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*City University of New York* PH.D. 1982

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# THE DEVELOPMENT OF CENTRAL NERVOUS SYSTEM LOCI THAT SUPPORT SELF-STIMULATION IN INFANT RATS

by

Theodore Dale Lithgow

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.

1982

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.

April 23, 1982 — date

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April 29, 1982 — date

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## Abstract

THE DEVELOPMENT OF CENTRAL NERVOUS SYSTEM LOCI THAT SUPPORT SELF-STIMULATION IN INFANT RATS

by

Theodore Dale Lithgow

Advisor: Dr. Gordon A. Barr

The development and maturation of the nervous system of the rat and other altricial species predominantly consists of postnatal events. Behavior developing according to specific ontogenetic timetables suggests an intricate quantifiable link with neural ontogeny. New, easily acquired behavioral tests would provide additional dependent measures to assess this neural development.

The purpose of the present research was to determine whether very young animals could learn to nudge a pole using direct electrical stimulation of the brain as a reinforcer, and whether that self-stimulation could be manipulated pharmacologically. By sampling various forebrain loci with this self-stimulation technique (Experiment 1), changes in responding by pups were noted following acute administration of d-amphetamine, a pharmacological agent classically employed to facilitate self-stimulation in adults (Experiment 2). Results are summarized as follows:

(1) A reliable stereotaxic method for chronic electrode or cannula implant in rats was developed with mortality or loss of implant each below 20%.

(2) Using a two-pole test apparatus designed and constructed in the laboratory, stimulus control of self-stimulation behavior was demonstrated in 27 and 31% of 7- and 10-day-old pups, respectively.

(3) Using only 10-day-old pups with electrodes targeted for the medial forebrain bundle (MFB), self-stimulation rates increased following 5.0 mg/kg of d-amphetamine, but not after saline or a 1.0 mg/kg dose. The 5.0 mg/kg treated pups showed both differential facilitation of responding and behavioral activation, while 1.0 mg/kg pups showed only the latter.

(4) Similar response patterns were shown for drug-treated pups when subject populations were separated on a histological basis, e.g., only pups with electrodes localized in the MFB or nucleus accumbens.

These findings suggest that the immature nervous system will mediate behaviors characteristic of the adult, and that this particular behavior is sensitive to acute pharmacological challenge and possibly chronic pharmacologic treatment. These results extend recent investigations regarding the degree of function by the nervous system during ontogeny, suggesting neonatal self-stimulation as a useful behavioral metric to reflect the functional maturity of the developing nervous system.

## Acknowledgements

There are clearly many dear friends and family without whom this work would not have been possible. If I have learned anything throughout this course of training, it is that one cannot do anything of substance alone. Since it is not possible to name the contribution of each and every individual, I have thanked most personally and record here special public acknowledgement.

First, I thank each of my committee members; namely, Drs. Gordon Barr, Richard Bodnar, Gerald Turkewitz, and Robert Thompson. I eagerly absorbed neuroanatomy from Rich, while I gained valuable developmental insights from Gerry, both of whom always seemed to be available for help when needed.

Quite honestly, I feel fortunate for the opportunity to have worked both for and with Bob Thompson, to whom I am indebted for instilling a keen appreciation for experimental method. The time and effort that he extended to me throughout both requisite curriculum and research gave shape to many ideas that may never have seen fruition without a little extra effort on his part. Though I consider myself as one of his students, he always sincerely treated me as a professional and colleague, and if only for that I am truly grateful.

Certainly each of knows the love and understanding given freely by those who care deeply about us. All of

this would not have been possible without personal friends, parents and relatives. When the strain was too much, the weight too heavy, or the nights endless, they were always there for whatever was needed. If it were possible, Jane's name would be included on this document, since she spent the better part of these last four years supporting me, both emotionally and physically. Her dreams of my completion and success were complemented only by my own. We both spent four, difficult, though frequently joyous, years together. Perhaps the most important statement that we can make about those years was that we shared them. In addition, I would especially like to thank Theodore M. and Gertrude Lithgow, who supported me as teachers, grandparents, friends, and loved ones. Their lives are a tribute to standards to live by, and represent many of the ideals that are important to me. One day I shall tell my own children how fortunate I truly was to share the love that they gave.

Of course, I cannot omit Ola 'Chuckles' Aroyewun, Chris 'Larry' Capuano, Stuart 'She's awesome' Reigler, and Diane Gerow, all of whom made the laboratory a professional, though sometimes bizarre, environment. Their time, effort, and constant bitching invested in the shaping of ideas pervades these studies. The majority of this manuscript was professionally typed and proofed by Diane, though what she has given to me and done for me extends beyond anything that I could return in kind.

Admittedly, I have saved the most important acknowledgement for last. I have thought a long time about how best to describe the sheer amount of time, effort, and soul put into this work by Gordon Barr. Trips to the laboratory at midnight, the smuggling of rats and perfused brains on the subways, calls at home or office meetings only to make sure that work was progressing, all characterize Gordon and the professionalism that he exemplifies. He was and is not only a tireless professional and mentor, but also a damn good friend and confidant. I had only to ask and he was there, early morning or late evening. If my students or peers have a fraction of the respect for me as much as we at Hunter College respect Gordon, then I will consider myself both fortunate and successful.

Socrates: Then anyone who leaves behind him a written manual, and likewise anyone who takes it over from him, on the supposition that such writing will provide something reliable and permanent, must be exceedingly simple minded...

Plato (424?-347 B.C.), Phaedrus 275 C.

Be obscure clearly.

Anon.

No matter what you plan to do, how you plan to do it, or with whom you plan to do it, I will support you.

T.T. Lithgow, Jr., 1982.

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## GENERAL INTRODUCTION

A major thrust in developmental neurobiology is a description of the complex interactions that ultimately result in the expression of the adult nervous system. An assessment of the coding of the nervous system by neurotransmitters, as well as the role of other genetic and environmental factors, is one attempt to describe these complex interactions. Recent investigations that have been conducted to explore the neurochemical basis of behavior attest to the practicality of this method. Specifically, changes in several behaviors, e.g., spontaneous motor activity (Gottlieb, 1973; Sedlacek, 1978), stereotyped behavior (Lal & Sourkes, 1973), aggression, (Kety, 1970, 1972), and others have been correlated with changes in the levels and metabolism of a few well known neurotransmitters such as norepinephrine (NE), dopamine (DA), serotonin (5-hydroxytryptamine, 5-HT), acetylcholine (ACh), and gamma-aminobutyric acid (GABA).

Reviews of the structural and histochemical composition of the brain during development emphasize the intimate relation that exists between the duration of postnatal neurogenesis and the degree and temporal course of the development of function and behavior (Lanier, Dunn, & Van Hartesveldt, 1976; Pradhan & Pradhan, 1980). Though neurogenesis or neurotransmitter maturation and ontogeny of behavior must be inextricably linked, until the last decade

(e.g., Gottlieb, 1973), little research attempted to delimit the ontogeny of each component. Studies on the developmental aspects of these two components (Pradhan & Pradhan, 1980) have generally taken one of two directions: either 1) examination of the development of certain neurotransmitters and their relation or correlation with the development of a behavior or, 2) study of long term neurobehavioral effects in subjects following an insult during their development with a chemical known to affect the metabolism of a neurotransmitter, the behavioral teratological experiment (Coyle, Wayner, & Singer, 1980; Thornburg & Moore, 1976; Slotkin & Thadani, 1980).

Recent texts (e.g., Cooper, Bloom, & Roth, 1978) and reviews of the nature of neurotransmission (Krnjevic, 1974) have implicated catecholamines not only as important behavioral determinants in adults, but as possible neurotrophins (Ramon y Cajal, 1928) that may guide the developing nerve fibers to their targets during ontogeny (Lawrence & Burden, 1973; Wendlandt, Crow, & Stirling, 1977) and as regulators of cellular differentiation during ontogenesis (Lauder & Bloom, 1974; Olson & Seiger, 1972; Porcher & Heller, 1972). Also, reward theorists have posited a role for catecholamines in central reward theories of motivation. In the last decade and a half, thinking about reward systems has been greatly influenced by the notion that there exists one or more central neural

systems which are specialized for, and which play a critical role in reward phenomena, a set of catecholamine-containing neurons (for reviews, see Crow, 1972, 1973; German & Bowden, 1974; Poshel & Ninteman, 1963; Stein, 1962). Most pharmacological treatments that affect catecholamines exert similar effects on DA and NE, two important catecholamines; hence, this lack of pharmacological specificity has made the distinction of which catecholamine is critical a difficult one (Stein, 1978). However, selective blockade of either NE synthesis (Stein, Belluzi, & Wise, 1977; Wise, Belluzi, & Stein, 1977; Wise & Stein, 1969, 1970) or DA receptors (for review, see Wise, 1978, 1981) marshalls support for both NE or DA, respectively, as the critical substrate for intracranial self-stimulation (ICSS) reward. In as much as the study of brain mechanisms of reinforcement has largely been dominated by ICSS studies, there has been far less interest in the ontogeny of such reinforcement mechanisms.

Here one is presented with an interesting paradox. On one hand, a plethora of histological and morphological data on monoamine development suggests that all of the requisite data for functional development are available. Regrettably, this neurological surfeit does little to explicate the role of these neurons in the ontogeny of behavior. As outlined below, at birth in the rat and other altricial species, young are born neurologically and behaviorally immature.

Neurologically, the brain is morphologically incomplete and increases in both weight and complexity during the postnatal period (for review, see Coyle, 1973, Tennyson, 1970). The development of catecholamine mechanisms, like growth and differentiation, are predominantly postnatal events. Most postnatal neuronal growth is that of dendritic and axonal proliferation and terminal synaptogenesis (Olson & Seiger, 1972). In fact, the newborn rat differs markedly from the adult both in the extent of development of the monoamine terminals and in the size and intensity of fluorescent cell bodies (Loizou, 1972; Loizou & Salt, 1970). Indeed, it is apparent that we have collected a great deal of information concerning the maturation of individual monoamine components (for review, see Lanier et al., 1976; Thornburg & Moore, 1976).

Behaviorally, neonatal rats display only integrated bulbospinal reflexes involved with respiration, crawling, righting, suckling, etc.; behavior modulated by the progressive evolution of more complex neural circuitry that exerts actions upon these spinal reflexes (Scheibel & Scheibel, 1971). These limited sensory and motor capabilities of the neonatal rat (Almli & Sudarshan, 1975; Bolles & Wood, 1964) have impeded studies of the development of learning and motivation in young animals. Until the recent demonstration of an operant design using neonatal rat pups (Johanson & Hall, 1979), behavioral

activation and motivational studies were limited to 1) dependent measures such as suckling (Blass, Beardsley, & Hall, 1979; Kenny & Blass, 1977), and maze running (Amsel, Letz, & Burdette, 1977), or 2) physical insult such as brain transections (Kornblith & Hall, 1979) or lesions (Aimli, 1978).

Knowing the developmental patterns and anatomy of neurotransmitter systems and developmental timetables of their synthetic machinery provides a basis for determining when particular neurotransmitters begin to function in neural communication. In the present studies, a new approach to understanding this functional development is proferred. The ICSS technique has been used as a method of assessing neurotransmitter function in adult animals. In light of the variety of nonspecific effects of drug or neurotoxin treatment in adult or neonatal animals, ICSS may be a method of choice in elucidating the time course of the functional maturation of neurotransmitters that mediate such ICSS behavior, provided that the neonatal animal can perform simple operants such as nudging a pole to obtain electrophysiological brain stimulation. The use of self-stimulation in the present design is predicated on the assumption that some neurotransmitters, e.g., NE or DA, do in fact mediate ICSS behavior. Since levels of these neurotransmitters and their synthetic, regulatory, and degration components are low at birth and increase steadily

to adulthood, this technique may provide an additional behavioral metric with which to determine when neurotransmitters are capable of mediating adultlike behaviors. The performance of these animals relative to the implant site should reflect the functional integrity of the nervous system. The development of reliable methods to stereotaxically implant and subsequently test immature animals in a paradigm that is amenable to acute pharmacological or teratological manipulation may provide additional insights into the ontogeny of neural functioning.

To attempt this assessment, many of the methodological and technical difficulties in neonatal implantation research, including both surgical and behavioral obstacles, needed to be overcome. Subsequently, 7- and 10-day-old rat pups were chronically implanted with bipolar electrodes, tested 16 to 24 hr postoperatively, and sacrificed immediately following testing. The specific objectives were:

1) To develop methods for the difficult procedure and unique problems of chronic electrode implantation in immature rat brains.

2) To develop an economical yet quantitative technique to measure an easily performed operant for young rat pups.

3) To determine the time course of the development of self-stimulation behavior. This assessment initially was performed in the medial forebrain bundle (MFB), which contains transmitters other than catecholamines but supports ICSS. The MFB implants were followed by an attempt to map behaviorally positive, neutral, or aversive sites in the forebrain.

4) To determine if 10-day-old rat pups were behaviorally responsive in the present paradigm to acute d-amphetamine treatment.

# PART I: DEVELOPMENT OF METHODS

## Stereotaxic Method for Chronic Electrode Implantation of Infant Rat Pups

Electrical and chemical stimulation of localized brain regions in adult animals has enjoyed wide favor among behaviorists, neurochemists, and electrophysiologists. Recent advances in developmental psychology and psychopharmacology have stimulated an interest in the developing brain of neonates, resulting in at least three stereotaxic atlases for neonatal and infant rats, of age 3 days (Heller, Hutchens, Kirkby, Karapas, & Fernandez, 1979), 10, 21, and 39 days (Sherwood & Timiras, 1970), and 1, 7, and 14 days (Valenstein, Case, & Valenstein, 1969). Recent developmental investigations have centered around whole brain transmitter depletion (Smith, Cooper, & Breese, 1973; Lytle, Shoemaker, Cottman, & Wurtman, 1972) lesion techniques (Almli, 1978; Almli & Fisher, 1977; Almli & Golden, 1976; Cheronis, Erinoff, Heller, & Hoffman, 1979; Johnson, Poplawsky, Lancaster, & Jackson, 1974; Johnson, Poplawsky, Bielauskas, & Liebert, 1972; Kornblith & Hall, 1979) or chronic electrode recording (Almli, Forbes, Henault, Velozo, & Morgane, 1980). Though ICSS has been reported for young domestic chicks (Andrews, 1967) and beagle puppies (Bacon & Wong, 1972), no brain stimulation studies were reported in the literature for neonates or

infant rats before the development and implementation of the the following method (Lithgow & Barr, 1982).

The rationale for the development of a technique to stimulate, either electrically or chemically, localized brain regions in young animals stems from studies demonstrating that early lesions of neocortical structures may produce behavioral changes that are different, or even opposite, to the changes produced by later lesions (e.g., Isaacson, 1968). In addition, numerous studies indicated that steroid hormones did not exert the same effect on the hypothalamus at different ages (e.g., Valenstein, 1968). Hence there was a need to generate an atlas in order to ablate, and possibly stimulate, specific hypothalamic nuclei in the very young animal (in conjunction with hormonal manipulations). Valenstein et al. (1969) summarily developed such a stereotaxic atlas by placing marker lesions and perfusing the animals for histology a few days later, as well as ancillary techniques for the 1, 7, and 14-day-old rat pup, primarily because this age period was believed to be critical for hormonal differentiation of hypothalamic mechanisms.

Following this stereotaxic development, several papers by Johnson and coworkers described both parameters for particular lesions and techniques for enhancing the survival of infants following surgery, as well as emphasizing the maintenance of animals for rather long

periods of time for behavioral observation during development. Specifically, these studies produced partial septal lesions (Johnson, 1972; Johnson, Bieliauskas, & Lancaster, 1973; Johnson et al., 1972), and caudate lesions (Johnson & Becker, 1973; Johnson, Poplawsky, Lancaster, & Jackson, 1974).

Procedures used in these studies described above were similar to or modifications of Valenstein et al. (1969). A brief communication (Johnson et al., 1974) later added data on amygdaloid and hippocampal lesions, as well as outlining a variety of specific problems and considerations in stereotaxic placements of subcortical lesions in 7-day-old rats, especially with respect to a great variability and irregularity in the spread of lesions relative to adults.

While Johnson et al. (1974) essentially used the procedure described by Valenstein et al. (1969), Sherwood and Timiras (1970) introduced a more detailed atlas of the developing rat brain and a stereotaxic method for electrode placement in 10-, 21-, and 39-day-old rat pups. Unfortunately, this procedure was difficult to apply to animals younger than 5 days of age (Johnson et al., 1974). While studying the functional development of the dopaminergic nigrostriatal projection, Heller and coworkers designed and described a head holder for stereotaxic electrode placement in the neonatal rat (Heller et al., 1979). Problems involved in holding the neonatal head in a

stereotaxic coordinate system were overcome by use of an elegant apparatus which held the head by means of a mouth bar anteriorly and a curved needle inserted in the foramen magnum posteriorly. The stereotaxic device and atlas that resulted from its application were used in 3- to 10-day-olds to successfully determine the age at which the biochemical responses of nigrostriatal neurons to physical (Erinoff & Heller, 1978; Cheronis et al., 1979) or pharmacological (Cheronis et al., 1979) manipulation were similar to those of adult neurons (Stock, Magnusson, & Anden, 1973).

Note that all aforementioned studies described stereotaxic techniques for lesion placement, and never for stimulation experiments, either electrical or chemical. Much of the inherent difficulty of such a stimulation technique lies in the fluctuant physical nature of pup morphology during growth and development.

As a prelude to the actual dissertation experiments, the present work describes a simple, inexpensive technique that can be employed for any of a variety of stereotaxic implants, such as chronic electrodes or cannulae. The proposed stereotaxic technique, wholly designed, modified and perfected in our laboratory, can be presented in two phases, i.e., construction of a form-fitted mold for holding the rat pup and the actual surgical implantation of the electrode or cannula.

## Method

**Subjects.** The subjects were the offspring of Long Evans Hooded rats (Blue Spruce Company, Altamont, N.Y.) mated in our laboratory. The parent animals were housed in plastic tubs or rectangular 10 gal. aquaria in a colony room with a 12 hr light/12 hr dark lighting cycle and maintained at 22 - 25° C. Dams were fed Purina Lab Chow and water ad libitum. Beginning 2 weeks after mating, cages were checked twice daily at approximately 0800 and 1700 hr. Pups found on that day at either time were termed 0 days of age. Litters were culled to eight pups on Day 1, by randomly discarding pups after runts were first removed. Beginning on the day of implant, pups were weighed and marked with indelible ink.

**Construction of form fitted mold.** The construction of the form fitted mold can itself be divided into two parts. In the first, a plastic soap-type dish or container (ca. 10 x 6 x 3 cm) is filled with fast setting dental stone (Modern Materials Dentstone, Henry Schein Inc., Port Washington, N.Y.) and form fitted to the general shape of the pup's body. The stone form is molded to the pup body dimensions by anesthetizing a pup, lubricating the pup body with grease or vaseline, and placing the pup into the wet, fast-setting stone for approximately 30 sec. This stone provides a permanent but only approximate mold that can be reused indefinitely for stereotaxic surgery. The second

part of the mold construction attempts to more firmly fit a pup for stereotaxic implantation. Following the removal of the pup the now permanent stone mold is filled with approximately 45-50 ml of a fast-setting alginate impression material (Caulk Jeltrate, L.D. Caulk Co.) into which a pup is similarly form-fitted, with the head in a level position with skull landmarks bregma and lambda in the same plane. The alginate may be mixed in any small, suitable container, in a ratio of approximately one part alginate to four parts cold water. Warmer water may be used to speed hardening. When the wet alginate is pushed over the sides of the pup's head, this hardened form offers a firm yet flexible mold that does not necessitate additional restraints to keep the head in proper position or alignment, though care must be taken to position the pup parallel to the sides of the plastic container to facilitate correct alignment in the stereotaxic instrument. Figure 1 presents a photograph of stone and alginate forms used at various ages. This alginate form may be reused repeatedly over the course of a single day. Pups to be implanted on the same day that are not exactly the same size as the original pup used for the mold construction will fit equally well as the original since the flexible alginate will generally conform to the shape of the new pup. However, its firm, flexible character depends upon a colloid that dehydrates, making the alginate unusable after 24 hrs.

Figure 1. Photograph of stone and alginate forms used to restrain and position pups for stereotaxic surgery. Molds at various stages of completion are shown for 3-, 7-, and 10-day-old pups.

10
7
5

Implantation Procedures. The pup to be implanted is anesthetized using slight alterations of Sodium Pentobarbital (Nembutal) doses from Sherwood and Timiras (1970) and placed into the form-fitted mold. The entire pup mold, including stone and alginate form, as well as the anesthetized pup, is positioned in a stereotaxic instrument (David Kopf Instruments, Model 1430). The entire arrangement is positioned beneath the stereotaxic instrument is shown in Figure 2. Frequently the plane between skull landmarks lambda and bregma is difficult to adjust exactly to the horizontal as the wet alginate sets. This adjustment may be facilitated after the mold has hardened by drilling holes at various heights in the sides of the plastic dish and into the inner stone base, through which the stereotaxic ear bars are placed to position the entire arrangement firmly in the stereotaxic instrument. Once the anesthetized and restrained pup is positioned beneath the stereotaxic instrument, the skin covering the cartilaginous skull is cut away to reveal the skull landmarks. Visualization of bregma is difficult but is aided by staining with a drop of commercially available green food coloring. At this point the plane between the two skull landmarks is adjusted to the horizontal by placing the ear bars in one set of the symmetrically drilled sets of holes in the stone base. The coordinates for implantation into 7- and 14-day-old pups are taken from Sherwood and Timiras (1970).

Figure 2. Photograph of stone and alginate composite mold positioned in the stereotaxic device for electrode implantation. Note that the horizontal plane is adjusted by positioning the plastic mold base in symmetrically pre-drilled holes, and by using the nose clamp and tooth bar to firmly set the mold in place.

Chronic Electrode/Cannula Placement. The skull of 10-day-old animals may be penetrated by pressure of the electrode only, without prior drilling (Heller et al., 1979). Our experience, however, like that of Johnson and coworkers (1974) has shown considerable compression of the cranium in the process which may result in distortion or inaccuracy in placement, as well as cortical or subcortical brain damage. Therefore, the accuracy in stereotaxic placement of electrodes (Plastic Products, MS303/2 and MS303/3) in the present method depends upon the integrity of the dura mater from which dorsal-ventral coordinates are measured. We elect to drill electrode holes, remeasure coordinates and implant.

Following the cutting of the skin to reveal the skull, two stainless steel screws (Small Parts Inc., 0 x 80 x 1/8 in.) are mounted the skull, approximately 5 mm from the electrode locus. The electrode base is then cemented to the screws and skull using a thin layer (ca 0.5 mm) of a resinous dental medium, Caulk Grip Cement (L.D. Caulk Co.). Unlike the rigid binding offered by standard dental acrylic, this cement gives a firm yet slightly pliable adherence to the skull. This slightly pliable property of the Grip Cement to the skull cannot be emphasized too strongly, since its binding properties offer a compromise between strong, rigid adherence and flexibility to the pressure and torque exerted upon the electrode when, for

example, the pup is connected to a suitable commutator for stimulation. As an alternative to the Caulk Grip Cement, we have used an ethyl cyanoacrylate cement (Permabond International Corp., Englewood, N.J.) with satisfactory results. In either case, this initial grip or cyanoacrylate cement layer is followed by a layer of standard dental acrylic (L.D. Caulk Co.) capping the entire skull and sides of the exposed electrode. No sutures are required. The entire implantation procedure requires approximately 30-45 min. for a practiced technician.

## Results

The implant procedure described above yields an economical simple technique for preparing very young pups for brain stimulation studies. This technique is useful when evaluated in terms of the small proportion of surgically related deaths or the number of electrodes lost after implant.

Table 1 presents data for rats implanted at four different ages. Of 154 animals implanted, only two lost electrode caps before testing. Only 27 pups (16.8%) died using the technique, though this percentage has recently been dramatically reduced (see Discussion).

Electrode targets included the MFB as well as a variety of forebrain structures. These loci have been plotted and described in detail below (see Experiment 1).

Table 1

Results for Pups Implanted at Four Different Ages

| | Pup Age (Days) | | | | |
|---|---|---|---|---|---|
| | 3 | 6-8 | 9-11 | 14-15 | Totals |
| | Total Number of Pups | | | | |
| Animals Implanted | 5 | 40 | 59 | 50 | 154 |
| Surgically Related Death | 0 | 5 | 16 | 6 | 27 |
| Faulty Electrode Cap | 0 | 2 | 0 | 0 | 2 |

Most placements were easily targeted in the medial-lateral and dorsal-ventral planes, while anterior-posterior localization was more difficult. Electrode placements were generally anterior of target structures, especially in 10-day-olds. This anterior localization underscores the importance of using valid criteria for bregma, e.g., as described by Valenstein et al. (1968), in rat pups with skull sutures that have not fully closed. In addition, these placements emphasize the importance of carefully measuring and adjusting the horizontal plane between lambda and bregma.

## Discussion

It is important to note that our procedure requires that animals be connected to commutators and tested only once following surgery. Other procedures that may require repeated connecting or disconnecting from electrical leads may yield a greater proportion of caps that are pulled or twisted off the animal's head.

By far the most important consideration in the procedure is the number of animals lost due to surgical complications or electrode placement. Neither Heller et al. (1979) nor Valenstein et al. (1969) reported the survival rate of their animals, but Johnson et al. (1974) found that for most experiments three times as many animals need to be produced in order to approximate the required number of experimental animals, after deaths and reclassifications on histological basis.

The majority of animal deaths were probably directly related to both the type and dose of anesthetic. In contrast to the present use of Nembutal (3.0 mg/100 g), other laboratories have employed slightly stronger doses of Nembutal (Sherwood & Timiras, 1970), sodium pentobarbital and chloralhydrate (Valenstein, 1961, 1969), or ether (Johnson et al., 1974; Heller et al., 1979). We have noted a significant reduction in the number of surgically related deaths if pups are administered a small dose (1.0 ml/100 g

of a 0.3% solution) of 3,3-methylethylglutarimide (Mikedimide, Ortho Chemical Co., Englewood, N.J.) postoperatively. In fact, a recently completed study in our laboratory that employed the use of Mikedimide postoperatively in 10-day-olds yielded only a single surgically related death in one animal of 56 implanted (see Experiment 2). However, data from animals administered such drugs, especially when such data has been collected from seizure or kindling thresholds, etc., should be interpreted cautiously. An effective, yet safe anesthetic for pups will inevitably reduce the number of animals lost in the present procedure.

The success of such a procedure as outlined in the present paper depends upon numerous factors mentioned above, as well as the familiarity of the technician with the method. The present procedure can be quite successful with very little practice and as such, offers a simple and economical technique for implanting chronic electrodes or cannulae in neonatal or infant rats. This stereotaxic procedure makes it possible to explore the neonatal rat brain with electrodes or cannulae for electrical or chemical stimulation, recording of neuronal activity, or stereotaxic placement of destructive lesions. Like Johnson et al. (1974), the degree of accuracy in placement appears to be slightly more variable (cf., Heller et al., 1979) to that seen in the adult.

## Operant Paradigm and Test Apparatus

The limited sensory and motor capabilities of the neonatal rat (Almli & Sudarshan, 1975; Bolles & Woods, 1964) make the development of learning and motivation difficult to study. Most animal studies have thus addressed questions that relate to the development of ingestive behavior and a number of related ontogenetic issues, including the mechanisms that control suckling, the processes underlying weaning, and the characteristics of developing motivational systems.

To the extent that suckling is a motivated act (Amsel, Burdette, & Letz, 1976; Kenny & Blass, 1977) this behavior has been a topic of choice in an ontogenetic analysis. Even so, suckling does not appear to be analogous to adult ingestion as there are few physiological controls of suckling (Hall, Cramer, & Blass, 1977; Hall & Rosenblatt, 1977). But suckling, like other complex behaviors, has both consummatory (Blass et al., 1979) and appetitive (Amsel et al., 1976; Kenny & Blass, 1977; Kenny, Stoloff, Bruno, & Blass, 1979) components.

The appetitive component has been divorced from the consummatory aspect by requiring neonatal rats to approach anesthetized dams from a distance in order to suckle. For example, the running time to approach an anesthetized mother in a straight alley was shown to decrease over successive trials (Amsel et al., 1976). In a Y-maze

discrimination task, 7- to 23-day-old pups preferred nonnutritive suckling of the anesthetized mother in one goal area relative to rooting into a gauze-covered ventrum in an alternate area (Kenny & Blass, 1977). In a similar Y-maze discrimination, nutritive suckling was preferred over nonnutritive suckling, reflected by pup's appetitive behavior at 17 and 21 days of age but not at 10 and 12 days (Kenny et al., 1979).

The neuroanatomical origins of ingestive behavior have also been investigated. Based on deficits in suckling behavior, lateral hypothalamic lesions in pre- or postweanling rats produced aphagia or adipsia similar to that seen in adults (Almli, 1978; Almli & Golden, 1976; Lytle & Campbell, 1975). Using an intraoral cannula implantation technique (Hall & Rosenblatt, 1977), milk intake was severely reduced in 3-day-old pups with diencephalic transections. In contrast, the behavioral activation that accompanies milk intake tended to decline as transections were made at more caudal, e.g., mesencephalic, levels (Kornblith & Hall, 1979). Interestingly, such a dissociation of feeding and deprivation-induced activation has been found in adult rats (Campbell & Baez, 1974; Mabry & Campbell, 1975).

Thus, these and other recent studies (Rudy & Cheatle, 1977; Brake, 1978) of the development of learning and motivation have revealed unexpected capabilities of infant

rats. Unfortunately, most paradigms such as those described above are suited only to pups at least 7 days old because younger animals do not readily locomote. But, even 1-day-old rat pups can learn an appetitive response and can use this response in making (and later reversing) a two-choice discrimination (Johanson & Hall, 1979). This experiment underscores the notions that not only are neonates, even newborns, sensitive to the consequences of their behavior, but given an operant that is age and task appropriate, the neonate may not only be activated or aroused, but also may respond differentially. Even so, such a procedure can create interpretational problems, owing to the fact that milk as a reinforcer may actually elicit the response (cf., Johanson & Teicher, 1980). Regrettably, these elegant though simplified behavioral measures that are age and task appropriate are also highly activating. Indeed, the activational component has been shown following milk reinforcement (Hall, 1979a,b), stroking or tail pinch (Sullivan & Brake, 1981), or self-stimulation (Moran et al., 1981; Moran, personal communication). Even so, this paradigm provides an excellent means of examining relationships between stimuli, responses, and reinforcers during the ontogeny of specific behaviors.

In the context that neonates may indeed be sensitive to the consequences of their behavior, the present study

based its operant design and methodological protocol on a series of papers by Hall and coworkers (Hall, 1979a, 1979b; Hall & Bryan, 1980; Johanson & Hall, 1980). Pups are initially more active in a cool environment than a warm environment (Fowler & Kellogg, 1975; Johanson, 1980) although they gradually become hypothermic and inactive at cool temperatures. Hall and Bryan (1980) noted that milk intake under these conditions was related to the length of food deprivation. More importantly, in terms of the present series of experiments, this milk intake in pups less than 9 days old was marked by mouthing, probing, rolling, and vigorous locomotion, but only when pups were fed at a warm temperature.

A subsequent study (Johanson & Hall, 1980) specified the nature of important thermal determinants on both ingestion and the behavioral activation induced by feeding. Though the deprivational state alone of the pup influenced the spontaneous frequency of mouthing, licking, and probing (Hall, 1979b) high temperatures stimulated even higher levels of locomotor activity, as well as mouthing and probing. In essence, deprivation and temperature interact to alter pups' activity. Deprived 3- and 6-day-old pups were more behaviorally responsive to high temperatures than were nondeprived pups. At 12 days of age, pups' activity levels were less dependent on the effects of environmental temperature and deprivation. In addition, low body

temperature per se did not account for suppressed behavior in pups fed at cool temperatures. Though body temperature did affect pups' responses to food, these effects were not as consistent as ambient temperature (Johanson & Hall, 1980).

Studies by Hall and coworkers have outlined factors that are prerequisite for a study of the ontogeny of a behavior. A simple task such as nudging or pushing a paddle (Hall, 1981) conducted in a warm, humidified chamber (e.g., Johanson & Hall, 1980) will certainly enhance the ability of the pup to demonstrate substantive, learned behavior on a particular task, such as the response requirement for direct electrical brain stimulation in the present experiment. The present procedure was an adaptation of Hall's operant design, modified for pups 7 and 10-14 days of age.

## Test Apparatus

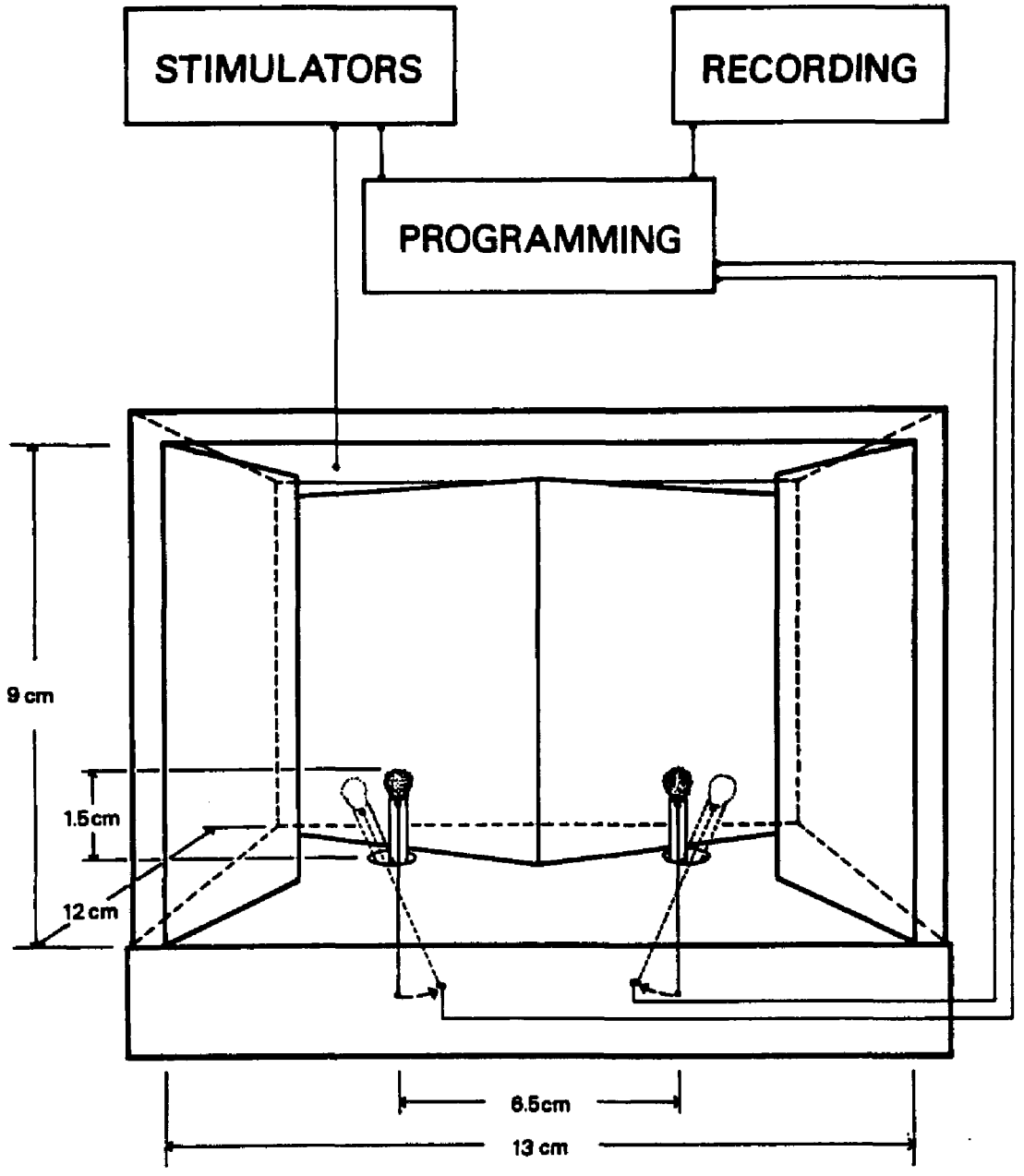
Figure 3 presents a diagram of the test chamber, manipulanda, and associated instrumentation. Following an 18-24 hr postoperative recovery period, pups younger than 15 days of age were placed in a 9 x 12 x 13 cm plexiglass chamber into which two gauze-covered stainless steel rods (1 mm diam) project approximately 2 cm vertically through the floor of the chamber to serve as response poles. Each pole is placed in a corner in the back of the cage relative to the chamber door and approximately 1 cm from the two

adjacent chamber walls. A layer of gauze covered the rear half of the chamber floor. The vertical poles exit the cage floor and extend through the aluminum base (ca 40 x 20 cm) on which the chamber is mounted. The aluminum base was drilled and tapped to hold a standard plastic lamp cover, through which the steel rod was seated by a soldered washer base and swiveled freely in any direction. Beneath the aluminum base was an inverted pendulum-type mounting for each pole that resulted in a switch closure whenever the pole was nudged so that its minimum excursion was approximately $10^{o}$ or more in any direction. The entire apparatus, i.e., chamber, aluminum base, and mounting for the omnidirectional switch, was mounted on legs approximately 12 cm in height. The entire testing apparatus was placed in a sound attenuating chamber and the ambient temperature was varied from room ($22^{o}$ C) to warm ($35^{o}$ C) temperatures using a small aquarium heater placed on the floor of the chamber.

## Testing Procedure

This arrangement allowed the manipulation of one of the two poles (S+) that enter the experimental chamber to result in the delivery of an electrical stimulus to the implanted pup via a commutator and electrode connector attached to the top of the chamber. The electrical stimulus was a 60 Hz, 250 msec pulse train of 125 uA intensity. Alternatively, manipulation of the other pole

Figure 3. Two pole apparatus and associated instrumentation used for testing 7- and 10-day-olds. The two scented response poles served as omnidirectional switches when nudged or manipulated by the pup.


STIMULATORS
RECORDING
PROGRAMMING
9 cm
1.5cm
12 cm
6.5cm
13 cm

(S-) had no effect, although responses were counted as for the S+ pole. To maximize the ability of the pups to discriminate the active from the inactive (S-) pole, these poles were scented with either lemon or sesame oil. Both odor and pole position were counterbalanced. The session was initiated by plugging the electrode connector in place, then positioning the pup into the center of the chamber midway between the two poles and facing the rear of the chamber. Responding by pups was not shaped. Responses were counted on both the active and inactive pole for each hour of the session. A t-test ($p<.05$) was used to compare significant differences between the two poles for the last 8 hr of the session. This test was not intended as a statistical test, but rather as an objective criterion by which to determine a response bias on the active or inactive pole. The aversiveness of the stimulation was behaviorally defined by the characteristic squealing, rolling, curling, and/or severe jumping or withdrawal from the response pole.

It was our experience that very young pups are impeded by thick electrode cables and commutators that generate considerable torque relative to the size of the pup. In fact, these animals worked well with electrode cables that have been stripped of the metal spring covering and plastic tube that encases the leads.

The total number of responses every 60 min for a 10 hr period were counted. This lengthy experimental session was necessary since the number of responses emitted by very young pups was dramatically reduced relative to older pups. In addition, a similar experimental arrangement (Johanson & Hall, 1979) with young pups required a 12 hr session for maximum effectiveness. An increase in the frequency of response that was merely the result of increased activity or nonspecific activation, was reflected in concomitant increases in the number of S- responses. In addition, significantly increased number of responses on S+ relative to S- would be evidence for differential responding.

# PART II: EXPERIMENTS

## Experiment 1.

## A mapping study of the medial forebrain bundle and forebrain sites that support self-stimulation.

Though behavior that is motivated by biological needs is easily reinforced by conventional rewards such as food or water, the physiological mechanisms that mediate such behavior are often difficult to identify and characterize. The discovery that rats given a brief electrical stimulus to specific brain loci learned to perform a similar, if not identical, response to obtain such stimulation (Olds & Milner, 1954) proferred a unique approach to the physiological basis of motivation. A specialized system or systems whose activation by ICSS was an attractive model, especially it that activation yielded behavioral effects comparable to behaviors motivated by biological needs.

Major unresolved problems in the underlying mechanisms and circuitry for ICSS have foiled numerous attempts to define or characterize ultimate neuronal substrates of reward (Wetzel, 1969; Wise, 1978). Recent inquiries into the precise roles of noradrenergic (NE) and dopaminergic (DA) systems have circumvented these problems. In general, abolition of ICSS by anatomical or pharmacological disruption of either NE or DA systems suggests that these systems both play an integral, mutual role in the mediation

of behavior mobilized and terminated by reward (Crow, 1972; Herberg, Stephens, & Franklin, 1976; Stein, 1978).

Although such important neurotransmitters are thought to play significant roles in the functioning of the fully integrated nervous system of the adult, the exact role played by the neurotransmitters in neural mechanisms and in the modulation of behavior patterns of the mammalian brain during pre- and postnatal development is still obscure. At birth in the rat and other altricial species, young are born neurologically and behaviorally immature, as discussed earlier (see **GENERAL INTRODUCTION**). Regrettably, the behavioral limitations outlined above have restricted the scope of ontogenetic investigations to a few quantifiable behaviors.

As discussed earlier, a new approach to understanding this functional development is proposed herein. The ICSS technique, while not physiological, has been used as an accepted method of assessing neural functioning and alterations of function in adult animals. Only a very limited number of investigations have attempted a determination of the onset of function (e.g., Cheronis et al., 1979; Erinoff & Heller, 1979) in morphologically and biochemically immature neural substrates. The demonstration of appetitive responding for direct electrical stimulation, presumably mediated by these neural systems, would be interesting in its own right, since these neurons are not histochemically mature.

A quantification of self-stimulation behavior likely to be affected by neural development may serve both as an informative index of an animal's behavioral capacity at different times and as a behavioral index of temporal parameters regarding the degree of functional integrity of these neural systems. Previous demonstrations of self-stimulation in young have been shown in rats (Moran et al., 1981), 5- to 6-day-old domestic chicks (Andrews, 1967) and 4- to 5-day-old beagle puppies (Bacon & Wong, 1972). These studies have all been restricted to the MFB or the lateral hypothalamus. Recently, Velley and Cardo (1978) have reported that ad libitum bilateral stimulation of the lateral hypothalamus in 15-day-old rats results in acquisition and extinction performance changes in a light-dark discrimination when tested 30 days after stimulation.

Using a stereotaxic procedure (Lithgow & Barr, 1982) developed as a prelude (Part I) to the following body of work, the present experiment tested the possibility that young rat pups of various ages could learn to respond for direct electrical brain stimulation. Pups were implanted not only in the MFB, which has traditionally been the site of choice for ICSS studies (Olds, 1969), but also in a variety of forebrain structures of 7- and 10-day-old rat pups, sites thought not to be neurologically mature until much later developmentally (e.g., Lanier et al., 1976).

## Methods

Subjects. The subjects were the offspring of Long-Evans Hooded rats (Blue Spruce Company) mated and maintained as described in PART I. Approximately 40 7-day-olds and 55 10-day-olds were used to map positive, neutral, and aversive sites.

Surgery. Anesthetic doses and the coordinates for implantation were taken from Sherwood and Timiras (1970). Seven and 10-day-old pups were implanted using a stereotaxic technique and surgical procedures described in detail earlier (see Part I). Recently we have used Dow-Corning 3110 RTV silicone rubber as a more permanent alternative to the alginate. Coordinates for specific target structures were altered to account for these slight deviations in target loci. All coordinates were devised with lambda and bregma skull landmarks in the same horizontal plane. For operating coordinates listed below, note that the anterior-posterior coordinate (AP) refers to the distance from bregma. Dorsal-ventral dimensions (DV) refer to the distance ventral to the dural surface. Medial-lateral coordinates (ML) refer to the distance from the midline. The actual coordinates were -1.5 to +1.5 mm AP, +1.5 to +1.9 mm ML, and 6.0 to 7.0 mm DV for 7-day-olds. Coordinates for 10-day-olds were -1.7 to +2.0 mm AP, +1.5 to 1.9 mm ML, and 6.5 to 7.4 mm DV.

Testing Procedure. Pups were tested as described in Part I.

Histology. Sacrifice was performed under Nembutal anesthesia and subjects were perfused intracardially with saline followed by buffered formalin (10% solution). The brains were paraffin-embedded and cut every 15 microns with every tenth section stained and mounted. All sites were projected and recorded onto the Sherwood and Timiras atlas (1970).

## Results

Behavioral. Results showed that 27 and 31% of 7- and 10-day-olds, respectively, self-stimulated when given the opportunity when tested in the two-pole apparatus. Pups did not show clear temperature-dependent effects between electrode sites in the two temperature conditions, though the 10-day-old rate in the warm temperature was significantly greater than that at room temperature.

Figure 4 presents a bargraph of the percent of positive animals for 7- and 10-day-old pups. The lowest proportion of positive animals was shown in 10-day-old pups tested at room temperature. While 50% of 10-day-olds in the warm condition reached a positive criterion, only 16% of room temperature pups reached the same criterion. Conversely, a slightly greater proportion of room temperature 7-day-olds were positive relative to warm condition pups.

Figure 4. Bargraph for the percent of pups that self-stimulated at the two different ages tested. The criteria for positive self-stimulation are described in the text.

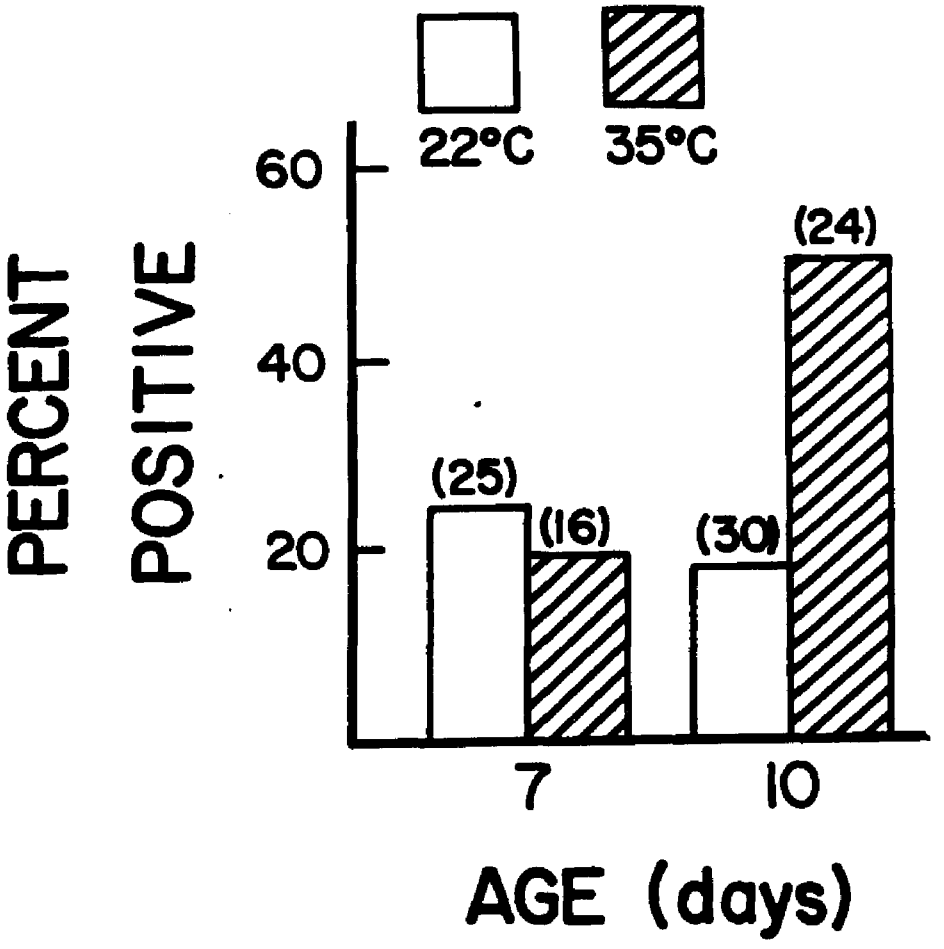

22°C
35°C
60
40
20
PERCENT POSITIVE
(25)
(16)
(30)
(24)
7
10
AGE (days)

Figure 5 presents response rate data only for positive animals, expressed as the difference between the mean number of S+ and S- responses for each hour. Figure 5 also shows data for both warm and room temperature conditions. In the two-pole chamber, response rates were highest for 10-day-olds tested in the warm condition, but lowest for 10-day-olds at room temperature. Ten-day-old response rates were greater in the warm condition relative to those at room temperature. However, this low 7-day-old warm rate and 10-day-old room rate represent only three and two positive animals, respectively, and should be treated cautiously. Positive animals exhibited a variety of different response topographies to maintain pole nudging. Approximately half of the pups nudged the poles with the nose or head, either locomoting against the pole directly and reversing momentarily, or positioning their head in front of the pole and using a type of treading response to maintain stimulation in bouts from 5-10 sec to approximately 1 hr. The other half of the pups used less conventional, though equally effective responses, such as positioning their bodies between the wall of the chamber and the pole, squirming and wriggling to displace the pole to the switch closure position. In almost every case, pups continued to sample both poles, even if pups were receiving a very high number of stimulations.

Figure 5. Response rate data for 7- and 10-day-old animals that self-stimulated in the two-pole apparatus. Responses were expressed as the difference between the mean number of S+ and S- responses for each hour.

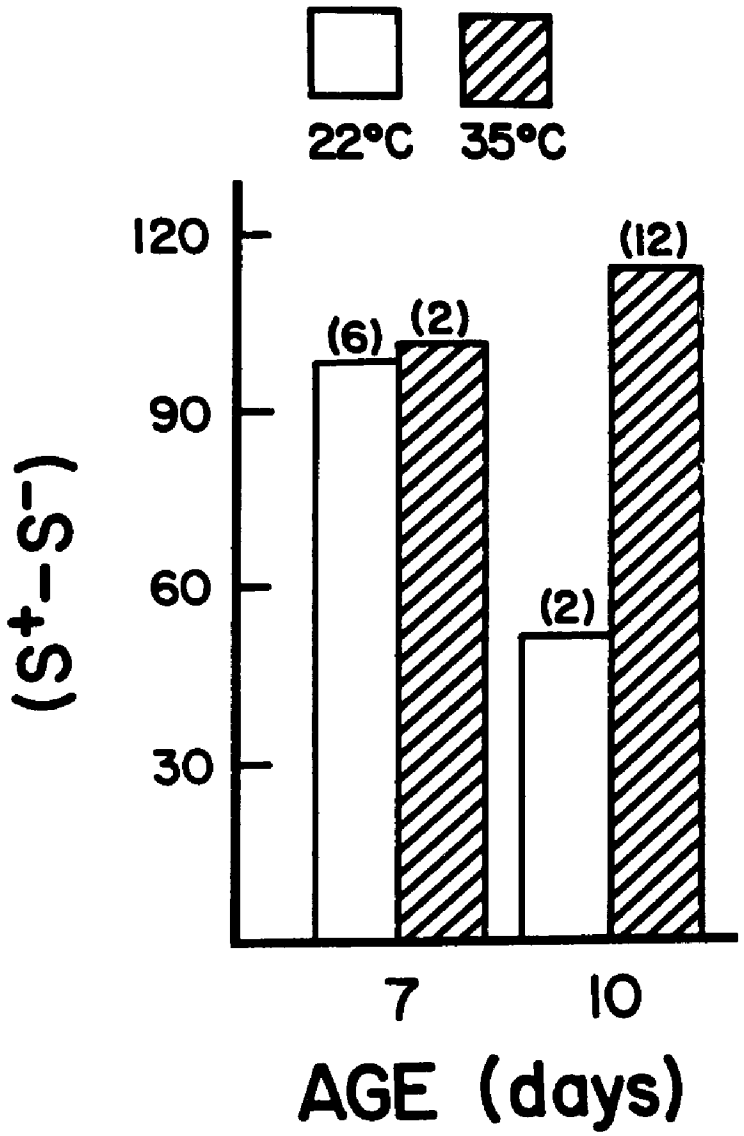
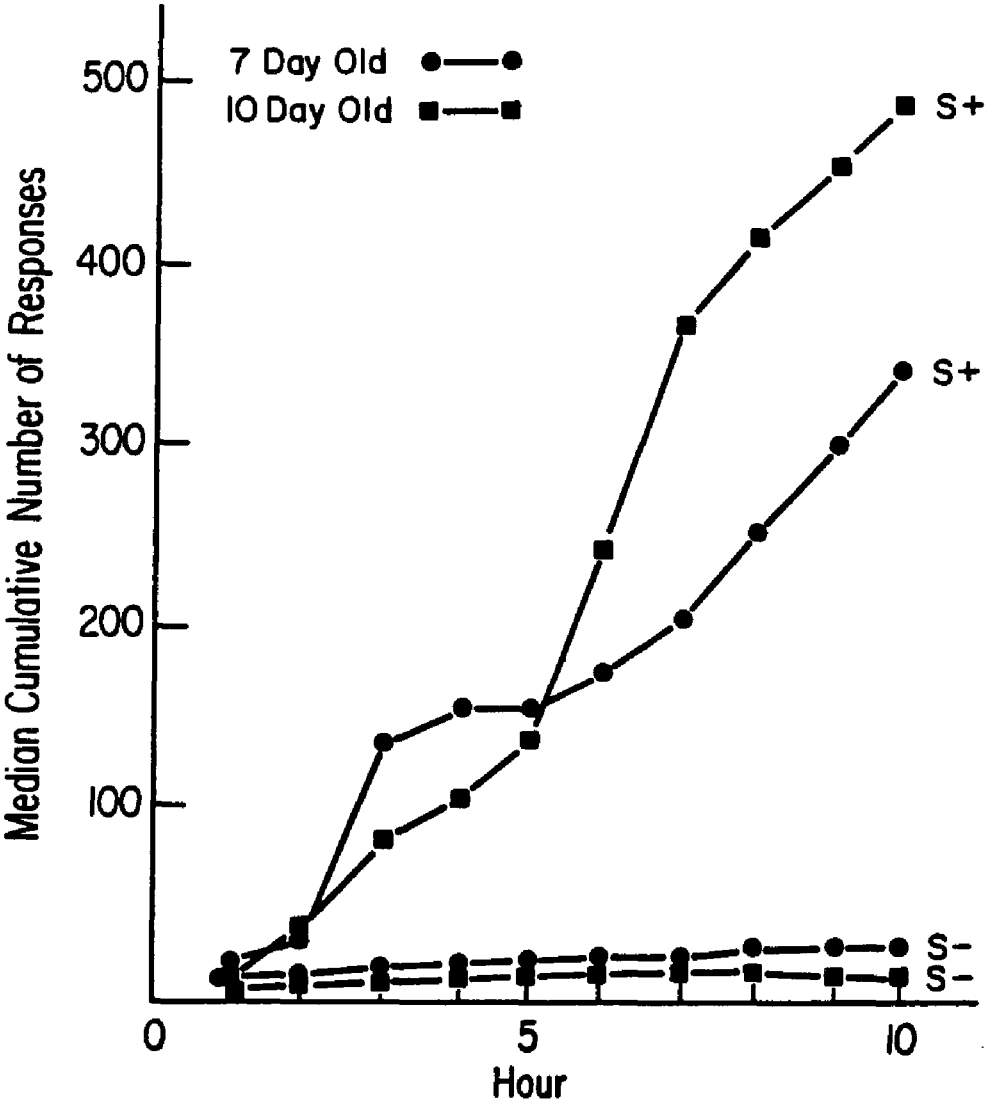

22°C
35°C
120
90
60
30
(S+-S-)
(6)
(2)
(2)
(12)
7
10
AGE (days)

Figure 6 presents a cumulative record of the median number of responses for each hour of the session, for both 7- and 10-day-old positive pups. Responses for both room and warm temperature groups were combined within ages. The plot shows rapid acceleration of responding on S+ for both ages between hours 2 and 3, followed by a gradual leveling off of response rates for both ages until hours 4 to 5. Then both plots are again positively accelerated between hours 5-8 and 4-7 for 7- and 10-day-olds, respectively. Responding on S- for both groups remained at a low, constant level throughout the session. The mean cumulative number of responses was also plotted; parametric statistics were performed on these hourly means. Both the figure and statistical analysis for the means are presented in Appendix 3.

Histological. Both 7- and 10-day-old rat pups learned the discrimination task with electrodes located in a number of forebrain sites. The majority of sites that were capable of supporting ICSS in 7-day-olds tended to be both lateral and dorsal to positive sites in 10-day-olds due to differences in operating coordinates. Figure 7 presents histology for 7-day-old pups. Generally, most positive sites were localized at the level of the medial and ventral thalamic nuclei anterior to the nucleus parafascicularis and posterior to the nucleus anterior dorsalis. Positive sites included the zona incerta, ventral and ventromedial

Figure 6. A cumulative record of the median number of responses for each hour of the 10 hr session. Only responses for positive, self-stimulating pups (see text for criteria) were included, with responses for both room and warm temperature groups combined.


7 Day Old
10 Day Old
S+
S+
S-
S-
500
400
300
200
100
0
5
10
Hour
Median Cumulative Number of Responses

thalamic nuclei, medial amygdaloid nucleus, and bed nucleus of the stria terminalis. Neutral sites mingled with positive sites in the nucleus accumbens and zona incerta, e.g., sections 2.0, 2.3, and 5.9.

Figure 8 presents histology for 10-day-old pups. Positive sites for 10-day-olds tended to be distributed throughout the frontal and prefrontal cortex, anterior to both the parataenialis and reuniens thalamic nuclei, as well as the paraventricular hypothalamic nucleus. Specifically these loci included six sites in the anterior MFB and olfactory tubercle, two sites just dorsal to pyriform cortex, two sites in the nucleus accumbens, and one site in the caudate-putamen just ventral to the anterior olfactory nucleus. Neutral sites interspersed positive sites in the accumbens and MFB, e.g., sections 4.7 and 5.6-7.0 of Figure 8. Table 2 presents a summary of implanted sites in 7- and 10-day-old pups.

Figure 7. Histology for 7-day-olds with sites plotted on the Sherwood and Timiras (1970) atlas. Positive sites are indicated by filled circles ( ● ), neutral by open circles ( ○ ), and aversive by hatched ( ▨ ) using behavioral criteria described in the text.

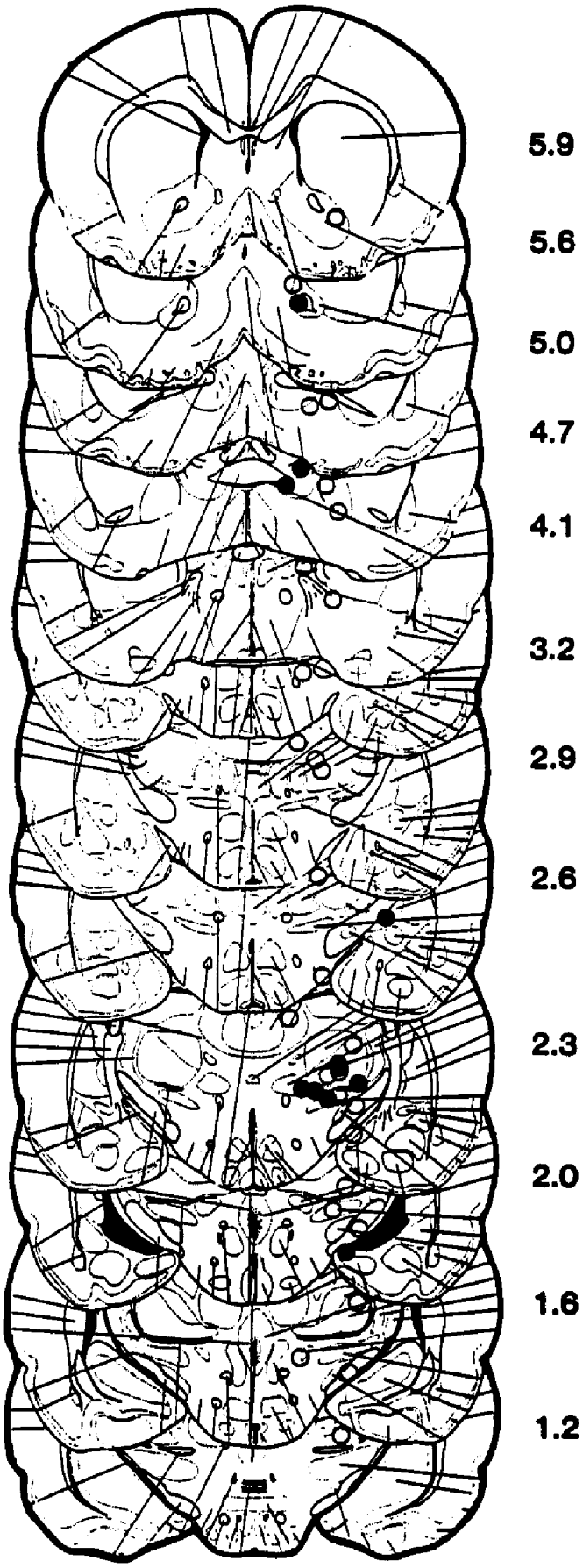
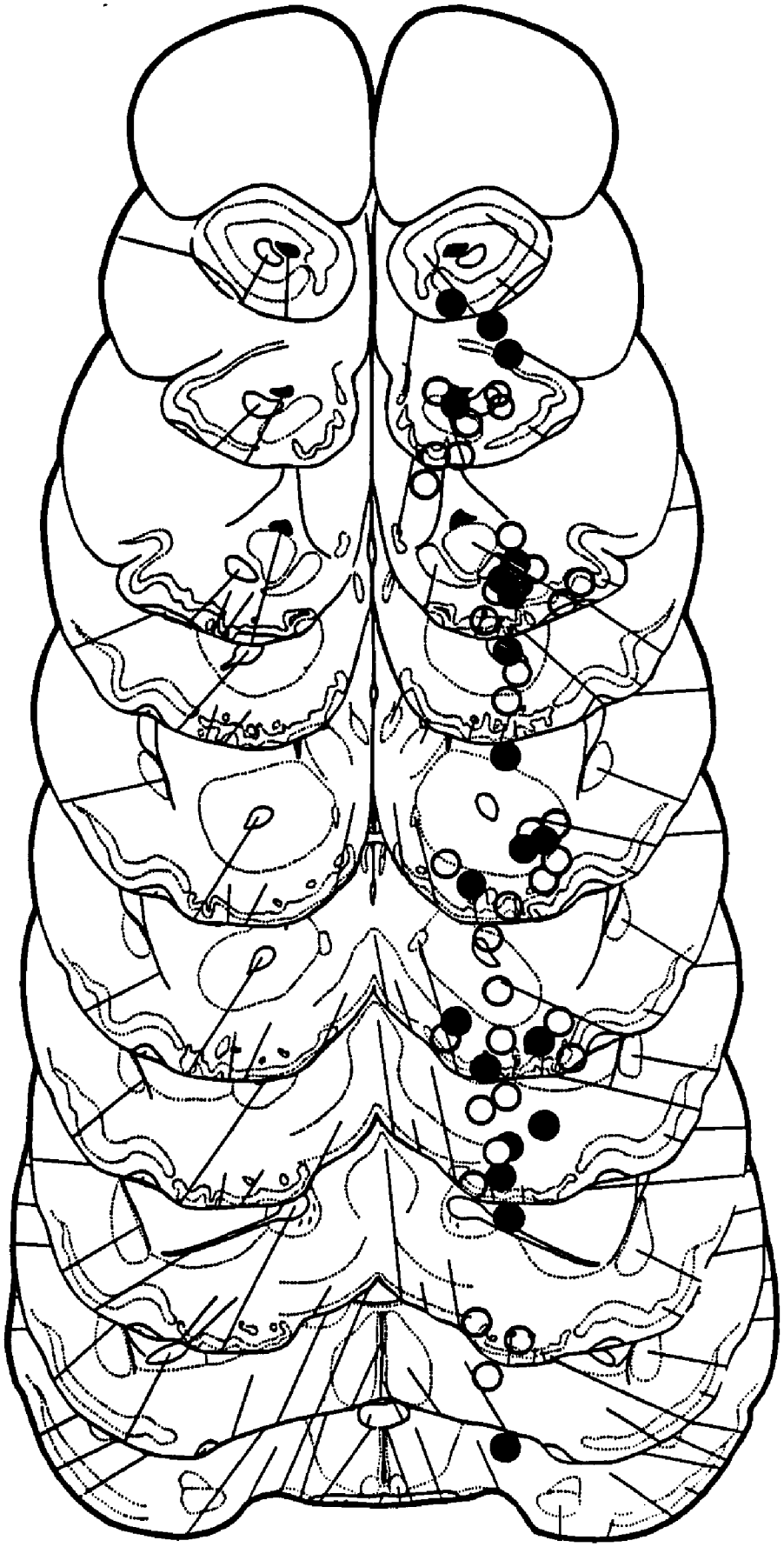

5.9
5.6
5.0
4.7
4.1
3.2
2.9
2.6
2.3
2.0
1.6
1.2

Figure 8. Histology for 10-day-olds. See Figure 7 for legend.



8.0

7.5

7.0

6.5

6.2

5.9

5.6

5.3

4.7

4.1

Table 2

Summary of Brain Loci Implanted with Bipolar Electrodes in 7- and 10-day-old Rat Pups

| | 7-day-olds | | 10-day-olds | |
|---|---|---|---|---|
| Locus | # Sites | # Pos | # Sites | # Pos |
| Anterior Commissure | 2 | 1 | 5 | 0 |
| Nucleus Accumbens | 2 | 0 | 8 | 3 |
| Amygdaloid Nucleus | | | | |
| Pars medialis | 2 | 1 | 0 | - |
| Anterior Olfactory Nuc | | | | |
| Pars lateralis | 0 | - | 1 | 1 |
| Pars medialis | 0 | - | 2 | 0 |
| Pars posterior | 0 | - | 2 | 0 |
| Basis Pedunculi | 3 | 0 | 0 | - |
| Bed Nuc Stria Terminalis | 2 | 2 | 0 | - |
| Caudate-Putamen | 0 | - | 1 | 1 |
| Corticohabenular Tract | 1 | 0 | 0 | - |
| Globus Pallidus | 3 | 1 | 0 | - |
| Medial Forebrain Bundle | 3 | 0 | 22 | 9 |
| Medial Prefrontal Cortex | 0 | - | 3 | 1 |
| Olfactory Tubercle | 0 | - | 9 | 2 |
| Piriform Cortex | 0 | - | 2 | 1 |
| Thalamic Nuclei | | | | |
| Nucleus Ventralis | 11 | 2 | 0 | - |
| Pars dorsomedialis | 1 | 0 | 0 | - |
| Pars medialis | 2 | 1 | 0 | - |
| Nucleus Medialis | 2 | 0 | 0 | - |
| Nucleus Reticularis | 1 | 0 | 0 | - |
| Zona Incerta | 4 | 2 | 0 | - |

## Discussion

Though both 7- and 10-day-old pups learned the discrimination task with electrodes in a number of forebrain sites that correspond remarkably well to those of the adult, the issue of reward as opposed to actual

behavioral activation is less clear. The correlation of positive sites in young pups with those in adults is consistent with the concept of reward mechanisms operating in the pup. Positive sites in 7-day-olds included zona incerta, and ventral and ventromedial thalamic nuclei, for which Wurtz and Olds (1963) have shown adult ICSS. Additional positive sites in 7-day-olds were congruent with adult data. These sites included medial amygdaloid nucleus (Edwards, Wishik, & Sinnamon, 1979) and nucleus of the stria terminalis (Olds, Travis, & Schwing, 1960).

Similarly, positive sites for 10-day-olds have supported ICSS in adults. The positive forebrain sites included the MFB and olfactory tubercle (Huang & Routtenberg, 1971; Phillips, Brooke, & Fibiger, 1975; Routtenberg, 1971), a site just dorsal to the pyriform cortex (Olds et al., 1960; Phillips et al., 1975), the nucleus accumbens, caudate-putamen, and anterior olfactory nucleus (Huang & Routtenberg, 1971; Olds et al., 1960; Routtenberg, 1971; Phillips et al., 1975).

Response data for 7-day-olds yielded a significant but low proportion of positive animals, despite a rather extensive mapping of forebrain sites. Limited locomotor related capacities do not readily account for this low positive proportion since mean hourly response rates for positive animals were actually slightly greater, though not significantly than those for 10-day-olds. We have

replicated this work using milk as a reinforcer with posterior tongue cannulae (Hall, 1979a; Hall & Rosenblatt, 1977) in food-deprived 7- and 10-day-old pups and have seen a proportion of 7-day-old pups that did not learn the operant in the two-pole chamber (unpublished observations). Therefore, the low proportion of positive sites may represent a minimum assessment in terms of the number of loci that may actually support ICSS in a slightly modified paradigm. Furthermore, our criterion for a positive site in the young animals was a probabalistic one, thus adding the possibility of sampling error in determining both positive and neutral sites.

Most importantly, it should be clear that the data presented here do not entirely support the actual demonstration of learning. Though the plot of cumulative responding indicated behavioral contrast between the active and the inactive poles, we switched neither olfactory cues nor active pole position, in order to determine whether pups would reverse responding to the previously neutral pole. Moreover, we did not demonstrate extinction curves within animals (e.g., Johanson & Hall, 1979). Admittedly, this protocol is a prerequisite to the demonstration of associative learning, though interpretational problems may still exist (e.g., Hall, 1979b; Johanson & Hall, 1979). However, the procedure in the present design was based upon additional considerations related to, for example, the time

and duration of deprivation and surgery, and the testing session. Each consideration presented inherently different interpretational problems. Clearly, a proportion of pups in the present experiment showed a greater degree of responding on the active relative to the inactive response pole. Since pups did continue to sample both poles throughout and pups exhibited a high degree of behavioral contrast in the presence of the two poles, one interpretation of these results is the learning of an appetitive response for access to direct electrical stimulation of specific brain loci.

However, these pups also exhibit a considerable amount of behavioral activation when stimulated. In fact, an alternative explanation is that the manifestation of this behavioral activation impugns the role of a distinct reward system or systems in the rat pup. This issue becomes particularly important in view of recent suggestions that any stimulus which elicits behavioral activation in pups may have reinforcing properties in and of itself (Sullivan & Brake,1981). Activation of young pups has been noted by others in self-stimulation (Moran et al., 1981; Moran, personal communication), milk reinforcement (Johanson & Hall, 1979; Hall, 1979a,b), administration of neurochemical precursors (Dewyngaert & Kellogg, 1974; Kellogg & Lundborg, 1972), and other pharmacologic (Campbell, Lytle, & Fibiger, 1969; Fibiger, Lytle, & Campbell, 1970) paradigms. Indeed,

this activational component may simply complement or supplement as yet undifferentiated reward systems. Presumably, activation mechanisms in the pup eventually lose their intrinsic ability to maintain behavior and are ultimately replaced by more specific reward mechanisms. Certainly, the conception of such a composite reward mechanism is congruent with the general increase in activity that is characteristic of conventionally reinforced behaviors in the adult animal (Reynolds, 1975). Not surprisingly, appetitive or even averse components, may comprise the activation component.

The behavioral data provided herein extends biochemical, histological, and recent pharmacological investigations on the ontogeny of neural systems. Catecholamine neurons, thought to be at least one critical link in self-stimulation pathways (German & Bowden, 1974), are comprised of storage, regulatory, and degradative components that mature at different rates (Porcher & Heller, 1972; Seiger & Olson, 1973; Lanier et al., 1976). The disjunctive nature of neural construction complicates attempts to explicate the role of each component in development. Accordingly, various manipulations have attempted to delimit the functional maturation of various neuronal components or of the neuron as a unit. For example, modification of the ontogeny of catecholamine neurons by receptor blockade (Mandell, Segal, Kuczencki, &

Knapp, 1972) or early administration of a catecholamine precursor (Dewyngaert & Kellogg, 1974; Kellogg & Lundborg, 1972) can induce temporary alterations in motor function. These effects have been measured in terms of hyperactivity in 7-day-olds (Kellogg & Lundborg, 1972) or rotational behavior in 10- and 14-day-olds (Dewyngaert & Kellogg, 1974).

In the adult animal, transection of the dopaminergic nigrostriatal pathway results in an acute elevation in the level of striatal DA (Walters, Roth, & Aghajanian, 1973). Presumably the elevation in DA is the result of a change in the kinetic state of tyrosine hydroxylase (TH), in turn mediated by a presynaptic DA receptor (Roth, Walters, Murrin, & Morgenroth, 1975). Axotomy of the neonatal nigrostriatal projection has shown adult-like increases in 8- to 10-day-olds but not 4- to 6-day-olds (Cheronis et al., 1979; Erinoff & Heller, 1978). Additional pharmacological intervention using an inhibitor of TH suggests that the abrupt development of the response to axotomy after the sixth or seventh day of age is the initiation of impulse traffic in the immature neuron. Hence, the degree of function in 10-day-old neural loci from the present work supports data from related pharmacological manipulations.

The neuroanatomical localization of function has also been attempted in developing rat pups. Citing a

dissociation of feeding and deprivation-induced activation in adult rats (Campbell & Baez, 1974; Mabry & Campbell, 1974), Kornblith and Hall (1979) noted a separation of ingestion and accompanying behavioral activation in the 3-day-old rat by discrete brain transections. Intake was severely reduced only in animals with diencephalic transections, while activity declined with more caudal transections. These data suggest that the behavioral activation noted in the present results may be physically separated in a food-rewarded paradigm as early as 3 days of age.

Thus, the degree to which neurons are functionally mature can be assessed in part, using morphological or histological changes (e.g., Olson, Seiger, & Fuxe, 1972) or a comparison of the response of neonates to surgical or pharmacological challenge relative to those obtained in adult animals.

Despite the conceptual problems associated with the actual behavioral mechanism in this paradigm, it is apparent that young rat pups differentially responded to stimulation, before neural components were fully mature, particularly in terms of adult levels of transmitters, receptor concentration, and regulation (Lanier et al., 1976). Of course, electrical stimulation of the brain is not physiological and the sites may not normally function. In fact, other pharmacological data (Cheronis et al., 1979)

have shown a stimulation of function in pathways that have not begun autogenous functioning. Regardless of the important issue of reward or behavioral activation, the application of this paradigm is useful, particularly in terms of providing a response measure that may serve as a tool with which to delimit the response of the immature nervous system. Subsequent research will attempt to more closely specify the nature of the relationship of these two possibly distinct, components.

## Experiment 2.

## Behavioral response for ICSS following acute pharmacological treatment with d-amphetamine in 10-day-old rats.

The relationship between neurochemical and behavioral development can be studied by administering drugs known to affect developing neurotransmitter systems in immature organisms. Specifically, drugs that selectively affect NE, DA, 5-HT, or ACh have effects on different behaviors at different times during development (Campbell & Spear, 1972; Feigley, 1974; Kellogg & Lundborg, 1972).

Early studies for potential support of a neurotransmitter-mediated ICSS theory concerned evidence that d-amphetamine facilitated ICSS behavior (Domino & Olds, 1972; Stein, 1964; Stein & Ray, 1959). Amphetamine

and methamphetamine generally increase rates of responding for brain stimulation (Domino & Olds, 1972; Horovitz & Carlton, 1962; Horovitz, Chow, & Carlton, 1962; Margules, 1969; Olds, 1959; M.E. Olds, 1970; Prahdan & Bowling, 1971; Stein, 1964; Stein & Ray, 1959). Low doses of amphetamine generally facilitate self-stimulation by increasing response rates at fixed stimulation intensities (for review, see Wise, 1978) or by decreasing the stimulation threshold (Stein & Ray, 1959). In addition, amphetamine facilitation has been demonstrated at electrodes that yielded low rates (Domino & Olds, 1972; Olds, 1970) or high rates by testing at current intensities near threshold (Horovitz & Carlton, 1962; Horovitz et al., 1962; Pradhan & Bowling, 1971; Stein, 1964). The increase in response rates has been attributed to selective action of amphetamine on the pathway(s) that mediate the rewarding effects of ICSS.

There is a predilection to view amphetamine as an agent which simply and directly facilitates self-stimulation. However, amphetamine may inhibit self-stimulation behavior depending upon a number of parameters that include electrode placement (Stark, Turk, Redman, & Henderson, 1969), amphetamine dose (Wise, Yokel, Hansson, & Gerber, 1977), stimulation current, or baseline response rate (for review, see Wauquier, 1976).

Biochemically-mediated changes in brain pathways have been postulated to account for the facilitation of responding following amphetamine administration. Amphetamine appears to enhance transmission at both dopaminergic and noradrenergic presynaptic terminals by potentiating release of DA and NE, respectively, as well as possibly enhancing transmission by inhibiting monoamine oxidase or blocking the presynaptic reuptake of DA and NE (Axelrod, 1970; Bloom & Giarman, 1968; Carlson, 1970; Sulser & Sanders-Bush, 1971). Not only does amphetamine potentiate release of these catecholamines and inhibit their degradation and reuptake, but amphetamine may also act as an agonist at catecholamine receptors (Feltz & DeChamplain, 1973). Furthermore, the depletion of catecholamines by reserpine treatment has been shown to markedly reduce the rate-enhancing effect of amphetamine (Stein, 1966).

Amphetamine has been widely used experimentally (for review, see Sanger & Blackman, 1976) though less frequently for purposes of an ontogenetic analysis (cf., Lytle et al., 1971; Marsden & Guldberg, 1973; Raskin & Campbell, 1981; Spear, Shalaby, & Brick, 1980). The present experiment addressed the question of whether immature neural pathways that mediate ICSS behavior are responsive to an acute pharmacological treatment with d-amphetamine. In light of the general support for a purported selective facilitation

of ICSS responding by acute amphetamine administration, the present design offers a unique opportunity to assess amphetamine-mediated effects in 10-day-old rat pups. Pups that respond at high or low rates may be compared directly. Similarly, threshold effects may be determined by comparing abrupt increases in amphetamine treated pups that do not respond regularly for ICSS before treatment.

## Method

**Subjects.** The subjects were the offspring of Long-Evans Hooded pregnant rats (Blue Spruce Company) housed and maintained as described earlier. Pups that were born to these dams were dated, sexed, and culled as described in **PART I**. In a simple bivalent design, 9- to 11-day-old pups implanted in forebrain sites that varied only in the AP coordinates were used. The sites were ones that exhibited a high proportion of positive animals by response criteria outlined in **Experiment 1**. All rat pups were implanted using the stereotaxic method described in **PART I** at coordinates +0.5 to +2.0 mm AP, +1.6 mm ML, and 6.5 mm DV. Implanted pups were given Mikedimide (1 ml/100 g of a 0.3% solution) postoperatively, and allowed to recover overnight.

**Procedure.** All pups were tested in the two-pole apparatus according to procedures described in **PART I**. At the beginning of the test session, pups were voided, weighed, and placed in the heated (35$^{o}$C) chamber, which

represented a 16 to 18 hr food deprivation since implantation. As before, responses were counted for the first 5 hr. Pups were then injected (ip) with saline (10 ml/kg) or d-amphetamine (1 mg or 5 mg/kg) and run for an additional 5 hr. These test doses were chosen following an examination of typical amphetamine doses in both neonatal and adult rat studies. A number of ontogenetic studies and typical doses are presented in Table 3. Pups were sacrificed and perfused as described earlier at the end of the second 5 hr. Approximately 4-6 pups were used from each of 10 litters. For the three test groups, 16, 20, and 20 pups were used for the 5 mg/kg, 1 mg/kg, and saline groups, respectively. Electrode sites were plotted on the Sherwood and Timiras (1970) atlas.

## Results

Rat pups in all three groups showed variable but similar response patterns for the first 5 hr., and response rates on both poles increased dramatically following amphetamine treatment, with selective increases on S+ for the higher dose of amphetamine.

Figure 9 presents the mean number of responses for hours 1 to 10 for saline and amphetamine-treated rat pups. The mean number of responses was not different for the three groups for the first 5 hr. All of the plots show peaks of responding on S+ at 7 hr. The figure shows evidence of both a generalized behavioral activation effect

Table 3

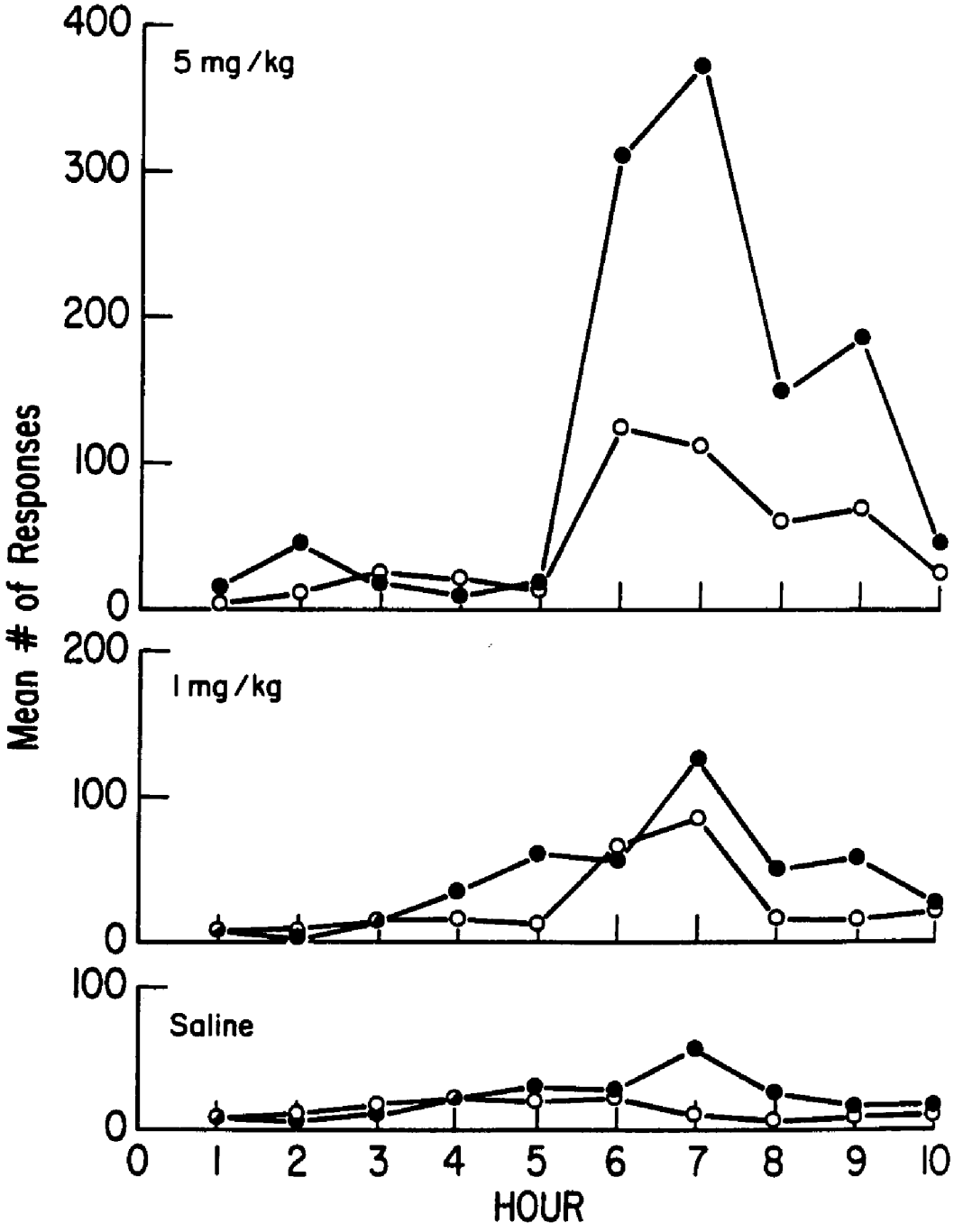
Typical Amphetamine Doses for Studies Using Rat Pups or Adults

| Study Topic | Investigator | Animal Age (days) | Amphetamine Dose (mg/kg) |
|---|---|---|---|
| Activity/Arousal | | | |
| | Bauer & Duncan, 1975 | 28-33, adults | 2.0, 5.0 |
| | Campbell et al., 1969 | 5-25 | 0.25-4.0 |
| | Lanier & Isaacson, 1977 | 18-49, adults | 2.0-10.0 |
| | Lytle et al., 1970 | 5-25 | 0.25-4.0 |
| | Sobrian et al., 1975 | 1-21 | 0.25,2.0 |
| | Spear et al., 1980 | 23-54, adults | 2.0-10.0 |
| Anorexia/Hyperphagia | | | |
| | Capuano et al., 1982 | 3-15 | 0.5- 2.0 |
| | Leshem, 1981 | 5-25 | 1.0 -9.0 |
| | Lytle et al., 1971 | 5-25 | 0.25-4.0 |
| | Raskin & Campbell, 1981 | 5-25 | 1.0, 2.0 |
| Chronic Amphetamine | | | |
| | Segal, 1975 | Adults | 0.5 -7.5 |
| Catecholamine Biosynthesis | | | |
| | Harris & Baldessarini, 1973 | Adults | 0.5 -5.0 |
| ICSS | | | |
| | Carey et al., 1974 | Adults | 0.1 -3.0 |
| | Wauquier & Niemegeers, 1974 | Adults | 0.16-2.5 |

shown by increases in responding on both poles for the two amphetamine groups, as well as a preferential facilitation of responding on the positive pole for the higher dose of amphetamine. Data were analyzed using a three-way analysis of variance with two trial factors (pole, hour) and one grouping factor (drug). The analysis of variance showed a significant difference between groups $F = 90.77$, $df=2,53$, $p<0.001$), S+ vs. S- ( $F = 16.48$, $df=1,53$, $p<0.001$), and hour ( $F = 14.41$, $df=9,477$, $p<0.001$). There were also significant interactions for pole by dose ( $F = 5.50$, $df=2,53$, $p<0.01$), pole by hour ( $F = 3.88$, $df=9,477$, $p<0.001$), hour by dose ( $F = 5.2$, $df=18,477$, $p<0.001$), and pole by hour by dose ( $F = 2.15$, $df=18,477$, $p<0.01$).

Group main effects were further analyzed. Post hoc tests were performed to determine if amphetamine increased overall responding on both poles and if there was a preferential facilitation of responding on S+. For dose by pole simple interaction effects, responding increased significantly on S+ for the 5mg ( $F =50.7$, $df=2,530$, $p<0.01$) and 1mg ($F =3.42$, $df=2,530$, $p<0.05$) groups. Responding on S- significantly increased only for the 5mg group ( $F =6.20$, $df=2,530$, $p<0.01$). Similarly, the dose by hour interaction showed significant increases in responding over time for the 5mg ( $F =103.3$, $df=2,530$, $p<0.01$) and 1mg ( $F =6.00$, $df=2,530$, $p<0.01$) groups, but not for saline.

Figure 9. The mean number of responses for hours 1 to 10 for saline and amphetamine treated rat pups. Pups were injected at 5 hours with either saline or d-amphetamine (1 mg/kg or 5 mg/kg).


400
300
200
100
0
5 mg/kg
200
100
0
1 mg/kg
100
0
Saline
Mean # of Responses
0 1 2 3 4 5 6 7 8 9 10
HOUR

Pups treated with 5.0 mg/kg of d-amphetamine exhibited significant increases in responding on both poles following drug administration. The drug treatment produced a significant increase in response rate on S+, shown by the difference in pre- and postinjection hourly means on S+ ( $F = 4.98$, $df=9,117$, $p < 0.001$) and S- ( $F = 11.20$, $df=9,117$, $p < 0.001$). Simple main effect post hoc tests showed significant differences in S+ and S- for hours 6-9 ( $F = 23.94$, 46.97, 5.42, 9.47, respectively, $df=19,530$, $p < 0.001$). Pups treated with 1.0 mg/kg of d-amphetamine showed a slight but not statistically significant increase in responding on S+ at 4 hr., which continued to increase following drug administration to maxima on both poles at 7 hr. An analysis of variance did not reveal significant differences between responding on either S+ or S- for pre- vs. postinjection. There were no response differences on or between S+ and S- following saline administration.

In summary, 5.0 mg/kg of d-amphetamine significantly facilitated overall responding on both S+ and S-, differentially increasing responding on S+ over S-. A 1.0 mg/kg dose facilitated responding only when responses were pooled across poles, but not for either S+ or S- independently. Further, there were no differences between S+ and S- responding after the 1.0 mg/kg dose. Saline administration had no effect on responding.

Figure 10. A typical cumulative record of responding by a 10-day-old pup administered 5 mg/kg d-amphetamine. Each unbroken record represents 30 min. The pup was injected at the arrow, and the record is read from the top down as the session progressed. Responses on S+ are shown as response steps by the pen, with S- responses shown by hash marks.

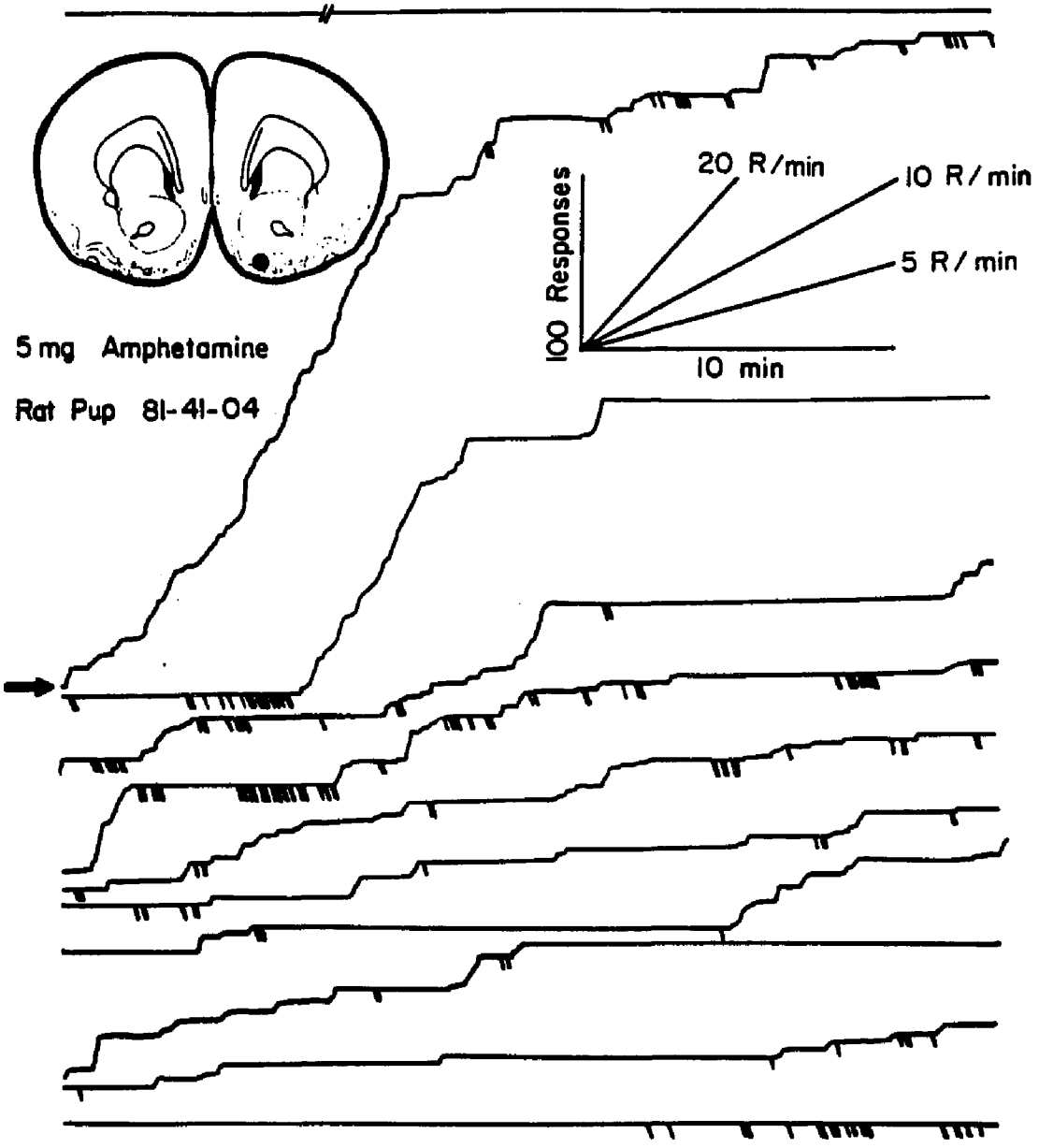

20 R/min
10 R/min
5 R/min
100 Responses
10 min
5 mg Amphetamine
Rat Pup 81-41-04

Figure 10 presents a typical cumulative record of responding by a 10-day-old pup administered 5.0 mg/kg of d-amphetamine. Histology for this pup showed the electrode site localized in the anterior MFB. This pup evidenced an extraordinary, abrupt facilitation of responding on S+ that began immediately with injection and continued for at least 3 hr. This precipitant increase in responding on S+ contrasts sharply with the low baseline rate before injection.

Figure 11 presents histology for the localization of electrode loci in saline and d-amphetamine treated groups. Sites for all three groups were plotted to show the magnitude of increase in responding on S+ by calculating the difference in the mean hourly rate for hours 6,7, and 8 compared to that during hours 4 and 5. These hours for rate comparisons were chosen from a post hoc analysis of responding shown in Figure 9. Projection loci were summarized in Table 4 and included sites in nucleus accumbens, anterior olfactory nucleus, anterior commissure, caudate-putamen, MFB, olfactory tubercle, prefrontal cortex, stria terminalis, and tractus diagonalis. Generally, more anterior sites, e.g., sections 6.2-7.5 mm, showed greater increases in responding following drug, though every site in the 5 mg group demonstrated a mean hourly increase between 10 and 1000 responses. Most sites in the MFB showed similar moderate to high facilitation of

Table 4

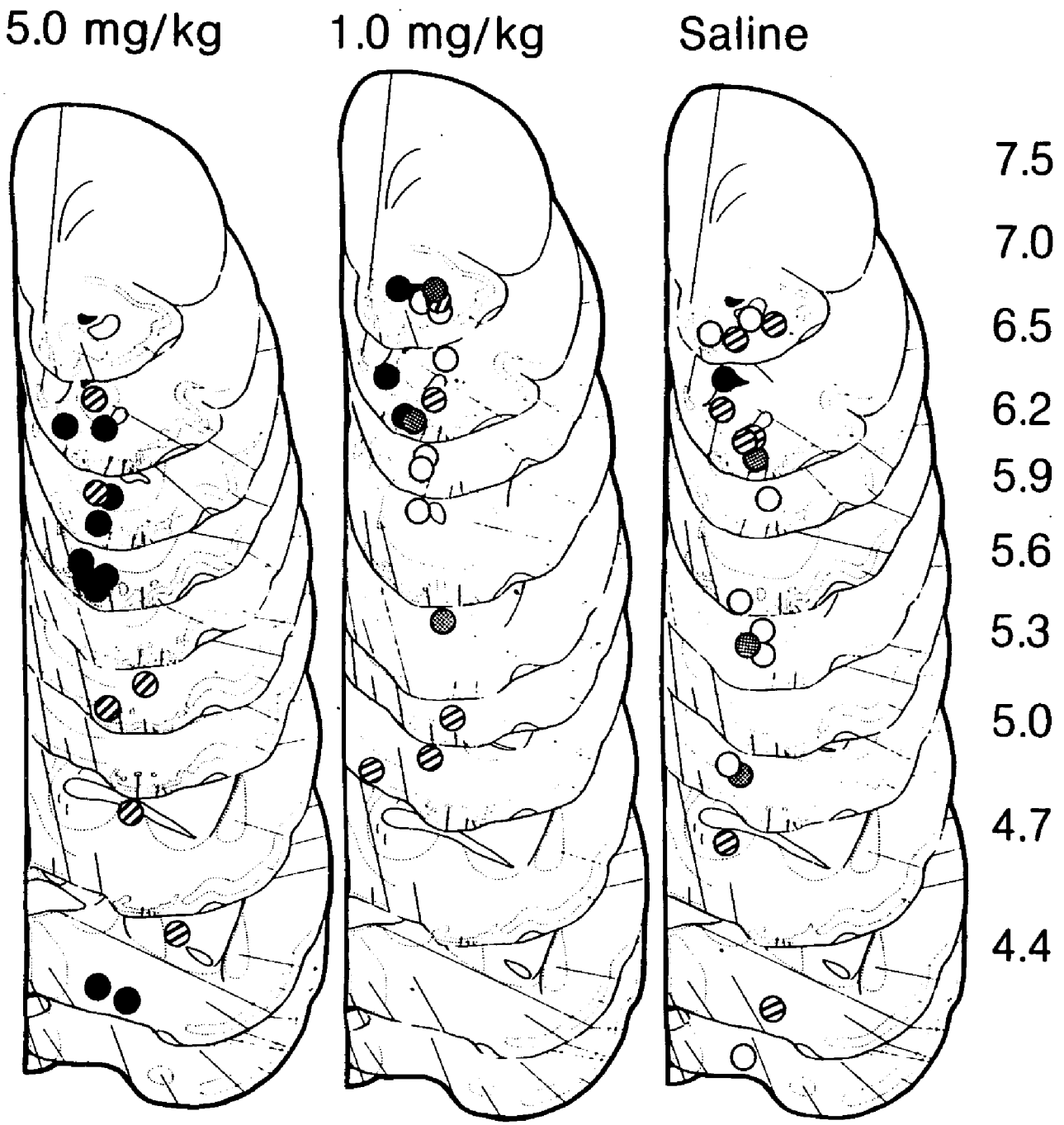
Summary of Implanted Brain Loci and Nudging Response on

on S+ Following Acute Treatment

| | Amphetamine | | | | Saline | |
|---|---|---|---|---|---|---|
| | 5mg/kg | | 1mg/kg | | | |
| Locus | # Sites | Mean Resp Increase* | # Sites | Mean Resp Increase | # Sites | Mean Resp Increase |
| Nucleus Accumbens | 3 | 158 | 2 | 1 | 1 | 0 |
| Anterior Commissure | 1 | 35 | 2 | 5 | 1 | 0 |
| Anterior Olfactory Nuc | | | | | | |
| Pars medialis | 1 | 186 | 1 | 416 | 1 | 38 |
| Pars posteriori | 1 | 314 | 1 | 222 | 2 | 0 |
| Bed Nuc Stria Term | 0 | - | 0 | - | 1 | 36 |
| Caudate-Putamen | 1 | 54 | 0 | - | 0 | - |
| Medial Forebrain Bundle | 7 | 268 | 7 | 27 | 9 | 6 |
| Prefrontal Cortex | 0 | - | 3 | 0 | 1 | 135 |
| Olfactory Tubercle | 2 | 579 | 1 | 9 | 2 | 1 |
| Piriform Cortex | 0 | - | 0 | - | 1 | 37 |

* 'Mean Resp Increase' refers to the increase in the mean number of responses for hours 6, 7, and 8 relative to that during hours 4 and 5.

Figure 11. Histology for the localization of electrode loci in saline and amphetamine-treated pups. Sites for all three groups were plotted to show the increase in responding by calculating the difference in the mean hourly rate for hours 6, 7, and 8 compared to that during hours 4 and 5. Rates are changes in S+ responding only. Open (  ), stipled (  ), hatched (  ), and filled () represent 0, 1-10, 10-100, and 100-1000 fold changes, respectively.


5.0 mg/kg
1.0 mg/kg
Saline
7.5
7.0
6.5
6.2
5.9
5.6
5.3
5.0
4.7
4.4

response rates while others, e.g., those in the nucleus accumbens or anterior commissure, were less consistent. In addition, sites in sections 5.0-5.6 mm showed a consistent moderate facilitation for both doses of amphetamine. More posterior brain sections, i.e., 4.4-5.0 mm did not contain enough sites to permit response rate comparisons between groups.

Electrode sites were also plotted for changes in responding on S-, before and after treatment. Results were generally similar, except that the number of 100-1000 fold differences were fewer.

The mean number of responses per hour for each of the three test groups was reanalyzed on a histological basis. For example, response rates were determined for animals with electrodes only in the MFB (n=23) and the anterior olfactory nucleus (n=5). These results were not different from those shown in Figure 9.

## Discussion

Pups treated with the 5 mg dose of amphetamine showed a dramatic and significant increase in responding following injection, thus extending facilitative effects demonstrated in adults (Domino & Olds, 1972; M.E. Olds, 1970; Stein, 1964). Clearly, responding increased differentially on S+ relative to S- for the 5 mg group but not for the 1 mg group. Though this lower dose did produce a similar increase in the variability of responding (data not shown),

the greater efficacy of the 5 mg dose was clear. The nature of the increase of the effectiveness of stimulation following amphetamine administration is not clear. Amphetamine may (a) enhance the rewarding properties of electrical stimulation by possibly lowering the threshold of stimulation or potentiation of its efficacy directly, or (b) behaviorally activate the pup and potentiate arousal properties induced by stimulation, activation that is unrelated to reinforcement per se. Either interpretation suggests that the effects are clearly related to direct electrical stimulation. There is empirical support for both interpretations of the data.

Stein and Ray (1959) demonstrated a lowering of threshold with d-amphetamine, using a stepwise current resetting technique. Numerous pups in the 5 mg group of the present design showed very low S+ response rates before treatment (e.g., see Figure 10) followed by rather dramatic though not entirely selective increases after injection. Thus, these abrupt increases in responding suggest that amphetamine may have both a facilitative and threshold-lowering effect in 10-day-olds in the present design, though the latter is an inference that requires further experimental validation.

Though pups exhibited a facilitation of responding for self-stimulation at this higher dose as studies in adults have shown (Stein, 1964), the drug also incresed responding

on the inactive pole. Stein demonstrated the facilitation of ICSS at threshold levels of intensity, suggesting that these effects were due to an increase in the rewarding properties of stimulation. Unlike Stein's work, the present study showed concomitant increases in behavioral activation evidenced by concurrent increases in responding on the inactive pole (e.g., Figures 9,10) and increases in locomotor behavior (observations not shown). Note however, that the two paradigms differed in a number of important respects, including the fact that Stein tested during extinction and the present design did not.

The generalized increase in behavioral activation supports earlier work (see Experiment 1) and other reports of infant self-stimulation without pharmacological treatment (Lew & Moran, 1981; Moran et al., 1981) as discussed earlier. Accordingly, another interpretation of these results is a potentiation of this effect by d-amphetamine that is independent of a possible facilitating effect or threshold-lowering of self-stimulation reward. Indeed, arousal-inducing properties of amphetamine administered to infant rats are evident at least as early as postnatal day 10 (Campbell et al., 1969; Fibiger et al., 1970).

Other important interactive factors may be operating to produce the facilitation of responding by these drug-treated pups. Hall (1979a) has shown that warm, food-

deprived pups are more active than non-deprived animals. Furthermore, adult rats increase response rates for ICSS following food deprivation (Carey, Goodall, & Lorens, 1975; J. Olds, 1962). Noting the apparent facilitation of responding in the present experiment following amphetamine, progressive increases in responding may reflect a potentiating, synergistic effect as a result of a drug-deprivation interaction. This possibility is especially acute when one considers that pups were tested at least 16 hr after removal from the dam.

Amphetamine has been used in other paradigms for purposes of an ontogenetic analysis. Adult rats show a substantial anorexic effect following amphetamine administration (Biel, 1970), and this effect has been reported in the 15-day-old (Lytle et al., 1971) and also in the 5-, 15-, and 25-day-old (Raskin & Campbell, 1981) when pups are 22 hr food-deprived before testing. Interestingly, 3- and 10-day-olds may actually increase milk intake following amphetamine administration when deprived for only 1-4 hr (Capuano, Barr, & Liebowitz, 1982).

Campbell et al. (1969) demonstrated that pups show a differential sensitivity to d-amphetamine that depends upon the age of the pup when testing is performed in a stabilimeter cage. Older pups show a 16-fold decrease in sensitivity relative to younger pups, while open field

tests show that 28- to 33-day-olds (Bauer & Duncan, 1975) and 34- to 38-day-olds (Lanier & Isaacson, 1977) are unresponsive to amphetamine. Notably, pups demonstrate a curvilinear sensitivity to amphetamine in such open field tests, with activity increased at postnatal days 18-22, unchanged at days 34-38, and either increased or decreased at 45-49 days, depending upon the test dose.

In adult rats systemic administration of amphetamine with a unilateral lesion of the dopaminergic nigrostriatal tract induces vigorous turning ipsilateral to the side of the lesions (Marsden & Guldberg, 1973) attributed to the increased release of DA by amphetamine from the intact side of the striatum (Ungerstedt, 1971). Similarly, pharmacological blockade or temporary inhibition of unilateral CA biosynthesis would produce the same turning ipsilateral to the injection site, especially when followed by systemic amphetamine treatment. Such induced turning is observed in 12-day-old rat pups but not younger, suggesting rapid postnatal development of the striatal dopaminergic system and the possible dopaminergic component of amphetamine stereotypy (Marsden & Guldberg, 1973) in the second postnatal week (White & Tapp, 1977). It is interesting that Erinoff and Heller (1978) show an adult-like response in the same nigrostriatal system by simply lesioning the pathway in 8-day-olds.

Thus the present paradigm provides an additional dependent measure with which to support very early postnatal behavioral responses to pharmacological treatment. The present data fit well with these investigations into the ontogeny of neural systems that mediate both self-stimulation and other behaviors motivated by conventional reinforcers.

## GENERAL DISCUSSION

In an attempt to view results for the stereotaxic method and the three preceding experiments into perspective, this discussion addresses important issues and implications enjoined by each section above. Specific problems of design and measurement have been discussed within each experiment.

Notable in Experiment 1 was the considerable effort with which the terms ICSS and self-stimulation were used in the context of specific reward/reinforcement vs. activation paradigms. Though adult animals were said to engage in ICSS behavior, pups or neonates 'self-stimulated'. The dichotomy is not an arbitrary nor capricious one, but it is in fact predicated on the literature which reviews ICSS (e.g., Olds & Fobes, 1981). The ICSS literature has always assigned a motivating, reinforcing role of direct electrical brain stimulation in adult animals. There is no

convincing evidence that such a reinforcing or reinforcement mechanism exists in very young animals. As discussed in Experiment 1 (see Discussion) this issue is particularly salient in view of the impressive behavioral activation by pups following tactile (Sullivan & Brake, 1981; Sullivan, personal communication), food-rewarded (Hall, 1979a,b), self-stimulation (Moran et al., 1981), and pharmacologic (Campbell et al., 1969) designs. Self-stimulation via direct electrical brain stimulation in pups may be a very different phenomenon in young, developing animals compared to that in adults. The consequences of and motivation for such appetitive behavior may not be comparable. As others have suggested (Hall, personal communication), this activational component may simply complement the as yet undifferentiated reward system or systems. Thus, a conservative interpretation of the results of direct brain stimulation herein requires a prudent examination of related work and implications for developing animals.

A recent study (Moran et al., 1981) attempted to overcome the difficulty in separating activational from reinforcing events. Using electrodes implanted only in the MFB and yoked controls, this study demonstrated that yoked pups did not learn to self-stimulate as did 'reinforced' pups. However, Moran et al.'s testing population was a screened population of subjects, pups that had all

uniformly exhibited behavioral activation after implantation and ad libitum electrical stimulation but before admission to the experimental group. Since Moran et al. used behavioral activation as a criteria for the inclusion of subjects into the experimental group, results did little to dissect the respective roles of activation vs. reward in neonatal learning. The pre-selection of subjects in no way minimizes or invalidates the import of such work, but the generality of those results may be suspect, especially since the present thesis showed that only a proportion of pups learned to self-stimulate in a very similar paradigm.

The apparent controversy surrounding activation vs. learning makes theoretical distinctions between either alternative difficult. Evidence for the arousal-potentiating actions of amphetamine have been reviewed herein (Campbell et al., 1969; Fibiger et al., 1970; Spear et al., 1980) and extended by the present work. However, a review of the ICSS adult literature shows similar ambiguity in an adequate description of reinforcement by ICSS. For example, Routtenberg (1968) proposed three systems as mediators of motivated behavior, two of which are relevant to the issue of behavioral activation and excitation. The activation of Arousal I, a system with anatomical origins in the reticular midbrain, produced neocortical arousal and 'organized' behavioral responses. Reward and incentive

were produced by activation of the Arousal II system, with anatomical origins in the ventral tegmentum and hypothalamic, hippocampal, and septal projections. Similarly, Valenstein (1969, 1970) envisioned the motivational element of direct hypothalamic stimulation as excitation of hypothalamic cell aggregates, the chief motivational element in most studies of ICSS. The facilitation of motor output by feedback activity to the stimulation of motor systems (via the hypothalamus) provided a mechanism for reinforcement; i.e., ICSS was reinforcing because of its capacity to activate motor outputs and provide feedback excitation. Lastly, Stein (1978) has postulated the existence of three distinct systems attuned for the mobilization, reinforcement, and termination of behavior. These three sytems are presumably modulated and coordinated more or less discretely by DA, NE, and opiate neurotransmitter systems, respectively. Though Stein's hypothesis is an interesting one, the hypothesis is untestable and little empirical data supports its existence at all. As stated by Olds and Fobes (1981), the trouble with all of these theories of motivation or arousal is the failure of the specification of the operation or dynamics of the way such systems work, except in the most speculative of terms.

The potentiation of behavioral activation was shown following the administration of d-amphetamine in Experiment

2 and the experiment that comprised Appendix 4. More importantly, these experiments showed dramatic, preferential facilitation of responding for direct electrical stimulation in 10-day-old rat pups. The shortcomings in Experiment 2 included the limitation of the number of amphetamine doses. However, when a few 10-day-olds were tested in this paradigm at greater (e.g., 10 mg/kg) doses, the mortality rate was quite high and unacceptable (unpublished observations). Since a significant difference in responding was noted at 5.0 mg/kg and not at 1.0 mg/kg or saline, a test dose that is an order of magnitude lower than 1.0 mg/kg may extend this initial bivalent study in terms of dose-response relationships. This additional test dose would show whether the infant response to self-stimulation is a monotonic function of the amphetamine dose. A 2.5 mg/kg dose may be an approximate threshold dose for response differences, judging from data accumulated herein. An additional limitation of the acute amphetamine study must be the variability in electrode loci between animals. Different electrode loci yield differences in response rates for ICSS in adults (for review, see German & Bowden, 1976) and amphetamine has different effects on ICSS at different sites (Fibiger, 1978). Fortunately, most sites were localized in the MFB, though the location of these sites varied in the anterior-posterior plane. Since each

experimental group sampled loci with a relatively similar anatomical proximity between groups, and since results within groups were fairly consistent, these results may be interpreted as a valid, reliable effect.

The added spoil of Experiment 2 was the demonstration of threshold-like effects following administration. The demonstration of this rather dramatic effect showed the surprizing sensitivity of this self-stimulation paradigm, even in 10-day-old pups. This effect was also shown in the chronic haloperidol study in Appendix 4.

The demonstration of these amphetamine-mediated effects in 10-day-olds herein, 5-day-olds (Raskin & Campbell, 1981), 15- and 25-day-olds (Lytle et al., 1969; Raskin & Campbell, 1981), and nondeprived 3-and 10-day-olds (Capuano, Barr, & Liebowitz, 1982) has prompted subsequent replication of the present self-stimulation work with 3-day-old subjects given acute d-amphetamine. Preliminary results show that these pups exhibit a similar preferential facilitation of responding on S+ (unpublished observations). Evidence for the DA theory of ICSS derives principally from (a) anatomical data which shows ICSS can be obtained from brain regions that contain predominantly DA neurons, (b) pharmacological data using neuroleptics, and (c) studies using permanent selective depletion of DA which eliminates ICSS. Since DA levels and neural DA constituents are so very low in 10-day-olds and virtually

non-existent in 3-day-olds (Lanier et al., 1974), this data seriously questions the applicability, even the validity of the DA theory of ICSS reinforcement in pups or neonates. This statement should not be interpreted to impugn the role of DA mediation of reinforcement for adult ICSS. It is possible that very low DA levels participate in the modulation of self-stimulation in these pups and that amphetamine administration potentiates or 'primes the pump' to enhance release or inhibit DA degradation at these early ages. However, the relatively low levels of DA, in contrast to the significant effect following amphetamine administration, suggest that this effect may be mediated by a system other than DA, such as the reticular activating system that is relatively more mature at birth (Carlton, 1963). Again, this hypothesis suggests a very important role for activation as a principle response determinant, possibly mediated by activation of the reticular activating system or other early developing neural structures, either directly or indirectly by amphetamine.

The majority of this present thesis has attempted to outline discontinuities in development, i.e., the onset of the development of infant self-stimulation presumably related to the onset of neuronal function. Gottlieb (1982, in press) has noted:

> As a matter of experimental strategy, the search for continuities in development has been emphasized somewhat at the expense of discontinuities...it is much more useful at this point in time to attempt to establish firm continuities in

> development than it is to prove...that functions arise entirely de novo...

Thus the present work may be seen in a larger perspective, relative to the expanse of literature that emphasizes continuity in development.

Gottlieb has briefly reviewed several exceptionally well-founded principles of development, including the principle of forward reference, the notion that the behavioral capabilities of newborn animals are typically so very well adapted to their usual life circumstances that they have a preadapted quality. Forward reference is conceptually related to the issue of precocious functional maturation, i.e., many prenatal and early postnatal phenomena are anticipatory or preparatory (Anokhin, 1964; (Carmichael, 1963; Coghill, 1929). For example, hungry 3-day-old rats deprived of nutritive suckling are capable of adult-like independent ingestion long before normal time of weaning at approximately 21 days (Hall & Bryan, 1980). This principle reiterates the view that early behavioral development, whether expressed as neurosensory or neuromuscular maturation, has a substantial impact upon later behavior. Though this descriptive principle of development has little explanatory value in and of itself, this principle underscores very recent findings that many behavioral capabilities of the neonate may arise during the prenatal phase (Johanson & Hall, 1979a, Teicher & Blass, 1977). In the present context, forward reference reminds

us that at the level of the unit of the nervous system, neural components may be detected, measured, even manipulated, before the onset of normal autogenous functioning. Our own investigatory manipulations may be an adequate stimulus to trigger the precocious onset of function.

The origins of developmental research may be traced to simple, naturalistic, and non-interventionist techniques which still continue to provide information on the ontogeny of behavior and function (for review, see Gottlieb, 1976). However, technological advances within the last few decades have required a degree of precision and accuracy heretofore not required of developmentalists. For example, it is not enough to empirically describe the development of a behavior; an adequate description must now include the mechanisms, neurological bases and components, levels of hormones or neurotransmitters, receptor populations, and so on. Indeed, it is ironic that our present degree of analytical sophistication does little to specify the nature of the interaction of these various components in behavior. Perhaps we suspect that as the degree of precision and measurement becomes more exacting, so will the specification of the manner in which these components interact.

It is clear that there is no analytical substitute for clever experimental manipulations. Our degree of

technological prowess must be used to supplement or complement skillful ontogenic research, but never to guide the course of such work. It is in this regard that we may someday explain rather than describe development.

## Appendix 1: A Review of ICSS Research

The study of brain mechanisms of reinforcement has largely been dominated by studies involving ICSS. The reward systems as suggested by brain stimulation studies have been assumed to represent systems which are normally activated by more natural reinforcers such as food for hungry animals. In the past decade and a half, thinking about reward systems in the brain has been greatly influenced in one form or another by the catecholamine (CA) hypothesis of brain reinforcement, i.e., there exists one or more central neural systems which are specified for, and which play a critical role in, reward phenomena, and that at least one critical link in the system or systems is a set of CA-containing neurons.

First suggested in the early 1960's (Poshel & Ninteman, 1963; Stein, 1962), classic lines of support for the CA hypothesis have been reviewed and documented (Crow, 1972, 1973; German & Bowden, 1974; Stein, Belluzi, & Wise, 1976). However, the CA hypothesis is still controversial owing to particular problems and strong objections (e.g., Amaral & Routtenberg, 1975; Clavier, Fibiger, & Phillips, 1976; Clavier & Routtenberg, 1974; 1976; Corbett, Skelton, & Wise, 1977).

It has been argued that ICSS is not only found in CA projection areas (Arbuthnott, Fuxe, & Ungerstedt, 1971; Crow, 1972a, 1972b, 1973; Dreese, 1966; German & Bowden,

1974; Stein, 1969; Stein & Wise, 1969) as originally mapped by Ungerstedt (1971) and more recently by Lindvall and Bjorklund (1974a, 1974b), but that it is proportional in strength to the concentration of CA neural elements at the electrode tip (German & Bowden, 1974). This generalization is based primarily on the fact that the strongest self-stimulation is seen with electrode placements along the path of the medial forebrain bundle (MFB) where there are overlapping projections of the mesocortical, and substantia nigra (SN) dopaminergic systems (Lindvall & Bjorklund, 1974a, 1974b).

Most data suggest that both of the DA systems, as well as the dorsal NE system, substantially overlap the areas that support ICSS (e.g., German & Bowden, 1974). Similarly, most CA treatments exert similar effects on DA and NE; hence it has been difficult to distinguish actions associated with one CA from those associated with another. However, selective blockade of NE synthesis can be accomplished by inhibiting dopamine-B-hydroxylase (DBH), the enzyme that converts DA to NE. Central or systemic administration of DBH inhibitors disulfiram, diethyldithiocarbamate (DDC), U-14,624, and fusaric acid abolished self-stimulation and eliminated the rate-enhancing action of amphetamine (Wise & Stein, 1969, 1970; Stein, Belluzi, & Wise, 1977; Wise, Belluzi, & Stein, 1977). Since DBH inhibitors leave brain DA levels

unaffected and since replenishment of depleted neurotransmitter stores by intraventricularly administered NE produces rapid and almost complete behavioral recovery (Wise & Stein, 1969, 1970; Stein et al., 1977; Wise et al., 1977, these experiments provide strong support for the conclusion that NE neurons are specifically involved in self-stimulation.

Other studies have demonstrated that response-contingent activation of NE systems is required for behavioral facilitation (Shaw & Rolls, 1976; Stein, Belluzi, Ritter, & Wise, 1974). These pharmacological studies fit nicely with the results of self-stimulation mapping studies on one hand and histochemical maps of NE pathways on the other (e.g., Crow, Spear, & Arbuthnott, 1972; Ritter & Stein, 1973).

These and other studies attest to the notion that the strength of the CA hypothesis is not the sometimes equivocal anatomical evidence itself, but is rather the convergence of anatomical findings with evidence from pharmacological studies. In general, drugs that inhibit CA function tend to disrupt ICSS, whereas drugs that enhance CA function tend to facilitate ICSS (German & Bowden, 1974; Stein, 1969; Wauquier, 1976).

Strong evidence for DA mediated ICSS behavior has also been demonstrated, especially when compared to conflicting reports regarding noradrenergic mediation. Self-

stimulation has been reported from areas of DA cell bodies, fibers, and terminals (Crow, 1972b, 1976; Phillips, Carter, & Fibiger, 1976; Stein, 1969).

The work of Routtenberg and his students (Huang & Routtenberg, 1971; Routtenberg & Malsbury, 1969) is cited in support of the view that SN cell bodies support self-stimulation, although most of their positive electrode placements in the ventral tegmental area were at the point where DA fibers from the SN (A9) and midline tegmental (A10) cell groups converge to ascend in the MFB (Routtenberg & Malsbury, 1969). Clearly, good ICSS behavior does occur with placements along the DA projections of the MFB (German & Bowden, 1974; Olds, 1970) where, of course, many other fiber pathways overlap.

In spite of negative or conflicting reports of caudate stimulation (Olds, 1970; Routtenberg, 1971), ICSS has also been reported for the DA terminal projections in nucleus accumbens (Phillips, Brooke, & Fibiger, 1975) and the caudate (Phillips et al., 1976). The distribution of positive sites in the caudate has been suggested by some to parallel the distribution of DA terminals (Phillips et al., 1976). However, the involvement of the nigral DA cells has been challenged (Belluzi et al., 1975; Clavier & Fibiger, 1977).

Since DA blockers also attenuate food-rewarded (Fibiger et al., 1976; Wise, Yokel, & de Wit, 1976) and

amphetamine-rewarded (Davis & Smith, 1975; Risner & Jones, 1976; Yokel & Wise, 1975, 1976) responding, it seems possible that some critical DA system plays a part in all or at least several positively-rewarded behaviors; such a critical system could be transynaptically activated in ICSS, even if stimulation fails to activate it directly.

ICSS behavior demonstrated in work with synthesis inhibitors and depletion (Wise & Stein, 1969), recovery of ICSS following pharmacological intervention (Franklin & Herberg, 1974, 1975; Wise & Stein, 1969), receptor blockers (German & Bowden, 1974; Wauquier, 1976), and indirect agonists (Poshel, 1969, 1976; Poshel & Ninteman, 1964; Crow, 1970) all suggest a coherent picture of DA mediation of ICSS. The effects of direct CA receptor stimulants have been studied only minimally and do not suggest a consistent picture (Berger et al., 1976; Herberg, Stephens, & Franklin, 1976; Wauquier, 1976).

The limitation of pharmacological evidence is, of course, that putative effects or non-effects of specific therapies may be artifacts of the debilitating effects of the drug. While some decrease in general activity and food rewarded lever pressing is seen even with low doses of DA blockers (Fibiger, Carter, & Phillips, 1976; Rolls, Kelly, & Shaw, 1974; Rolls, Rolls, Kelly, Shaw, Wood, & Dale, 1974), several facts suggest that DA receptor blockers can block the rewarding effects of stimulation selectively, at

doses lower than those required to produce motor impairment (Fouriezos & Wise, 1976; Rolls et al., 1974). The pattern of response change seen in tests after pretreatment with DA receptor blockers suggests that these agents specifically block neural systems critical for the rewarding property of stimulation.

Perhaps the most provocative correlational evidence in support of DA involvement in ICSS comes from studies of cortical placements. Self-stimulation is seen with electrode sites in medial and sulcal frontal cortex (Routtenberg, 1971; Routtenberg & Sloan, 1972), in entorhinal cortex (Collier, Kutzman, & Routtenberg, 1977) and in anterior cingulate cortex. DA terminals have been found in these but not other cortical areas (Hokfelt, Fuxe, Johansson, & Ljungdahl, 1974; Theirry, Stinus, Blanc, & Glowinski, 1973; Theirry, Tassin, Blanc, & Glowinski, 1976). Thus, ICSS sites are nicely correlated with cortical DA terminals: In entorhinal cortex high rates of ICSS seem associated with sites where DA terminals are clustered on cell groupings, and low rates are associated with sites where DA terminal density is low.

To reiterate, the CA hypothesis of reward holds that there are specialized systems in the brain which mediate the control of behavior by rewarding events, and that at least one critical link in these specialized reward systems involves a CA pathway. The CA hypothesis is based on

studies of ICSS. Two major lines of evidence have been critical: 1) anatomical evidence suggesting that possible ICSS sites are always near one or another CA pathway, and that the best sites are near the most densely aggregated major CA systems, and 2) pharmacological evidence that ICSS is disrupted by drugs which interfere with CA function and is generally facilitated by drugs which augment CA function. There are shortcomings for each category.

## Appendix 2.

## Pilot study of the development of ICSS using 16-, 25-, and 34-day-old rats.

### Methods

**Subjects.** The subjects were the offspring of Long-Evans Hooded rats (Blue Spruce Company) mated and maintained as described in PART I.

**Surgery.** Anesthetic doses and the coordinates for implantation were taken from Sherwood and Timiras (1970). All coordinates were devised with lambda and bregma skull landmarks in the same horizontal plane. For operating coordinates listed below, note that the anterior-posterior coordinate (AP) refers to the distance from bregma. Dorsal-ventral dimensions (DV) refer to the distance ventral to the dural surface. Medial-lateral coordinates (ML) refer to the distance from the midline. Actual operating coordinates are presented in Table 5. All pups were mounted directly in the stereotaxic instrument (David Kopf Instruments, Model 1430).

**Testing.** The electrical stimulus for all pups during testing was a 60 Hz, 125uA train of 250msec duration. Animals older than 15 days, like adult animals, were shaped to barpress in a standard operant chamber with a single bar. Approximately 16 to 24 hr postoperatively, these pups were placed in a standard operant, continuous reinforcement, barpress paradigm for constant current ICSS.

Table 5

Coordinates for MBF Implants in Pups of Various Ages

| Age of Pup (Days) | Reference AP | ML | DV |
|---|---|---|---|
| | Coordinates (mm) | | |
| 16 | -1.5 | +1.9 | 7.3 |
| 25 | -0.3 | +1.9 | 7.6 |
| 34 | -0.3 | +1.9 | 7.7 |

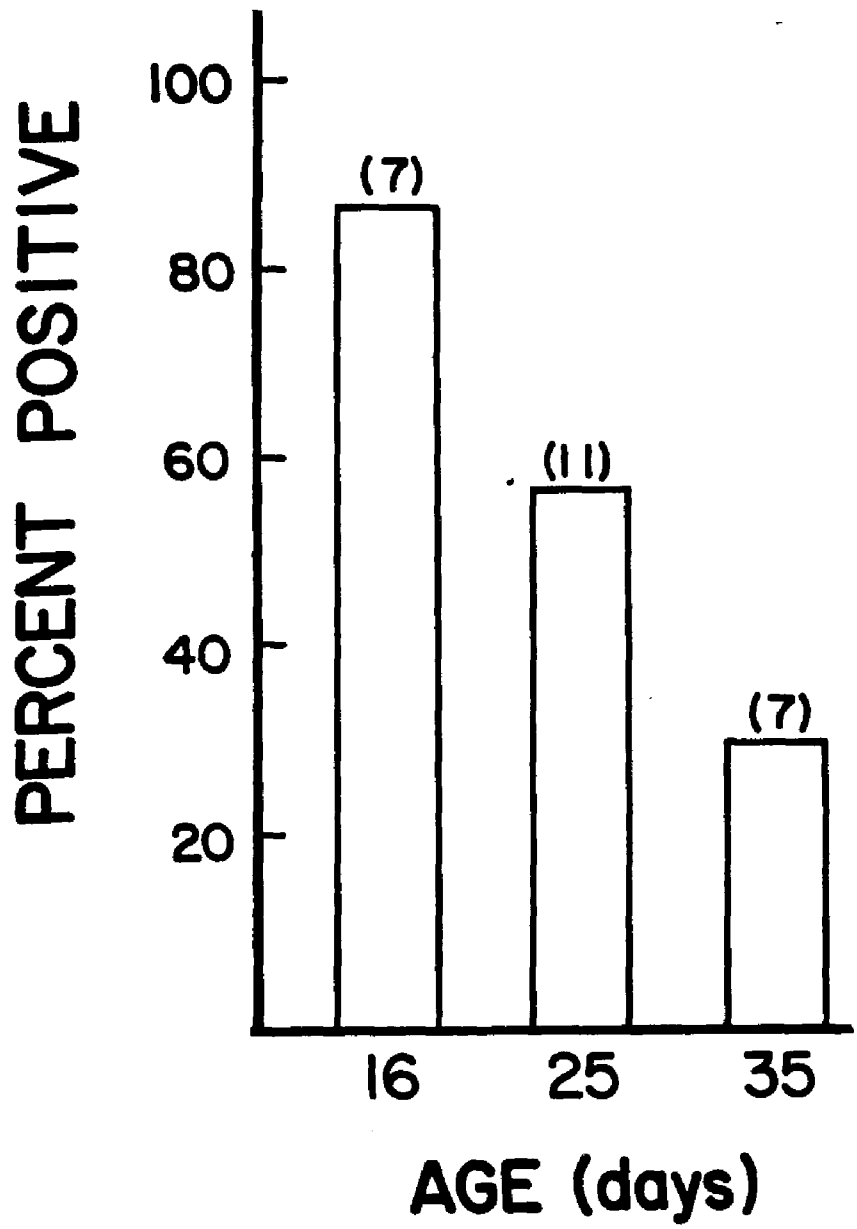
Pups were given a 10-15 min shaping and warm-up period, followed by a 50 min experimental session, where the number of barpresses in 10 min intervals was automatically counted and recorded. A pup was judged positive if the response rate exceeded 200 responses/hr.

Histology. Sacrifice was performed under Nembutal anesthesia and subjects were perfused intracardially with saline followed by buffered formalin (10% solution). Frozen 50 micron sections were cut and stained with cresyl violet to examine cell bodies or stained with a modified Kluver Barrera method for visualization of fibers (Wolf, 1971).

## Results

Behavioral. Figure 12 presents the proportion of positive rat pups for all three ages. The highest

Figure 12. The proportion of positive (self-stimulating) rat pups for three different ages. Pups were tested in a standard operant chamber in which only the force requirement for a barpress was altered.


PERCENT POSITIVE
100
80
60
40
20
(7)
(11)
(7)
16
25
35
AGE (days)

proportion of positive animals were 16-day-olds, but the few number of pups that were tested precludes a direct comparison between ages.

Figure 13 presents response rates for the three different ages. Response rates were greatest for 34-day-olds in the barpress paradigm, though the variability is high, owing to the very small sample (n=2) of positive animals. The 25-day-old pups showed moderate rates of 1120 responses/hr, intermediate between 34- and 16-day-olds, that showed a low to moderate self-stimulation rate, 573.5 responses/hr.

Histological. Figures 14, 15, and 16 present histological data for 34-, 25-, and 16-day-old rat pups, respectively. For 34-day-olds, two neutral sites are shown in both the MFB and optic tract, while a fifth neutral site is localized just ventral to the premammillary nucleus of the hypothalamus. Two positive sites are shown in the internal capsule and the periphery of the MFB or dorsal supraoptic nucleus. Two positive, two neutral, and one aversive site are localized in the MFB of 25-day-olds. The remaining two neutral sites bordered the medial amygdaloid or subthalamic nucleus. An additional aversive site is shown medial to the basis pedunculi, but this same site is positive just anteriorly. For 16-day-olds, all eight positive sites, as well as six neutral sites are shown in the MFB. The remaining neutral sites included the internal

Figure 13. Response rates only for rat pups that self-stimulated. The 'ns' are the number of positive animals in the sample shown, and bars are standard error of the mean (+SEM).

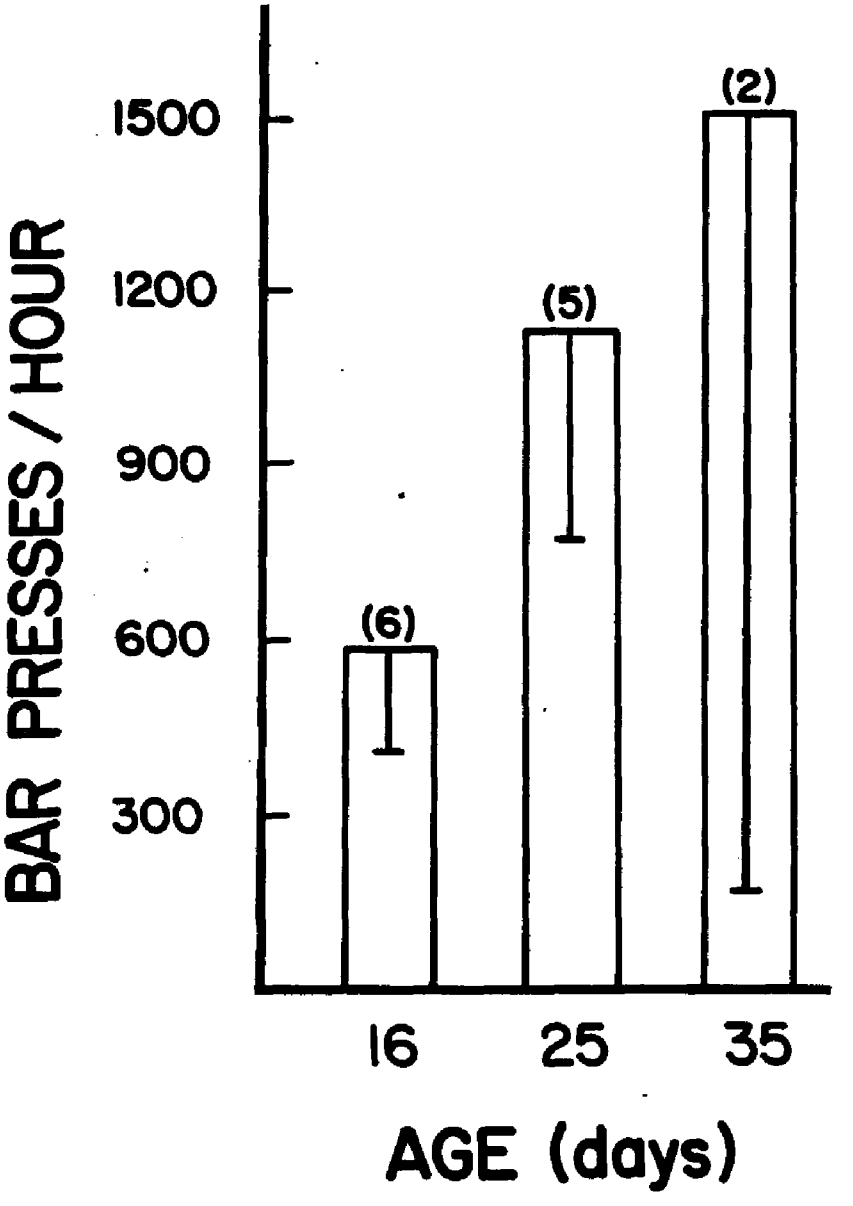

BAR PRESSES / HOUR
1500
1200
900
600
300
(6)
(5)
(2)
16
25
35
AGE (days)

Figure 14. Histology for 16-day-olds, plotted on the Sherwood and Timiras (1970) atlas. Positive sites are indicated by filled circles (●), neutral by open circles (○), and aversive by hatched (◍).

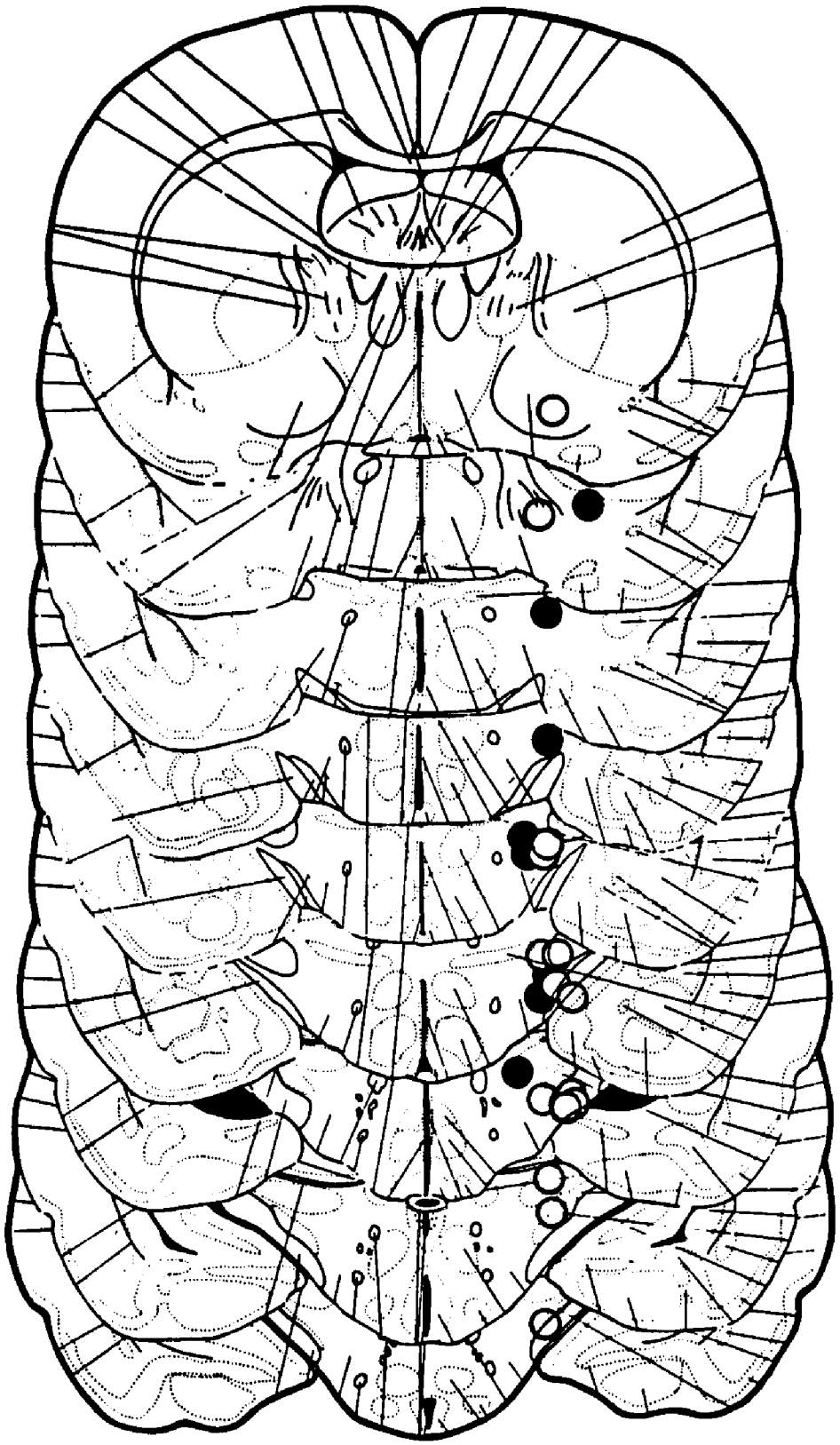

5.6
5.3
4.7
4.4
4.1
3.8
3.2
2.9
2.6

Figure 15. Histology for 25-day-olds. See Figure 14 for legend.

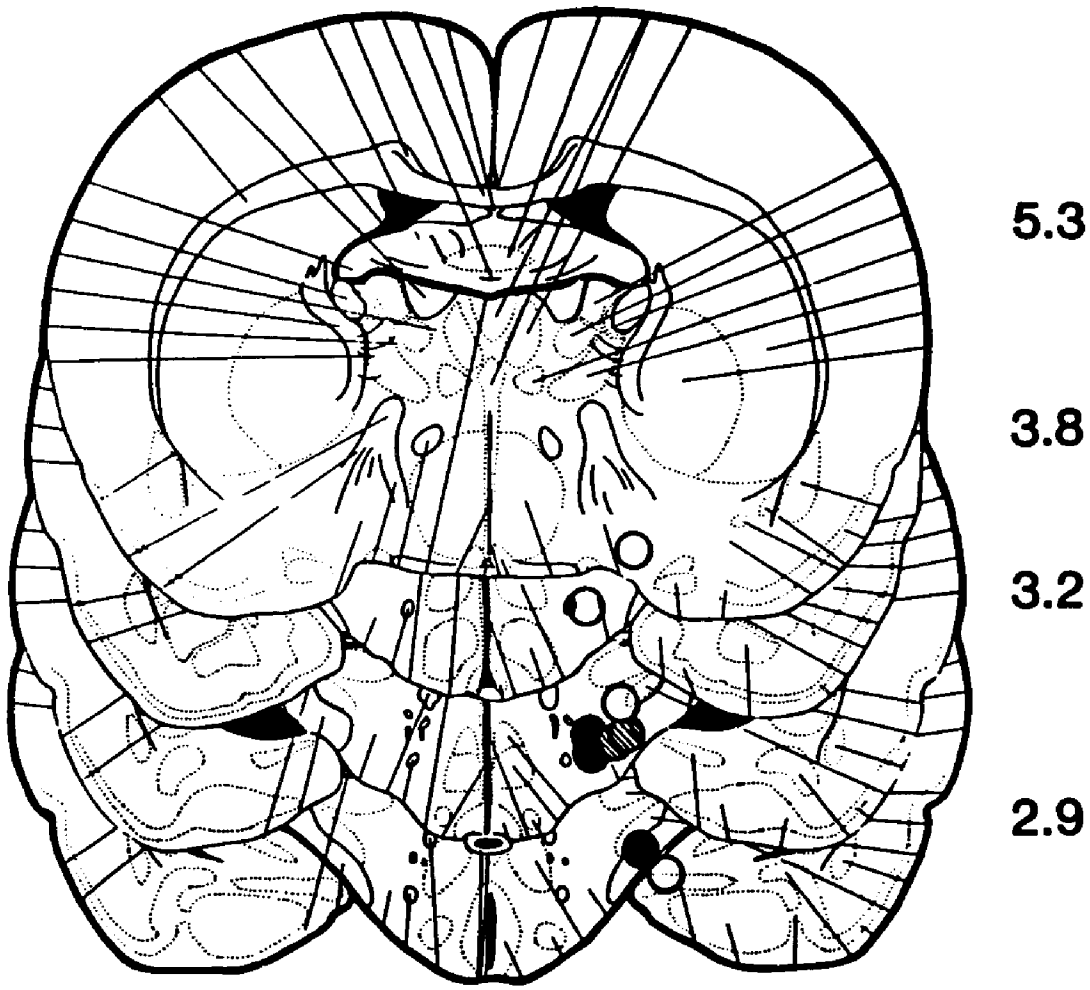
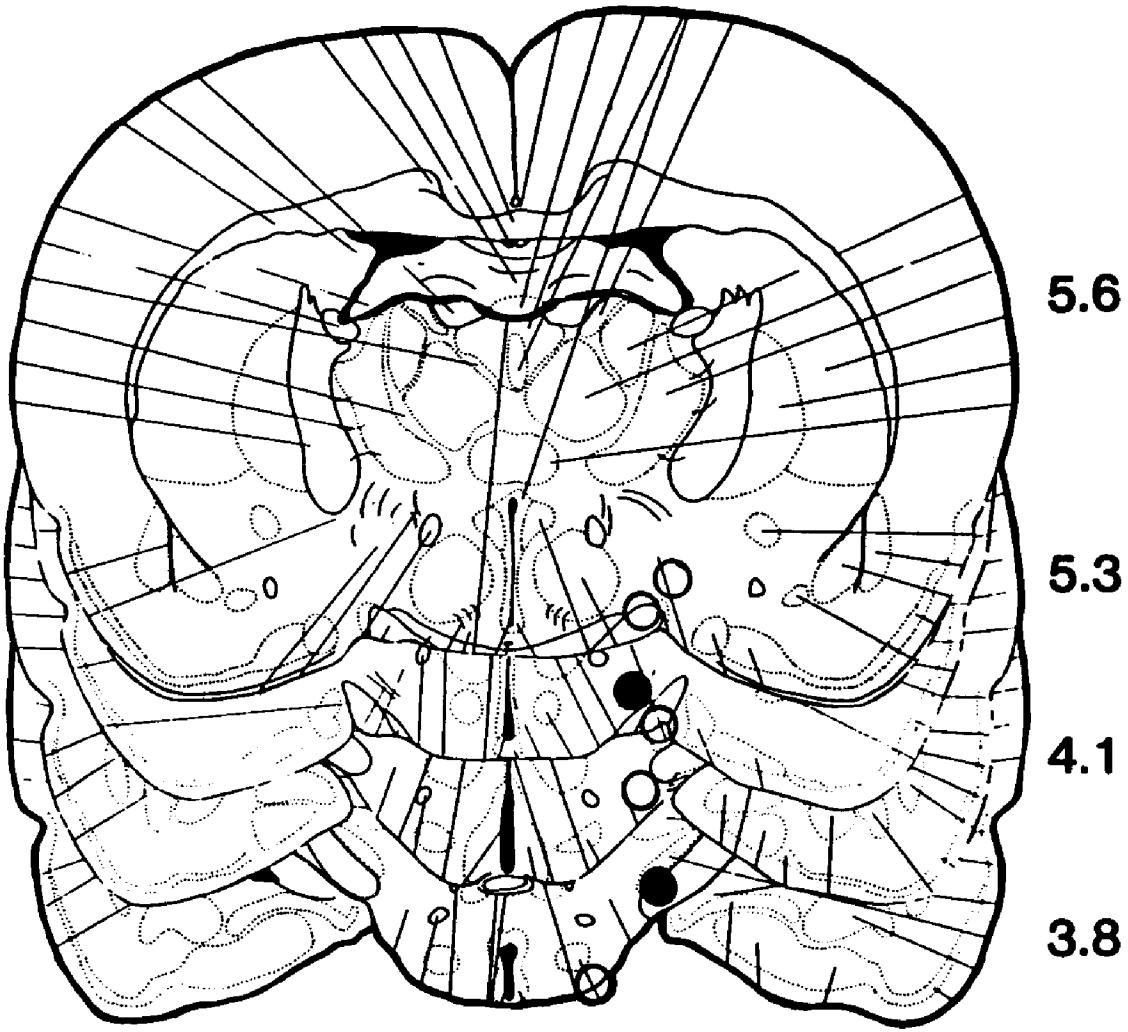

5.3
3.8
3.2
2.9

Figure 16. Histology for 34-day-olds. See Figure 14 for legend.


5.6
5.3
4.1
3.8

capsule, optic tract, basis peduncularis, zona incerta, and subthalamic nucleus. In general, 16-day-old histology showed a trend for positive sites in anterior brain sections, relative to neutral sites. Further, these positive sites tended to be slightly more medial to neutral sites.

## Discussion

While younger pups self-stimulated when given an opportunity in a task commensurate with their size and locomotor capabilities (see Experiment 1), 16- to 34-day-olds self-stimulated in a standard barpress paradigm. The fact that pups older than 10 days of age self-stimulated in a conventional barpress paradigm asserts a rewarding role of electrical stimulation akin to that in adult animals. Pups from 16 to 34 days of age learned to self-stimulate when electrodes were located in MFB and adjacent structures such as the internal capsule and supraoptic nucleus. Notably, most positive sites tended to be slightly more medial relative to neutral sites, though any given site did not reliably show positive responding when compared between animals. Differences in responding by 16-day-olds in the two paradigms merit attention. The high proportion of positive 16-day-old animals attests to the ease with which these pups learned to respond in the barpress paradigm. The surprisingly low proportion of 16-day-olds in the two-pole chamber (data not shown) contrasted sharply with the

same age barpress data. Response data from these and slightly younger pups, e.g., 13- and 14-day-olds (unpublished observations), suggest that the two-pole chamber as used here is best suited for 7- to 10-day-olds, and may not be as reliable for older animals, especially those older than 16 days. Stable response rates by 16-day-olds in the barpress paradigm support this notion. The lower reliability may be related to very different response topographies used by older pups, unrelated to for example, pole nudging.

## Appendix 3.

## Mean hourly response data for 7- and 10-day-olds

Eleven of 40 7-day-olds (27%) and 17 of 55 10-day-olds (31%) exhibited a greater total number of responses on the S+ vs. S- pole for the hours 2 to 10 of the session. These proportions of 7- and 10-day-old pups were significantly ($X^2$ = 42.6, and 77.7, respectively, $p < 0.05$) greater than chance. Figure 17 presents the mean cumulative number of responses for 7- and 10-day-old pups that self-stimulated for the 10 hr session. Results have been combined for warm and room temperature groups. Mean response rates for the 10 hr session were calculated using that portion of the session that appeared relatively linear in Figure 17. The 7-day-old pups showed an initially high response rate on S+, largely due to 3500 responses by a single subject in the first hour which quickly shifted to relatively uniform response rate, approximately 68 responses/hr for hours 1 to 8. This moderate response rate differs considerably from the very low S- rate of 21 responses/hr. Unlike 7-day-olds, the 10-day-olds showed a gradual separation of S+ vs. S- rates. Ten-day-olds exhibited a relatively uniform response rate, approximately 140 responses/hr, on S+ for hours 2 to 9, compared to a low rate of 21 responses/hr on S-. Response rates tended to decrease for both ages, beginning at 9 hr. For 7-day-olds, an analysis of variance with repeated measures showed a significant effect of hour

( $F = 6.50$, $p < 0.001$, $df = 9,72$) and pole by hour interaction ( $F = 4.22$, $p < 0.001$, $df = 9,72$), computed over the entire 10 hour session. Ten-day-olds showed significant main effects of pole ( $F = 15.15$, $p < 0.01$, $df = 1,15$), hour ( $F = 19.97$, $p < 0.001$, $df = 9,135$), and a pole by hour interaction ( $F = 16.78$, $p < 0.001$, $df = 9,135$). Table 6 presents mean hourly response rates for 7- and 10-day-old pups, calculated for the first and second 5 hr of the 10 hr session. The table shows the expected increase in S+ responding over the session for 10-day-olds, while responding on S- remained at a low and unchanged level. Though responding on S- for 7-day-olds decreased as predicted, these pups curiously evidenced an unexpected decrease on S+ as well. However,

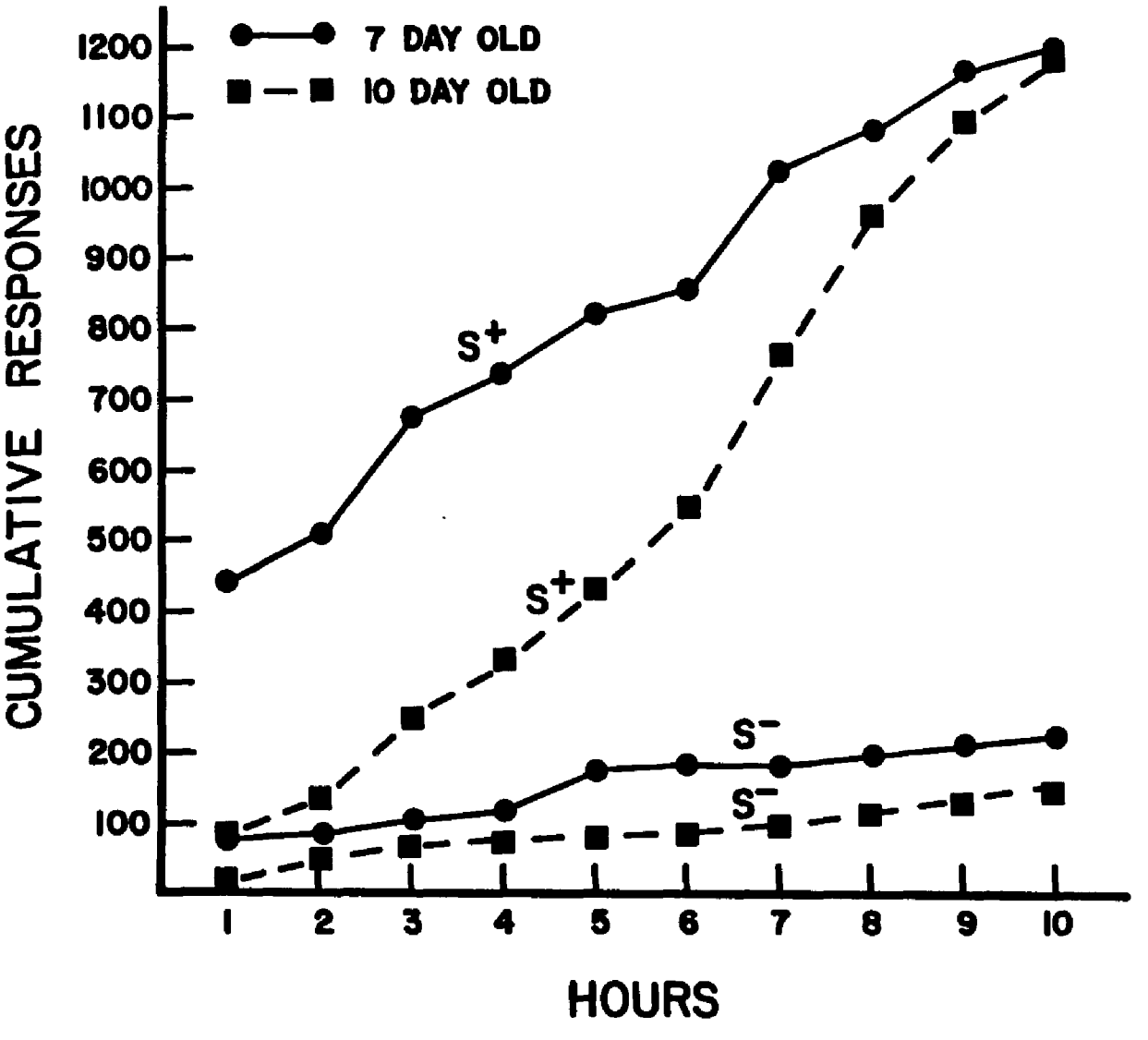
Table 6

Mean Hourly Response Rates for 7- and 10-day-old Pups

| | Pole | |
|---|---|---|
| | S+ | S- |
| | Mean Number of Responses (+SEM) | |
| 7-Day-Olds | | |
| Hours | | |
| 0-5 | 149.0 (107.0) | 39.0 (28.2) |
| 5-10 | 81.6 (47.0) | 6.1 (3.5) |
| 10-Day-Olds | | |
| Hours | | |
| 0-5 | 86.0 (32.6) | 13.4 (10.8) |
| 5-10 | 157.2 (58.4) | 13.8 (8.2) |

responding on S+ was still much greater than that on S-.

Figure 17. Mean cumulative number of responses for 7- and 10-day-old pups that self-stimulated. Response rates on both poles are shown for both ages. The greater 7-day-old rate was largely due to the inordinately large number of responses by a single subject in the first hour of the session.


7 DAY OLD
10 DAY OLD
CUMULATIVE RESPONSES
1200
1100
1000
900
800
700
600
500
400
300
200
100
S+
S+
S−
S−
1
2
3
4
5
6
7
8
9
10
HOURS

## Appendix 4.

## Teratology of 10-day-old rat pups: Response to acute d-amphetamine following chronic prenatal haloperidol.

The immature developing organism has been used to study long term effects of drugs administered early in ontogeny. It is clear that a number of drugs of abuse or chronically administered psychoactive drugs given in doses which are not generally associated with teratogenesis are able to cause developmental alterations at the neurochemical level (Slotkin & Thadani, 1980). Neurotransmitter systems in the developing brain and autonomic nervous system appear to be sensitive to a variety of drugs, especially those shown to exert direct actions upon functioning neurons. Data that is reviewed in the present introduction outlines the development of neuronal components and mechanisms that may be influenced by chronic drug exposure during ontogeny. However, the following discussion highlights the development of DA neural components since the present experiment attempts to determine behavioral alterations related to changes in DA receptors.

Two basic issues that have guided research on the development of the CNS are when do specific neuronal pathways form in the immature brain (Coyle, 1977; Lanier et al., 1976; Thornburg & Moore, 1976), and when does

effective neurotransmission among the pathway components begin (Cheronis et al., 1979; Erinoff & Heller, 1978; Pradhan & Pradhan, 1980). Recent technological advances in histofluorescent and radioactive labeling techniques have directly addressed the former issue, while far fewer studies have surmounted problems posed by the latter.

With respect to the latter issue, the developing brain of the intact animal does not allow easy identification of the beginning of functional neurotransmission. Since a clear determination of the ontogeny of transsynaptic communication is difficult in the CNS, this problem has been addressed by studies of the peripheral autonomic nervous system. Such studies utilizing reflex sympathetic responses have shown that stimulation of the splanchnic nerve to the adrenal medulla (Bartolome & Slotkin, 1976; Slotkin, 1973) and sympathetic cardiac innervation (Bartolome, Lau, & Slotkin, 1976; Weksstein, 1965) does not produce either secretion of catecholamines or induction of the rate-limiting enzyme tyrosine hydroxylase (TH) in rats less than 7 days old (for review, see Slotkin & Thadani, 1980). Interestingly, sympathetic sensitivity to direct nonneural stimulation by nicotine is present at birth (Bareis & Slotkin, 1979).

On the other hand, new histofluorescent, radioactive, and immunocytochemical techniques have rapidly advanced the detailing of specific neuronal pathway formation. The

activity of catecholamine related enzymes and levels of endogenous amines in the neocortex are relatively low throughout the neonatal period (Lanier, et al., 1976; Porcher & Heller, 1972). Though these pathways histofluoresce from very early in gestation (Lauder & Bloom, 1974), the limited development of monoamine-containing neurons reflects the general immaturity of the rat or rabbit CNS. Much evidence suggests that the central noradrenergic and GABA systems develop and mature earlier than the dopaminergic systems (Coyle, 1977; Coyle & Enna, 1976; Kellogg & Lundborg, 1972; Lauder & Bloom, 1974).

Two major dopaminergic pathways, the nigrostriatal tract and the mesolimbic tract, terminate in the striatum and portions of the limbic forebrain, respectively. Histofluorescent studies have shown that dopaminergic nerve terminals develop earlier in limbic relative to striatal terminals (Olson et al., 1972). Within the nigrostriatal system, dopaminergic receptors in the striatal pathway mature functionally before receptors in the striatum (Cheronis et al., 1979; Erinoff & Heller, 1978).

At birth, dopaminergic neurons with cell bodies in the midbrain form less than 1% of the total synaptic contacts present in the adult cerebral cortex (Coyle & Molliver, 1977; Molliver & Kristt, 1975). Specific neuroleptic binding to DA receptors in the corpus striatum is 10-15% of the adult levels and remains unchanged until the seventh

postnatal day (Pardo, Creese, Burt, & Snyder, 1977). During the first week of postnatal life presynaptic markers generally develop faster than postsynaptic DA receptors (Coyle & Campochiro, 1976; Keller, Bartholini, & Pletscher, 1973). The second postnatal week is characterized by a doubling of receptor density, followed by a tripling by the end of four weeks (Coyle & Campochiro, 1976; Pardo et al., 1977). Admittedly, it is unlikely that the quantification of receptor ontogeny in the striatum presents an entirely representative scheme of dopaminergic development, particularly since each region of the brain develops according to its own developmental timetable (Benjamins & McKann, 1972). Not surprisingly, the maturation of neurotransmitter systems, e.g., DA, varies across and even within brain regions, though ostensibly such development preserves the general theme of a caudal-rostral sequence (Benjamins & McKann, 1972; Jacobson, 1978, p. 58; Lanier et al., 1976). Accordingly, differences in the rate of maturation of the dopaminergic system in different brain regions may account for differences in vulnerability of those regions to early drug effects (see Rosengarten & Friedhoff, 1980, p. 613).

Data summarized above highlights the relatively late development of DA-mediated neurotransmitter systems. As such, changes in the functioning of these dopaminergic systems appear to be amenable to similar pharmacologic

manipulation and concomitant behavioral effects (e.g., Rosengarten & Friedhoff, 1979a, b, c; Barr, Gibbons, Alheid, Bridger, Turkewitz, Rosengarten, & Friedhoff, 1979).

The increased responsiveness of DA systems in clinical disease states, e.g., manifest as tardive dyskinesia and related extrapyramidal movement disorders (Baldessarini, 1979) or schizophrenia (Friedhoff, 1977), can be simulated in the laboratory. Biochemically, chronic administration of DA antagonists, neuroleptics, produces increased binding of labeled DA ligands, which reflects an increase in the number of receptors without a change in their affinity (Burt, Creese, & Snyder, 1977; Muller & Seeman, 1977; Tarsy & Baldessarini, 1974). Behavioral changes in adult animals after withdrawal from chronic neuroleptics include hyperactivity (Spear et al., 1980; Thornburg & Moore, 1975), an enhanced response to DA agonist induced stereotypy and locomotor activity (Asper, Baggiolini, Burki, Lauener, Ruch, & Stille, 1973; Makman, Gardner, Thal, Hirschorn, Seiger, & Bhargava, 1980; Muller & Seeman, 1977), an attenuated response to the cataleptic actions of a DA antagonist (Spear et al., 1980; Thornburg & Moore, 1975), and increases in ICSS (Seeger, Gardner, & Bridger, 1981).

There is strong evidence that dopaminergic brain substrates are important, though not exclusive, mediators

of ICSS reward (Breese & Cooper, 1976; Cooper, Cott, & Breese, 1974; Wise, 1976, 1978) though serious challenges to the so-called DA hypothesis exist (Belluzi, Wise, Ritter, & Stein, 1975; Wise, 1969; Wise & Stein, 1970). Accordingly, neuroleptics have been employed to attempt to pharmacologically specify the neuroanatomical substrates of ICSS reward (Wauquier, 1976; Wauquier & Niemegeers, 1972; Fouriezos & Wise, 1976). In addition to the use of haloperidol as a pharmacological tool to assess critical substrates of ICSS reward, recent work suggests that the neuroleptic haloperidol has possible teratogenic effects in the offspring of chronically treated dams (Engel & Lundborg, 1974; Lundborg, 1972; Spear et al., 1972).

These chronically treated pups exhibit hyperactivity (Spear et al., 1980; Vorhees, Brunner, & Butcher, 1979) and various behavioral anomalies (Ahlenius, Brown, Engel, & Lundborg, 1973; Ahlenius, Engel, & Lundborg, 1975; Lundborg, 1972; Spear et al., 1980). Compensatory mechanisms occurring in response to chronic neuroleptics during development appear to be different from those of adulthood (Spear et al., 1980).

The present experiment was based on an initial report (Rosengarten & Friedhoff, 1979a) and subsequent presentations of data (Rosengarten & Friedhoff, 1979b,c) that showed biochemical changes secondary to chronic prenatal neuroleptic treatment with haloperidol. This

treatment produced a decrease in specific $^{3}$H-spiroperidol and $^{3}$H-N-propylapomorphine binding in caudate of pups exposed to these agents during the last 16 days of gestation. Decreased binding was produced by a decrease in receptor number rather than a change in affinity. Offspring of chronic haloperidol-treated dams have shown a persistent decreased behavioral response to apomorphine with this concomitant decrement in the density of DA receptors.

Experiment 2 in the present report suggested this self-stimulation paradigm as a rather sensitive behavioral metric with which to measure the functioning of infant neural systems, showing a significant and dramatic facilitation of response rates for amphetamine-treated (5 mg/kg) 10-day-old pups relative to saline controls. Therefore, the deficit in receptor number demonstrated by Rosengarten and Friedhoff has important implications in the context of behavioral teratology, especially in the present paradigm. The present self-stimulation paradigm was used in a teratological design in order to determine changes in self-stimulation behavior following acute DA agonist administration to pups treated prenatally with chronic haloperidol.

## Method

Subjects. Approximately 3 to 5 days before mating of haloperidol and vehicle-treated animals, eight untreated

Long Evans hooded females were mated to serve as foster mothers in the laboratory colony described earlier. Pups that were born to foster mothers remained with the foster mothers until the birth of experimental pups from treated females. Vaginal smears were taken each morning until a positive sperm count was found at which time the forthcoming litter was designated 0 days of conceptual age. A second group of eight females was mated for the two drug groups. At the litter's fifth embryonic day, and continuing to full term, four females were injected (sc) with haloperidol (1.0 mg/kg) at approximately 10:00 hr each morning, while the remaining four received an equivalent volume of the drug vehicle. Pups were fostered as near as possible to 24 hrs after parturition on day 1. A total of 20 pups were used in each of haloperidol- and vehicle-treated groups. The two drugs were coded so that prenatal injections of dams were blind to the Experimenter.

Procedure. Only 9- to 11-day-old pups were implanted and tested using a similar design as in Experiment 2. Response rate and histology were collected and analyzed in the same manner, except that pups were run for an initial 8 hr before d-amphetamine challenge (5 mg/kg). This longer pre-injection period allowed closer examination of possible performance differences between groups before the administration of amphetamine.

In addition to the females mated to produce pups for behavioral analysis in the self-stimulation paradigm, two haloperidol,. two vehicle, and four females to serve as foster mothers were mated for subsequent receptor binding analysis. An identical experimental protocol was used, with pups fostered to untreated dams at birth, but pups were sacrificed at 16 days postnatally to determine H-spiroperidol binding to whole brain. Results of the receptor binding have not been completed, but will appear in subsequent work.

## Results

Offspring of vehicle- (VEH) and haloperidol-treated (HAL) dams showed dramatic, significant increases in the mean number of responses on both S+ and S- after d-amphetamine administration. Though there were no significant differences in the performance between groups over the session, HAL pups showed a tendency to self-stimulate at the elevated response level for a longer period after amphetamine, relative to VEH pups.

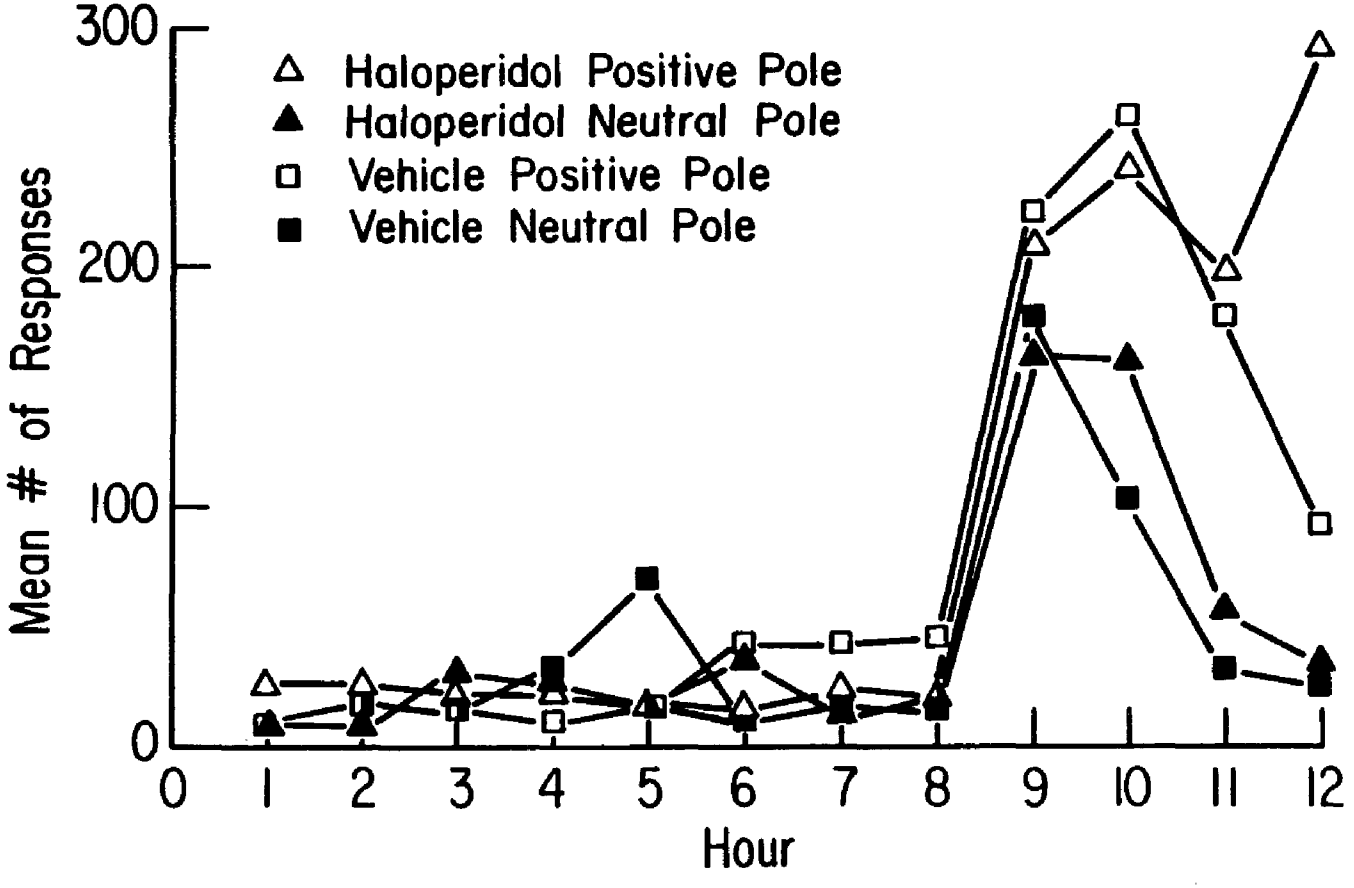
**Behavioral.** Overall response data was analyzed using a 3-way analysis of variance with repeated measures using two trial factors (hour, pole) and one grouping factor (drug). There was no difference in the mean number of responses/hr between VEH and HAL groups or for any drug by trial factor interaction. As in Experiment 1 and Experiment 2, significant effects of pole ( $F = 6.07$,

df=1,38, p 0.02) and hour ( F =11.83, df=11,418, p <0.001) were shown for both groups combined, with a significant interaction of pole by hour ( F =2.24, df=11,418, p <0.02). An analysis of the pole by hour interaction by a simple main effect did not show a significant response change at any hour.

Figure 18 presents the mean number of responses/hr for VEH and HAL groups. The figure shows similar low response rates for both groups on S+ and S- for the 8 hr baseline that preceded d-amphetamine challenge. Pre-injection rates were slightly greater, though not significantly, on S+ vs. S- for the 8 hr. baseline period. Mean S+ and S- response rates were 22.4 and 20.2 for haloperidol-treated and 24.9 and 23.9 responses/hr. for vehicle-treated animals, respectively. The mean number of responses on S+ was greater than that on S- for both groups. Maxima on S+ were shown at 10 and 12 hours for VEH and HAL pups, while maxima for both groups on S- were shown at 9 hours.

Figure 19 presents a cumulative record of a typical HAL pup for the last 7 hours of the 12 hr session. Note the limited number of responses on both S+ and S- before amphetamine challenge at 8 hr, followed by an abrupt increase in responding on S+. Responses on S- dropped out after amphetamine for this animal. As the number of S+ responses declined, e.g., hours 10-12, responses on S- began to increase in frequency. The differential rate-

Figure 18. The mean number of responses per hour for vehicle- and haloperidol-treated pups. Pups were injected at 8 hours.


Haloperidol Positive Pole
Haloperidol Neutral Pole
Vehicle Positive Pole
Vehicle Neutral Pole
Mean # of Responses
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Hour

enhancing effect of amphetamine appeared to last for approximately 3 hr by inspection of the figure.

Histology. Figure 20 presents histological data from VEH and HAL pups. As in Experiment 2, data were plotted to show the increase in responding for the difference in the mean hourly rate for hours 9, 10, and 11 compared to that during hours 7 and 8. The figure shows sites for VEH pups in the MFB, olfactory nucleus and tubercle, medial prefrontal sulcus. HAL pups exhibited electrode sites in the MFB, nucleus accumbens, olfactory nucleus, medial prefrontal sulcus, and a site on the border of the anterior commissure and nucleus accumbens.

Litter Size and Mortality. Table 7 presents litter size and mortality data from offspring of foster, vehicle- and haloperidol-treated dams. Parametric statistical tests were used when the variance between groups was equivalent, while nonparametric tests were used if one or more groups exhibited variances that were not homogeneous. Both the number of pups born and the mortality rate were not significantly different between groups. There were no significant differences between the weights of females and males within each group, but female HAL pups were significantly lighter than foster (Kruskal-Wallis test, $p < 0.04$) and VEH pups ($p < 0.01$). Male VEH pups were also significantly heavier than HAL (Newman-Keuls test, $p < 0.01$) and foster pups ($p < 0.05$).

Figure 19. A typical cumulative record for a haloperidol-treated pup for the last 7 hours of a 12 hour session. See Figure 13 for details, but note that an unbroken horizontal pen excursion represents 1 hour. The first two top lines have been condensed to show 2 hours each.

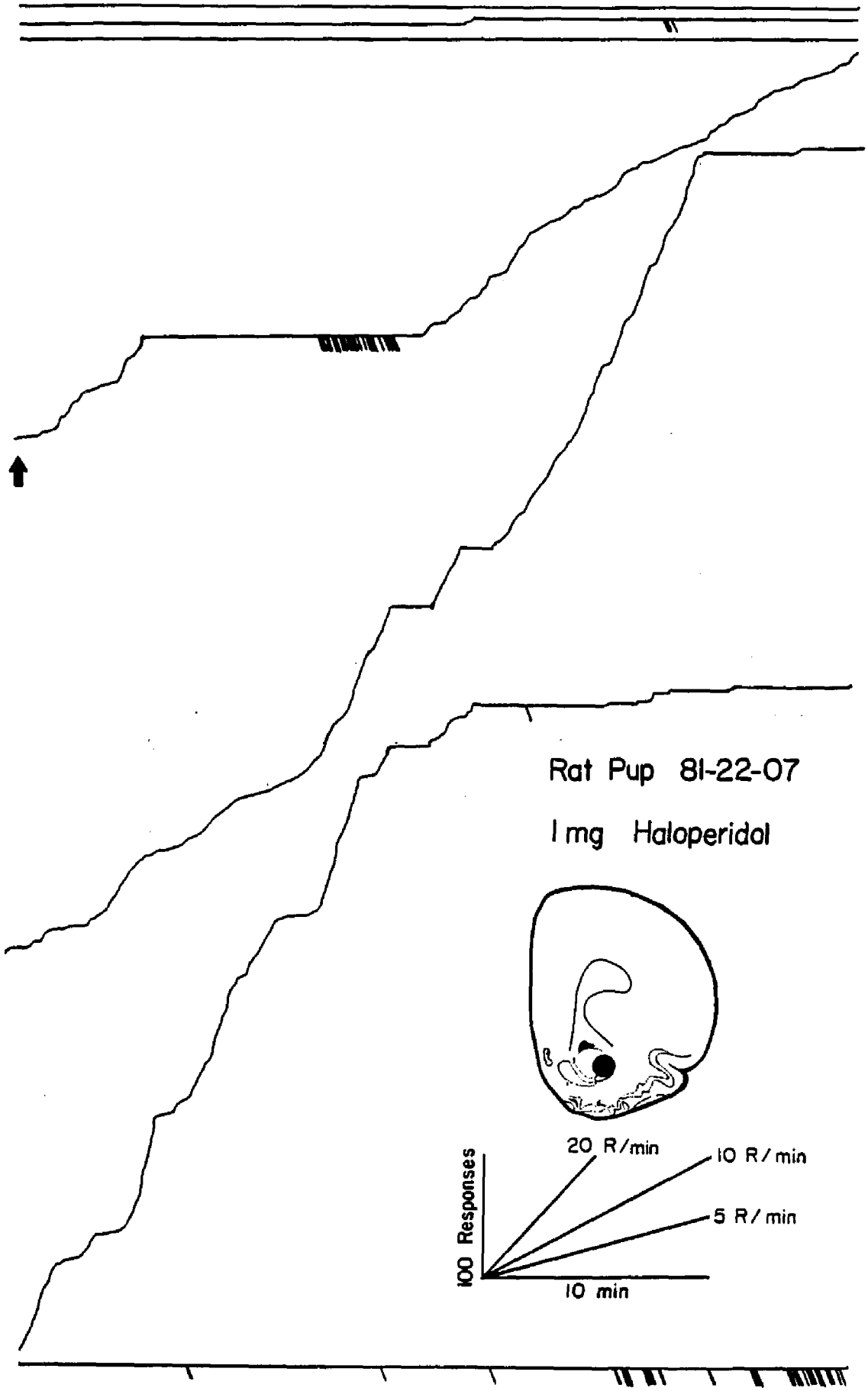

Rat Pup 81-22-07
1 mg Haloperidol
20 R/min
10 R/min
5 R/min
100 Responses
10 min

Figure 20. Histological data for vehicle- and haloperidol-treated pups. See Figure 11 for legend.

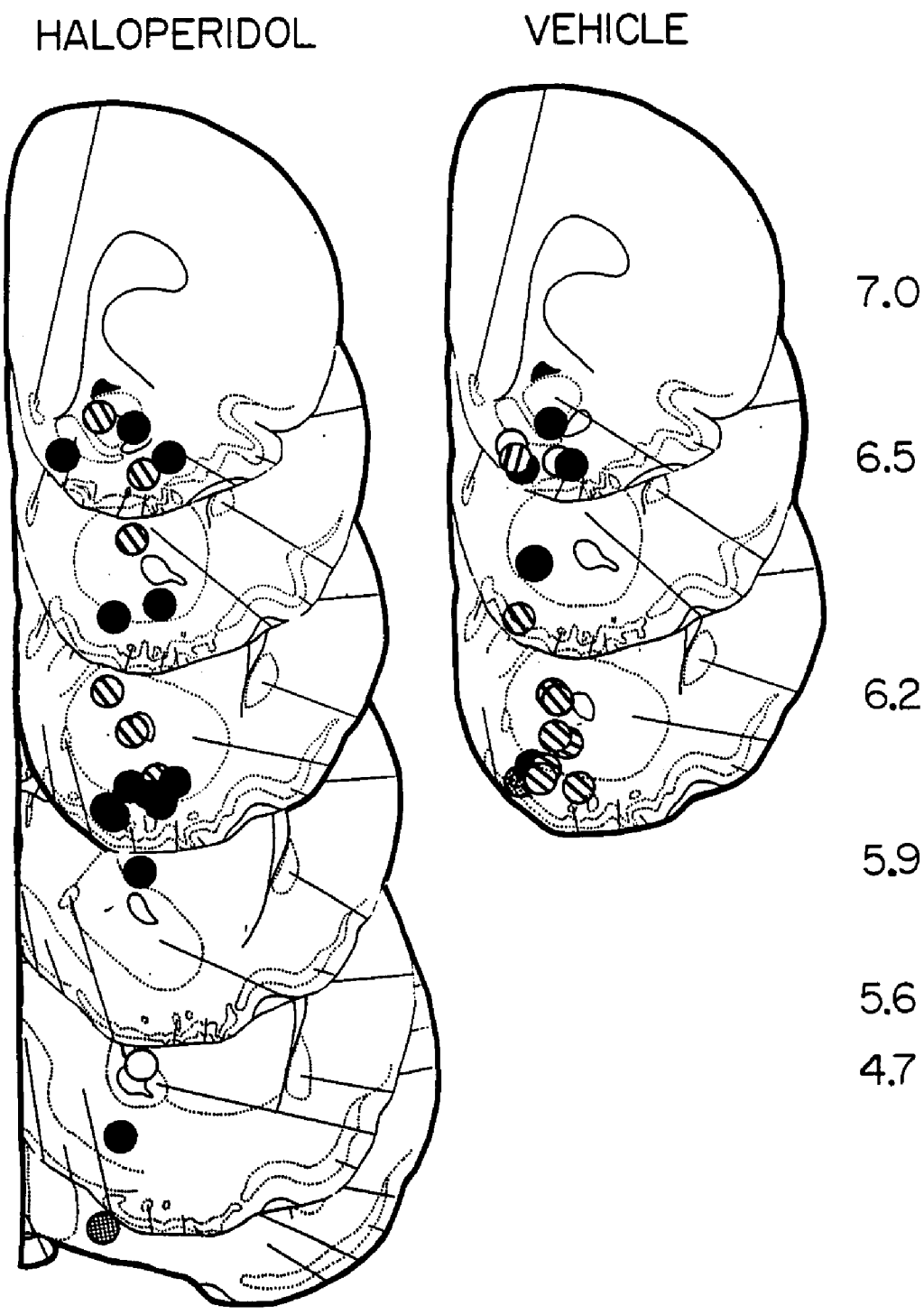

HALOPERIDOL
VEHICLE
7.0
6.5
6.2
5.9
5.6
4.7

Table 7

Litter Data from Offspring of Treated and Untreated Dams

| Group | Foster | | Vehicle | | Haloperidol | |
|---|---|---|---|---|---|---|
| | Mean # of Pups/Litter | | | | | |
| Litter Size | 10.8 | | 10.7 | | 9.0 | |
| Mortality | 0.6 | | 1.7 | | 1.5 | |
| Sex | Male | Female | Male | Female | Male | Female |
| | 6.8 | 4.8 | 4.3 | 6.5 | 3.8 | 6.0 |
| | Total Number | | | | | |
| | 28 | 23 | 14 | 22 | 21 | 30 |
| | Grams | | | | | |
| Mean Weight | 6.5 | 6.4 | 7.1 | 7.0 | 6.1 | 5.9 |

## Discussion

Results from the present experiment have shown that 10-day-old offspring of rat dams chronically treated with haloperidol or an equivalent volume of vehicle increased their response rate in a simple discrimination task for direct electrical stimulation following the administration of d-amphetamine. Though the rate of responding on both manipulanda significantly increased with that on S+ greater than responding on S-, no significant difference was shown between groups. Pups from haloperidol-treated dams tended

to exhibit increased responding for a longer period of time following amphetamine administration relative to control offspring, though this difference was not statistically significant.

The lack of a significant difference between groups was not anticipated, especially in light of recent results by related investigations using similar experimental designs. Using a 2.5 mg/kg dose of haloperidol, Rosengarten and Friedhoff (1979) demonstrated that offspring of chronically treated pregnant dams showed a decrease in behavioral responsiveness to the DA agonist apormorphine by 28- and 35-day-olds, coincident with a decrease in the apparent density of DA receptors using labeled $^{3}$H-spiroperidol. Behavioral tests performed 8-61 days postnatally revealed generalized hyperactivity in open field testing and decreased sensitivity to apomorphine and amphetamine (Barr et al., 1979). Using a similar protocol to that of Rosengarten and Friedhoff, a decrease in the behavioral response to apomorphine was shown in 10-day-old offspring, but not in 15- or 30-day-olds (Madsen, Campbell, & Baldessarini, 1981). Madsen et al. suggested that differences in findings of the two studies may be attributed to residual haloperidol in neonatal rat tissues after parturition, thus accounting for transient rather than persistent postpartum behavioral and altered binding effects. This explanation was based on the premise that

drug metabolizing pathways may be less efficient in newborns than adults (e.g., Tredger & Chhabra, 1980) and that the behavioral response of the rat appears to be sensitive to smaller amounts of haloperidol in the brain at later times after chronic administration (Campbell, Hershel, Cohen, & Baldessarini, 1980). Thus, carryover effects of haloperidol may have contributed to transient antiapomorphine effects. It is also possible that residual haloperidol may have antagonized the apomorphine-mediated effect by inhibiting action at the receptor site itself or by inhibiting entry of the DA agonist into the CNS (Westerink & Horn, 1979), but the absence of a definitive time course effect (Madsen et al., 1981) militates against such a hypothesis. Such a carryover effect in the present investigation would presumably result in a decreased self-stimulation rate or a response pattern with a time course of shorter duration, since neuroleptics uniformly decrease self-stimulation rates (Wise, 1978, 1981). Since responding by HAL pups did not differ from VEH pups, a carryover effect by residual haloperidol was unlikely in the present results. In fact, HAL pups tended to stimulate for a longer time after amphetamine, relative to controls.

Chronic pre- and postnatal haloperidol administration during development has been shown to induce long term behavioral and psychopharmacological effects in related studies (e.g., Spear et al., 1980), effects which appear to

be specific to the age of testing. These chronically treated offspring exhibit hyperactivity in the open field and increased hole-poke behavior when tested at 24 or 47-48 days postnatally, relative to same age controls, but not at days 35-36. Similarly, 25-day-olds are less sensitive to amphetamine-induced matrix crosses, but 37-day-olds are not. Matrix-crossing data from older 49-day-olds is ambiguous. Haloperidol-treated pups are also more sensitive to the cataleptic actions of an acute injection of haloperidol relative to controls when tested at the early and late testing periods, but not during the middle testing period. Of course, 'normal' untreated developing animals are very different behaviorally (e.g., Amsel, 1979; Spear et al., 1980) and with respect to the psychopharmacological response between age groups (Infurna & Spear, 1977; Spear, 1979; Spear et al., 1980). In fact, much literature has shown differences in the response to amphetamine between animals of different ages, with middle age pups less sensitive than younger or older pups (Bauer & Duncan, 1975; Infurna & Spear, 1979; Lanier & Isaacson, 1977; Spear et al., 1980).

Psychopharmacological effects of chronic haloperidol treatment during development appear to be different from those in adulthood. Treated adult animals may show hyperactivity (Vorhees et al., 1979), an accentuated response to amphetamine with an attenuated response to

haloperidol (Spear et al., 1980; Thornburg & Moore, 1975), while developmentally treated pups show an attenuated response to amphetamine and an accentuated response to haloperidol when tested at either 23-30 or 47-54 days postnatally. The pattern of behavioral and pharmacological responsiveness to haloperidol treatment during development may reflect a variety of compensatory changes. These changes may include variations in neurotransmitter levels, synthetic, or utilization rates (Engel & Lundborg, 1974), brain growth (Bartolome, Seidler, Andersen, & Slotkin, 1976) and innervation (Coyle & Campochiaro, 1976; Dorner, Straught, Wenzel, Kvetnansky, & Murgas, 1977). At present, the specification of which changes are critical for normal behavioral functioning has not been determined.

Other investigations, in addition to haloperidol studies (Lundborg, 1972), have shown that numerous neuroleptics can cause gross behavioral anomalies. For example, penfluoridol or pimozide administered to nursing rat dams impairs acquisition of a conditioned avoidance response in 4-week-old offspring (Ahlenius et al., 1973; Ahlenius et al., 1975). Chlorpromazine administered early in gestation to pregnant dams impairs maze-learning behavior in young, but not when administered late in gestation (Hoffeld & Webster, 1965; Murai, 1963). Similar teratological results were found using reserpine or meprobamate (Hoffeld & Webster, 1965; Kletzkin,

Wojeiechowski, & Margolin, 1964; Werboff & Havlena, 1962; Werboff & Kesner, 1963).

The biochemical basis of behavioral changes observed in offspring of penfluoridol-treated mothers has been examined by measuring the accumulation of DOPA after pharmacological inhibition of aromatic amino acid decarboxylase (AAAD) or by measurement of the disappearance of DA after inhibition of its synthesis. Initial results failed (Engel & Lundborg, 1974) but follow-up studies showed decreased DA synthesis by the former manipulation and evidence of reduced nerve impulse activity in mesolimbic DA neurons by the latter (Engel & Lundborg, 1976). This neural anomalie, i.e., decreased impulse flow measured via the method above, is significant since mechanical or pharmacological inhibition of central DA neurons in adults is typically followed by an increase in DA synthesis (Carlsson, 1975). This finding suggests a disturbed feedback mechanism caused by treatment with DA receptor antagonists early in development (Engel & Carlsson, 1976), a finding which alludes to similar conclusions regarding chronic haloperidol studies (Spear et al., 1980).

These studies have a direct relevance to the present investigation, since amphetamine administration to offspring can counteract the behavioral impairment of conditioned avoidance behavior (Ahlenius et al., 1975).

Indeed, response deficits or response changes by neuroleptic-exposed pups in the present experiment may have been ameliorated by amphetamine administration since responding by haloperidol-treated pups was not different from that of controls. Even so, response data between groups before amphetamine administration did not show differences as would be predicted by this hypothesis. In addition, the present work did not investigate variations in haloperidol or amphetamine doses, and concomitant effects in responding, important factors in any drug related study. An alternative explanation of present results is that this paradigm was simply not sensitive to changes in responding following prenatal exposure to chronic haloperidol administration. Validation of this hypothesis requires additional studies that manipulate neuroleptic dose, amphetamine dose, and parameters of stimulation or responding.

The present report may be regarded as an additional attempt to assess the behavioral teratological effect of chronic haloperidol administration during gestation. Coyle et al. (1980) have suggested that there is presently not a sufficient data base to make specific recommendations pertaining to the selection of a specific set of behavioral tests for behavioral teratology. Thus the present study may be viewed as a step in the development of a set of adequate criteria for such an assessment.

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