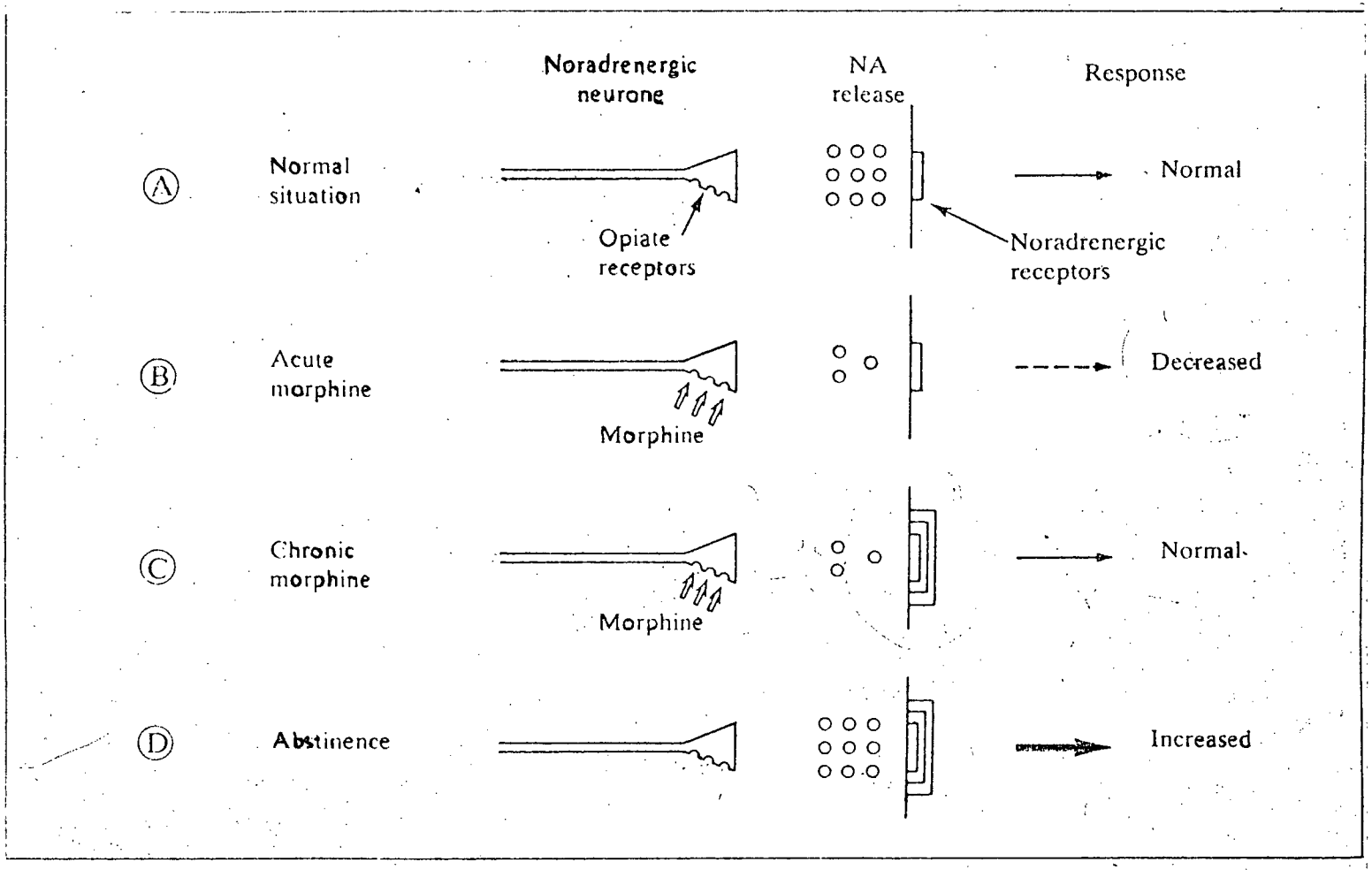


Fig. 1. Model for the effects of acute and chronic morphine treatment on noradrenergic transmission[8].

Llorens et al (1978) found that rats chronically treated with morphine developed a hypersensitivity of postsynaptic cells to noradrenergic input, as indicated by an increased responsiveness to NA and isoprenaline. This finding was partly attributed to an increased number of β-adrenergic receptors. The authors suggested that this hypersensitivity to NA could account for the development of tolerance to the action of morphine.

Puri, Volicer and Lal (1977) tested the supersensitivity hypothesis biochemically by measuring the changes in striatal dopamine turnover after administration of apomorphine in morphine dependent rats. They found that chronic morphine treatment did not alter the striatal DA turnover, however, the ability of apomorphine to decrease DA turnover was significantly enhanced in 72-hour withdrawn morphine dependent animals. In that it has been suggested that apomorphine decreases dopamine turnover by stimulating dopamine receptors, eliciting a compensatory decrease in presynaptic utilization and release of dopamine, it would appear that this data suggests a supersensitization of postsynaptic DA receptors.

Puri and Lal (1974) further hypothesized that if morphine dependency were characterized by supersensitivity of DA receptors, there should be a reduction in pharmacological responsiveness to drugs that block DA receptors, such as haloperidol. After acute injection of morphine or haloperidol, there was a marked increase in the rate of DA depletion after inhibition of its synthesis with AMPT and hence a significant increase in turnover. However, animals made dependent on morphine showed tolerance to the effect of morphine or haloperidol on DA turnover. Catalepsy was used as the behavioural correlate to the above neurochemical findings. Acute administration of haloperidol or morphine resulted in catalepsy in non-dependent animals, but the behavioural effects to haloperidol or morphine were not present in morphine dependent rats. The observation that the supersensitive

DA receptors reduce the effectiveness of drugs that act by blocking DA receptors (i.e. haloperidol) was interpreted by the authors as support for the development of supersensitive DA receptors during narcotic dependence.

Smee and Overstreet (1976) reported a significant increase in oral cage-oriented stereotyped behaviour in chronic morphine treated animals when challenged with apomorphine, but no difference in saline treated animals. When pimozide, a DA antagonist was administered to chronic morphine treated rats, the depressive effects on activity were reduced.

Further evidence in support of this theory has been provided by Baume, Patey, Marcais, Protais, Constentin and Schwartz (1979). They tested whether several features of the typical dopaminergic hypersensitivity syndrome observed following blockade of DA receptors by haloperidol could be detected following chronic administration of morphine. A significant increase in the behavioural responsiveness to apomorphine was observed on a climbing test, as well as a significant decrease in HVA levels. Therefore, both behavioural and biochemical data supported the theory of hypersensitivity to DA after sustained blockade of DA receptors.

A DA-sensitive adenylate cyclase, found in the striatum has been suggested as the DA receptor (Clouet and Iwatsubo, 1975a). Inasmuch as the stimulation of DA-sensitive adenylate cyclase can be inhibited in vitro by neuroleptic receptor blockers, which also increase DA turnover, the effect of narcotic analgesics on basal and DA-sensitive adenylate cyclase in striatal nerve ending preparations were examined to determine whether opiates have a direct effect on the DA receptor. It has found that morphine had no effect on DA-sensitive adenylate cyclase in crude ruptured nerve endings from the rat striatum, and it was therefore suggested that morphine, unlike haloperidol, does not react directly with the "dopamine receptor". When, however, adenylate cyclase was measured in a smaller tissue sample, a fraction con-

taining ruptured synaptosomal contents and portions of the pre- and post-synaptic membrane, striatal DA-sensitive adenylate cyclase was increased, while basal adenylate cyclase remained constant, following chronic morphine treatment. It is therefore suggested that the increased DA sensitivity of adenylate cyclase in the striatum of morphine tolerance rats is related to the supersensitivity to DA agonists (i.e. apomorphine) found in behavioural experiments.

Considering the evidence heretofore presented supporting the theory of postsynaptic receptor supersensitivity as an explanation for the mechanisms underlying morphine tolerance, it was decided to test the theory by studying several behavioural effects of chronic morphine administration in rats.

STUDY I. EFFECT OF DB-6-OHDA LESIONS ON TOLERANCE DEVELOPMENT TO MORPHINE-INDUCED LOCOMOTOR ACTIVITY.

INTRODUCTION

Morphine Tolerance and Locomotor Activity:

Morphine produces antinociceptive actions, as well as effects on locomotor activity. Following acute injection, morphine produces a biphasic effect on locomotor activity, characterized by an initial depressant phase, followed by a stimulatory phase, approximately 2-3 hours post-injection (Babbini and Davis, 1972).

Hosoya, Oguri and Akita (1963) observed tolerance development to the effects of chronic morphine administration on spontaneous locomotor activity, specifically to the initial sedative effect, by the third day of testing. Although the authors claimed that tolerance occurred only to the sedative effects of morphine on locomotor activity, they noted that the excitatory effects also became enhanced by repeated morphine administration. This finding has since been confirmed by Vasko and Domino (1978) and Smee and Overstreet (1976).

Catecholamines have been implicated in morphine's action on locomotor activity. Eidelberg and Schwartz (1970) reported that α-methylparatyrosine (AMPT), a catecholamine synthesis inhibitor, prevented the hyperactivity evidenced by morphine tolerant rats, and that this effect was reversed by pre-injection of L-dopa. These results in morphine tolerant rats were confirmed by Davis, Babbini and Khalsa (1972) and Buxbaum, Yarbrough and Carter (1973). Further support for these findings following acute morphine administration was paralleled by Oka and Hosoya (1976) and Carroll and Sharp (1972). In addition, Carroll and Sharp (1972) provided further evidence that the morphine induced activation response in mice is modified by

drugs that affect catecholamines, with the observations that inhibition of monoamine oxidase activity by pargyline potentiated acute morphine-induced hyperactivity and blockade of α-adrenoreceptors with phentolamine and phenoxybenzamine reduced the response. Herman (1970) provided evidence that intraventricular NA increases locomotor activity in rats, whereas Maj, Grabowska and Mogilnicka (1971) suggested that motor stimulation appears only if DA levels are raised and if NA levels are approximately normal. Estler (1973) reported that morphine caused marked excitation in mice and that simultaneous treatment with the α-sympatholytic drug, phenoxybenzamine, abolished this effect.

Recent research, however, has implicated a more dominant role for DA than NA in morphine induced locomotor hyperactivity. Broekkamp, Phillips and Cools (1979) reported increased levels of spontaneous locomotor activity in animals receiving intracerebral injections of the long acting synthetic enkephalin analogue ($D\text{-}ala^2$) - Met^5enkephalinamide (AME) into the dopaminergic A10 region of the ventral tegmental area (VTA). Carroll and Sharp (1972) reported that haloperidol completely blocked the activation response of mice to acute morphine, while chlorpromazine had a significant but less potent effect. It has been reported that these two drugs have equal α-adrenergic blocking activity (Anden, Butcher, Corrodi, Fuxe and Ungerstedt, 1970), but that haloperidol is ten times more active than chlorpromazine against central DA mechanisms. It was therefore suggested that catecholamines are involved in the normal activation response of mice to acute morphine injection, but that the dopaminergic mechanism is of primary importance. Kuschinsky and Hornykiewicz (1974) and Kuschinsky (1976) reported that DA stimulation is responsible for morphine-induced locomotor stimulation, inasmuch as pre-treatment with AMPT abolished the morphine effect, which could easily be rested by L-dopa, but not DOPS (a precursor of NA).

In addition, morphine produced an increase in striatal HVA and bilateral electrolytic lesions of the striatum significantly decreased morphine's stimulant effect on locomotor activity. Furthermore, these authors pointed out that drugs such as L-dopa, apomorphine and amphetamine, which are thought to act primarily via central DA mechanisms produce marked hyperactivity, whereas NA agonists (DOPS, clonidine) do not produce motor stimulation. The authors therefore inferred that the brain amine primiarly involved in morphine-induced locomotor activity is DA and that the noradrenergic system may be involved in regulating the sensitivity of the effector system(s) through which DA exerts its effect.

Smee and Overstreet (1976) hypothesized that the biphasic behavioural changes of morphine on locomotor activity are related to the initial blockade of dopamine transmission and that this results in an increase in dopamine synthesis and turnover which outlasts the blockade. Chronic treatment results in postsynaptic DA receptors becoming supersensitive, as evidenced by a supersensitive stereotypy response to amphetamine and apomorphine, and a subsensitive response to pimozide after chronic morphine treatment.

On the basis of the above literature, it is possible to conclude that catecholamines are somehow implicated in the expression of morphine-induced locomotor activity, as well as the mechanisms underlying the development of tolerance, although clear differentiation of the specific roles of the DA and NA systems is as yet incomplete. Previously, the selective neurotoxin 6-hydroxydopamine has been used in defining the role of catecholaminergic neurons in morphine-mediated behaviour. 6-OHDA produces selective destruction of catecholaminergic nerve endings and cell bodies in the central nervous system (see review by Fibiger, Fibiger and Zis, 1973), and specific injection of the drug into the vicinity of the dorsal noradrenergic projection

(DB) substantially depletes forebrain NA and increases significantly the content of NA in the cerebellum and spinal cord (Mason, Roberts and Fibiger, 1975). This procedure results in a slight, but nonsignificant effect on DA levels and therefore enables the dissociation of the NA and DA systems in morphine-mediated behaviour. Roberts, Mason and Fibiger (1978) reported that 6-OHDA lesions to this ascending NA projection potentiated the locomotor depressant effects of morphine when it is acutely administered. In addition, it has been reported that the same lesion potentiated morphine-induced catalepsy (Mason, Roberts and Fibiger, 1978). Considering this evidence, and prior findings of tolerance development to the suppressant effects of morphine on locomotor activity (Hosoya et al, 1963), it was hypothesized that NA may mediate some aspects of tolerance development following chronic morphine administration. It was therefore decided to explore the effects of a 6-OHDA lesion to the dorsal noradrenergic projection on morphine tolerance development, by studying changes in spontaneous locomotor activity with chronic morphine treatment.

METHODS

Subjects:

A total of forty male Wistar albino rats from Woodlyn Farms, Guelph, Ontario were used in the following series of studies. Using pentobarbital (Nembutal) anesthesia, one group (n=20), weighing 290 - 330 grams, received bilateral injections of 6-hydroxydopamine (4 µg/2 µl) expressed as the base (6-OHDA-HBr, Regis) in 0.9% saline containing 0.3 mg/ml ascorbic acid in the dorsal noradrenergic projection. An injection rate of 2 µl/5 min through a 32 gauge 10 µl Hamilton syringe was maintained. Following the injection, the syringe was left in place for 2 min to allow for any diffusion. The stereotaxic coordinates were A.P. + 2.6, from the interaural line; M.L. ± 1.1 mm from the midline and D.V. + 3.7 mm from the interaural line, with the animal's head positioned in a Kopf stereotaxic and the incisor bar adjusted 4.2 mm below the interaural line. Control animals (n=20) were identically lesioned, with the exception that only ascorbic saline was injected.

Drugs:

Doses of morphine sulphate were expressed in terms of salts. The solutions were made with physiological saline and injected IP at room temperature.

Apparatus:

Locomotor activity: Spontaneous locomotor activity was recorded in six circular photoactometer cages (BRS Foringer #PAC-ool), measuring 61 cm in diameter, and 43 cm in height. Each cage contained 12 photocell sensor units placed equidistant around the wall of the cage. Interruption of the photocell beams was automatically recorded on electromechanical counters (BRS Foringer #POS-112), which printed cumulated scores and then reset to zero every 10 minutes. Room temperature and lighting conditions were main-

tained in a constant state throughout the duration of the study.

Procedure:

Following surgery, all animals received ad libitum food and water, and were housed individually. A 12 hour dark-light cycle was maintained throughout the experiment. Behavioural testing commenced two weeks after the lesions, to permit completion of anterograde degeneration (Ross and Reis,1974).

Animals from each of the two lesioned groups were randomly assigned to two drug conditions, 1) morphine, or 2) saline, thereby defining four groups:

1. dorsal bundle-6-hydroxydopamine (DB-6-OHDA) lesioned, morphine injected;
2. dorsal bundle-6-hydroxydopamine (DB-6-OHDA) lesioned, saline injected;
3. vehicle, morphine injected and 4. vehicle, saline-injected.

Before behavioural testing began, all animals received three days of pre-handling to reduce stress associated with the injection procedure.

All animals received the appropriate daily IP drug injection (either saline or morphine) throughout the study. Each day, morphine sulphate was dissolved in physiological saline (0.9%) and a dose of 25 mg/kg of body weight was injected to the appropriate groups of animals. All injections were given in a volume of 1 mg/kg. The drug solutions were prepared each morning and between the morning and afternoon injections, were wrapped in light insensitive plastic and stored in a refrigerator. Body weight data were recorded each day, and organized according to mean group weights. The data were analyzed using a 3 factor repeated measures ANOVA (subjects), using a simple main effects model (groups). Significant differences between groups were tested using the Duncan Multiple Range Test, $p < .05$.

To facilitate behavioural testing, two injection schedules were introduced. One group, consisting of one-half of the subjects from each of the

four groups previously described, was injected between 10:30 and 11:30 A.M., and the remaining animals were injected between 1:30 and 2:30 P.M. Each animal was maintained on its own strict injection schedule, receiving the same drug injection once daily at the same time each day for an 18 day period.

Only 12 animals could be tested daily on the locomotor apparatus (6 in the A.M., and 6 in the P.M.), and the initiation of the injection schedules was staggered over a three day period so as to accommodate activity testing on the first day of drug treatment for all animals. Therefore, although all animals were injected once daily, spontaneous locomotor activity was recorded every three days for each animal, i.e. day 1, day 4, day 7, day 10, etc. until each animal had undergone testing for six sessions, each three days apart. At least one animal from each of the four lesion/drug groups participated in every testing situation.

Animals were injected in their home cages on the days they were not tested in the activity cages.

Locomotor activity: Animals were placed individually in the activity cages at either 9:00 A.M., or 1:30 P.M. and their spontaneous locomotor activity was recorded for one hour. This constituted the habituation phase. Following this, animals were removed from their cages, injected IP with either saline or morphine (25 mg/kg), according to their group designation and then replaced in their original cages. Activity levels were then recorded for a three hour period, at which time animals were removed and immediately returned to their home cages.

Data from each of the four groups was organized according to sessions, where session 1 corresponded to data from all animals collected on the first day of drug injection; session 2 corresponded to day 4 of drug injection;

session 3 corresponded to day 7 of drug injection, etc., until data for 6 sessions (corresponding to day 16) had been collected. Data from the one hour habituation period was summed across the 6 ten minute periods for each group and analyzed across days using a Three Factor Repeated Measures Analysis of Variance (Subjects) with the simple main effects model (groups). Significant differences between groups were tested using the Duncan Post Hoc Multiple Range Test, $p < .05$.

Post-injection activity data were organized according to 20 minute periods (=9 variables) and analyzed using the above technique. The same data were also subdivided into two phases: the initial hypoactive phase corresponded to data from the first 4 ten minute periods summed for each group and the subsequent hyperactive phase corresponded to the last 140 minutes of testing (ten minute periods 5-18 summed for each group). Data were analyzed according to the methods outlined above.

Biochemistry:

Upon completion of the behavioural testing, six animals from each of the dorsal bundle-6-OHDA lesioned groups and four animals from each of the vehicle groups were sacrificed by cervical fracture. The brains were immediately dissected on ice, as previously described (Roberts, Zis and Fibiger, 1975). Noradrenaline levels were then measured in the hippocampus and cerebral cortex, and dopamine was measured in the striatum by the method of McGeer and McGeer (1962). Student's *t* Test was used to analyze the data.

RESULTS

Habituation Data:

Figure 1 shows the habituation data of the four groups across days. Statistical analysis revealed a significant group effect $F=7.15$, $df=5, 145$, $p < .001$; and a significant group x days interaction $F=2.05$, $df=15, 145$, $p < .02$.

The Duncan Multiple Range Test indicated that no significant group differences were observed on Day 1 (Fig. 1). By day 4 of drug treatment, however, the vehicle-morphine group had demonstrated significantly less locomotor activity than the other three groups (Fig. 1). On days 7, 10, 13 and 16 of drug treatment, the DB-6-OHDA-morphine and the vehicle-morphine groups demonstrated significantly less locomotor activity during habituation than did the DB-6-OHDA-saline and vehicle saline groups (Fig. 1).

Post-Injection Data:

Figures 2 and 3 show the post-injection locomotor activity of the four groups over days, organized according to the hypoactive and hyperactive phases.

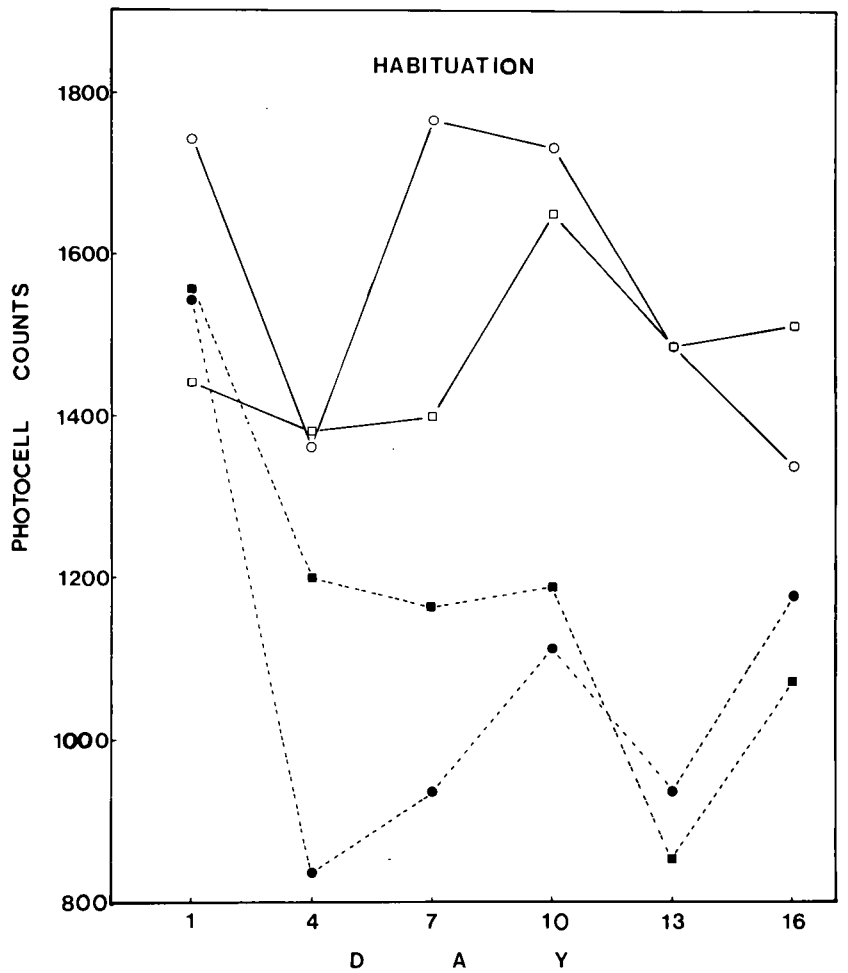
Hypoactive Phase:

A significant days effect was observed, $F=23.32$, $df=5, 145$, $p < .001$, as well as a significant group x days interaction, $F=8.26$, $df=15, 145$, $p < .001$. The Duncan Multiple Range Test indicated that the 6-OHDA-saline and vehicle-saline groups demonstrated no significant change in their locomotor activity over the days tested, whereas the DB-6-OHDA-morphine and vehicle-morphine groups demonstrated a significant increase in activity over days tested.

On day 1 of drug injection (Fig. 2), the Duncan Multiple Range Test indicated that both the DB-6-OHDA-morphine and the vehicle-morphine groups

Figure 2

Mean locomotor activity during a 1 hour pre-injection habituation phase for days 1,4,7,10,13 and 16 of drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.


HABITUATION
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1800
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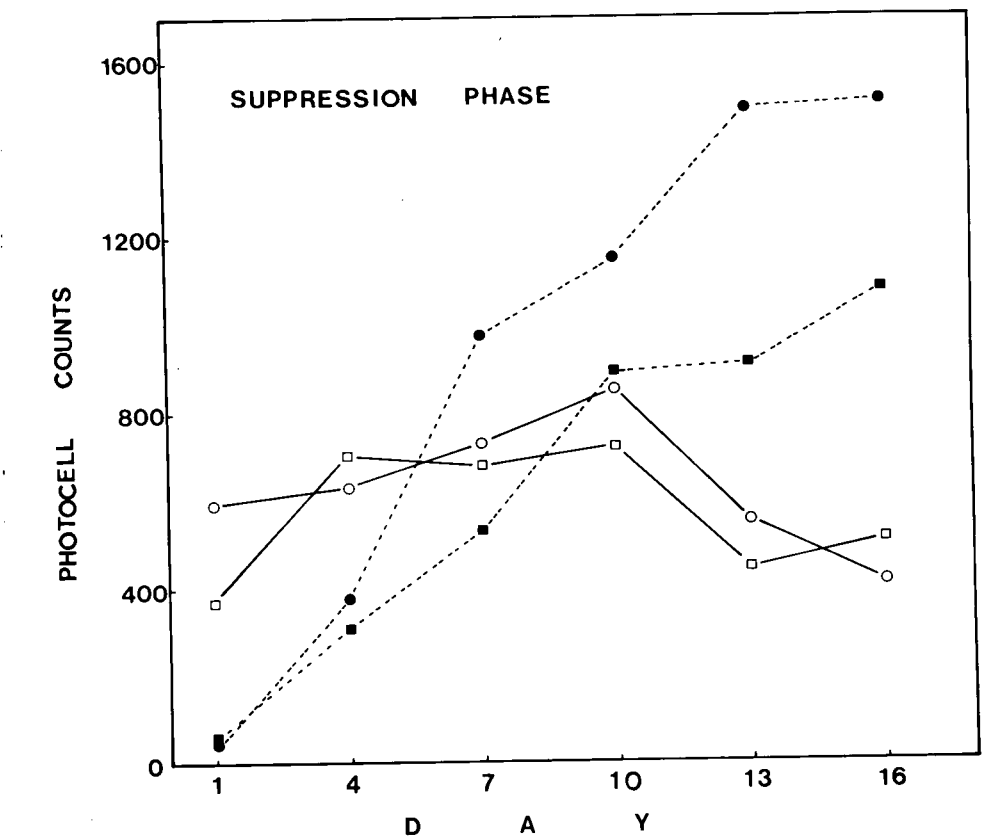
1000
800
1
4
7
10
13
16
D A Y

Figure 3

Mean locomotor activity during the hypoactive phase for days 1,4,7,10,13 and 16 of drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.


SUPPRESSION PHASE
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demonstrated significant hypoactivity when compared to the DB-6-OHDA-saline and vehicle-saline groups, characteristic of morphine's initial action on locomotor activity in acutely injected animals.

Repeated morphine administration resulted in the development of tolerance to the hypoactive phase, as indicated by the gradual increase in activity levels for both the DB-6-OHDA-morphine and the vehicle-morphine groups when tested on day 4 of drug treatment (Fig. 2). More rapid tolerance development to the hypoactive phase is evidence by the vehicle-morphine group than the DB-6-OHDA-morphine group, as evidence by their activity levels on day 7 and day 10 of chronic drug treatment and by the significantly higher levels of locomotor activity displayed by the vehicle-morphine animals when compared to the saline injected groups on day 10 (Fig. 2).

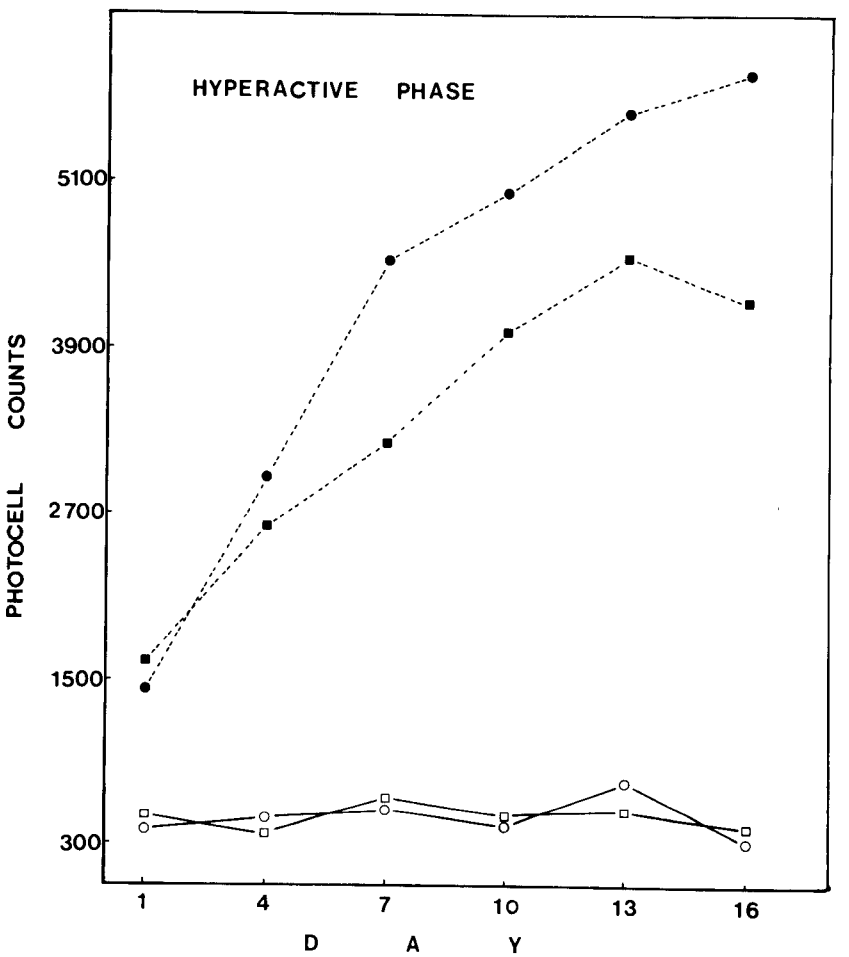
Complete tolerance to the depressant effects of morphine on locomotor activity was displayed by both the DB-6-OHDA-morphine and vehicle-morphine groups on days 13 and 16 of chronic morphine treatment, in that both groups showed significantly higher levels of locomotor activity than the two saline injected groups (Fig. 2). In addition, the activity levels of the vehicle-morphine group were significantly higher than those of the saline injected groups as well as the DB-6-OHDA-morphine greater, indicating greater tolerance development in the vehicle-morphine group (Fig. 2).

Hyperactive Phase:

The Duncan Multiple Range Test indicated that on day 1, both the vehicle-morphine and the DB-6-OHDA-morphine groups demonstrated significantly higher activity levels than the vehicle-saline and DB-6-OHDA-saline groups, characteristic of the 2nd phase of morphine biphasic action on locomotor activity. This finding was also observed on day 4 of drug

Figure 4

Mean locomotor activity during the hyperactive phase for days 1,4,7,10,13 and 16 of drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle saline group, N=8.


HYPERACTIVE PHASE
PHOTOCELL COUNTS
5100
3900
2700
1500
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1
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7
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16
D A Y

treatment with the added observation that animals from the DB-6-OHDA-morphine and vehicle-morphine groups showed an enhanced level of hyperactivity that increased steadily as morphine administration continued, and is therefore referred to as "reverse tolerance" (Fig. 3). It was also observed that although the enhanced hyperactivity was evident for both morphine-injected groups, this effect was significantly less marked in the DB-6-OHDA-morphine than the vehicle-morphine group on days 7, 10, 13 and 16 of testing (Fig. 3).

Figures, 4, 5 and 6 show the time course of the post-injection locomotor activity of the 4 groups for days 1, 7 and 16 of drug injection. Statistical analysis summarized in Appendix I, revealed an overall significant group effect except for the first 2 variables (40 minutes), and a significant days effect on all 9 variables (180 minutes), with a significant increase in activity over days, regardless of group. A significant group x days interaction was observed for all but the last 40 minutes (variables 8 and 9) of testing.

The Duncan Multiple Range Test revealed that on day 1, both the vehicle-morphine and DB-6-OHDA-morphine groups displayed suppressed levels of activity for the first 20 minute period, characteristic of the biphasic action of morphine on locomotor activity (Fig. 4). No significant differences in activity were evident for the next 2 hours. However, at 120-140 minutes post-injection, both the vehicle morphine and the DB-6-OHDA-morphine groups showed significantly more activity than the vehicle-saline and DB-6-OHDA-saline groups (Fig. 4).

By day 7 (Fig. 5), both the vehicle-morphine and the DB-6-OHDA-morphine groups displayed some tolerance to the suppressant effect of morphine. Statistical analysis revealed that although the morphine injected groups did not demonstrate significantly higher levels of activity than the saline injected groups during the hypoactive phase (0-40 minutes), the vehicle-mor-

phine group demonstrated significantly higher overall locomotor activity than the DB-6-OHDA-morphine group.

During the hyperactive phase (50-180 minutes post-injection), the vehicle-morphine and the DB-6-OHDA-morphine groups showed significantly higher levels of locomotor activity than the vehicle-saline and DB-6-OHDA-saline groups. In addition, a dissociation in activity occurred between the vehicle-morphine and DB-6-OHDA-morphine groups, whereby the vehicle-morphine group displayed a significantly higher activity overall than the DB-6-OHDA-morphine group throughout the hyperactive phase.

By day 16 (Fig. 6), both the vehicle-morphine and the DB-6-OHDA-morphine groups demonstrated significantly higher levels of locomotor activity than the vehicle-saline and DB-6-OHDA-saline groups indicating that both groups were completely tolerant to the suppressant effects of morphine. In addition, a dissociation in activity again occurred between the vehicle-morphine and the DB-6-OHDA-morphine groups, whereby the vehicle-morphine group displayed significantly higher activity than the DB-6-OHDA-morphine group during the hypoactive phase.

In reviewing the hyperactive phase on day 16 (Fig. 6), statistical analysis revealed that, as with day 7, the vehicle-morphine and DB-6-OHDA-morphine groups demonstrated significantly higher levels of locomotor activity than the saline injected groups, with a dissociation in activity between the vehicle-morphine and DB-6-OHDA-morphine groups, whereby the vehicle-morphine group displayed significantly higher levels of activity than the DB-6-OHDA-morphine group during the hyperactive phase.

Weight Data:

Figure 7 shows the mean body weight data of the 4 groups for days 1, 6, 12 and 18 of drug injection. Statistical analysis revealed a significant days effect $F=16.45$, $df=17, 510$, $p < .001$, and a significant group x days

Figure 5

Mean locomotor activity during the 3 hour post-injection period on day 1 of drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.

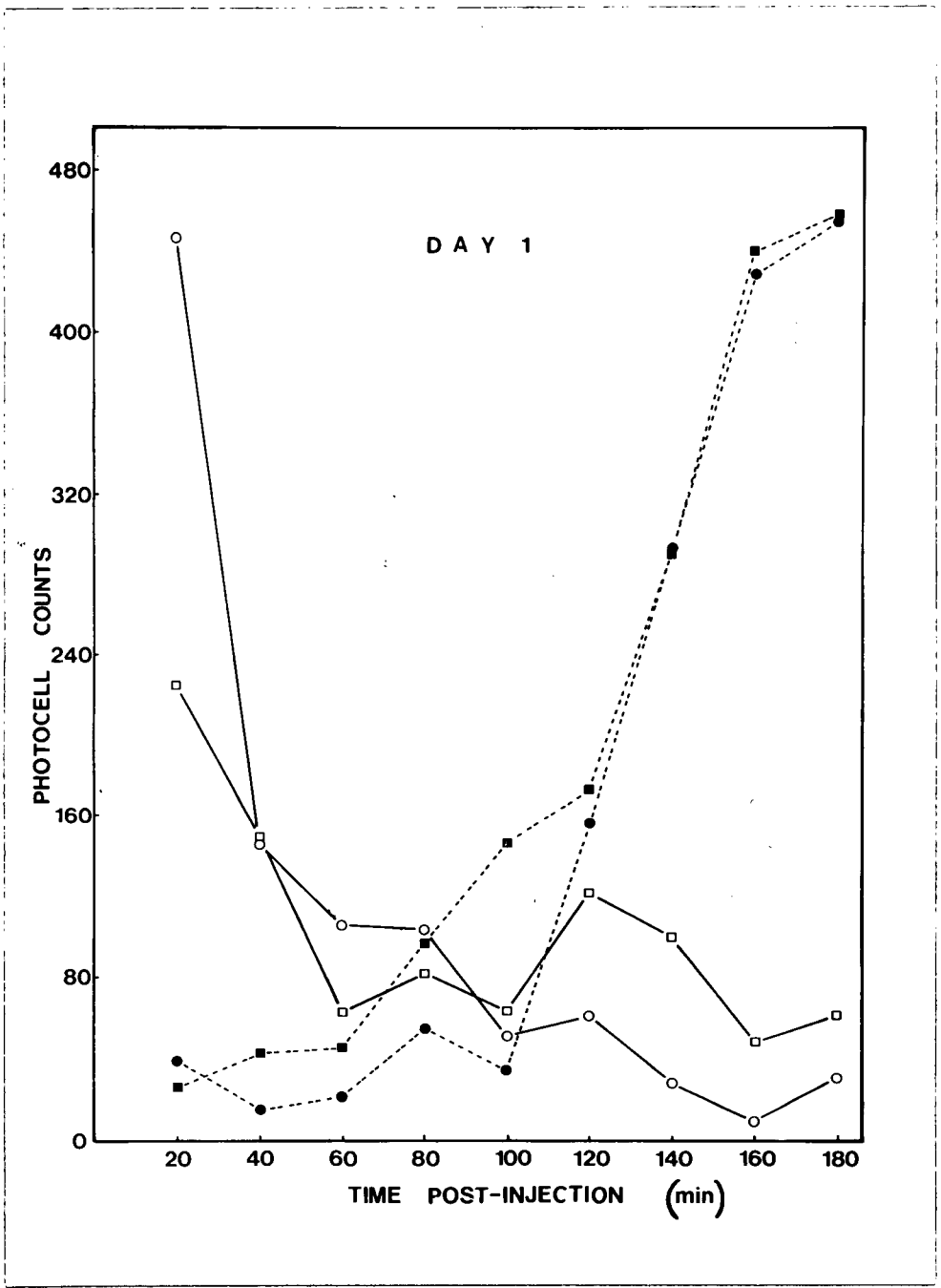

DAY 1
PHOTOCELL COUNTS
480
400
320
240
160
80
0
20
40
60
80
100
120
140
160
180
TIME POST-INJECTION (min)

Figure 6

Mean locomotor activity during the 3 hour post-injection period on day 7 of drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.

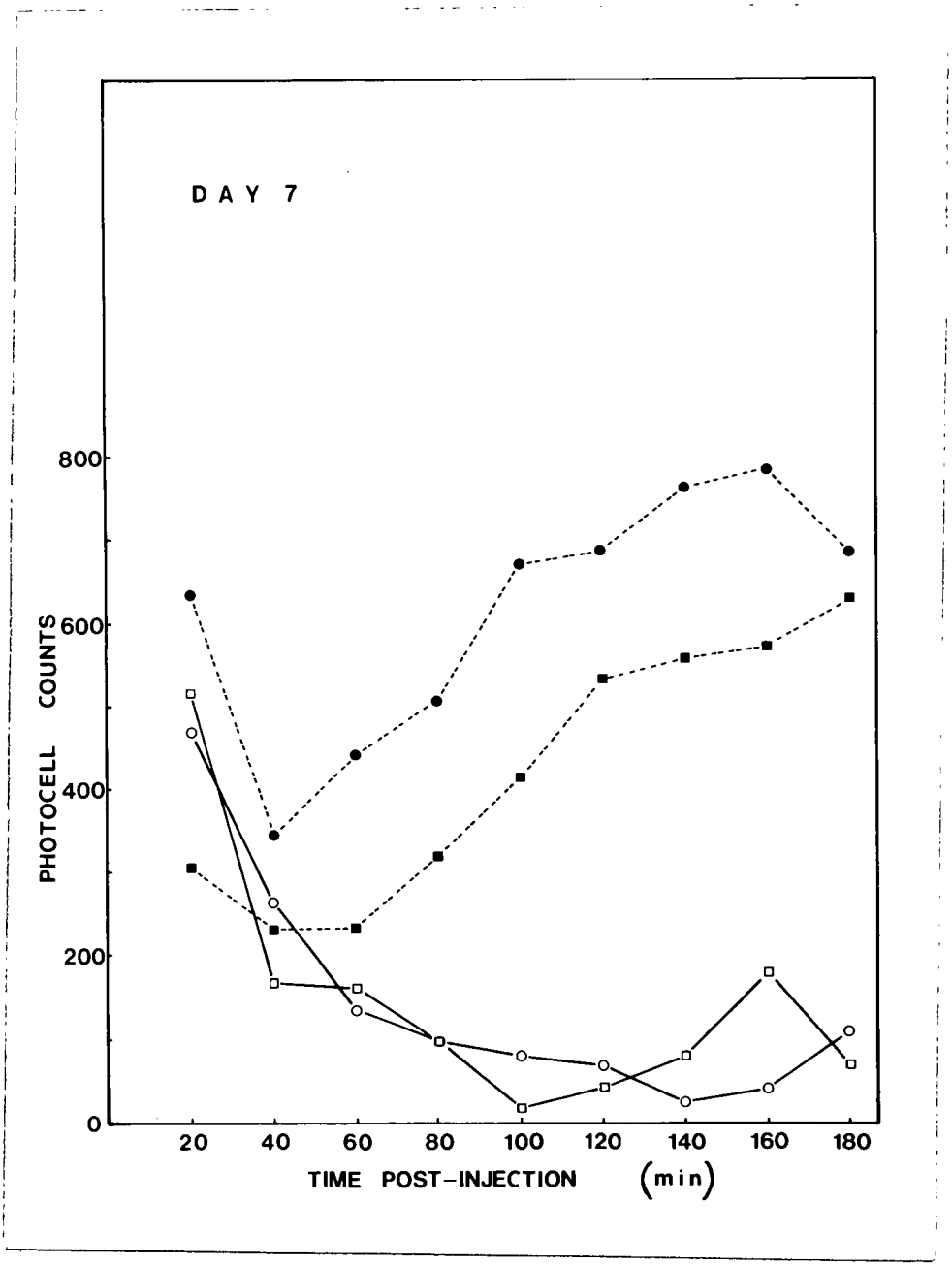

DAY 7
PHOTOCELL COUNTS
800
600
400
200
0
20
40
60
80
100
120
140
160
180
TIME POST-INJECTION (min)

Figure 7

Mean locomotor activity during the 3 hour post-injection period on day 16 for drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.

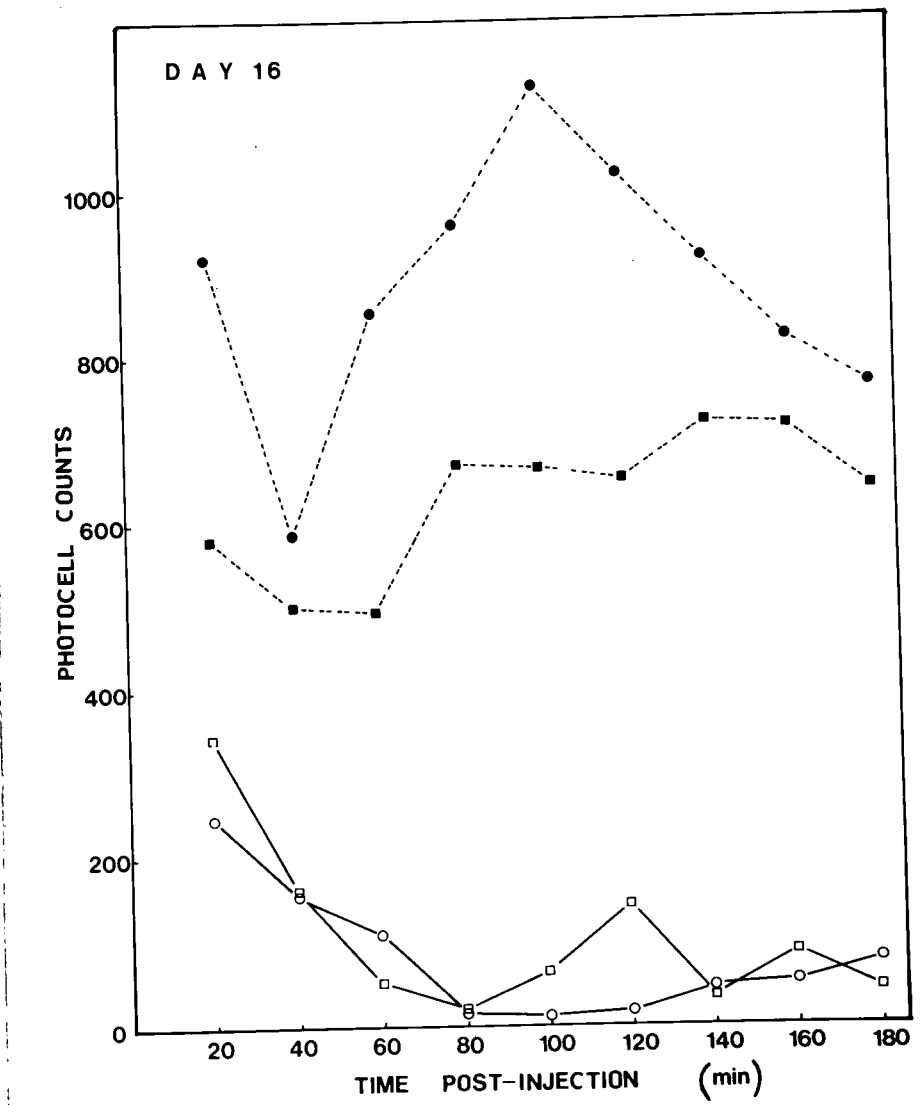
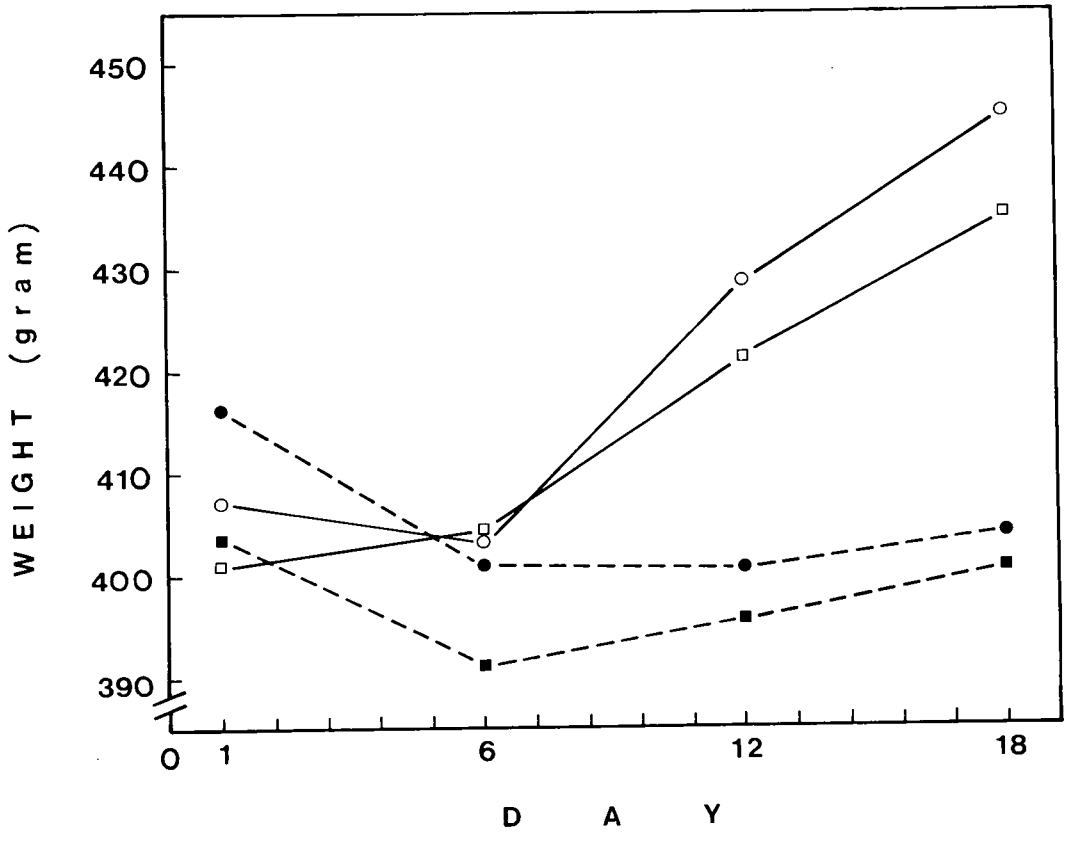

DAY 16
PHOTOCELL COUNTS
1000
800
600
400
200
0
20
40
60
80
100
120
140
160
180
TIME POST-INJECTION (min)

Figure 8

Mean body weight data on days 1,6,12 and 18 of drug injection. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.


WEIGHT (gram)
450
440
430
420
410
400
390
0
1
6
12
18
DAY

interaction F=7.20, df=51, 510, $p < .001$. The Duncan Multiple Range Test indicated that on day 1 and day 6, there were no significant group differences; however, on days 12 and 18, the DB-6-OHDA-morphine and vehicle-morphine groups weighed significantly less than the DB-6-OHDA-saline and vehicle-saline groups.

Biochemistry:

The effect of the 6-OHDA lesion on brain noradrenaline and dopamine levels is summarized in Table 1 . Animals in the saline injected group that received bilateral injections in the dorsal noradrenergic bundle showed significant depletion of hippocampus/cortex noradrenaline levels to 6.34% of vehicle-saline group, $t = 12.55$, $p < .002$.

Animals in the morphine injected group that received bilateral injections in the dorsal noradrenergic bundle, showed significant depletion of the hippocampus/cortex noradrenaline levels to 3.00% of the vehicle-morphine group, $t = 24.2$, $p < .002$.

A small, but nonsignificant, effect on striatal dopamine levels was measured in both 6-OHDA lesioned groups.

TABLE I

Effect of bilateral 6-OHDA injections into the dorsal NA projection on NA levels in the hippocampus/cortex and caudate DA levels.

	NA µg/g Cortex/hippocampus	DA µg/g Caudate
Vehicle-morphine N=4	.450±.030	9.46±2.50
DB-6-OHDA-morphine N=6	0.0135±.003	12.92±1.36
% of control	3.00%	136.58%
Vehicle-saline N=4	.410±.050	12.49±1.83
DB-6-OHDA-saline N=6	.026±.006	11.84±0.86
% of control	6.34%	94.80%

DISCUSSION

Morphine Tolerance and Locomotor Activity:

The effect of injections of 6-OHDA into the dorsal noradrenergic projection was evaluated with respect to tolerance development in chronic morphine treated animals. Inasmuch as animals display a biphasic response to acute morphine injections, characterized by an initial suppressant or hypoactive and a subsequent hyperactive phase, and that tolerance can be shown to develop to the suppressant effects (Hosoya et al, 1963; Vasko and Domino, 1978; and Smee and Overstreet, 1976), it was decided to employ the suppressant phase of morphine induced locomotor activity as an index of tolerance development.

On day 1 of drug administration, animals from the DB-6-OHDA-morphine and the vehicle-morphine groups demonstrated the characteristic hypoactive phase. With repeated injections and further behavioural testing, these animals displayed rapid tolerance development to the hypoactive phase, as indicated by the gradual increase in activity levels for both morphine injected groups. With continued drug administration, both the DB-6-OHDA-morphine and the vehicle-morphine groups demonstrated increasing levels of locomotor activity when compared to the two saline-injected groups, indicating increasing tolerance to the depressant effects of morphine on locomotor activity. In addition, the rate and degree of tolerance development to the depressant effects of morphine on locomotor activity was different in the two groups in that the vehicle-morphine group displayed significantly higher levels of hyperactivity than the DB-6-OHDA-morphine group on days 13 and 16 of chronic morphine administration.

When changes in the initial depressant or hypoactive phase of locomotor activity are used as an index of tolerance development, it can be

concluded from these data that with chronic morphine treatment, the DB-6-OHDA lesion resulted in potentiation of the hypoactive phase of locomotor activity indicating that the rate of development and degree of tolerance were impeded.

It should be noted that it was reported previously that DB-6-OHDA lesions potentiated catalepsy and locomotor hypoactivity following acute morphine injections (Roberts et al, 1978; Mason et al, 1978) and these behavioural findings were not replicated in this study (see Figure 4 = day 1 of drug injection). One possible explanation for this negative finding is that the higher dose of morphine used in this study may have resulted in a "bottoming-out effect". Alternatively, closer consideration of the study of Roberts et al (1978) reveals that the same groups of animals underwent behavioural testing following 3 drug doses of morphine, separated by 6 drug free days. There is evidence that animals pre-treated with morphine and subjected to a delay period will still exhibit tolerance when injections are re-established (Siegel, 1975; Cochin and Kornetsky, 1964). It therefore appears that the design employed by these authors may have introduced a tolerance effect which was overlooked in the interpretation of the data. This criticism, however, does not apply to data obtained using a dose of 10 mg/kg morphine, in that this represented the first drug injection, however, this dose is substantially lower than the dose employed in the present study (25 mg/kg), thereby making comparison of these data difficult. Nonetheless, in the present study, the initial injection of a dose of 25 mg/kg morphine resulted in the characteristic hypoactive phase with no potentiated depressant phase for the DB-6-OHDA-morphine group and therefore it is felt that group differences between the vehicle-morphine and the DB-6-OHDA-morphine groups and their relation to tolerance development can be made assuming equal baseline behaviour for the two groups.

Similar findings were observed for the hyperactive phase. With chronic morphine treatment, both groups displayed increasing levels of hyperactivity, or "reverse tolerance". By day 7, the vehicle-morphine group demonstrated significantly higher levels of activity than the DB-6-OHDA group and again this effect was replicated on days 10, 13 and 16.

Weight changes during chronic morphine treatment have been used as an index of tolerance development (Mucha, Kalant and Linseman, 1979). In accordance with previous findings, it was observed that morphine caused an initial suppression of weight gain, followed by a gradual, but less marked increase, when compared to saline treated groups. No significant differences were observed when comparing the DB-6-OHDA-morphine and vehicle-morphine groups, indicating that perhaps this behavioural measure is a less sensitive indicator of tolerance development.

In conclusion, the findings that DB-6-OHDA lesions resulted in the enhancement of the hypoactive phase of locomotor activity, thereby resulting in a slower rate and a lesser degree of tolerance development, implicates a role for noradrenaline in the mediation of tolerance development to chronic morphine treatment. Similarly, the stimulatory effects of morphine become enhanced with repeated morphine administration, but this effect is significantly less pronounced in the DB-6-OHDA lesioned animals. Interpretation of these findings is detailed in the General Discussion.

STUDY II. THE EFFECT OF A 6-OHDA LESION TO THE DORSAL NORADRENERGIC BUNDLE ON WITHDRAWAL IN MORPHINE-DEPENDENT RATS.

INTRODUCTION

The role of catecholamines in the abstinence syndrome in morphine-dependent animals has been examined previously (Huidobro, Contreras and Croxatto, 1963; Schwartz and Eidelberg, 1970; and Maruyama and Takemori, 1973). Maruyama and Takemori (1973) implicated noradrenaline and dopamine in the abstinence syndrome in mice with the observation that disulfiram and AMPT caused a significant inhibition of naloxone-induced jumping. It was therefore concluded that the full expression of the abstinence syndrome in morphine-dependent mice required the integrity of the central store of catecholamines.

Similar findings were reported by Schwartz and Eidelberg (1970). They reported that AMPT reduced wet dog shakes and hypothermia induced by nalorphine administration to morphine-dependent rats. In addition, Watanabe (1971) reported that intraventricular pretreatment with noradrenaline or dopamine ameliorated levallorphan induced withdrawal.

However, contraditory evidence to the above results also exists. Gunne, Jonsson and Fuxe (1969) reported that withdrawal signs of tremor, piloerection, irritability and diarrhea induced by nalorphine were not modified by pretreatment with AMPT. Segal, Deneau and Seevers (1972) reported that methyldopa did not alter the morphine abstinence syndrome in monkeys, whereas Pozeulo and Kerr (1972) reported that AMPT inhibited the withdrawal syndrome in morphine-dependent monkeys.

Friedler, Bhargahava, Quock and Way (1972) reported that precipiated abstinence, as measured by nalozone-induced withdrawal, jumping was enhanced by 6-OHDA pretreatment intracerebrally, and that weight loss after abrupt

withdrawal was also increased by 6-OHDA pretreatment. The enhanced jumping response was explained according to the theory of denervation supersensitivity: if physical dependency is a manifestation of central denervation supersensitivity, the withdrawal phenomena would reflect a state of rebound hyperexcitability.

There is some evidence in the literature that noradrenaline may play a more important role than dopamine in the expression of withdrawal. Herz, Blasig and Papeschi (1974) reported that selective inhibition of NA synthesis by FLA-63 resulted in a reduction in withdrawal intensity, whereas desipramine, a drug that specifically inhibits NA reuptake mechanisms aggravated the withdrawal syndrome. In addition, it was observed that the antagonism of withdrawal with AMPT was reversed with L-dopa only when the synthesis of NA was not prevented by inhibition of DA-beta-hydroxylase. It was therefore concluded that NA is more highly involved in the manifestation of the morphine-withdrawal syndrome. Cicero, Meyer and Bell (1974) demonstrated that noradrenergic blocking agents (i.e. phenoxybenzamine) caused a dose-dependent suppression of wet dog shakes and diarrhea - two behavioural characteristics of naloxone-induced withdrawal.

Recently, clonidine, which is the most powerful of the α_2 agonists known to inhibit the firing of locus coeruleus (LC) neurons has been reported to suppress the symptoms of opiate withdrawal in humans (Gold, Redmond and Kleber, 1978). Aghajanian (1978) reported that tolerance developed to the inhibitory effect of morphine on the firing of LC neurons and that direct application of naloxone to LC neurons by microiontophoresis induced a withdrawal response of > 100% activation of firing. During periods of naloxone-induced opiate-receptor blockade and withdrawal activation, the microiontophoresis of clonidine was able to depress the LC cell firing to below baseline rates. This study also indicated that morphine and clonidine act

at independent LC cell-receptors, in that naloxone antagonized morphine, but not the α-blocker piperoxane antagonized clonidine, but not morphine. Therefore, it was suggested that since morphine and clonidine act on independent receptors with the LC, but have similar depressant effect on overall LC cell activity, clonidine might suppress certain symptoms of opiate withdrawal by means of a parallel, but independent action on cell activity.

Laverty and Roth (1979) and Crawley, Laverty and Roth (1979) paralleled the electrophysiological results of Aghajanian (1978) with biochemical findings. Laverty and Roth (1979) reported that NA turnover, as measured by AMPT depletion of NA, increases during naloxone precipitated withdrawal and that this increase is attenuated by clonidine. In addition, Crawley et al (1979) reported that 3-methoxy-4-hydroxy-phenethyleneglycol (MHPG), a NA metabolite, increased during naloxone-induced withdrawal and clonidine reversed this increase. These results taken together serve to implicate a primary role for NA in withdrawal.

In studies where withdrawal is precipitated by saline substitution, or by narcotic antagonists, weight loss has been shown to be a reliable index (Tilson, Rech, Stolman, 1973; Akera and Brody, 1967; Hosoya et al, 1963; and Friedler et al, 1972). It was therefore of interest to examine the effect of this lesion on the withdrawal response, using weight loss as an indicator (Study IIa).

In addition, a growing tendency for some animals to display irritability on handling and injection was observed during chronic morphine administration, and therefore a rating scale was developed and applied to evaluate the effects of the DB-6-OHDA lesion on chronic morphine-induced irritability (Study IIa).

Also, the fact that there is considerable literature that implicates

catecholamine in the morphine abstinence syndrome, prompted an examination of the effect of 6-OHDA lesions of the dorsal noradrenergic bundle on narcotic antagonist-induced withdrawal in morphine dependent rats, using the measurement technique of Blasig, Herz, Reinhold and Zieglansberger (1973) and Linseman (1975), (Study IIb).

STUDY IIa

METHODS

Irritability Rating Scale:

It was observed that animals from Study I began to show irritability on handling and injection during the course of the study. The animals' behaviour during injection was therefore rated starting day 14 of the study according to the following scale:

0 = Calm, passive behaviour during handling and injection.

1 = Crying when removed by the tail from the home cage.

2 = Crying when removed by the tail from the home cage and crying or mild wriggling during the IP injection.

3 = Crying when removed by the tail from the home cage and extreme struggling during the IP injection.

These data obtained were analyzed using the Mann Whitney U Test.

Withdrawal:

After 24 days of once daily IP injections of 25 mg/kg to both the DB-6-OHDA-morphine and the vehicle-morphine groups and completion of the behavioural testing for experiments I, II and IIIb, injections were discontinued and body weight of all animals was recorded once daily at 12:00 noon for a 5 day period. The mean group body weight data were used as an index of withdrawal and were analyzed using a Three Factor Repeated Measures Analysis of Variance (subjects) with a simple main effects model (groups). Significant differences between groups were tested using the Duncan Multiple Range Test, $p < .05$.

STUDY IIb

METHODS

Subjects:

A separate group of forty animals was used in the following experiment. The same procedure for lesioning and group determination was used, as previously described in Experiment I.

Drugs:

Doses of morphine sulphate and naltrexone hydrochloride were expressed in terms of the salts. The solutions were made with physiological saline and injected IP at room temperature.

Procedure:

All animals received the appropriate daily IP drug injection (either saline or morphine) throughout the study. Each day, morphine sulphate was dissolved in physiological saline (0.9%). All injections were given IP in a volume of 1 ml/kg. Drug solutions were prepared each day, wrapped in light insensitive plastic, and stored in a refrigerator.

Animals from the DB-6-OHDA-morphine and vehicle-morphine groups were put on the IP injection schedule as presented in Table II.

Once animals were receiving 100 mg/kg daily, or higher, injections were made twice daily to prevent animals from entering withdrawal between injections.

Animals from the DB-6-OHDA-saline and vehicle-saline groups were injected according to the above schedule, except that animals from these groups were injected with only physiological saline.

On day 25, five animals from each of the DB-6-OHDA-morphine and the vehicle-morphine groups underwent naltrexone precipitated withdrawal.

TABLE II

Summary of the morphine injection procedure for induction of physical dependence

Day	IP Injection Dose (mg/kg)
1	2.5
3	5
5	10
7-14 inclusive	20
15	40
16	40
17	60
18	60
19	80
20	80
21	100
22	100
23	100
24	160
25	160
26	200

Withdrawal Testing:

Animals from each of the DB-6-OHDA-morphine and the vehicle-morphine groups were alternately injected IP with morphine (100 mg/kg) every 30 minutes. One half hour after this injection, animals received an injection of naltrexone (2 mg/kg), and their behaviour during withdrawal was observed for 3 ten minute periods immediately following their naltrexone injection. Animals were placed in a cardboard box (40x40x65 cm) for observation. The order of testing was balanced across both groups, with an equal proportion of animals in each group tested after similar morphine-naltrexone intervals and after the same amount of morphine experience.

A scoring procedure similar to that of Blasig, Herz, Reinhold and Zieglansberger (1973) and Linseman (1975) was used. The incidence of the following behaviours was counted: circling (complete circles within the box, an index of locomotor activity), rearing (an index of exploratory behaviour, jumping (leaping onto the edge of the box, four feet off the ground at the same time), wet dog shakes, teeth chattering (episodes), and writhing (abdominal stretching).

The presence of the following signs was checked every 10 minutes: scream-on-touch, hostility on handling, ptosis, eye twitching, rhinorrhea, lacrimation, diarrhea and penile erection. A score of 1 was awarded when the sign was present, and 0 if the sign was not.

Data from the counted signs were analyzed for each 10 minute period using an analysis of variance and data from the signs present were summed for the 3 ten minute periods, with scores ranging from 0-3, depending on whether or not the signs were consistently present during the 30 minute period, and the data were then analyzed using Student's *t* test.

STUDY IIa

RESULTS

Irritability Data:

Results of the Irritability Rating Scale were analyzed using the Mann Whitney U Test and are presented in Table III. Statistical analysis revealed that the DB-6-OHDA-morphine group was significantly more irritable on all rating days than the vehicle-morphine group, $p < .01$. In comparing the DB-6-OHDA-saline and the vehicle-saline groups, no significant group difference was observed, nor was a significant group difference observed when comparing the vehicle-morphine and vehicle-saline groups, indicating that irritability could not be attributed solely to a lesion, or drug effect.

Withdrawal Weight Data:

Figure 8 shows the results of the mean body weight measurements taken for the last day of morphine injection (corresponding to day 24) and for 5 days following abrupt discontinuation of daily morphine injections. Statistical analysis revealed a significant day effect, $F=101.31$, $df=4, 56$, $p < .001$, but no significant group x day interaction, $F < 1.57$, indicated significant weight changes over time, but no group differences between the vehicle and DB-6-OHDA lesioned animals. Although both the morphine injected groups had lost weight by 48 hours following the last injection, the Student's t test revealed no significant differences between groups ($t < 1.31$).

Figure 9

Mean body weight data on the last day of morphine injection (day 24) and for 5 days following discontinuation of daily morphine injections. Closed squares = DB-6-OHDA-morphine group, N=10; Closed circles = vehicle-morphine group, N=10.

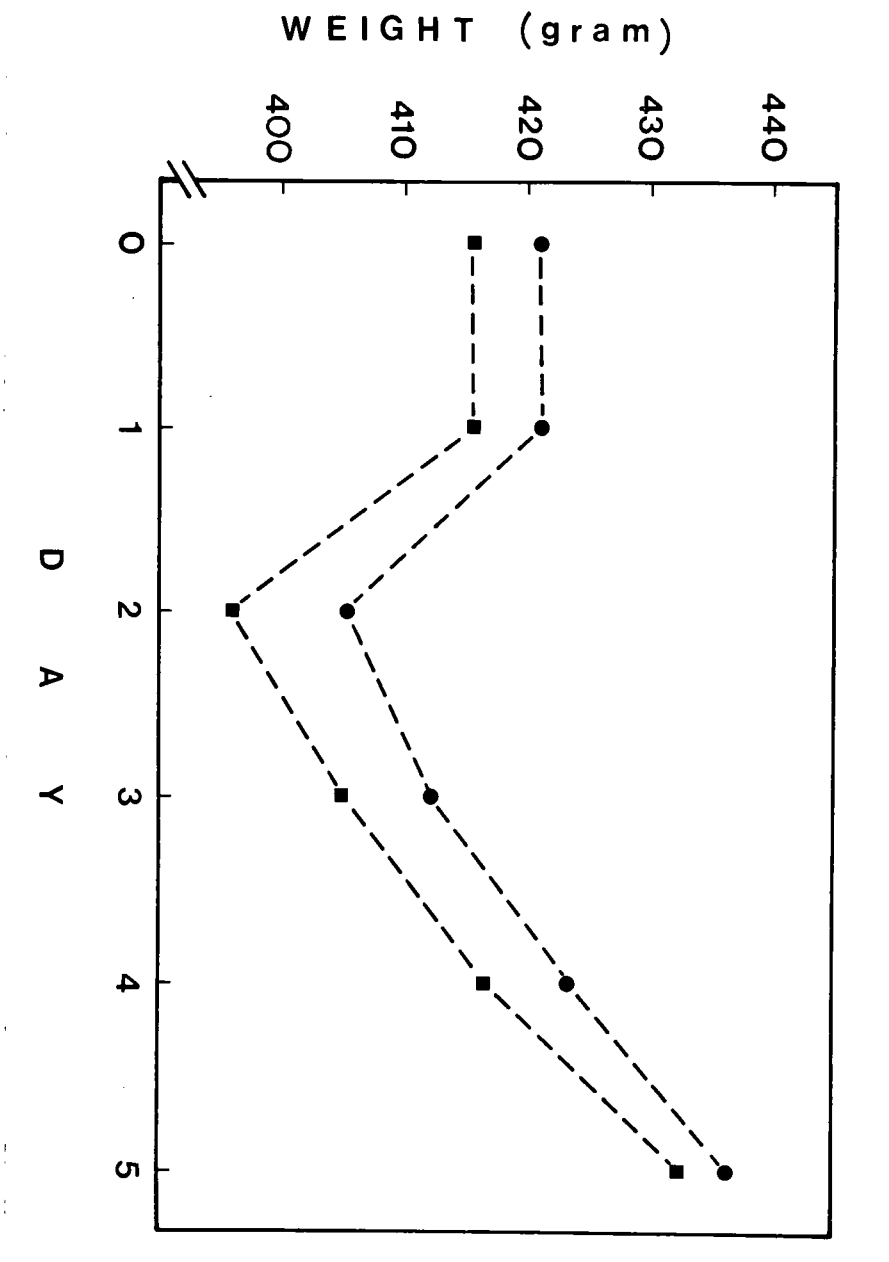

WEIGHT (gram)
400
410
420
430
440
0
1
2
3
4
5
D A Y

TABLE III

Irritability Data

	Vehicle-saline	DB-6-OHDA-saline	Vehicle-morphine	DB-6-OHDA-morphine
Days				
1	.25	1.0	.33	1.33*
5	0	.25	0	1.11*
8	0	0	0	.78*
10	0	0	.22	1.63*

* Indicates a significant difference between the DB-6-OHDA-morphine and vehicle-morphine groups, $p < .01$.

Table III: Mean group scores for irritability on days 1, 5, 8 and 10 of rating using the irritability rating scale.

STUDY IIb

RESULTS

Data from the naltrexone precipitated withdrawal are presented in Tables IV&V & Appendix II. The analysis of variance revealed no significant group differences ($F < 2.23$) and no significant group x time interaction term, ($F < .28$). However, a significant time difference was observed $F=24.38$, $df=2, 24$, $p < .001$, indicating that counted signs decreased significantly over time, regardless of group. Of the counted signs, only circling and rearing, jumping and teeth chattering were observed in the two morphine groups. No incidence of wet dog shakes or writhing were ever noted in any animals during the observation period. The analysis of the checked signs revealed no significant group differences on any parameter.

TABLE IV

Withdrawal-Counted Signs

		DB-6-OHDA-Morphine	Vehicle-Morphine
Circling & tearing	0-10 min	16.22	10.0
	10-20 min	3.89	.40
	20-30 min	3.78	.20
Jumping	0-10 min	25.67	17.6
	10-20 min	15.56	6.4
	20-30 min	5.78	4.0
Teeth chattering	0-10 min	8.56	9.0
	10-20 min	.44	1.60
	20-30 min	0	.20

Table IV: Mean group scores of counted signs present during three 10 minute periods immediately following IP injection of naltrexone (2 mg/kg).

TABLE V

Withdrawal-Present Signs

	DB-6-OHDA-Morphine	Vehicle-Morphine
Screaming on touch	2.20	2.33
Hostility on handling	2.60	2.78
Ptosis	3.00	2.56
Eye twitching	2.60	2.56
Rhinorrhea	2.80	2.33
Lacrimation	0	0
Diarrhea	1.40	1.22
Penile erection	2.20	.78

Table V: Mean group scores of present signs summed for a 30 minute period immediately following IP injection of naltrexone (2 mg/kg).

DISCUSSION

A growing tendency for some animals to display irritability on handling and injection was observed, and therefore a rating scale was developed and applied during the injection procedure. Results indicate that the DB-6-OHDA lesioned animals displayed the most irritability and in that these animals were significantly more irritable than the vehicle-morphine group and the lesion did not produce significant irritability in saline injected animals, it can be concluded that the lesion, in interaction with chronic morphine treatment was responsible for the observed irritability. These results are in accordance with those presented by Friedler et al (1972) who reported difficulty in handling morphine dependent animals injected with 6-OHDA intracerebrally.

Naltrexone precipitated withdrawal and weight loss after abrupt withdrawal were not affected by 6-OHDA injections into the dorsal noradrenergic bundle. These injections resulted in a very substantial depletion of cortex/hippocampus NA, and using this test of withdrawal, it appears that forebrain noradrenaline may not be involved in the expression of withdrawal.

It has been suggested previously that dopamine may be more important than NA in the expression of withdrawal. Lal and Puri (1972) reported that morphine withdrawal aggression was blocked by drugs which block dopamine receptors (haloperidol) and enhanced by drugs which stimulate DA activity (apomorphine, amphetamine, levo-dopa). Gianutsos, Hynes, Puri, Drawbaugh and Lal (1974) reported that withdrawal aggression measured 30 days after the last morphine injection was blocked by morphine or lesions of the nigrostriatal bundle. Aggression was reinstated when the lesioned animals were treated with a small dose of apomorphine. Apomorphine also reduced the turnover of dopamine in 30-day withdrawn animals at doses which were ineffective

in non-dependent rats. These results are interpreted according to the theory of dopamine supersensitivity.

Maruyama and Takemori (1973) reported that repletion of dopamine levels (AMPT + DOPA treatment) partially restores withdrawal jumping in morphine-dependent animals, while repletion of NA levels (AMPT + DOPS treatment) does not. Lal, Puri and Karkelas (1971) reported that haloperidol, a DA receptor blocker, markedly reduces withdrawal in rats and humans.

Several explanations have been proposed for the discrepancies between the role of DA and NA in withdrawal (Herz et al, 1974; Collier, Francis and Schneider, 1972), included the following: a) often only one sign of morphine withdrawal is considered, i.e. naloxone-induced jumping in mice; b) withdrawal is precipitated in animals that have developed quite different degrees of dependence; and c) the action of drugs that modify withdrawal depends on the time at which the drug is administered in the course of dependency development and withdrawal.

In conclusion, it appears that 6-OHDA depletion of forebrain noradrenaline is involved in some tests of withdrawal but not others, and these findings indicate a nondominant role for NA and may lend indirect support to the literature supporting a dopaminergic influence. Further discussion of these data are made in the General Discussion.

STUDY III. A STUDY OF THE PSYCHOPHARMACOLOGICAL INTERACTION OF MORPHINE AND AMPHETAMINE

INTRODUCTION

Amphetamine is a well known stimulant drug that releases catecholamines. There is also evidence that various behavioural effects of morphine (i.e. locomotor activity) are mediated by catecholamine systems, in that catecholamine synthesis inhibitors (AMPT and (bis-(1-methyl-4-homopiperazinyl-thiocarbanyl) disulphide) (FLA-63)), DA receptor blocking drugs (spiramide and pimozide) and noradrenaline receptor blocking drugs (aceperone and phenoxybenzamine) have been reported to antagonize morphine induced excitation (Ayhan and Randrup, 1973).

Ayhan and Randrup (1973) reported that the behavioural profiles of morphine and amphetamine induced excitation were different, inasmuch as morphine stimulated some items of behaviour, including motor activity, grooming, eating and drinking, whereas amphetamine stimulated motor activity and learning, but had no effect, or inhibition on grooming, eating and drinking. Also morphine caused an increase in brain levels of HVA and DOPAC, whereas amphetamine caused a decrease in DOPAC and an increase in HVA (Fukui et al, 1972; Roffler-Tarlov, Sharman and Tegerdine, 1971).

Further interaction studies between the behavioural actions of morphine and amphetamine indicate a mutual antagonism with respect to stereotyped behaviour (Fog, 1970) and enhancement of the analgesic potency of morphine by amphetamine (Sprague and Takemori, 1978).

Finally, Broekkamp (unpublished data), has reported increased levels of spontaneous locomotor activity in animals receiving either an IP injection of amphetamine, or intracerebral (IC) injection of enkephalin into the dopaminergic A10 region, with a further potentiation of the locomotor hyperacti-

vity in animals receiving injections of enkephalin (IC) and amphetamine (IP) together.

Since some behavioural effects of both amphetamine and morphine are mediated by the catecholamine system, it was decided to examine the effects of acute injection of amphetamine and morphine together and separately on spontaneous locomotor activity in rats.

Smee and Overstreet (1976) have presented evidence using acute injections of amphetamine following chronic morphine injections in support of the "postsynaptic DA supersensitivity" hypothesis. They reported an increase in oral cage-directed stereotyped behaviour in chronic morphine treated animals following administration of d-amphetamine. It was therefore concluded that morphine treated animals demonstrated a supersensitivity to d-amphetamine.

Considering the literature heretofore presented supporting the theory of postsynaptic DA receptor supersensitivity as explanation for the mechanisms underlying morphine tolerance, it was decided to further test the theory by studying the effect of an acute injection of d-amphetamine on the spontaneous locomotor activity of chronically treated morphine rats. In addition, the effect of DB-6-OHDA lesions on the behavioural interaction of amphetamine and morphine was also evaluated.

STUDY IIIa. INTERACTION BETWEEN THE BEHAVIOURAL EFFECTS OF ACUTE MORPHINE AND D-AMPHETAMINE ADMINISTRATION ON LOCOMOTOR ACTIVITY IN RATS.

METHODS

Subjects:

A separate group of 40 naive Wistar albino rats from Woodlyn Farms, Guelph, Ontario were used in this study. In that it was of interest to study the hyperactive phase of morphine induced locomotor activity in interaction with amphetamine induced hyperactivity, a pilot study was conducted, and the time course of a dose of 2.5 mg/kg morphine was determined. The results indicated that the hypoactive phase was of duration of .5 hour and was followed by a hyperactive phase of 1.5 hours. Therefore, it was decided to inject d-amphetamine .5 hour following the initial morphine injection, in order to adequately assess the combined effects of the drugs on locomotor hyperactivity. Animals were therefore assigned randomly to one of the four following drug groups: 1. saline, amphetamine (.5 mg/kg); 2. morphine (2.5 mg/kg), saline; 3. morphine (2.5 mg/kg), amphetamine (.5 mg/kg); and 4. saline, saline. All animals received two IP drug injections 30 minutes apart and all testing occurred between 10:00 A.M. and 2:30 P.M., daily.

Apparatus:

Locomotor Activity: Spontaneous locomotor activity was recorded in six circular photoactometer cages, as described in Experiment 1.

Procedure:

All animals were housed individually and given free access to food and water. A 12 hour dark-light cycle was maintained throughout the duration of the experiment.

Locomotor Activity:

Six animals representing two drug treatment groups were individually

tested in the locomotor activity apparatus starting at 10:00 A.M. The following day, another 6 animals representing the other two drug groups were tested. This procedure of testing alternate drug groups continued until an n=10 was reached for each group.

Animals were placed in the locomotor activity cages for a one hour habituation period, whereupon each animal was removed, given its first IP injection and replaced in its wooden carrying cage for 0.5 hour. At this time, the second IP injection was delivered and the animal was returned to the photoactometer apparatus where spontaneous locomotor activity was recorded for a three hour period. At the completion of this phase of testing, all animals were returned to their home cages.

Post-injection data were organized according to 18 minute trials and analyzed using a Three Factor Analysis of Variance (subjects), with the simple main effects model (groups). Significant differences between groups were tested using the Duncan Post Hoc Multiple Range Test, $p < .05$.

STUDY IIIa

RESULTS

Figure 9 shows the time course of the locomotor responses of the 4 drug groups. Statistical analysis indicated: a significant group effect, $F=21.60$, $df=3, 36$, $p < .001$; a significant time effect, $F=11.89$, $df=9, 324$, $p < .001$; and a significant groups x time interaction, $F=2.79$, $df=27, 324$, $p < .001$. The Duncan Multiple Range Test indicated that the saline/amphetamine and the morphine/amphetamine groups demonstrated a gradual decrease in activity over time, whereas the morphine/saline groups showed no change. The saline/saline group showed a significantly higher activity in the first 36 minutes (2 trials), but thereafter showed no significant difference in activity over time.

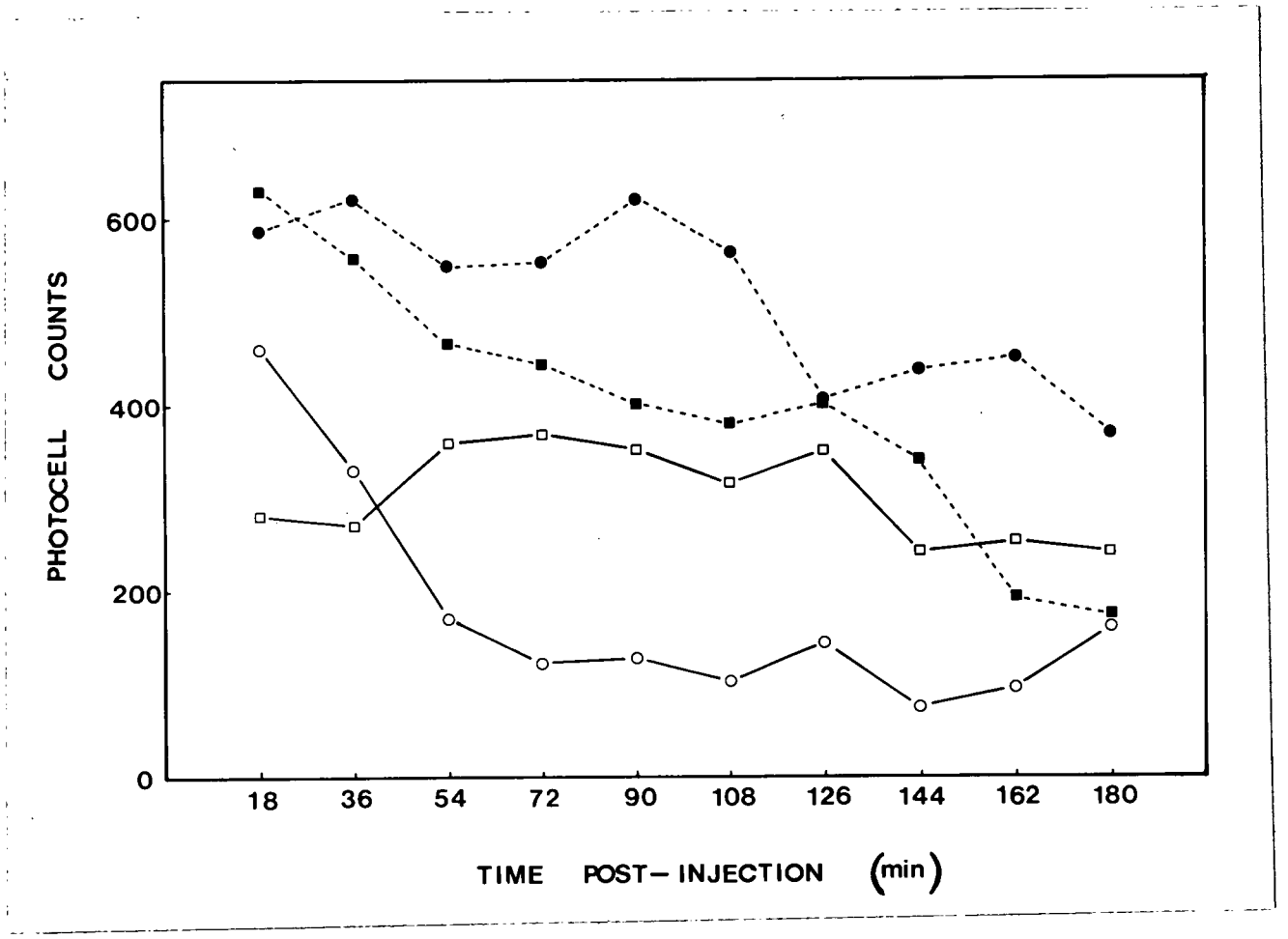
In accordance with the biphasic action of morphine on locomotor activity, animals in the morphine/saline groups initially demonstrated significantly less activity than the other three groups. It is perhaps noteworthy that the pilot study indicated the hypoactive response was of a duration of 30 minutes, but when locomotor testing commenced 30 minutes after the morphine injection, animals still demonstrated the initial hypoactive phase. This perhaps indicates an environmental influence on the behavioural actions of morphine on locomotor activity. Following this, animals of the morphine/saline group demonstrated the characteristic hyperactive phase, maintaining significantly higher levels of locomotor activity than the saline/saline group for the next 2 hours.

Characteristic of the stimulant action of amphetamine, animals of the amphetamine/saline group demonstrated significantly higher levels of locomotor activity than the saline/saline group for approximately 2 hours post-injection.

Of particular interest was the behaviour of those animals who received a first injection of morphine and a second injection 30 minutes later of amphetamine. The Duncan Multiple Range Test revealed that overall, the morphine/amphetamine group demonstrated significantly higher levels of locomotor activity than the other three groups. These animals initially demonstrated significantly higher levels of locomotor activity than the saline/saline and the morphine/saline groups, but the same level of hyperactivity as the amphetamine/saline group. However, by 72-108 and 144-162 minutes post-injection, animals of the morphine/amphetamine group demonstrated significantly higher levels of locomotor activity than the other three drug groups, including the amphetamine/saline group. By the completion of the 3 hour testing session, the morphine/amphetamine group still demonstrated significantly higher levels of locomotor activity than the saline/amphetamine and saline/saline groups.

Figure 10

Mean locomotor activity during the 3 hour post-injection period. Closed squares = saline/amphetamine, N=10; Open squares = morphine/saline, N=10; Closed circles = morphine/amphetamine, N=10; Open circles = saline/saline, N=10 per group.


PHOTOCELL COUNTS
600
400
200
0
18
36
54
72
90
108
126
144
162
180
TIME POST– INJECTION (min)

STUDY IIIb. THE EFFECT OF DB-6-OHDA LESIONS IN MORPHINE TOLERANT RATS ON LOCOMOTOR ACTIVITY FOLLOWING ACUTE AMPHETAMINE ADMINISTRATION

METHODS

Subjects and Procedure:

At the completion of the previous study, all animals had been injected with either morphine or saline for a 21 day period. According to the locomotor testing schedule previously described, animals from all four groups were retested in the photocell cages. Following the one hour standard habituation period, animals from all four groups received an IP injection of d-amphetamine (1 mg/kg) according to body weight and were then replaced in the photocell cages. Their spontaneous locomotor activity was then recorded for a three hour period (as previously described). Animals were then removed from the photocell cages, given their appropriate daily IP injections of saline or morphine, and returned to their home cages.

Data from the one hour habituation period was summed across the 6 ten minute trials for each group and analyzed using a One Way Analysis of Variance. Significant between group differences were tested using the Duncan Post Hoc Multiple Range Test, $p < .05$.

Post-injection data were organized according to 20 minute periods (=9 variables) and analyzed using a Three Factor Repeated Measures Analysis of Variance (subjects) with the simple main effects model (groups). Significant differences between groups were again tested using the Duncan Post Hoc Multiple Range Test, $p < .05$.

Drugs:

Doses of morphine sulphate and d-amphetamine sulphate were expressed in terms of the salts. The solutions were prepared daily with physiological saline and injected IP at room temperature, in a volume of 1 kg/ml.

STUDY IIIb

RESULTS

Habituation Data:

Figure 10 shows the time course of the locomotor activity during the habituation period for the four groups. Statistical analysis revealed a significant between groups difference, $F=17.007$, $df=3$, $p < .001$. The Duncan Multiple Range Test indicated that the DB-6-OHDA-morphine group showed overall significantly less locomotor activity than the vehicle-morphine group, which in turn showed significantly less locomotor activity than the DB-6-OHDA-saline and vehicle-saline groups.

Post-Injection Data:

Figure 11 shows the time course of the locomotor activity of the four groups, post-injection. Statistical analysis indicates a significant group effect, $F=21.60$, $df=3, 36$, $p < .001$, a significant time effect, $F=11.89$, $df=9, 324$, $p < .001$ and a significant groups x time interaction, $F=2.79$, $df=27, 324$, $p < .001$. The Duncan Multiple Range Test indicated that all four groups showed a decrease in activity over time.

No significant group difference was revealed for the first 20 minutes post-injection. However, for the following one hour period, animals that had undergone chronic morphine pretreatment, regardless of lesion, demonstrated significantly higher levels of locomotor activity than those animals from both groups with saline pretreatment.

Figure 11

Mean locomotor activity during a 1 hour pre-injection habituation phase prior to acute amphetamine injections. See Study IIIb. Closed squares = DB-6-OHDA-morphine group, N=10; Open squares = DB-6-OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.

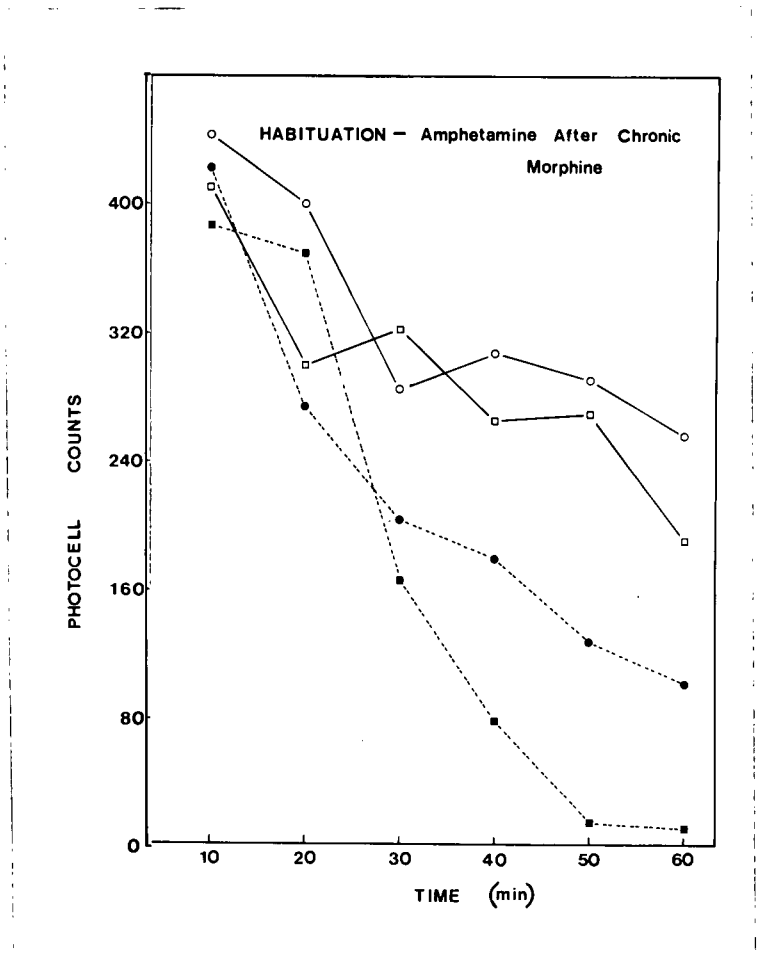
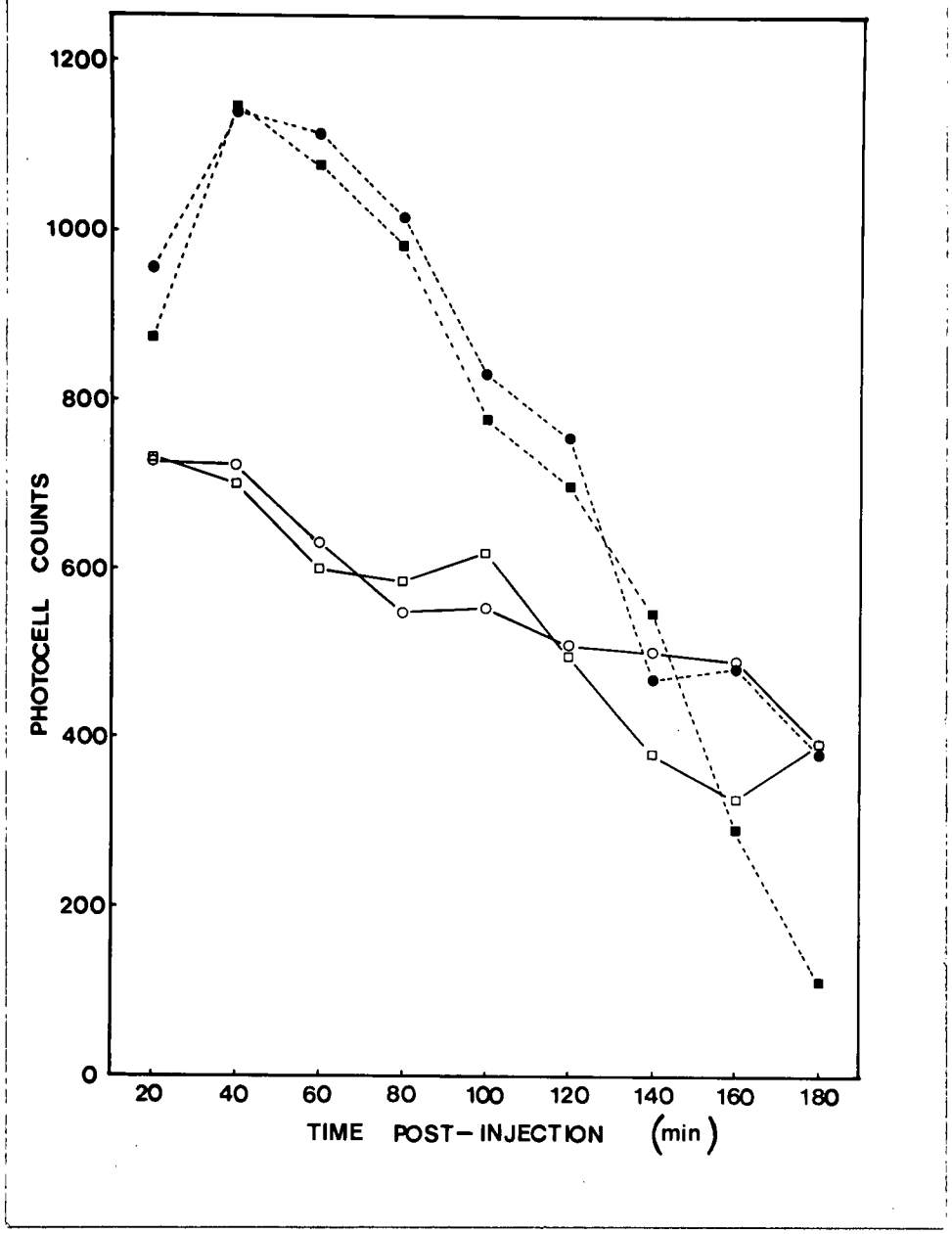

HABITUATION – Amphetamine After Chronic Morphine
PHOTOCELL COUNTS
400
320
240
160
80
0
10
20
30
40
50
60
TIME (min)

Figure 12

Mean locomotor activity during a 3 hour period following acute amphetamine injection. Closed squares = DB-6OHDA-morphine group, N=10; Open squares = DB-6OHDA-saline group, N=8; Closed circles = vehicle-morphine group, N=10; Open circles = vehicle-saline group, N=8.


1200
1000
800
600
400
200
0
PHOTOCELL COUNTS
20
40
60
80
100
120
140
160
180
TIME POST-INJECTION (min)

DISCUSSION

Amphetamine/Morphine Interaction:

Acute IP injection of a low dose of amphetamine (.5 mg/kg) followed 30 minutes later by acute injection of a low dose of morphine (2.5 mg/kg) resulted in significantly higher levels of locomotor activity than observed in animals receiving either ampehtamine (.5 mg/kg) or morphine (2.5 mg/kg) injection.

As previously noted, it was recently reported that opiates produce behavioural excitation by activating DA neurons (Carroll and Sharp, 1972) and that injection of the long acting synthetic enkephalin analogue (D-ala^2) - Met^{5}enkephalinamide (AME) into the nucleus accumbens and the dopaminergic A-10 area of the ventral tegmental area (VTA), produced an increase in locomotor activity (Pert and Sivit, 1977; Broekkamp et al, 1979). The nucleus accumbens is one of the projection areas of DA cells in the VTA, and there is also evidence of involvement of this mesolimbic DA pathway in the stimulant effects of amphetamine (Kelley, Seviour and Iversen, 1975). One may therefore hypothesize that the enhancement of locomotor activity with acute amphetamine + morphine injections is related to increased activation of dopaminergic systems in the mesolimbic pathway.

The potentiation of spontaneous locomotor hyperactivity following an acute amphetamine injection (1 mg/kg) also was observed in chronic morphine pretreated animals when compared to saline pretreated animals in accordance with results previously implicating a synergistic role of amphetamine and morphine (Smee and Overstreet, 1976; Ahyan and Randrup, 1973). Inasmuch as this behavioural effect was unaffected by a DB-6-OHDA lesion, it seems likely that it is mediated by another transmitter system, possibly DA. It should be noted that these results cannot be attributed to a ceiling effect,

in that it was demonstrated previously that animals who received bilateral injections of kainic acid into the striatum displayed activity count levels as high as 900 counts/10 min following an injection of 1 mg/kg amphetamine (Pisa, Sanberg and Fibiger, 1979), whereas count levels in the present study reached a maximum of approximately 1200 counts/20 min. Furthermore, injections of 2 mg/kg into animals with the same lesion resulted in activity levels of 1100/10 min (Mason and Fibiger, 1978).

In addition, our findings that a DB-6-OHDA lesion did not affect the amphetamine-induced potentiation of locomotor hyperactivity following acute and chronic morphine do not lend support to the hypothesis that NA mediates a modulatory, or transynaptic role in DA-mediated locomotor stimulation (Maj et al, 1971; Kuschinsky and Hornykiewicz, 1974).

It is possible that morphine and amphetamine may be acting on two completely different brain systems that interact, or alternatively, it has been suggested that both act on the same DA neuronal system. Smee and Overstreet (1976) observed enhanced amphetamine induced stereotypy in animals that had received chronic morphine pretreatment, when compared to saline pretreated animals and they ascribed these findings to supersensitive postsynaptic DA receptors, resulting from chronic morphine treatment. Based on this evidence and other previous findings implicating postsynaptic DA receptor supersensitivity in the effects of chronic morphine treatment (Puri et al, 1977; Puri and Lal, 1974; Baume et al, 1979; and, Clouet and Iwatsubo, 1975a), it seems reasonable to ascribe this mechanism to our observation of enhanced amphetamine-induced locomotor activity in animals chronically pretreated with morphine.

GENERAL DISCUSSION

Several behavioural effects of morphine were studied following specific injections of 6-OHDA into the dorsal noradrenergic bundle, in an attempt to evaluate the interaction of morphine with the catecholamine systems. Studies I and II explored the effects of chronic morphine on the development of tolerance and physical dependence, respectively. A state of tolerance is said to be reached when after repeated use, a given dose of drug produces a decreased effect, or when increased doses must be taken to obtain the effects of the original dose. Physical dependence results when repeated administration of a drug alters the physiological state and necessitates the continued use of the drug to prevent withdrawal (Jaffe and Martin, 1975).

Firstly, the effect of DB-6-OHDA lesions on tolerance was assessed employing changes in the hypoactive phase of morphine-induced locomotor activity as an index of tolerance development. It was observed that a role for the catecholamine systems, specifically for NA can be implicated in tolerance development to chronic morphine treatment, in that injection of 6-OHDA into the dorsal noradrenergic bundle resulted in a slower rate and a lesser degree of tolerance development to the hypoactive phase of morphine-induced locomotor activity. Several models can be proposed in relation to the data obtained, all of which maintain the basic premise that the biphasic action of morphine on locomotor activity can be attributed to the interaction of the NA and DA systems.

Theoretical Considerations:

There is much evidence that motor stimulation is mediated by increased catecholamine transmission, specifically of DA (Kuschinsky, 1976; Creese and Iversen, 1973; Moore, 1977; Roberts et al, 1978), i.e. the central stimulant action of amphetamine is blocked primarily by drugs which have DA-receptor blocking properties (i.e. pimozide), but not drugs which block α- and β-adrenergic receptors (i.e. phentolamine or propanolol). In addition, the central stimulant actions of amphetamine were blocked in animals with selective loss of DA neurons, but not altered in animals with loss of NA neurons. Also, the hypothesis that the stimulatory effects of morphine are mediated by the DA system has been reviewed earlier (see Introduction). The present finding that acute amphetamine administration following acute or chronic morphine pre-treatment resulted in potentiated hyperactive responses, regardless of the DB-6-OHDA lesion, further implicates a role for DA in the mediation of the stimulatory effects of morphine.

In addition, it has been suggested that the biphasic actions of morphine on locomotor activity may involve more than one neurotransmitter substance (Vasko and Domino, 1978; Mason et al, 1978). Roberts et al (1978) reported that 6-OHDA-induced depletion of forebrain NA caused potentiation of the depressant actions of acute morphine administration, whereas the stimulant actions appeared independent of noradrenergic mechanisms. Buxbaum et al (1973) observed that hyperactivity observed after acute administration of morphine was antagonized by treatments that deplete brain catecholamines and that hypoactivity produced by high doses of morphine was antagonized by treatment that deplete serotonin levels. The authors suggested that the effect of morphine on locomotor activity is dependent upon a balance between the two systems. Of particular interest, is the evidence of Broekkamp, Van de Bogaard, Heijnen, Rops, Cools, and Van Rossum (1975) that intracerebral

injections of morphine separated the excitatory and inhibitory effects of morphine. They reported that pure inhibitory effects were obtained from the central grey substance surrounding the aqueduct and locus coeruleus, whereas pure excitatory effects were measured after injections into the posterior hypothalamus and ventral tegmental area. Based on the above findings, it seems reasonable to ascribe the biphasic effect of morphine on locomotor activity to an interaction between the hypoactive response mediated by the NA system,and the hyperactive response mediated by the DA system. The behavioural effects on locomotor activity following chronic morphine administration can then be explained as follows: initially the hypoactive phase characterized by a decrease in NA transmission,is dominant and masks the DA influence. The secondary response of hyperactivity represents the increasing dominance of the DA system and is characterized by increased release of DA. According to this formulation, the DB-6-OHDA lesioned animals display a lower level of hyperactivity following chronic morphine treatment because they show a potentiated NA-mediated hypoactive response,which when added to the secondary DA-mediated hyperactive response,results in the observed response of a lower level of hyperactivity.

With this basic premise in mind, several models can be proposed for the effects of chronic and acute morphine or locomotor activity. The first is perhaps the most parsimonious and interprets the data according to the theory of "disuse supersensitivity", initially proposed by Collier (1965) and Jaffe and Sharpless (1968), and more recently summarized by Llorens et al (1978). Acute morphine injections result in presynaptic noradrenergic inhibition, which results in decreased NA transmission. With chronic morphine administration, long-term presynaptic inhibition results in a compensatory increase in the responsiveness of the postsynaptic cell to NA. It should be noted that 6-OHDA lesions into the fibers of the dorsal noradrenergic bundle result in widespread depletion of forebrain noradrenaline and that

destruction of nerve fibers may be partially compensated for by supersensitive postsynaptic NA receptors, due to denervation supersensitivity. Therefore, according to this model, because the DB-6-OHDA animals would already have postsynaptic NA supersensitivity, one would hypothesize that an acute injection would result in decreased or no observed hypoactivity, indicating tolerance development to the hypoactive phase with an acute morphine injection. This prediction is, of course, contrary to the observed findings of hypoactivity in lesioned animals following an acute injection of morphine.

Secondly, when considering the response to chronic morphine, if tolerance development is attributed to increased postsynaptic NA receptor supersensitivity, as Llorens et al (1979) suggest, and DB-6-OHDA animals have developed postsynaptic NA supersensitivity prior to the beginning of chronic morphine administration, it is not possible to make predictions with respect to tolerance development to the hypoactive phase when the response to acute morphine injection is considered. It therefore appears that the mere consideration of postsynaptic supersensitivity is inadequate to explain the development of tolerance following repeated morphine administration.

The second model is similar to the first presented, in that it postulates a role for increased postsynaptic NA supersensitivity following chronic morphine administration. In addition, it makes two assumptions: 1) there is a ceiling effect regulating the amount of postsynaptic receptor proliferation (supersensitivity) that can occur in the lesioned and non-lesioned animals, and 2) in the DB-6-OHDA animals, increased postsynaptic NA receptor supersensitivity is outweighed by the presence of substantially fewer NA nerve terminals (as a result of the 6-OHDA lesion), resulting in an overall reduction in NA tone. Inclusion of the presence of decreased NA tone affords an alternate explanation when interpreting the behavioural effects of the DB-6-OHDA lesion, especially with regard to tolerance develop-

ment following chronic morphine treatment. In this case, the model would predict that decreased NA tone would result in less tolerance development in the DB-6-OHDA group or increased levels of hypoactivity in relation to the vehicle-morphine group with repeated morphine administration. This prediction is supported by the present findings.

The model is perhaps less effective in predicting the acute actions of morphine. Acute morphine injection in non-lesioned animals results in the initial hypoactive locomotor response, as a result of decreased NA release. The model postulates that the DB-6-OHDA lesion results in decreased NA tone. It is difficult on the basis of the model to qualitatively compare the differences in tone between the non-lesioned and DB lesioned animals upon receipt of acute morphine injection. One possible recourse is to consider the observed findings that both groups display the same degree of hypoactivity following acute injection of a dose of 25 mg/kg morphine and assume that the two groups therefore have approximately the same degree of reduction in NA tone. It appears, however, that further biochemical investigation is necessary to fully evaluate the effects of a DB-6-OHDA lesion on NA release and turnover in the forebrain, so as to avoid making inferences about the biochemical changes following acute morphine treatment solely from behavioural evidence.

In summary, it appears that the second model, which includes consideration of NA tone as well as NA postsynaptic supersensitivity, is somewhat ineffective in predicting the effects of acute morphine injection, but is the model better suited as an explanation for the behavioural effects of chronic morphine observed in control and DB-6-OHDA lesioned animals.

An alternative and perhaps equally important consideration is the effect of DB-6-OHDA lesions on cerebellar and spinal cord levels of NA. Atweh and Kuhar (1977b) have demonstrated opiate receptors in the spinal cord using autoradiographic identification of stereospecific (^{3}H) dispre-norphine (a potent opiate antagonist) binding sites and Garcin and Coyle (1977) reported that peripheral treatment of newborn rats with 6-OHDA significantly increased the levels of NA and opiate receptor binding in the cerebellum, suggesting that opiate receptors in the cerebellum may be localized on NA fibers innervating this region.

It has been reported that DB-6-OHDA lesions significantly increase the levels of NA in the cerebellum and spinal cord (Mason et al, 1978; U'Prichard, Reisine, Mason, Fibiger and Yamamura, in press). Recent investigation has revealed that the increased levels of NA result in a compensatory decrease in the number of β-adrenergic receptors available for NA to act upon (U'Prichard et al, in press). It is suggested that cerebellar receptor sites postsynaptic to NA terminals can become desensitized or "subsensitive" as a result of increased NA levels (U'Prichard et al, in press). A third model for the acute and chronic effects of morphine can therefore be proposed, incorporating these neuronal changes in the spinal cord following a DB-6-OHDA lesion.

In considering the non-lesioned animals, acute morphine results in decreased catecholamine release, and a concomitant increase in turnover.

With chronic morphine administration, non-lesioned animals demonstrate tolerance to the effect of morphine on turnover (Puri and Lal, 1974; Gauchy et al, 1973; see review by Clouet and Iwatsubo, 1975b) as well as postsynaptic supersensitivity (Collier, 1965; Jaffe and Sharpless, 1968; Llorens et al, 1978). Therefore, chronic morphine administration results in increased NA tone following morphine injection, manifested behaviourally by decreased levels of locomotor hypoactivity. In considering the response of the DB-6-OHDA group to chronic morphine treatment, it is assumed that the DB-6-OHDA group shows a similar tolerance response to turnover however, DB-6-OHDA lesions also result in increased levels of NA in the cerebellum and spinal cord with the concomitant reduction in the number of β-adrenergic receptors available for NA to act upon. It can therefore be hypothesized that chronic morphine treatment would result in the development of less supersensitivity in the DB-6-OHDA group due to the presence initially of a lesser number of postsynaptic receptors as a result of the lesion. This would result in lower NA tone, manifested behaviourally by prolongation of the locomotor hypoactive response. These predictions were again in accordance with the observed behavioural responses following chronic morphine treatment, and provide support for the hypothesis that the initial hypoactive phase of morphine-induced locomotor activity is mediated by the NA system.

As with the models presented earlier, this model is less adequate in explaining the effects of acute morphine administration in the naïve animal. The neurochemical effects of the DB-6-OHDA lesion in the spinal cord and cerebellum have already been described, namely an increase in NA levels and a reduction in the number of postsynaptic β-adrenergic receptors. However, it is not possible to predict the effects of acute morphine treatment on NA tone solely from these observations. For this reason, predictions are made according to the behavioural data observed. Locomotor hypoactivity has been

cited as a behavioural correlate of the reduction in release of NA, and the same levels of hypoactivity were observed in both the lesions and non-lesioned animals following acute morphine treatment. It is therefore assumed based on the present behavioural observations, that the DB-6-OHDA and control groups showed the same reduction in NA release following acute morphine administration.

It is possible to apply one final interpretation to the data based on the observed neurochemical changes in the spinal cord and cerebellum following a DB-6-OHDA lesion described earlier. It has been reported that acute morphine administration results in increased levels of MHPG, a major metabolite of brain NA (Roffman et al, 1979) as well as decreases in brain NA following intraventricular injection of morphine (Watanabe, 1971). In addition, it has been reported that acute administration of an analgesic dose of morphine increases the concentration of another NA metabolite, normetanephrine (NM) in the dorsal half of the spinal cord (Shiomi and Takagi, 1974; Takagi, Shiomi, Kuraishi, Fukui and Ueda, 1979) and that animals that underwent chronic morphine treatment showed tolerance to the NM increase. It has therefore been suggested that morphine accelerates the release of NA from descending noradrenergic fibers (Shiomi and Takagi, 1974). On this basis, it seems possible that the behavioural effects of acute morphine, specifically the suppressant phase of locomotor activity, may be mediated by an increase in NA release in the spinal cord and cerebellum. The observation of tolerance to the increase in turnover following chronic morphine treatment (Shiomi and Takagi, 1974; Puri and Lal, 1974; Gauchy et al, 1973; see review by Clouet and Iwatsubo, 1975) correlates with the behavioural observation of tolerance to the hypoactive phase of morphine-induced locomotor activity. It should be noted that these biochemical results are inconsistent with physiological evidence presented by Aghajanian (1978) that morphine causes inhibition of

the spontaneous firing rate of NA cells in the locus coeruleus. However, comparison of the actions of amphetamine using the same biochemical and physiological techniques has yielded the same inconsistencies. There is much evidence that the behavioural stimulant effects of amphetamine are mediated by increased release of DA (Kuschinsky, 1976; Creese and Iversen, 1973; Moore, 1977; and Roberts et al, 1978); however, physiological studies indicate that d-amphetamine inhibits the firing of DA neurons in the zona compacta and ventral tegmental area (Bunney, Aghajanian and Roth, 1973). Therefore, perhaps the actions of morphine on NA neurons can be viewed as similar to those of amphetamine on DA neurons. On this basis it can be hypothesized that morphine administration inhibits the spontaneous firing rate of LC cells, as well as mediates an increase in the release of NA by an action on NA terminals. With these assumptions in mind the behavioural effects of morphine can be explained as follows: as earlier stated, the DB-6-OHDA lesion results in increased levels of NA in the brain and spinal cord (U'Prichard et al, in press) when compared to controls. If increased levels of NA in the spinal cord of the DB-6-OHDA lesioned animals result in an enhanced release of NA, it can be hypothesized that the DB-6-OHDA animals should demonstrate more pronounced hypoactivity and a lesser degree of tolerance development following chronic morphine administration. However, it should be noted that this model is speculative, inasmuch as little if any evidence is currently available on the effects of acute and chronic morphine on NA release and turnover in the spinal cord and cerebellum.

In summary, several models have been presented in an attempt to explain the observed behavioural findings. No one model adequately predicts or explains the behavioural and biochemical effects of acute and chronic morphine administration. However, each one provides a framework in which to discuss the data obtained. It is obvious that further research is necessary before

a truly satisfactory model can be proposed, and that all assumptions regarding the biochemical effects of the DB-6-OHDA lesion on NA release and turnover in the spinal cord, cerebellum and forebrain following acute and chronic morphine should be empirically tested. One essential experiment currently underway is the evaluation of the effects of depletion of spinal and cerebellar NA, so as to more clearly delineate the individual roles of alterations in NA levels in the spinal cord/cerebellum and the forebrain in mediating the effects of acute and chronic morphine administration.

The 2nd study tested the effect of a DB-6-OHDA lesion on the development of physical dependence. The classical index of physical dependence is withdrawal, and the degree of physical dependence is measured by the severity of withdrawal (Jaffe and Martin, 1975). There are several techniques available for measuring withdrawal: firstly, chronic morphine pretreated animals can be injected with an opiate antagonist (i.e. naloxone) and the incidence of a series of withdrawal behaviours measured. A second common manifestation of withdrawal is weight loss following discontinuation of morphine treatment. In addition, in the studies outlined above, irritability associated with handling and injection and locomotor activity levels during the habituation period were also attributed to a withdrawal response and therefore used as indices of physical dependence.

The effect of the DB-6-OHDA lesion on physical dependence was assessed by measuring naltrexone-induce withdrawal in lesioned and vehicle animals that had received chronic morphine treatment. Results indicate that although NA is important in tolerance development it does not mediate a dominant role in physical dependence, as manifested in naltrexone-precipitated withdrawal. Some effects of the lesion on other measurements of dependence, however, were observed, specifically on irritability associated with the handling and injection procedure. Consideration of the habituation data also revealed information about the effect of DB-6-OHDA lesions on physical dependence.

Habituation Data:

The Morphine Tolerance and the Amphetamine After Chronic Morphine studies (Study IIIb) occurred in the chronological order listed and therefore enable observation of the behaviour of the animals during the pre-injection habituation periods over an extended period of morphine treatment.

Initially in session 1 of the Morphine Tolerance Study, no significant differences were observed. However, by session 2, the vehicle-morphine group demonstrated significantly less locomotor activity than the other three groups, especially during the last 30 minute period. In sessions 3-6 inclusive, the DB-6-OHDA-morphine and the vehicle-morphine groups demonstrated significantly less activity than the DB-6-OHDA-saline and vehicle-saline groups. This group difference in activity was again especially pronounced during the last 30 minute period.

This trend of morphine injected animals demonstrating less activity during the habituation period was also evident in subsequent studies. During the final experiment of the series, representing prolonged morphine treatment (see Study IIIb - Amphetamine After Chronic Morphine) another group dissociation became evident, whereby the DB-6-OHDA-morphine group demonstrated significantly less locomotor activity than the vehicle-morphine group, which in turn demonstrated significantly less activity than the DB-6-OHDA-saline and vehicle-saline groups.

In reviewing these findings, it appears that chronic morphine treated animals displayed significantly lower levels of locomotor activity during the habituation phase than the saline treated animals. In addition, the DB-6-OHDA lesion potentiated this trend towards suppression of locomotor activity of the morphine treated groups during the habituation period. Perhaps this behaviour can be attributed to a withdrawal effect, inasmuch as the 1 hour pre-injection habituation period represents the final stages

of the 24 hour interval between injections. This is consistent with the results of the irritability data, wherein animals of the vehicle-morphine and DB-6-OHDA-morphine groups demonstrated significant irritability on handling and injection during the course of the experiment, with an enhanced effect in the DB-6-OHDA-morphine group (see Study IIa; Friedler et al, 1972). Taken together, these data suggest that both groups were undergoing withdrawal, with the DB-6-OHDA-morphine group showing a somewhat more severe reaction. It should be noted, however, that the finding of increased withdrawal, with the DB-6-OHDA-morphine group was not reproduced when consideration was made of other indices, namely, naltrexone precipitated withdrawal. Some explanation for these discrepant findings can be sought from consideration of the schedules employed for the induction of dependence. The irritability and habituation data were used as indices of dependence in animals that received once daily injections of a dose of 25 mg/kg morphine, whereas animals who underwent naltrexone-precipitated withdrawal, received steady dose-increments, until the daily dose reached 200 mg/kg. Perhaps inconsistencies in behavioural findings can be explained by these differences in the morphine injection schedule prior to withdrawal testing, and therefore reflect different degrees of physical dependence. In effect, a schedule of once daily injections of a stable dose of 25 mg/kg for a 24 day period, may be sufficient for tolerance development, but reflect only a slight degree of physical dependence. Furthermore, although the data are not presented, animals that underwent this schedule of injection, exhibited no signs of withdrawal when challenged with naloxone, again indicating that the animals responded to only some tests of withdrawal. Conversely, the second injection schedule which consisted of a rapid induction procedure may have resulted in highly dependent animals. The nonsignificant group differences may therefore be attributed to a 'ceiling effect'.

It seems possible that the behavioural indices of physical dependence may be affected by the drug administration procedure, and that certain behavioural measures (i.e. habituation and irritability data) may correlate with the early phases in the development of physical dependence, whereas, antagonist-precipitated withdrawal may be a better index of a highly dependent state. Physical dependence should perhaps be viewed as a non-static process and consideration should be given to its dynamic properties when utilizing its behavioural indices. It is therefore suggested, that further research is necessary to define the properties of physical dependence as well as design behavioural measures that adequately characterize its development, before the role of the catecholamine systems can be adequately assessed.

It is also noteworthy that depletion of forebrain NA by a 6-OHDA lesion affected tolerance development in relation to locomotor activity, but was without effect when considering classical symptoms of withdrawal. Several explanations can be offered for a lesion effect on tolerance, but not physical dependence. Firstly, withdrawal testing is used as an index of physical dependence and the relation of tolerance to withdrawal is not clearly understood, nor are there behavioural tests available that clearly delineate their respective properties.

Recently, Mucha et al (1979) attempted to evaluate the relation of tolerance to physical dependence by measuring withdrawal responses and relating them to tolerance development in relation to tailflick analgesia. It was reported that only some withdrawal signs correlate well with tolerance measurement and therefore it was concluded that the choice of responses to measure tolerance and dependence determines whether the phenomena are related. It is possible therefore, that our choice of behavioural tests were responsible for our inconsistent findings.

Secondly, it is possible that physical dependence and tolerance are, in fact, somewhat independent yet related phenomena and that a noradrenergic system may be involved in one process but not the other. At this time, therefore, it appears that further investigation is necessary to evaluate the relation of tolerance and physical dependence and their respective underlying mechanisms of action.

Lastly, the interaction of amphetamine and morphine with the DA system was assessed by studying the behavioural effects of amphetamine in animals following either acute or chronic morphine treatment. These results indicated that amphetamine-induced locomotor hyperactivity was potentiated following acute and chronic morphine treatment. This potentiation was unaffected by a DB-6-OHDA lesion in chronically morphine pretreated animals, and it is therefore suggested that another transmitter system, probably dopamine, mediates this effect.

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APPENDIX I

Summary of Analysis of Variance for Post-Injection Data for Days 1,7 and 16 of Drug Injection

	df	SS	F	p
variable 1 (0-20 minutes)				
Groups	3	620707.8	2.32	p< .01
Subjects	30	2673663.62		
Trials	2	2591391.82	25.20	p< .001
Trials x Groups	6	2834717.05	9.19	p< .001
Trials x Subjects	55	2828282.74		
variable 2 (20-40 minutes)				
Groups	3	311951.40	1.49	ns[a]
Subjects	30	2093091.67		
Trials	2	1356526.61	16.04	p< .001
Trials x Groups	6	1042320.75	4.11	p< .001
Trials x Subjects	55	2324462.47		
variable 3 (40-60 minutes)				
Groups	3	1677314.76	7.08	ns[a]
Subjects	30	2369349.80		
Trials	2	2003864.43	20.56	p< .001
Trials x Groups	6	1896825.99	6.49	p< .001
Trials x Subjects	55	2680505.07		
variable 4 (60-80 minutes)				
Groups	3	3241853.34	21.26	p< .001
Subjects	30	1524948.51		
Trials	2	2296370.11	18.93	p< .001
Trials x Groups	6	2736636.83	7.52	p< .001
Trials x Subjects	55	3335353.39		
variable 5 (80-100 minutes)				
Groups	3	5341340.59	44.64	p< .001
Subjects	30	1196556.56		
Trials	2	3095094.91	58.94	p< .001
Trials x Groups	6	3280828.94	20.82	p< .001
Trials x Subjects	55	1444208.82		
variable 6 (100-120 minutes)				
Groups	3	5310304.06	43.18	p< .001
Subjects	30	1229677.85		
Trials	2	2228487.28	34.69	p< .001
Trials x Groups	6	2209702.56	11.47	p< .001
Trials x Subjects	55	1766420.99		

Cont'd...

Appendix I - cont'd

	df	SS	F	p
	variable 7 (120-140 minutes)			
Groups	3	7023907.77	56.78	p< .001
Subjects	30	1237025.37		
Trials	2	1433510.26	35.43	p< .001
Trials x Groups	6	1276187.81	10.51	p< .001
Trials x Subjects	55	1112581.43		
	variable 8 (140-160 minutes)			
Groups	3	7626961.19	99.80	p< .001
Subjects	30	764246.26		
Trials	2	844191.14	12.86	p< .001
Trials x Groups	6	407070.30	2.07	ns$^{\alpha}$
Trials x Subjects	55	1804817.57		
	variable 9 (160-180 minutes)			
Groups	3	6960158.30	79.62	p< .001
Subjects	30	874121.82		
Trials	2	444568.84	11.34	p< .001
Trials x Groups	6	215171.31	1.83	ns$^{\alpha}$
Trials x Subjects	55	1078048.19		

ns$^{\alpha}$ = not significant

APPENDIX II

Summary of Analysis of Variance for Withdrawal - Counted Signs

	df	SS	F	P
variable 1 (0-10 minutes)				
Groups	1	245.38	2.23	ns[α]
Subjects	12	1318.27		
Trials	2	3813.90	24.38	p< .001
Trials x Groups	2	44.05	.28	ns[α]
Trials x Subjects	24	1877.38		
variable 2 (10-20 minutes)				
Groups	1	386.79	1.63	ns[α]
Subjects	12	2840.67		
Trials	2	2205.19	14.95	p< .001
Trials x Groups	2	101.97	.69	ns[α]
Trials x Subjects	24	1769.51		
variable 3 (30-30 minutes)				
Groups	1	3.47	0.09	ns[α]
Subjects	12	479.60		
Trials	2	639.57	10.62	p< .001
Trials x Groups	2	1.58	.03	ns[α]
Trials x Subjects	24	722.84		

ns[α] = not significant