Order Number 8820906

# The role of the dopaminergic system in behavioral activation of infant rats

Viscardi, Eun-Jee, Ph.D.

City University of New York, 1988

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

# THE ROLE OF THE DOPAMINERGIC SYSTEM IN BEHAVIORAL ACTIVATION OF INFANT RATS

by

Eun-Jee Viscardi

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1988

This manuscript has been read and accepted by the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy

21. Dec. '87
date

Gordon A. Barr
Gordon A. Barr, Ph. D.,
Chair of Examining Committee

December 21, 1987
date

Herbert D. Saltzstein
Herbert Saltzstein, Ph. D.,
Executive Officer

Eliot L. Gardner, Ph. D.

Myron A. Hofer, Ph. D.

Michael Potegal, Ph. D.

Gerald Turkewitz, Ph. D.

Supervisory Committee

The City University of New York

## Abstract

THE ROLE OF THE DOPAMINERGIC SYSTEM IN BEHAVIORAL ACTIVATION OF INFANT RATS

by

Eun-Jee Viscardi

Advisor: Professor Gordon A. Barr

Young rat pups become behaviorally activated, emitting a series of responses that includes mouthing, licking, pawing, and locomotion, following either reinforcing exteroceptive stimuli such as milk, or electrical stimulation of the medial forebrain bundle. To characterize the role of striatal and mesolimbic systems in the behavioral activation of infant rats, the behavioral responses of 3-day-old rats were observed following brief electrical stimulation of the caudate nucleus (CD) or the nucleus accumbens (NA).

Bipolar teflon coated stainless steel electrodes, bared at the tip, were implanted in the CD or the NA of 3-day-old pups. Testing occurred 16 to 20 hours after surgery. Following a 5 minute adaptation period and a 2 minute baseline observation period, behavior was recorded over 6 one minute intervals during which biphasic square wave stimulation (500 msec train of 50 pulses with a pulse width of 2 msec) was delivered every 10 seconds during every other minute. Current was 60

uA, although inactive sites were tested up to 1000 uA. The behavior of the pup was recorded during each minute. Following testing, pups were overdosed with a barbiturate and perfused intracardially. Frozen brain sections were stained for Nissl substance and the electrode placements verified independent of the behavioral data. Stimulation of the NA (N=12) resulted in behavioral activation, while stimulation of the CD (N=15) produced locomotion only.

To assess first the role of dopamine (DA) systems in behavioral activation, and second the differential effect of typical and atypical neuroleptics on the striatal and mesolimbic DA systems, subsequent studies tested whether elicited behavioral activation could be blocked by the dopamine antagonists haloperidol (HAL) and clozapine (CLOZ). HAL (0.05 and 0.2 mg/kg) and CLOZ (0.5 and 2.5 mg/kg) produced comparable effects in blocking stimulation induced activation.

These results suggest the involvement of the mesolimbic system in the behavioral activation that accompanies reinforcement in infant rats, and a possible role of dopamine mediating this behavioral activation.

Acknowledgments

I would like to sincerely thank my research advisor Dr. Gordon Barr for his continuous support of my scientific growth throughout my graduate program at Hunter College. His flexibility and compassion with regard to my unique circumstances, and his persistence in keeping my work on track, is warmly appreciated, for without it, completion of my dissertation would not have been possible.

I would also like to thank Drs. Eliot Gardner, Myron Hofer, Michael Potegal, and Gerald Turkewitz for their guidance, and for serving on my dissertation committee.

I would like to extend my sincere gratitude to Dr. George Rebec for introducing me to brain research, and for providing me with laboratory facilities at Indiana University, Dr. Jeff Alberts for his expertise and continuous supply of subjects, and Dr. Shiela Chase for her support when important decisions were needed.

I want to thank Bill Paredes for providing me with the important equipment and information needed to begin my research.

I also want to thank Drs. Dwight German, and Sam Speciale for their comments and input during the final drafts of my dissertation.

I want to extend my warmest gratitude to Anthony Viscardi, my loving husband, whose patience and creative contributions aided me every step along the way.

In general, I would like to thank my friends Joan, Leslie, Edda, Jim and Chris for their support throughout my graduate years.

This work is dedicated to the future of my children as young scientists, John and Susan Lee.

Table of Contents

# List of Tables

List of Figures

# PART I: GENERAL INTRODUCTION

## Significance of Behavioral Activation in Rat Pups

Rats, as well as other altricial species are neurologically and behaviorally immature at birth (Coyle, 1973, 1977), with limited sensory and motor capabilities (Almli & Fisher, 1977; Bolles & Woods, 1964). Rat pups are blind and deaf during the first two postnatal weeks, and their major sensory abilities are somatosensory and olfactory. However, neonates are sensitive to the consequences of their behavior, and they have shown remarkable capacities to form and retain new associations, and given an operant that is age and task appropriate, they respond differentially. For example, one-day-old rat pups exhibit operant learning by pressing a lever to receive intraoral milk infusions (Johanson & Hall, 1979). Three- day-old rats prefer novel odors paired with milk or suckling (Brake, 1981; Johanson & Teicher, 1980), and they are able to attach on a chemically lavaged female in the presence of an artificial odor, provided the pup had previously been stimulated in the presence of that odor (Pedersen et al., 1982). Seven-day-old pups learn spatial discrimination using suckling as an incentive (Kenny & Blass, 1977).

A feature common to these demonstrations of early

learning is that infants are behaviorally activated. Therefore, when typically inactive 3-day-old rat pups are given milk, they become behaviorally activated, usually making mouthing and licking movements and more general body movements such as stretching, kicking, probing, rolling, and locomotion (Hall, 1979). In addition to milk, these behaviors are elicited by various natural stimuli that include maternal licking (Rosenblatt & Lehrman, 1963), stepping, retrieving (Hofer, 1975) and home cage odor (Bulut & Altman, 1974). Infant rats are behaviorally activated by specific experimental manipulations such as food deprivation, maternal separation, testing at high ambient temperatures, administration of amphetamine (AMP)(Hofer, 1980), L-dopa (Kellogg & Lundborg 1972), by electrical brain stimulation of the medial forebrain bundle (MFB)(Lithgow & Barr,1984; Moran et al., 1981), or by pinching a pup's tail (Szechtman & Hall, 1976). Therefore, electrical stimulation administered to 3-day-old rat pups through electrodes directed at the MFB at the level of the lateral hypothalamus reliably elicit behavioral activation that include mouthing, licking, probing, pawing, gaping, rolling, stretching, lordosis, and locomotion (Moran et al., 1983).

The above natural and experimental stimuli that are

accompanied by a series of ingestive and non-ingestive behavioral activation have been successfully used in learning situations. The key question is then whether the behavioral modification produced in the rat pups was due to a specific property of the reinforcer used or the behavioral activation per se.

The importance of behavioral activation was demonstrated by Pedersen et al. (1982). In 3-day-old albino rats, vigorous stimulation by tactile stimulation such as stroking with a soft artist's brush, or pharmacological stimulation with AMP in the presence of an odor enabled the odor to elicit attachment to a washed nipple. They indicated that behaviorally activating a pup in the presence of an odor may be sufficient to alter the pup's subsequent behavior toward the odor, and presented evidence which suggests that in situations when a reinforcer is not behaviorally activating, pups do not learn. In the absence of stroking, almost 40% of Caesarean-delivered pups died within the first hour after delivery, possibly as a result of nipple attachment failure (Pedersen et al., 1982).

Indeed, it appears that any activating stimulus at this neonatal age could be used in a learning situation. Sullivan and Brake (1981) demonstrated classical

conditioning to an odor when paired with activating stimuli such as milk, stroking, the odor of rat saliva, and even seemingly noxious stimuli such as a strong tail pinch, suggesting that behavioral activation may be a necessary characteristic of the stimulus-response sequence to produce a behavioral modification in rat pups less than 1-week old. The results from intracranial self stimulation (ICSS) studies are consistent with this view by showing behavioral activation in rat pups during ICSS (Moran et al., 1983).

The tight link between behaviorally activating stimuli and rewards is unique to infancy, since the behavioral activation by MFB stimulation (Moran et al., 1983) or by oral infusion of milk (Hall, 1979; Johanson & Hall, 1979) is apparent in young pups less than 10-days-old, but declines by the end of two weeks. For example, Moran et al. (1981) have shown ICSS in 3-day-old rats. In a subsequent study, they demonstrated behavioral activation in 3-day-old pups when electrical stimulation was administered in the MFB. However, the behavioral activation but not the ICSS elicited from the MFB stimulation declined by the end of two weeks of age.

The fact that activating stimuli may provide a basis for neonatal learning critical for survival, and its uniqueness to infancy suggest that behavioral activation

may have important adaptive significance to the neonate. Later in life, as behavioral activation become less important, portions of the nervous system mediating these behaviors may be suppressed or overlaid by later developing portions of the nervous system that are responsible for mediating adult-typical behavior patterns.

Nevertheless, despite the importance of behvioral activation at this age, little is known about the brain substrates or mechanism critical for the mediation of behavioral activation. As indicated earlier, the behavioral activation in infant rat pups was shown when electrical stimulation was administered through electrodes at the MFB. However, the MFB is a complex heterogenous fiber system. The ascending MFB is comprised of cholinergic (Shute & Lewis, 1966), noradrenergic, dopaminergic and serotonergic axons (Ungerstedt, 1971). Therefore, stimulation of the MFB could be very general and perhaps all the neurotransmitter systems which course through the MFB are involved. The purpose of the present experiment was to investigate not only which neurotransmitter systems are critical for the behavioral activation in pups, but also the brain areas critical for this behavioral activation.

Although there has been little systematic investigation of the nervous system substrates of behavioral activation per se, a growing body of evidence from neuroanatomical, pharmacological, behavioral and brain stimulation studies implicate the role of the dopamine (DA) system in behavioral activation and reward. The role of DA system in the mediation of psychomotor activity is well documented. Across a wide range of species, AMP, an indirect DA agonist, elicits a dose-dependent increase in psychomotor activity and a series of repetitive stereotypic behaviors (Randrup & Scheel-Kruger, 1966). In addition, DA (Crow, 1972; Roberts et al, 1975) has been implicated in ICSS behavior. ICSS behaviors following pharmacological intervention with DA antagonists (Mogenson et al, 1979; Phillips et al., 1975; 1976), and studies with direct and indirect DA agonists (Baxter et al., 1972), suggest a coherent picture of DA mediation of ICSS.

A number of studies using infant rats have also implicated the DA system in the mediation of behavioral activation (Sobrian et al., 1975) and reward (Pedersen et al., 1982) and have shown the coincidence of the behavioral activation and reward in young pups. In addition, the ICSS from the NA increase with DA agonists (Lithgow & Barr, 1984).

To characterize the role of the DA systems in the behavioral activation of infant rats, two major DA projection sites, the caudate nucleus (CD) and the nucleus accumbens (NA), were stimulated electrically in the infant rats whose dopaminergic development of these two sites appear to be different from adult rats. The CD and the NA were the sites of interest since they have been implicated in locomotion and other stereotypic behaviors such as probing, head bobbing, mouthing, licking in adult rats. In this work, behavioral activation is operationally defined as an increase in behaviors including mouthing, licking, pawing, and locomotion. This subset of behaviors were chosen, because they are commonly seen in rat pups when they are behaviorally activated either by natural activating stimuli or by electrical stimulation of the MFB.

## Anatomy and Significance of Dopamine System

Since DA was first identified in the central nervous system (Carlsson et al., 1958) a great deal of experimental behavioral work has been done, and it is now thought that the DA system may play an important role in the neural circuitry underlying locomotor activity (Beninger, 1983; Iversen & Koob, 1977; Stinsus et al., 1980), ingestive behavior (Swanson & Mogenson, 1981; Ungerstedt, 1971), and self stimulation (Broekkamp

et al., 1979) as well as in the etiology of schizophrenia (Snyder et al., 1974). The DA pathways have been described in considerable detail (Lindvall & Bjorklund, 1978). Three major ascending DA systems are the nigrostriatal, mesolimbic and mesocortical pathways. The nigrostriatal dopaminergic pathway arises from the substantia nigra and terminates in the CD (Lindvall, 1979; Lindvall & Bjorklund, 1978; Ungerstedt, 1971). DA-containing cells which project to the CD are located mainly within the substantia nigra pars compacta (SNC), and accounts for about 70% of the total brain content of DA. The mesolimbic dopaminergic projections innervate diverse areas of the limbic forebrain including the olfactory tubercle, septum, stria terminalis, amygdala and the NA (De France & Yoshihara, 1975; Ungerstedt, 1971). Their DA cell bodies reside mainly in the ventral tegmental area (VTA), A10 cell group. With the advent of more sensitive histochemical fluorescent techniques, the mesocortical DA pathway was discovered (Berger, 1977). The mesocortical DA projection originates from cell bodies in both the SNC and VTA. These cell groups innervate the entorhinal cortex, perirhinal and piriform cortex, and regions of the frontal cortex (Fuxe et al., 1974; Lindvall et al., 1974). Of the other DA pathways are the

incertohypothalamic and periventricular systems and originate in hypothalamic areas (Bjorklund et al., 1975; Lindvall and Bjorklund, 1978).

Development of the central DA system is far from complete in 3-day-old pups. However, the mesolimbic and mesocortical DA system is relatively mature at 3 days of age. DA receptors in the NA are dense (Murrin et al., 1985), and tyrosine hydroxylase activity in the ventral tegmental area is about 75% of adult levels (Loren et al., 1976; Schmidt et al.,1982). Also, DA terminals in the prefrontal cortex at birth are well developed (Schmidt et al., 1982). This contrasts with less well developed DA projections to the CD (Coyle & Campochiaro, 1976; Loren et al., 1976; Pardo et al., 1977) and cingulate cortex (Loren et al., 1976), as well as the later maturing noradrenergic (NE) system (Kirksey et al., 1978, Loren et al., 1976). DA concentration in the CD is only 15 to 40 percent of adult levels (Schmidt et al., 1982). DA receptors are found at birth in the CD and the NA and increase in density with age (Murrin, 1982; Schmidt et al., 1982). At birth, receptors are found throughout the striatum with the most dense region being in the dorsolateral aspect of the striatum. This dorsolateral density is no longer seen at 21 days (Murrin et al., 1985).

## Dopamine System and Psychomotor Activity

The role of DA system in the mediation of psychomotor activity is well documented (Randrup & Scheel-Kruger, 1966; Rolls & Kelly, 1972). Across a wide range of species, AMP elicits a dose-dependent increase in psychomotor activity and a series of repetitive stereotypic behaviors. Following small doses (less than 1.0mg/kg), for example, AMP increases locomotor acitvity with periodic sniffing and licking in adult rats (Beninger, 1983; Randrup & Scheel-Kruger, 1966). With increasing doses of AMP, locomotor activity is enhanced and the oral behaviors become more repetitive, and the more intense behaviors such as head bobbing are also recruited. In rat pups, hyperactivity induced by maternal deprivation was enhanced by AMP while pretreatment of reserpine, which is known to deplete DA storage, prevented the development of hyperactivity (Hofer, 1980).

It is widely believed that AMP facilitates DA transmission by 1) directly releasing DA from neuronal terminals (Besson et al., 1971), 2) blocking the neuronal reuptake of DA (Fuxe & Ungerstedt, 1970; Glowinski et al.,1966), and 3) inhibiting the intra- and extra-cellular degradative enzyme monoamine oxidase (Glowinski et al., 1966). Although facilitation of both

dopaminergic and NE neurotransmission has been implicated in the pharmacologic actions of AMP, the DA systems are believed to play the predominant role in mediating the behavioral responses of AMP (Cole, 1978). Support for this viewpoint derives from the fact that selective inhibition of NE synthesis is relatively ineffective in blocking AMP-induced psychomotor activities (Randrup & Scheel-kruger, 1966). In addition, destruction of DA-containing neurons by 6-hydroxydopamine (6-OHDA), a potent neurotoxin for catecholamine-containing neurons, prevents the expression of AMP-induced psychomotor activity. In contrast, selective inhibition of the NE system does not antagonize these behaviors (Roberts et al., 1975).

## Dopamine System and Learning

A great deal of evidence that implicates DA in reward effect comes from ICSS studies (Bozarth & Wise, 1980; German & Bowden, 1974; Wise, 1978). While not identical to more natural reinforcers such as food for a hungry animal, there are enough similarities that it has become a valuable tool for studying the reward system. Animals work to receive ICSS just as hungry animals work for food. Effective ICSS sites have been reported for DA cell bodies, fibers, and terminals (Arbuthnott et al., 1971; Clavier & Fibiger, 1977; Crow, 1972; German &

Bowden, 1974; Wise, 1978) including the NA (Lyness et al., 1979; Robertson & Mogenson, 1978; Taylor & Robbins, 1985) and the CD (Phillips et al., 1976).

The evidence for DA (Crow, 1972; Roberts et al, 1975) mediated ICSS behavior has been demonstrated, especially when compared to conflicting reports regarding NE mediation (Arbuthnott et al., 1971; Roberts et al., 1975). The difficulty of demonstating self-stimulation using electrodes placed caudal to the dopaminergic cell-bodies in the ventral NE bundle or its corresponding cell groups is interpreted as support for the DA hypothesis (Clavier et al., 1976; Clavier & Routtenberg, 1976). It is probable that the activation resulting from ICSS of the MFB might be a result of stimulation of the ascending DA fibers, since the strongest self-stimulation is seen with electrode placements along the path of the MFB where there are overlapping projections of the mesocortical and substantia nigra dopaminergic systems (German & Bowden, 1974).

Additional evidence in support of the DA involvement in ICSS comes from studies of cortical placements. Self-stimulation is seen with electrode sites in medial and sulcal frontal cortex (Routtenberg & Sloan, 1972), in entorhinal cortex (Collier & Kutzman,

1977; Routtenberg & Sloan, 1972) and in anterior cingulate cortex where DA terminals have been found (Mercuri et al., 1985).

The DA theory of reward is controversial in that the optimal stimulation frequency for self-stimulation is higher than maximal stimulation frequencies for DA release, refractory periods of self-stimulation fibers are shorter than for DA neurons, conduction velocities differ, and the actual site of stimulation need not be dopaminergic (Clavier et al., 1976; Corbett et al., 1977; Gallistel et al., 1969). However, strong indications that the reward system is dependent on the DA system comes from pharmacological studies. ICSS behaviors following pharmacological intervention with DA antagonists (Mogenson et al, 1979; Phillips et al., 1975; 1976), and studies with direct and indirect DA agonists (Baxter et al., 1972), suggest a coherent picture of DA mediation of ICSS. AMP and methamphetamine facilitate ICSS (Broekkamp et al., 1975; Bulut & Altman, 1974; Chiueh & Moore, 1974; German & Bowden, 1974; Pickens & Harris, 1968; Spyraki et al., 1982; Wise, 1978) by increasing response rates or decreasing the stimulation threshold (Kofman et al., 1985; Stein & Ray, 1959). The enhancement of ICSS has been attributed to selective action of AMP on the DA

pathways that mediate the rewarding effects of ICSS.

Several observations suggest that DA antagonists can block the rewarding effects of stimulation selectively at doses lower than those required to produce motor impairment. The pattern of response change seen in tests after pretreatment with DA antagonists suggests that these agents specifically block neural systems critical for the rewarding property of stimulation (Stellar et al., 1983; Wauquier & Niemegeers, 1972) even though some decreases in general activity are seen with DA antagonists.

A number of studies using infant rats have also implicated the DA system in the mediation of behavioral activation (Sobrian et al., 1975) and reward (Pedersen et al., 1982) and also have shown the coincidence of behavioral activation and reward in young pups. For example, Moran et al. (1981) have shown ICSS in 3-day-old rats. However, the behavioral activation but not the ICSS elicited from the MFB stimulation declined by the end of two weeks of age (Moran et al., 1983). As development progresses, these immature behavioral activations from rewarding stimuli may be replaced by more organized goal directed responses such as bar pressing.

Lithgow and Barr (1984) reported positive ICSS

sites including NA in 7- and 10-day-old rats. Additional positive sites in 7-day olds were congruent with adult data and included the medial amygdaloid nucleus and the nucleus of the stria terminalis. These pups also exhibited considerable behavioral activation during ICSS. In their subsequent study, an increase in self-stimulation was demonstrated by administering various doses of AMP (1.0, and 5.0 mg/kg) and cocaine (10.0, and 30.0mg/kg), a DA re-uptake inhibitor, in 3- and 10-day-old rats (Barr & Lithgow, 1986). In addition, AMP effectively induced odor conditioning of suckling behavior in infant rats (Pedersen et al., 1982). Caffeine, a stimulant that does not affect the DA system directly, was not effective in this experiment.

The nigro-striatal and mesolimbic DA pathways have been implicated for different components of DA induced behaviors (Cole, 1978). It has been suggested that the NA modulates the animal's general tendency to move, whereas the CD is involved in motor stereotypies. Bilateral 6-OHDA lesions of the CD reduce all or most components of AMP-induced stereotypy, leaving locomotion unaltered (Costall et al., 1975; Costall & Naylor, 1976, 1977; Fray et al., 1980; Jackson et al., 1975; Pijnenberg et al., 1973, 1975, 1976), whereas direct

infusion of AMP into the CD elicit stereotypic behaviors (Cools & Van Rossum, 1976). Conversely, 6-OHDA lesions of the NA blocked the heightened locomotor response produced by AMP, while stereotypy remained constant (Kelly & Iversen, 1976). Direct application of AMP into this site produces locomotion but not stereotypy (Jackson et al., 1975). At this time the major functional difference between the CD and the NA revealed by behavioral and pharmacological studies in the rat remains without clearly defined anatomical counterparts (Fallon et al., 1978; Simon et al.,1979).

## PART II: ELECTRICAL STIMULATION STUDY

### Method

#### Subjects

Twenty-seven 3-day-old Sprague-Dawley rats, born and reared in the Indiana University colony, weighing from eight to ten grams, were used as subjects. One group of pups had electrodes implanted in the NA (N=12), while the other group had electrodes implanted in the CD (N=15). Pups were used only once. Litters containing less than eight pups were not used. The day of birth was designated as postnatal day 0. Litters resided with their own dam in polypropylene maternity cages (48x20x26Cm) on a substrate of pine shavings. The colony was maintained on a 16:8 hour light/dark cycle,

with Purina Rat Chow and water available ad lib.

Surgery

Electrode units were constructed prior to the time of implantation. Two teflon-coated stainless steel wires (0.1 mm, A-M systems, Inc.) bared at the tip were used for the electrode. The bipolar electrodes were soldered into individual gold-plated connectors that were mounted in a plastic frame. A Kopf stereotaxic device modified for neonatal surgery (Heller et al., 1979) by adding a neonatal head holder was used as a surgical stage. Pups were isolated from their mothers and were anesthesized by methoxyflurane (Pitman-Moore, Inc.) inhalation before stereotaxic surgery. Following a midsaggital scalp incision to expose the calvarium, the animal was inserted in the head holder, first being positioned so that the mouth bar is at the angle of the jaw. The needle (1.5mm) was inserted in the foramen magnum. The foramen needle stem was then secured to the face plate by means of a binding screw to adjust the frontal zero plane passing through the bregma, and the midline plane passing through the sagittal sinus. Following the incision, the hole was drilled in the skull but not the dura overlying the right CD (2.0mm anterior, 2.5mm lateral to bregma and 3.5mm ventral from dura) or the right NA (1.8mm anterior, 1.2mm lateral to

bregma and 4.0mm ventral to dura). Coordinates were modified from the Sherwood and Timiras' atlas (1970). The electrode was lowered and was cemented (Caulk Grip cement and standard dental acrylic) to the skull after the dura was gently incised. From all subjects used, 55% of the electrode tips were located either in the NA or the CD. The pups were kept warm (32 $^{o}$ C) and humid in a incubator after surgery.

Testing Procedure

Pups were stimulatd 16 to 20 hrs post-operatively on a felt-covered surface in a 32 $^{o}$ C environment. Temperature was maintained at 32 $^{o}$ C by a heat lamp. The humidity was not controlled. Immediately prior to testing, all pups were voided, weighed, and injected intraperitoneally (IP) with 0.9% saline solution and numbered on their back with a marking pen for easy identification of each animal. Pups were placed individually in a felt-covered container and were allowed to recover from injection handling for 20 minutes.

Following 5 min adaptation and a 2-min baseline observation, each behavior was recorded over 6-one minute intervals during which biphasic square wave stimulation (500msec train of 50 pulses with a pulse width of 2 msec) was delivered every 10 sec during every

other minute. A biphasic constant current stimulator built in this lab from a modification of Schaefer et al.'s circuit design (1981) was used to administer a 500 msec train of biphasic square wave stimulation. Current administered was 60 uA, although inactive sites were tested up to 1000 uA. The stimulation parameters, such as pulse frequency, train duration, stimulus intensity, and the stimulation schedule used in this experiment were the same stimulation found effective for self-stimulation in neonatal rats (Moran et al., 1981). The frequency of each behavior of the pup was recorded during each minute. The recorded behavioral categories were: mouthing, repeated opening and closing of the mouth or other rapid oral movements that do not include licking; licking, movement of the tongue outside the mouth; pawing, wiping the forepaw along the head or snout; locomotion, movement of either the forepaws or hindpaws. At the end of the stimulation session, IP injection of AMP (2.5mg/kg) was given to each pup to insure the capability of behavioral activation.

Following testing, pups were overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline and 10% formalin. Frozen brain sections (60 um) were stained in cresyl violet for Nissl substance and electrode placements were verified independent of the

behavioral data. The results from recorded frequency of each behavior during pre- and post-stimulation was compared using a two way analysis of variance (ANOVA) with repeated measures to assess the main effects of stimulation and stimulation sites as well as their interactions and behavioral trend from repeated stimulations. The F-test for simple effect was followed to run individual comparisons where indicated. Also the Pearson product-moment correlation was applied to determine the relationship between recorded behaviors.

## Results

Following 500 msec stimulation trains to the NA, pups displayed reliable behavioral activation, while stimulation of the CD and other areas such as the diagonal band of Broca or the lateral septum did not produce behavioral activation. When not stimulated, baseline mouthing, licking, pawing and locomotion behaviors of the tested pups were not significantly different between groups, and the pups were typically inactive. Intra-accumbens stimulation (N=12) produced a dramatic and reliable behavioral activation while intra-caudate stimulation (N=15) produced weak and variable results. While electrical stimulation frequently caused stretch responses as well as rolling and ear wiggling,

the most predominant behaviors were mouthing, licking, pawing and locomotion when the stimulating electrodes were in the NA. The behavioral activation elicited by electrical stimulation of the NA appeared to be different from the enhanced locomotion resulting from stimulation of the CD, and included appetitive behaviors, such as mouthing, pawing, and licking. These behaviors were elicited reliably and all behaviors appeared in virtually all of the pups tested whose electrodes were in the NA. The behaviors occurred immediately after stimulation initiation and ended with the end of stimulation. Occasionally, the onset of elicited behavior was delayed and the elicited behavioral responses often persisted well after stimulation ended. In addition, the behavioral responses were noted during off-stimulation minutes. Behavioral data of pups in which the electrode placment was not in the NA or the CD were not included in the results.

Mouthing Mixed two-way ANOVA revealed main effects of both stimulation, $F(3,75)=8.69$, $p < .001$, and group effects, $F(1,25)= 22.75$, $p < .001$, with a significant interaction effect, $F(3,75)=4.84$, $p < .005$, between stimulation and group variables. The NA and the CD were significantly different in stimulation-induced mouthing.

From Figure 1, it is clear that the NA group was highly activated by electrical stimulation by the marked increase in mouthing. Mouthing behavior occurred much more frequently upon stimulation of the NA compared to the CD. In response to stimulation, young pups exhibited mouthing behavior which occurred immediately after the onset of stimulation and decayed rapidly when the stimulation ended. Mouthing was elicited reliably and appeared in virtually all of the pups tested whose electrode was in the NA. However, stimulation of the CD did not induce mouthing. The CD group was virtually identical to their baseline behavior in mouthing following stimulation. F-test for simple effects was followed to determine the trend of 3 stimulation trials. The analyses revealed significant increase of mouthing behavior when each stimulation minute of mouthing behavior was compared to baseline mouthing behavior, F values between 3.66 and 5.33, p between $<.05$ and $<.01$. There was no significant differnce among stimulation trials. Histological data on the NA and the CD are presented in Figure 2. The sequence of brain sites runs from rostral to caudal. The numbers shown in this figure represent the number of mouthing behaviors which occurred during 3 stimulation minutes at the particular electrode placement. Each placement represents a single

Figure 1. Mean ±SEM of the number of occurrences of mouthing behavior in response to three stimulation trials in the nucleus accumbens and the caudate nucleus.

# Mouthing

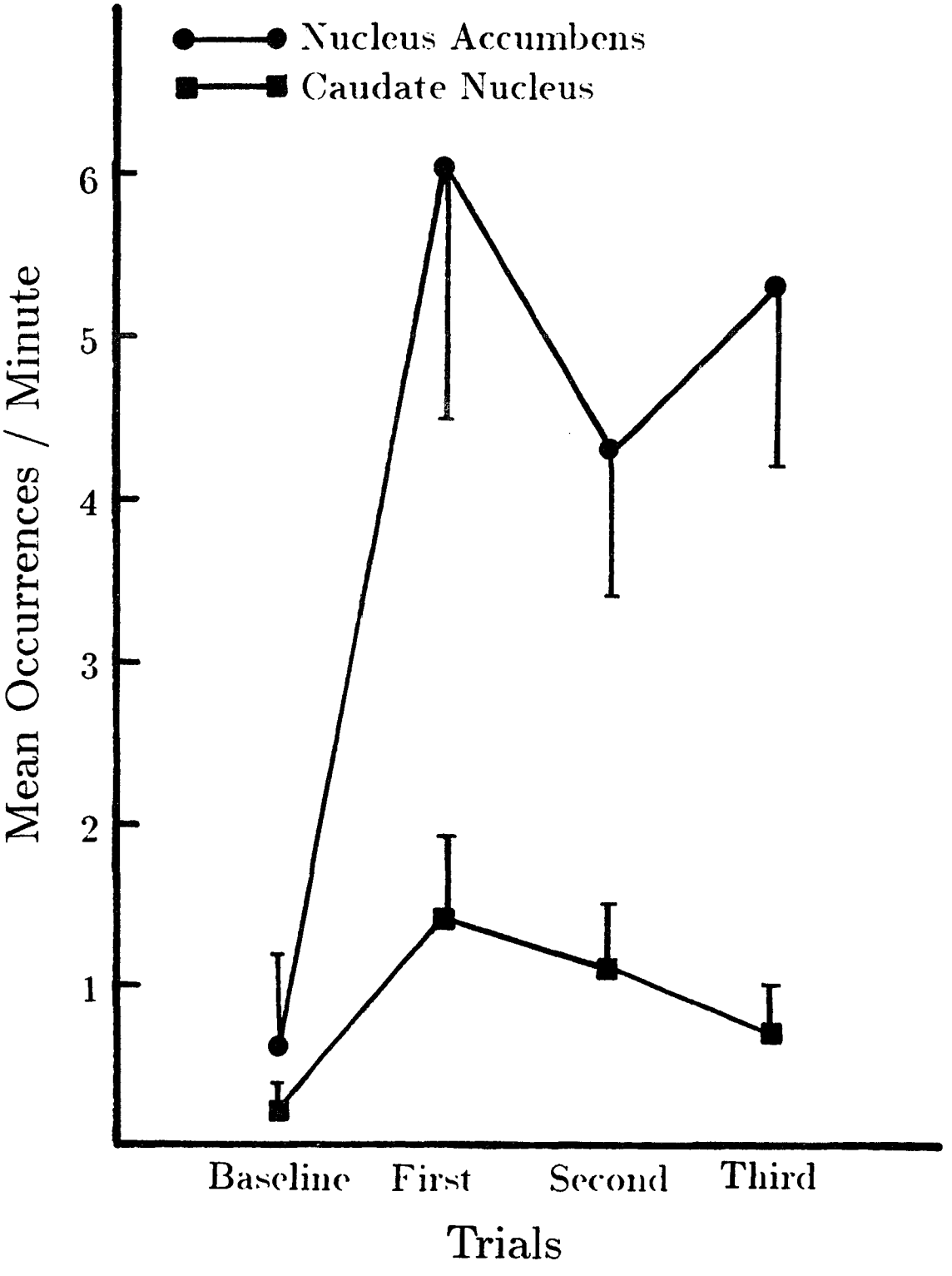
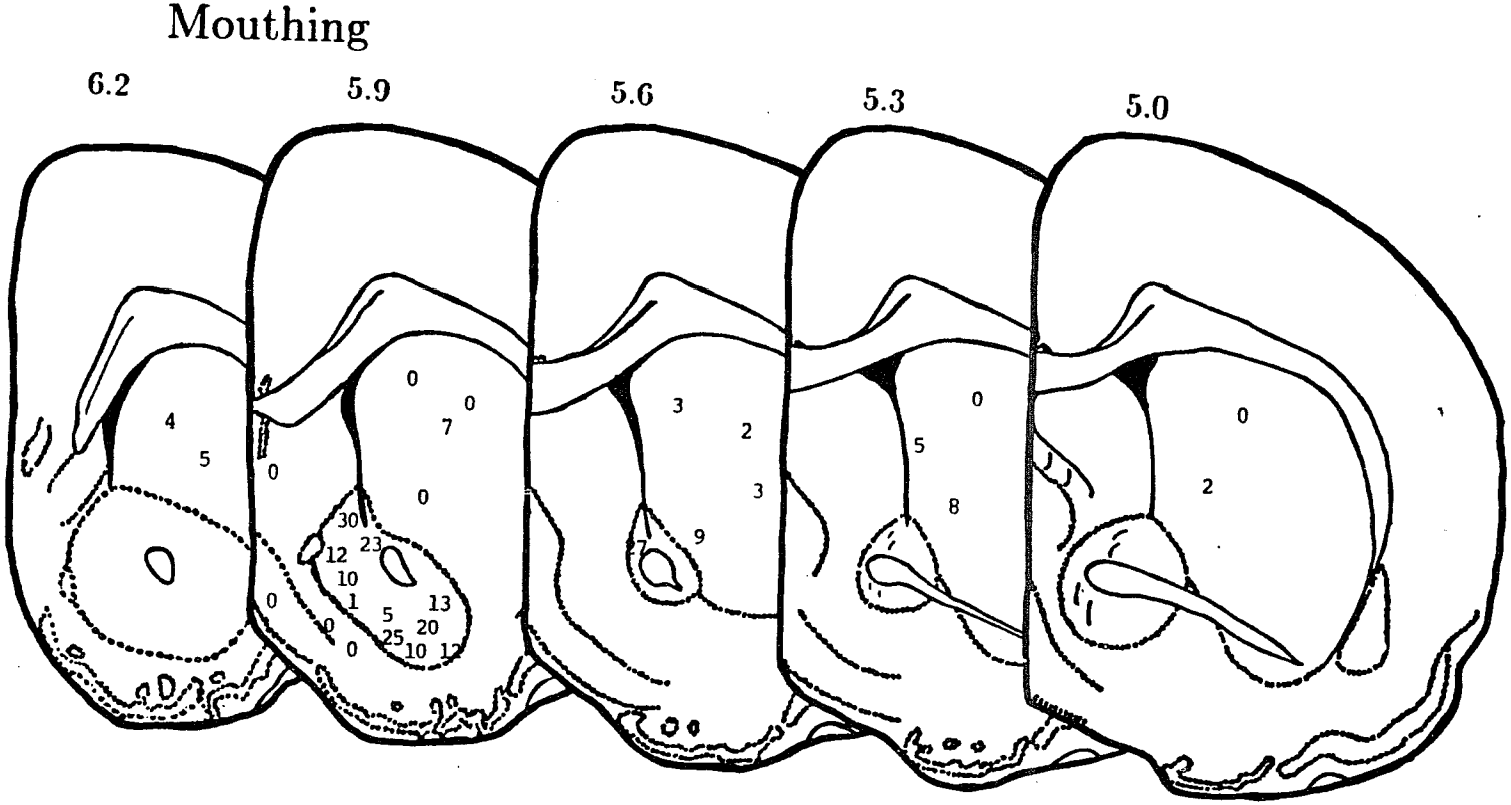

Nucleus Accumbens
Caudate Nucleus
Mean Occurrences / Minute
6
5
4
3
2
1
Baseline
First
Second
Third
Trials

Figure 2. Histological analysis of stimulation effects on mouthing behavior in three day olds. The numbers at the top of the sections correspond to the anterior posterior co-ordinates from the Sherwood and Timiras atlas for 10 day olds. The numbers in the sections represent the number of mouthing behavior occurred during three stimulation minutes at the particular electrode placement.


Mouthing
6.2
5.9
5.6
5.3
5.0
4
5
0
0
0
7
0
0
30
12
23
10
1
13
5
20
25
10
12
0
0
0
0
3
2
3
7
9
0
5
8
0
2

animal. The maximum number of mouthing behaviors observed in response to stimulation of the CD was 8-9 occurences. In comparison, the maximum number of mouthing behavior seen in the pups stimulated in the NA was around 30 times. The average number of mouthing behavior in the CD and the NA were 4 and 16, respectively.

Pawing The same pattern of result was found in pawing behavior. Mixed two-way ANOVA revealed significant main effects of both stimulation, $F(3, 75)=4.42$, $p < .01$, and group effects, $F(1, 25)= 5.55$, $p < .05$, without a significant interaction effect. As was the case in the mouthing behavior, there was a significant group difference. Pawing also was elicited reliably and appeared in virtually all of the pups tested whose electrodes were in the NA but not in the CD. As can be seen in Figure 3, pawing behavior occurred much more frequently upon stimulation of the NA compared to the CD. The F-test for simple effects indicated a strong stimulation effect for the NA, and a significant increase of pawing when each stimulation trial was compared to baseline behavior, F between 3.42 and 5.84, p between $<.05$ and $< .01$. There was no significant difference among stimulation trials. Histological data on the NA and the CD are presented in Figure 4. The

Figure 3. Mean ± SEM of the number of occurrences of pawing behavior in response to three stimulation trials in the nucleus accumbens and the caudate nucleus.

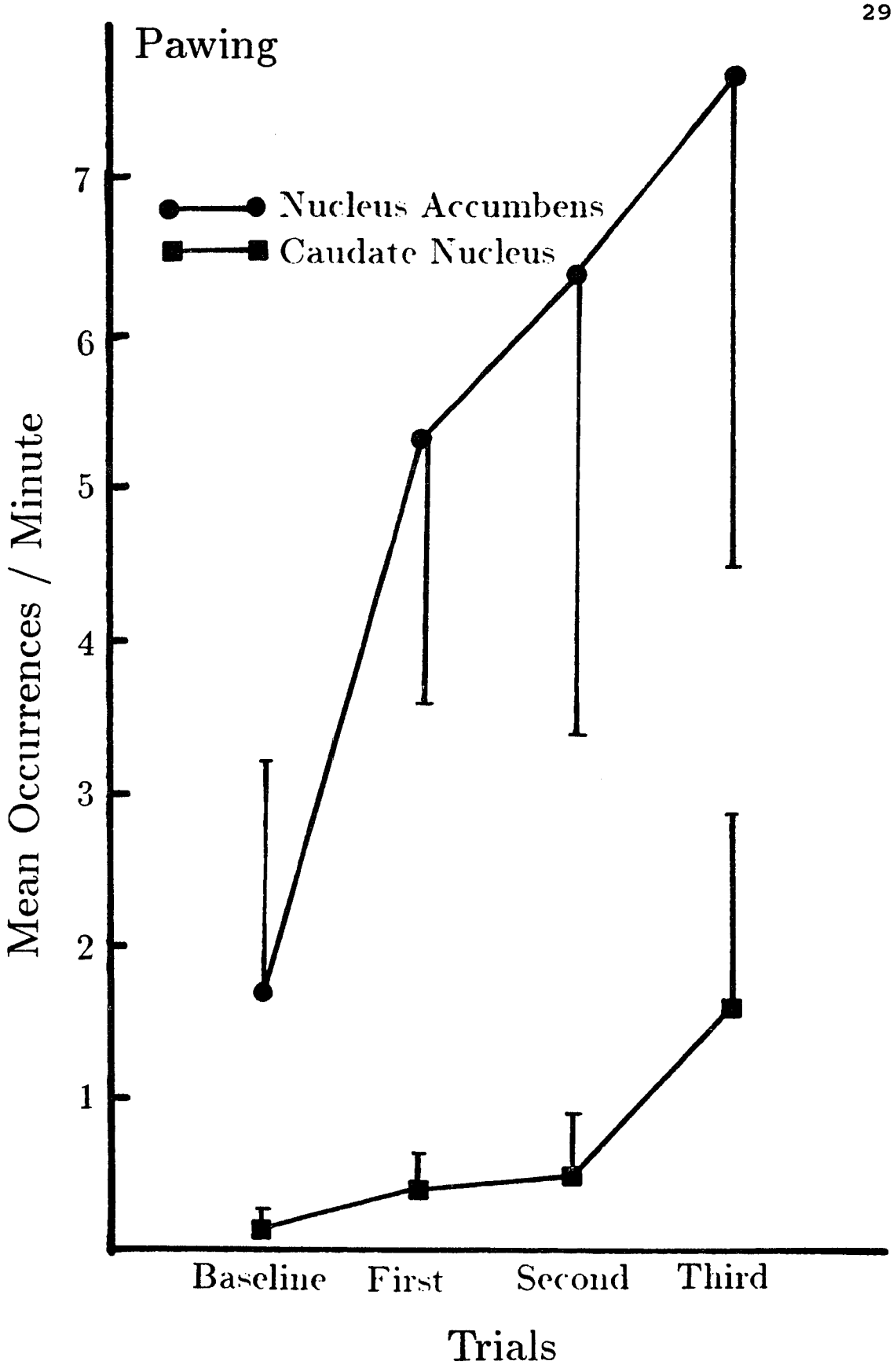
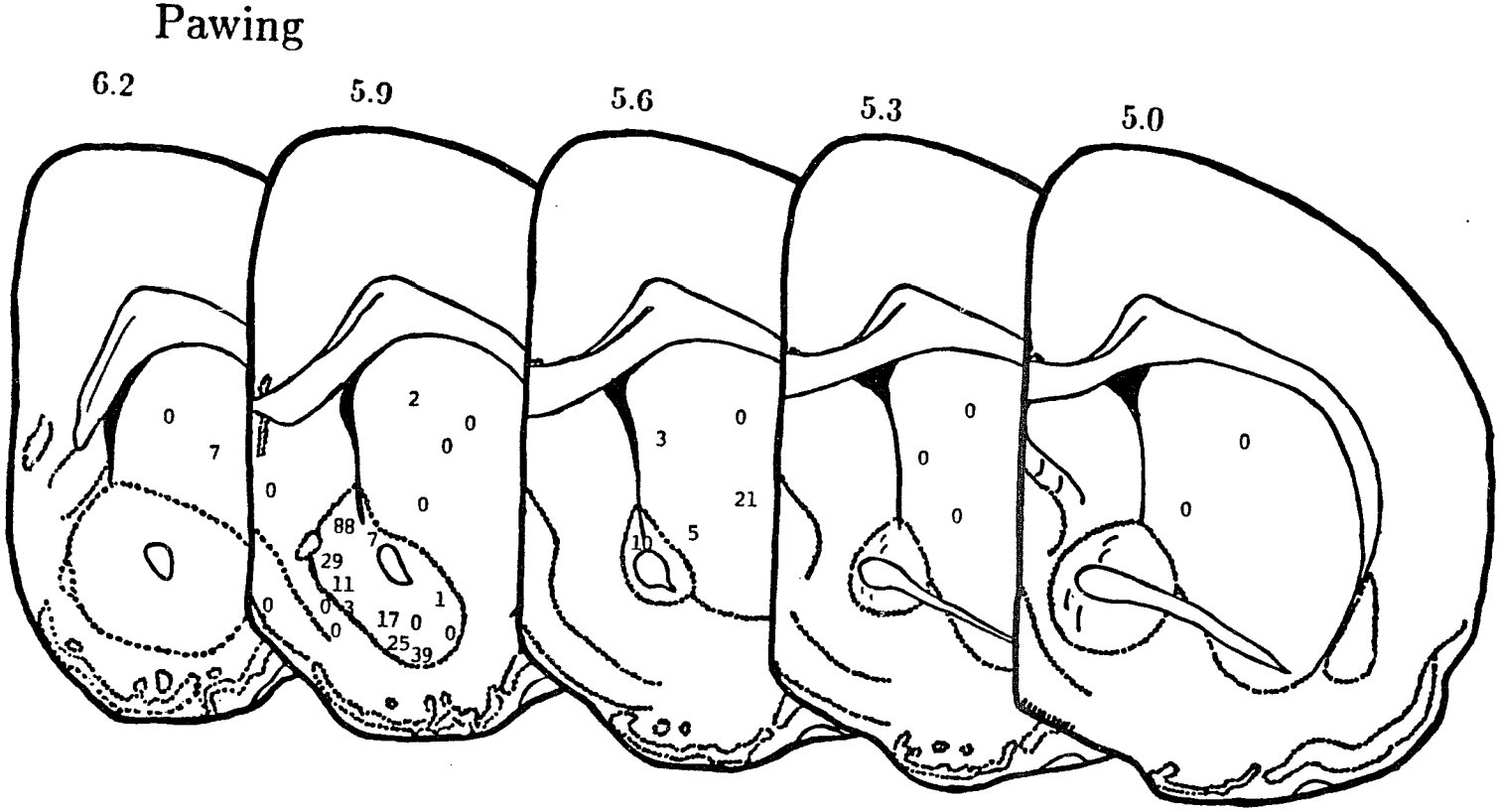

Pawing
Nucleus Accumbens
Caudate Nucleus
Mean Occurrences / Minute
7
6
5
4
3
2
1
Baseline
First
Second
Third
Trials

Figure 4. Histological analysis of stimulation effects on pawing behavior in three day olds. The numbers at the top of the sections correspond to the anterior posterior co-ordinates from the Sherwood and Timiras atlas for 10 day olds. The numbers in the sections represent the number of pawing behavior occurred during 3 stimulation minutes at the particular electrode placement.


Pawing
6.2
5.9
5.6
5.3
5.0
0
7
0
88
7
29
11
0
3
0
17
0
25
39
0
1
0
2
0
0
0
3
0
21
10
5
0
0
0
0
0

number of pawing behaviors elicited by the respective electrode placements was plotted in the same pups. Again the numbers shown in this graph represent the number of pawing behaviors that occurred during 3 stimulation minutes at the particular electrode placement. The maximum number of the pawing behavior observed in response to stimulation was 21 and 88 for the CD and the NA, respectively. Average number of pawing behaviors during three stimulation minutes in the CD and the NA were 2 and 14, respectively.

Licking Most of the licking behavior was preceded or accompanied by mouthing, and appeared to represent a heightened level of mouthing. Licking showed no stimulation or interaction effect, but did show a slight group effect, $F(1, 75)=3.3$, $p < .10$. There was a stimulation effect in the NA group, $F(1, 3)=2.08$, $p < .01$, but not in the CD group. Figure 5 shows the licking behavior occurrences before and after stimulations in both groups. Although the stimulation effect on licking behavior was not as robust as mouthing or pawing, there was an increasing trend in the NA group. In Figure 6, numbers in the nuclei represent the number of licking behaviors elicited by the respective electrode placements. The maximum number of the behaviors observed in response to stimulation was 12 and

Figure 5. Mean ± SEM of the number of occurrences of licking behavior in response to three stimulation trials in the nucleus accumbens and the caudate nucleus.

# Licking

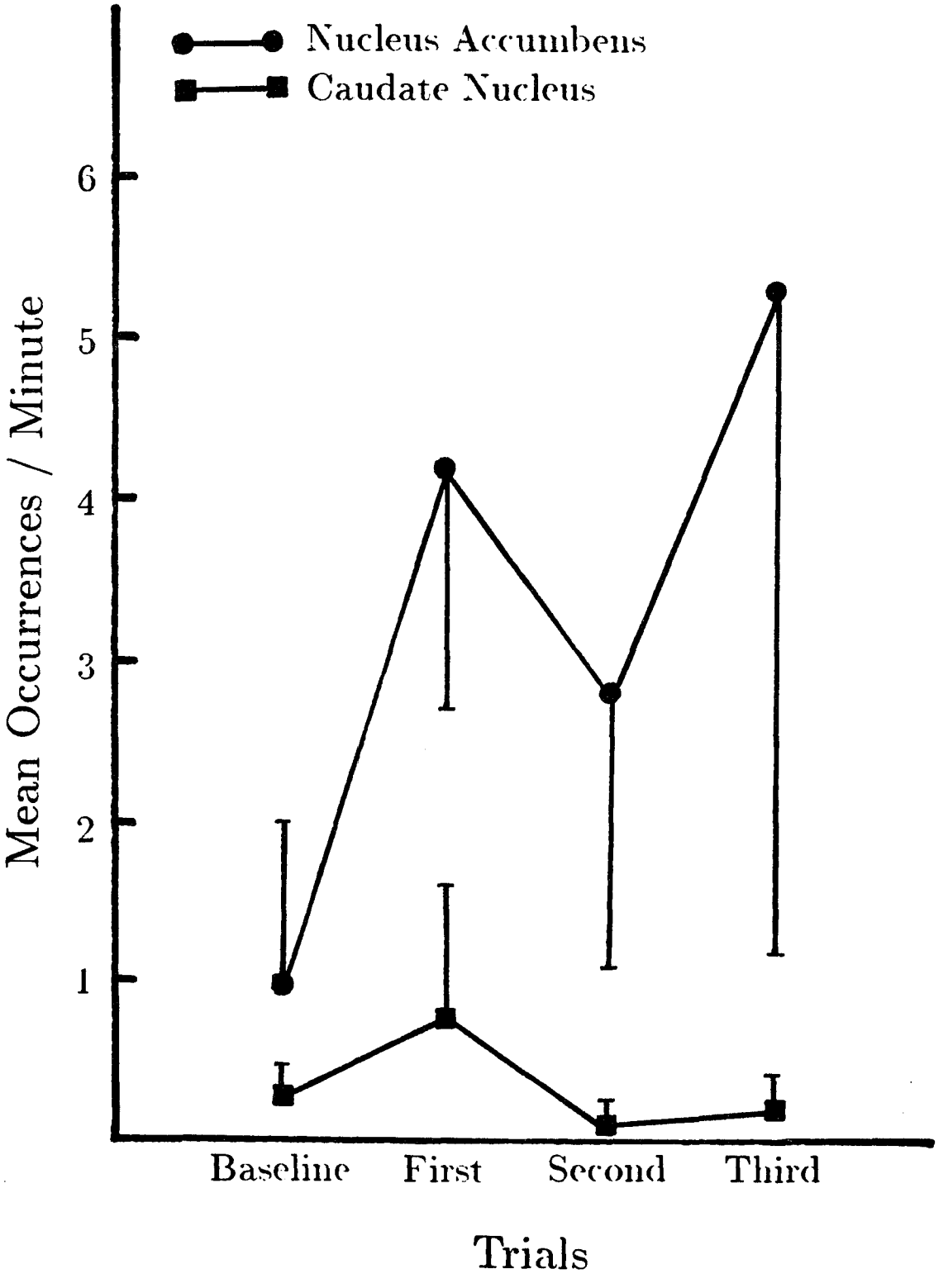
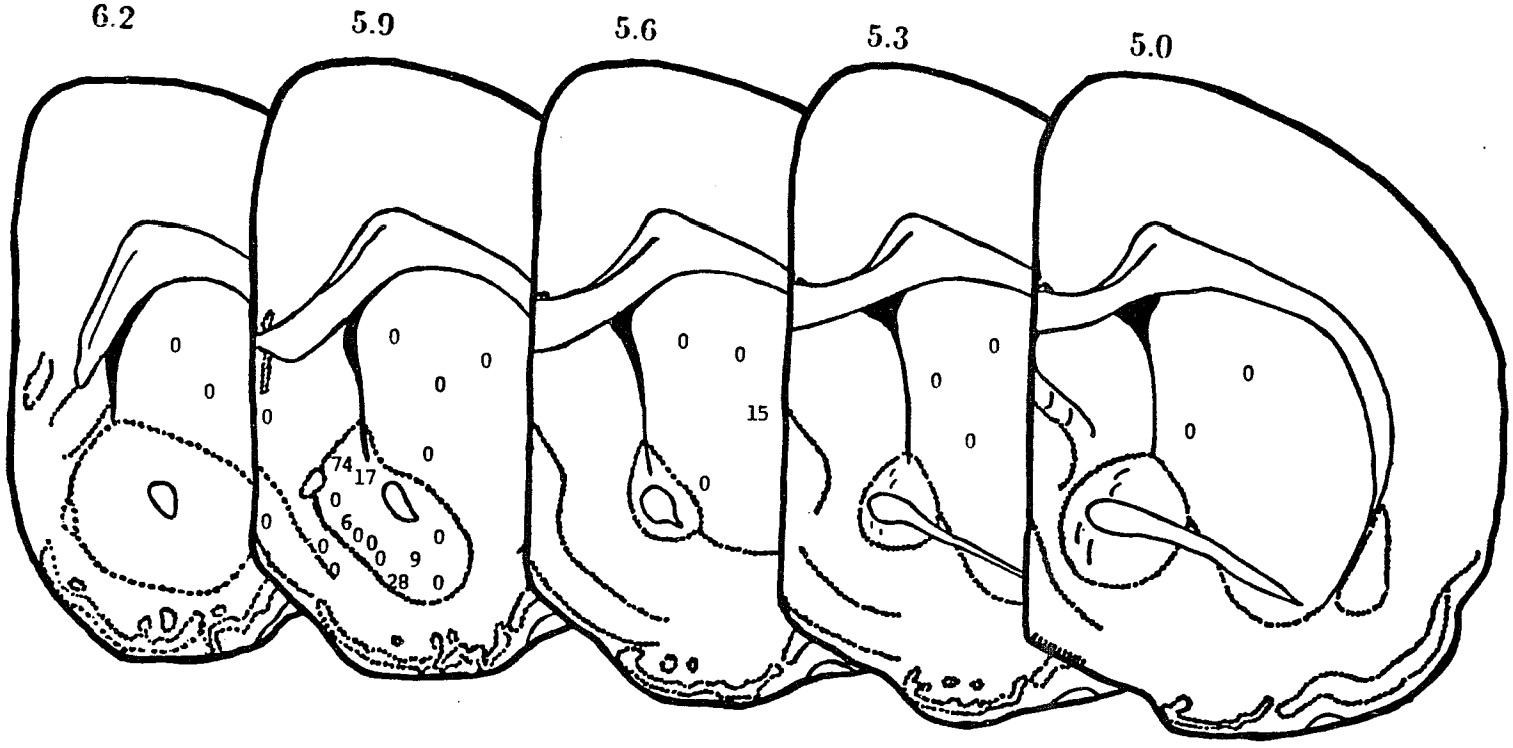

Nucleus Accumbens
Caudate Nucleus
Mean Occurrences / Minute
6
5
4
3
2
1
Baseline
First
Second
Third
Trials

Figure 6. Histological analysis of stimulation effects on licking behavior. The numbers at the top of the sections correspond to the anterior posterior co-ordinates from the Sherwood and Timiras atlas for 10 day olds. The numbers in the sections represent the number of licking behavior occurred during 3 stimulation minutes at the particular electrode placement.

Licking

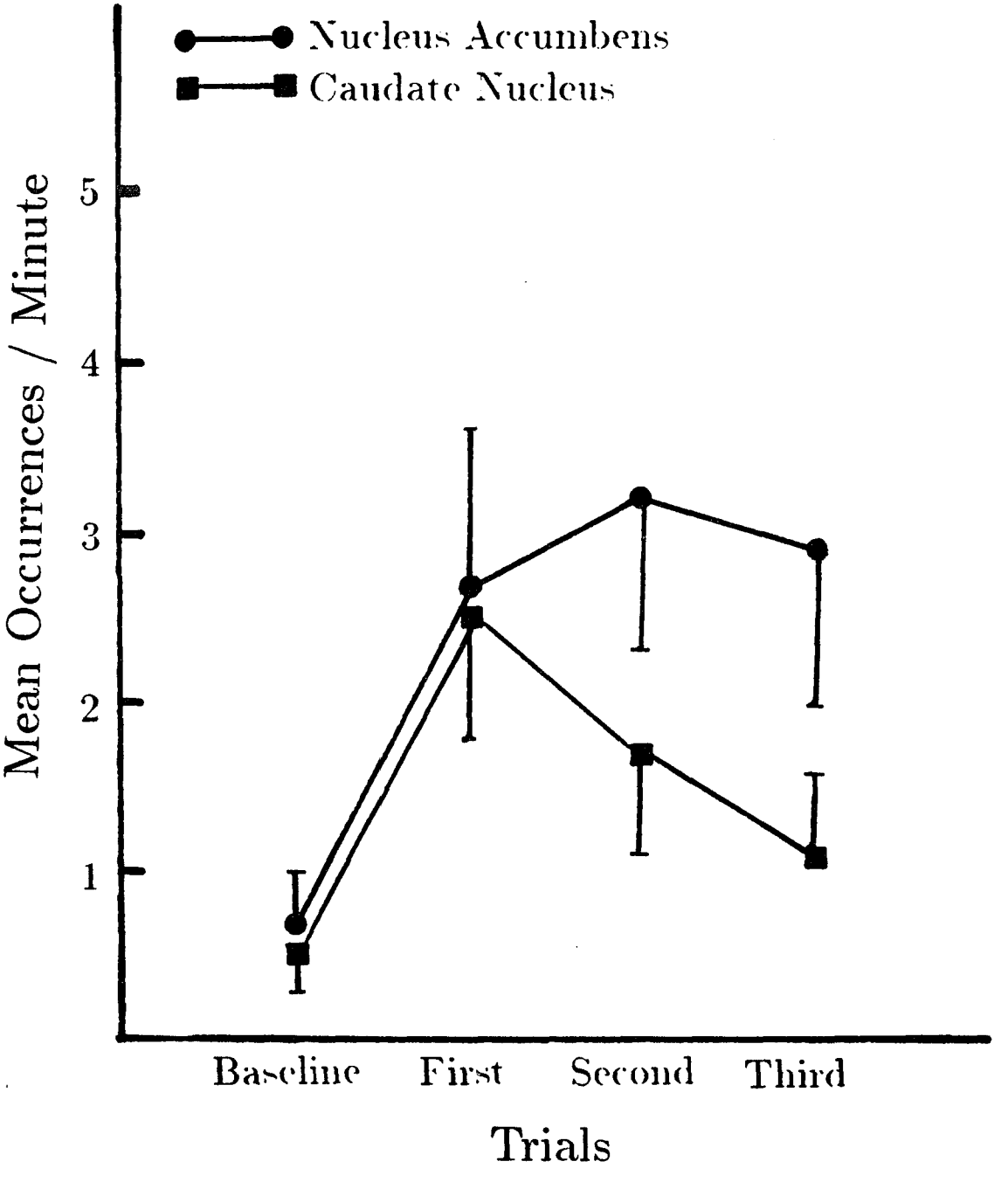


74 for the CD and the NA, respectively. One of the pups whose electrode was implanted in the NA had high licking behavior before stimulation, possibly due to a piece of pine shaving stuck on the pup's tongue. When stimulated, the licking behavior increased in this same pup. The average number of licking behaviors during three stimulation minutes in the CD and the NA were 1 and 9 respectively.

Locomotion In contrast to the previous behaviors, locomotion showed stimulation effects, $F(3, 75)=7.37$, $p < .001$, without group or interaction effects. Locomotion was seen following stimulation of both the NA and the CD. Locomotor behaviors between the NA and CD groups were not significantly different. In Figure 7, the effect of stimulation on locomotor behavior is plotted. As can be seen in this figure, locomotor behavior increased upon stimulation of the NA and the CD. The F-test for simple effects indicated a strong stimulation effect for the NA, $F(1, 3)=5.5$, $p < .005$, and a significant increase of locomotion when each stimulation trial was compared to baseline behavior, F between 1.92 and 2.42, p between $< .05$ and $< .01$. There was no significant difference among stimulation trials. The stimulation effect in the CD was also significant, $F(1, 3)=3.74$, $p < .05$. The histology data

Figure 7. Mean ± SEM of the number of occurrences of locomotor behavior in response to three stimulation trials in the nucleus accumbens and the caudate nucleus.

# Locomotion


Nucleus Accumbens
Caudate Nucleus
Mean Occurrences / Minute
5
4
3
2
1
Baseline
First
Second
Third
Trials

for locomotion is shown in Figure 8. The maximum number of locomotor behaviors observed in response to stimulation was 18 and 28 for the CD and the NA, respectively. The average number of locomotor behaviors during the three stimulation minutes in the CD and the NA were 4 and 7, respectively.

A summary of the behavioral results is shown in Figure 9. With regard to mouthing, pawing and licking, the difference in activation was significant between the two nuclei. However, elicited locomotion was not different between the two sites. All pups were activated by an AMP injection at the end of the experiment demonstrating their capacity to behaviorally activate. Figure 10 shows the mean number of mouthing, licking, pawing, and locomotor behaviors combined during three stimulation minutes in the NA and the CD. As can be seen in this summary graph, electrical stimulation of the NA caused a dramatic and reliable behavioral activation while stimulation of the CD produced variable results.

The Pearson product-moment correlation revealed a substantial relation between mouthing, pawing, and licking without significant correlation to locomotion. The correlation coefficients are listed in Table 1.

Figure 8. Histological analysis of stimulation effects on locomotor behavior in three day olds. The numbers at the top of the sections correcpond to the anterior posterior co-ordinates from the Sherwood and Timiras atlas for 10 day olds. The numbers in the sections represent the number of locomotor behavior occurred during 3 stimulation minutes at the particular electrode placement.

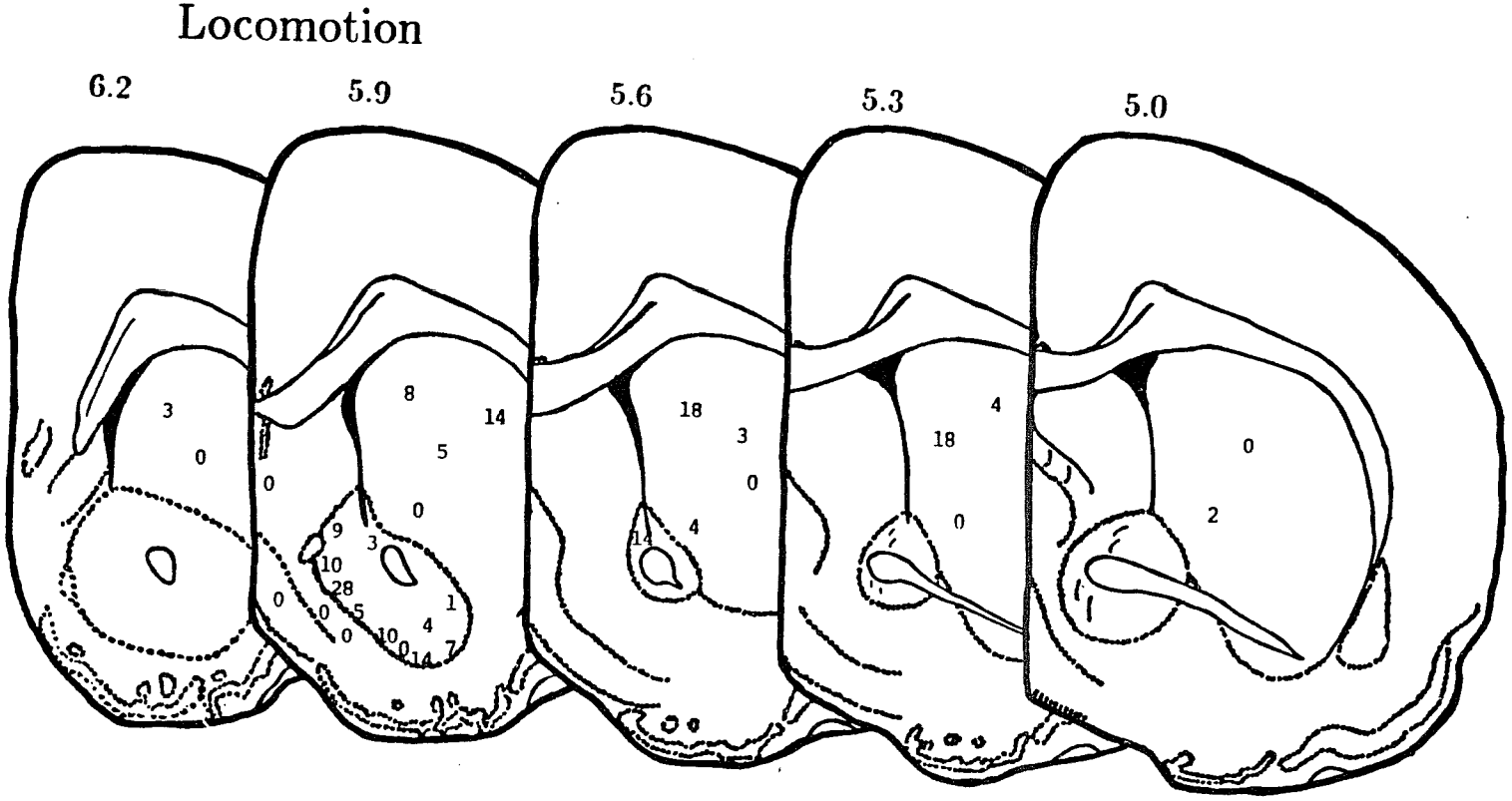

Locomotion
6.2
5.9
5.6
5.3
5.0
3
0
0
8
14
5
0
9
3
10
28
0
0
5
1
0
10
4
0
14
7
18
3
0
4
14
4
18
0
0
2

Figure 9. Mean ± SEM of the number of occurrences of each behavior in response to stimulation of the nucleus accumbens and the caudate nucleus.

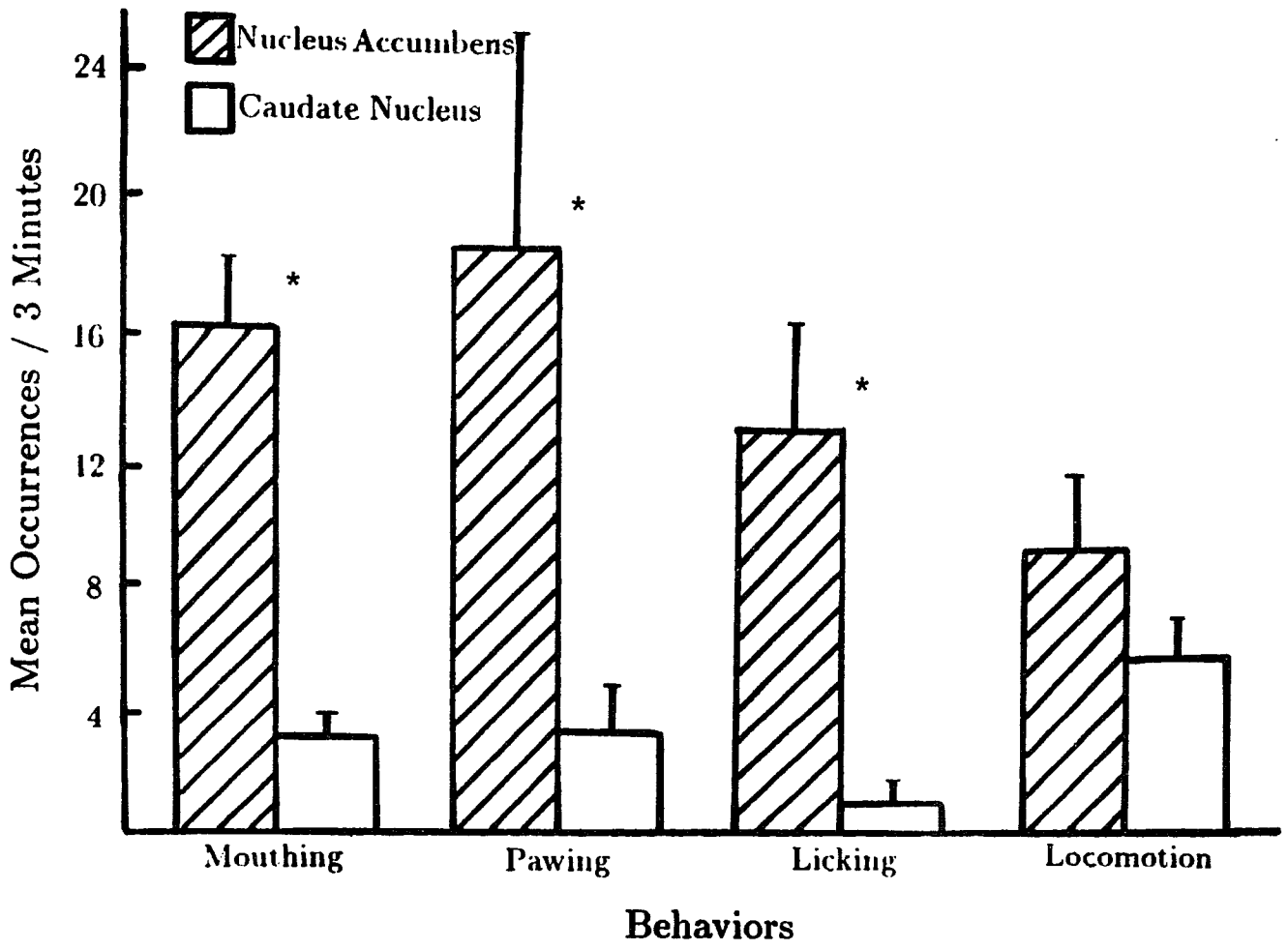

Nucleus Accumbens
Caudate Nucleus
Mean Occurrences / 3 Minutes
24
20
16
12
8
4
*
*
*
Mouthing
Pawing
Licking
Locomotion
Behaviors

Figure 10. Mean ± SEM of the number of occurrences of all four active behaviors in response to stimulation in the nucleus accumbens and the caudate nucleus.

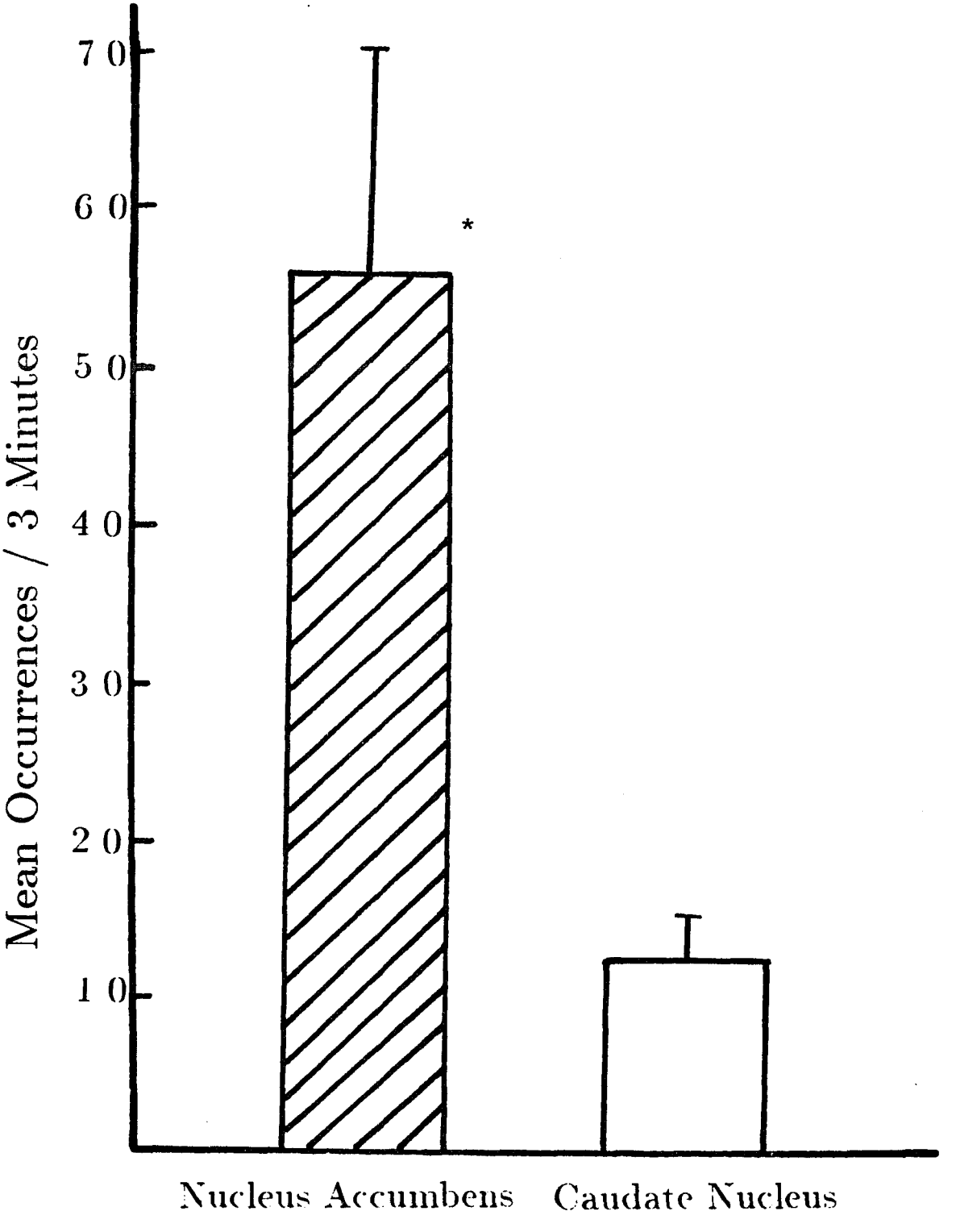

Mean Occurrences / 3 Minutes
7 0
6 0
5 0
4 0
3 0
2 0
1 0
*
Nucleus Accumbens
Caudate Nucleus

Table 1.

| | Mouthing | Licking | Pawing | Locomotion |
|---|---|---|---|---|
| Mouthing | | 0.71* | 0.51 | -0.12 |
| Licking | | | 0.91** | 0.01 |
| Pawing | | | | 0.11 |

Note * $p < 0.01$

** $p < 0.001$

## Discussion

Following electrical stimulation to the NA, pups displayed reliable behavioral activation, while stimulation of the CD and other areas such as the diagonal band of Broca or the lateral septum did not produce behavioral activation. Electrical stimulation of the NA caused a dramatic and reliable change in the behaviors of 3-day-old pups. While electrical stimulation frequently caused stretch responses as well as rolling and ear wiggling, the most predominant behaviors were mouthing, licking, pawing and locomotion when the stimulating electrodes were in the NA. The behavioral activation elicited by stimulation of the NA was similar to the behaviors seen in response to some other forms of conventional rewarding stimuli, such as milk, by exihibiting high levels of oral activity as well as locomotion.

The elicited behavioral activation was different

from behaviors seen in response to some other forms of activating stimulation in the pup, and was initiated and maintained in the absence of any relevant goal objects. Also, the elicited behavioral responses in the pups were different from adult stimulation data. In adults, the behavioral responses are relatively concurrent with stimulation and environmental cues and a training period plays a major role in the elicited behaviors (Valenstein et al., 1970; Valenstein et al., 1968). The elicited behavioral activation in the infant rats were less stimulation bound, and frequently was exhibited during non-stimulation minutes. Occasionally, the onset of elicited behavior was delayed, and elicited behaviors persisted well after stimulation has ended.

In addition, the elicited behaviors were different from adult CD and NA stimulation data. Stimulation of the NA and the CD induce locomotion and repetitive stereotypic movements respectively in adults. The CD groups in rat pups were virtually identical to their baseline behavior in mouthing, licking, and pawing following stimulation. The apparent lack of behavioral activation from the CD stimulation might be from the immature nigro-striatal DA system at this age. Also, the different behavioral results from the stimulation of these sites might result from an immature

neurotransmitter system at this age. This elicited behavioral activation in infant rats may become more coherent and controlled with age as with other behaviors such as feeding behavior or MFB elicited behaviors (Hall, 1979; Moran et al., 1983).

The correlation results revealed close relationships among mouthing, licking and pawing behaviors. Locomotion showed no correlation with any of these behaviors. The mouthing, licking, and pawing behaviors were specifically elicited by stimulation of the NA while locomotion was not specific to this site. This might suggest that mouthing, licking and pawing behaviors are possibly from the same neurotransmitter system in the NA, while locomotion might be mediated by a different neurotransmitter system which is present in the NA.

It is unlikely that these animals were not capable of behavioral activation, because the injection of AMP (2.5mg/kg) at the end of each experiment induced reliable behavioral activation in every pup. Elicited behavioral activations can not be explained on the basis of temperature or other peripheral effects alone, since rat pups responded differentially depending on stimulation sites.

The results from this experiment are consistent

with the hypothesis that the behavioral activation following electrical stimulation of the NA in rat pups depends upon the early maturing mesolimbic DA system. The results, in fact, are quite similar to those found by Stellar and coworkers (1985) showing the behavioral activation during learning trials while chronic guide cannula were implanted in the NA. If the DA system is involved in behavioral activation, DA antagonists should reduce elicited behavioral activation.

## PART III: PSYCHOPHARMACOLOGICAL STUDY

### Introduction

In the previous experiment, the electrical stimulation of the NA was observed to increase mouthing, pawing, licking and locomotor behaviors in 3-day-old pups. However, electrical stimulation is not selective as far as involved neurotransmitter systems are concerned, since the electrical stimulation is not specific to the area, but could be the result of stimulating a few neurotrnasmitter systems which are present in the NA.

The evidence from neuroanatomical, pharmacological, and behavioral studies in addition to the present electrical stimulation results implicate the role of the DA system in the elicited behavioral activation. Therefore the specificity of the DA system in the

behavioral activation was further investigated using psychopharmacological techniques. Ideally, it would be most convincing to show that a DA antagonist reduces behavioral activation while a DA agonist increases behavioral activation. Since all tested infant rats activated by IP injections of AMP in the first experiment, the present study focused on the effectiveness of DA antagonists in reducing or inhibiting stimulation-induced behavioral activation of the infant rats.

There is abundant evidence that antipsychotic drugs block DA transmission. They inhibit DA sensitive adenylate cyclase (Clement-Comier & Robinson, 1977) and increase the DA synthesis rate and utilization (Carlsson & Lindqvist, 1963). They reverse the suppression of activity of DA neurons caused by DA agonists (Rebec et al., 1979), and block DA agonist-induced sterotypic behaviors (Randrup & Munkvad, 1974) as well as brain self-stimulation in rats, dogs, and monkeys by blocking DA receptors.

The major questions asked in this experiment were whether DA antagonists block the stimulation induced behavioral activation, and if so, whether different classes of DA antagonists, which have been shown to block DA agonist-induced behaviors differentially,

affect the components of behavioral responses elicited by electrical stimulation of the NA and the CD differentially. The specificity of the DA system on the behavioral activation in infant rats were investigated by administerng typical or atypical DA antagonists and recording the effects of these drugs on elicited behavioral activation.

## Actions of Neuroleptics

Antipsychotic drugs act primarily by blocking DA neurotransmission, while little action is exerted at NE synapses. For instance, antipsychotic neuroleptics cause an increase in the synthesis and release of DA (Antelman et al., 1976) and a subsequent increase in the brain levels of DA metabolites (Carlsson & Lindqvist, 1963) by directly blocking DA receptors. Comparable effects are not observed to the same extent in NE-containing sites. Secondly, adenylate cyclase activity, commonly used as an index of post-synaptic DA receptor stimulation (Greengard, 1974) declines to a greater extent in DA-containing sites than in NE systems following administration of antipsychotic drugs (Clement-Comier & Robinson, 1977; Miller, 1976). Moreover, biochemical studies have demonstrated that the antipsychotic neuroleptics are specific conformational complements of the DA molecule (Horn & Snyder, 1971),

providing additional support that antipsychotic bind specifically to DA receptors. This evidence is consistent with the fact that antipsychotics often produce Parkinsonian-like side effects (Gerlach et al., 1975) that are commonly associated with imparied transmission at dopaminergic, but not at NE synapses.

## Classification of Neuroleptics

Antipsychotic neuroleptics which have been the best available pharmacological treatment for schizophrenia can be divided into two categories based primarily on their behavioral effects. Classical neuroleptics, such as haloperidol (HAL), pimozide, and chlorpromazine, produce an extrapyramidal syndrome, such as Parkinsonian-like motor dysfunction or tardive dyskinesia, whereas the atypical neuroleptics such as clozapine (CLOZ), thioridazine, and l-sulpiride, are relatively devoid of these extrapyramidal symptoms.

Parkinson's disease, characterized by rigid posture and a severe impairment in the initiation of movement (Hornykiewicz, 1977) is associated with a significant loss of DA cells in the substantia nigra. Tardive dyskinesia most often involves the mouth, lips, and tongue and sometimes extends to the limbs or trunk, has been suggested from chemical denervation of central DA neurons following prolonged blockade of dopaminergic

transmission and subsequent development of post-synaptic supersensitivity (Klawans, 1973). Part of the evidence supporting this hypothesis is that administration of L-dopa can exacerbate tardive dyskinesia (Klawans & Mckedall, 1971) and that drugs which reduce dopaminergic activity, such as neuroleptics, can at least temporarily alleviate the symptoms of tardive dyskinesia (Kobayashi, 1977). Thioridazine, and to a lesser extent sulpiride, induces chewing movement when given repeatedly while CLOZ does not induce these repetitive behaviors (Gunne et al., 1986).

These antipsychotic drugs have also been differentiated according to their ability to block different components of the behavioral response to AMP. HAL, a representative classical antipsychotic drug, abolishes the locomotion and focused stereotypy produced by AMP (Stanley & Wilk, 1977). CLOZ appears to act selectively on mesolimbic neurons (Anden & Stock, 1973), since acute administration of this drug blocks motor activity produced by low doses of apomorphine (APO), a direct DA agonist, and AMP, but not the focused stereotypy (Ljungberg & Ungestedt, 1978; Iversen & Koob, 1977). Rats withdrawn from chronic CLOZ have been reported to respond to APO with a selective increase in motor activity (Gianutsos & Moore, 1977; Ljungberg &

Ungerstedt, 1978; Smith & Davis, 1976), by selectively increasing DA receptor sensitivity in the NA and other regions of the mesolimbic system but not in the CD (Ljungberg & Ungerstedt, 1978).

In addition, atypical neuroleptics produce a greater increase of DA metabolites in the NA than in the CD (Anden & Stock, 1973). No difference in metabolite levels were found when typical antipsychotics were tested. This indicates a preferential action of CLOZ in the NA, a finding supported by turnover studies. Also these drugs showed differential effect on the SNC and the VTA DA neurons which project largely to the CD and the NA respectively (White & Wang, 1983). They differ in their potencies for D2 DA receptors depending on the brain region. CLOZ or thioridazine has higher affinity to D2 sites in the limbic tissue than the CD, while HAL is equipotent in these brain regions (Borison & Diamond, 1983). The affinity to 5-HT2 serotonin receptors also differs and CLOZ is much higher compared to HAL (Altar et al., 1986; Fillion et al., 1978; Nelson et al., 1978; Peroutka & Snyder, 1979).

Contrary evidence, however, argues against the regional selectivity of the neuroleptics. A few studies reported that CLOZ and HAL elevated DA metabolites in the CD and the NA to a similar extent (Stanley & Wilk,

1977; Westerink & Korf, 1975). In addition, CLOZ and HAL are reported to produce a similar effect on single unit activity in both the CD and the NA (Rebec et al., 1980). Furthermore, both drugs block the neuronal response to AMP and APO in both nuclei (Rebec et al., 1979). Since the affinity of neuroleptics for muscarinic receptor bindings in the brain varies inversely with the propensity of a drug to elicit extrapyramidal effects (Snyder, 1974), a more likely explanation is the anticholinergic properties of CLOZ and thioridazine, as contrasted with HAL. It is well known that anticholinergics can counteract neuroleptic-induced side effects.

## Method

### Subjects

The subjects were 3-day-old Sprague-Dawley rats housed and maintained as described previously. All rat pups were implanted using the stereotaxic method described in the electrical stimulation study. To test the effect of DA antagonists on the elicited behavioral activation, one group of rat pups were implanted in the NA and pretreated with 0.2 mg/kg HAL (N=5), 0.05 mg/kg HAL (N=5), 2.5 mg/kg CLOZ (N=4), or 0.5 mg/kg CLOZ (N=8), while other group of rat pups were implanted in the CD and pretreated with 0.2 mg/kg HAL (N=7), or 2.5

mg/kg CLOZ (N=4) before electrical stimulation.

Testing Procedure

Testing occurred 16 to 20 hours after surgery. Pups were pre-tested on a felt covered container in a 32 °C environment by giving three pulse trains at 60 uA separated by a 30 sec delay to determine the effects of electrical stimulation on behavioral activation. A pulse train was the same as the first experiment and consisted of 50, 500 msec pulses with a pulse width of 2 msec. Again, these stimulation parameters were chosen because it has been shown that these stimulation parameters were effective for self-stimulation and behavioral activation in the MFB in neonatal rats (Moran et al., 1981). The pups which responded with oral behaviors or other behaviors including locomotion to two out of three stimulation trains were included in the test procedure. Pups that did not respond to this current (60uA) were not tested further.

At the beginning of the test session, pups were voided, weighed, and numbered on their back with a marking pen for easy identification of each animal. Each subject received an IP injection of 0.9% saline vehicle solution or doses of 0.5 or 2.5 mg/kg CLOZ, 0.05 or 0.2 mg/kg HAL, 20 min before the test session began. During this period, pups were allowed to recover from

injection handling. The injection was arranged so the identity of the treatment was not known to the observer. The observer was blind to the injected doses as well as the site of implant. These doses were chosen to include doses known to have behavioral effects in adult animals, as well as infant animals. Most of the data available to date have been obtained from experiments using rats with electrodes chronically implanted in the MFB at the level of the hypothalamus. For example the ED50 for inhibiting brain self stimulation behavior in rats were 0.047 mg/kg and 13.2 mg/kg subcutaneously for HAL and CLOZ respectively (Fielding & Lal, 1978). The doses necessary to cause 50% inhibition of avoidance behavior were 1.03 mg/kg and 11.73 mg/kg for HAL and CLOZ respectively (Fielding & Lal, 1978). The effect of neuroleptics lasted a few hours to several days. Low doses of typical neuroleptics were selected to avoid catalepsy. From pilot studies, the above doses eliminated elicited behavioral activation. Therefore, the neuroleptic doses were lowered until reliable behavioral activation data was obtained. These typical neuroleptic doses in rat pups did not produce catalepsy (Spear & Ristine, 1982) when tested using the procedure by Baez et al. (1976).

Following 5 min adaptation and a 2 min baseline

observation, behaviors were recorded over 6-one minute intervals during which biphasic square wave stimulation was delivered every 10 seconds during every other minute. Pups were sacrificed and perfused as described earlier at the end of the experiment. No more than 2 pups were used from a litter. The electrode sites were plotted on the Sherwood and Timiras (1970) atlas.

In order to compare the effects of each DA antagonist pretreatment on pups from stimulation, the data for the three stimulation minutes were pooled since there was no significant differences in the recorded behavioral measures among stimulations by the F-test for simple effect analysis in the first experiment, and the effects of each antipsychotic dose on each behavioral measure were compared by a nonparametric Mann-Whitney U test. The Mann-Whitney U test was chosen because the distribution of the data did not meet parametric test assumptions.

To investigate the effect of neuroleptics on motivational or motor function of infant rats, separate groups of non-implanted 3-day-old rat pups were tested for milk consumption. All pups were removed from their mother and placed with their littermates in an incubator for 23 hours before testing. At the time of testing, each pup was removed from the incubator, weighed,

labeled with a magic marker, injected IP with 0.5 mg/kg HAL, 5.0 mg/kg CLOZ or saline. The effect of urinary excretion on weight change was minimized by stroking the anogenital area with a Q-tip to stimulate urination prior to injection. The milk intake session began 20 min after the injection and lasted one hour. Pups were allowed to drink freely from a gauze pad saturated with milk (Half and Half). The ambient temperature was maintained at 32 $^{o}$C during this period. At the end of test session, body weights of the pups were recorded again after pups were gently dried by a gauze pad.

## Results

HAL and CLOZ produced comparable effects blocking stimulation induced activation. Injection of typical antipsychotic HAL (0.2 mg/kg, N=5) effectively prevented stimulation induced mouthing (U (5, 12)= 2 or 58, p=.003), pawing (U (5, 12)=7 or 53, p=.015), and locomotion (U (5, 12)=3.5 or 56.5, p=.005), in the pups whose electrodes were in the NA. Mouthing (U (4, 12)=8.5 or 39.5, p=.006) and pawing (U (4,12)=7 or 41, p=.039) behaviors were inhibited even at the low dose of HAL (.05 mg/kg, N=4). Locomotion, however was not blocked by this dose. Injection of atypical antipsychotic CLOZ (2.5 mg/kg, N=4) also prevented mouthing (U (4, 12)=1 or 47, p=.005), pawing ( U (4,

12)=8.5 or 39.5, p=.06), and locomotion (U (4, 12)=2 or 46, p=.008), when the electrodes were implanted in the NA. The low dose of CLOZ (0.5 mg/kg, N=8) also effectively inhibited mouthing (U (8, 12)=2 or 94, p < .001) and pawing (U (8, 12)=12 or 84, p=.005). Locomotion in contrast was not inhibited at this low dose. Figure 11 presents the number of mouthing responses for 3 stimulation minutes for drug-treated and control rat pups. As shown in figure 11, mouthing was inhibited by each tested dose of atypical and typical antipsychotics. Mouthing was significantly inhibited by pretreatment of typical antipsychotic 0.05 and 0.2 mg/kg HAL. The atypical antipsychotics 0.5 and 2.5 mg/kg CLOZ produced a comparable effect and inhibited mouthing significantly when compared to the control group. Figure 12 presents the number of pawing responses for 3 stimulation minutes for drug-treated and control rat pups. Pawing also was significantly inhibited by pretreatment of the typical antipsychotic 0.05 and 0.2 mg/kg HAL. The atypical antipsychotic 0.5 and 2.5 mg/kg CLOZ produced comparable effects and inhibited pawing significantly when compared to the control group. Figure 13 presents the number of licking responses for 3 stimulation minutes for neuroleptic-treated and control groups. Licking was not

Figure 11. Mean ± SEM of mouthing behavior in the neuroleptics-treated and control groups.

# Mouthing

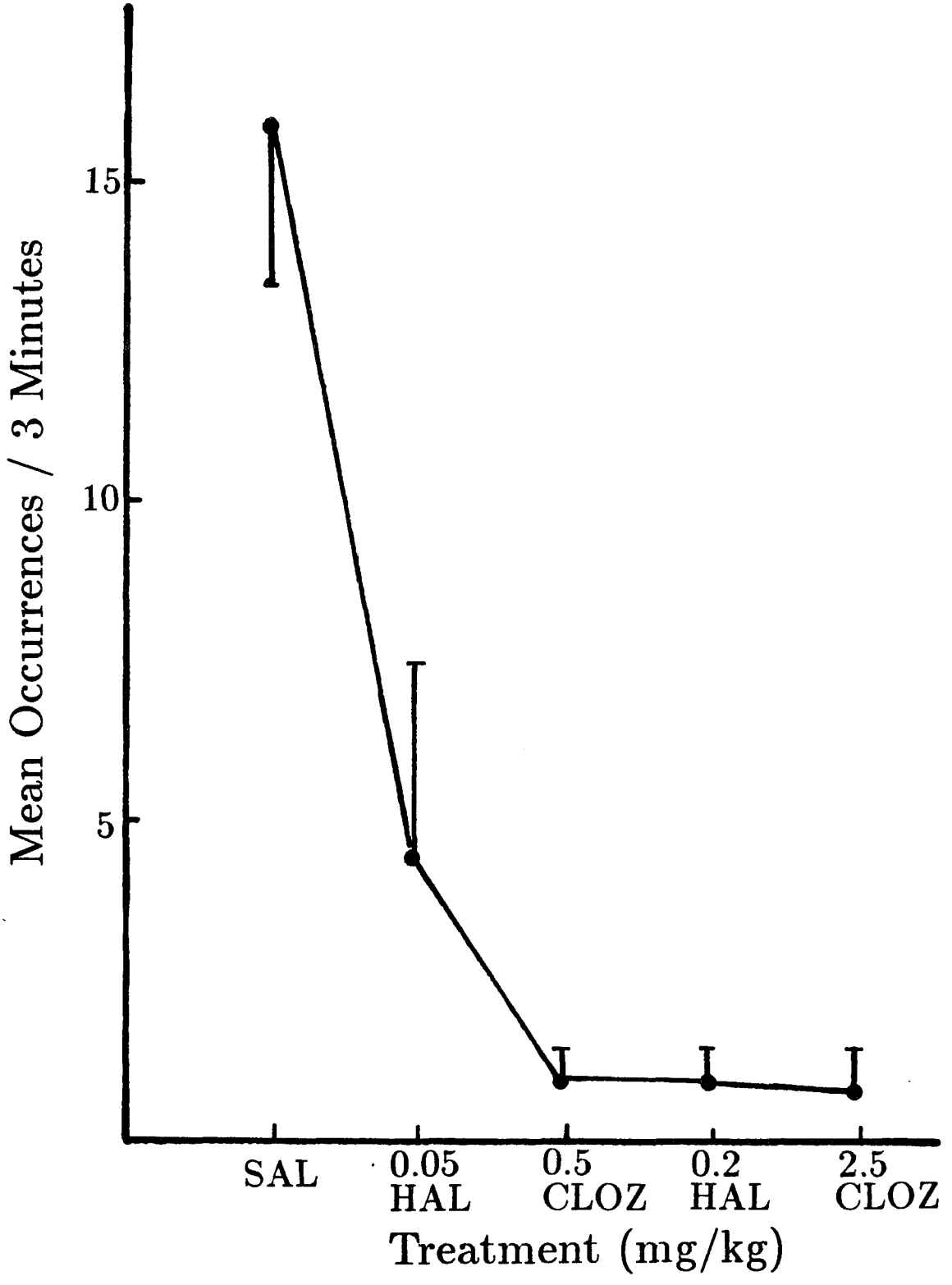

Mean Occurrences / 3 Minutes
15
10
5
SAL
0.05 HAL
0.5 CLOZ
0.2 HAL
2.5 CLOZ
Treatment (mg/kg)

Figure 12. Mean ± SEM of pawing behavior in the neuroleptics-treated and control groups.

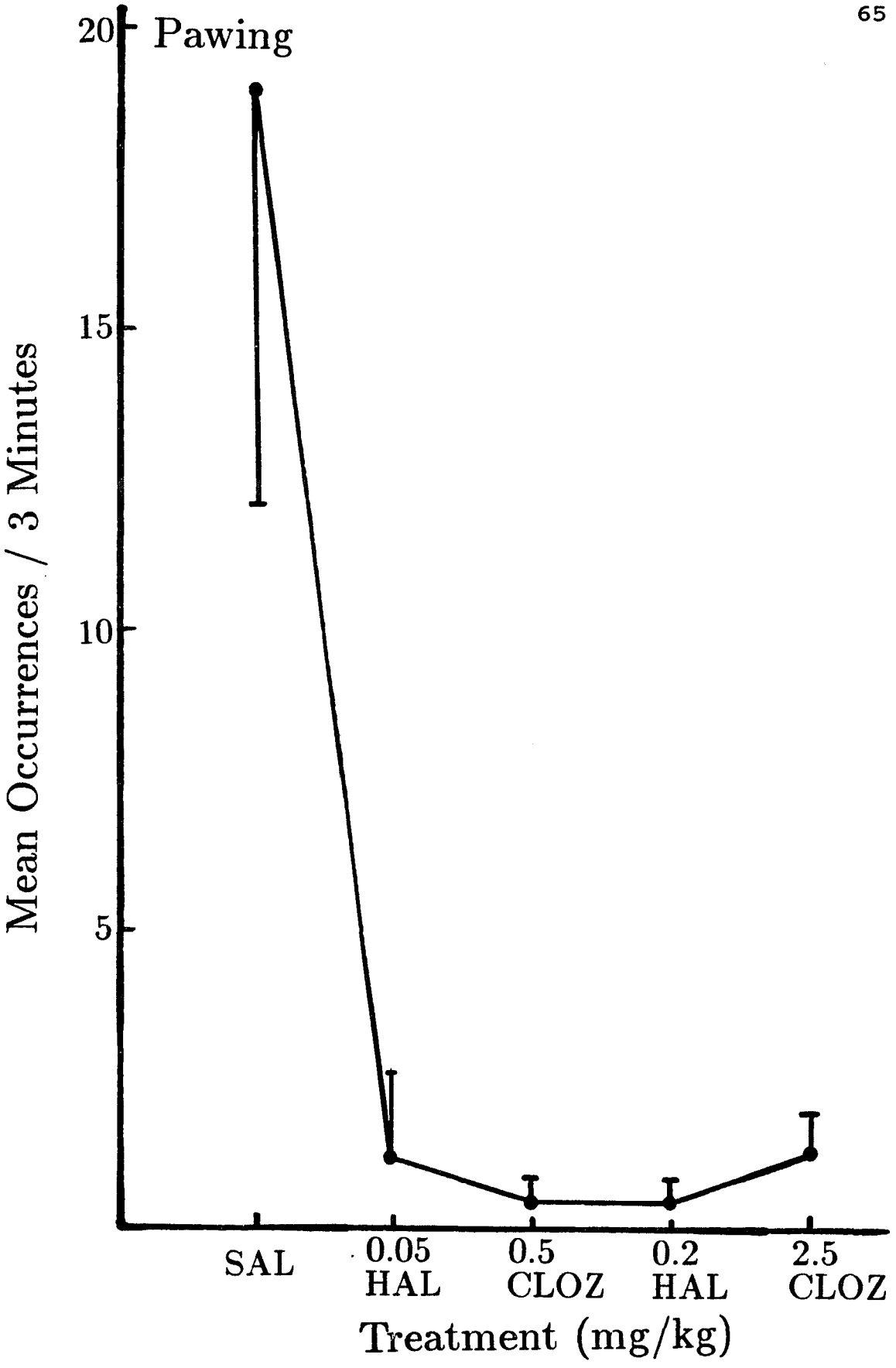
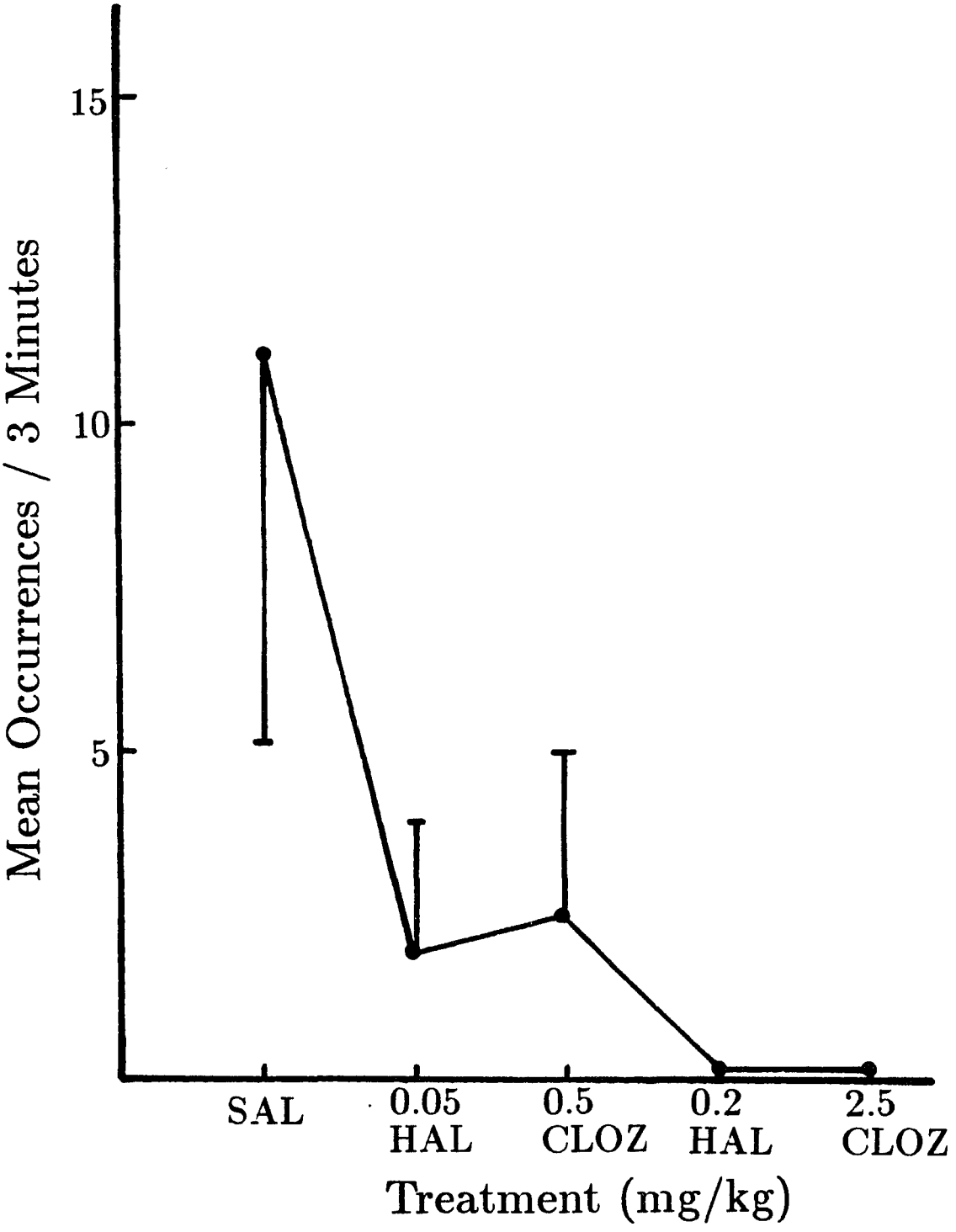

Pawing
20
15
10
5
Mean Occurrences / 3 Minutes
SAL
0.05 HAL
0.5 CLOZ
0.2 HAL
2.5 CLOZ
Treatment (mg/kg)

Figure 13. Mean ± SEM of licking behavior in the neuroleptic-treated and control groups.

# Licking


15
10
5
Mean Occurrences / 3 Minutes
SAL
0.05 HAL
0.5 CLOZ
0.2 HAL
2.5 CLOZ
Treatment (mg/kg)

significantly inhibited by tested doses, but the figure shows the decreasing trend of licking behavior by antipsychotic neuroleptics. Note that the increase of licking behavior was not as robust as mouthing or pawing in the stimulation study. Figure 14 shows the number of responses for 3 stimulation minutes for drug-treated and control rat pups. As can be seen in this figure, stimulation induced locomotion was inhibited only by 0.2 mg/kg HAL or 2.5 mg/kg CLOZ when the electrodes were in the NA. A summary of the drug data is shown in Figure 15. The HAL group was virtually identical to CLOZ in inhibiting these behaviors. The behavioral activation was significantly reduced by both drugs. Doses of 0.05 to 0.2 mg/kg HAL were effective in inhibiting stimulation-induced mouthing and pawing. Doses of 0.5 to 2.5 mg/kg CLOZ produced comparable effects and blocked stimulation induced mouthing and pawing. Stimulation induced locomotion was inhibited only by 0.2 mg/kg HAL (N=5) or 2.5 mg/kg CLOZ (N=4) when the electrodes were in the NA. Figure 16 presents the number of responses for 3 stimulation minutes for drug-treated and control rat pups, with electrodes implanted in the CD. The locomotion induced by stimulation of the CD was not significantly inhibited even at 0.2 mg/kg HAL (N=7) or 2.5 mg/kg CLOZ (N=4). Figure 17 shows the

Figure 14. Mean ± SEM of locomotor behavior in the neuroleptic-treated and control groups.

# Locomotion

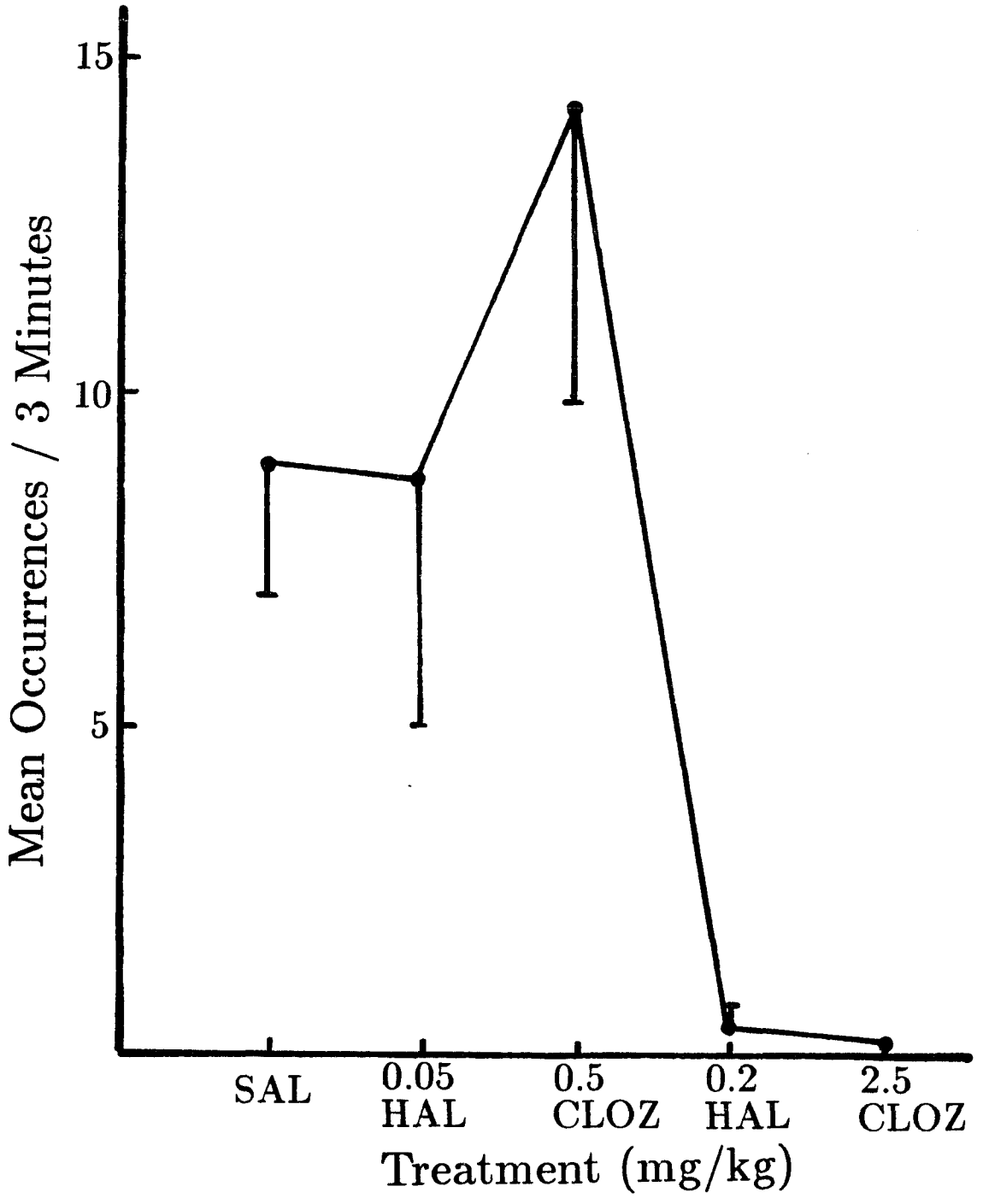

15
10
5
Mean Occurrences / 3 Minutes
SAL
0.05 HAL
0.5 CLOZ
0.2 HAL
2.5 CLOZ
Treatment (mg/kg)

Figure 15. Mean ± SEM of the number of occurrences in the neuroleptic-treated and the control group when the electrodes were implanted in the nucleus accumbens.

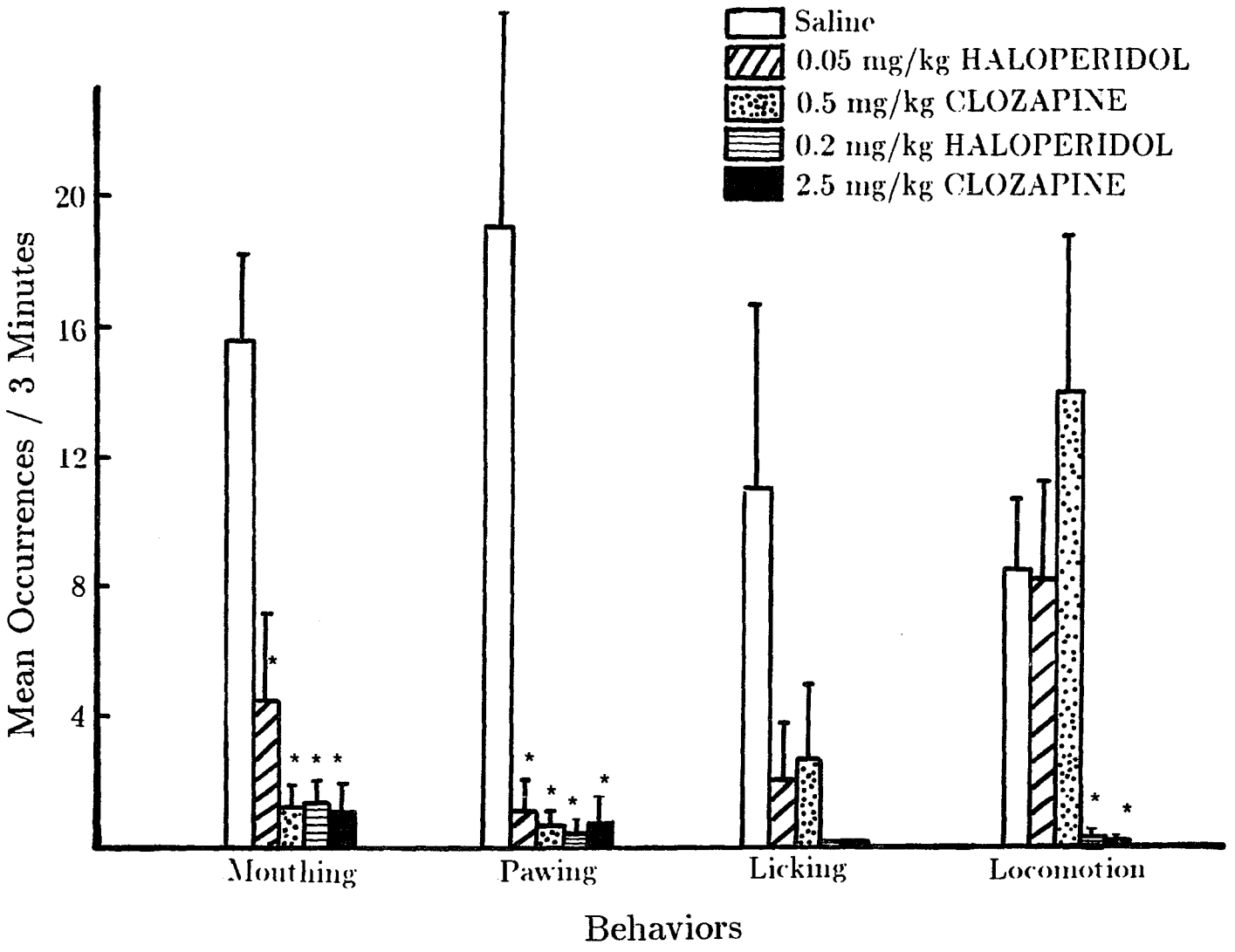

Saline
0.05 mg/kg HALOPERIDOL
0.5 mg/kg CLOZAPINE
0.2 mg/kg HALOPERIDOL
2.5 mg/kg CLOZAPINE
Mean Occurrences / 3 Minutes
20
16
12
8
4
Mouthing
Pawing
Licking
Locomotion
Behaviors

Figure 16. Mean ± SEM of locomotor behavior elicited from the CD in the neuroleptic-treated and control groups.

# Locomotion

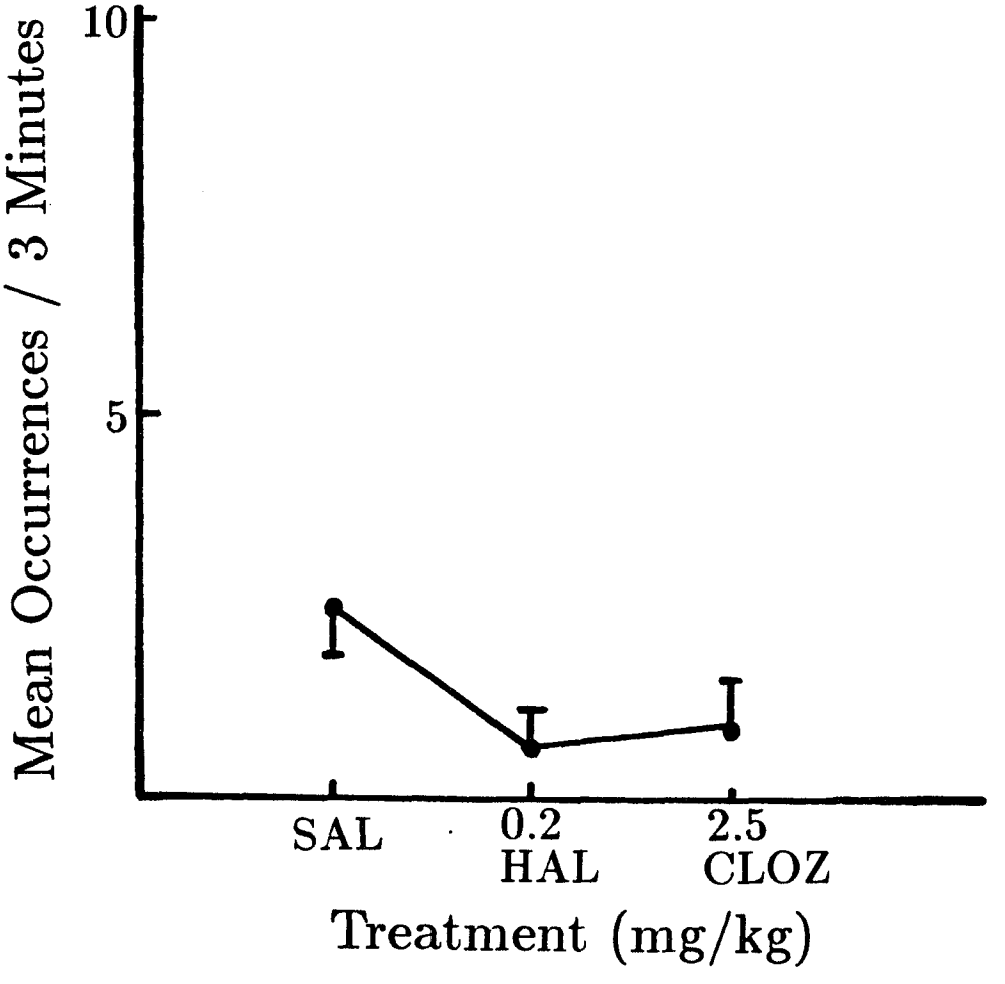
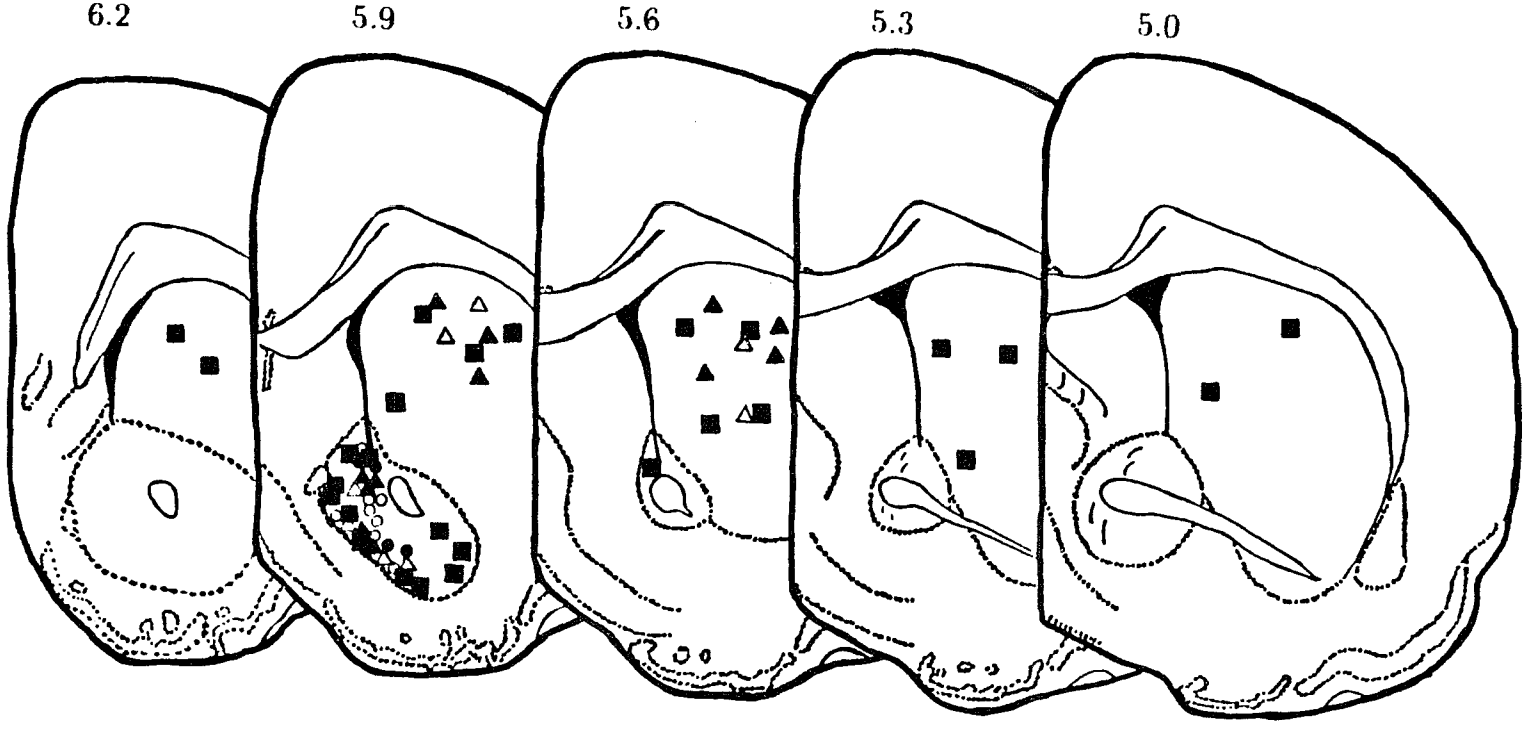

Mean Occurrences / 3 Minutes
10
5
SAL
0.2
HAL
2.5
CLOZ
Treatment (mg/kg)

Figure 17. Histology for the localization of electrode loci in the saline and drug treated pups.


■ Saline
▲ 0.2 mg/kg HALOPERIDOL
△ 2.5 mg/kg CLOZAPINE
o 0.5 mg/kg CLOZAPINE
• 0.05 mg/kg HALOPERIDOL
6.2
5.9
5.6
5.3
5.0

histological results of the drug groups.

Milk intake data revealed that drug treatment did not affect the amount of milk consumed by rat pups. The pups drank from a milk saturated gauze pad and became bloated with milk by the end of one hour, as indicated by their extended abdomen, which was whitish in color. The results of this experiment are summarized in Table 1. Each entry is the mean ±SEM milk intake measured in grams.

Table 2

Effects of Haloperidol and Clozapine on Milk Intake of Non-implanted Rat Pups

| Saline (N=16) | Clozapine (N=2) (5.0 mg/kg) | Haloperidol (N=18) (0.5 mg/kg) |
|---|---|---|
| 0.21±0.04 | 0.20±0.01 | 0.20±0.05 |

Note - Each entry is the mean (±SEM) milk intake measured in grams for 23 hour deprived pups allowed one hour access to a saturated gauze pad of milk.

The mean increase of body weight was similar to Raskin and Campbell (1981), and approximately increased 3 % from the original body weight. In the subsequent experiment the CLOZ (5.0 mg/kg) and the saline groups were tested for milk intake, since the sample size of the CLOZ group was small in the first milk intake experiment. The mean values (±SEM) were identical in the saline (N=10) and the CLOZ (N=11) groups

(0.01±0.02).

## Discussion

Both typical and atypical neuroleptics, HAL and CLOZ were observed to inhibit stimulation-induced behavioral activation of 3-day-old rat pups. HAL and CLOZ produced comparable effects and blocked elicited behaviors. Behaviors such as mouthing and pawing were very sensitive to DA antagonists and were blocked by very low doses of HAL or CLOZ. Locomotion elicited by stimulation of the NA however, was blocked only by higher doses of either neuroleptic, while locomotion induced by stimulation of the CD was not significantly inhibited by the same doses. These results might be from a less developed nigro-striatal DA system as compared to the mesolimbic DA system. Consistent with this interpretation, various neural sites differ in sensitivity to AMP when infant rats were allowed to self stimulate (Barr & Lithgow, 1986). Therefore it is possible that the pharmacologic action of DA antagonists may shift among regions. Confirmation of this interpretation would require further studies in which independent measures of DA receptors are taken, and DA metabolism in the other brain sites are assayed or manipulated directly.

The reduction or complete inhibition of behavioral

activation induced by these antagonists did not appear to be a result of a debilitating effect of the drugs or to be due to any alteration in motivation since drug treatment did not affect the amount of milk consumed by rat pups. Also, no catalepsy was reported, even at the high dose of 1.0 mg/kg HAL (Spear & Ristine, 1982).

It is unlikely that the inhibition from HAL and CLOZ on elicited behavioral activation are from their effect on other neurotransmitter systems, such as their effect on 5-HT2 serotonin receptors. The fact that the higher doses of CLOZ compared to HAL were needed to inhibit elicited behavioral activation argue against the involvement of 5-HT2 receptors, since the affinity of 5-HT2 receptors is reported to be much higher in CLOZ as compared to HAL (Peroutka & Snyder, 1979). In addition, while HAL suppresses the elicited behavioral activation, it does not suppress suckling responses which are presumably mediated by the serotonin system. Serotonergic agonists such as quipazine increase suckling behavior while serotonin antagonists such as methysergide, methiothepin and metergoline inhibit suckling. Spear and Ristine (1982) reported inhibition of suckling by serotonin antagonists while DA antangonists were not effective inhibiting this behavior.

## PART IV: GENERAL DISCUSSION

The present study reported the effects of electrical stimulation on behavioral activation, using 3-day-old rats with electrodes implanted in the NA and the CD, two major DA projection areas. Rat pups responded differentially, depending on stimulation sites. The behavioral activation induced by electrical stimulation of the NA appeared to be different from the enhanced locomotion resulting from stimulation of the CD, and included appetitive behaviors, such as mouthing, pawing, and licking. The elicited behavioral activation was blocked by pretreatment of DA antagonists. Both typical and atypical neuroleptics produced comparable effects and blocked elicited behaviors.

These results are consistent with the hypothesis that the DA system is critical in the mediation of behavioral activation in infant rats. In particular, the mesolimbic DA system appears to play an important role in behavioral activation. Evidence that the DA system develops earlier than the NE system (Loren et al., 1976), as well as the earlier development of the mesolimbic DA system compared to the nigro-striatal system as indicated by early development of tyrosine hydroxylase activity in the VTA (McGeer et al., 1976),

are consistent with the view that the mesolimbic DA system is involved in behavioral activation. Considering the site or early development of the mesolimbic DA pathway, the DA system is sufficient to mediate behavioral activation at this age when this system is electrically activated. The lack of elicited behavioral activation from the CD implant may be a result of a less developed DA system at this age.

The findings that behavioral activation accompany ICSS in the MFB (Moran et al., 1983), and that ICSS in the NA is responsive to AMP's actions at lower doses (Barr & Lithgow, 1986), brain sites that support self-stimulation in adults correspond well to the findings at this age (Barr & Lithgow, 1986; Lithgow & Barr, 1984), and the preferential effect on neuroleptics in the NA in reducing the reward effect (Stellar et al., 1983, 1985; Taylor & Robbins, 1984, 1985), as well as the results from this experiment, suggest an interesting possibility that behavioral activation and rewards in the infants are mediated by the same neurotransmitter system, such as the mesolimbic DA system.

In adult rats, the mesolimbic DA system has been implicated with the reward system (Fibiger, 1978; Wise, 1980). Seeger and Gardner (1979) reported an increase in the self-stimulation rate in adult rats with electrodes

implanted into the VTA, the source of mesolimbic DA projections, following chronic HAL treatment, suggesting the involvement of the mesolimbic DA system in the reward system. Also, bilateral injections of AMP into the NA increased ICSS (Broekkamp et al., 1975). Furthermore, impairment of a learning task was greater when the the neuroleptic, cis-flupenthixol, was injected into the NA as compared to the CD (Stellar et al., 1985). Taylor and Robbins (1984, 1985) also recently presented evidence showing involvement of the mesolimbic DA system in reward. The 6-OHDA lesions of the NA but not the CD, attenuated the reinforcement effect produced by intra-accumbens AMP infusion.

The rather undifferentiated behavioral activations in infant rats may represent a reward system in developing brains before neural components are fully mature in terms of neurotransmitter levels and receptor types and numbers. This elicited behavioral activation in infant rats may become more coherent and controlled with age as with other behaviors such as feeding behavior or MFB elicited behaviors (Hall, 1979; Moran et al., 1983). By the end of the second week of age, visual and auditory systems are relatively mature (Gottlieb, 1971; Rose, 1968; Crowly & Hepp-Raymond, 1966), cortical inhibitory mechanisms develop (Hicks & D'Amato, 1975),

and development of dopaminergic pathways has increased (Szechtman & Hall, 1980). Behavioral activation mechanisms in the pup eventually lose their intrinsic ability to maintain behavior and may be replaced by more specific reward mechanisms by later developing portions of the nervous system. Another possibility is that other neurotransmitter systems are so dominant after two weeks, that they overshadow behavioral activation. A combination of these factors might inhibit behavioral activation in older rats in a reward situation.

Nevertheless, the present findings do not demonstrate the rewarding properties of behavioral activation in infant rats. However, in light of previous research from this laboratory on the role of the AMP in self stimulation, differential sensitivity of this drug depending on the neural sites, and the concomittant behavioral activation in infant rats, it could be postulated that the behavioral activation could be coterminous as reinforcement in infant rats. For example, it would be interesting to find out if a neutral stimuli, such as an odor, could become reinforcing itself after the pups are stimulated in the NA in the presence of the same odor. The pups might develop preference to that odor if the behavioral

activation is reinforcing in and of itself. Alternatively, if behavioral activation is blocked during brain stimulation, no learning should take place.

Clearly, more anatomical mapping studies are needed to assess the relative roles of the DA system in behavioral activation. However, it is unlikely that either self-stimulation or behavioral activation in rat pups is mediated by another system.

## References

Ahlenius, S., Brown, R., Engel, J., and Lundborg, P. (1973). Learning deficits in 4 weeks old offspring of the nursing mothers treated with the neuroleptic drug penfluridol. Naunyn-Schmiedeberg's Archives of Pharmacology, 279, 31-37.

Almli, C. R., and Fisher, R. S. (1977). Infant rats: Sensorimotor ontogeny and effect of substantia nigra destruction. Brain Research Bulletin, 2, 425-459.

Altar, C.A., Wasley, A.M., Neale, R.F., and Stone, G.A. (1986). Typical and atypical antipsychotic occupancy of D2 and S2 receptors: An autoradiographic analysis in rat brain. Brain Research Bulletin, 16, 517-525.

Anden, N.E, and Stock, G. (1973). Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system. Journal of Pharmacy and Pharmacology, 25, 346-348.

Antelman, S. M., Szechtman, H., Chin, P., and Fisher, A. E. (1976). Inhibition of tyrosine hydroxylase but not dopamine-B-hydroxylase facilitates the action of behaviorally ineffective doses of neuroleptics. Journal of Pharmacy and Pharmacology, 28, 66-88.

Arbuthnott, G. W., Crow, T. J., Fuxe, K., Olson, L., and Ungerstedt, U. (1970). Depletion of catecholamines in vivo induced by electrical stimulation of central monoamine pathways. Brain Research, 24, 471-483.

Arbuthnott, G. W., Fuxe, K., and Ungerstedt, U. (1971). Central catecholamine turnover and self stimulation behavior. Brain Research, 27, 406-413.

Baez, L. A., Eskridge, N. K., and Schein, R. (1976). Postnatal development of dopaminergic and cholinergic catalepsy in the rat. European Journal of Pharmacology, 36, 155-162.

Bagshaw, E. V., and Evans, M. H. (1976). Measurement of current spread from microelectrodes when stimulating within the central nervous system. Experimental Brain Research, 25, 391-400.

Barr, G. A., and Lee, E. H. (1985). Neural bases of reward and activation in infant rats. Paper presented at the psychobiology of stress and emotion symposium, New Orleans, Louisiana.

Barr, G. A., and Lithgow, T. (1986). Pharmaco-ontogeny of reward: Enhancement of self-stimulation by d-amphetamine and cocaine in 3- and 10-day-old rats. Developmental Brain Research, 24, 193-202.

Baxter, B. L., Gluckman, M. I., Stein, L., and Scerni, R. A. (1972). Self injection of apomorphine in the rat: Positive reinforcement by a dopamine receptor stimulant. Pharmacology, Biochemistry and Behavior, 2, 387-392.

Beninger, R. J. (1983). The role of dopamine in locomotor activity and learning. Brain Research Review, 6, 173-196.

Berger, B. (1977). Histochemical identification and localization of dopaminergic axons in rat and human cerebral cortex. Advances in Biochemical Pharmacology, 16, 13-20.

Besson, J. J., Cheramy, A., Feltz, P., and Glowinski, J. (1971). Dopamine: Spontaneous and drug-induced release from the caudate nucleus in the cat. Brain Research, 32, 407-424.

Bjorklund, A., Lindvall, O., and Nobin, A. (1975). Evidence of an incertohypothalamic dopamine neurone system in the rat. Brain research, 89, 29-42.

Bolles, R. C., and Woods, P. J. (1964). The ontogeny of behavior in the albino rat. Animal Behavior, 21, 427-440.

Borison, R.L.,and Diamond, B.I. (1983). Regional selectivity of neuroleptic drugs: An argument for site selectivity. Brain Research Bulletin, 11, 215-218.

Bozarth, M. A., and Wise R. A. (1980). Intracranial self-administration as a technique to study the reward properties of drugs of abuse. Pharmacology, Biochemistry and Behavior, 13, 245-247.

Bozarth, M. A., and Wise, R. A. (1981). Heroin reward is dependent on a dopaminergic substrate. Life Science, 29, 1881-1886.

Brake, S. C. (1981). Suckling infant rats learn a preference for a novel olfactory stimulus paired with milk delivery. Science, 211, 506-508.

Broekkamp, C. L. E., Pijnenberg, A. J. J., Cools, A. R., and Van Rossum, J. M. (1975). The effect of microinjection of amphetamine into the neostriatum and the nucleus accumbens on self stimulation behavior. Psychopharmacologia, 42, 179-183.

Bulut, G. F., and Altman, J. (1974). Spatial and tactile discrimination learning in infant rats motivated by homing. Developmental Psychobiology, 7, 465-473.

Carlsson, A., and Lindqvist, M. (1963). Effects of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacologica et Toxicologica, 17, 140-148.

Carlsson, A., Lindqvist, M., Magnusson, T., and Walldeck, B. (1958). On the presence of 3-hydroxytyramine in brain. Science, 127, 471.

Chiueh, D. D., and Moore, K. E. (1974). In vivo release of endogenously synthesized catecholamines from the cat brain evoked by electrical stimulation and by amphetamine. Journal of Neurochemistry, 23, 159-168.

Clavier, R. M., and Fibiger, H. C. (1977). On the role of ascending catecholaminergic projections in intracranial self-stimulation of the substantia nigra. Brain Research, 131, 271-286.

Clavier, R. M., Fibiger, H. C., and Phillips, A. G. (1976). Evidence that self-stimulation of the region of the locus coeruleus in rats does not depend upon noradrenergic projections to telencephalon. Brain Research, 113, 71-81.

Clavier, R. M., and Routtenberg, A. (1976). Brain stem self-stimulation attenuated by lesions of medial forebrain bundle but not by lesions of brainstem norepinephrine systems. Brain Research, 101, 251-271.

Clement-Cormier, Y. C., and Robinson, G. A. (1977). Adenylate cyclase from various dopaminergic areas of the brain and the action of antipsychotic drugs. Biochemical Pharmacology, 26, 1719-1722.

Cole, S. O. (1978). Brain mechanisms of amphetamine-induced anorexia, locomotion and stereotypy: A review. Neuroscience and Biobehavioral Reviews, 2, 89-100.

Collier, T. J., Kutzman, S., and Routtenberg, A. (1977). Intracranial self-stimulation derived from the entorhinal cortex. Brain Research, 137, 188-196.

Cools, A. R. (1973). Chemical and electrical stimulation of the caudate nucleus in freely moving cats: the role of dopamine. Brain Research, 58, 437-451.

Cools, A. R., and Van Rossum, J. J. (1976). Excitation-mediating and inhibition-mediating dopamine receptors: A new concept towards a better understanding of electrophysiological, biochemical, pharmacological, functional and clinical data. Psychopharmacology, 45, 243-254.

Cooper, B. R., Cott, J. M., and Breese, G. R. (1974). Effects of catecholamine-depleting drugs and amphetamine on self-stimulation of brain following various 6-hydroxy-dopamine treatments. Psychopharmacologia, 37, 235-248.

Corbett, D., Skelton, R.w., and Wise, R.A. (1977). Dorsal bundle lesions fail to disrupt self-stimulation from the region of locus coeruleus. Brain Research, 133, 37-44.

Costall, B., Marsden, C. D. Naylor, R. J., and Pycock, C. J. (1977). Stereotyped behavior patterns and hyperactivity induced by amphetamine and apomorphine after discrete 6-hydroxydopamine lesions of extrapyramidal and mesolimbic nuclei. Brain Research, 123, 89-111.

Costall,B., and Naylor, R. J. (1976). Antagonism of the hyperactivity induced by dopamine applied intracerebrally to the nucleus accumbens septi by typical neuroleptics and by clozapine, sulpiride and thioridazine. European Journal of Pharmacology, 35, 161-168.

Costall,B., and Naylor, R. J. (1975). The behavioral effects of dopamine applied intracerebraly to areas of the mesolimbic system. European Journal of Pharmacology, 32, 87-92.

Costall, B., and Olley, J. E. (1971). Cholinergic- and neuroleptic induced catalepsy: Modification by lesions in the caudate putamen. Neuropharmacology, 10, 297-306.

Coyle, J. T. (1977). Biochemical aspects of neurotransmission in developing brain. International Review of Neurobiology, 20, 65-104.

Coyle, J. T., and Campochiaro, P. (1976). Ontogenesis of dopaminergic-cholinergic interactions in the rat striatum: A neurochemical study. Journal of Neurochemistry, 27, 673-678.

Coyle, J. T., and Henry, D. (1973). Catecholamines in fetal and newborn rat brain. Journal of Neurochemistry, 21, 61-67.

Coyle, J. T., and Molliver, M. E. (1977). Major innervation of newborn rat cortex by monoaminergic neurons. Science, 196, 444-447.

Crow, T. J. (1972). A map of the rat mesencephalon for electrical self-stimulation. Brain Research, 36, 265-273.

Crowley, D. E., and Hepp-Raymond, M. C. (1966). Development of cochlear function in the ear of the infant rat. Journal of Comparative and Physiological Psychology, 62, 427-430.

De France, J. F., and Yoshihara, H. (1975). Limbic input to the nucleus accumbens septi. Brain Research, 1975, 90, 159-163.

Dewyngaert, M., and Kellogg, C. (1974). Effects of early L-DOPA administration on the ontogeny of motor functions in the rat. Brain Research, 73, 175-179.

Fallon, J. H. Riley, J. N., and Moore, R. Y. (1978). Substantia nigra dopamine neurons: Separate populations project to neostriatum and allocortex. Neuroscience Letters, 7, 157-162.

Fibiger, H. C. (1978). Drugs and reinforcement mechanisms: A critical review of the catecholamine theory. Annual Review of Pharmacology and Toxicology, 18, 37-56.

Fielding, S., and Lal, H. (1978). Behavioral actions of neuroleptics. In L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.), Handbook of Psychopharmacology: Biochemical studies of CNS receptors (pp.91-128). New York: Plenum Press.

Fillion, G., Rousselle, J. C.,Fillion, M.P., Beaudoin, D., Goiny, M., Deniau, J.M., and Jacob, J. (1978). High-affinity binding of 3H-5-hydroxytryptamine to brain synaptosomal membranes: comparison with 3H lysergic acid diethylamide binding. Molecular Pharmacology, 14, 50-59.

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

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

Fuxe, K., Hokfelt, T., Johansson, O., Jonsson, G., Lindbrink, P., and Ljngdahl, A. (1974). The origin of the dopamine nerve terminals in limbic and frontal cortex. Evidence of meso-cortico dopamine neurons. Brain Research, 82, 349-355.

Fuxe , K., and Ungerstedt, U. (1970). Histochemical, biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. In E. Costa and S. Garattini (Eds.), Amphetamines and related compounds New York: Raven Press.

Gallistel, C.R., Rolls, E.T., and Greene, D. (1969). Neuron function inferred from behavioral and electrophysiological estimates of refractory period. Science, 166, 1028-1030.

Gerlach, J., Thorsen, K., and Fog, R. (1975). Extrapyramidal reactions and amine metabolites in cerebrospinal fluid during haloperidol and clozapine treatment of schizophrenic patients. Psychopharmacologia, 40, 341-350.

German, D. C., and Bowden, D. M. (1974) Catecholamine system as the neural substrate for intracranial self-stimulation: A hypothesis. Brain Research, 73, 381-419.

Gianutsos, G., and Moore, K. E. (1977). Dopaminergic supersensitivity in striatum and olfactory tubercle following chronic administration of haloperidol or clozapine. Life Sciences, 20, 1585-1592.

Glowinski, J., Axelrod, J., and Iversen, L. L. (1966). Regional studies of catecholamines in the rat brain IV. Effects of drugs on the disposition and metabolism of 3-H-norepinephrine and 3-H-dopamine. Journal of Pharmacology and Experimental Therapeutics, 153, 30-41.

Gottlieb, G. (1971). Ontogenesis of sensory functions in birds and mammals. In E. Tobach, L. Aaronson and E. Shaw (Eds.), The biopsychology of development (pp. 67-128). New York: Academic Press.

Greengard, P. (1974). Molecular studies on the nature of the dopamine receptor in the caudate nucleus of the mammalian brain. In P. Seeman and G.M. Brown (Eds.), Frontiers in neurology and neuroscience research (pp. 12-15). Toronto: University of Toronto Press.

Gunne, L.M., Andersson, U., Bondesson, U., and Johansson, P. (1986). Spontaneous chewing movements in rats during acute and chronic antipsychotic drug administration. Pharmacology, Biochemistry, and Behavior, 25, 897-901.

Hall, W.G. (1979). Feeding and behavioral activation in infant rats. Science, 205, 206-209.

Heffner, T. G., Heller, A., Miller, F. E., Kotke, C., and Seiden, L. S. (1983). Locomotor hyperactivity in neonatal rats following electrolytic lesions of mesocortical dopamine neurons. Developmental Brain Research, 9, 29-38.

Heller, A., Hutchens, J. O., Kirby, M. L., Karapas, F., and Fernandez, C. (1979). Stereotaxic electrode placement in the neonatal rat. Journal of Neuroscience Methods, 1, 41-76.

Hicks, S. P., and D'Amato, C. J. (1975). Motor sensory cortex-corticospinal system and developing locomotion and placing in rats. American Journal of Anatomy, 143, 1-42.

Hofer, M. A. (1975). Studies on how early maternal separation produces behavioral change in young rats. Psychosomatic Medicine, 37, 245-264.

Hofer, M. A. (1980). Effects of reserpine and amphetamine on the development of hyperactivity in maternally deprived rat pups. Psychosomatic Medicine, 42, 513-520.

Horn, A. S., and Snyder, S. H. (1971). Chlorpromazine and dopamine: Conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs. Proceedings of the National Academy of Sciences of the United States of America, 68, 2325-2328.

Hornykiewicz, O. (1977). Biogenic amines in the central nervous system. In P.J. Vinken and G.W. Bruyn (Eds.), Metabolic and deficiency diseases of the nervous system, Part III (pp. 459-483). Amsterdam: North-Holland.

Horwitz, J. Heller, A., and Hoffman, P. C. (1982). The effect of development of thermoregulatory fuction on the biochemical assessment of the ontogeny of neonatal dopaminergic neuronal activity. Brain Research, 235, 245-252.

Iversen, S. D., and Koob, G. F. (1977). Behavioral implications of dopaminergic neurons in the mesolimic system. In E. Costa and G.L. Gessa (Eds.), Nonstriatal dopaminergic neurons (pp. 209-214). New York: Raven Press.

Jackson, D. M., Anden, N. E., and Dahlstrom, A. (1975). A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. Psychopharmacology, 45, 139-149.

Johanson, I. B., and Hall, W. G. (1979). Appetitive learning in 1-day-old rat pups. Science, 1979, 205, 419-420.

Johanson, I. B., and Hall, W. G. (1982). Appetitive conditioning in neonatal rats: Conditioned orientation to a novel odor. Developmental Psychobiology, 15, 379-397.

Johanson, I. B., and Teicher, M. H. (1980). Conditioning of an odor preference in 3-day-old rats. Behavioral and Neural Biology, 29, 132-136.

Jones, D. L., Mogenson, G. J., and Wu, M. (1981). Injections of dopaminergic, cholinergic, serotoninergic and gabaergic drugs into the nucleus accumbens: Effects on locomotor activity in the rat. Neuropharmacology, 20, 29-37.

Kellogg, C., and Lundborg, P. (1972). Ontogenetic variation in responses to L-dopa and monoamine receptor-stimulating agents. Psychopharmacology, 23, 187-200.

Kenny, J. T., and Blass, E. M. (1977). Suckling as incentive to instrumental learning in preweanling rats. Science, 196, 898-899.

Kelly, P., and Iversen, S. (1976). Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in rat. European Journal of Pharmacology, 40, 45-56.

Kirksey, D. R., Seidler, F. J., and Slotkin, T. A. (1978). Ontogeny of (-)3H norepinephrine uptake properties of synaptic vesicles of rat brain. Brain Research, 150, 367-375.

Klawans, H. L.,Jr. (1973). The pharmacology of tardive dyskinesias. American Journal of Psychiatry, 130, 82-86.

Klawans, H. L., Jr., and McKedall, R. R. (1971). Observations on the effect of levodopa on tardive lingual-facial-buccal dyskinesia. Journal of Neurological Sciences, 14, 189-192.

Kobayashi, R. M. (1977). Drug therapy of tardive dyskinesia. New England Journal of Medicine, 296, 257-260.

Kofman, O., Yeomans, J., and Whitefield, S. (1985). Elevation of medial forebrain self-stimulation thresholds following micro-injections of dopamine in ventral tegmentum. Neuroscience Abstract, 11, 718.

Kula, N. S., Baldessarini, R. J., Bromley, S., and Neumeyer, J. (1985). Effects of isomers of apomorphines on dopamine receptors in striatal and limbic tissue of rat brain. Life Sciences, 37, 1051-1958.

Lal, S., and Sourkes, T. (1973). Ontogeny of stereotyped behavior induced by apomorphine and amphetamine in the rat. Archives of International Pharmacodynamics and Therapeutics, 202, 171-182.

Lauder, J. M., and Bloom, F. E. (1974). Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation. Journal of Comparative Neurology, 155, 469-482.

Lee, E. H., and Barr, G. A. (1985). Behavioral activation in 3-day-old rats produced by electrical stimulation of the nucleus accumbens but not the neostriatum. Neuroscience Abstract, 11, 495.

Lindvall, O (1979). Dopamine pathways in the rat brain. In A.S. Horn, J. Korf and B.H.C. Westerink (Eds.), The neurobiology of dopamine (pp. 319-342). London: Academic Press,

Lindvall, O., and Bjorklund, A. (1978). Anatomy of the dopaminergic neuron systems in the rat brain. In P.J. Roberts, G.N. Woodruff and L.L. Iverson (Eds.), Dopamine (pp.1-24). New York: Raven Press.

Lindvall, O., Bjorklund, A., Moore, R., and Stenevi, U. (1974). Mesencephalic dopamine neurons projecting to neocortex. Brain Research, 81, 325-331.

Lithgow, T., and Barr, G. A. (1984). Self stimulation in 7- and 10-day-old rats. Behavioral Neuroscience, 98, 479-436.

Ljungberg, T., and Ungerstedt, U. (1978). Classification of neuroleptic drugs according to their ability to inhibit apomorphine-induced locomotion and gnawing: Evidence for two different mechanisms of action. Psychopharmacology, 56, 239-247.

Loren, I., Bjorklund, A., and Lindvall, O. (1976). The catecholamine systems in the developing rat brain: Improved visualization by a modified glyoxylic acid-formaldehyde method. Brain Research, 117, 313-318.

Lyness, W. H., Friedle, N. M., and Moore, K. E. (1979). Destruction of dopaminergic nerve terminals in nucleus accumbens: Effects on d-amphetamine self-administration. Pharmacology, Biochemistry and Behavior, 11, 553-556.

McGeer, E. G., Parkinson, J., and McGeer, P. L. (1976). Neonatal enzymic development in the interpeduncular nucleus and surrounding ventral tegmentum. Experimental Neurology, 53, 109-114.

Mercuri, N., Calabresi, P., Stanzione, P., and Bernardi, G. (1985). Electrical stimulation of mesencephalic cell groups (A9-A10) produces monosynaptic excitatory potentials in rat frontal cortex. Brain Research, 338, 192-195.

Miller, R. J. (1976). Comparison of the inhibitory effects of neuroleptic drugs on adenylate cyclase in rat tissues stimulated by dopamine, noradrenaline, and glucagon. Biochemical Pharmacology, 25, 537-541.

Mogenson, G. J., Takigawa, M., Robertson, J. A., and Wu, M. (1979). Self stimulation of the nucleus accumbens and ventral tegmental area of Tsai attenuated by microinjections of spiroperidol into the nucleus accumbens. Brain Research, 171, 249-259.

Moran, T. H., Lew, M. F., and Blass, E. M. (1981). Intracranial self-stimulation in 3-day-old rats. Science, 214, 1366-1368.

Moran, T. H., Schwartz, G. J., and Blass, E. M. (1983). Organized behavioral responses to lateral hypothalamic electrical stimulation in infant rats. Journal of Neuroscience, 3, 10-19.

Murrin, L. C. (1982). In vivo studies of dopamine receptor ontogeny. Life Sciences, 31, 971-980.

Murrin, L. C. Gibbens, D. L., and Ferrer, J. R. (1985). Ontogeny of dopamine, serotonin and spirodecanone receptors in rat forebrain - An autoradiographic study. Developmental Brain Research, 23, 91-109.

Nelson, D.L., Herbert A., Bourgoin, S., Glowinski, J., and Hamon, M. (1978). Characteristics of central 5-HT receptors and their adaptative changes following intracerbral 5,7-dihydroxytryptamine administration in the rat. Molecular Pharmacology, 14, 983-995.

Pardo, J. V., Creese, I., Burt, D. R., and Snyder, S. H. (1977). Ontogenesis of dopamine receptor binding in the corpus striatum of the rat. Brain Research, 125, 376-382.

Pedersen, P. E., Williams, C. L., and Blass, E. M. (1982). Activation and odor conditioning of suckling behavior in 3-day-old albino rat. Journal of Experimental Psychology, 8, 329-341.

Peroutka, S., and Snyder, S. (1979). Multiple serotonin receptors: Differential binding of 3H-5-hydrotryptamine, 3H-lysergic acid diethylamide, and 3H-spiroperidol. Molecular Pharmacology, 16, 687-699.

Phillips, A. G., Brooke, S. M., and Fibiger, H. C. (1975). Effects of amphetamine isomers and neuroleptics on self-stimulation from the nucleus accumbens and dorsal noradrenergic bundle. Brain Research, 85, 13-22.

Phillips, A. G., Carter, D. A., and Fibiger, H. C. (1976). Dopaminergic substrates of intracranial self-stimulation in the caudate-putamen. Brain Research, 104, 221-232.

Pickens, R., and Harris, W. C. (1968). Selfpstimulation of d-amphetamine by rats. Psychopharmacologica, 12, 158-163.

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

Pijnenberg, A. J. J., Honig, W. H. M., and Van Rossum, J. M. (1975). Antagonism of apomorphine and d-amphetamine-induced stereotyped behavior by injection of low doses of haloperidol into the caudate nucleus and nucleus accumbens. Psychopharmacologia, 45, 65-71.

Pijnenburg, A. J. J., and Van Rossum, J. M. (1973). Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. Journal of Pharmacy and Pharmacology, 25, 1003-1005.

Randrup, A., and Munkvad, I. (1974). Pharmacology and physiology of stereotyped behavior. Journal of Psychiatric Research, 11, 1-10.

Randrup, A. , and Scheel-Kruger, J. (1966). Diethyldithiocarbamate and amphetamine stereotyped behavior. Journal of Pharmacy and Pharmacology, 18, 752.

Raskin, L. A., and Campbell, B.A. (1981). Ontogeny of amphetamine anorexia in rats: A behavioral analysis. Journal of Comparative and Physiological Psychology, 95, 425-435.

Rebec, G. V., Bashore, T. R., Zimmerman, K. S., and Alloway, K. D. (1979). Classical and atypical antipsychotic drugs: Differential antagonism of amphetamine and apomorphine-induced alterations of spontaneous neuronal activity in the neostriatum and nucleus accumbens. Pharmacology, Biochemistry and Behavior, 11, 529-538.

Roberts, D. C. S., Zis, A. P., and Fibiger, H. C. (1975). Ascending catecholaminergic pathways and amphetamine-induced locomotor activity: Importance of dopamine and apparent non-involvement of norepinephrine. Brain Research, 93, 441-454.

Robertson, A., and Mogenson, G. J. (1978). Evidence for a role for dopamine in self-stimulation of the nucleus accumbens of the rat. Canadian Journal of Psychology, 32, 67-76.

Rolls, E. T. (1971). Contrasting effects of hypothalamic and nucleus accumbens septi self-stimulation on brain stem single unit activity and cortical arousal. Brain Research, 31, 275-285.

Rolls, E. T., and Kelly P. H. (1972). Neural basis of stimulus-bound locomotor activity in the rat. Journal of Comparative and Physiological Psychology, 81, 173-182.

Rose , G. H. (1968). Development of visually evoked electrocortical responses in the rat. Developmental Psychobiology, 1, 35-40.

Rosenblatt, J. S., and Lehrman, D. S. (1963). Maternal behavior of laboratory rat. In: H. L. Rheingold (Ed.), Maternal behavior in mammals. New York: Wiley.

Routtenberg, A., and Malsbury, C. (1969). Brainstem pathways of reward. Journal of Comparative and Physiological Psychology, 68, 22-30.

Routtenberg, A., and Sloan, M. (1972). Self-stimulation in the frontal cortex of rattus norvegicus. Behavioral Biology, 7, 567-572.

Rudy, J. W., and Cheatle, M. D. (1977). Odor-aversion learning by neonatal rats. Science, 198, 845-846.

Schaefer, G. J., Bonsall, R. W., and Michael, R. P. (1981). An easily constructed biphasic constant-current stimulator for intracranial self-stimulation. Physiology and Behavior, 25, 163-165.

Schmidt, R. H., Bjorklund, A., Lindvall, O., and Loren, I. (1982). Prefrontal cortex: Dense dopaminergic input in the newborn rat. Brain Research, 281, 222-228.

Seeger, T. F., and Gardner, E. L. (1979). Enhancement of self-stimulation behavior in rats and monkeys after chronic neuroleptic treatment: Evidence for mesolimbic supersensitivity. Brain Research, 175, 49-57.

Shaywitz, B.A., Yager, R.D., and Klopper, J.H. (1976). Selective brain dopamine depletion in developing rats: An experimental model of minimal brain dysfunction. Science, 191, 305-308.

Sherwood, N. M., and Timiras, P. S. (1970). A Stereotaxic Atlas of the Developing Rat Brain. Berkeley: University of California Press.

Shute, C.C.D., and Lewis, P.R. (1966). Cholinergic and monoaminergic pathways in the hypothalamus. British Medical Bulletin, 22, 221-226.

Simon, H., Stinus, L., Tassin, J. P., Lavielle, S., Blanc, G. , Thierry, A.M., Glowinski, J. and Le Moal, M. (1979). Is the dopaminergic mesocorticolimbic system necessary for intracranial self-stimulation? Biochemical and behavioral studies from A10 cell bodies and terminals. Behavioral and Neural Biology, 27, 125-145.

Smith, R. C., and Davis, J. M. (1976). Behavioral evidence for supersensitivity after chronic administration of haloperidol, clozapine and thioridazine. Life Sciences, 19, 725-732.

Snyder, S. H., Banerju, S. P., Yamamura, H. I., and Greenberg, D. (1974). Drugs, neurotransmitters and schizophrenia. Science, 184, 1243-1253.

Sobrian, S. K., Weltman, M., and Pappas, B. A. (1975). Neonatal locomotor and long-term behavioral effects of d-amphetamine in the rat. Developmental Psychobiology, 8, 241-250.

Spear, L., and Ristine, L. A. (1982). Suckling behavior in neonatal rats: Psychopharmacological investigations. Journal of Comparative and Physiological Psychology, 96, 244-255.

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

Stanley, M., and Wilk, S. (1977). The effect of antipsychotic drugs and their clinically inactive analogs on dopaminergic metabolism European Journal of Pharmacology, 44, 293-302.

Stein, L, and Ray, O. S. (1959). Self-regulation of brain current intensity in the rat. Science, 130, 570-572.

Stellar, J. R., Corbett, D., and Hamilton, A.L. (1985). Forebrain map of dopamine's relevance to lateral hypothalamic stimulation reward as based on intracranial neuroleptic injection. Neuroscience Abstract, 11, 48.

Stellar, J. R., Kelley, A. E., and Corbett, D. (1983). Effects of peripheral and central dopamine blockade on lateral hypothalamic self-stimulation: Evidence for both reward and motor deficit. Pharmacolology Biochemistry and Behavior, 18, 433-442.

Stinsus, L., Koob, G. F., Ling, N., Bloom, F. E., and Le Moal, M. (1980). Locomotor activation induced by infusion of endorphins into the ventral tegmental area: Evidence for opiate-dopamine interactions. Proceedings of the National Academy of Sciences of the United States of America, 77, 2323-2327.

Sullivan, R., and Brake, S. C. (1981). Reinforcement and activation in infant rats. Paper presented at the meeting of the International Society for Developmental Psychobiology, New Orleans, Louisiana.

Swanson, L. W., and Cowan, W. M. (1975). A note on the connections and development of nucleus accumbens. Brain Research, 92, 324-330.

Swanson, L. W., and Mogenson, G. J. (1981). Neural mechanisms for the functional coupling of autonomic, endocrine, and somatomotor responses in adaptive behavior. Brain Research Reviews, 3, 1-33.

Szechtman, H., and Hall, W. G. (1976). Ontogeny of oral behavior induced by tail pinch and electrical stimulation of the tail in rats. Journal of Comparative Psychology, 94, 436-445.

Taylor, J. R., and Robbins, T. W. (1985). 6-hydroxydopamine lesions of the nucleus acccumbens, but not caudate nucleus, attenuate enhanced responding for conditioned reinforcement produced by infusions of intra-accumbens d-amphetamine. Neuroscience Abstract, 11, 5.

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

Troiano, R., and Siegel, A. (1978). Efferent connections of the basal forebrain in the cat: The nucleus accumbens. Experimental Neurology, 61, 185-197.

Ungerstedt, U. (1971). Stereotxic mapping of monoamine pathways in the rat brain. Acta Physiologica Scandinavica, 82, 1-48.

Ungerstedt, U. (1971). Aphagia and adipsia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiologica Scandinavica Supplement, 367, 95-121.

Uzbekov, M.G., Murphy, S., and Rose, S.P.R. (1979). Ontogenesis of serotonin "receptors" in different regions of rat brain. Brain Research, 168, 195-199.

Vaccarino, F. J., Bloom, F. E., and KooB, G. F. (1985). Blockade of nucleus accumbens opiate receptors attenuates intravenous heroine reward in the rat. Psychopharmacology, 86, 37-42.

Valenstein, E.S., Cox, V.C., and Kakolewski, J.W. (1968). Modification of motivated behavior elicited by electrical stimulation of the hypothalamus. Science, 159, 1119-1120.

Valenstein, E.S., Cox, V.C., and Kalolewski, J.W. (1970). Reexamination of the role of the hypothalamus in motivation. Psychology Review, 77, 16-31.

Von Hungen, K., Roberts, S., and Hill, D.F. (1974). Development and regional variations in neurotransmitter sensitive adenylate cyclase systems in cell-free preparations of rat brain. Journal of Neurochemistry, 22, 811-819.

Wauquier, A., and Niemegeers, C. J. E. (1972). Intracranial self-stimulation in rats as a function of various stimulation parameters. II. The influence of haloperidol, pimozide, and pipamperone on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacologia, 27,191-202.

Westerink, B. H. C., and Korf, J. (1975). Influence of drugs on striatal and limbic homovanillic acid concentration in the rat brain. European Journal of Pharmacology, 33, 31-40.

White, F. J., and Wang, R. Y. (1983). Differential effects of classical and atypical antipsychotic drugs on A9 and A10 dopamine neurons. Science, 221, 1054-1057.

White, F. J., and Wang, R. Y. (1986). Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. Journal of Neuroscience, 6, 274-280.

Wise, R. A. (1978). Catecholamine theories of reward: A critical review. Brain Research, 152, 215-247.

Wise, R. A. (1980). Actions of drugs of abuse on brain reward systems. Pharmacology, Biochemistry and Behavior, 13, Suppl. 1, 213-223.