BRAINSTEM SITES OF ACTION FOR L-DOPA-INDUCED LOCOMOTION IN NEONATAL RATS

By

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# TABLE OF CONTENTS

ACKNOWLEDGMENTS .......................................................... ii

ABSTRACT ................................................................. iv

REVIEW OF LITERATURE. ................................................... 1
  Development of Locomotion ........................................ 2
  Pharmacology of Locomotion in Neonatal Rats .................. 4
  Locomotion and Brainstem Neuroanatomy ......................... 12
    Forebrain Systems ............................................. 12
    Midbrain Systems ............................................. 15
    Medullary Systems ............................................. 29
    Spinal Cord Systems .......................................... 32
  Summary .................................................................. 34

EFFECTS OF TRANSECTION ON L-DOPA-INDUCED 
AIR-STEPPING IN NEONATAL RATS .................................... 37
  Experiment 1 ...................................................... 37
  Experiment 2 ...................................................... 51
  Experiment 3 ...................................................... 63

GENERAL DISCUSSION .................................................... 79

REFERENCES ............................................................. 86

BIOGRAPHICAL SKETCH .................................................. 99
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Administration of L-3,4-dihydroxyphenylalanine (L-DOPA) to neonatal rats induces behavioral activation. When such animals are suspended in the air, this behavior is expressed as stereotyped locomotor activity known as air-stepping. The site of action for the initiation of air-stepping by L-DOPA is unknown. The present studies were conducted in order to determine the gross neuroanatomical location of the site of action for L-DOPA in the neonatal rat. To this end, three experiments were conducted in order to assess the effects of three levels of midbrain transection on the expression of L-DOPA-induced air-stepping in five day-old rats. In the first experiment, transection at the precollicular/postmammillary level did not prevent L-DOPA-induced air-stepping; however, pretreatment of both sham-operated and transected subjects with either the dopamine D1 antagonist SCH 23390 or the dopamine D2 antagonist spiperone prevented L-DOPA-induced air-stepping. In the second experiment, a transection was made midcollicular dorsally and caudal to the substantia nigra and ventral tegmental areas ventrally, in order to determine if the expression of L-DOPA-induced air-stepping was dependent on local activity in these midbrain dopaminergic structures. This level of transection did not
prevent L-DOPA-induced air-stepping; however, a greater proportion of the air-stepping that was elicited in the transected animals was atypical in some features of the limb movements. In the third experiment, the transection was placed caudal to the inferior colliculus and angled to the pontine-mesencephalic border. Transection of the midbrain at this level disrupted the expression of L-DOPA-induced air-stepping, implying that structures of the caudal midbrain and their descending projections must be intact for L-DOPA-induced air-stepping to occur.

The results of these experiments suggest that (1) the expression of L-DOPA-induced air-stepping in the neonatal rat is dependent on the integrity of some structure and its descending influence from the caudal midbrain; (2) air-stepping is not dependent on locally induced activity of L-DOPA within the major dopaminergic cell groups of the midbrain, although these structures may have some regulatory influence on limb activity in the intact animal; and (3) the neural mechanisms involved in the production of L-DOPA-induced air-stepping probably involve dopaminergic synapses at some point in the pathway.
REVIEW OF LITERATURE

The purpose of this research was to determine grossly the site of action for L-DOPA-induced locomotion in the neonatal rat. One means of understanding what neuroanatomical structures are involved in producing selected behaviors is to use a lesion approach. By removing certain areas of the brain and examining the effects of such removal on the expression of a particular behavior, one can make limited assumptions regarding the functional importance of that structure for the behavior. Previous studies have shown that administration of catecholamine agonists to neonatal rats can induce behavioral activation both on a surface and in the air. The locomotor activation elicited by peripheral injection of L-DOPA is stereotyped, robust, and, if subjects are tested in a permissive environment, of long duration. Although numerous studies have examined the behavioral effects induced by L-DOPA, the site of action for this drug in eliciting locomotion remains unknown. For the purpose of determining where L-DOPA has its site of action, the three experiments presented in this paper were conducted. For each experiment, a transection was made at a different level of the brainstem, and the subsequent effects on L-DOPA-induced locomotion were assessed. Subjects were tested in a permissive environment to minimize the effects that support of body weight would impose on young animals. In order to explain the rationale behind these experiments, some background literature will be cited in regards to other work that has been done to examine 1) the development of locomotor behavior tested with different paradigms in the intact pre-weanling rat; 2) the pharmacology of locomotion induced by catecholaminergic agonists; and 3) the neuroanatomy of the brainstem as it relates to locomotion in the neonatal rat.
Development of Locomotion

One of the first developmental studies to focus on locomotor behavior was that of Preyer in 1885 (cited in Bekoff, 1981). Other studies of the development of locomotor behavior followed. During the early 1900s the individual works of George E. Coghill, William Windle, and Paul Weiss each contributed greatly to the understanding of neural substrates of locomotion. Further advancement of the field came in the 1960s with the experimental embryology experiments done by Victor Hamburger. This work, plus technical advances in neurophysiology at that time, revitalized the study of developmental neurobiology, and since then numerous researchers have added to the field of knowledge (see reviews Bekoff, 1981; Provine, 1986).

In any study of a behavior, it is important to understand how that behavior is expressed under normal circumstances for the species under investigation. Detailed information was provided for the preweanling rat by Bolles and Woods (1964) and Altman and Sudarshan (1975). These studies examined in detail the expression of locomotion on a surface and other behaviors during ontogeny in the laboratory rat. There are several discrepancies between these two studies in the time course for appearance of several behaviors; however, the sequences of events are in general agreement, and differences may be due to the different strains of rat used in each study (Sprague Dawley and Purdue Wistar). In addition, it is not clear whether the day of birth was counted as day 0 or day 1 in reporting the ages of the animals. For clarity in referring to stage of gestation and postnatal ages in the present study, some conventions have been adopted. Gestation of the Sprague Dawley rat is considered to be 22 days. Prenatal age will be represented by the embryonic day (i.e., E1-21), and postnatal age will be referred to as the postnatal day (i.e., P5), with P0 being the day of birth.
The rat is born in a relatively immature developmental state. At birth, rat pups are blind and hairless, and cannot maintain core body temperature outside the nest. They are not able to locomote effectively or raise their heads or bodies from a surface (Bolles & Woods, 1964; Altman & Sudarshan, 1975). The first expression of locomotor behavior on a surface appears within 2-3 days after birth and primarily involves the forelimbs, which are used as paddles to drag the body and hind limbs (Bolles & Woods, 1964). This action does not result in much forward locomotion; often the hindquarters remain anchored to the surface, resulting in laterally directed movements described as "pivoting" (Altman & Sudarshan, 1975). Crawling, in which both forelimb and hind limb girdles engage in paddling motions, is seen within the first week. The body is not raised from the surface, probably due to the inability of the limbs to support the weight of the animal, and the belly supports most of the weight (Bolles & Woods, 1964; Altman & Sudarshan, 1975). The pups begin walking when they are able to support their own weight and stand, at about P10 (Bolles & Woods, 1964).

Studies of the development of locomotion have been hampered by the muscular immaturity of neonatal animals. It is difficult to measure the locomotor abilities of an animal that does not engage in much spontaneous locomotion or support its own body weight. However, it cannot be assumed that the differences in behavior seen between young and adult animals are due to absence of a neural substrate in the young animals. In order to overcome these limitations, some investigators have chosen to study the locomotor abilities of young animals in a more permissive environment (Fentress, 1978). Bekoff and Trainer (1979) and Bekoff and Lau (1980) examined the ability of neonatal and prenatal rats to engage in interlimb coordination during swimming. The animals were analyzed in water to "minimize any effect of weak limb muscles in very young rats" (Bekoff & Trainer, 1979, p. 1). In addition, immersion in water has an activating effect, so that the animals under observation tend to move for more prolonged periods of time than do those on a surface. In this permissive environment, interlimb coordination was
seen between homonymous (same limb girdle) and homolateral (same side, fore- and hind) limb pairs at P1 (Bekoff & Trainer, 1979), and between homologous limbs at E20 (Bekoff & Lau, 1980). These studies demonstrated that early neonatal and even prenatal rats are capable of a higher degree of coordination than was thought possible, because of the permissiveness of the testing environment that was used. The capacity of the developing nervous system to produce mature forms of limb coordination therefore precedes the expression of that coordination during locomotion on a surface.

Pharmacology of Locomotion in Neonatal Rats

Pharmacological manipulations can be used to gain information about the organization and functional features of the neural substrates of locomotion through ontogeny. Administration of some drugs can elicit different or even opposite behavioral reactions in neonates than in older pups or adult animals. One explanation for such discrepancies could be that there are differences in the underlying neural substrates upon which the drug acts. Other factors may also be involved, such as metabolic differences in enzyme activity, or immaturity of peripheral systems that effect the behavior that the drug elicits, but are not directly influenced by the drug themselves. For example, in young animals muscle strength and the ability to support body weight are important factors in producing effective locomotion, but muscle strength in itself may not be affected by the administration of drugs that induce locomotion. By using a pharmacological approach, such as treatment with selective agonists and antagonists, the roles of particular neurotransmitter systems in the production of locomotion can be assessed.

It has long been known that locomotion can be reliably elicited, even in surgically reduced preparations, via systemic application of catecholamine agonists. One such agonist is L-DOPA. Systemically administered L-DOPA is actively transported across the blood brain barrier and taken up by catecholaminergic neurons where it is subsequently
decarboxylated into dopamine (DA) and, in those cells which contain dopamine beta hydroxylase, hydroxylated into noradrenaline (NA). In cells that contain the enzyme phenylethanolamine-N-methyl transferase the product of L-DOPA metabolism is epinephrine. The locomotor effects of L-DOPA have been studied in a number of species and different surgical preparations (Hornykiewicz, 1966; Grillner, 1969, 1973; Stromberg, 1970; Stromberg & Svensson, 1970; Kellogg & Lundborg, 1972a; Randall & Giniger, 1974; Iwahara, Van Hartesveldt, Garcia-Rill, & Skinner, 1991b; Stehouwer & Van Hartesveldt, 1991; Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991; McCrea, Stehouwer, & Van Hartesveldt, in press).

In the neonate, L-DOPA induces increased locomotor activity, while similar doses in the adult rat suppress spontaneous activity, and only administration of very high doses results in locomotion following initial suppression of activity (Hornykiewicz, 1966; Kellogg & Lundborg, 1972a). These differences in behavioral effects imply that changes may occur in the roles played by catecholamine systems in the modulation of locomotion, and that the effects of L-DOPA administration reflect these changes behaviorally. Kellogg and Lundborg (1972a) examined the ontogeny of the effects of L-DOPA and several other monoamine receptor-stimulating substances from P1 to weanling age in the laboratory rat. They found that animals as young as one day of age were activated motorically by injection of L-DOPA. Intense crawling and head raising were observed following administration of this drug, at P1, P4, and P7. At P14, L-DOPA induced sporadic running with intermittent periods of inactivity, during which the animals appeared stuporous. An arched-back posture with abducted hind legs was frequently observed at this age, in addition to oral stereotyped behavior. At P21, the animals displayed the arch-back posture, but did not show the same degree of increased motor activity seen in the younger animals.

Pretreatment of older animals with the peripheral dopa decarboxylase inhibitor MK-486 followed by L-DOPA injection resulted in increased activity and aggressive
behavior, whereas in younger pups the same treatment simply increased the duration of the locomotor response (Kellogg & Lundborg, 1972a). Peripheral dopa decarboxylase metabolizes systemically injected L-DOPA, resulting in less of the drug being available to cross the blood-brain barrier. This enzyme is not present at adult levels until late in ontogeny and is much reduced in very young pups (Kellogg & Lundborg, 1972b). Lower peripheral levels of this enzyme would leave more drug available to cross the blood-brain barrier, be metabolized into DA and NA, and exert central effects in younger animals. In addition, it was found that peripherally administered L-DOPA resulted in significant increases in levels of both DA and NA in the brains of P1, P4, and P7 subjects, but the increases were not significantly different from that of control animals at P14 and P21. Activity levels were correlated positively with the levels of central catecholamines. Based on these findings, it seems likely that changes in the response to L-DOPA and other catecholaminergic agents could be due to differences in the activity of peripheral decarboxylase, specifically, that in younger animals the decarboxylase activity is lower and allows more L-DOPA to cross the blood-brain barrier. The increased activity of the peripheral decarboxylase enzyme in older animals results in less L-DOPA entering the central nervous system to be converted into catecholamines, and consequently less catecholamine-activated locomotion.

Kellogg and Lundborg (1972a) also tested the effects of the selective noradrenergic agonist clonidine on locomotor activity in young rats. This drug was found to stimulate crawling in P1, P4, and P7 animals that was similar in appearance to that induced by L-DOPA, but did not induce head-raising and was "slower and less frantic" (p. 195) than was seen following L-DOPA treatment. In addition, it was found that a low dose of haloperidol, primarily a dopamine antagonist, blocked clonidine-induced locomotion in P7 rats, but only increased the latency of the response in younger animals. Administration of the dopaminergic receptor agonist apomorphine appeared to induce only a slight head tremor in P1 animals, and slight forward crawling at P4 and P7.
However, when clonidine and apomorphine were administered together, the induced behavior in P1, P4, and P7 pups appeared to be indistinguishable from that induced by L-DOPA. It appears from these findings that the locomotion induced by L-DOPA may be dependent on stimulation of both dopaminergic and noradrenergic receptor activity.

Similar effects of catecholamine agonists in animals of early and late neonatal ages were demonstrated in a study by Camp and Rudy (1987). This study examined the effects of various catecholaminergic agents on the "behavioral activation" of preweanling rat pups. Behavioral activation, in this case, was defined as "generalized motor responses" or "rolling, curling, wall climbing" (Camp & Rudy, 1987, p. 317). It was found that both specific activation of the dopamine system by apomorphine and nonspecific activation by L-DOPA elicited increased locomotion in P6 rat pups, and this activation was blocked by administration of haloperidol. Paradoxically, L-DOPA administration to P12 pups was found to depress behavioral activation. It was hypothesized that behavioral activation is elicited through the DA system, and that some qualitative change occurs in the neural mechanisms underlying this activation during the second week of life.

Camp and Rudy (1987) also examined the role of the noradrenergic system in behavioral activation. Administration of either the nonselective alpha agonist phenylephrine or the alpha antagonist labetalol had no effect on activity measures, while the non-selective beta adrenergic agonist isoproterenol and the beta adrenergic antagonist propranolol both elicited increased activity in the 6 day-old pups. However, in adult rats beta adrenergic agonists and antagonists (isoprenaline and propranolol, respectively) cause behavioral sedation (see references in Camp & Rudy, 1987). It was concluded that, similar to the DA system, selective pharmacological manipulations of the NA system can elicit locomotor behavior in neonates. In the process of maturation, changes occur in both systems such that administration of catecholaminergic drugs increases activity in young animals, but depresses activity in older ones. It is interesting to note that in this study, the noradrenergic alpha agonist phenylephrine did not result in increased activation; however,
in other studies the noradrenergic alpha-2 agonist clonidine elicits locomotor activity in neonatal rats (Kellogg & Lundborg, 1972a; Reinstein & Isaacson, 1977; Spear & Brick, 1979).

From the previous reports, it is clear that locomotion on a surface in developing rats can be elicited by effects of drugs on either the DA or NA systems. In addition, the involvement of these systems changes over the course of development in that the same drugs that cause activation in young animals cause suppression of activation or a different activity (such as stereotypy or aggression) in older animals. The results of these studies imply that L-DOPA may in fact be activating both NA and DA systems to elicit locomotion, and that in older animals, L-DOPA induces behaviors other than locomotion.

There are other examples of research that have implicated both catecholamines in the behavioral effects of L-DOPA. In adult mice pretreated with the monoamine oxidase inhibitor nialamide to prevent the catabolism of monoamines, central levels of both NA and DA were increased, as was locomotor activity. In a different condition in the same study, a DA beta-hydroxylase inhibitor, FLA-63, was administered following nialamide treatment. This treatment resulted in moderate reduction of the central NA level, while DA levels remained high and motor activity was only slightly reduced. Administration of synthesis inhibitors to nialamide-pretreated animals caused decreases in both the monoamine content of the brains and motor activity (Svensson & Waldeck, 1970). Other studies have examined the effects of various catecholaminergic drugs on motor activity. Apomorphine, a dopamine receptor agonist, as mentioned previously, elicits slight increases in locomotion in neonatal rats but in older pups this drug elicits more stereotyped mouthing behaviors (Lai & Sourkes, 1973; Shalaby & Spear, 1980; Camp & Rudy, 1987). Similar to apomorphine, amphetamine, a mixed catecholamine agonist, induces locomotor behavior in neonates, but with increasing age, produces more stereotyped sniffing, licking, and biting behaviors (Lai & Sourkes, 1973). Clonidine, a noradrenergic alpha-2 receptor agonist, also elicits increased motor activity in younger
neonates (Kellogg & Lundborg, 1972a; Reinstein & Isaacson, 1977; Spear & Brick, 1979) while cocaine, an indirect noradrenergic agonist, increases locomotion and wall climbing (Spear & Brick, 1979).

The fact that young rats are able to express locomotor behavior if tested in a permissive environment was established by studies in which a swimming paradigm was used to allow animals to express activation without having to support their own body weight. More recently, air-stepping has been used as another permissive method for testing locomotion. This paradigm allows for testing behavior without requiring support of body weight and without the activating effects of immersion in water. Instead, prolonged episodes of locomotion can be elicited pharmacologically in this paradigm. That young animals can be activated pharmacologically not only affords the researcher extended periods of time in which to observe the details of locomotion in whatever environment is chosen for testing, but also implies that the neural substrates affected by the administered drug can be activated.

An advantage that the air-stepping paradigm has over swimming studies is that resistance or drag on the limbs as they are moved through the water is not a factor in air-stepping. With this method, animals of various ages are suspended in slings that leave the limbs free to move (with the neuraxis approximately perpendicular to the ground), and can be injected with substances that have been shown to induce locomotor activity on the ground, such as L-DOPA (Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991; McCrea, Stehouwer, & Van Hartesveldt, in press). Van Hartesveldt et al. (1991) have examined the effects of L-DOPA on air-stepping across a range of ages and, recently, across ranges of dose and age (McCrea, Stehouwer, & Van Hartesveldt, in press) and with kinematic analysis of limb coordination (Stehouwer, McCrea, & Van Hartesveldt, in press). These more recent studies were conducted on rat pups at P0, P5, P10, P15, and P20 and examined the effects of several doses of L-DOPA (25, 50, 75, and 100 mg/kg). It was found that the duration of time the subjects spent air-stepping generally increased with
dose of the drug at each age that was studied, and that L-DOPA induced locomotor activity in the air at all the ages studied. These results differ with those of studies in which L-DOPA-induced activation was quantified using locomotion on a surface as a measure. Kellogg and Lundborg (1972) found that at P21, rat pups that have received L-DOPA appeared to be "quite placid" (p. 195). Some aspect of the air-stepping paradigm appears to allow for release of locomotor activity that is suppressed in animals that are tested on a surface.

In addition to measuring the duration of locomotor activity in the pups, McCrea et al. (in press) examined the variations in gait that were found in pups of different ages that had been activated by L-DOPA. Air-stepping was expressed primarily as forelimb alternation in P0 pups; as a diagonal progression pattern of all four limbs in P5 pups; and became more variable in the older pups, consisting of gaits that resembled adult-like swimming, terrestrial galloping, and a peculiar variation in which one forelimb was dropped out of the diagonal progression pattern of movement to remain extended forward (McCrea, Stehouwer, & Van Hartesveldt, in press). This particular pattern of limb movement is not seen during spontaneous swimming or terrestrial locomotion. In addition, the gaits that were observed were dependent on the dose of L-DOPA, such that in P0 rats, in which the predominant gait is characterized by alternation of only the forelimbs, the highest dose elicited a diagonal progression pattern of all four limbs. In P15 rats, the lowest dose of L-DOPA elicited no galloping, but the highest dose elicited galloping in every subject.

While the dose of L-DOPA affected the type of gait that was elicited at some ages, Stehouwer et al. (in press) found that dose did not affect the rate of diagonal progression stepping that occurred within an age group. This result is in contrast to those found in studies in which electrical stimulation is used to elicit locomotor activity in neonatal rat in vitro brainstem-spinal cord preparations (Atsuta, Garcia-Rill, & Skinner, 1988). In these reduced preparations, increasing the current levels induced a faster step rate. These
results suggest that different mechanisms may be involved in the locomotion elicited by L-
DOPA in intact pups and electrical stimulation in the \textit{in vitro} preparation.

Studies such as these have shown that L-DOPA can elicit locomotor activity on a
surface or in a permissive environment such as during air-stepping. L-DOPA probably
induces air-stepping by the same means that it elicits locomotion on a surface, although
contact with the substrate during locomotion on a surface alters the expression of
behavior. This activation may involve both DA and NA systems. One means of
examining the roles of each neurotransmitter system on locomotion is to use selective
agonists to reproduce the response. However, pilot work in this laboratory has shown
that exact replication of the characteristics of L-DOPA-elicited air-stepping is difficult to
achieve by administration of selective agonists. Although many catecholaminergic
substances produce activation in suspended animals, none reproduce the same stereotyped
and long-lasting locomotion that is elicited by L-DOPA. The only exception might be
clonidine, which elicits fairly stereotyped air-stepping behavior in P20 rats (in contrast to
the findings of Kellogg and Lundborg, 1972a, in which this drug elicits suppression of
surface activity in P21 rats). However, clonidine-induced activity has not yet been
compared systematically to the air-stepping induced by L-DOPA, and may be slightly
different in some aspects, as has been shown for locomotion on a surface in younger
animals.

Indirect evidence for the involvement of both NA and DA systems in producing
the behavioral effects of L-DOPA comes from studies in which air-stepping in P5 rats was
blocked by pretreatment with selective DA receptor antagonists (Sickles, Stehouwer, &
Van Hartesveldt, 1992) and with selective noradrenergic receptor antagonists (Taylor,
Sickles, Stehouwer, & Van Hartesveldt, in press). Since direct receptor antagonists to
either catecholamine system can block the locomotion induced by L-DOPA, it is likely that
both DA and NA systems are involved in L-DOPA-induced air-stepping.
Locomotion and Brainstem Neuroanatomy

Not only is it unknown how each of the monoamine neurotransmitter systems is involved in L-DOPA-induced air-stepping, but it is also unclear where in the brain the drug acts. There are many regions of the central nervous system from which electrical or chemical stimulation can elicit rhythmic stepping activity in a variety of experimental preparations. L-DOPA may elicit locomotor behavior through direct or indirect actions on a number of locomotor regions. Studies related to the neuroanatomy of locomotion will be presented in this section, and the findings will be discussed in terms of four major regions of the brain; the forebrain, the midbrain, the medulla, and the spinal cord.

For discussions of neuroanatomy, and throughout this paper, the nomenclature of Paxinos (1985a, 1985b) will be used, and reference to the nomenclature of Dahlstrom and Fuxe (1964) will also be given for monoamine systems where appropriate. All neuroanatomical information presented here is in reference to the adult rat, unless otherwise noted. The organization of locomotor control systems in this section will be partially based on that used by H.G.J.M. Kuypers in "A New Look at the Organization of the Motor System," in Progress in Brain Research, vol. 57, Anatomy of Descending Pathways to the Spinal Cord (1982).

Forebrain Locomotor Systems

The motor cortex may be considered the highest level of hierarchical motor control (Ghez, 1991). Its primary importance for locomotor behavior lies in initiation of voluntary movements and in performing sensory gating functions during activity (Garcia-Rill, 1986). The motor cortex projects to brainstem nuclei and also directly to the spinal cord via the corticospinal tract. In the adult rat this tract arises primarily from pyramidal cells of layer 5B of the primary somatosensory cortex and from the adjacent agranular motor cortex (Wise & Jones, 1977). The processes course through the forebrain via the internal capsule
and then merge to form the cerebral peduncles at the lateral aspects of the midbrain. At this point, fibers branch off to the substantia nigra (SN), the ventral tegmental area (VTA) and other midbrain structures (Paxinos, 1985a). The peduncles then disperse to become the longitudinal fibers of the pons. At the caudal extent of the pons, the fibers reconverge to form the pyramids. In the caudal medulla, a majority of the fibers decussate to the contralateral side of the brainstem and descend in the ventral part of the dorsal columns (Brown, 1971). Remaining fibers descend ipsilaterally in the ventral funiculus (Vahlsing & Feringa, 1980). The axons of the corticospinal tract synapse at all levels of the spinal cord in the dorsal horn (Brown, 1971) and especially at the levels of the cervical and lumbar enlargements, in laminae 7 and 8 (Donatelle, 1976).

In the prenatal rat, the first corticospinal fibers reach the midbrain by E18, the pons by E19 and the pyramidal decussation by E20 (Schreyer & Jones, 1981). Fibers enter the rostral end of the cervical segment of the spinal cord on E21 or P0, and reach the caudal cervical segments around P3. By P5, there is evidence that axons of the corticospinal tract extend as far as the white matter of upper lumbar segments of the cord (Donatelle, 1976; Leong, 1983). This growth has been related to behavioral development of tactile placing reactions in the rat. Forelimb placing is first observed in intact animals between 4 and 7 days postnatally. Hind limb placing first appears between P9 and P13, at which time corticospinal axons can be found in the coccygeal segments of the spinal cord (Donatelle, 1976).

Although axons have been found at the lumbar level of the spinal cord at P5, the innervation of gray matter does not take place immediately. For example, the tract is present at the cervical level on P0, but fibers do not enter the gray matter at that level until 3 days later, when the leading descending fibers of the tract have just begun to grow into the upper thoracic segments (Jones, Schreyer & Wise, 1982; Donatelle, 1976). In addition, myelination does not begin in the cord until after P11 (Jones, Schreyer & Wise, 1982). Although present in the upper lumbar white matter by P5, it is unlikely that the
corticospinal tract is involved in the production of L-DOPA-induced air-stepping at that age. If the general finding holds for the entire cord that initial appearance of the fibers in a segment precedes innervation of the gray matter by 2-3 days, then at P5 innervation of the lower cervical segments is just beginning to take place.

The diencephalic locomotor region is an area from which electrical stimulation elicits rhythmic stepping on a treadmill in anesthetized cats (Waller, 1940) and rats (Sinnamon, 1984). The area is comprised of the ventral-posterior and lateral hypothalamus, including the median forebrain bundle (Orlovskii, 1970; Sinnamon, Lee, Adams, & Stopford, 1984) and portions of the thalamus (Ross & Sinnamon, 1984). This area can also include the subthalamic locomotor region as described by Grillner (1981) and Shik et al. (1966). Sinnamon (1984) mapped the extent of the regions in which electrical stimulation elicited well-coordinated quadruped locomotion in the rat. He found that of the sites that were positive for inducing stepping in the diencephalon, the more dorsal sites induced stepping in both forelimbs and hind limbs, while the more ventral sites induced only hind limb stepping. In addition, a subsequent study found that stepping induced by stimulation of the lateral hypothalamus was dependent on the presence of an ipsilateral pathway through the dorsal VTA (Sinnamon, Lee, Adams, & Stopford, 1984).

L-DOPA could be taken up by and converted to DA in several groups of dopaminergic cells located in the diencephalon, designated A11-A14 (Dahlstrom & Fuxe, 1964). Dopaminergic (and non-dopaminergic) projections to the spinal cord arise from one of these diencephalic DA groups, A11. These cells are distributed in the caudal thalamus, zona incerta and posterior dorsal hypothalamus (Bjorklund & Skagerberg, 1979, Bjorklund & Lindvall, 1984). The pathway descends through the midbrain, where the fibers from A11 are joined by other dopaminergic fibers from SN, to travel in the dorsal longitudinal fasciculus to lamina 1, the central canal, and the dorsolateral funiculus (Lindvall & Bjorklund, 1983). Dopaminergic axons can be found throughout the length of the cord, but are most dense in the areas of the intermediolateral cell column and the
central canal at thoracic and lumbar levels (Commissiong, Galli, & Neff, 1978a; Lindvall & Bjorklund, 1983; Bjorklund & Lindvall, 1984). Developmentally, the hypothalamic cell groups A11-A14 appear later than the other DA cell groups in the midbrain, and are not detectable until E18 or later (Seiger & Olson, 1973).

With regard to the influence of these forebrain areas on L-DOPA-induced air-stepping, studies by Iwahara et al. (1991b) and Stehouwer and Van Hartesveldt (1991) determined that precollicular/postmammillary transection does not prevent L-DOPA-induced air-stepping in neonatal rats. The locomotion induced in this manner was not different from that seen in intact animals, either in limb coordination or in gait. These experiments indicate that diencephalic locomotor-inducing regions are not necessary for the production of L-DOPA-induced air-stepping. However, the efferents from this region that synapse in more caudal structures may still have active terminals which could be involved in the production of air-stepping in the acute decerebrate preparation. In addition, it is obvious from these experiments that an intact motor cortex is not required for L-DOPA-elicited air-stepping, and since the midbrain projections of this system are not catecholaminergic, it is unlikely that any efferent terminals remaining after transection are activated following L-DOPA injection.

Midbrain Systems

The midbrain locomotor region (MLR) is a physiologically defined area in the dorsal posterior midbrain just ventral to the inferior colliculus and surrounding the brachium conjunctivum. In the rat, the MLR is coextensive dorsally with the lateral and caudal cuneiform nucleus, and more ventrally with the pedunculopontine tegmental nucleus or PPTg (Grillner, 1981; Sinnamon, 1984; Skinner & Garcia-Rill, 1984; Ross & Sinnamon, 1984). Numerous studies have examined the effects of electrical stimulation of the MLR on locomotor activity (Brudzynski, Houghton, Brownlee, & Mogenson, 1986; Milner & Mogerson, 1988; Atsuta, Garcia-Rill, & Skinner, 1990; Kinjo et al. 1990). Repeated high frequency, low amplitude current stimulation of this area in the midbrain
induces locomotor activity in intact (Sterman & Fairchild, 1966) and decerebrate cats (Shik, Severin, & Orlovskii, 1966; Grillner & Shik, 1973), and in intact (Depoortere, DiScala, & Sandner, 1990) and decerebrate rats (Skinner & Garcia-Rill, 1984) on a treadmill. In addition, injections of several chemical substances, such as kainic acid (Milner & Mogenson, 1988), L-glutamate (Brudzynski, Houghton, Brownlee, & Mogenson, 1986), N-methyl D-aspartate (NMDA), substance P (Garcia-Rill et al. 1990; Kinjo et al. 1990), and gamma-aminobutyric acid (GABA) antagonists (Garcia-Rill, Skinner, & Fitzgerald, 1985; Milner & Mogenson, 1988; Kinjo et al. 1990) into the MLR can also induce locomotor activity.

Studies of the role of the MLR in locomotion have generally employed the paradigm used in the seminal work of Shik et al. (1966), in which a decerebrate, lightly anaesthetized cat was suspended over a treadmill that could be moved at controlled speeds. The head of the animal was fixed in a stereotaxic instrument, and electrodes were implanted in the area 3-4 mm below the surface of the inferior colliculus, either unilaterally or bilaterally. Without stimulation, the animals were completely immobile. However, with electrical stimulation, subjects almost immediately began to step on the treadmill, usually with the hind limbs first, then the forelimbs. The animals were able to adjust the speed of their gaits to changes in the speed of the treadmill, and if stimulation was increased in strength, they would increase speed or even change gaits from walking to galloping. The results of this experiment demonstrated that neither cortical motor centers nor the subthalamic locomotion-inducing site was required to elicit coordinated step patterns. However, if the decerebration level passed rostrally to the subthalamic locomotor region, the animals could engage in spontaneous locomotion.

In the intact cat, electrical stimulation of the MLR also affects locomotor activity. Stimulation of the cuneiform nucleus in intact, unanesthetized cats increases speed of locomotion in a trained runway task. The range of stimulation that effectively produced locomotion was found to be similar between subjects, with currents below a certain
strength having no effects, and currents higher than the effective range causing disruption of the locomotion (Sterman & Fairchild, 1966).

Other studies have examined the effects of electrical stimulation of the MLR in rats. The decerebrate non-anesthetized rat can be induced to locomote on a treadmill following electrical stimulation of the MLR. This preparation differed from that of the cat in two ways. First, MLR stimulation could evoke treadmill stepping only if the decerebration level was placed rostrally to the SN. Likewise, locomotion induced by stimulation of the subthalamic locomotor region is also dependent on the integrity of areas in the ventral midbrain, including the SN (Grillner, 1981). The subthalamic region has been suggested to have a tonic influence providing for modulation of locomotor output of the MLR in the rat (Grillner, 1981; Skinner & Garcia-Rill, 1984). A second difference was that areas of the anterior PPTg contained locomotion-producing sites in the rat, while similar sites were ineffective in the cat (Skinner & Garcia-Rill, 1984).

MLR stimulation induces locomotor activity in intact rats as well. Sinnamon (1984) and Ross and Sinnamon (1984) suspended lightly anaesthetized rats over a treadmill, and applied electrical stimulation to various areas of the brainstem. Well-coordinated stepping of all four limbs could be elicited by dorsal midbrain stimulation in the areas of the inferior collicular commissure, the lateral central gray, the cuneiform nucleus and the PPTg. Stimulation of some ventral midbrain areas also resulted in locomotion, including the VTA and areas surrounding the medial lemniscus, and appeared to have some regional specificity, in that sites ventral to the lemniscus supported hind limb stepping, while dorsal sites supported forelimb stepping (Ross & Sinnamon, 1984; Sinnamon, 1984).

Depoortere et al. (1990) also employed MLR stimulation in anaesthetized intact rats on a treadmill. The investigators compared such treadmill locomotion to activity induced by MLR stimulation in intact freely moving rats. In the free-moving condition, MLR stimulation elicits escape reactions, including "frantic locomotion followed by
explosive jumps" (Depoortere, DiScala, & Sandner, 1990, p. 723) which appear to represent reactions to an aversive stimulus. They have proposed that the locomotor activity induced on a treadmill in anaesthetized or decerebrate rats constitutes a generalized motor response to the aversive effects generated by MLR stimulation. In the intact rat, MLR stimulation elicits an escape response.

An alternative preparation to that of treadmill stepping was initially developed by Otsuka and Konishi (1974) and has been recently adapted for study of locomotion (Smith & Feldman, 1987). An in vitro isolated brainstem-spinal cord preparation from the newborn rat (0-5 days of age) has yielded much information regarding the chemical control of locomotion in neonatal animals. Due to its small size and lack of myelin, this preparation can survive for several hours on diffused metabolites and gas from the bath solution (Cazalets, Sqalli-Houssaini, & Clarac, 1992). The lack of a blood-brain barrier allows for all drugs added to the bath to reach the nervous system. Neuroactive substances can therefore be applied directly to the tissue, an advantage over using intact animals, in which the blood brain barrier may prevent the diffusion of many substances into the brain (Smith & Feldman, 1987; Cazalets, Sqalli-Houssaini, & Clarac, 1992). A disadvantage of using this preparation is that the tissue must be kept at a low temperature to prolong survival, which is likely to decrease the rate of metabolic processes that are normally in effect in the intact animal. The activity that occurs following stimulation is quantified by recording from the ventral roots or individual motor neurons (fictive locomotion), or by electromyographic (EMG) recordings in preparations with hind limbs attached (Smith, Feldman, & Schmidt, 1988). Several studies have utilized the hind limb-attached preparation to examine the effects of electrical stimulation of the MLR on locomotion (Atsuta, Garcia-Rill, & Skinner, 1988, 1990). Results of such studies have shown that electrical stimulation of the MLR in the brainstem-spinal cord in vitro preparation induces rhythmic movements that appear similar to the step cycles of adult rats on a treadmill. Similar to that of the adult rat, the step cycle frequency could be
increased with higher amplitude stimulation, but unlike those of the adult, alternating step movements could not be driven to a gallop-like gait in the neonates. It is also of interest to note that stimulation of the pyramids (corticospinal tract) in this preparation did not result in locomotion (Atsuta, Garcia-Rill, & Skinner, 1988).

Numerous chemical applications have also been found to stimulate locomotor activity in the neonatal rat in vitro preparation (Smith, Feldman, & Schmidt, 1988; Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991; Cazalets, Sqalli-Houssaini, & Clarac, 1992). Studies in which fictive locomotion was quantified and EMG recordings of hind limb-attached preparations were made have shown that a number of chemicals that induce locomotion in adult rats also induce locomotion in the neonatal in vitro preparation. Chemicals can be added to the bath of selected local areas by use of a petroleum jelly barrier between the brain and the spinal cord. Application of GABA antagonists, substance P (a peptide), NMDA, and other excitatory amino acids such as glutamic acid induce locomotor activity when applied to the region of the preparation containing the MLR. Applications of both NMDA and GABA antagonists induced long-lasting effects on locomotion, with NMDA inducing prolonged episodes of stepping movements, while GABA antagonists induced briefer and more episodic periods of limb alternations. The activity elicited in this manner in the hind limb-attached preparations has been referred to as "airstepping" (Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991); however, airstepping elicited in this preparation, in which the hind limbs are attached to a spinal cord and alternating from a supine position, should not be confused with air-stepping referred to in this paper, in which a whole animal is suspended above a surface in a sling.

A limited amount of work has been done regarding locomotion in decerebrate animals using the air-stepping paradigm. Iwahara et al. (1991) demonstrated that decerebration at the precollicular, midnigral level in pups aged 0 to 22 days resulted in no spontaneous locomotor activity. The subjects remained fairly inactive, but if stimulated with tail or pinna pinch, would engage in brief scratch reflexes and some limb alternation.
When suspended in the air, the animals were limp and inactive. Within ten minutes following injection with 100 mg/kg of L-DOPA, the subjects showed dorsal flexion, with elevation of the head and tail. Air-stepping usually began shortly after the initiation of dorsal flexion, and continued for thirty to ninety minutes. Both the step rates and the patterns of locomotion expressed were similar to that seen in the intact air-stepping rat pup (Van Hartesveldt, Sickles, Porter & Stehouwer, 1991).

The afferents and efferents of the MLR have been studied in the cat (Noga, Kriellaars, & Jordan, 1991) and rat (Garcia-Rill, Skinner, Conrad, Mosley, & Campbell, 1986; Garcia-Rill & Skinner, 1987b), and have been found to be fairly similar. The area corresponding to the PPTg in the rat receives afferents from the globus pallidus, entopeduncular nucleus, subthalamic nucleus, lateral hypothalamus (Paxinos, 1985b) and the SN (Hedreen, 1971; Garcia-Rill, Skinner, Conrad, Mosley, & Campbell, 1986). Ascending efferents project to the globus pallidus, entopeduncular nucleus, the subthalamic nucleus, and centro median nucleus of the thalamus (Paxinos, 1985b). Midbrain projections extend to the SN, and a descending afferent projects to the gigantocellular reticular nucleus of the medulla (Garcia-Rill, 1986; Garcia-Rill, Skinner, Conrad, Mosley, & Campbell, 1986). Cells of the PPTg are considered part of the pontine reticular formation, and as such, contribute a cholinergic projection to the reticulospinal tract (Jackson & Crossman, 1983; Spann & Grofova, 1984). The cuneiform nucleus, also considered as part of the mesencephalic reticular formation, projects along with efferents of the deep mesencephalic nucleus via the ventral funiculus as far as the upper thoracic segments of the spinal cord to synapse in lamina 7 (Paxinos, 1985b).

The neuroanatomy of several systems is relevant to the locomotion induced by stimulation of the MLR, including the rubrospinal, vestibulospinal, tectospinal and ceruleospinal tracts, the raphe-spinal system and dopaminergic projections to the spinal cord (Grillner, 1981). These systems will be described in terms of their presumed
functions in the adult rat, and the neuroanatomy will be described in terms of
developmental status in the neonatal rat.

The rubrospinal tract. The lateral pathway from the brainstem is primarily
composed of descending fibers from the red nucleus, but also includes fibers from cranial
nerve nuclei VII, VIII and XII, and the cuneate and gracile nuclei (Ghez, 1991). This
pathway descends in the dorsolateral columns, and terminates mainly in the dorsal and
lateral parts of the intermediate zone (Kuypers, 1964). Because of the nature of its
connections in the spinal cord, this pathway is thought to be responsible for maintaining
balance and posture through control of distal muscles (Kuypers, 1982). However,
unilateral lesions of the red nucleus in the adult rat have no effect on turning tendencies,
facilities in limb use, or righting reflex, and do not induce ataxia during walking
(Papaioannou, 1971).

In the neonatal rat, Shieh et al. (1983) examined the projections of the rubrospinal
tract using HRP administration. The results of that study showed that the rubrospinal
tract extends to the lumbosacral segments of the spinal cord on the day of birth. In
addition, it was found that the projections were somatotopically organized; axons that
terminated in the cervical region of the spinal cord originated from cells located in the
dorsal and dorsomedial areas of the red nucleus, while those that projected to the
lumbosacral segments originated from cells of the ventral and ventrolateral red nucleus
(Shieh, Leong, & Wong, 1983). It is unknown if these projections have established
functional synapses in the neonatal rat. However, in the normal infant rat it is obvious that
very little postural tonus is demonstrated until about P3-P4 (Bolles & Woods, 1964). It is
possible that with pharmacological manipulations, the system may be activated and
produce behavioral effects.

The vestibulospinal tract. This system has two divisions, the lateral vestibulospinal
tract or LVST and medial vestibulospinal tract or MVST. The LVST in the adult projects
to all levels of the spinal cord, and terminates primarily in lamina 8, with additional
synapses in laminae 7 and 9. There is also evidence for direct connections with extensor motor neurons in the lumbosacral part of the cord (Paxinos, 1985b). Fibers of the MVST descend in the spinal cord close to the ventral midline. They extend only to the midthoracic level of the cord, and also terminate in laminae 7, 8, and 9. Because of the extent of these projections, this pathway is thought to influence motor neurons of the neck instead of the limbs (Paxinos, 1985b). Both components of the vestibulospinal system are believed to be involved in control of posture and balance (Ghez, 1991).

In the P2 rat, the vestibular nuclear complex shows similar density and labeling following HRP administration into the lumbosacral cord to that seen in adults (Leong, Shieh, & Wong, 1984). It may be inferred that this system is not only anatomically in place in the newborn rat, but functional as well, since newborn animals will attempt to right when placed on their backs, and although inefficient in their methods, are capable of this behavior (Bolles & Woods, 1964; Altman & Sudarshan, 1975).

The tectospinal tract. This tract arises from neurons in the intermediate and deep gray layers of the superior colliculus. The fibers cross to the contralateral side at the level of the midbrain, and descend though the brainstem just ventral to the medial longitudinal fasciculus (Paxinos, 1985b). In the rat, the tectospinal system innervates only the upper 2-3 cervical segments of the spinal cord, and terminates in the ventral horn, lamina 8 (Waldron & Gwyn, 1969). In the adult, this system controls coordination of head and eye movements (Ghez, 1991).

In the P2 rat, Leong et al. (1984) found that following HRP injection into the cervical spinal cord, there was indistinct labeling of only a very few cells in the deep and intermediate layers of the superior colliculus. This result indicates that few fibers from this nucleus have reached their targets in the spinal cord by this age.

The ceruleospinal system. The ceruleospinal system arises from cells in the locus ceruleus, subceruleus, and the lateral tegmental groups, areas A5 and A7. The projections descend ipsilaterally with the central tegmental tract to the spinal cord (Commissiong,
Hellstrom, & Neff, 1978b; Bjorklund & Lindvall, 1984; Paxinos, 1985b). Some fibers leave the tract and terminate in the medullary reticular formation, while the majority continue caudally in the ventrolateral columns to all levels of the spinal cord (Moore & Bloom, 1979; Commissiong, 1983b). Terminals appear bilaterally in the ventral horn, although the majority of the terminals remain ipsilateral in the intermediate gray and the ventral half of the dorsal horn, but are especially dense in the areas of the cervical and lumbar enlargements (Kuypers, 1982; Bjorklund & Lindvall, 1984; Paxinos, 1985b; Rajaofetra et al. 1992). Terminals from the subceruleus are found in the intermediolateral cell column (Paxinos, 1985b).

In addition to the ceruleospinal connections, there are other extensive efferents from the LC, the majority of which ascend to various forebrain structures. However, many midbrain and brainstem structures also receive projections from the LC and subceruleus, including the SN/VTA, cerebellar cortex, the sensory and spinal trigeminal nuclei, the cochlear nuclei, the pontine nuclei, the central gray, the interpeduncular nucleus and the dorsal raphe (Fuxe, 1965; Bjorklund & Lindvall, 1984; Paxinos, 1985b). Projections of the lateral tegmental nuclei, A5 and A7, and the dorsal medullary nuclei, A1-A3, innervate several cranial nerve nuclei, the ventral mesencephalic central gray, the pontine nucleus, the parabrachial nuclei, the VTA and the inferior olivary nucleus (Bjorklund & Lindvall, 1984).

Afferents to the noradrenergic nuclei are also numerous. Midbrain and brainstem structures that project to these nuclei are the cuneiform nucleus (MLR), the lateral reticular nucleus, and the central gray. A large serotonergic projection arises from the dorsal raphe, and a fairly substantial non-dopaminergic projection along with a light DA projection arises from the VTA. In addition, there are afferents from the marginal zone of the dorsal horn, the solitary tract, the vestibular nucleus, and the deep cerebellar nuclei (Degueurce & Milon, 1983; Paxinos, 1985b).
Activation of the noradrenergic system appears to increase the responsiveness of both alpha and gamma motor neurons (Commissiong, 1981) to locomotion-inducing stimuli, by inducing a long-lasting decrease in the threshold for motor neuron activation (White & Neuman, 1980; Kuypers, 1982). In the acute spinaly-transected cat, activation of noradrenergic receptors produces changes in reflex activity, by decreasing short latency and increasing long latency flexor-reflex afferent discharges (Anden, Jukes, Lundberg, & Vyklicky, 1966b; Jankowska, Jukes, Lund, & Lundberg, 1967; Kiehn, Hultborn, & Conway, 1992). In addition, noradrenergic activation elicits stepping movements in the same preparation and walking occurs if the animal is suspended over a treadmill or moved over a surface (Maling & Acheson, 1946; Jankowska, Jukes, Lund, & Lundberg, 1967; Grillner, 1969; Forssberg & Grillner, 1973; Grillner & Shik, 1973; Barbeau & Rossignol, 1991). Kuypers (1982) has proposed that the aminergic brainstem pathways may, under limbic control, exert motivational drive for the execution of motor activity such as that involved in flight or fight behaviors. This hypothesis has found recent support in the work of Depoortere et al. (1990), in which MLR stimulation in freely-moving rats has been found to elicit escape reactions. In addition, the work of Brudzynski et al. (1992) has shown that locomotor activity elicited by DA injection into the nucleus accumbens can be significantly decreased by lesion or temporary blocking of synaptic transmission in the MLR. These results suggest a functional connection between limbic regions and the MLR in the initiation of locomotor activity.

Various sources indicate that the LC and associated noradrenergic structures have differentiated and migrated to their respective final positions in the rat brainstem by E18 (Seiger & Olson, 1973; Coyle, 1974; Specht, Pickel, Joh, & Reis, 1981). In addition, evidence has been found that these cells are functional prenatally. The enzymes responsible for NA metabolism (tyrosine hydroxylase, dopa decarboxylase, and dopamine beta hydroxylase) are present by E15 (Coyle & Henry, 1973; Coyle, 1974). The high-affinity uptake mechanism for NA is present in terminals by E18 (Coyle & Axelrod, 1971).
Although very few NA terminals are present in the forebrain at birth (Coyle, 1974), NA is detectable in the spinal cord at E18, with terminals visible in the ventral horn at birth (Commissiong, 1983a). It is likely that noradrenergic innervation of the spinal cord is capable of synaptic function at birth in the rat.

The raphe-spinal system. The raphe nuclei extend from the level of the interpeduncular nucleus of the midbrain to the level of the pyramidal decussation and are the major source of 5-HT in the brain. Briefly, the raphe nuclei are subdivided into the following groups: the caudal linear nucleus (CLi), which contains both serotonergic and dopaminergic cells; the dorsal raphe (DR or B5-B7), which has the most dense aggregation of serotonergic cells of all the raphe nuclei; the median raphe (MnR or B8); the raphe pontis nucleus (RPn or B4); and the three groups which have substantial projections to the spinal cord, the raphe magnus (RMg or B3), raphe obscurus (ROb or B2), and raphe pallidus (RPa or B1, Paxinos, 1985b).

Retrograde tracing studies have shown that all the raphe nuclei except the MnR project to the spinal cord to some degree. The three groups from which most of the spinal projections arise will be discussed here. These nuclei, along with other serotonergic projections from associated reticular nuclei form two groups of descending pathways. The first descending group arises from serotonergic cells of the RMg and the pars alpha of the gigantocellular reticular nucleus. These cells project in the dorsolateral columns to the dorsal horn and are involved in endogenous pain modulation. The second descending group of projections is formed from cells in the ROb and RPa. This group travels in the ventral columns to the ventral and intermediolateral horns (Loewy & McKellar, 1981; Bowker, Westlund, Sullivan, & Coulter, 1982) and may be involved in behavioral activation.

Other efferents of the raphe system are complex, since virtually all areas of the brain receive 5-HT innervation to some degree (Paxinos, 1985b). Non-serotonergic cells also appear to project from the raphe, but less is known about their target structures or the
neurotransmitters involved. Midbrain targets of serotonergic innervation have been determined, although exact localization of cells of origin is difficult, since the innervation of the brainstem is dense. Serotonergic fibers terminate in the tectum, the central gray, the VTA, the RLi and CLi, the interpeduncular complex, the SN, the LC, and the reticular formation, especially in the areas of A8 and the parabrachial nuclei. In the caudal brainstem, raphe projections innervate cranial nerve nuclei and the inferior olive. The cerebellum also receives 5-HT innervation, in the deep nuclei and the cortex (Dahlstrom & Fuxe, 1965; Fuxe, 1965; Paxinos, 1985b).

Afferent projections to the raphe complex are as diverse as the efferents. Afferents from brainstem nuclei to the DR arise from the LC, as mentioned in the previous section, also from the laterodorsal tegmental nucleus, the parabrachial nuclei, the pontine central gray, SN, medullary reticular formation and other raphe nuclei. In addition to receiving similar projections from these areas, the MnR also receives projections from the interpeduncular nucleus, and medullary catecholamine groups A1 and A3. The medullary raphe groups, RMg, ROb and RPa receive major afferents from the reticular formation and midbrain central gray. In addition to these, the RMg receives afferents from the MnR and DR, as well as neurotensin afferents from the central gray and cuneiform nucleus (Paxinos, 1985b).

The functions of the raphe-spinal serotonin system are associated with modulation of pain signals from the spinal cord, as stated above, and also with a behavioral syndrome characterized by increased motor activity such as tremors, forepaw treading and rigidity (Jacobs & Klemfuss, 1975). In addition, as was found with NA, application of 5-HT to lumbosacral motor neurons has been found to induce long-lasting decreases in the threshold of excitability (White & Neuman, 1980), while depletion of 5-HT in spinalized rats prevents L-DOPA induced activation of alpha motor neurons (Commissiong, 1981). Barbeau and Rossignol (1991) proposed that 5-HT agonists in the spinal cord induce long-lasting changes in motor neuron excitability, thereby influencing the final output of
locomotor patterns. A recent study by Cazalets et al. (1992) found that 5-HT or certain excitatory amino acids, when applied to the bath of isolated brainstem-spinal cord preparations from neonatal rats, resulted in fictive locomotion as recorded from the ventral roots.

The raphe nuclei appear early in prenatal development. Groups B4-B9 are found by fluorescence histochemical techniques as early as E12 in the rat. The groups with major spinal projections, B1-B3, appear somewhat later, at E14. By E21, the B1-B3 complex demonstrates large descending axon bundles to the spinal cord (Seiger & Olson, 1973). The relationship between locomotor activation by L-DOPA and the 5-HT system remains unclear.

The dopamine systems. In the adult rat, dopaminergic structures are concentrated in the ventral midbrain tegmentum and include: the substantia nigra (SN or A9); the ventral tegmental area (VTA or A10) and its subdivisions, the paranigral and parabrachial pigmented nuclei; the interfascicular nucleus, the rostral and caudal linear nuclei of the raphe (RLi and CLi); the peripeduncular nucleus; and the retrorubral field (RRF or A8, Bjorklund & Lindvall, 1984). In addition to these midbrain groups, there are several groups of dopaminergic cells located in the diencephalon, designated A11-A14 (Dahlstrom & Fuxe, 1964) as mentioned previously. The efferents of midbrain dopaminergic nuclei form several pathways. For the purposes of this paper, the nigrostriatal, mesolimbic and mesocortical pathways need not be described, since decerebration experiments have shown that L-DOPA-induced air-stepping is not dependent on any of these pathways. Instead, the local and descending projections of the dopaminergic structures will be discussed.

There is evidence for a dopaminergic spinal cord projection from the SN (Commissiong, Galli, & Neff, 1978a; Commissiong, Gentleman, & Neff, 1979). The pathway possibly descends through the midbrain in the dorsal longitudinal fasciculus to travel in the spinal cord in lamina 1, the area surrounding the central canal, and the
dorsolateral funiculus (Lindvall & Bjorklund, 1983). The axons can be found throughout the length of the cord, but are most dense in the areas of the intermediolateral cell column and surrounding the central canal at thoracic and lumbar levels (Commissiong, Galli, & Neff, 1978a; Lindvall & Bjorklund, 1983; Bjorklund & Lindvall, 1984).

In addition to the above-mentioned projections to the spinal cord, the SN/VTA has moderate dopaminergic projections to the MnR and a "light" projection from the VTA to the LC (Beckstead, Domesick, & Nauta, 1979; Degueurce & Milon, 1983). Non-dopaminergic local and descending projections are somewhat more extensive than the dopaminergic efferents. There is a projection from the SN to the deep layers of the superior colliculus (Gerfen, Staines, Arbuthnot, & Fibiger, 1982). Tegmental projections from the SN/VTA include those to the PPTg, the central gray, the deep mesencephalic nucleus, the parabrachial nuclei, the LC and the dorsal and median raphe (Hedreen, 1971; Hopkins & Niessen, 1976; Beckstead, Domesick, & Nauta, 1979; Reavill, Leigh, Jenner & Marsden, 1981; Gerfen, Staines, Arbuthnot, & Fibiger, 1982; Swanson, 1982).

In the developing animal, dopaminergic groups A8-A10 appear around E13 (Seiger & Olson, 1973). These cells have reached their respective positions in the brain, and resemble adult distributions by E18 (Specht, Pickel, Joh, & Reis, 1981). Dopamine was found in the spinal cord by E20 and increased slowly after that time, with the highest concentrations being found in the thoracic region throughout neonatal life (Commissiong, 1983b). Despite the slow pattern of development seen postnatally (Commissiong, 1983a; Santana, Rodriguez, Afonso, & Arevalo, 1992), it appears from several reports that dopaminergic neurons are functionally active at birth (Loizou, 1969; Coyle & Henry, 1973; Coyle, 1974; Commissiong, 1983a; Santana, Rodriguez, Afonso, & Arevalo, 1992). Some investigators have advanced the hypothesis that DA and NA neurons serve a trophic function during development for other later maturing structures of the nervous system (Lawrence, & Burden, 1973; Seiger & Olson, 1973; Lauder & Bloom, 1974). In terms of L-DOPA-induced activation, the normal functional significance of these systems...
in vivo in the neonatal rat may not be as important as the fact that they are in place and can be activated pharmacologically to produce locomotor behavior in the neonatal rat.

Medullary Systems

Continuous with the MLR is another locomotion-inducing area of the brain, the pontobulbar locomotor region (PLR). This area extends ventrally in the lateral tegmentum from the caudal edge of the MLR through the dorsolateral medulla into the cervical regions of the dorsal horn, and appears to be partially coextensive with the caudal reticular nucleus of the pons or PnC (Ross & Sinnamon, 1984; Garcia-Rill & Skinner, 1987a; Noga, Kriellaars, & Jordan, 1991). Stimulation of either the PLR or MLR causes a field potential in the other, indicating there are connections between the two areas (see references in Grillner, 1981). A second area from which locomotion can be elicited with electrical stimulation is in the medioventral medulla, an area that roughly corresponds to, but is not necessarily limited to, the gigantocellular reticular nucleus (Garcia-Rill & Skinner, 1987a; Kinjo et al. 1990; Noga, Kriellaars, & Jordan, 1991). The MLR projects to this region in the cat (Garcia-Rill & Skinner, 1987a, 1987b) and the rat (Garcia-Rill, Skinner, Conrad, Mosley, & Campbell, 1986).

Treadmill studies using adult cats have examined the role of the medioventral medulla in eliciting locomotion. It was found that both low amplitude high frequency electrical stimulation or local injections of substance P or cholinergic agonists elicited locomotion on a treadmill. This activity, in both cases, could be blocked by local injections of cholinergic antagonists into the medioventral medulla (Garcia-Rill & Skinner, 1987a). In addition, approximately one-third of the cells in the area were found to project to the spinal cord, and half of those cells in turn received input from the MLR (Garcia-Rill & Skinner, 1987b). The medioventral medulla has been suggested to serve as a relay site through which MLR activation is passed on to the spinal cord. This projection does not appear to be dependent on any connections with the PLR, since a lesion of the lateral
tegmentum or dorsal half of the spinal cord (in which the PLR lies) does not abolish MLR-stimulated locomotion (Noga, Kriellaars, & Jordan, 1991).

Studies of medioventral medulla-induced treadmill locomotion in the rat have found that the area has similar connections to those found in the cat (Ross & Sinnamon, 1984; Kinjo et al. 1990). Using an anesthetized intact rat, Ross and Sinnamon (1984) demonstrated that electrical stimulation of the ventral reticular nucleus of the medulla (MdV), the gigantocellular reticular nucleus (Gi) and the trigeminal system all induced locomotor activity, although with different combinations of limbs. Kinjo et al. (1990) examined the effects of electrical stimulation of decerebrate rats on a treadmill and found that the locomotion-inducing sites in the rat were similar in extent to those of the cat. In addition, chemical activation of locomotion in the same preparation could be induced with local injections of cholinergic agonists, GABA antagonists, substance P and NMDA.

In the neonate, the medioventral medulla has been shown to be similar to that of the adult rat in relative size and functional capability by use of in vitro brainstem spinal cord preparations (Atsuta, Garcia-Rill, & Skinner, 1988, 1990; Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991). Electrical stimulation of the medioventral medulla was found to elicit adult-like patterns of EMG recordings in the hind limb-attached preparation (Atsuta, Garcia-Rill, & Skinner, 1988, 1990). Chemical activation of locomotion from the medulla was also similar to that found in treadmill studies in the adult rat. Again, cholinergic agonists, GABA antagonists, substance P and NMDA elicited EMG-recorded hind limb activity; in addition, application of 5-HT to the bath also elicited rhythmic stepping (Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991). These studies have demonstrated that brainstem systems involved in control of spinal locomotion generators are present in the newborn animal, and that stimulation of these systems can produce adult-like patterns of stepping (Atsuta, Garcia-Rill, & Skinner, 1988).

The reticulospinal projections from the medulla have been proposed to be a crucial link between locomotion-inducing regions and the spinal cord. Locomotion induced by
stimulation of the ventral posterior hypothalamus, the MLR (Orlovskii, 1970) and the PLR (Noga, Kriellaars, & Jordan, 1991) in each case involves synaptic contacts in medullary reticular nuclei. The oral pontine reticular nucleus (PnO), the PnC, and the Gi in particular have been implicated in control of somatic motor functions (Kuypers, 1982; Paxinos, 1985b). Nomenclature used to refer to reticular nuclei is, unfortunately, not uniform from study to study. The confusion created by this situation is compounded by the fact that the reticular formation is a large and diffuse collection of cells with variable cytoarchitecture and functions (Paxinos, 1985b). However, it seems clear that within the medulla, two fairly distinct areas from which stimulation can elicit locomotion have been described, the medioventral medulla and the PLR. These two areas both apparently project to spinal cord locomotion-generating circuits via different routes, the medioventral area through the ventral longitudinal fasciculus, and the PLR via the dorsal longitudinal fasciculus (Noga, Kriellaars, & Jordan, 1991). Trigeminal stimulation, which also elicits a locomotor reaction, appears to act through efferents to the PLR system, while the primary projections of the MLR are transmitted through the medioventral medulla (Garcia-Rill, Skinner, Conrad, Mosley, & Campbell, 1986; Noga, Kriellaars, & Jordan, 1991).

The medullary reticulospinal projections arise primarily from the ventral reticular nucleus of the medulla (MdV), the PnC, PnO, and the Gi. The MdV projections have been studied in the cat, and in that species have been found to extend as far as the lower thoracic level and travel ipsilaterally in the dorsolateral columns (Paxinos, 1985b). Projections from the Gi and the pontine reticular nuclei course bilaterally in the ventral and lateral funiculi to terminate throughout the length of the spinal cord (Holstege & Kuypers, 1982). Gi projections contain serotoninergic and catecholaminergic fibers (Dahlstrom & Fuxe, 1964; Kuypers, 1982; Paxinos, 1985b).

Although little specific information could be found regarding development of the reticulospinal systems in the neonatal rat, Leong et al. (1984) determined that in the P2 rat the midline and lateral reticular nuclear complexes appear to have similar density and
pattern of labeling to that of adults, following HRP injection at the level of the lumbosacral cord. In addition, large reticular neurons can be found by E12, appear to be differentiated by E14, and may send axonal processes into the dorsal columns at that time (Das & Hine, 1972). Evidence from neonatal rat in vitro studies in particular indicate that the reticulospinal system is capable of relaying locomotor-inducing signals from more rostral brain regions at birth.

**Spinal Cord Systems**

Locomotion has been studied at the spinal cord level in numerous species (for review see Grillner, 1981). The spinally transected cat is a preparation that has been used frequently in studies of spinal cord-generated locomotion. The general paradigm is similar to the one used by Shik et al. (1966) with decerebrate cats, in that the animals are suspended over a treadmill belt, but also receive transections at various levels of the spinal cord. Testing has been done in acute and chronic conditions, with locomotion induced by electrical stimulation and a number of chemicals, but for the purposes of brevity this paper will discuss manipulations in acute spinalized and in vitro conditions.

The acute spinalized cat shows neither postural nor locomotor activity. However, if the animal is suspended over a treadmill and receives nociceptive stimulation of the perineal region (Sherrington, 1910), electrical stimulation of the cord (Roaf & Sherrington, 1910), or injection of certain catecholaminergic agonists (Grillner, 1969, 1973; Forssberg & Grillner, 1973), it will step. Numerous experiments have examined the effects of L-DOPA in adult spinalized cats tested on a treadmill (Anden, Jukes, & Lundberg, 1966a; Anden, Jukes, Lundberg, & Vyklicky, 1966b; Forssberg & Grillner, 1973; Grillner, 1973; Pearson & Rossignol, 1991). In these studies, the locomotor effects of L-DOPA have been attributed to its conversion to NA in terminals of brainstem afferents to the spinal cord. This idea is supported by the fact that clonidine also elicits locomotion in the spinalized animal on a treadmill (Forssberg & Grillner, 1973), although clonidine may not have identical actions to L-DOPA on locomotor pattern generators.
(Pearson & Rossignol, 1991). Some of these studies predate experimental evidence for dopaminergic innervation of the spinal cord (Commissiong, Galli, & Neff, 1978a; Commissiong, Gentleman, & Neff, 1979; Gentleman, Parenti, Commissiong, & Neff, 1981; Commissiong, 1983a), and their arguments that the locomotor effects of L-DOPA are mediated through the noradrenergic terminals of the spinal cord presume an absence of dopaminergic terminals in the cord.

Studies in the neonatal rat have been conducted using the in vitro reduced preparation (Kudo & Yamada, 1987; Smith, Feldman, & Schmidt, 1988; Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991; Iwahara, Atsuta, Garcia-Rill, & Skinner, 1991a). Using ventral root recordings and hind limb-attached preparations, it has been shown that both electrical and chemical stimulation of the spinal cord evokes locomotor activity. Electrical stimulation of the lumbar enlargement in either the dorsal columns or the ventral funiculus produced locomotor activity as determined by EMG recording (Iwahara, Atsuta, Garcia-Rill, & Skinner, 1991a). In the same preparation, bath application of DA, aspartate, glutamate (Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991), NMDA (Kudo & Yamada, 1987; Atsuta, Abraham, Iwahara, Garcia-Rill, & Skinner, 1991), and cholinergic agonists (Smith, Feldman, & Schmidt, 1988) all elicited stepping activity.

Application of L-DOPA to a spinal cord bath preparation induces fictive swimming in the lamprey eel (Cohen & Wallen, 1980; Poon, 1980). The actions of L-DOPA in this application have been attributed to its action directly at receptor sites as an excitatory amino acid. L-DOPA does not induce locomotor activity when applied to an in vitro neonatal rat spinal cord bath preparation (Garcia-Rill, personal communication). However, in mid-thoracic spinalized neonatal rats in the air-stepping paradigm, L-DOPA induces stepping in the forelimbs but not in the hind limbs (Iwahara, Van Hartesveldt, Garcia-Rill, & Skinner, 1991b; Stehouwer & Van Hartesveldt, 1991). It is not known whether the hind limbs of such animals would step if placed on a treadmill, as in spinalized
adult cats. The two paradigms differ in several ways. Treadmill stepping has the added dimensions of tactile stimulation from the foot pads and proprioceptive effects of partial weight-bearing. Also, some treadmill studies employ a monoamine oxidase inhibitor in addition to L-DOPA injection (Pearson & Rossignol, 1991), which would affect not only the NA and DA systems, but also serotonin and adrenaline activity as well. With these factors in mind, it is difficult to interpret the differences in the effects of L-DOPA at the spinal cord level between adult spinalized cats and neonatal spinalized rats. Without further experiments it cannot be ruled out that the differences in L-DOPA-elicited activity in spinalized cats on a treadmill and neonatal rats during air-stepping may be due to species differences in neuroanatomy alone.

Summary

The implications of the foregoing studies are that the activational effects of L-DOPA could involve both DA and NA systems. Regarding the role of epinephrine, little can be concluded about its possible role in L-DOPA induced locomotion. Epinephrine cells can be found in the C1 and C2 cell groups of the lateral tegmentum and dorsal medulla, respectively, and have been shown to project primarily to the hypothalamus, but also to the LC, where epinephrine release inhibits firing. The functional importance of epinephrine in the central nervous system is thought to be related to neuroendocrine mechanisms and blood pressure regulation (Cooper, Bloom, & Roth, 1991). The role of this catecholamine in L-DOPA-induced locomotion (if any) is unclear.

L-DOPA could exert its effects via increased DA and NA activity at a number of locomotion-inducing sites in the brainstem. L-DOPA induces air-stepping in decerebrate neonatal rats that is similar to that of intact animals, but it does not induce air-stepping in the hind limbs of subjects with midthoracic transection. These results imply that midbrain or brainstem catecholaminergic cell groups and their projections are involved in air-stepping. The most likely areas of the mesencephalon to be sites of action for L-DOPA, by virtue of their dense catecholaminergic cell populations, are the LC and the SN/VTA
complex. Both of these areas are also implicated by their extensive neuronal connections, locally and to the spinal cord. L-DOPA may act to initiate air-stepping in the neonatal rat by increasing catecholaminergic activity within dopaminergic structures such as the SN/VTA, to influence downstream structures via dopaminergic or non-dopaminergic efferents. Alternatively, L-DOPA could act by increasing NA release from the many projections of the LC, activating other midbrain structures such as the raphe nuclei or reticular formation, or acting directly on the spinal cord via ceruleospinal projections. These two major catecholamine-containing structures can be easily separated by transection surgery, allowing for the assessment of L-DOPA-induced activity in a preparation that retains both the major DA and NA structures of the midbrain, or in a further reduced preparation that retains only the more caudally located NA structures. Through the use of transections at varying levels of the midbrain, and subsequent examination of the effects of such transections on air-stepping, a gross localization of the site of action for L-DOPA can be determined. This method would have some advantages over that of the in vitro technique, since the effects of transection on gait variations could be assessed in both forelimbs and hind limbs.

The present study examined the effects on L-DOPA-induced air-stepping of three progressively more caudal transections through the midbrains of five day-old rat pups. Each level of transection separated different areas of the brainstem from more rostral influences, and allowed for assessment of the behavioral effects of L-DOPA in centrally reduced preparations. These experiments were designed to determine if L-DOPA acts to initiate air-stepping from some level of the midbrain. It is the hypothesis of this study that L-DOPA-induced air-stepping is dependent on localized actions in midbrain catecholamine structures, and that if substantial portions of these structures are removed, the actions of systemically injected L-DOPA will be blocked.

Previous work has demonstrated that DA D1 and D2 antagonists block L-DOPA-induced air-stepping in intact neonatal rats (Sickles, Stehouwer, & Van Hartesveldt,
Since the greatest concentrations of DA receptors are found in the basal ganglia, the ability of these neurotransmitters to block L-DOPA-induced air-stepping may be localized to that area. It was suspected that in the decerebrate subject these antagonists would no longer be able to block air-stepping. Precollicular/postmammillary transected subjects were therefore pre-treated with selective D1 and D2 antagonists to assess the effects of these substances on air-stepping.

Because L-DOPA is largely thought to act in the Parkinson patient through nigrostriatal projections, it was assumed that L-DOPA-induced air-stepping might also involve the major dopaminergic structures of the midbrain, the SN and VTA. Transection of the midbrain through the midtectum and caudal to the SN was conducted, to assess the effects on air-stepping of the removal of the major DA-producing nuclei.

Finally, the effects of a third transection level on air-stepping were assessed to determine if air-stepping could be elicited in subjects in which the majority of DA structures were separated from the caudal portions of the brainstem. If L-DOPA-induced air-stepping could be elicited in this preparation, it is likely that the action of this drug is primarily within caudal midbrain or brainstem structures, such as the noradrenergic cells of the reticular formation and LC.
EFFECTS OF TRANSECTION ON L-DOPA-INDUCED AIR-STEPPING IN NEONATAL RATS

Experiment 1

Introduction

That systemic L-DOPA induces increased locomotor activity in neonatal rats is well established (Kellogg & Lundborg, 1972; Randall & Giniger, 1974; Camp & Rudy, 1987; Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991). It has also been established that L-DOPA is converted into both DA and NA in the brain. Increases in brain content of each of these neurotransmitters result following L-DOPA administration (Hornykiewicz, 1966; Kellogg and Lundborg, 1972; Camp & Rudy, 1987), but the roles of each system in relation to L-DOPA-induced air-stepping are not known. A previous study conducted in this laboratory (Sickles, Stehouwer, & Van Hartesveldt, 1992) examined whether selective DA receptor antagonists could block L-DOPA-induced air-stepping, thereby implying a role for DA release in the expression of this behavior. Results of that study revealed that both the D1 receptor antagonist SCH 23390 and the D2 receptor antagonist spiperone independently blocked L-DOPA-induced air-stepping in five day-old rats. The basal ganglia of the forebrain are a major target for projections from midbrain DA cells, and contain a dense concentration of DA receptors even in the neonatal rat (Rao, Molinoff, & Joyce, 1991). The basal ganglia, then, are a likely site of action for DA antagonists in the modulation of L-DOPA-induced air-stepping. If DA antagonists indeed act in the forebrain to block L-DOPA-induced air-stepping, then the efficacy would be compromised in decerebrate subjects.
In this experiment the level of transection was precollicular dorsally and postmammillary ventrally. This level of transection removes the telencephalon and diencephalon and severs descending projections from both, while the midbrain DA groups retain both local and descending projections. Subjects with precollicular transections have been previously shown to exhibit air-stepping in response to L-DOPA administration that is indistinguishable from that of intact animals (Iwahara, Van Hartesveldt, Garcia-Rill, & Skinner, 1991b; Stehouwer & Van Hartesveldt, 1991). Precollicular/postmammillary transection removes the area in which the highest concentrations of DA receptors are found, the basal ganglia. It has been shown previously that both D1 and D2 receptor antagonists block L-DOPA-induced air-stepping in intact animals (Sickles, Stehouwer, & Van Hartesveldt, 1992). In the intact animal, DA antagonists are assumed to act at the level of the forebrain to block the actions of DA. If these antagonists block air-stepping in intact animals at the level of the forebrain, then their capacity to block air-stepping in decerebrate subjects would be compromised. Therefore, the abilities of the dopamine D1 antagonist SCH 23390 and D2 antagonist spiperone to block L-DOPA induced air-stepping were assessed in these precollicular-transected neonatal rats.

Doses for the antagonists were chosen based on previous studies (Sickles, Stehouwer, & Van Hartesveldt, 1992). Pilot work in our laboratory determined doses that completely blocked air-stepping induced by 100 mg/kg L-DOPA in intact animals. Rat pups were used on postnatal day five (P5), since previous work in our laboratory has shown that following a 100 mg/kg dose of L-DOPA, animals at this age demonstrate fairly invariant diagonal progression of limb movements of long duration. In addition, fewer P5 subjects fail to air-step in response to L-DOPA injection than older pups (Van Hartesveldt, Sickles, Porter, & Stehouwer, 1991) and pilot work for this experiment showed that P5 pups were better able to withstand cold anesthesia and to recover from decerebration than were older pups.
Materials and Methods

Subjects  A total of 54 five-day-old Sprague Dawley rat pups bred in our laboratory from Charles River dams and sires were used for this experiment. Females were placed in group cages with males and checked daily for the presence of sperm by vaginal lavage. Sperm-positive females were transferred to single cages until day 18 of gestation, when they were placed in solid bottom plastic breeding cages with sani-chip bedding. Animals were permitted ad libitum access to food and water at all times. Time of birth of litters was noted to within 12 hours, and day of birth was counted as postnatal day 0 (P0). On P2, pups were culled to litters of 10, with equal numbers of males and females in each group when possible. Pups remained with their dams and litter mates in breeding cages until the day of testing. Colony rooms were maintained on a 12:12 light-dark cycle, with lights on at 0700 hours and off at 1900 hours. Rooms were kept at a constant ambient temperature of 21°C.

Procedure  All pups were tested on P5, as described above. On the day of testing, subjects were removed from their home cage, weighed, and placed in a warmed container prior to anesthesia and surgery. Only pups weighing at least 14 but no more than 16 grams were used as subjects, in order to control for differences in brain size which would complicate placement of the transection. One litter per day was tested, and no more than 2 subjects per condition were used from any one litter. Pups were anesthetized by immersion up to the neck in a container of crushed ice and tap water (cold anesthesia) and were carefully monitored to ensure that their noses remained above the surface of the water. No subject was in the water for more than 4 minutes. Pups were removed from the container and prepared for surgery when an anesthetized state was apparent, tested by a light pinch of the tail.

Following anesthesia, subjects were placed on an operating platform and a longitudinal incision was made in the skin approximately over the tectum. The cranial surface was cleaned and dried with a cotton swab, and a burr hole of approximately 0.5 by
1.0 mm was made in the skull just rostral to bregma and approximately 2 mm to the right of midline using a dentist's drill. Transections were made by inserting a hand-held knife blade into the burr hole, slowly lowering the tip of the blade down the left side of the inner cranium, and drawing it up the right side and out of the burr hole. The knife blade was made in this laboratory from a steel drug spatula ground at one end to a slightly curved knife edge approximately 5 mm long, 1 mm wide, and 0.5 mm thick. Total length of the tool including the handle was 14 cm. Time of transection was noted, and the incision was sutured. Sham-operated animals were used as controls for surgery and anesthesia, and received identical treatment with the exception of the knife cut.

Subjects were returned to the warmed container, and allowed a minimum recovery period of 90 minutes. Recovery was assessed by the administration of several behavioral tests. Following the minimum recovery period, and just prior to placement in the testing chamber, each pup was removed from the recovery container and placed on a flat surface at room temperature. Each pup was observed for 1 minute and the appearance of any spontaneous locomotion was noted. If no attempts at locomotion were observed, the pup received a light tail pinch, and any locomotor activity was noted. Pups were then placed on their backs for approximately 30 seconds, and righting or any attempt to right was noted. These tests were conducted to determine if the surgery resulted in general inactivity. Pups that did not engage in locomotion or attempt to right following stimulation were excluded from the experiment, as were any pups that were pale and unresponsive or appeared to have uncontrolled bleeding from the transection site.

Following the post-recovery tests, the subjects received an injection of either 16 mg/kg SCH 23390, 6 mg/kg spiperone, or a vehicle solution. Next, a thin strip of adhesive tape was carefully wrapped around the trunk of each animal, so as to ensure free movement of all limbs. The pups were positioned in the slings with their trunks approximately horizontal to the ground. Pups were then suspended in a temperature-controlled testing chamber kept at 31-33°C for the duration of the experiment. Following
placement in the testing chamber, pups received an injection of L-DOPA (100 mg/kg) or a vehicle injection. L-DOPA injections were given at specific intervals following the antagonist injections so that the behavioral effects of the two drugs would coincide. L-DOPA (or the vehicle) was administered 15 minutes after SCH 23390 and 45 minutes after spiperone. Optimal injection times were determined by previous work (Sickles, Stehouwer, & Van Hartesveldt, 1992). All injections were made subcutaneously in the nape of the neck at a volume of 0.01 ml/g body weight, and all injected solutions were buffered to a pH of approximately 6.7.

A total of nine treatment groups were tested using this preparation. For each of the 2 antagonists, there were 3 conditions: (1) decerebrate receiving the antagonist and L-DOPA; (2) sham-operated receiving the antagonist and L-DOPA; and (3) decerebrate receiving antagonist and vehicle injections. In the other 3 conditions pups received vehicle injections instead of antagonist injections: (1) decerebrate receiving vehicle and L-DOPA; (2) sham-operated receiving vehicle and L-DOPA; and finally (3) decerebrate receiving 2 vehicle injections.

Videotaping began immediately following the second injection. Each of the treatment groups had 6 subjects, and each subject was videotaped for 1 hour. In the chamber, the subjects were suspended over a mirror that was tilted to a 45° angle so as to afford both a rostral and a ventral view of each pup to the camera. Tapes were made using a Panasonic CCTV camera and Panasonic AG 1230 video recorder.

Analysis. Behavioral analysis consisted of measuring the amount of time each subject spent engaged in each of the following five categories of activity: (1) inactive: no movement; (2) non-stereotyped active behavior: any movement that was not stereotyped and did not involve rigid posture; (3) extension/tremor: dorsal flexion of the body and extension of the limbs, sometimes accompanied by a body tremor; (4) typical air-stepping: stereotyped locomotor activity with alignment of the head, body and tail, lack of lateral head movements, and alternation of all four limbs in a diagonal progression pattern; (5)
atypical air-stepping: stereotyped locomotor activity with alignment of the head, body and tail that lacks one or more of the limb movements characteristic of typical air-stepping.

Each subject's activity was scored by a trained observer who was blind as to the treatment condition of the subject, and randomly selected scores were analyzed a second time to ensure reliability of the scoring procedure. Tapes were scored at twice normal speed, and the computer clock was accurate to the second, so that accuracy of the final measured durations was within 2 seconds of real time. Data for each behavior category were analyzed by one-way ANOVA. Pairwise comparisons were made using Duncan's Multiple Range Test.

**Histology** Following testing, the pups were deeply anesthetized using sodium pentobarbital (0.01 ml per pup) and transcardially perfused, first with heparinized saline and then with 10% buffered formalin (approximately 5 ml each solution). Brains were removed and kept refrigerated in formalin for 4 days; they were then examined for gross localization of the transection site. The brains that appeared to have the correct transection level were placed in 30% sucrose formalin for 3 days, then sectioned in the sagittal plane at 90 microns. The entire width of the brainstem was cut and alternate slices kept for histological verification. Slides were stained with thionin for identification of structures.

All transection levels were verified by histology, and subjects were chosen based on correct location of transection (figures 1 and 11). For each animal, it was determined if the transection was located perpendicular to the midline and placed dorsally at the rostral edge of the superior colliculus. The ventral portion of the cut was checked to determine if it was located at the rostral edge of the SN laterally, and just caudal to the mammillary bodies medially. Deviations of even a portion of a millimeter in placement of the transection resulted in rejection of the subject from the experiment. In addition, each tissue sample was examined for the presence of bleeding at the level of the cut. Excessive
amounts of blood in the tissue sample, or damage to any of the structures caudal to the level of the cut also resulted in the subject's being rejected from the experiment.

Results

In intact sham-operated and decerebrate animals that did not receive antagonist pretreatment, L-DOPA induced air-stepping that was similar in both duration and appearance. These two groups of subjects, the vehicle/L-DOPA/decerebrate and the vehicle/L-DOPA/sham-operated were the only subjects that engaged in typical air-stepping. There were no statistically significant differences between these two groups in the mean amounts of time spent engaging in either typical or atypical air-stepping, although variance was greater for the decerebrate group. Nor was there a significant difference in the amount of time these two groups spent engaged in non-stereotyped activity. These data support previous findings that precollicular transection produces no obvious deleterious effects on L-DOPA-induced air-stepping.

Post-recovery testing Of the 36 subjects that received precollicular transection, 13 exhibited spontaneous locomotion when placed on a surface at room temperature, while 6 out of 18 of the sham-operated animals engaged in spontaneous locomotion. However, following a mild nociceptive stimulus such as a tail pinch, 31/36 of the transected and all 18 of the sham-operated animals demonstrated locomotor activation. This activity consisted of movements similar to those described by Altman and Sudarshan (1975) for locomotion in neonatal rat pups. The animals attempted to crawl, primarily using their forelimbs and dragging their abdomens across the surface. These attempts to locomote were not prolonged. The animals would generally make several attempts to pull themselves across the surface, then appeared to rest or fall asleep. When placed on their backs, all of the animals attempted to right, and nearly all were successful in righting within 30 seconds (33/36 transected and 18/18 sham-operated).

SCH 23390 Administration of the D1 antagonist SCH 23390 effectively blocked L-DOPA-induced air-stepping in P5 rats. One-way ANOVA revealed significant effects
of condition on typical air-stepping (F=99.34, d.f.=5,35, p<0.01), atypical air-stepping (F=5.93, d.f.=5,35, p<0.01) and non-stereotyped active behavior (F=8.48, d.f.=5,35, p<0.01).

In the groups that were pre-treated with SCH 23390 and given L-DOPA for the second injection, both air-stepping and atypical air-stepping were completely blocked. This was the case for both the decerebrate and the sham-operated conditions. However, these subjects still engaged in non-stereotyped active behavior, with the decerebrate group engaging in more active behavior than the sham-operated group (p<0.05). In addition, the SCH 23390/vehicle/decerebrate group was significantly more active than the vehicle/vehicle/decerebrate group (p<0.05). In no group was there complete sedation of the subjects, since all groups engaged in non-stereotyped active behavior (see table 1 for means and standard errors and figure 2 for graph).

**Spiperone.** As with the D1 antagonist, pre-treatment with the D2 antagonist spiperone blocked L-DOPA-induced air-stepping. Spiperone treatment significantly affected typical air-stepping (F=98.08, d.f.=5,35, p<0.01), atypical air-stepping (F=3.46, d.f.=5,35, p<0.05), and non-stereotyped active behavior (F=3.38, d.f.=5,35, p<0.05). Air-stepping was blocked by pre-treatment with spiperone in both sham-operated and decerebrate groups. Atypical air-stepping, however, was not completely blocked in subjects in the spiperone/L-DOPA/sham-operated condition. These subjects engaged in significantly more atypical air-stepping than did the spiperone/L-DOPA/decerebrate subjects (p<0.01), but the amount of atypical air-stepping in this group was not different from that of either the vehicle/L-DOPA/sham-operated or vehicle/L-DOPA/decerebrate groups (see table 2 for means and standard errors and figure 3 for graph).

Non-stereotyped active behavior was demonstrated consistently in all but the spiperone/vehicle/decerebrate group, which was significantly less active than the spiperone/L-DOPA/sham-operated and spiperone/L-DOPA/decerebrate groups (p's<0.01) as well as the vehicle/L-DOPA/sham-operated group (p<0.05). There were no differences
in the amount of time subjects engaged in non-stereotyped active behavior among any of
the other groups (figure 3).

Analysis  Repeated scores of individual subjects' locomotor activity generally had
total durations that were within 5 minutes of the original scores.

Histology  The precollicular transection surgery did not result in excessive
amounts of subcranial bleeding or damage to structures located near the cut. This level of
transection removed the pretectal area, the posterior nucleus of the thalamus, and some of
the rostral cells of the VTA. The cut extended to the ventral surface of the brain just
caudal to the mammillary bodies, at the anterior edge of the interpeduncular fossa.

Discussion

Precollicular transection of P5 rats did not result in substantial change in the air-
stepping response to L-DOPA. These data support those of Iwahara et al. (1991b) and
Stehouwer and Van Hartesveldt (1991), in which precollicular-transected subjects were
found to engage in L-DOPA-induced air-stepping that was indistinguishable from that of
intact controls with respect to topography; that is, pattern of limb movements, posture,
and rate of stepping. However, transection does appear to increase the variability between
subjects in the duration of air-stepping induced by L-DOPA treatment. Individual subjects
that have received precollicular transection show a wider range of total durations of air-
stepping than do intact animals. Some possible explanations for this increase in variability
include variations in the amount of intracranial bleeding, slight differences in the location
of the transection site, or different degrees of damage done to tissue proximal to the
transection site.

This experiment showed that pretreatment with either the D1 antagonist SCH
23390 or the D2 antagonist spiperone completely blocked L-DOPA-induced air-stepping
in precollicular-transected P5 rats. This effect was not the result of an overall sedation,
since subjects in all groups (with the exception of the spiperone/L-DOPA/decerebrate
group) engaged in non-stereotyped active behavior, but was instead specific to the L-
DOPA-induced stereotypy. It can be assumed then, that modulation of L-DOPA-induced locomotor output is not effected through forebrain DA receptors, at least in the neonatal rat. These data provide evidence for modulation of L-DOPA-induced air-stepping by DA receptors within the midbrain and/or brainstem areas.

Figure 1: Illustration of level of precollricular/postmammillary transection in P5 rat. The section is parasagittal, showing the approximate locations of the subthalamic nucleus (STN), the substantia nigra (SN), area A8, and the pedunculopontine tegmental nucleus (PPTg).
Table 1: Statistical data for control and experimental groups pre-treated with the D1 antagonist SCH 23390. For each of the behaviors listed, the table shows the number of subjects out of 6 that demonstrated the behavior, the group mean in minutes, and the standard error of the mean. Treatment groups are labeled according to the series of drug injections received and whether they were sham-operated or decerebrate. SCH = 16 mg/kg SCH 23390; L-D = 100 mg/kg L-DOPA; VEH = vehicle injection; S = sham-operated; and D = decerebrate.

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<th>SEM</th>
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<td>0</td>
<td>--</td>
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<tr>
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<td>Non-Stereotyped Active</td>
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<td>0</td>
<td>--</td>
</tr>
<tr>
<td>VEH/VEH/D</td>
<td>Non-Stereotyped Active</td>
<td>0/6</td>
<td>0</td>
<td>--</td>
</tr>
</tbody>
</table>
Figure 2: Graph showing total mean durations of air-stepping, atypical air-stepping, and active behavior for each of the 6 conditions tested. 0=vehicle injection; 16=16 mg/kg SCH 23390; 100=100 mg/kg L-DOPA; D=decerebrate; S=sham-operated control.
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<th>ATYPICAL AIR-STEPPING</th>
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Table 2: Statistical data for control groups and experimental groups pre-treated with the D2 antagonist spiperone. For each of the behaviors listed, the table shows the number of subjects out of 6 that demonstrated the behavior, the group mean in minutes, and the standard error of the mean. Treatment groups are labeled according to the series of drug injections received and whether they were sham-operated or decerebrate. SPIP = 1 mg/kg spiperone; L-D = 100 mg/kg L-DOPA; VEH = vehicle injection; S = sham-operated; and D = decerebrate.
Figure 3: Graph showing total mean durations of air-stepping, atypical air-stepping, and active behavior for each of the 6 conditions tested. 0=vehicle injection; 6=6 mg/kg spiperone; 100=100 mg/kg L-DOPA; D=decerebrate; S=sham-operated control.
Experiment 2

Introduction

The SN/VTA complex contains the densest accumulation of dopaminergic cells in the brain. It is therefore likely that peripherally injected L-DOPA crosses the blood-brain barrier and is taken up by these cells, converted to DA, and released. Through the activation of local circuits within the SN/VTA, possibly via dendritic release of DA (Cheramy, Leviel, & Glowinski, 1981) and subsequent effects on descending efferents to other locomotion-related nuclei, it is possible that this complex comprises the primary site of action for L-DOPA (Robertson & Robertson, 1988). In the previous experiment, it was shown that L-DOPA elicits air-stepping in five day-old precollicular transected rats that is similar in topography and pharmacology to that of intact sham-operated subjects, and furthermore, that this activity can be blocked by pretreatment with either the D1 antagonist SCH 23390 or the D2 antagonist spiperone. Since blocking either of these receptor subtypes affects the behavior, it seems likely that the release of DA and its subsequent effects on postsynaptic receptors must take place at some point in the series of synaptic events that lead to air-stepping. Dopaminergic receptors can be found in the SN at birth in the rat (Sales, Martres, Bouthenet, & Schwartz, 1989; Murrin & Zeng, 1990; Rao, Molinoff, & Joyce, 1991). If the SN is the site of uptake and metabolism of L-DOPA into DA, and release of DA within local circuits of the SN/VTA complex is responsible for inducing air-stepping, then transection caudal to the level of this structure should prevent L-DOPA from inducing air-stepping.

The substantia nigra or area A9 (Dahlstrom & Fuxe, 1964) is located in the ventral tegmentum of the midbrain. Its two major subdivisions are the pars compacta (SNC), which is densely populated by dopaminergic cells, and the pars reticulata (SNR), an area that is cell poor, containing primarily neuropil and a few GABA and DA cells (Paxinos, 1985a). Continuous with and medial to the SN is the ventral tegmental area, or area A10.
The VTA contains dopaminergic and cholecystokinin-containing cells (Hokfelt et al. 1978). Together, these structures project rostrally to various nuclei of the forebrain, via mesotelencephalic pathways. The ascending pathways are much more extensive and more widely studied in relation to locomotion; however, there are several descending projections from the SN/VTA as well. In the adult rat, a moderate dopaminergic projection is known to exist to the median raphe (MnR) and a "light" projection from the VTA to the LC (Swanson, 1982; Degueurce & Milon, 1983). There are also non-dopaminergic projections from these nuclei. Approximately 99% of the VTA projection to the LC is non-dopaminergic, and non-dopaminergic efferents from the SNR project to the superior colliculus, the dorsolateral central gray, mesencephalic reticular formation, pedunculopontine tegmental nucleus (PPTg) and the LC.

There are dopaminergic cells in several other nuclei throughout the brainstem, the largest group being the retrorubral fields, or area A8. This group of DA cells extends caudally and dorsally from the caudal end of the SN. In addition, DA cells can be found in the deep mesencephalic nucleus and the caudal linear nucleus of the raphe (Paxinos, 1985a). All of these nuclei lie at least partially caudal to the level of transection that is caudal to the SN/VTA complex, and cells in these structures may take up L-DOPA and metabolize it to DA.

Previous work has shown that air-stepping can be blocked by pretreatment with either the noradrenergic alpha 1 antagonist prazosin, or the alpha 2 antagonist idazoxan (Taylor, Sickles, Stehouwer, & Van Hartesveldt, 1994). This result implies that L-DOPA-induced air-stepping may be dependent on release of both DA and NA. In the midcollicular-transected subject, the major noradrenergic cell structures lie caudal to the level of transection. These structures have extensive projections throughout the brain, both ascending and descending. L-DOPA could exert its effects on locomotion via uptake and conversion to NA, having synergistic effects with L-DOPA-induced activity in DA systems, or by being part of the series of synaptic events that take place in the initiation of
air-stepping. In addition, Grillner (1981) presented evidence that electrically-induced locomotion from the MLR occurs only if the ventral rostral part of the brainstem is intact. This area includes the SN.

In this experiment, the role of the SN/VTA complex in the production of L-DOPA-induced air-stepping was examined. Two transection levels were used, one at a precollicular/postmammillary level, as a control condition for transection surgery, and one at a midcollicular/postnigral level, to determine the effect of loss of the SN/VTA complex on L-DOPA-induced air-stepping.

Materials and Methods

Subjects. A total of 23 Sprague Dawley rat pups, bred in our laboratory as described previously (see Materials and Methods, experiment 1), were used for the following experiment.

Procedure. Testing was carried out on P5. Subjects were removed from the home cage, weighed, and placed in a warmed container. Only animals weighing between 14 and 16 grams were used. Pups received cold anesthesia and when an anesthetized state was apparent, were placed on an operating platform. A longitudinal incision was made in the skin over the tectum, the cranial surface was cleaned and dried with a cotton swab, and a burr hole of approximately 0.5 by 1.0 mm was made in the skull. Three conditions were used in this experiment, necessitating two different placements of the burr hole. The first condition required a precollicular/postmammillary transection, so the hole was placed just rostral to bregma and approximately 2 mm to the right of midline. The second condition required a midcollicular/postnigral transection. The burr hole was made midway between bregma and lambda sutures, just to the right of midline. To achieve the correct angle of transection, the pups' heads were placed over a support which allowed for a downward tilt of the head during the knife cut. The third condition was a sham operation. In this case about half of the subjects received the burr hole rostral to bregma, and half between bregma and lambda. Subsequent analysis revealed no effect on the animals' behavior due
to the location of the burr hole in sham-operated subjects, so data from these subjects were collapsed. Transections were made by insertion of a thin knife blade into the burr hole as described in experiment 1. Time of transection was noted, and the incision was sutured.

Following surgery, the subjects were replaced in the warm container for a recovery period of at least 90 minutes. As described previously, pups were assessed for spontaneous locomotor activity following recovery, and reactions to tail pinch were recorded, as were attempts at righting. Pups that did not attempt to right or locomote following stimulation were excluded from the experiment, as were pups that were pale and inactive or appeared to have excessive bleeding. Subjects were wrapped carefully around the midsection with adhesive tape, and suspended in the testing chamber so that their bodies were perpendicular to the ground. An injection of 100 mg/kg L-DOPA was made subcutaneously in the nape of the neck through petroleum jelly, to minimize leakage at the injection site. Subjects were videotaped for 1 hour following injection (as described previously).

**Analysis.** Videotaped sessions were scored using a computer program to measure the amount of time that each subject spent engaged in each of the following five behaviors: (1) inactive: no movement; (2) non-stereotyped active behavior: any movement that was not stereotyped and did not involve rigid posture; (3) extension/tremor: dorsal flexion of the body and extension of the limbs, sometimes accompanied by a body tremor; (4) typical air-stepping: stereotyped locomotor activity with alignment of the head, body and tail, lack of lateral head movements, and alternation of all four limbs in a diagonal progression pattern; (5) atypical air-stepping: stereotyped locomotor activity with alignment of the head, body and tail that lacks one or more of the limb movements characteristic of air-stepping.

Each subject was scored by a trained observer who was blind to the treatment condition of the subjects, and analysis was repeated for several randomly selected subjects
to ensure reliability of scoring methods. Data were analyzed using one-way ANOVA. Pairwise comparisons were made using Duncan's Multiple Range Test.

**Histology.** Following testing, brains were removed as previously described (see Materials and Methods, experiment 1). All transection levels were verified by histology, and subjects were chosen based on correct location of transection. For precollicular transections the tissue was assessed as described in experiment 1. For subjects receiving midcollicular transections, it was determined if the transection was located perpendicular to the midline and placed dorsally at the midpoint of the tectum (see figures 4 and 11). The ventral portion of the cut was checked to determine if it was located at the caudal edge of the SN. If the cut removed any portion of the rostral tip of the pons, the subject was excluded from the experiment. Deviations of even less than a millimeter in placement of the transection resulted in rejection of the subject from the experiment. In addition, each tissue sample was examined for the presence of bleeding at the level of the cut. Excessive amounts of blood in the tissue sample, or damage to any of the structures caudal to the cut also resulted in the subject being rejected from the experiment.

**Results**

**Post-recovery testing.** Of the 6 subjects that received precollicular transection, only 1 exhibited spontaneous locomotion when placed on a surface at room temperature. Of the 9 midcollicular transected subjects, 6 engaged in spontaneous surface locomotion, as did 4 out of the 8 sham-operated animals. Following a mild nociceptive stimulus such as a tail pinch, every subject, including transected and sham-operated animals, demonstrated surface locomotion. This activity consisted of movements similar to those described by Altman and Sudarshan (1975) for locomotion on a surface in neonatal rat pups (see Results, experiment 1). When placed on their backs, all of the animals attempted to right. Of the precollicular-transected animals 3/6 succeeded in righting themselves within 30 seconds, as did 8/9 of the pups with midcollicular transections. All 8 of the sham-operated animals succeeded in righting themselves.
L-DOPA-induced air-stepping was not blocked in subjects with midcollicular/postnigral transections. However, a significant effect of surgical condition was found (F=4.82, d.f.=2,22, p<0.05) with the midcollicular transection group engaging in less typical air-stepping than the intact group. One outlier in the midcollicular transection group engaged in no air-stepping, but instead had 26 minutes of extension/tremor activity. The precollicular transection group was not significantly different from either the midcollicular transection group or the intact group in duration of typical air-stepping. For this experiment, a measure of total air-stepping was obtained by adding the duration of typical air-stepping to the duration of atypical air-stepping for each subject. Analysis of variance for this measure revealed no significant differences among the three conditions in total amount of stereotyped air-stepping activity (see table 3 for means and standard errors, and figure 5 for graph).

There were no significant differences in the durations of inactivity, non-stereotyped active behavior, extension/tremor or atypical air-stepping among the three experimental conditions. Atypical air-stepping was evaluated descriptively for each subject that was scored. In each group, the topography of the behavior was similar. The most frequently occurring characteristic of atypical air-stepping in either the intact or the transected subjects was irregular movement of the hind limbs. The limbs appeared to alternate with uneven step rates, both within the hind limb girdle and when compared to the forelimbs. Occasionally the limb movements within a girdle switched from an alternating pattern to an in-phase movement. Frequently one or both of the hind limbs ceased to move. The arrested limb would generally be held in a slightly extended position behind the animal, sometimes for several step cycles at a time. There was variation in the amplitude of the steps as well, the amount of distance between the points of greatest extension and greatest flexion of the limb. Most of the subjects demonstrated a shift to smaller amplitude steps at the end of the experiment in both the hind limbs and the forelimbs, sometimes involving
movement only in the most distal joints. All of these variations appeared in all of the groups with fairly comparable frequency.

**Analysis** Repeated scores of individual subjects' locomotor activity generally had total durations that were within 5 minutes of the original scores.

**Histology** The histology results of this experiment revealed that the conditions of the precollicular and midcollicular transection groups were similar in that there was minimal bleeding and structures caudal to the transections were intact. The appearance of the precollicular transection level in these subjects was also similar to those of experiment 1 (see Results). In subjects that received a midcollicular transection, the cut was located dorsally at the midpoint of the tectum, and passed through the central gray to the caudal edge of the SN. Medially the cut passed through the CLI and caudal to the VTA (see figure 4a).

**Discussion**

Transection caudal to the SN in the P5 rat does not prevent L-DOPA-induced air-stepping. Although there was a significant difference in the amount of typical air-stepping in the midcollicular transection group compared to the sham-operated group (p<0.05), this may have been due to an outlier that engaged in 26 minutes of extension/tremor activity and no typical air-stepping. It is possible that this outlier deflated the air-stepping mean for the group so as to result in the difference observed. There was no obvious anomaly in the histology of this individual subject to account for its unusual behavior, but it must be noted that transection does appear to result in increased variability of behavior. All other subjects in the midcollicular transection group appeared to behave similarly to subjects in either of the other conditions.

A precollicular/postmammillary transection condition was included in this study to control for the effects of transection surgery. In addition, this preparation controlled for severing the descending projections from the superior colliculus to the spinal cord, which are thought to influence posture of the head and upper body. Descending projections
from the superior colliculus are left intact by precollicular transection, but are severed in the midcollicular/postnigral preparation. The loss of this projection could affect posture in the air-stepping animal. However, qualitative assessment of atypical air-stepping revealed no differences in posture between the two groups, so it is assumed that this projection is not necessary for the appearance of stereotyped dorsal flexion or head posture in the air-stepping rat.

No topographical differences were found for any measured behavior between the precollicular and the midcollicular transection groups. Nor were there any differences in the topography of behavior of either transection group compared to the sham-operated animals. All subjects demonstrated similar characteristics of atypical air-stepping, and when atypical and typical air-stepping were added together as a total measure of stereotyped, drug-induced behavior, there were no significant differences in duration. It is important to note that following transection surgery, subjects in each of the three conditions attempted to right when placed on their backs, and exhibited generalized activation (vocalization, attempts to crawl) in reaction to mild nociceptive stimuli such as a tail pinch. These results show that transection surgery did not cause the animals to become unresponsive or completely inactive.

The SN/VTA complex is the most dense aggregation of DA cells in the brain, but transection through the midbrain caudal to this structure does not prevent L-DOPA-induced air-stepping. If systemic L-DOPA induces air-stepping through conversion and release of DA, which seems likely since the behavior can be blocked with direct DA receptor antagonists (see experiment 1), then other dopaminergic cells located caudal to the level of a midcollicular transection must be considered. There are several midbrain groups of DA cells, although none are as dense or large in area as the SN/VTA. The largest nucleus of DA cells caudal to the SN is area A8. This area extends caudally and dorsally from the caudal pole of the SNR towards the base of the inferior colliculus. In relation to other midbrain structures, it is large but diffuse in the neonate, and can only be
readily discerned in tissue by immunocytochemistry for the enzyme tyrosine hydroxylase. The majority of the known efferents from A8 project rostrally with other midbrain DA cells as part of the mesolimbic and mesocortical pathways. Not much is known about descending efferents from this area. However, A8 cells are near the PPTg, an area of the caudal midbrain that is contained within the physiologically defined midbrain locomotor region of the rat (Skinner & Garcia-Rill, 1984). Although not demonstrated in the rat, catecholaminergic cells have been found within the MLR of the cat, in close proximity to points from which electrical stimulation elicits treadmill stepping (Steeves, Jordan, & Lake, 1975). The dopaminergic cells of A8 may elicit or modulate locomotor activity via their influence on efferent cells of the MLR.

In addition to A8, another dopaminergic structure of the caudal midbrain is the caudal linear nucleus of the raphe (CLi), a midline structure which extends dorsally and caudally from the rostral linear nucleus (which lies medial to the VTA), to the rostro-ventral pole of the dorsal raphe. The CLi contains both dopaminergic and serotonergic cells and has projections to the VTA, amygdala, nucleus accumbens, olfactory tubercle and other forebrain structures as part of the mesolimbic system. In addition to these ascending efferents, the CLi also has a moderate projection to the MnR (Paxinos, 1985a).

It is possible that DA projections descending from a rostral midbrain structure may remain functional for hours after transection surgery, and that L-DOPA could act at these terminals. There is evidence for a "light" dopaminergic projection from VTA to the LC (Swanson, 1982). Dopamine released from the distal remains of the VTA projections could serve to initiate or modulate activation of the LC or other brainstem nuclei. Through these projections, midbrain dopaminergic activity could affect descending projections from the LC to the spinal cord. There is also evidence for a dopaminergic projection from the SN to the spinal cord (Commissiong, Gentleman, & Neff, 1979) which could be involved in motor functions.
Figure 4a: Illustration of the level of a midcollicular/postnigral transection in a P5 rat. The section is approximately midsagittal, and shows the locations of the ventral tegmental area (VTA) and the caudal linear nucleus (CLi).

Figure 4b: Illustration of the level of a midcollicular/postnigral transection in the P5 rat. The section is parasagittal, and shows the locations of the ventral tegmental area (VTA) and medial portions of area A8.
### TABLE 3
DATA FOR EXPERIMENT 2

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Table 3: Statistical data for sham-operated and experimental groups. For each of the behaviors listed, the table shows the number of subjects out of the total that demonstrated the behavior, the group mean for each behavior in minutes, and the standard error of the mean.
Figure 5: Graph of mean durations of total air-stepping showing proportions of total air-stepping that were typical and atypical for each condition. Group means for total air-stepping are not significantly different.
Experiment 3

Introduction

In the previous experiment, it was shown that L-DOPA-induced air-stepping could be elicited in neonatal rats that had received midcollicular/postnigral transection. Transection at this level disconnects the major midbrain DA groups from more caudal structures. It is clear that local actions in these nuclei are not required for the production of air-stepping, but it is possible that descending projections from these groups may still be capable of uptake and metabolism of L-DOPA, and release of catecholamines. Descending dopaminergic projections from the VTA have already been discussed (see Discussion, experiment 2). Other descending projections that may still be functional are thought to arise from GABAergic cells in the SNR. It seems unlikely that terminals of these non-dopaminergic cells would take up and convert L-DOPA to DA, although the possibility of direct effects of L-DOPA on post-synaptic D2 receptors (Nakamura et al. 1994; Yue, Nakamura, Ueda, & Misu, 1994) or beta-adrenergic receptors (Goshima, Kubo, & Misu, 1986; Misu, Goshima, & Kubo, 1986; Goshima, Nakamura, Ohno, & Misu, 1991) cannot be ruled out by the experiments discussed here.

There are other DA groups in the midbrain that may be capable of affecting locomotor activity induced by L-DOPA. Caudal portions of area A8 lie within the physiologically defined MLR. Little is known about the projections of A8, but its proximity to sites from which electrical stimulation can elicit locomotor activity in some preparations makes it a possible candidate for the site of action for L-DOPA in the initiation of air-stepping. In addition to A8, a midline group of DA cells, the CLi, extends dorsally and caudally from the rostral linear raphe nucleus to the ventral surface of the dorsal raphe nucleus. Although sparse, these cells are in a position to affect the descending projections of the dorsal raphe, which has extensive reciprocal connections to the LC and also projects to all layers of the spinal cord (Paxinos, 1985b).
By disconnecting the major dopaminergic cell groups from more caudal structures, the previous experiment ruled out the possibility that the most dense population of DA cells in the brain, the SN/VTA complex, was the sole site of action for L-DOPA-induced air-stepping. However, other studies have shown that DA receptor antagonists block L-DOPA-induced air-stepping in both intact (Sickles, Stehouwer, & Van Hartesveldt, 1992) and decerebrate (see experiment 1) subjects, implying that release of DA is important at some point in the series of synaptic events that lead to air-stepping. If local DA circuits of the SN/VTA complex are not necessary for this activation to occur, then DA must be released from some other site, such as A8 or the CLi. If release of DA from these cells is important to the activation of air-stepping, and if these nuclei could also be disconnected from more caudal structures by some level of transection, then it is likely that air-stepping would be blocked in such a preparation.

In this experiment, the effect of postcollicular/postnigral transection on L-DOPA-induced air-stepping was assessed in neonatal rats. A transection located caudal to the tectum dorsally and angled to the pontine-mesencephalic border ventrally disconnects the structures of the rostral portion of the midbrain, including A8 and the majority of the CLi, from caudal midbrain or brainstem structures. This transection level is also caudal to the majority of the area of the MLR, although a portion of the PPTg lies caudal to the line of transection. Transection at this level effectively separates all the midbrain dopaminergic cell groups from more caudal structures, and retains the majority of noradrenergic cell groups of the brainstem.

Materials and Methods

 Subjects. A total of 23 Sprague Dawley rat pups bred in our laboratory as previously described (see Materials and Methods, experiment 1) were used for the following experiment.

 Procedure. On the day of testing, pups were removed from the home cage, weighed and placed in a warmed container. Only subjects weighing between 14 and 16
grams were used. Cold anesthesia was used (as described previously) and when anesthetized, subjects were placed on an operating platform. Surgery was conducted as described in experiment 1, with the exception that the pups' heads were placed over a support to achieve a downward tilt of the head for the knife cut. The burr hole was located just rostral to lambda suture, and slightly to the right of midline, and the knife tip was tilted forward toward the ventral surface at an angle of approximately 30°. Two conditions were used for this experiment: 1) a postcollicular/postnigral transection condition; and 2) a sham-operated condition which received similar surgery except for the knife cut.

Following surgery, the pups were placed in a warm container for recovery from anesthesia for a minimum of 90 minutes. Just prior to testing, pups were removed from the recovery container, and assessed for recovery from surgery and anesthesia. All pups were tested for spontaneous locomotion on a flat surface at room temperature, and if no spontaneous locomotion occurred, pups received a mild nociceptive stimulus, such as a light tail pinch. Animals were also tested for attempts to right when placed on their backs. The reactions of each pup to the post-recovery testing were recorded. Following post-recovery testing, pups were suspended (as described in experiment 1) in the testing chamber and videotaped for 30 minutes prior to L-DOPA injection. At the end of 30 minutes, the pups were injected with 100 mg/kg of L-DOPA subcutaneously in the nape of the neck in a volume of 0.01 ml/g body weight. Subjects were videotaped for 1 hour after L-DOPA injection (as described previously).

Analysis. Videotaped sessions were scored as mentioned previously. There were four categories of behavior included in the analysis: (1) inactive: no movement; (2) non-stereotyped active behavior: any movement that was not stereotyped and did not involve rigid posture; (3) typical air-stepping: stereotyped locomotor activity with alignment of the head, body and tail, lack of lateral head movements, and alternation of all four limbs in a diagonal progression pattern; (4) atypical air-stepping: stereotyped locomotor activity
with alignment of head, body and tail, that lacks one or more of the limb movements characteristic of diagonal progression in the P5 subject.

Subjects were scored by a trained observer who was blind to the treatment condition, and randomly selected scores were repeated to ensure reliability. Data were analyzed using one-way ANOVA. Pairwise comparisons were made using Duncan's Multiple Range Test.

Histology. Following testing, subjects were deeply anesthetized and perfused as described previously. Brains were kept in formalin for 1-2 days, and then placed in 30% sucrose formalin until they sank (usually overnight). Brains were sectioned in the sagittal plane on a freezing stage microtome into 50 micron slices. The entire width of the brainstem was sectioned and alternate sections mounted directly onto slides. All transection levels were verified by histology, and subjects were chosen based on correct location of transection (see figures 6 and 11). The sections were assessed to determine whether the location of the cut was at the caudal surface of the inferior colliculus dorsally, and angled ventrally to a point between the caudal edge of the SN. If the cut removed any portion of the pons, or retained any portion of the inferior colliculus, the subjects were rejected. The tissue was stained with thionin for identification of the brain regions.

Several intact subjects were also perfused and the brains processed for tyrosine hydroxylase immunocytochemistry (TH) and counter-stained for NADPH diaphorase. The use of tyrosine hydroxylase immunocytochemistry provided firsthand neuroanatomical information on the locations of catecholamine structures in the neonatal rat, which was necessary in order to determine where various structures were located in relation to the transection levels. Animals were perfused with heparinized saline, followed by 4% paraformaldehyde in phosphate buffered saline (PBS). Brains were removed and post-fixed at 4°C for 12 hours. Sections were cut at 50 microns, collected in PBS, and blocked for 1 hour in 50 mM Tris chloride buffer. Following several rinses, the tissue was incubated in a 1:1000 dilution of TH antibody (rabbit; Chemicon International, Temecula,
CA) at 4°C for a minimum of 16 hours. The tissue was rinsed and incubated at room temperature for 1 hour in biotinylated secondary antibody (goat anti-rabbit, Zymed Laboratories, San Francisco, CA), rinsed again, and the reaction product developed in streptavidin peroxide conjugate and diaminobenzidene.

NADPH diaphorase staining is a useful method for visualization of the cholinergic cells of the PPTg (Vincent, Satoh, Armstrong, & Fibiger, 1983; Skinner, Conrad, Henderson, Gilmore, & Garcia-Rill, 1989) which is coextensive with the MLR in the rat (Skinner & Garcia-Rill, 1984). Following TH procedure, the sections were incubated at room temperature for several minutes in 50 mM Tris chloride buffer with 1 mM NADPH and 0.1 mM nitroblue tetrazolium (a blue stain for the reaction product). Sections were then rinsed, mounted, air-dried, dehydrated and coverslipped.

Results

Post-recovery testing. Of the 14 subjects that received postcollicular transection, only 1 exhibited spontaneous locomotion when placed on a surface at room temperature, and only 3 out of the 9 sham-operated animals engaged in spontaneous surface locomotion. However, following a mild nociceptive stimulus such as a tail pinch, 13/14 of the transected and all 9 of the sham-operated animals demonstrated surface locomotion. This activity consisted of movements similar to those described by Altman and Sudarshan (1975) for surface locomotion in neonatal rat pups (see Results, experiment 1). When placed on their backs, all of the subjects attempted to right, and 10/14 of the transected animals succeeded in righting themselves within 30 seconds, while all 9 of the sham-operated animals succeeded in righting themselves.

Postcollicular/postnigral transection profoundly disrupted the expression of L-DOPA-induced air-stepping in the P5 rat. Transection at this level significantly decreased the mean duration of typical air-stepping (F=608.02, d.f.=1,21, p<0.01) and reduced the number of pups engaging in typical air-stepping. Only 2 of the 14 transected subjects engaged in any typical air-stepping at all, and this was of extremely brief duration in both
cases. The latency to the onset of typical air-stepping in these two animals was also affected, with postcollicular transection resulting in a significantly longer latency to air-stepping than was seen in sham-operated subjects (F=32.03, d.f.=1,9, p<0.01).

Postcollicular/postnigral transection did not affect the mean duration of atypical air-stepping as compared to the sham-operated group. However, of the 14 subjects in the transected group, 6 showed atypical air-stepping, and of those 6 subjects, 3 had total durations of over 30 minutes. The remaining 3 subjects had total durations of less than 7 minutes each. The only 2 subjects in the transected group that showed typical air-stepping also showed more than 30 minutes of total atypical air-stepping each. In the sham-operated group, all 9 subjects engaged in atypical air-stepping, with 2 subjects having over 20 minutes total duration and the remaining 7 with less than 12 minutes each. Although there was no group difference in the total duration of atypical air-stepping between the 2 conditions, only a few of the transected subjects engaged in any form of stereotyped locomotor activity, and the behavior of these individuals, although not adequately reflecting the behavior of the group, was responsible for the similarity of the mean durations for atypical air-stepping (see table 4 for means and standard errors, and figure 7 for graph).

Qualitative assessment of the types of atypical air-stepping observed in each subject revealed mixed results. Similar to the findings of the previous experiment, the most common topographical irregularities during stereotyped locomotor activity occurred in the hind limbs. Both sham-operated and postcollicular-transected animals demonstrated hind limb irregularities. These consisted of either uneven alternation or no movement at all in the hind limbs. Irregular alternation patterns consisted of the hind limbs moving in and out of phase with each other and with the forelimbs, and often appeared to be moving at different speeds, both within the hind limb girdle and compared to the forelimbs. Frequently these changes in alternation were accompanied by a brief arrest of either one or
both of the hind limbs, which would be held immobile and slightly extended from the body for varying numbers of step cycles.

However, postcollicular transection caused atypical activity in the forelimbs, as well, whereas in sham-operated animals, atypical air-stepping was predominantly the result of hind limb irregularities. In all the animals, there was a tendency toward smaller forelimb and hind limb steps later in the session, but in the transected animals, nearly all forelimb alternation throughout the duration of the experiment appeared to be slow and of low amplitude. Also, the atypical air-stepping of postcollicular-transected animals included phase changes on the forelimb movements, while there was virtually no regular cycling of the hind limbs in any of these animals. In the 3 transected animals that engaged in periods of atypical air-stepping in excess of 30 minutes, the topography of the behavior was more similar to that of sham-operated animals than to other transected animals. Specifically, the forelimb movements were of larger amplitude, and more regular alternation than was seen in the other transected subjects.

**Analysis** Repeated scores of individual subjects' locomotor activity generally had total durations that were within 5 minutes of the original scores.

**Histology** The postcollicular transection surgery did not result in excessive amounts of subcranial bleeding or damage to structures located near the cut. This level of transection removed the entire tectal area, the SN/VTA complex, and the cells of area A8. Medially, the CLi was removed. The transection retained the LC and the caudal portion of the PPTg. The level of transection approximately bisected the PPTg lengthwise, removing the rostro-ventral portion of the nucleus that is partly coextensive with the TH immunoreactive cells of the SN and area A8 (figure 10).

**Discussion**

The results of this experiment indicate that some structural component that is crucial to activation of L-DOPA-induced air-stepping lies caudal to the level of a midcollicular/postnigral transection, since air-stepping is blocked when the transection
level is postcollicular and postnigral. However, there was substantial individual variability in the responses of the transected animals. Three of the 14 transected animals engaged in substantial amounts of atypical air-stepping behavior, in excess of 30 minutes each. This atypical air-stepping was more similar to that seen in the intact animals than to other transected subjects, in that it consisted of regular forelimb alternation with irregular hind limb movements. It is possible that some differential effects of the surgery may have occurred in these animals. Minor variations in the location of the cut (the entire midbrain of a P5 rat is approximately 3-4 mm in length, and the width of the knife is about 0.5 mm) and varying amounts of bleeding at the lesion site may differentially affect nearby tissue. These variations in the surgical effects may not be easily discerned by histological inspection, but may nevertheless affect the tissue and therefore result in greater variation among behaviors of individual subjects. Proximity of the transection level to the critical areas involved in inducing air-stepping could also cause variable effects in the expression of the behavior.

If L-DOPA produces air-stepping through conversion to and subsequent release of DA and/or NA, as indicated by the results of selective antagonist studies (experiment 1; Sickles, Stehouwer, & Van Hartesveldt, 1992; Taylor, Sickles, Stehouwer, & Van Hartesveldt, 1994; Taylor, in preparation), then it is logical to presume that catecholamine cell groups are a likely site of action for this drug. The effects of midcollicular transection on L-DOPA-induced air-stepping (see experiment 2) reveal that the major midbrain DA groups (the SN and VTA) are not needed for L-DOPA to produce air-stepping. However, postcollicular transection, which is caudal to the dopaminergic structures A8 and CLI, does prevent air-stepping. It is possible that L-DOPA has its site of action in one of these areas.

However, the TH immunoreactive cells of these nuclei are rather sparse (see figures 8 & 9). Their populations of DA cells and their locations in relation to midbrain areas that are known to be involved in locomotion (such as the MLR) and to structures
that have substantial projections to the spinal cord (such as the dorsal raphe and the LC) are the most substantial evidence for these structures being involved in the initiation of air-stepping by L-DOPA. Although there is no direct evidence for projections from A8 or CLi to the LC, there is evidence that the dorsal raphe projects to both of these structures (Paxinos, 1985b), and reciprocal connections are not unlikely. The CLi does have a known projection to the MnR, which in turn has projections to the medullary reticular formation (Paxinos, 1985b). In addition, histology results from the present study have revealed that the ventral-most portions of area A8 and the PPTg are coextensive, and that cells of each of these nuclei could be in contact with each other (figure 10). If so, such a connection could constitute a possible pathway by which dopaminergic cells could influence the output of the MLR. Such influence would be compromised following a postcollicular transection, which would remove many of the terminals, but possibly not all of them. Variable numbers of remaining dopaminergic synapses in the MLR could account for some of the variability seen in the behaviors of the transected subjects.

Other nuclei of the caudal midbrain may be more likely candidates as the sites of action for L-DOPA. The LC (including areas A6, A7, and A4) which has substantial projections throughout the brain and spinal cord, is located just caudal to the level of a postcollicular transection. It is conceivable that this structure, through metabolism of L-DOPA to NA, could exert effects on DA cells of A8 or CLi through collaterals of ascending projections. This action could take place in addition to increased activation of LC projections to the spinal cord, and both activities might be required to produce integrated air-stepping. In this manner, both DA and NA receptor antagonists could conceivably block air-stepping. There is no evidence for NA receptors on dopaminergic cells of area A8 nor in the CLi, but little is known about the neuroanatomy of these areas. It would be possible to rule out this explanation of the actions of L-DOPA through the LC by examining receptor binding characteristics of these two dopaminergic areas.
Figure 6: Illustration of level of postcollicular/postnigral transection in P5 rat. The section is parasagittal, showing the approximate locations of the VTA/SN border, area A8 and the pedunculopontine tegmental nucleus (PPTg).
<table>
<thead>
<tr>
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<th>Sham-Operated Controls</th>
<th>Postcollicular Transected</th>
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<tr>
<td>Durations:</td>
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<tr>
<td>Inactive</td>
<td>9/9 8 1.4</td>
<td>14/14 33 4.9</td>
</tr>
<tr>
<td>Active</td>
<td>9/9 9 1.8</td>
<td>14/14 20 3.9</td>
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<tr>
<td>Air-step</td>
<td>9/9 33 1.7</td>
<td>2/14 0 0.1</td>
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<tr>
<td>Atypical air-step</td>
<td>9/9 9 2.4</td>
<td>6/14 8 3.7</td>
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<tr>
<td>Latency</td>
<td>9/9 8 0.9</td>
<td>2/14 21 2.4</td>
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Table 4: Statistical data for sham-operated and experimental groups. For each of the behaviors listed, the table shows the number of subjects out of the total that demonstrated the behavior, the group mean for each behavior in minutes, and the standard error of the mean. Latency measures were to first episode of typical air-stepping.
Air-Stepping and Atypical Air-Stepping in Post-Collicular Transected Pups

Figure 7: Graph of mean durations of total air-stepping showing proportions of total air-stepping that were typical and atypical for each condition. Group means for total air-stepping are significantly different (p<0.01) for durations of total and typical air-stepping.
Figure 8: Photograph of tyrosine-hydroxylase immunoreactive cells (brown color) located in the ventral tegental area and caudal linear nucleus. Section is sagittal and just lateral to midline. The immunoreactive area is large in comparison to the size of the midbrain, but contains few cells.
Figure 9: Photograph of parasagittal section of the midbrain of a P5 rat, double stained for tyrosine-hydroxylase immunoreactivity (brown color) and NADPH diaphorase (blue color). The location of area A8 is pictured, extending from the caudal pole of the substantia nigra. The cholinergic cells of the pedunculopontine tegmental nucleus can also be seen extending from the caudal edge of the nigra, with some of the cells of these nuclei intermingled.
Figure 10: Higher magnification of tyrosine hydroxylase-immunoreactive dopaminergic cells of area A8 (brown) and NADPH diaphorase stained cholinergic cells of the pedunculopontine tegmental nucleus (blue). The proximity of the cells of these two nuclei are evident, implying that a possible functional relationship may exist between dopaminergic cells of the midbrain and cells that are contained within the MLR.
Figure 11: Parasagittal section of P5 rat (forebrain partially removed) stained for tyrosine hydroxylase immunoreactivity (brown) and NADPH diaphorase staining (blue). Levels of each transection are depicted in relation to the stained structures: (1) precollicular/postmammillary, (2) midcollicular/postnigral, and (3) postcollicular/postnigral.
GENERAL DISCUSSION

The previous studies have employed a lesion approach to grossly localize the site of action for L-DOPA-induced air-stepping in the neonatal rat. Use of a lesion approach, specifically, the use of transections, allowed for assessment of the locomotion-inducing effects of L-DOPA following removal of rostral regions of the brain. Transection through three different levels of the midbrain revealed that the induction of air-stepping by L-DOPA is dependent on the integrity of a structure or structures located caudally to the level of a midcollicular/postnigral transection.

There are some disadvantages to using a lesion design. First, the level of transection by no means gives specific information regarding the structures that are involved in inducing the response. However, once it has been determined which structures must be intact for the response to occur, further studies can be conducted using finer methods of localization, for example central injections, or more selective chemical or electrolytic lesions. Second, transection at any particular level removes all the brain structures rostral to that level, any of which could be directly or indirectly involved in producing the drug-induced behavior. This is an important factor to consider when a transection blocks the expression of behavior. If behavior is blocked by transecting at a certain level, it may be that the reduced preparation is incapable of expressing any behavior due to the effects of shock or loss of some non-specific influence on behavior in general. In the previous studies, several tests were given to the subjects following transection surgery to determine the extent to which the surgery had debilitating effects on general activity. Although fewer than half of the transected animals at any level showed spontaneous surface locomotion, nearly all demonstrated attempts to right and engaged in
brief locomotor activity following a slightly nociceptive stimulus (tail pinch). These animals were not behaviorally suppressed, but instead demonstrated the capacity to react to stimuli by moving their limbs, vocalizing and attempting to right when placed on their backs.

A third concern for interpretation of lesion studies is in regards to fibers of passage. It is possible that the distal fibers of rostral dopaminergic structures, the diencephalic group A11, for example, could remain active following transection. The terminals of such fibers could take up and convert L-DOPA to DA and release it tonically, which could exert locomotor-inducing effects in the spinal cord. However, it would be expected that such terminals would be retained following a spinal cord transection as well, since the axons extend throughout the length of the cord (Commissiong, Galli, & Neff, 1978a; Lindvall & Bjorklund, 1983; Bjorklund & Lindvall, 1984). Following postcollicular (experiment 3) or midthoracic transection (Iwahara, Van Hartesveldt, Garcia-Rill, & Skinner, 1991b; Stehouwer & Van Hartesveldt, 1991), L-DOPA does not induce air-stepping. In either case, the terminals of the diencephalic DA groups are present in the spinal cord, and may even remain functional following transection, but do not appear to be the sole site of action for L-DOPA-induced air-stepping.

Previous lesion studies have determined that L-DOPA does not induce air-stepping in the hind limbs of the spinally-transected neonatal rat (Iwahara, Van Hartesveldt, Garcia-Rill, & Skinner, 1991b; Stehouwer & Van Hartesveldt, 1991) which implies that L-DOPA does not act at the level of the spinal cord to induce locomotion in this paradigm. However, it is possible that L-DOPA does have its primary site of action at the level of the spinal cord, but requires some additional descending influence from the area of the caudal midbrain that is disrupted following midthoracic or postcollicular transection. This influence may be through a tonically active descending projection, or the influence could itself be activated or potentiated by the peripheral injection of L-DOPA. These hypotheses could be tested with several experiments. The first question to address may
be; does L-DOPA have its site of action in the spinal cord, but also require a non-specific descending influence from the brainstem in order to produce air-stepping? If L-DOPA were administered intrathecally at the lumbar level in intact subjects, then tonic descending influences would be intact, while L-DOPA effects would be restricted to the lower cord. The production of air-stepping in this preparation would indicate that some tonic descending influence is required for the production of air-stepping, and that this influence was removed by the midthoracic and postcollicular transections. In addition, it could be inferred from this result that L-DOPA does not affect the descending influence at more rostral regions, since its effects would be restricted to the spinal cord in this preparation.

More specific information regarding the nature of such a tonic descending influence could be obtained from combining electrical stimulation of discrete areas of the brainstem with intrathecal administration of L-DOPA. Recent evidence suggests that there may be a serotonergic component to the production of L-DOPA-induced air-stepping (Stehouwer, personal communication). All the raphe nuclei except MnR project to the spinal cord. Electrical stimulation of any of these nuclei in combination with intrathecal L-DOPA administration could elicit air-stepping. Alternatively, serotonin could be given intrathecally following transection at the midthoracic level, and L-DOPA injected systemically. Production of air-stepping in either of these preparations could indicate that the serotonergic projections to the spinal cord are important for the production of air-stepping. If the electrical stimulation studies indicate that a particular serotonergic nucleus is required for the production of air-stepping following intrathecal L-DOPA, it would then be possible to destroy the site selectively. In an animal with this lesion, L-DOPA should not induce air-stepping following peripheral or intrathecal administration.

If air-stepping did not occur in an intact animal given L-DOPA intrathecally, it would lend support to the hypothesis that the site of action for the drug lies somewhat more anteriorly, or that sites of action exist in both the spinal cord and the brainstem. The
The next question to address would then be; must L-DOPA act in both the brainstem and the spinal cord simultaneously to produce air-stepping, or is the site of action exclusively located rostral to the spinal cord? To address this question, the most expedient method would be central injection of L-DOPA into the brainstem. Because of the results of experiments 2 and 3, the most likely region in which to inject the drug would be the caudal midbrain. In addition, decerebration of the subjects at the precollicular/postmammillary level would ensure that no forebrain influences would affect the behavior. This approach would limit the actions of L-DOPA to the brainstem while preventing direct effects of L-DOPA on the spinal cord. If air-stepping were induced in this preparation, it would provide evidence that the site of action for this behavior lies solely within the midbrain.

However, when L-DOPA is applied to the brainstem bath of the in vitro brainstem-spinal cord preparation, it does not induce air-stepping (Garcia-Rill, personal communication). This indicates that air-stepping would be an unlikely result of midbrain injection of L-DOPA into intact animals. In addition, certain technical difficulties might hinder the successful completion of such an experiment. L-DOPA is difficult to get into solution at a pH that is acceptable for use in central injections, especially at higher doses. Furthermore, the total rostro-caudal extent of the midbrain in the P5 pup is approximately 4 mm. In such a small region it would be difficult to give a discrete injection that would affect only certain structures. It could be that the pharmacological activity of L-DOPA in one area of the midbrain could be cancelled out by an opposite action in another area. In this case, the absence of air-stepping following L-DOPA injection would not provide conclusive information as to the site of action for L-DOPA.

It is important to understand where the sites of action for L-DOPA lie in the neonatal rat, but it is also important to know how L-DOPA-induced air-stepping relates to other forms of experimentally-induced and spontaneous locomotion such as treadmill stepping. Comparisons of the results of studies using the air-stepping paradigm and of those using treadmill stepping are difficult for a number of reasons. Studies of treadmill
stepping have used adult rats, while air-stepping studies use preweanling rats. Treadmill stepping studies frequently have employed electrical stimulation, while similar studies have not yet been conducted using an air-stepping paradigm. In addition, numerous treadmill studies have examined the chronic effects of transections on locomotion, while air-stepping studies have used acute preparations. Further experiments must be done in order to make conclusive statements regarding the similarities or differences in these two paradigms. The treadmill paradigm could be applied to L-DOPA-induced activity in the neonate. One example of such an experiment would be to test whether subjects with a midthoracic or postcollicular transection would engage in stepping if they were suspended over a treadmill. This condition would be more analogous to that of the spinal cat or adult rat, both of which exhibit stepping following decerebration or transection of the spinal cord if injected with L-DOPA and suspended over a treadmill (Anden et al. 1964).

Treadmill stepping might provide afferent input that could act in addition to the spinal effects of L-DOPA to induce air-stepping in the transected neonate.

Conversely, the air-stepping paradigm could be used to test the methods of eliciting treadmill locomotion. Electrical and chemical stimulation of various sites in the midbrain or brainstem may also be found to induce air-stepping. It would be of interest to examine whether the behavior induced by stimulating the MLR or medioventral medulla (regions in which various chemicals or electrical stimulation can induce treadmill stepping), was similar in topography to that of L-DOPA-induced air-stepping.

Whether the locomotion-inducing effects of L-DOPA are tested on the ground or in the air, this drug appears to have some unique qualities in comparison to other catecholaminergic agonists. The effects of L-DOPA may not be completely due to its conversion to catecholamines. Recent evidence has accumulated that L-DOPA may be released endogenously and act as a neurotransmitter itself, to stereoselectively potentiate the effect of beta adrenergic receptor activation (Goshima, Nakamura, Ohno, & Misu, 1991; Nakamura et al. 1994; Yue, Nakamura, Ueda, & Misu, 1994). Transmitter-like
endogenous release has been demonstrated from striatal tissue, with actions possibly mediated through a receptor site selective for L-DOPA (Nakamura, Goshima, Yue, Miyamae, & Misu, 1992; Misu & Goshima, 1993). Numerous pilot studies conducted in this laboratory have attempted to recreate the stereotyped activity seen during L-DOPA-induced air-stepping by administration of various NA or DA receptor agonists. However, none of the agonists, either alone or combination with other agonists, was able to reproduce the highly stereotyped and long-lasting effects induced by L-DOPA. Therefore, L-DOPA may produce its unique behavioral effects by having pharmacological actions other than or in addition to the increase of DA and NA activity.

L-DOPA may have effects on systems other than the catecholamines. Peripherally administered L-DOPA is actively transported across the blood-brain barrier by a common pathway with aromatic amino acids. This uptake is competitive, and some evidence exists for this transporter mechanism not only being functional, but even more active in neonatal rats than in adults (Guroff & Udenfriend, 1964). High plasma levels of L-DOPA may competitively interfere with the uptake of other amino acids, thereby limiting the available substrates needed to synthesize other neurotransmitters. Although effects of L-DOPA uptake were not specifically measured, Guroff and Udenfriend (1964) found that injection of phenylalanine lowered the amount of brain serotonin produced from an injected dose of 5-hydroxytryptophan, the precursor for 5-HT. L-DOPA could have similar effects to that of phenylalanine, and subsequently lower the amount of available substrate for 5-HT formation. An additional means by which L-DOPA could have effects on the 5-HT system is through uptake into serotonergic neurons. The enzyme 5-hydroxytryptophan decarboxylase is the same structurally to to dopa decarboxylase, the enzyme responsible for the formation of DA from L-DOPA, and could act on L-DOPA (Carlsson, 1970). In this way, high brain concentrations of L-DOPA could decrease serotonergic activity by reacting with the available enzyme for synthesis of 5-HT, in addition to decreasing the amount of amino acid precursor available (Stromberg & Svensson, 1970). These subtle
effects on the 5-HT system may be an important part of the means by which L-DOPA induces air-stepping.

L-DOPA clearly has unique effects on the neonatal nervous system. Exactly how those effects are produced remains unknown. However, the results of the foregoing experiments demonstrate that the integrity of the caudal brainstem is crucial to the production of L-DOPA-induced air-stepping. In addition, these studies provide the rationale and direction for further research regarding the localization of the site of action for L-DOPA in the neonatal rat.
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BIOGRAPHICAL SKETCH

Anne Elizabeth McCrea was born in Philadelphia, Pennsylvania, on February 23, 1961. She graduated from Wilson Area High School in Easton, Pennsylvania, in 1979. She completed her undergraduate work at the Pennsylvania State University, University Park, Pennsylvania, in August 1983, when she was awarded her B.S. in psychology with distinction. Following graduation, she obtained a position as a Behavior Modification Programmer for the Psychology Department of the Woodbine Developmental Center, in Woodbine New Jersey, where she worked with developmentally disabled institutionalized clients. She began her graduate research work at the University of Florida in 1988 with Dr. Carol Van Hartesveldt, in the area of developmental psychobiology. Upon receiving her doctoral degree in August 1994, she plans to continue research as a postdoctoral research fellow in developmental teratology with Dr. Rose Booze at the University of Kentucky in Lexington.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of a scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Carol Van Hartesveldt, Chairman
Professor of Psychology

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Neil E. Rowland
Professor of Psychology

Marc N. Branch
Professor of Psychology
I certify that I have read this study and that in my opinion it conforms to acceptable standards of a scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Roger L. Reep
Associate Professor of Veterinary Medicine

This dissertation was submitted to the Graduate Faculty of the Department of Psychology in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1994

Dean, Graduate School