Shapiro, Raymond Marc

NIGRO-STRIATAL ASYMMETRY AND ROTATIONAL BEHAVIOR IN RATS: NEUROCHEMICAL, ANATOMICAL, AND FUNCTIONAL EVIDENCE FOR A TWO POPULATION MODEL

City University of New York PH.D. 1986

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy ✓
5. Pages with black marks, not original copy ✓
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements ✓
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received ✓
16. Other _____

_____

_____

University
Microfilms
International

Nigro-Striatal Asymmetry and Rotational Behavior in Rats: Neurochemical, Anatomical, and Functional Evidence for a Two Population Model

Raymond M. Shapiro

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

1986

This manuscript has been read and accepted for the Graduate Faculty in the Biomedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

January 13, 1986
date

Chairman of Examining Committee
Stanley D. Glick, Ph.D., M.D.

January 16, 1986
date

Executive Officer
Terry A. Krulwich, Ph.D.

Lindsay B. Hough, Ph.D.

Joseph Goldfarb, Ph.D.

Peter J. Knott, Ph.D.

Richard E. Heikkila, Ph.D.

Supervisory Committee

The City University of New York

Abstract

NIGRO-STRIATAL ASYMMETRY AND ROTATIONAL BEHAVIOR IN RATS: NEUROCHEMICAL, ANATOMICAL, AND FUNCTIONAL EVIDENCE FOR A TWO POPULATION MODEL

by

Raymond M. Shapiro

Adviser: Professor Stanley D. Glick

The role of the dopaminergic nigro-striatal pathways in turning, or rotational, behavior in rats has been studied since the mid-1960s when it became clear that dysfunction in this system was related to the clinical manifestations of Parkinson's Disease in humans. Since then, turning behavior has been considered to be the result of asymmetric dopaminergic activity in the nigro-striatal pathways of the rat: The currently accepted model for turning considers the side away from which an animal turns (i.e., the contralateral side) to be the side containing the striatum with the higher dopamine concentration and the greater post-synaptic dopaminergic receptor activity. This model developed primarily from work that was done using rats with 6-hydroxydopamine-induced unilateral striatal dopamine depletions, but also from work with unlesioned animals as well.

In this thesis, the combined use of biochemical, anatomical, and behavioral techniques resulted in the development of a somewhat more complex model than the one that is currently accepted. Using the Vmax for dopamine uptake in vitro as a

measure of dopaminergic nerve terminal density in the two striata, two groups of rats were identified on the basis of their spontaneous nocturnal rotational behavior. One group circled predominantly away from the side containing the striatum with the greater dopaminergic innervation, and the other circled predominantly towards the side containing the striatum with the greater dopaminergic innervation; but for both groups, the amount of rotational behavior was correlated with the size of the innervation asymmetry. While the current model for rotational behavior might have predicted this correlation for the group that rotated away from the side with the greater dopaminergic innervation, there is at present no model that would have predicted the second correlation; thus, this second correlation was regarded as evidence implying the existence of another "kind," or population, of rat.

Subsequent experiments were designed to test the validity of the proposed two population model. Neurochemical, anatomical, and functional differences were found between the striata of the two proposed populations of rats. For example, bilateral striatal dopamine turnover, as reflected by the ratios of dopamine metabolites to dopamine, was greater in the population that circles towards the side containing the striatum with the higher dopamine concentration. In addition, the difference between the contralateral and ipsilateral striatal serotonin concentrations is smaller in this group of rats. Evidence

for anatomical differences between the two populations came from measurements of dissected striatal tissue weights, and from tracings of enlarged projections of coronal cross-sections through the striatum. Additional support for the proposed two population model came from the results of a study examining the behavioral effects of partial unilateral 6OHDA-induced striatal dopamine depletions on the side towards which the rats normally turned. Although the neurochemical effects of the lesions were virtually identical in the two groups, two groups of responders were clearly identified: In one group, turning toward the lesioned side was increased compared to controls, and in the other group turning toward the lesioned aide was unchanged or was actually decreased.

The data are discussed in terms of their relevance to the role of striatal dopamine in turning behavior, and in terms of their relevance to Parkinson's Disease, in which two sub-groups of responders to L-Dopa therapy have been identified.

ACKNOWLEDGEMENTS

This dissertation is dedicated to Jayne Freedman Shapiro, my wife, to whom words do not do justice.

There are many others to whom I am indebted. It is a pleasure to express my heartfelt gratitude to:

Dr. Terry Krulwich and the Medical Scientist Training Program, for giving me the opportunity to attend graduate school. Dr. Krulwich and Mrs. Senta Frank displayed considerable flexibility and thoughtfulness in helping me deal with numerous practical matters that arose during my graduate training.

My family, for their relentless love and support through various and unpredictable ups and downs.

Ed Gaffney, Aaron Kutz, Bunny Levere, and Dr. Steve Keller for expressing their faith in me, particularly at times when I did not have very much faith in myself.

Kelly Drew, Dan Togasaki, and Bob Lyon, fellow students, for fruitful discussions, and for helping to maintain a friendly, supportive working environment.

Pat Hinds, Ron Guido, Nancy Camarota (M.V.H.P.L.C., 1984/5), and Kay Su for innumerable contributions that made my work easier than it otherwise would have been.

Drs. Jerry Hruska and Mary Notter, and Drs. Steve Keller, Steve Schleifer, and Marvin Stein, for stimulating my interest in basic research.

The members of my Supervisory Committee, and Dr. Jeff Carlson, for their interest in my work, and for their interest in me. The countless insights of Drs. Lindsay Hough and Joe Goldfarb over the past three years were especially appreciated.

Dr. Stan Glick, patient teacher, wise mentor, and accepting friend, for transforming an amorphous jumble of misdirected energy into a young scientist. "If you wanted the sky, I would write across the sky . . . ."

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CNS	central nervous system
Contra	contralateral
CSF	cerebrospinal fluid
DA	dopamine
DOPAC	dihydroxyphenylacetic acid
5HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptamine (serotonin)
HVA	homovanillic acid
Ipsi	ipsilateral
LSD	D-lysergic acid diethylamide
NE	norepinephrine
6OHDA	6-hydroxydopamine
prot	protein

CHAPTER ONE

INTRODUCTION

> It has become clear that within the last 15 years a new field has developed, that is, the study of the biological foundations of cerebral dominance. It is now obvious that cerebral asymmetry is of major importance in every branch of biological science and in every branch of medicine. The field has the potential for revolutionary new approaches to many aspects of biology in respect to normal function, superior talents, and disease.
>
> The development of animal models of asymmetry . . . should lead to novel approaches for understanding the nature of the human mind. I believe that we are on the threshold of what can be described without hyperbole as a major revolution in biology. (Geschwind, 1985, pg 276)

These are the bold words of the eminent neurologist, Norman Geschwind* to whom much of the credit will be given if such a revolution does indeed take place. Geschwind's faith in the importance of the study of cerebral asymmetry was based to a large degree on recent advances in the study of non-human species, and much of that work will be reviewed below. First, though, it is appropriate to consider some of the evidence for cerebral asymmetry in humans, particularly since the study of hemispheric differences in humans is so much older than it is in animals.

Cerebral Asymmetry in Humans.

Scientific interest in cerebral asymmetry began with the clinical observation of the strong association between right sided hemiparesis and the loss of the ability to speak (expressive, or Broca's aphasia). Whether it was Dax (1836, cited in Kolb and Whishaw, 1980) or Broca in the 1860s (e.g., Broca, 1865, cited in Eling, 1984) who first drew public attention to this association is somewhat controversial, but it is clear that widespread interest in the phenomenon did

*Dr. Geschwind died suddenly, on November 4, 1984.

not develop until Broca's autopsy findings were reported: in every one of his original series of eight cases, the same region of the left cerebral hemisphere -- corresponding to roughly two-thirds of the inferior frontal gyrus -- was affected.

Even though Broca specifically cautioned otherwise (see Eling, 1984), after his report he was generally credited with advancing the widely accepted notion that the same hemisphere was "dominant" for the control of both language and handedness; thus, it was left for others (e.g., Subirana, 1958; Luria, 1970) to subsequently "prove" him wrong, and show that the left hemisphere is "dominant" for speech in most left handers as well.

After Broca's findings were reported Wernicke described another type of aphasia -- now known as a receptive or Wernicke's aphasia -- which was caused by damage to the posterior peri-Sylvian region of the left hemisphere (Wernicke, 1874, cited in RG Robinson, 1985). Thus, from this and other work it became clear that damage to the left cerebral hemisphere was much more likely to impair language abilities than was comparable damage to the right hemisphere. But if the left hemisphere was specialized for language what was the right hemisphere specialized for? One possibility advocated by some in the earlier part of the 20th century was that the right, or "minor," hemisphere was essentially dormant and that its functions were controlled by the "dominant" left hemisphere. By the 1950s, however, from the

results of work done on patients with brain damage localized to the right hemisphere, it became accepted that the right hemisphere, too, had its own "special" skills, for example, involving spatial relations and musical talent. Later, in the 1960s, Sperry confirmed and extended these observations in his work with patients who had undergone split-brain surgery for the treatment of intractable epileptic seizures. In addition, Sperry's results indicated that the right hemisphere, despite its paucity of verbal capacities compared to the left hemisphere, was endowed with a consciousness and will of its own (e.g., Sperry et al., 1969; Damasio, 1982).

Despite the above observations it was widely assumed that there was no anatomical basis for functional differences between the two hemispheres. Even though there had in fact been sporadic reports of a number of anatomical asymmetries they were apparently ignored (Galaburda, 1984). Concentrated interest in anatomical asymmetry did not develop until Geschwind and Levitsky's (1968) report that a region typically involved in Wernicke's aphasia was usually larger on the left than on the right, a finding that has since been replicated in numerous laboratories (e.g., Wada et al., 1975). Interestingly, it is in this region that choline acetyltransferase activity has been found to be greater on the left than on the right in adults (Amaducci et al., 1981), although this asymmetry is reversed in the fetus (Bracco et al., 1984). Other anatomical asymmetries in the human brain have also been described, for example, in Broca's area (Falzi et al., 1982) and in the course and extent of the Sylvian

fissure (LeMay, 1984).

The investigation of differences between left and right handed individuals has been a major focus of research for investigators interested in cerebral asymmetry (Herron, 1980). While it is still debated whether or not left handedness reflects the results of early brain damage and is therefore in some way pathological (Bishop, 1983; Bradshaw-McAnulty, et al., 1984; Satz et al., 1985), there is no doubt that left handed and ambidextrous individuals are different from right handers. Anatomically, for example, it has recently been shown that the corpus callosum of left and mixed handers is 11% thicker than in right handers, a size difference that "could represent as many as 25 million fibers" (Witelson, 1985, pg 665). This finding may indicate that there is more extensive inter-hemispheric communication in these individuals than in the right handed majority. If this turns out to be true then it could provide an anatomical basis for the repeated observation that left handers as a group show less hemispheric superiority on a variety of neuropsychological tasks (Bryden, 1982). Witelson's results might also account for the clinical observation that strokes tend to be less debilitating in left handers.

One of the last studies Geschwind was involved with resulted in the observation that left handers and their families had higher rates of learning disorders, immune disorders, and migraine than right handers and their families (Geschwind and Behan, 1982). In addition, left and right

handers have recently been reported to differ in their EEG responses to a variety of drugs (Irwin, 1985), a finding Geschwind surely would have taken some satisfaction in since he had been "impressed with the very high rate of anomalous drug reactions among left handers and their families" (Geschwind, 1985, pg 265). The possibility that cerebral asymmetry may play a role in the effects of psycho-pharmacological agents (Frumkin and Grim, 1981) calls to mind the work of Serafetinides, who found that most of the perceptual phenomena associated with injections of LSD-25 were altered by right but not by left temporal lobectomy in patients who had received the drug pre- and post-operatively (Serafetinides, 1965). The discovery that neurochemical asymmetry exists in the human brain, and that some neurochemical asymmetries are probably related to handedness (Glick et al., 1982), provides hope that the neurobiological mechanisms responsible for these curious but unexplained findings will some day be understood.

Specialization/Dominance vs. Asymmetry/Lateralization.

Because the study of hemispheric inequalities began with the work involving speech, and because speech is such a clear cut example of cerebral specialization, most of the thinking about, and research in, this field has been colored by what is known about the neural control of language processes. As a result the terms "cerebral specialization" and "cerebral dominance," which are essentially equivalent, can easily be confused with the more general terms "cerebral asymmetry" and

"cerebral lateralization." Since the capacity for language is among the highest neural processes to have evolved (arguably the highest), compared to other processes under CNS control its specialized physiology may well turn out to be more the exception than the rule. Thus, the search for analogous circumscribed regions of the left or right hemisphere that are clearly responsible for the control of some specified function is likely to result in failure. Nevertheless, many investigators are continuing the search, mostly in humans but also in animals; and as a result, for these workers the terms "cerebral specialization," "cerebral dominance," "cerebral lateralization," and "cerebral asymmetry" are all roughly equivalent. But the results of other types of experiments clearly indicate that the study of cerebral asymmetries and asymmetric cerebral processes involves more than the identification of loci in the two hemispheres which, when treated (e.g., lesioned), result in unequal behavioral effects. For example, Direnfeld et al. (1984) have identified differences between the CSF neurochemistry of left and right sided unilateral Parkinson's Disease patients. And Friedland et al. (1985), using positron emission studies with [^{18}F]fluorodeoxyglucose to measure glucose metabolism, have found that patients with (presumed) Alzheimer's disease have larger cortical asymmetries than controls, with the asymmetries favoring neither hemisphere. Furthermore, in rats, Crespi and Jouvet (1984) have found that injections of 5-hydroxytryptophan (5HTP) or serotonin (5HT) into the dorsal raphe on the left

side reduce 5-hydroxyindolacetic acid (5HIAA) release in both the left and the right striatum, while comparable injections on the right side elicit increased 5HIAA release in both striata. Such phenomena cannot be discussed in terms of cerebral specialization or in terms of cerebral dominance, but clearly they are relevant to the field of cerebral asymmetry. Indeed, my own feeling is that the issue of hemispheric "specialties" may turn out to be among the least interesting of the questions yet to be resolved about the two cerebral hemispheres and their interrelationships.

Cerebral Asymmetry in Animals.

From brain stem to neocortex, anatomical and functional differences between the left and right cerebral hemispheres have been documented in a wide variety of species. For example, the left habenula is larger than the right habenula in fish, amphibia, and lizards, as well as in moles (Kemali, 1984). Asymmetries in the rat hippocampus (Diamond et al., 1982) and neocortex (Diamond, 1984; Kolb et al., 1982; Sherman and Galaburda, 1984) have also been reported: In general, the left neocortex is thicker in females and the right neocortex is thicker in males. At present, the basis for these asymmetries is unknown -- e.g., whether they are due to side differences in neuron size, neuron number, glia, etc. To date the only documented hemispheric asymmetry in an identified cell type is a left>right mast cell asymmetry in the rat thalamus (Goldschmidt et al., 1984).

The functional significance of these anatomical

asymmetries remains obscure. Only in primates can we even venture a guess, since in primates, when anatomical asymmetries have been found they usually resemble asymmetries in humans that have been found in language associated regions (Sherman et al., 1982); thus, these regions may serve language-type functions in primates. Such a notion is not as unlikely as it may appear at first since two groups have now reported the experimentally induced impairment of communication skills after left, but not right, temporal lobe lesions (Dewson, 1977; Heffner and Heffner, 1984).

In rats many neurochemical asymmetries have been found (TE Robinson et al., 1985). For example, Oke et al. (1980) have found NE asymmetries in the rat thalamus, which is notable because they also found thalamic NE asymmetries in the human brain (Oke et al., 1978). Starr and Kilpatrick (1981) did an extensive investigation of regional GABA concentrations and turnover rates. They found no significant side differences in GABA levels, but left>right turnover asymmetries were present in striatum, ventromedial thalamus, and ventral tegmentum; right>left turnover asymmetries were present in substantia nigra, superior colliculus, and nucleus accumbens. Using a modification of Sokoloff's 2-deoxyglucose incorporation technique Ross et al. (1981, 1982) have documented metabolic asymmetries in virtually all regions of the rat brain, and these asymmetries were shown to change with age from infancy on to adulthood. In addition, there is now good evidence for the existence of a sex difference in a

striatal D2 binding site asymmetry: Males have more binding on the left (Schneider et al., 1982; Drew et al., in press) while females have more binding on the right (Drew et al., in press). Most recently, left>right free fatty acid content asymmetries have been documented in the brains of mice (Pediconi and Rodriguez de Turco, 1984) and rats (Ginobili de Martinez et al., 1985).

As with the anatomical asymmetries that have been detected, essentially nothing is known about the functional importance of the above left vs. right neurochemical asymmetries in the rat brain. On the other hand, something is known about what can alter some left/right asymmetries. For example, as noted above regional asymmetries in 2-deoxyglucose incorporation change with age; in addition, amphetamine has been shown to reverse the normal left>right 2-deoxyglucose incorporation asymmetries in frontal cortex and in hippocampus (Glick et al., 1979). Thus, while it may not be clear what the functions of the normal frontal cortex and hippocampus asymmetries are, these results suggest that amphetamine's neuropsychological effects are, at least in part, caused by its actions on these asymmetries. In addition, the hemispheric asymmetries in free fatty acid content noted above can also be altered. Bilaterally administered electroconvulsive shock increases free fatty acids on both sides, but more so on the right than on the left; and right hemispheric levels return to normal faster than do left hemispheric levels. Whether the endogenous asymmetry itself, or the differential hemispheric

responsiveness to electroconvulsive shock are related to the pathogenesis and/or treatment of seizure disorders or of affective disorders is an exciting possibility. Primary involvement of right hemispheric dysfunction in depression has long been speculated about, on the basis of (i) the differential effects on mood of unilateral intra-carotid barbiturate injections (Terzian, 1967) and lesions (Sackeim et al., 1982), although this issue remains controversial (Gainotti, 1983); (ii) the approximately equal efficacy of ECT administered over the right hemisphere alone, compared to bilateral ECT (Abrams and Fink, 1984; Gregory, et al., 1985); and (iii) the sporadic reports of depression-associated neurological symptoms on the left side of the body that cleared with successful treatment (Cutler et al., 1981; Freeman et al., 1985).

Additional evidence exists for functional asymmetry in the non-human animal brain: In an extensive series of studies RG Robinson and colleagues have demonstrated that locomotor activity is increased after lesioning the right, but not the left, neocortex with middle cerebral artery occlusions (Robinson and Coyle, 1980), with 6OHDA (Robinson and Stitt, 1981), with kainic acid (Kubos et al., 1982), with cortical undercuts (Kubos and Robinson, 1984a), by making surgical circumscriptions or "islands" (Kubos and Robinson, 1984b), and with remarkably small (1 mm^2) cortical suctions (Pearlson et al., 1984; Moran et al., 1984a); but not with the serotonergic neurotoxin 5,7-dihydroxytryptamine (Black and

Robinson, 1985). With the exception of the cortical circumscription lesions, which decrease it, none of these lesions, when made on the left side, have a significant effect on motor activity. The mechanism underlying this functional asymmetry is not clear; but since the lesion's behavioral effect is related to its anterior-posterior coordinate (Pearlson et al., 1984) as well as its size (Moran et al., 1984b) it has been proposed that a critical role is played by the noradrenergic projection from the locus coeruleus to the neocortex, which is known to course posteriorly from the anterior pole of the frontal cortex. The fact that the anterior-posterior location of the lesion is a determining factor in its behavioral effect is interesting because it is consistent with a similar observation in humans of the relationship between anterior-posterior location of a stroke and the subsequent development of behavioral sequelae (RG Robinson et al., 1985).

The results of experiments by Denenberg and colleagues also suggest functional differences between the left and right hemispheres; but these results are not entirely consistent with those of RG Robinson. While Denenberg et al. (1979) have found that right neocortical lesions increase motor activity more than left neocortical lesions do, they have found this to be the case only for those rats subjected to brief (3 min) daily isolation periods as pups prior to weaning; rats that were not disturbed as pups also increased their motor activity following unilateral lesioning, but there was no difference between the effects of left and right

hemispheric lesions. These results raise the interesting possibility that the housing conditions at the breeding farm from which RG Robinson obtains his rats may be less than serene. In any event, in another experiment by Denenberg's group Garbanati et al. (1983) showed that large left, but not right, neocortical lesions increase muricide rates, but, again, only among the rats that had experienced the daily isolation periods as pups. These and other results have been interpreted as indicating that early experience can induce, or increase, functional lateralization in the rat brain (Denenberg, 1984).

Behavior is not the only function that is differentially sensitive to the effects of left and right neocortical lesions. Over the past decade interest has grown rapidly in the functional interrelationship between the immune and central nervous systems (Besedovsky et al., 1977; Guillemin, 1985; Blalock and Smith, 1985). Adding to the complexity of this already complicated field, Renoux et al. (1983, 1984) have now demonstrated in mice that large lesions of the left and right cortex have remarkably different effects on measures of immunocompetence: Left sided lesions impair T-cell-dependent responses while right sided lesions enhance them. By contrast, B-cell and macrophage responses are not affected by left or right cortical lesions, which is consistent with the results of others indicating that B-cells and macrophages are much less sensitive to neural modulation than are T-cells (Schleifer and Shapiro, in press).

Non-cortical structures also appear to be functionally asymmetric. In the rat hippocampus the threshold current required to support self-stimulation is lower on the left than on the right; and LSD administered systemically preferentially affects self-stimulation on the left, while phencyclidine preferentially affects self-stimulation on the right (Glick, 1983). [It should be noted that LSD's greater effect on self-stimulation in the left hippocampus is in apparent conflict with the observations of Serafetinides in humans (pg 6).] In the hypothalamus Nordeen and Yahr (1982) have shown that estradiol pellets implanted in female newborns on the left (in the preoptic area) result in defeminized behavior, while right-sided implants (in the ventromedial nucleus) result in masculinized behavior. Whether this result is related to the right>left leutinizing hormone releasing factor (LHRF) asymmetry that has been demonstrated in females (Gerendai et al., 1978) is, of course, not known. Regardless, while bilateral ovariectomy had previously been shown to reduce hypothalamic LHRF content when the left and right sides are pooled (Wheaton and McCann, 1976), Gerendai (1984) has now reported this reduction to be due entirely to the decrease on the right side; left hypothalamic LHRF content is unchanged. Additional evidence for functional asymmetry in the rat hypothalamus comes from Gerendai's comparison of blood prolactin levels in male rats following unilateral mastectomy on the left and right sides. Whereas left mastectomy decreases prolactin levels right mastectomy increases prolactin levels (Gerendai, 1984).

Lastly, caudal hypothalamic lesions on the left, but not the right, have been shown to decrease mitotic activity in both lobes of the thyroid gland (Lewinski et al., 1982).

Rotational Behavior.

Widespread interest in rotational behavior -- also known as circling or turning behavior -- developed in the late 1960s as the result of an effort to develop an animal model for Parkinson's Disease. The history of this disease is well known. The clinical syndrome, which is characterized by resting tremor, bradykinesia, "cogwheel" rigidity, and a distinctive, stooped gait, was originally described by the English neurologist, James Parkinson (1817). A century later, Lewy described characteristic, eosinophilic inclusion bodies in the pigmented cells of the substantia nigra and locus coeruleus of patients who had died with the disease. Further evidence for the involvement of the substantia nigra in the pathogenesis of parkinsonism came from the surge in autopsy material that became available after the global epidemic of post-encephalitic parkinsonism that followed World War I. The critical role of DA in Parkinson's Disease was established when it became clear that (i) the DA precursor, L-Dopa, provides effective symptomatic relief (Birkmayer and Hornykiewicz, 1961, cited in Schulz, 1980), (ii) the nigro-striatal projection is largely dopaminergic (Anden et al., 1964), and (iii) the parkinsonian brain is DA deficient (Hornykiewicz, 1966).

With the development of 6OHDA, a neurotoxin relatively

selective for catecholaminergic neurons (e.g., Jonsson, 1976) it became possible to attempt to model in animals what was considered to be the essential neuropathology of Parkinson's Disease, that is, the loss of DAergic neurons in the substantia nigra. The logic in this line of thought was proved sound when it was shown that bilateral intra-nigral injections of 6OHDA did indeed produce some of the predicted effects: biochemically, forebrain DA concentrations were depleted, and behaviorally, treated animals became hypokinetic, although no tremor or rigidity were detected (e.g., Ungerstedt, 1971a). Unfortunately, these animals also became aphagic and adipsic , and were therefore difficult to keep alive. Nevertheless, these initial results were encouraging since at least one major symptom of the clinical syndrome, retarded movement, had been successfully modeled. Later it was found that unilateral 6OHDA-induced striatal DA depletions did not result in as severe aphagia and adipsia, so animals treated in this way could be maintained without force feeding them. Such animals were reported to rotate, or circle, (i) towards the lesioned side in response to the systemic administration of amphetamine (Ungerstedt, 1971b); and (ii) away from the lesioned side in response to apomorphine or L-Dopa (Ungerstedt, 1971c). Amphetamine's effects were attributed to its releasing DA in the "intact" striatum while the effects of apomorphine and L-Dopa were considered to be due to their acting predominantly in the striatum on the 6OHDA-treated side, on super-sensitive post-

synaptic DA receptors.

Since its introduction the model and numerous variations of it (e.g., Giambalvo and Snodgrass, 1978) have been employed in countless studies of the pharmacology and physiology of the basal ganglia (Pycock, 1980; Schultz, 1982). Nevertheless, numerous groups have reported data that are not entirely consistent with the model's predictions. For example, Tye et al. (1977) and Hodge and Butcher (1979) have presented convincing evidence that destruction of nigro-striatal projections alone is not sufficient for post-synaptic agonists to subsequently elicit circling away from the lesioned side. Tye et al. (1977) also showed that DA antagonists do not necessarily block apomorphine-induced circling: clozapine, for example, actually enhanced it. In addition, Gardner et al. (1980) have reported that apomorphine elicits rotation either towards or away from the lesioned side depending on which of two medial-lateral coordinates are employed for the intra-nigral 6OHDA injections; this, despite the fact that there is no difference between the extent of the induced DA depletions. And Heikkila et al. (1981) have clearly demonstrated that there is no close relationship between the extent of a 6OHDA lesion and the amount of amphetamine-induced turning toward the lesioned side. Despite these inconsistencies, as well as others (e.g., Cools, 1977; Vaccarino and Franklin, 1982a, b), it would probably be difficult to overestimate the acceptance of the unilaterally lesioned rotating rodent in modern neurobiology, not only as a tool for the screening of DA

drugs (e.g., Arnt and Hyttel, 1984), but also as a system from which conclusions may be drawn about the normal workings of the basal ganglia in intact animals (e.g., Starr and Summerhayes, 1985).

Although it was initially assumed that normal rats would not turn in circles, it was subsequently demonstrated that naive, unlesioned rats have drug-induced turning preferences that are consistent in direction and correlated in magnitude when rats are retested with the same dose of the same drug (e.g., Jerussi and Glick, 1976; Fleisher and Glick, 1979; Glick et al., 1983). Rotational behavior in unlesioned rats has been elicited by amphetamine (Jerussi and Glick, 1974, 1976; Thiebot and Soubrie, 1979; Becker et al., 1982; TE Robinson et al., 1980; Hyde and Jerussi, 1983), apomorphine (Jerussi and Glick, 1975; Hyde and Jerussi, 1983), scopolamine (Fleisher and Glick, 1979), L-Dopa (Jerussi and Glick, 1976), phencyclidine (Glick et al., 1980), morphine (Glick and Morihisa, 1976), and cocaine (Glick et al., 1983). It has also been shown that rats rotate spontaneously at night, during the active portion of their circadian cycle (Glick and Cox, 1978), and the direction of this circling is, for the vast majority of rats tested, in the same direction as that elicited by amphetamine during the day.

Because the direction of turning behavior is generally stable the two cerebral hemispheres can be defined on the basis of a rat's directional bias. Throughout this and the following chapters the side towards which a rat makes most of

its turns, either spontaneously at night or in response to amphetamine, will be referred to as the "ipsilateral" side, and the side away from which a rat makes most of its turns will be referred to as the "contralateral" side. Thus, the "ipsilateral" and "contralateral" cerebral hemispheres are, respectively, on the sides towards and away from which a rat rotates. The equally descriptive, but less neutral terms, "dominant hemisphere" and "non-dominant hemisphere," will be avoided.

As is the case with lesioned rats the role of striatal DA in the turning behavior of unlesioned rats is also incompletely understood. Zimmerberg et al. (1974), using a T-maze, and Dark et al. (1984), using a Dashiell maze, found that females with >60% side preferences had significantly more striatal DA on the side away from which they preferred to turn, that is, the contralateral side. In Zimmerberg's experiment only females were used, and only those females with side preferences >60% (which included >95% of those screened). In Dark's experiment 8 of the 15 females used had side preferences <60%, and in these rats there was no significant DA asymmetry. In males, according to the results of Dark et al., there is no evidence for any normal striatal DA asymmetry, although because only 2 of 14 had side preferences >60%, it could not be determined whether there was a DA asymmetry in these rats. In response to a high dose of amphetamine (20 mg/kg) the normal contralateral>ipsilateral asymmetry in females increases to approximately 25% (Glick et al., 1974; Jerussi and Glick, 1976; TE Robinson et al.,

1980); but in males, again, there is still no significant asymmetry (Robinson et al., 1980). On the other hand, in response to a low dose of amphetamine (1.5-2.0 mg/kg) the normal DA asymmetry in females apparently reverses (Jerussi and Glick, 1976), while in males a contralateral>ipsilateral asymmetry is produced (Yamamoto and Freed, 1984). Thus, the relationship between striatal DA and turning in unlesioned rats remains somewhat unclear.

The foregoing discussion notwithstanding, identifying functional differences between the contralateral and ipsilateral hemispheres has been a major and, perhaps surprisingly, successful focus of research in the laboratories of Glick and TE Robinson. The threshold current required to support self-stimulation behavior in the lateral hypothalamus is lower on the contralateral side than on the ipsilateral side (Glick et al., 1980); and while systemically administered amphetamine preferentially affects self-stimulation in the contralateral hypothalamus (Glick et al., 1981), morphine, at low doses, preferentially affects self-stimulation in the ipsilateral hypothalamus (Glick et al., 1983).

Glick et al. (1979) also found differences between the contralateral and ipsilateral hemispheres using their modified 2-deoxyglucose technique: The ipsilateral midbrain incorporated more deoxyglucose than the contralateral midbrain, and acute administration of amphetamine did not affect this asymmetry. On the other hand, amphetamine

produced a contralateral>ipsilateral asymmetry in the striatum that was not present in saline-treated controls.

Unilateral electrolytic lesions in the ipsilateral and contralateral striatum have been shown to have opposite effects on a variety of behaviors. Whereas ipsilateral lesions increase, contralateral lesions decrease: (i) morphine self-administration (Glick and Cox, 1980), (ii) performance on a passive avoidance task (Rothman and Glick, 1976), (iii) performance on a differential-reinforcement-of-low-rate task (Glick and Cox, 1976), and (iv) amphetamine-induced rotation (Glick and Cox, 1978). One of the most significant aspects about these results to consider is this: Had pre-lesion rotational bias not been taken into account the conclusion reached in each of these experiments would have been that unilateral lesions of the striatum have no statistically significant effects on any of these behaviors, since the behavioral endpoints went up in some rats and down in some rats.

Similar phenomena have been documented by TE Robinson and colleagues. They compared the effects of partial striatal DA depletions induced by injections of 6OHDA into the contralateral or ipsilateral substantia nigra (TE Robinson and Becker, 1983; TE Robinson et al., 1985). While rats with ipsilateral lesions almost always turned toward the lesioned side post-operatively, most rats with contralateral lesions did not turn toward the lesioned side unless the 6OHDA-induced striatal DA asymmetry was greater than 90%. They also found that body weight regulation was more severely

disrupted by contralateral lesions than by ipsilateral lesions (Robinson et al., 1985). Mittleman et al. (1985) have also found that 6OHDA lesions of the contralateral striatum, nucleus accumbens, or olfactory tubercle are more effective than comparable lesions on the ipsilateral side in disrupting feeding evoked by electrical stimulation of the lateral hypothalamus.

Thus, in summary, differences clearly exist not only between the left and right cerebral hemispheres but also between the contralateral and ipsilateral cerebral hemispheres. Parenthetically, it is obvious that rotational behavior is not the only way that one could define a "contralateral" and "ipsilateral" side and hemisphere. For example, in future work, paw preference on a particular task, or direction of rotation in response to some other class of drugs, might be used. The number of possible ways for defining the two cerebral hemispheres is probably limitless; the possible functional significance of differences between the hemispheres defined according to other criteria, of course, remains to be seen. Camp et al. (1984) have also addressed this issue.

In any event, considering all the results discussed in this brief review, Geschwind's claim (pg 2), that "cerebral asymmetry is of major importance in every branch of biological science and in every branch of medicine," would seem to be less improbable than it may have on first reading.

The Present Investigation.

The first experiment was conducted in order to determine: (i) whether there are significant side differences in the number, or density, of nigro-striatal DA terminals; and (ii) whether the side differences measured are related to nocturnal rotational behavior. DA uptake *in vitro* was measured in crude mitochondrial pellets (Gray and Whittaker, 1962; Kuhar, 1973) obtained from the striata of both cerebral hemispheres, and Vmax was used as the measure of DA terminals. If Vmax asymmetries could be documented it would be the first clear evidence of neural asymmetry in the rat brain.

The results of this first experiment led to the development of a "two population" model for rat rotational behavior, and subsequent work was designed to test this model.

CHAPTER TWO

STRIATAL DOPAMINE UPTAKE ASYMMETRIES AND ROTATIONAL BEHAVIOR IN UNLESIONED RATS: REVISING THE MODEL?

MATERIALS AND METHODS

Sprague-Dawley rats weighing 225-250 gms at the start of the experiment were obtained from Perfection Breeders (Douglassville, PA), and were housed in pairs in standard hanging steel cages (Hoelting) with food and water continuously available (lights on/off: 7:00 a.m./7:00 p.m.).

Each rat was tested once for spontaneous nocturnal rotational behavior (Glick and Cox, 1978) between the hours of 4:00 p.m. and 10:00 a.m. in a cylindrical automated rotometer (diameter=29.5 cm). The rotometer detects quarter turns via photocell activation and records a full turn when four consecutive quarter turns are made in the same direction, regardless of the time it takes to complete the turn (Greenstein and Glick, 1975). "Extra quarter turns" are those quarter turns that do not contribute to a full turn (cf. Ross and Glick, 1981). Rotational behavior was quantitated according to the following formulae:

$$\text{net rotations} = \text{full turns in preferred direction} - \text{full turns in non-preferred direction}$$

$$\%\ \text{preference} = \frac{\text{full turns in preferred direction}}{\text{total full turns}} \times 100$$

$$\text{extra quarter turns} = \text{total quarter turns} - (4 \times \text{total full turns})$$

$$\%\ \text{turning} = \frac{(4 \times \text{total full turns})}{\text{total quarter turns}} \times 100$$

The % preference and % turning parameters allow comparisons betwen rats independent of total activity. Subsequent

experimentation was carried out without knowledge of the behavioral data.

The two buffers used in the uptake procedure were prepared fresh each day. The sucrose buffer consisted of 0.31 M sucrose, 0.01 M dextrose, pH 7.4 with 100 uM TRIS. The Krebs-Ringer-phosphate buffer, pH 7.4, consisted of 122 mM NaCl, 4.8 mM KCl, 972 uM $CaCl_2 \cdot 2H_2O$, 15 mM Na_2HPO_4, 50 mM pargyline, 1.14 mM L-ascorbic acid, 162 uM EDTA, and 10.1 mM dextrose, continuously bubbled with 100% O_2.

One to 15 days after behavioral testing two rats were removed from their home cage between 10:00 a.m. and 12:00 noon, weighed (males: 230-415 gms; females 230-300 gms) and decapitated. Left and right corpora striata (caudate-putamen and globus pallidus) were dissected over ice, weighed (mean$\pm$SEM = 39.7$\pm$0.5 mg, N=90, no sex or side differences), and immediately placed into an iced, 4 ml glass homogenizing vessel containing 1 ml sucrose buffer. Further manipulations were done in a 4°C cold room.

Individual striata were homogenized with 8 strokes of a smooth teflon pestle in 40 vols (w/v) of sucrose buffer and centrifuged for 10 min at 900 X g. The supernatant fraction was then centrifuged for 30 min at 17,750 X g. The resulting (P2) pellet was resuspended in 30 vols of sucrose buffer. Fifty ul aliquots of the P2 resuspension, containing 78.2$\pm$0.7 ug protein (Lowry et al., 1951; with BSA as standard; no sex or side differences), were added to 0.94 ml ice cold Krebs-Ringer-phosphate buffer in 16 ml glass test tubes, and preincubated for 5 min in a 37°C shaker bath (98 cycles/min).

Ten ul ^{3}H-DA (New England Nuclear NET-094; specific activity = 25.9 Ci/mmol; 92.3 nCi/tube) were added and the incubation was allowed to continue for 1 min, during which uptake was linear. Incubation was terminated by vacuum filtering (20 psi) the contents of the tube over 0.65 um mixed cellulose ester membranes (Millipore DAWP2500; cf. Shoemaker and Nickolson, 1983) and washing immediately with 7 ml Krebs-Ringer-phosphate buffer (37°C; cf. Levi and Raiteri, 1973; Shoemaker and Nickolson, 1983). Filtering and washing took less than 5 sec. Radioactivity was determined 24 hr later (counting efficiency = 59.7% by external standard). For each striatum, temperature-dependent uptake at each DA concentration was calculated by subtracting cpm in the sample kept on ice from cpm in the sample at 37°C. Km and Vmax values were obtained from curvilinear hyperbolic (Parker and Waud, 1971) and linear Eadie-Hofstee least squares fits of the data. Only the Km and Vmax values obtained from the hyperbolic fitting procedure are presented since the two procedures yielded virtually identical results.

RESULTS

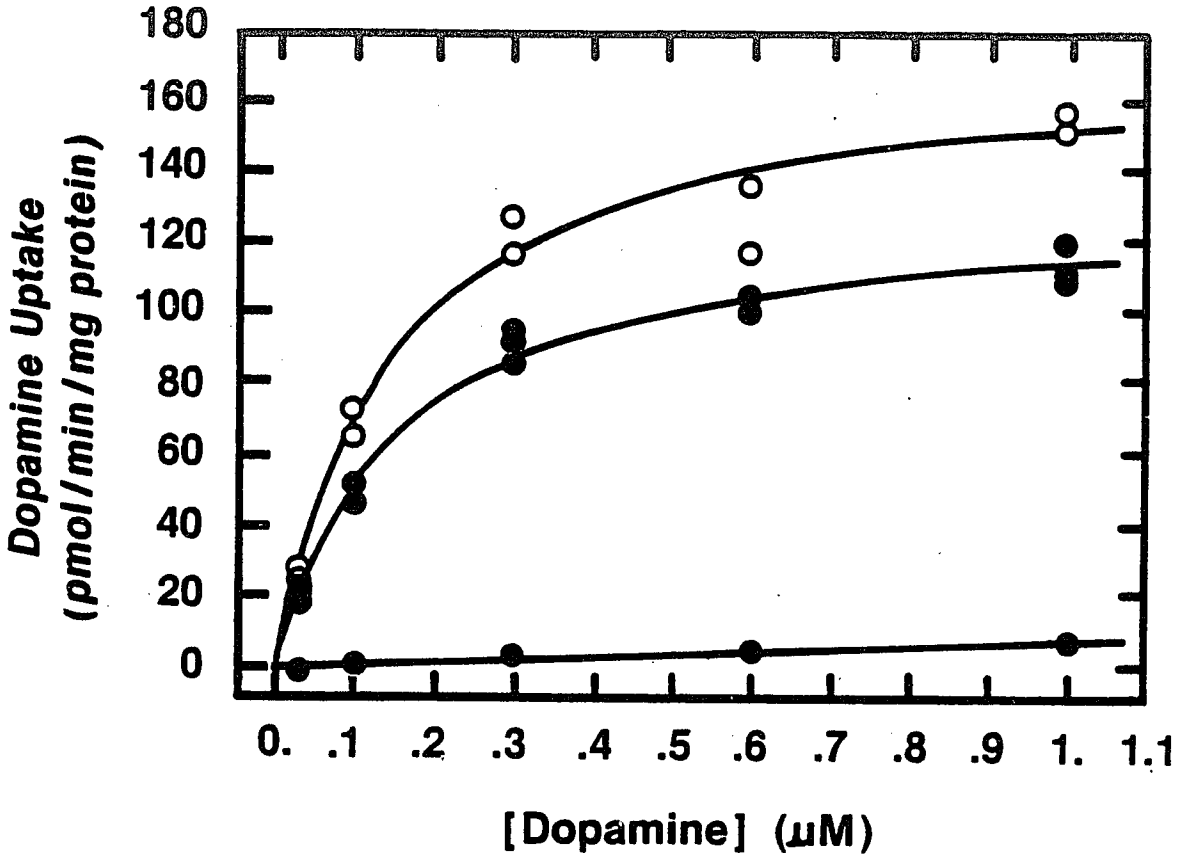
Sex Difference in DA Uptake.

Figure 2.1 shows the relationship between DA concentration and initial velocity of uptake for one rat. For the initial analysis, Km and Vmax values from the left and right striatum of each rat were averaged and the two sexes compared. As shown in Table 2.1, while there was no sex difference in mean Vmax, Km was significantly greater for the

Figure 2.1. Relationship between DA concentration and uptake velocity in the crude mitochondrial fraction of the two striata of one rat. This female made 240 and 75 full turns to the left and right, respectively; thus, the left and right cerebral hemispheres contained, respectively, the ipsilateral (open circles) and contralateral (solid circles) striata. Since Vmax is higher in the ipsilateral striatum this is an Ipsi>Contra rat.

(a) Hyperbolic curvilinear fit (Parker and Waud, 1971) of the temperature-dependent DA uptake data. Uptake and/or binding on ice was determined for both sides individually and resulted in the two overlapping lines at the bottom of the graph. Ipsilateral striatum: Km$\pm$SEM=0.15$\pm$0.02 uM; Vmax=131.3$\pm$4.2 pmol/min/mg protein; Contralateral striatum: Km=0.15$\pm$0.02 uM; Vmax=174.4$\pm$7.1 pmol/min/mg protein.

(b) Eadie-Hofstee transformations of the temperature-dependent DA uptake curves in (a). Ipsilateral striatum: Km=0.16$\pm$0.01 uM; Vmax=131.5$\pm$5.0 pmol/min/mg protein (r=0.97, p<0.0001); Contralateral striatum: Km=0.16$\pm$0.01 uM; Vmax=178.4$\pm$7.9 pmol/min/mg protein (r=0.96; p<0.0001).


Dopamine Uptake
(pmol/min/mg protein)
180
160
140
120
100
80
60
40
20
0
0.
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.
1.1
[Dopamine] (μM)

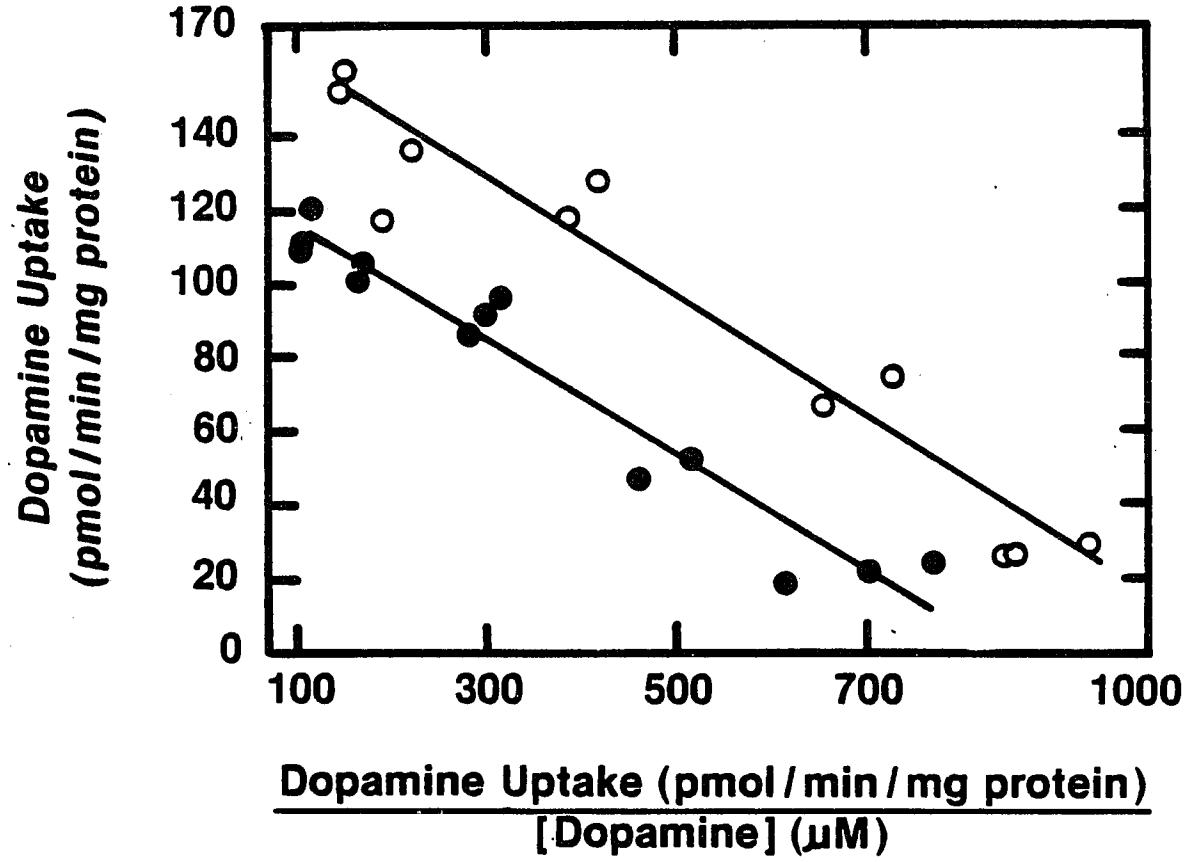

Dopamine Uptake
(pmol/min/mg protein)
170
140
120
100
80
60
40
20
0
100
300
500
700
1000
Dopamine Uptake (pmol / min / mg protein)
[Dopamine] (μM)

TABLE 2.1. Dopamine uptake in the crude mitochondrial fraction from striata of male and female rats. Km and Vmax were determined separately for the left and right striatum of each rat, averaged across sides, and then across rats to obtain the displayed mean (±SEM) parameter estimates for each sex.

Sex (N)	Km[1] ([DA]X10^{-7} M)	Vmax (pmol/min/mg protein)
Males (19)	1.51±0.04	180.6±7.07
Females (26)	1.81±0.07	185.4±6.92

[1]Significant sex difference in Km ($p<0.005$, t test).

females than for the males.

Relationship Between Vmax Asymmetry and Direction of Turning.

Paired t tests revealed no significant differences between either the contralateral and ipsilateral, or the left and right Km for either sex. On the other hand, Table 2.2 shows that the contralateral Vmax is greater than the ipsilateral Vmax for the females, while for the males there is a non-significant trend suggesting an ipsilateral>contralateral asymmetry. Neither sex had a significant left-right Vmax asymmetry.

The statistical significance of each individual rat's Vmax asymmetry was determined by performing t tests on the Y-intercepts obtained from the Eadie-Hofstee plots (Snedecor and Cochran, 1967, pg 435). Twenty of the 26 females had significant Vmax asymmetries, 16 of which were higher on the contralateral side and four of which were higher on the ipsilateral side. Nine of the 19 males had significant Vmax asymmetries, two of which were higher on the contralateral side and seven on the ipsilateral side. The frequency distribution of all rats (sexes combined) with respect to their contralateral/ipsilateral Vmax ratios is therefore bimodal (Figure 2.2), suggesting that there may actually be two different kinds of rats: those that rotate away from (Contra>Ipsi rats) and those that rotate toward (Ipsi>Contra rats) the side containing the striatum with the higher Vmax for DA uptake. Further analysis revealed that there was almost perfect overlap between the males and females with

TABLE 2.2. Mean Vmax values (±SEM pmol DA/min/mg protein) and Vmax asymmetries for DA uptake in crude mitochondrial fractions of rat striata.

Side[1]	Males (N=19)	Females (N=26)
Contralateral	175.7±8.0	190.4±7.8
Ipsilateral	185.5±7.4	180.5±6.7
Difference[2]	-9.8±6.0	9.9±4.5
High	192.7±7.8	196.1±7.1
Low	168.5±6.6	174.8±7.0
Difference[3]	24.2±3.0	21.4±2.4
Left	177.5±7.3	183.6±7.1
Right	183.7±8.1	187.3±7.6
Difference[4]	-6.2±6.3	3.7±4.9

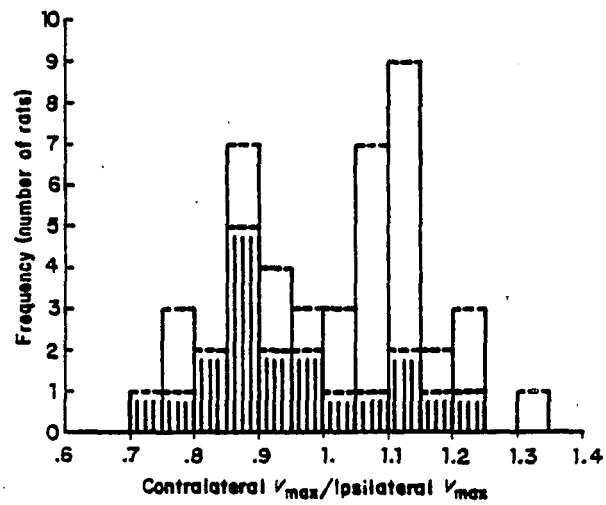
[1]"High" and "low" refer to the sides containing the striatum with the higher and lower Vmax, respectively, regardless of turning preference.

[2]Contralateral and ipsilateral values significantly different from each other in females only; $p<0.05$, paired t test.

[3]High and low values significantly different from each other in both sexes; $p<0.001$, paired t tests.

[4]No significant left-right differences in either sex.

FIGURE 2.2. Frequency distribution of contralateral/ipsilateral Vmax asymmetries (striped bars: males; open bars: females).


Frequency (number of rats)
0
1
2
3
4
5
6
7
8
9
10
.6
.7
.8
.9
1.
1.1
1.2
1.3
1.4
Contralateral V_{max}/Ipsilateral V_{max}

respect to the mean values for both the contralateral Vmax and ipsilateral Vmax in the two proposed populations of rats; and that while the mean ipsilateral Vmax values are virtually identical in the two populations (Figure 2.3; Contra>Ipsi rats: 180.3±6.8 pmol/min/mg prot; Ipsi>Contra rats: 185.6±7.3 pmol/min/mg prot), the 25 Contra>Ipsi rats can be distinguished from the 20 Ipsi>Contra rats by their significantly greater mean contralateral Vmax [Contra>Ipsi rats: 201.9±7.1 pmol/min/mg prot; Ipsi>Contra rats: 162.0±6.5 pmol/min/mg prot; $p<0.0025$, Scheffe test for unplanned, post-hoc comparisons following significant ($p<0.002$) ANOVA (Snedecor and Cochran, 1967, pg 271)]. In addition, the difference between the contralateral and ipsilateral sides was correlated with the contralateral Vmax ($r=0.51$, $p<0.001$), but not with the ipsilateral Vmax ($r=-0.05$, ns).

Correlations Between Vmax Asymmetries and Nocturnal Rotation.

Figure 2.4 shows a positive correlation between the contralateral/ipsilateral Vmax asymmetries and rats' rotational preferences among the 25 Contra>Ipsi rats ($r=0.45$, $p<0.025$), and a similar though inverse correlation among the 20 Ipsi>Contra rats ($r=-0.49$, $p<0.029$). When considered irrespective of rotational direction, the relationship appeared to be the same: Thus, there was also a significant correlation ($r=0.50$, $p<0.001$) between the absolute Vmax asymmetry (high side/low side) and % preference for both groups combined (Figure 2.5). When "net rotations" was used as the measure of nocturnal lateralized activity, the relationship with the absolute Vmax asymmetry was similar but was complicated by the fact that

FIGURE 2.3. Comparison of the mean (±SEM) contralateral and ipsilateral Vmax's among the males and females assigned to the Contra>Ipsi ("C>I") and Ipsi>Contra ("I>C") groups.

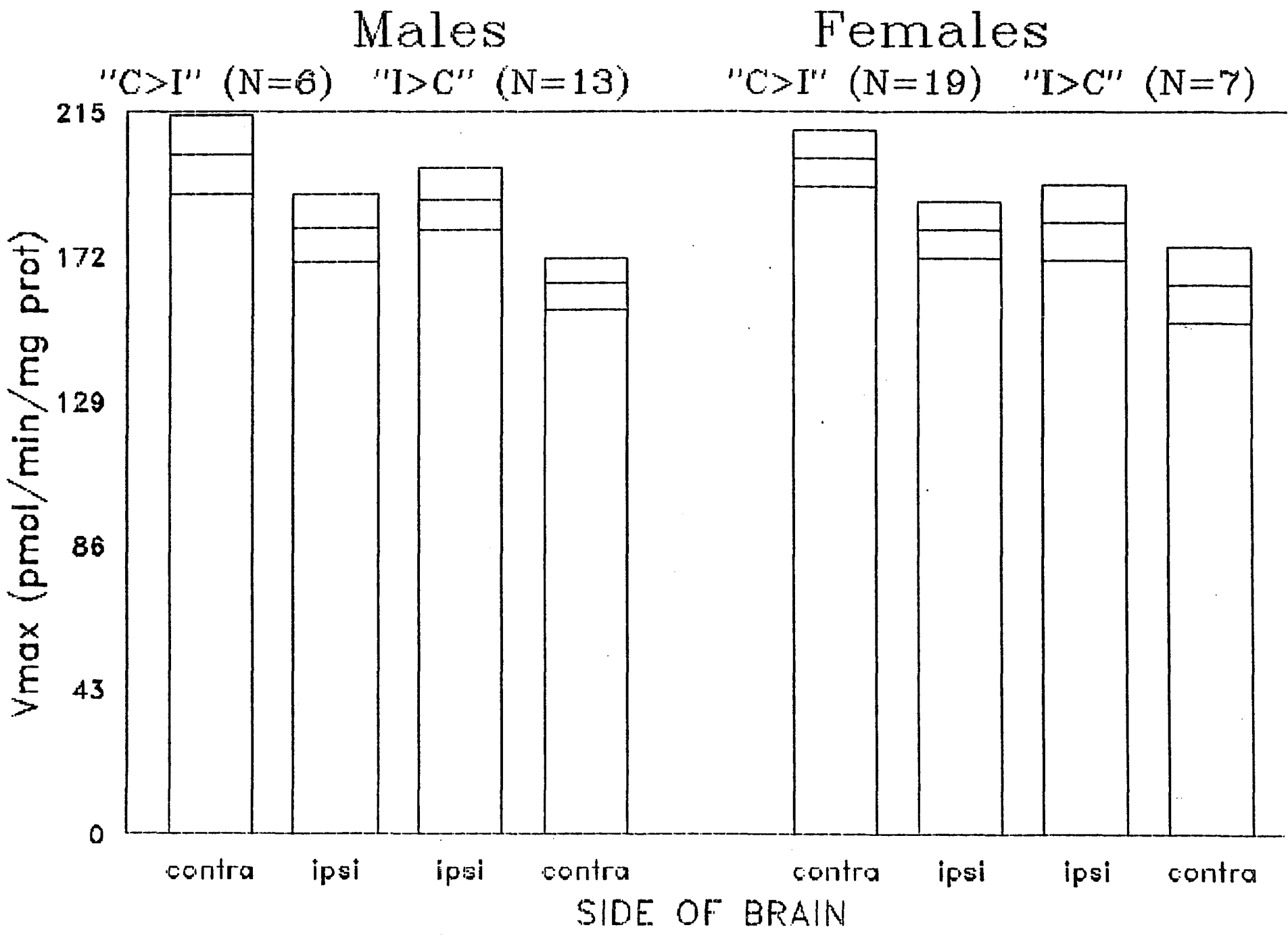
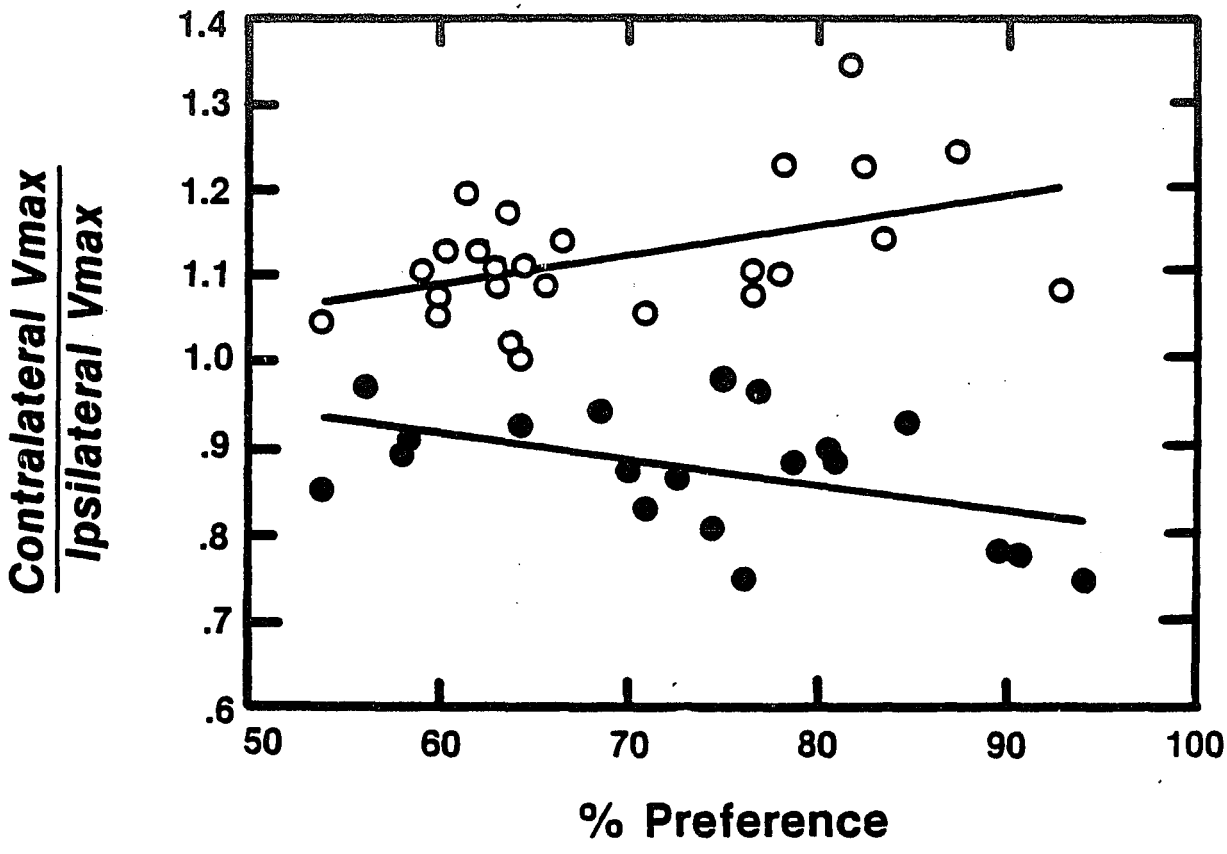
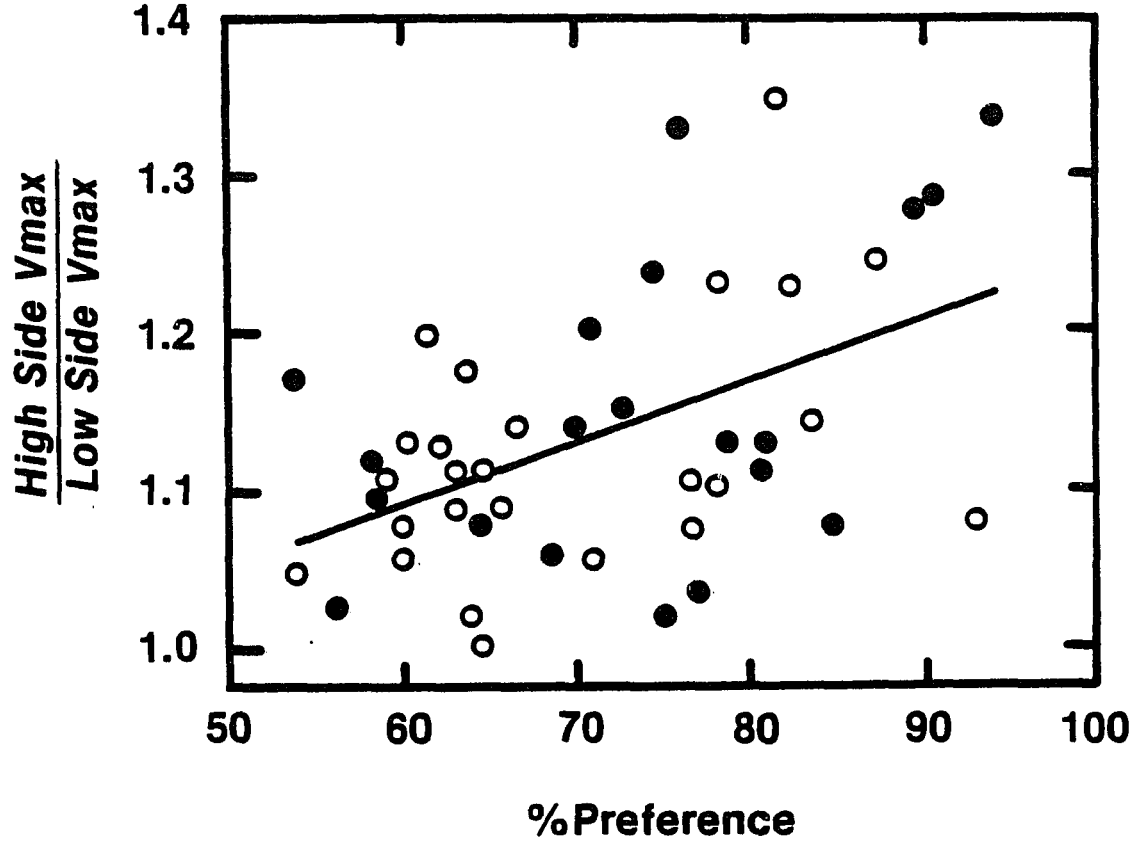

Males
Females
"C>I" (N=6)
"I>C" (N=13)
"C>I" (N=19)
"I>C" (N=7)
215
172
129
86
43
0
Vmax (pmol/min/mg prot)
contra
ipsi
ipsi
contra
contra
ipsi
ipsi
contra
SIDE OF BRAIN

FIGURE 2.4. Correlation between contralateral/ipsilateral DA uptake Vmax asymmetry and spontaneous nocturnal rotational behavior. As described in the text Contra>Ipsi rats (open circles; $r=0.45$, $p<0.023$) and Ipsi>Contra rats (solid circles; $r=-0.49$, $p<0.029$) are defined on the basis of whether the contralateral or ipsilateral striatum had the higher Vmax, respectively. Note that the slopes of the two fitted lines are equal in magnitude (0.0033 vs. -0.0030) though opposite in sign.

FIGURE 2.5. Correlation of the absolute DA uptake Vmax asymmetry (high side/low side) with spontaneous nocturnal rotational behavior for the Contra>Ipsi rats (open circles) and Ipsi>Contra rats (solid circles) regardless of which striatum had the higher Vmax and which way the rat preferred to turn; $r=0.50$, $p<0.001$. Slope of fitted line = 0.0039.


Contralateral Vmax / Ipsilateral Vmax
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
50
60
70
80
90
100
% Preference


High Side Vmax / Low Side Vmax
1.4
1.3
1.2
1.1
1.0
50
60
70
80
90
100
%Preference

females display a larger range in the magnitude of their net rotations than do males (Table 2.3) and the data were analyzed separately for the two sexes (males: $r=0.61$, $p<0.005$; females: $r=0.43$, $p<0.03$).

% Turning: A Behavioral Difference Between Contra>Ipsi and Ipsi>Contra Rats.

The behavioral data for all the rats are summarized in Table 2.3. The % turning score appears to be a behavioral measure that differentiates between Contra>Ipsi and Ipsi>Contra rats. Contra>Ipsi females had significantly higher % turning scores than Ipsi>Contra females, whereas Ipsi>Contra males had significantly higher % turning scores than Contra>Ipsi males. Contra>Ipsi and Ipsi> Contra rats did not differ with respect to any other rotational parameters; nor did left and right rotators differ with respect to any parameter, neurochemical or behavioral.

DISCUSSION

The present data suggest that there may be a somewhat more complex relationship than had previously been suspected between the DA innervation of the striatum and rotational behavior. These results also demonstrate a sex difference in the Km for DA uptake in rat striatum: the male carrier site apparently has a higher affinity for DA than does that of the female. This finding adds to a growing list of differences between the sexes that likely reflect gonadal influences on brain neurochemistry (Miller, 1983), and may help to explain why females are more sensitive than males to amphetamine

TABLE 2.3. Summary of behavioral data (Mean±SEM). Rats are classified according to sex, and whether the contralateral (Contra>Ipsi rats; "C>I") or ipsilateral (Ipsi>Contra rats; "I>C") cerebral hemisphere contained the striatum with the higher Vmax for dopamine uptake.

	Males			Females		
	C>I	I>C	Total	C>I	I>C	Total
	(N=6)	(N=13)	(N=19)	(N=19)	(N=7)	(N=26)
Net Full Turns	20±7	30±7	27±5	79±19	61±21	74±14
% Preference	65±4	73±3	70±3	71±2	76±4	73±3
Extra Quarter Turns	775±306	368±49	496±106	553±49	897±251	645±85
% Turning[1]	28±4	42±2	38±2	52±2	37±5	48±3

[1]Difference between Contra>Ipsi and Ipsi>Contra rats in both sexes; males: $p<0.06$; females: $p<0.025$, Scheffe test for unplanned, post-hoc comparisons following significant ($p<0.0001$) ANOVA (Snedecor and Cochran, 1967, page 271).

(Brass and Glick, 1981; Becker et al., 1982) and cocaine (Glick et al., 1983).

The Vmax for DA uptake is significantly greater in the contralateral than in the ipsilateral striatum for female rats as a group (Table 2.2) but not for males as a group. Since DA uptake and DA content are correlated (Tassin et al., 1976), these data are consistent with what is already known about the relationship between striatal DA content and circling in the two sexes: females as a group have higher DA levels contralateral to their turning preferences whereas males as a group do not (see Chapter One).

Despite the above sex difference in the contralateral vs. ipsilateral Vmax asymmetry, the analysis of Vmax asymmetries in individual rats suggested that the DAergic innervation to the two striata can be asymmetric in males as well as in females. Indeed, the absolute (high/low) Vmax asymmetry is as large in males as it is in females (Table 2.2), and in both sexes the striatum having the higher Vmax is contralateral to turning preferences in some rats and ipsilateral to turning preferences in other rats. This bimodal distribution suggests the existence of two contiguous populations of rats. In one population (the Contra>Ipsi rats), dominated by females, the larger the contralateral/ipsilateral asymmetry the stronger the rotational behavior (Figure 2.4). In the other population (the Ipsi>Contra rats) dominated by males, the larger the ipsilateral/contralateral asymmetry, the stronger the rotational behavior (Figure 2.4). Alternatively, these data could be interpreted as indicating

that the degree of striatal DAergic asymmetry determines only the strength of a rat's turning preference, and not the direction itself. However, the two population model is supported by the findings that Contra>Ipsi and Ipsi>Contra rats can be distinguished on the basis of both a behavioral parameter (% turning; Table 2.3) and a neuro-chemical parameter (the contralateral Vmax). The Contra>Ipsi rats also had a higher mean Km than the Ipsi>Contra rats ($1.78\pm0.07 \times 10^{-7}$ M vs. $1.57\pm0.06 \times 10^{-7}$ M, respectively; $p<0.05$, $\underline{t}$ test), but this is most likely attributable to the relative distribution of the two sexes in the two populations.

These data do not support the prevailing view of the relationship between striatal DA and turning behavior -- i.e., that the DA input to one striatum always serves to "push" the rat towards the other side. The present data support a somewhat more complex model, in which DA can be either excitatory or inhibitory with respect to circling behavior (i.e., "push" or "pull"); and whether an animal circles towards or away from the side containing the striatum with the greater DA innervation would depend on which of the two mutually antagonistic systems predominates in the two striata. This notion, of course, that DA may have two mutually antagonistic functions in (perhaps, different regions of) the striatum, is not a new one (Frigyesi and Purpura, 1967; Cools, 1977; Cools and van Rossum, 1980; Stoof and Kebabian, 1984; Schoener and Elkins, 1984; Sonsalla et

al., 1984); nor is the notion that mutually antagonistic subsystems exist within the DAergic nigro-striatal system with respect to circling behavior (Tye et al., 1977; Vaccarino and Franklin, 1982a, b; Hirschhorn et al., 1983; Gratton and Wise, 1985). In addition, it has also been proposed that striatal output is organized into counter-balancing excitatory and inhibitory sub-systems (Groves, 1983; Sonsalla et al., 1984). Thus, it seems clear that the neurobiological basis for turning behavior may not be as simple as is commonly believed. The experiments described in the following chapters were designed in order to test the present two population model.

CHAPTER THREE

DOPAMINE, SEROTONIN AND METABOLITES IN STRIATUM AND PREFRONTAL CORTEX: QUALIFIED SUPPORT FOR TWO POPULATION MODEL

The results of the experiment described in the previous chapter can be summarized as follows: First, nocturnal circling behavior was correlated with the absolute magnitude of the striatal Vmax asymmetry (Vmax in the high side/Vmax in the low side), regardless of which way the rat turned and which striatum had the higher Vmax. On this basis two groups, or "populations," of rats were defined: Contra>Ipsi rats were those with Vmax higher on the contralateral side (i.e., the side away from which the rat made most of its turns), and Ipsi>Contra rats were those with Vmax higher on the ipsilateral side. Second, the two proposed populations of rats could be distinguished statistically on the basis of one neurochemical parameter and one behavioral parameter. Whereas the mean ipsilateral Vmax was virtually identical in the two groups, the contralateral Vmax was greater in the Contra>Ipsi rats than in the Ipsi>Contra rats. Behaviorally, female Contra>Ipsi rats had higher % turning scores than female Ipsi>Contra rats, and male Contra>Ipsi rats had lower % turning scores than male Ipsi>Contra rats.

In the present study concentrations of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5HT), and 5-hydroxyindoleacetic acid (5HIAA) were determined bilaterally in the striata and medial prefrontal cortices (PFC) of rats previously tested for nocturnal circling behavior. The relationships between nocturnal circling behavior and striatal DA, DOPAC, and HVA have never been examined in the same group of rats; the relationship between

striatal 5HT and 5HIAA and nocturnal circling behavior has never been investigated. As an initial test of the proposed two population model of rotational behavior, it was hypothesized that striatal DA levels would be related to nocturnal circling behavior in a similar way to that described above for DA Vmax values in the two striata. Whether rats classified as belonging to the "Contra>Ipsi" and "Ipsi>Contra" populations differed with respect to any of the other neurochemical measures was also investigated.

Medial prefrontal cortex concentrations of DA, DOPAC, 5HT, and 5HIAA were measured in order to determine whether (i) there were any asymmetries between the left and right or contralateral and ipsilateral hemispheres, (ii) there were correlations between side differences and circling behavior, (iii) there were neurochemical differences in PFC between "Contra>Ipsi" and "Ipsi>Contra" rats (classified on the basis of striatal DA), and (iv) there were correlations between side differences in striatum and PFC. This last possibility was investigated since correlations between neurochemical asymmetries have been demonstrated in human brains (Glick et al., 1982).

MATERIALS AND METHODS

Male and female Sprague-Dawley rats weighing 225-250 g at the start of the experiment were obtained from Zivic-Miller Laboratories (Allison Park, PA). They were housed in groups of 2-4 in plastic cages with free access to food and water (lights on/off: 7:00 a.m./7:00 p.m.). Rats were tested

once for spontaneous nocturnal rotational behavior, and fifteen days later they were sacrificed by decapitation between 12:00 noon and 6:00 p.m. Brains were removed in less than two minutes and cooled in pentane (-5°C in NaCl-ice water) for 1 min. Using a rat brain matrix (Activational Systems #RBM4000C) a coronal cut was made corresponding to Koenig and Klippel (1963) level A10300; then medial PFC (8.0±0.4 mg, N=62) was dissected with a single razor cut using the forceps minor of the corpus callosum and the olfactory tract as lateral and ventral borders, respectively. This dissection is essentially identical to that described by Roth et al. (1983). The order of dissection was randomized such that on any given day half the pairs of PFC and half of the pairs of striata were first dissected on the left.

Tissue was weighed and sonicated for 30 sec in 0.5 ml 0.1 N perchloric acid containing 205.45 pM dihydroxybenzylamine as internal standard, and 0.1% L-cysteine and 2.6 mM EDTA as antioxidants. Samples were prepared for chromatography by centrifuging the sonicates at 32000 g for 15 min (Beckman J2-21, 4°C), and filtering the resulting supernatant through 0.2 um regenerated cellulose membranes (Schleicher & Schuell) at 800 g for 5 min (IEC Centra-7, 22°C). External standards, stable for at least three weeks, were prepared daily from the following refrigerated 1-1.5 mM stock solutions: DA, DOPAC, and HVA in 0.1 N perchloric acid/0.1% L-cysteine; 5HT and 5HIAA in normal saline/0.1% L-cysteine, as described by Renner and Luine (1984).

DA, DOPAC, HVA, 5HT, and 5HIAA were quantitated using an

HPLC-ECD system consisting of a BioRad AS-48 refrigerated (9°C) autoinjector, a 20 ul fixed loop injection valve (Rheodyne #7010), a dual piston pump (BioAnalytical Systems #PM-30A), a pulse dampener, a temperature controller (22°C; BAS #LC22A-23A), a guard column (2 cm; Upchurch #C-130B) packed with 10 um C18/Ultrapack (Altex #244232), and a C-18 reverse phase Biophase column (250 X 4.6 mm; BAS #6017). A potential of +0.8 V was maintained between the glassy carbon working (BAS #TL-5A) and Ag/AgCl reference electrodes with a BAS LC-4B/17 amperometric controller (1 nA full scale), and chromatograms were recorded and analyzed with an HP3392A integrator.

Mobile phase (pH 2.8; adapted from BAS LCEC Application Note No. 15) consisted of 0.645 mM sodium octyl sulfate (Eastman), 222.2 mM monochloroacetic acid, 116.9 mM NaOH, and 766.3 uM EDTA in 10% methanol (Fisher, HPLC grade). Prior to use it was filtered under vacuum through a 0.2 um Metricel membrane filter (Gellman #60585) and degassed. With a flow rate of 1 ml/min, back pressure was approximately 2000 psi, and complete chromatograms were obtained every 40 min. The lower limit of detection was 0.05 pmol for DA, DOPAC, HVA, AND 5HIAA, and 0.1 pmol for 5HT.

Data were analyzed with the Human Systems Dynamics ANOVA II and STATS Plus programs. Pairs of values were routinely excluded if one of the pair was greater than 2.5 standard deviations away from the mean for that side, which included the PFC DOPAC values from two males and two females that fell

below the limit of detection.

RESULTS

Relationship Between Neurochemical Side Differences in Striatum and Circling.

There were no significant correlations between any asymmetries (contralateral side/ipsilateral side or high side/low side) and circling behavior, either with net rotations or % preference. In addition, paired t tests revealed no statistically significant hemispheric asymmetries for any of the neurochemical parameters measured, either between the contralateral and ipsilateral or between the left and right striata. The data for each monoamine and metabolite, and for the ratios DOPAC/DA, HVA/DA, and 5HIAA/5HT, are summarized in Table 3.1 according to sex and predominant direction of nocturnal rotation. Data for each neurochemical parameter were analyzed with a two factor (sex X direction of rotation) ANOVA nested across sides of the brain (contralateral and ipsilateral). Significant effects were obtained for DOPAC/DA (for sex, $p<0.001$, and for direction of rotation $p<0.05$) and for 5HIAA/5HT (significant sex X side interaction).

% Turning is Different in "Contra>Ipsi" and "Ipsi>Contra" Rats.

The nocturnal rotational behavior of the rats used in the present study are summarized in Table 3.2 according to sex and direction of rotation. In addition, as was done in the previous chapter using the Vmax for DA uptake, rats were designated "Contra>Ipsi" or "Ipsi>Contra" depending on which

TABLE 3.1. Monoamine and metabolite levels (ug/gm; mean±SEM) in the contralateral ("Contra") and ipsilateral ("Ipsi") striata of rats grouped according to sex and predominant direction of nocturnal rotation; group N's in parentheses.

	Females				Males			
Direction of Rotation:	Left		Right		Left		Right	
Side:	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				DA				
	9.620	9.157	8.838	8.908	8.865	9.224	8.284	8.253
	±.367	±.281	±.450	±.421	±.311	±.277	±.730	±.798
	(8)		(7)		(12)		(4)	
				DOPAC				
	1.182	1.167	1.130	1.195	1.227	1.254	1.244	1.229
	±.064	±.053	±.051	±.068	±.046	± .049	±.084	±.091
	(8)		(8)		(12)		(4)	
				DOPAC/DA[1]				
	0.123	0.128	0.130	0.130	0.139	0.136	0.151	0.150
	±.004	±.006	±.004	±.003	±.004	±.003	±.004	±.004
	(8)		(7)		(12)		(4)	
				HVA				
	0.744	0.772	0.730	0.760	0.776	0.833	0.729	0.749
	±.037	±.048	±.041	±.049	±.054	±.057	±.067	±.079
	(8)		(8)		(12)		(4)	
				HVA/DA				
	0.078	0.084	0.086	0.082	0.087	0.090	0.089	0.091
	±.004	±.004	±.006	±.005	±.005	±.005	±.006	±.005
	(8)		(7)		(12)		(4)	

[1]Significant effects of sex ($p<0.001$) and direction of rotation ($p<0.05$).

TABLE 3.1 (cont'd).

	Females				Males			
Direction of Rotation:	Left		Right		Left		Right	
Side:	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				5HT				
	0.710	0.726	0.699	0.691	0.704	0.683	0.641	0.608
	±.031	±.036	±.027	±.039	±.013	±.020	±.046	±.039
	(8)		(5)		(12)		(4)	
				5HIAA				
	0.574	0.559	0.529	0.518	0.517	0.516	0.479	0.485
	±.027	±.017	±.029	±.027	±.019	±.021	±.041	±.035
	(8)		(6)		(11)		(4)	
				5HIAA/5HT[2]				
	0.809	0.775	0.735	0.730	0.736	0.756	0.748	0.797
	±.020	±.021	±.038	±.041	±.028	±.024	±.045	±.024
	(8)		(5)		(12)		(4)	

[2]Significant sex X side interaction ($p<0.02$).

TABLE 3.2. Rotational behavior (mean±SEM) summarized according to sex, direction of rotation, and whether the contralateral ("C>I") or ipsilateral ("I>C") cerebral hemisphere contained the striatum with the higher DA concentration.

	Females				Males			
	Direction of Rotation		"Population"		Direction of Rotation		"Population"	
	Left	Right	I>C	C>I	Left	Right	I>C	C>I
N	8	8	4	11	12	4	10	6
Net Rotations	19	10	26	11	15	10	14	16
	±6	±3	±10	±4	±4	±6	±4	±6
Preference	63	60	66	60	61	63	62	61
	±4	±3	±5	±3	±2	±4	±2	±3
% Turning[1]	38	35	47	33	46	56	49	41
	±4	±3	±7	±3	±4	±5	±5	±4

[1]Significant effect of "population" ($p<0.05$).

striatum had the higher DA concentration. This was done in order to compare the % turning scores of the "Contra>Ipsi" and "Ipsi>Contra" males and females to see whether results like those found in the previous chapter would be obtained; this despite the fact that no significant side differences in striatal DA had been found. Two factor (sex X "population") ANOVA revealed a significant "population" effect but no interactions; while the "Ipsi>Contra" rats as a whole had higher % turning scores the difference was significant only for the females ($p<0.05$, $\underline{t}$ test).

The nocturnal rotational behavior of the rats used in the present study differed markedly from that of the rats used in the uptake experiment (compare Tables 3.2 and 2.3). Rats used in the present experiment made fewer net rotations (females: $p<0.005$; males: $p<0.07$), and had lower % preference scores (females: $p<0.01$; males: $p<0.05$); in addition, the females had lower % turning scores ($p<0.02$) and the males had higher % turning scores ($p<0.05$).

Differences in Striatal Neurochemistry Between "Contra>Ipsi" and "Ipsi>Contra" Rats.

The neurochemical data from the striata of the 17 "Contra>Ipsi" and 14 "Ipsi>Contra" rats are summarized in Table 3.3. The two "populations" were compared with respect to each neurochemical parameter via single factor ANOVAs nested across the contralateral and ipsilateral sides. The results of the analysis on DA levels were virtually identical to those obtained in the preceding chapter for Vmax values:

TABLE 3.3. Striatal monoamine and metabolite levels (mean ±SEM) in the contralateral ("Contra") and ipsilateral ("Ipsi") cerebral hemispheres of rats classified according to whether the contralateral ("Contra>Ipsi" rats) or ipsilateral ("Ipsi>Contra" rats) hemisphere contained the striatum with the higher DA concentration; group N's in parentheses.

"Population:"	Contra>Ipsi		Ipsi>Contra	
Side:	Contra	Ipsi	Ipsi	Contra
		DA[1]		
	9.516	9.052	8.959	8.326
	±.272	±.298	±.216	±.231
	(17)		(14)	
		DOPAC		
	1.192	1.190	1.229	1.180
	±.042	±.044	±.040	±.038
	(17)		(14)	
		DOPAC/DA[2]		
	0.128	0.132	0.137	0.142
	±.003	±.003	±.003	±.003
	(17)		(14)	
		HVA		
	0.725	0.747	0.841	0.782
	±.035	±.041	±.039	±.038
	(17)		(14)	
		HVA/DA[3]		
	0.077	0.081	0.094	0.094
	±.003	±.003	±.003	±.004
	(17)		(14)	

[1]Significant "population" X side interaction ($p<0.001$).
[2]Significant "population" effect ($p<0.05$) and "population" X side interaction ($p<0.001$).
[3]Significant "population" effect ($p<0.001$).

TABLE 3.3 (cont'd).

"Population:"	Contra>Ipsi		Ipsi>Contra	
Side:	Contra	Ipsi	Ipsi	Contra
		5HT[4]		
	0.703 ±.017 (16)	0.672 ±.021	0.702 ±.024 (13)	0.687 ±.018
		5HIAA		
	0.544 ±.021 (17)	0.525 ±.018	0.524 ±.017 (14)	0.516 ±.015
		5HIAA/5HT		
	0.763 ±.023 (16)	0.772 ±.018	0.751 ±.021 (13)	0.752 ±.023

[4]Significant "population" X side interaction ($p<0.05$).

that is, (i) while there was a significant "population" X side interaction ($p<0.001$) due to the significant difference between the contralateral sides ($p<0.005$, t test), the mean ipsilateral values were differed by only 1%; and (ii) the difference between the contralateral and ipsilateral sides was correlated with the contralateral DA level ($r=0.47$, $p<0.01$), but not with the ipsilateral DA level ($r=-0.12$, ns). Further analysis revealed significant "population" effects for the ratios, DOPAC/DA and HVA/DA, and significant "population" X side interactions for DOPAC/DA and 5HT (see Table 3.3).

Relationship Between Neurochemical Side Differences in Medial Prefrontal Cortex and Circling.

As was the case in the striatum, there were no significant correlations between any neurochemical asymmetries in the PFC (contralateral side/ipsilateral side or high side/low side) and circling behavior, either with net rotations or % preference. However, in contrast to the striatum two statistically significant hemispheric differences were detected in PFC: in the males left PFC contained an average of 64.0 ($\pm$23.8) % more DA than right PFC ($p<0.05$, paired t test); and in the females left PFC contained an average of 9.7 ($\pm$3.8) % more 5HT than right PFC ($p<0.05$, paired t test).

Table 3.4 summarizes the neurochemical data from the PFC. As was done for the striatum, two factor (sex X direction of rotation) ANOVAs nested across the two cerebral

hemispheres were used to analyze the neurochemical measures. Two statistically significant ANOVAs were obtained. For DA there was a significant side effect; for 5HT right rotators had higher 5HT levels than left rotators, and there were two significant interactions: sex X side and sex X direction of rotation X side.

Comparison of Medial Prefrontal Cortex Neurochemistry in "Contra>Ipsi" and "Ipsi>Contra" Rats.

PFC neurochemistry data from the 17 "Contra>Ipsi" and 14 "Ipsi>Contra" rats (defined on the basis of the DA side differences in striatum) are summarized in Table 3.5. Possible differences between the two proposed populations of rats were analyzed in the same way that they were for striatum (Table 2.3), i.e., with a one way ANOVA on the two "populations" with the contralateral and ipsilateral sides nested. The analysis revealed a significant "population" X side interaction for DOPAC/DA: whereas the "Contra>Ipsi" rats had higher DOPAC/DA ratios in the ipsilateral PFC the "Ipsi>Contra" rats had higher DOPAC/DA ratios in the contralateral PFC.

DISCUSSION

No Evidence for Neurochemical Asymmetry in Striatum.

In the present study, no evidence was obtained in either sex for the existence of neurochemical asymmetries in rat striatum. The lack of a DA asymmetry in males was not unexpected since four previous studies failed to detect a

TABLE 3.4. Monoamine and metabolite levels (mean±SEM) in the left and right medial prefrontal cortices of rats grouped according to sex and predominant direction of nocturnal rotation; group N's in parentheses.

	Females				Males			
Direction of Rotation:	Left		Right		Left		Right	
Side:	Left	Right	Left	Right	Left	Right	Left	Right
				DA[1]				
	0.156 ±.022	0.153 ±.020	0.196 ±.028	0.162 ±.026	0.236 ±.037	0.151 ±.015	0.242 ±.052	0.192 ±.044
	(8)		(8)		(11)		(4)	
				DOPAC				
	0.092 ±.013	0.082 ±.013	0.084 ±.013	0.067 ±.006	0.097 ±.012	0.079 ±.009	0.104 ±.015	0.083 ±.022
	(5)		(6)		(9)		(4)	
				DOPAC/DA				
	0.658 ±.154	0.538 ±.128	0.483 ±.056	0.557 ±.064	0.501 ±.069	0.570 ±.095	0.501 ±.119	0.499 ±.187
	(5)		(6)		(9)		(4)	
				5HT[2]				
	0.621 ±.041	0.610 ±.045	0.769 ±.036	0.655 ±.025	0.616 ±.020	0.618 ±.026	0.651 ±.010	0.678 ±.030
	(8)		(7)		(9)		(4)	

[1]Significant side effect ($p<0.05$); paired t tests for each sex revealed significant left>right asymmetry in the males (0.075±.034; $p<0.05$) but not the females (0.019±.021; ns).

[2]Significant effect of direction of rotation ($p<0.035$), and significant sex X side ($p<0.01$) and sex X direction of rotation X side ($p<0.025$) interactions. Paired t tests for each sex revealed significant left>right asymmetry in the females (0.059±.023; $p<0.05$) but not the males (-0.010 ±.014; ns).

TABLE 3.4 (cont'd).

	Females				Males			
Direction of Rotation:	Left		Right		Left		Right	
Side:	Left	Right	Left	Right	Left	Right	Left	Right
				5HIAA				
	0.241	0.251	0.296	0.249	0.229	0.232	0.232	0.248
	±.020	±.024	±.025	±.011	±.015	±.014	±.027	±.005
	(8)		(7)		(9)		(4)	
				5HIAA/5HT				
	0.391	0.414	0.385	0.385	0.372	0.377	0.357	0.369
	±.028	±.035	±.031	±.023	±.018	±.018	±.040	±.022
	(8)		(7)		(9)		(4)	

TABLE 3.5. Monoamine and metabolite levels (ug/gm; mean±SEM) in the contralateral ("Contra") and ipsilateral ("Ipsi") medial prefrontal cortices of rats classified according to whether the contralateral ("Contra>Ipsi" rats) or ipsilateral ("Ipsi>Contra" rats) cerebral hemisphere contained the striatum with the higher DA concentration; group N's in parentheses.

"Population"	Contra>Ipsi		Ipsi>Contra	
Side:	Contra	Ipsi	Ipsi	Contra
		DA		
	0.181 ±.018	0.161 ±.019	0.226 ±.028	0.168 ±.019
	(17)		(14)	
		DOPAC		
	0.081 ±.008	0.088 ±.009	0.084 ±.010	0.089 ±.008
	(12)		(12)	
		DOPAC/DA[1]		
	0.490 ±.068	0.670 ±.077	0.425 ±.048	0.570 ±.069
	(12)		(12)	
		5HT		
	0.662 ±.033	0.627 ±.023	0.648 ±.019	0.653 ±.024
	(16)		(12)	
		5HIAA		
	0.272 ±.016	0.240 ±.011	0.241 ±.011	0.229 ±.015
	(16)		(12)	

[1]Significant "population" X side interaction ($p<0.02$).

TABLE 3.5 (cont'd).

"Population"	Contra>Ipsi		Ipsi>Contra	
Side:	Contra	Ipsi	Ipsi	Contra
		5HIAA/5HT		
	0.413	0.387	0.371	0.352
	±.018	±.018	±.014	±.020
	(16)		(12)	

contralateral-ipsilateral asymmetry in males (Robinson et al., 1980; Dark et al., 1984; Yamamoto and Freed, 1984, Table 2: "Untrained Controls (Saline-Untrained-Saline)"; Camp et al., 1984).

The lack of a significant DA asymmetry in the females was absolutely unexpected. This result conflicts with previous work from this laboratory and requires a carefully considered explanation. The original report by Zimmerberg et al. (1974) of an endogenous DA asymmetry in rat striatum was based on three results. Using female rats as subjects they found that: (i) rats exhibiting more than a 60% side preference in 10 T-maze trials had a mean contralateral>ipsilateral striatal DA asymmetry of 10-15%, when they were sacrificed immediately after behavioral testing; (ii) rats sacrificed 10 days after behavioral testing also had 10-15% contralateral> ipsilateral striatal DA asymmetries; and (iii) experimentally naive rats (i.e., no side preference determined) had similar 10-15% DA asymmetries (high side/low side). Thus, the conclusion of Zimmerberg et al., that normal rats have an endogenous contralateral>ipsilateral asymmetry of striatal DA that averages 10-15% certainly seems justified.

How then can the present results be understood? The work of Dark et al. (1984) would appear to be illuminating. These workers found no striatal DA asymmetry in a group of 15 females tested for side preferences in a Dashiell maze 24 hours prior to sacrifice. However, when only the 7 females with side preferences greater than 60% were examined it was

found that they did, in fact, have a significant DA asymmetry, with the contralateral striatum containing more than the ipsilateral striatum; in contrast, the other 8 females still had no statistically significant asymmetry. Thus, since Zimmerberg et al. studied only those (female) rats with (T-maze) % preference scores greater than 60, it would appear that the conclusions drawn from their results may have been flawed, due to the exclusion of a substantial proportion of the (female) rat population from the sample studied -- i.e., those rats with weak side preferences. However, this does not appear to be the case, because (i) Zimmerberg et al. report that only one out of 31 rats (3%) tested in their T-maze failed to reach the 60% criterion required for inclusion in their study; and (ii) as stated above, an unselected, experimentally naive group of rats included in their study also had a comparable 10-15% mean striatal DA asymmetry. The most parsimonious explanation, then, for the difference between the results of Zimmerberg et al. and those of Dark et al. is that the populations of rats sampled in the two studies were different. Whereas Zimmerberg et al. studied a population of rats made up almost entirely of rats with strong side preferences, Dark et al. sampled a population consisting of rats with strong and weak side preferences in approximately equal proportions. This conclusion is based, of course, on the assumption that side preference results in a T-maze and in a Dashiell maze -- which is, in effect, a series of T-mazes -- are roughly comparable. Assuming that the preceding argument is valid it

suggests that considerable differences may exist between the Sprague-Dawley rats supplied by different breeding laboratories, at least with respect to behavioral and neurochemical asymmetry. This is not a new idea since significant differences between outbred Sprague-Dawley rats from different suppliers have previously been documented (e.g., Ezerman and Kromer, 1985).

The rotational behavior of both the male and the female rats used in the present study differed considerably from that of the rats used in the uptake experiment described in the preceding chapter. Like the rats used by Dark et al. compared to the rats used by Zimmerberg et al., the rats used in the present study showed considerably less evidence of behavioral asymmetry than the rats used in the uptake experiment: they made fewer net rotations and had lower % preference scores. It seems likely, then, that in the present experiment the failure to observe a significant DA asymmetry between the contralateral and ipsilateral striata may have been due to the low behavioral asymmetries characteristic of the rats studied. Whether the differences in % turning scores between the two groups of rats also played a role is unknown.

The two population model proposed in Chapter Two would, of course, also account for the differences between the results of the present experiment and those of Zimmerberg et al. (1974) and Dark et al. (1984), as well as the Vmax data in Chapter Two. According to the two population model

the results of these experiments would simply depend on the proportion of Contra>Ipsi and Ipsi>Contra males and females that happened to be sampled in the various experiments. Taken together, the data from these experiments indicate that while females are more likely than males to have higher DA concentrations in the contralateral striatum (i.e., to be Contra>Ipsi rats; see Table 3.6) both males as well as females can have DA concentrations higher in either the contralateral or ipsilateral striatum; and the results of any one particular experiment would depend on the proportion of Contra>Ipsi and Ipsi>Contra rats that happened to be sampled in that experiment. Before this conclusion can be reached with confidence, however, additional experimental evidence in support of the two population model is needed.

Two additional asymmetries that have been reported previously were not replicated in the present study. First, Jerussi et al (1977) found higher DOPAC levels in the ipsilateral striata of a group of females previously tested for rotational behavior either nocturnally or in response to amphetamine. It is unlikely that the previous amphetamine treatment in some of the rats was responsible for the DOPAC asymmetry since the large group of females to be described later (Chapter Five) was treated (twice) with amphetamine and no striatal DOPAC asymmetry was detected. Either breeder differences or the two population model, as discussed above, could account for the discrepancy in these results.

The other striatal asymmetry that was not replicated in the present study was a left>right 5HT asymmetry reported by

TABLE 3.6. Rats from three experiments classified according to whether the contralateral ("Contra>Ipsi") or ipsilateral ("Ipsi>Contra") cerebral hemisphere contained the striatum with the higher DA concentration or Vmax for DA uptake. In addition to the two experiments reported in this volume, the one by Dark et al. (1984) is the only one in the literature in which data are reported for rats of both sexes that were, aside from the determination of side preferences, experimentally naive.

		Contra>Ipsi Rats	Ipsi>Contra Rats	Total
Source: Chapter Two Breeder: Perfection Chi-Squared = 6.07; p<0.025	Males	6 (32%)	13 (68%)	19
	Females	19 (73%)	7 (27%)	26
	Total	25 (56%)	20 (44%)	45
Source: Chapter Three Breeder: Zivic-Miller Chi-Squared = 5.59; p<0.025	Males	6 (38%)	10 (62%)	16
	Females	11 (73%)	4 (27%)	15
	Total	17 (55%)	14 (45%)	31
Source: Dark et al. (1984) Breeder: Simonsen Chi-Squared = 0.04; p>0.75	Males	8 (57%)	6 (43%)	14
	Females	7 (47%)	8 (53%)	15
	Total	15 (52%)	14 (48%)	29
Above Three Experiments Combined Chi-Squared = 5.74; p<0.025	Males	20 (41%)	29 (59%)	49
	Females	37 (66%)	19 (34%)	56
	Total	57 (54%)	48 (46%)	105

Rosen et al. (1984) in a group of male and female (combined) Purdue-Wistar rats. Possible explanations for the difference in these 5HT results include the difference in strain and the differences in the behavioral testing animals received prior to sacrifice.

The Two Population Model: Correlations With Nocturnal Circling?

Based on the results of the uptake experiment described in the preceding chapter, five statistically significant correlations between nocturnal circling and striatal DA asymmetry were expected in the present experiment. As summarized in Table 3.7, none of these five correlations was significant. The simplest explanation would be that DA concentrations and Vmax for DA uptake are not related closely enough for statistically significant correlations to have been expected. After all, the neurochemical-behavioral correlations detected in the uptake experiment, while statistically significant, were not very high. Another possibility is that the lack of any significant correlations in the present study was due to the considerably smaller range in both net rotations and % preference scores in the present experiment (Table 3.7). If the correlations with the Vmax asymmetries found in Chapter 2 were to become non-significant when rats with behavioral scores outside those found in the present experiment are excluded from the analysis, then it would argue against ruling out the possibility that these correlations also would have been significant with the DA

TABLE 3.7. Comparison of the neurochemical-behavioral correlations in the previous (Chapter Two) and the present (Chapter Three) experiments. Striatal DAergic asymmetry was measured with Vmax ratios in the previous experiment and with DA concentration ratios in the present experiment. None of the statistically significant correlations from the previous experiment were found in the present experiment. However, the correlations from the previous experiment are no longer statistically significant when recalculated using only those rats whose behavioral scores were within the range of those used in the present experiment (right-most column).

Correlation		Chapter Two	Chapter Three	Chapter Two
Contralateral Side/ Ipsilateral Side vs. Preference for "Contra>Ipsi" Rats only [Fig. 2.2].	Preference Range:	54-93	51-76	54-75
	N:	25	17	20
	r:	0.45	0.30	0.23
	p:	<0.025	>0.20	>0.20
	Slope:	0.0033	0.0021	0.0018
	Intercept:	0.889	0.930	0.984
Contralateral Side/ Ipsilateral Side vs. Preference for "Ipsi>Contra" Rats only [Fig. 2.2].	Preference Range:	54-94	52-80	54-80
	N:	20	14	14
	r:	-0.49	-0.36	-0.21
	p:	<0.025	>0.20	>0.20
	Slope:	-0.0030	-0.0023	-0.0017
	Intercept:	1.097	0.787	1.003
High Side/Low Side vs. Preference for all rats combined [Fig. 2.3].	Preference Range:	54-94	51-80	54-80
	N:	45	31	34
	r:	0.50	0.02	0.25
	p:	<0.001	>0.5	>0.1
	Slope:	0.0039	0.0001	0.0023
	Intercept:	1.061	1.058	0.957

TABLE 3.7 (cont'd).

		Chapter Two	Chapter Three	Chapter Two
Correlation				
High Side/Low Side vs. Net Rotations for Females only [pp 32-33].	Net Rotations Range:	9-276	1-53	9-49
	N:	26	15	16
	r:	0.43	0.02	0.25
	p:	<0.03	>0.50	>0.10
	Slope:	0.0005	0.0001	0.0023
	Intercept:	1.092	1.058	0.957
High Side/Low Side vs. Net Rotations for Males only [pp 32-33].	Net Rotations Range:	1-105	2-45	2-41
	N:	19	16	16
	r:	0.61	-0.39	0.26
	p:	<0.01	>0.10	>0.20
	Slope:	0.0022	-0.0020	0.0016
	Intercept:	1.088	1.112	1.098

asymmetries in this experiment had rats with a large enough range of turning scores been studied. This indeed turned out to be the case: as shown in Table 3.7, none of the five correlations is statistically significant after removing those rats with turning scores outside the corresponding range from the present experiment.

The Two Population Model and % Turning.

In the preceding chapter % turning scores were higher in Ipsi>Contra than in Contra>Ipsi males; and were lower in Ipsi>Contra than in Contra>Ipsi females. By contrast, in the present experiment, with rats designated "Contra>Ipsi" or "Ipsi>Contra" based on striatal DA content, % turning scores were higher in the "Ipsi>Contra" rats as a whole, but significantly higher only for the females (Table 3.2). These two sets of results are completely different and they either suggest that there is an interesting difference between breeders, or, more likely, that % turning is not a very useful behavioral parameter with which to differentiate "Contra>Ipsi" and "Ipsi>Contra" rats.

The Two Population Model: DA Content in Contralateral and Ipsilateral Striatum.

Figure 3.1 depicts the mean contralateral and ipsilateral Vmax values of the Contra>Ipsi and Ipsi>Contra rats from the preceding chapter (males and females combined). As shown in the figure, while the mean ipsilateral Vmax was virtually identical in the two groups of rats (differing by

3%), the mean contralateral Vmax values were significantly different (25%). It was this result which (i) provided additional evidence for the existence of two populations of rats, and (ii) suggested that there may be a positive correlation between the contralateral Vmax and the contralateral-ipsilateral Vmax difference, which there was ($r=0.51$, $p<0.001$).

Figure 3.2 depicts the relationship between the mean contralateral and ipsilateral DA levels of the "Contra>Ipsi" and "Ipsi>Contra" rats used in the present study. The similarity between the graphs in Figures 3.1 and 3.2 is striking. Statistically, the results were also essentially identical: While the mean ipsilateral DA levels differed very little (1%) the mean contralateral DA levels differed significantly by 14% in the two groups of rats ($p<0.05$). In addition, as was the case for the Vmax values the contralateral DA concentration was positively correlated with the difference between the contralateral and ipsilateral DA levels ($r=0.47$, $p<0.01$), while there was no such correlation for the ipsilateral DA concentration ($r=0.17$, ns).

In light of the marked differences between the rotational behavior of the two groups of rats studied in the two experiments, the similarity between the results depicted in Figures 3.1 and 3.2 was somewhat surprising; nevertheless, it constitutes critical evidence in support of the proposed two population model. It suggests that Ipsi>Contra and Contra>Ipsi rats were, in fact, present in both experiments despite the fact that the mean rotational behavior was

greater in the uptake experiment; that is, that regardless of all the additional factors -- besides striatal DA side differences -- that ultimately determine a group of rats' rotational behavior, some rats will show the "Contra>Ipsi" pattern of DA asymmetry (Figure 3.2, left side) and some rats will show the "Ipsi>Contra" pattern (Figure 3.2, right side). This prediction was tested by selecting those rats from the uptake experiment whose net rotations and % preference scores were within the range observed in the present experiment, and again comparing the Contra>Ipsi and Ipsi>Contra rats with respect to their mean contralateral and ipsilateral Vmax values. Sixteen males and 14 females were included in this re-analysis. Compared to the results summarized in Figure 3.1, the results of this re-analysis, as shown in Figure 3.3, were essentially unaffected: Again, the mean ipsilateral Vmax's were virtually identical (differing by 1%) while the mean contralateral Vmax's differed significantly, by 22% ($p<0.01$). In addition, the contralateral-ipsilateral difference between Vmax's was still positively correlated with the contralateral Vmax ($r=0.50$, $p<0.005$), but not with the ipsilateral Vmax ($r=0.08$, ns). These results suggest that Ipsi> Contra and Contra>Ipsi rats are present in any group of rats regardless of how much turning they may do and regardless of how large the overall striatal DA asymmetry may be.

The Two Population Model: Tentative Extrapolation to Humans.

Since both the striatal Vmax and DA concentration data yielded similar contralateral>ipsilateral=ipsilateral>contra-

lateral patterns, an initial attempt was made to determine if the same kind of patterns might be present in the human brain. DA concentrations from the left and right caudate, putamen, and globus pallidus of 14 humans were obtained from the data generated by Rossor et al. (1980) and reanalyzed by Glick et al. (1982). For each of the three structures, subjects were divided into two groups -- Left>Right and Right>Left -- based on whether the left or right side had the higher DA concentration; then, the mean left and right DA concentrations were determined for the two groups, and the results are summarized in Figures 3.4 (caudate), 3.5 (putamen), and 3.6 (globus pallidus). While the number of subjects included in the groups is very small the relationship between the right and left sides in the two groups must be considered highly reminiscent of the relationships depicted in Figures 3.1-3.3, especially in putamen, where the right sides are significantly different ($p<0.05$). These results suggest that the contralateral and ipsilateral hemispheres in rats and the right and left hemispheres in humans seem to share common characteristics, at least with respect to DAergic function in the basal ganglia. For example, it seems the variability on the ipsilateral side of the rats, and the right side of the humans was larger than on the contralateral and left sides, respectively. Perhaps the development of certain characteristics is more tightly regulated in one hemisphere than in the other. Why this might

(text continues on pg 87)

FIGURE 3.1. Comparison of the two proposed populations of rats from Chapter Two with respect to their mean (±SEM) contralateral and ipsilateral Vmax values (pmol DA/min/mg protein). This is essentially the same graph as that pictured in Fig. 2.3 except that the sexes are pooled. The contralateral sides are significantly different ($p<0.0025$, Scheffe test for unplanned, post-hoc comparisons following significant ($p<0.002$) ANOVA (Snedecor and Cochran, 1967, pg 271)).

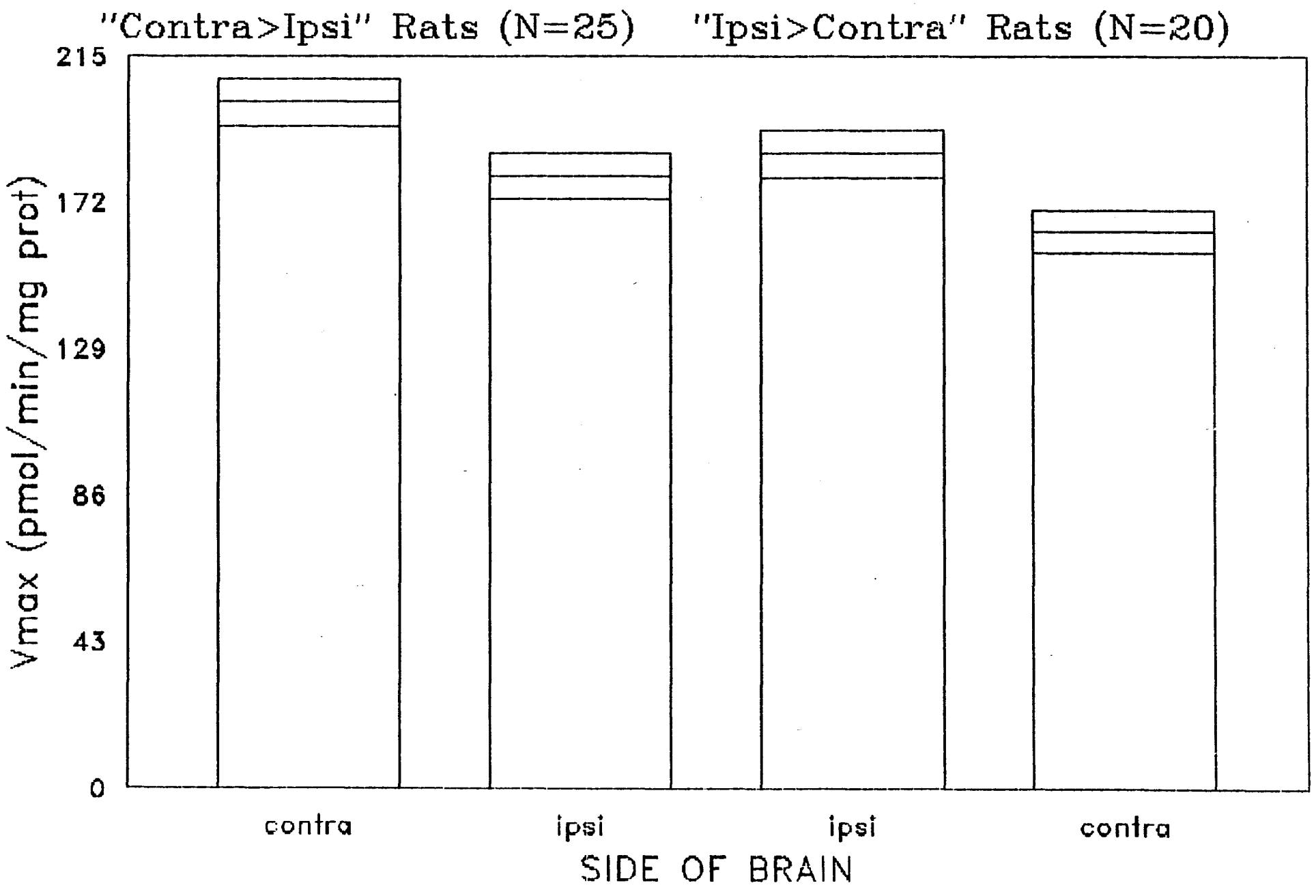

"Contra>Ipsi" Rats (N=25)
"Ipsi>Contra" Rats (N=20)
215
172
129
86
43
0
Vmax (pmol/min/mg prot)
contra
ipsi
ipsi
contra
SIDE OF BRAIN

FIGURE 3.2. Comparison of the two proposed populations of rats from the present chapter with respect to their mean contralateral and ipsilateral DA concentrations (ug/gm). The contralateral sides are significantly different ($p<0.05$, t test).

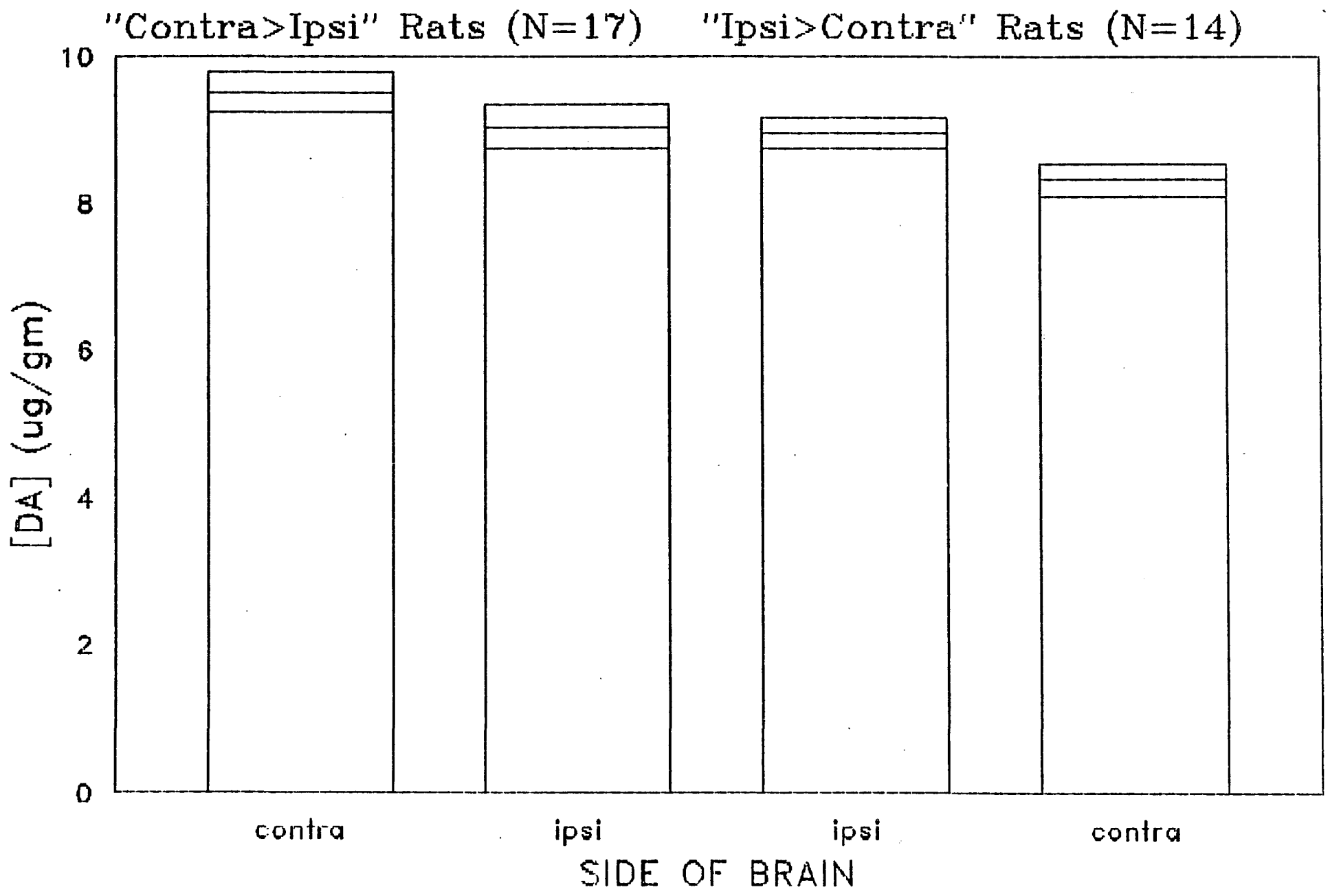

"Contra>Ipsi" Rats (N=17)
"Ipsi>Contra" Rats (N=14)
10
8
6
4
2
0
[DA] (ug/gm)
contra
ipsi
ipsi
contra
SIDE OF BRAIN

FIGURE 3.3. Using only those rats from Chapter Two whose rotational behavior was within the range of the rats from the present chapter, comparison of the two populations of rats from Chapter Two with respect to their mean (±SEM) contralateral and ipsilateral Vmax values (pmol DA/min/mg protein). The contralateral sides are still significantly different ($p<0.05$, t test).

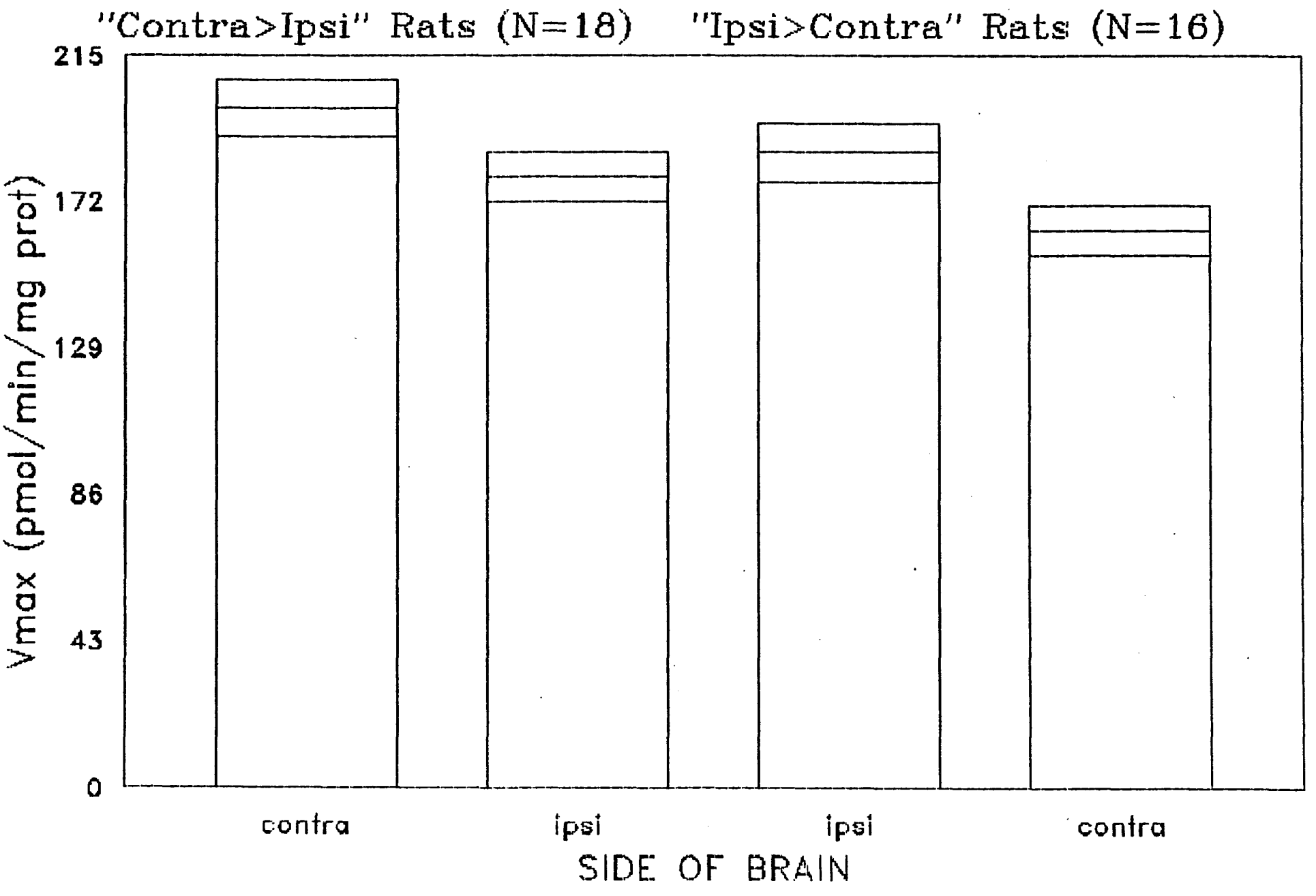

"Contra>Ipsi" Rats (N=18)
"Ipsi>Contra" Rats (N=16)
215
172
129
86
43
0
Vmax (pmol/min/mg prot)
contra
ipsi
ipsi
contra
SIDE OF BRAIN

FIGURE 3.4. Comparison of two groups of humans with respect to their mean (±SEM) right and left caudate nucleus DA concentrations. Subjects were assigned to the "Right>Left" or "Left>Right" groups based on whether the right or the left caudate had the higher DA concentration.

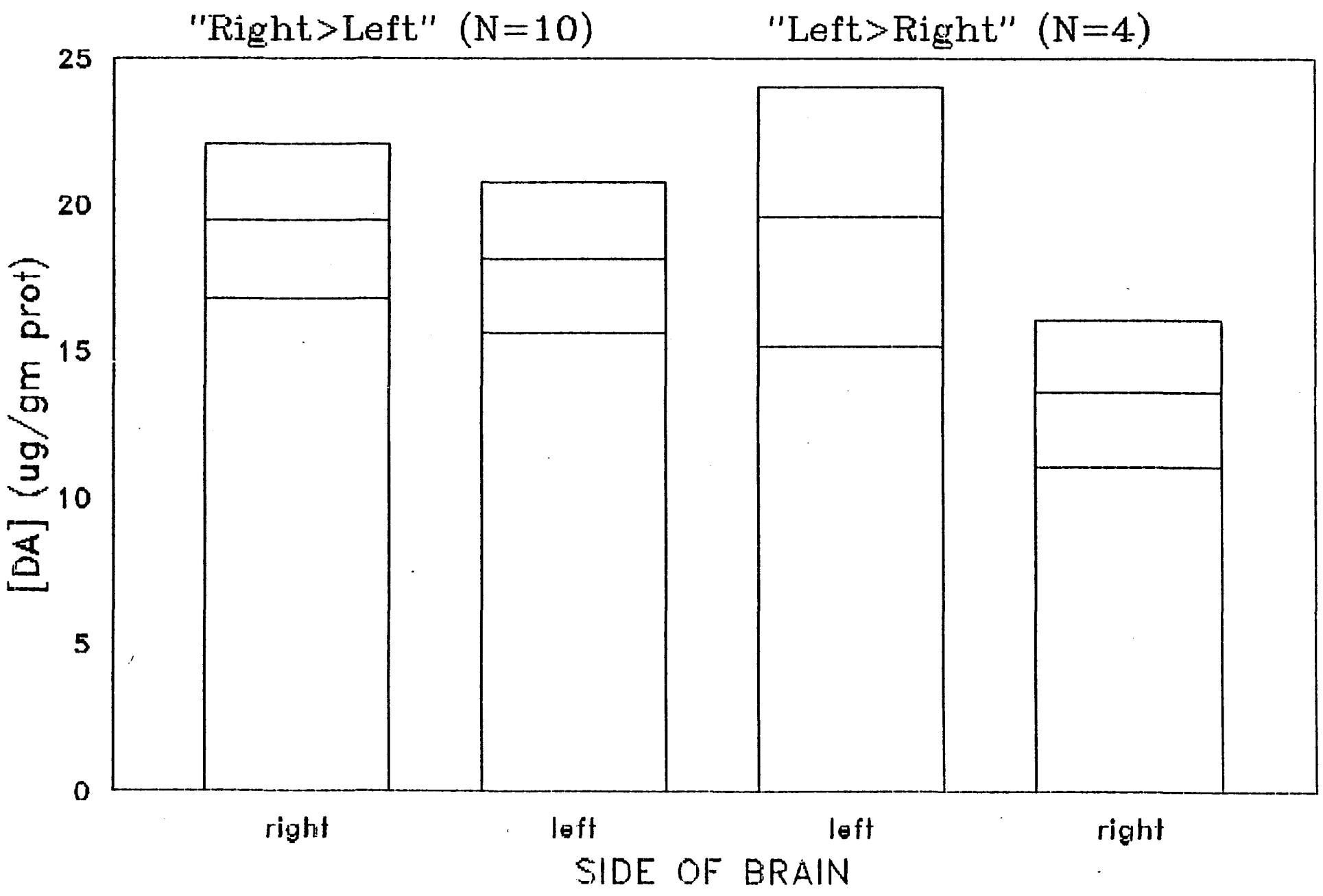

"Right>Left" (N=10)
"Left>Right" (N=4)
25
20
15
10
5
0
[DA] (ug/gm prot)
right
left
left
right
SIDE OF BRAIN

FIGURE 3.5. Comparison of two groups of humans with respect to their mean (±SEM) right and left putamen DA concentrations. Subjects were assigned to the "Right>Left" or "Left>Right" groups based on whether the right or the left putamen had the higher DA concentration. The right sides are significantly different ($p<0.05$, t test).

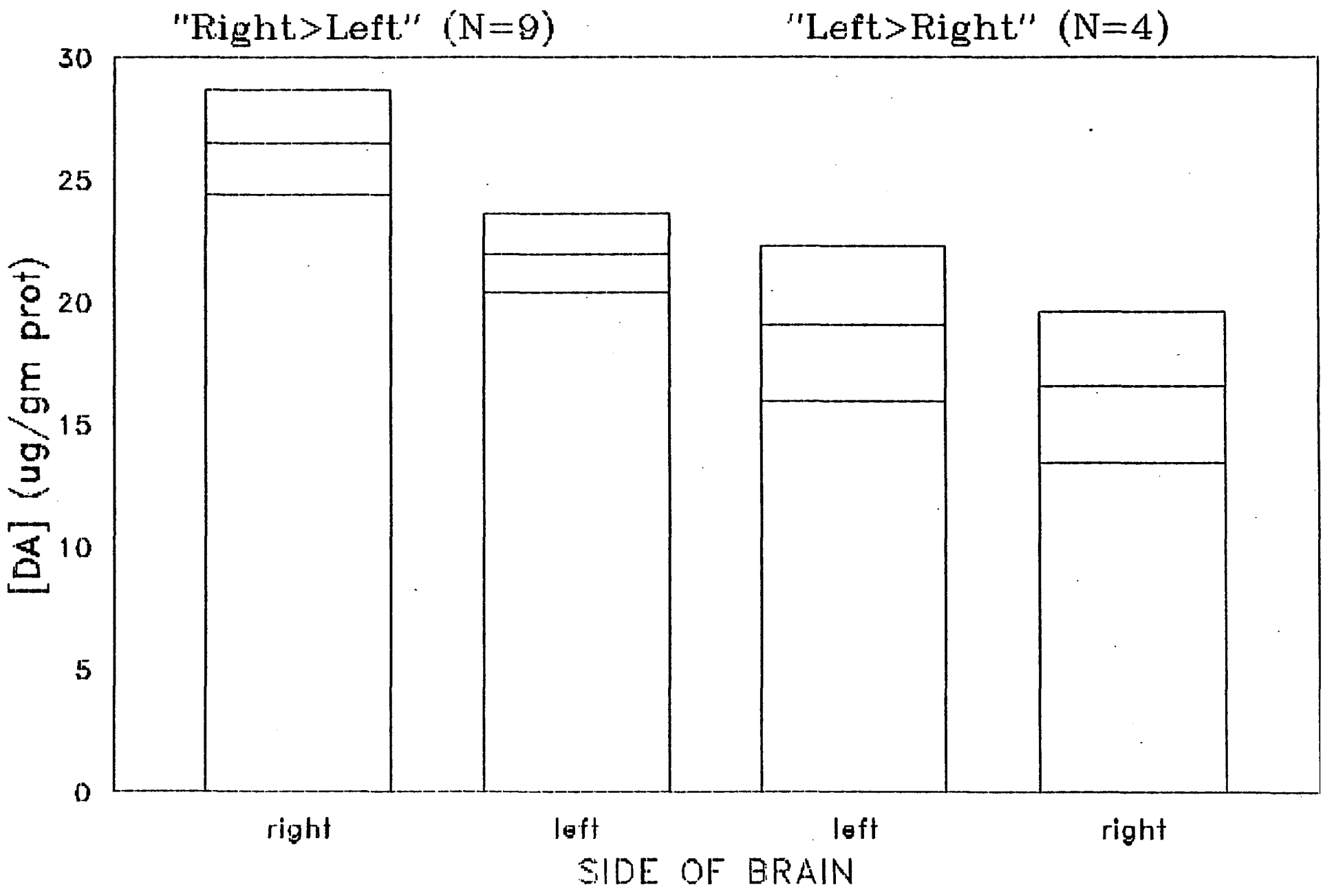
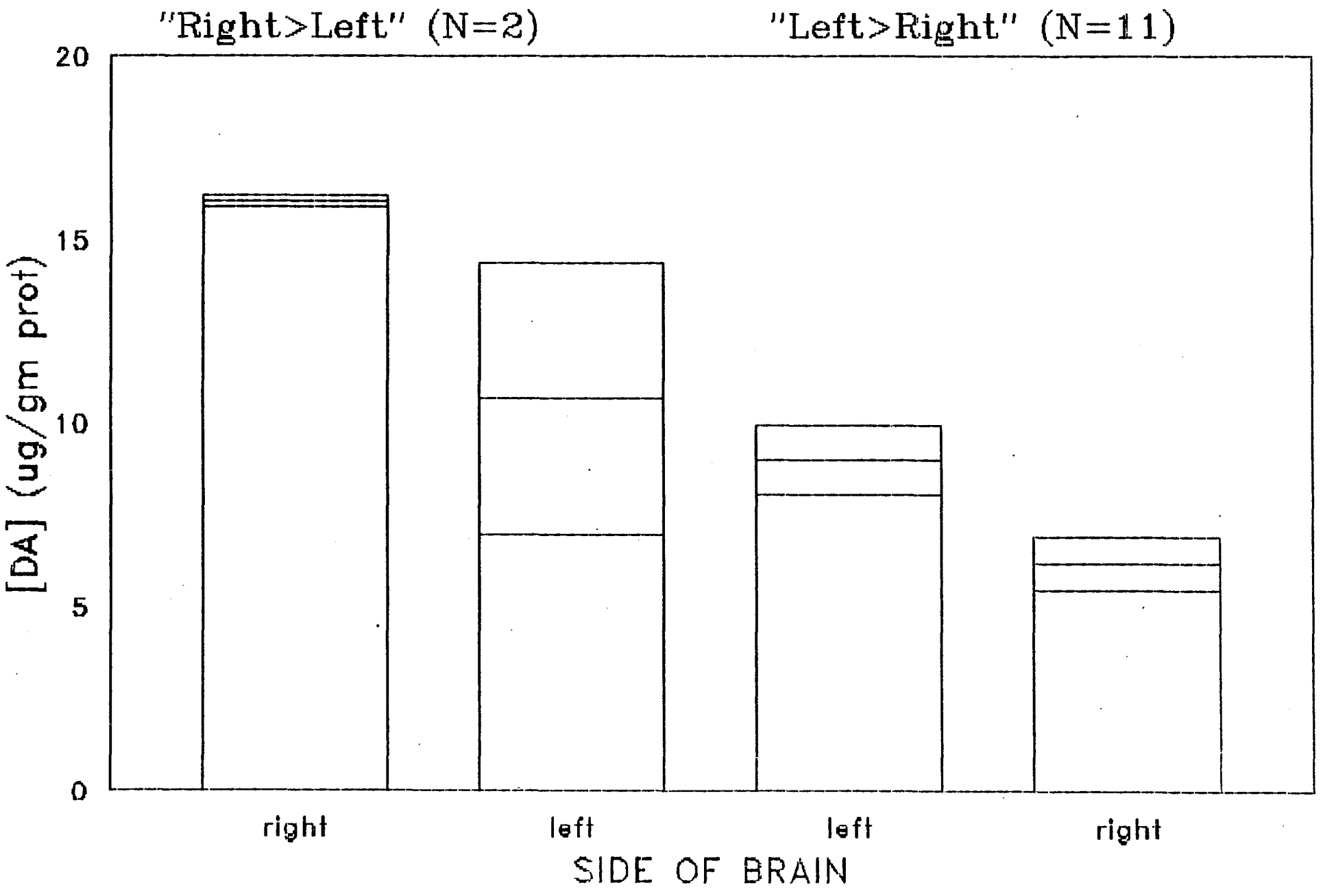

"Right>Left" (N=9)
"Left>Right" (N=4)
30
25
20
15
10
5
0
[DA] (ug/gm prot)
right
left
left
right
SIDE OF BRAIN

FIGURE 3.6. Comparison of two groups of humans with respect to their mean (±SEM) right and left globus pallidus DA concentrations. Subjects were assigned to the "Right>Left" or "Left>Right" groups based on whether the right or the left globus pallidus had the higher DA concentration.


"Right>Left" (N=2)
"Left>Right" (N=11)
20
15
10
5
0
[DA] (ug/gm prot)
right
left
left
right
SIDE OF BRAIN

occur is, of course, unclear at this time. One possiblity, suggested by the correlation between the contralateral DA level and the contralateral-ipsilateral difference, is that the ipsilateral side completes its development only after the contralateral side has completed its development, and it does so in a such way that some optimal bilateral level of DAergic function is maintained. Such a mechanism might be expected to operate in other paired nuclei as well.

The Two Population Model: Neurochemical Differences Between "Contra>Ipsi" and "Ipsi>Contra" Rats.

The basic tenet of the proposed two population model is that some rats circle predominantly toward the side with the greater DA innervation and some rats circle predominantly away from the side with the greater DA innervation. The results of the present study, as discussed above, provide qualified support for the model. Assuming that the model is valid, an important question to address is why the behavior of Ipsi>Contra and Contra>Ipsi rats is different; that is, given DA asymmetries of similar magnitudes, why does one group of rats circle in one direction and the other group in the other direction. Certainly, as discussed above, the striatal DA concentration on the contralateral side is different in the two groups of rats; but in the present experiment other neurochemical differences between the two groups were detected (Tables 3.3 and 3.5), and these may eventually shed additional light on why the two groups of rats behave as they do. The ratios of both DA metabolites --

DOPAC and HVA -- to DA were higher bilaterally in the striata of "Ipsi>Contra" than "Contra>Ipsi" rats. If these ratios reflect turnover rates (Roth et al., 1977; Hefti et al., 1985) then this result indicates that DA turnover is greater in both striata of "Ipsi>Contra" rats. One possible correlate (since it is impossible to know which is cause and which is consequence) of such a difference may be differences between the two groups in the number, type and/or ratio of pre- and post-synaptic striatal DA receptors; and such a possibility is certainly amenable to experimental verification.

The other three significant effects emerging from the analyses summarized in Tables 3.3 and 3.5 were interaction effects -- i.e., between "Contra>Ipsi" and "Ipsi>Contra" "populations" and side of the brain. Interestingly, the same interaction was significant for DOPAC/DA in both the striatum and the PFC, which suggests that similar regulatory mechanisms may be responsible in both structures. Impossible to interpret is the significant striatal 5HT interaction since the role of striatal 5HT in circling is still unclear. Still, this difference between the two "populations" is worth noting since we will refer to it again in the next chapter -- the same interaction term will emerge when two groups of rats whose behavioral responses to 6OHDA were different are compared.

Is the Distinction Between "Contra>Ipsi" and "Ipsi>Contra" Rats the Result of a Dissection Artifact?

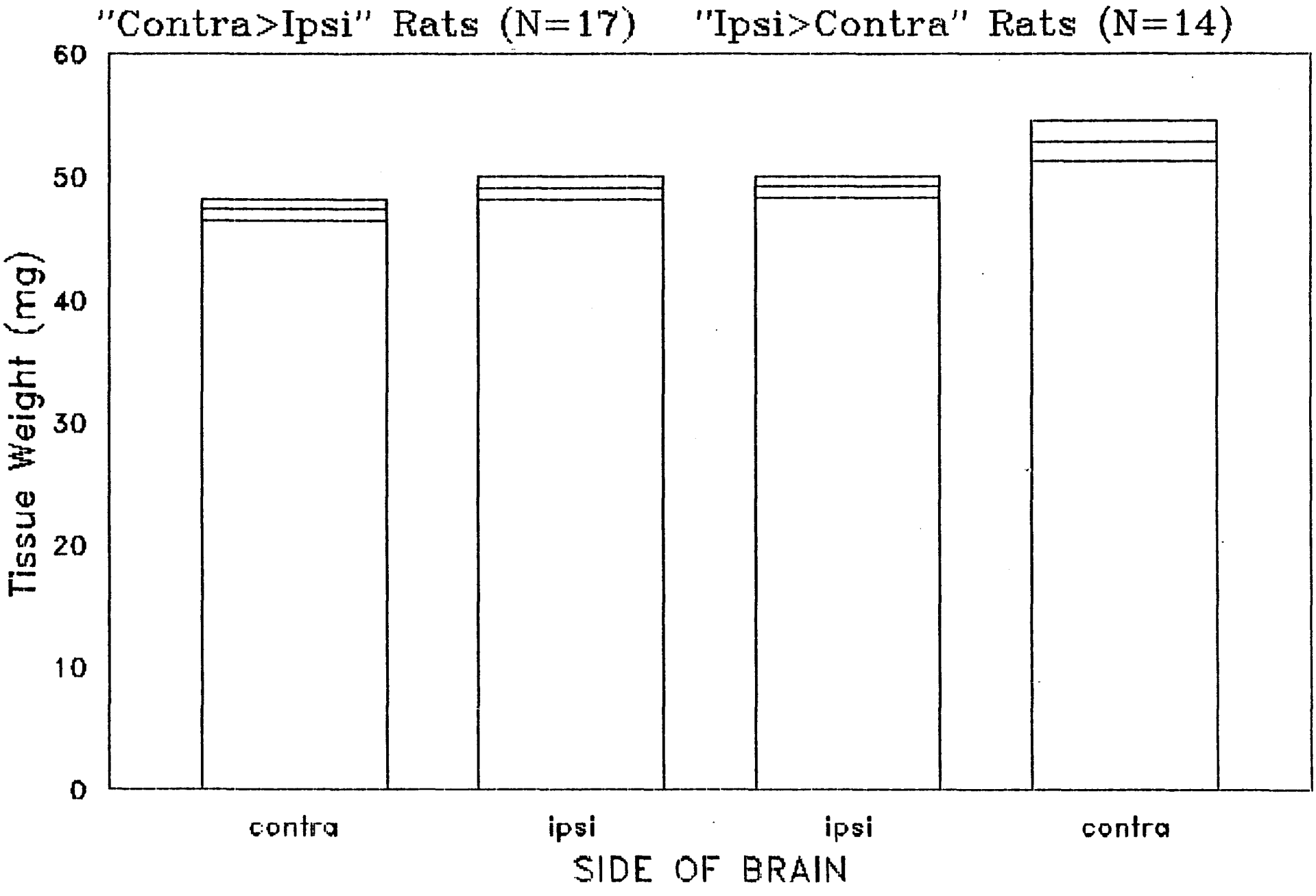
Two separate, though related, results suggested that the classification of the two "populations" in the present experiment -- i.e., on the basis of whether the contralateral or ipsilateral striatum had the higher DA concentration -- may have had more to do with side differences in the dissections than in actual differences between the striata in DA concentration. First, it was found that the observed striatal DA asymmetry was very highly correlated ($r=-0.91$, $p<0.001$) with the striatal dissection asymmetry; in other words, when there was no difference between the weights of the dissected striata there was also no difference between the DA concentrations in the two striata. Second, while the weights of the ipsilateral striata did not differ in the two "populations," the contralateral striata of the "Ipsi>Contra" rats were significantly heavier than those of the "Contra> Ipsi" rats (Figure 3.7). Thus, either the dissections were accurate enough to detect true differences between the weights of the contralateral striata in the two "populations," or, alternatively, an unexpected bias was somehow introduced into the dissection procedure which resulted in the observed, presumably spurious, difference between the two "populations." It can be argued that since similar correlations were not observed for DA in PFC, or for 5HT in striatum or PFC, the dissections were, in fact, accurate; still, due to the nature of brain dissection, this result is, admittedly, difficult to believe without corroborative data.

This issue will be addressed experimentally in the next chapter.

Neurochemical Asymmetry in Medial Prefrontal Cortex.

Using male rats from a Wistar-derived strain, Slopsema et al. (1982) previously reported a left>right DA asymmetry in medial PFC. While their method of dissection employed two micropunches per side on frozen tissue my dissection included a much larger piece of tissue. Still, to the extent that they can be compared, the present result and that of Slopsema et al. were identical: While they did not report their mean left/right DA asymmetry, their mean left DA concentration was 46.3% greater than the mean right DA concentration, which is the same as our comparable result. Nevertheless, these two results conflict with those of TE Robinson et al. (1980) who found no left-right DA asymmetry in medial frontal cortex in Sprague-Dawley-derived Holtzman rats. These discrepant results may be due to strain differences; however, since the DA concentrations reported by Robinson et al. are approximately 30% of those reported here, it is also possible that their dissections were larger than mine (tissue weights were not reported) thereby including more DA-poor tissue, which might have effectively obscured an asymmetry that was actually present. There is also another possibility: In light of the known involvement of mesocortical DA neurons in stress responses (Glowinski et al., 1984) it is tempting to speculate that the lack of a DA asymmetry in the study by TE Robinson et al. was related to the stress that accompanied

FIGURE 3.7. Comparison of the two proposed populations of rats from the present chapter with respect to their mean (±SEM) contralateral and ipsilateral corpus striatum weights (mg). The contralateral sides are significantly different ($p<0.05$, t test).


"Contra>Ipsi" Rats (N=17)
"Ipsi>Contra" Rats (N=14)
60
50
40
30
20
10
0
Tissue Weight (mg)
contra
ipsi
ipsi
contra
SIDE OF BRAIN

the saline injection and behavioral testing each of their rats received immediately prior to sacrifice; perhaps the two PFCs are affected differently by stress.

CHAPTER FOUR

ANATOMICAL SUPPORT FOR THE TWO POPULATION MODEL

With one potentially significant qualifier, the results of the experiment described in the previous chapter provide considerable support for the proposed two population model of rat rotational behavior. That qualifier concerns the accuracy of the striatal dissections. Rats in the previous experiment were classified as belonging to "Contra>Ipsi" or "Ipsi> Contra" groups depending on which striatum had the higher DA concentration (ug/gm wet weight). Based on the analogous result of the uptake experiment described in Chapter 3, it was predicted that while the mean ipsilateral DA concentrations would not differ in the two groups of rats, the mean contralateral DA concentration would be significantly higher in the "Contra>Ipsi" group. This prediction was confirmed experimentally (Figure 3.2). What was totally unexpected was that the same relationship, in reverse, would exist for striatum weights: the mean contralateral striatum weight was significantly higher in the "Ipsi>Contra" rats than in the "Contra>Ipsi" rats, while the mean ipsilateral weights for both groups were the same (Figure 3.7). Thus, it was important to determine with greater certainty whether (i) the dissections were, in fact, accurate and there actually is a relationship between the size of the two striata and the direction of rotation; or (ii) the dissections in the previous experiment were, for unknown reasons, biased with respect to animals' turning preferences. If this latter possibility were the case, the accuracy of the previous experiment's results would have to be questioned.

MATERIALS AND METHODS

The present experiment actually antedated the other work in this volume; it was originally carried out for reasons unrelated to the two population model. Young adult rats (14 females, 13 males; Perfection Breeders, Douglassville, PA) were tested once for nocturnal rotational behavior and decapitated within two weeks. Histological sections were prepared according to the procedure described by Shapiro et al. (1983): The brain is removed from the skull and placed on a microscope slide, ventral suface down with the long axis parallel to that of the slide. It is quick-frozen by carefully submerging the slide, with the brain on it, into a 60 ml beaker containing CCl_2F_2 (UCON 12, Union Carbide, NY) for 45 sec. The slide (with the brain now temporarily frozen onto it) is placed onto the variable inclined plane described by Herberg and Franklin (1973) and a cut is made anterior to the cerebellum. The brain is then taken off the slide and mounted on a chuck in a cryostat (-15°C). Serial 20 um coronal sections are made, dried on a Fisher (#12-594) slide warmer at 40°C, and stained with Sudan black B and neutral red.

The circumference of left and right caudate-putamen and globus pallidus was traced onto white paper from enlarged (X15) projections of the sections, and the areas of the tracings were then measured with a compensating polar planimeter (Keuffel and Esser, Teterboro, NJ, #62-0005). Cross-sectional areas were measured at fifteen evenly spaced levels

of the corpus striatum, but for this study the data from only two of those levels were analyzed (Figures 4.1a and b).

At each level rats were classified as belonging to one of two groups based on whether the contralateral or ipsilateral striatum had the larger area. Thus, some rats could -- and did -- belong to one group at one A-P level and the other group at the other A-P level. In order to minimize confusion these two groups were designated "Anat C>I" and "Anat I>C." This was done because the hypothesis under test was that the relationship between the mean contralateral and ipsilateral areas would parallel that between the contralateral and ipsilateral weights depicted in Figure 3.7, and, as previously discussed, this latter relationship was <u>opposite</u> to that found for the mean contralateral and ipsilateral DA concentrations. Thus, a "Ipsi>Contra" rat, classified on the basis of DA concentrations in the two striata, should correspond to an "Anat C>I" rat from the present experiment.

RESULTS

The data for the A-P levels shown in Figures 4.1a and 4.1b are summarized in Figures 4.2a and 4.2b, respectively. Six rats designated as "Anat C>I" rats at the first level (Fig. 4.1a) were "Anat I>C" rats at the second level (Fig. 4.1b); five rats designated as "Anat I>C" rats at the second level were "Anat C>I" rats at the first level. Thus, of the 22 rats included in Fig. 4.2b, 11 (50%) switched groups compared to Fig. 4.2a.

(text continues on pg 104)

FIGURE 4.1. Photographs of the two anterior-posterior levels from which corpus striatum areas were measured. The cross-section on top (Fig. 4.1a) corresponds to the A-P level 1.4 mm from the atlas of Pellegrino et al. (1979); on the bottom (Fig. 4.1b) the cross-section corresponds to A-P level 2.6 mm.

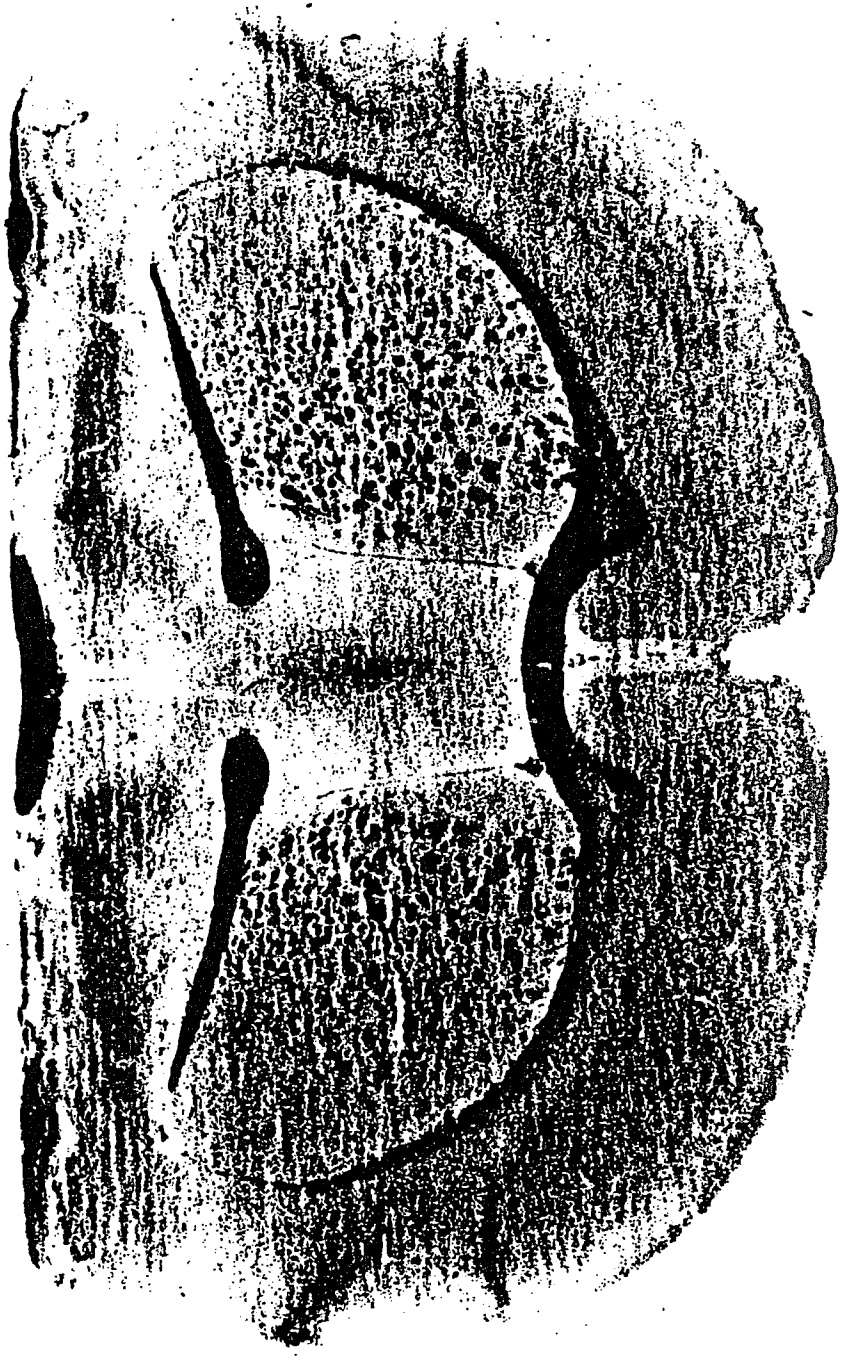
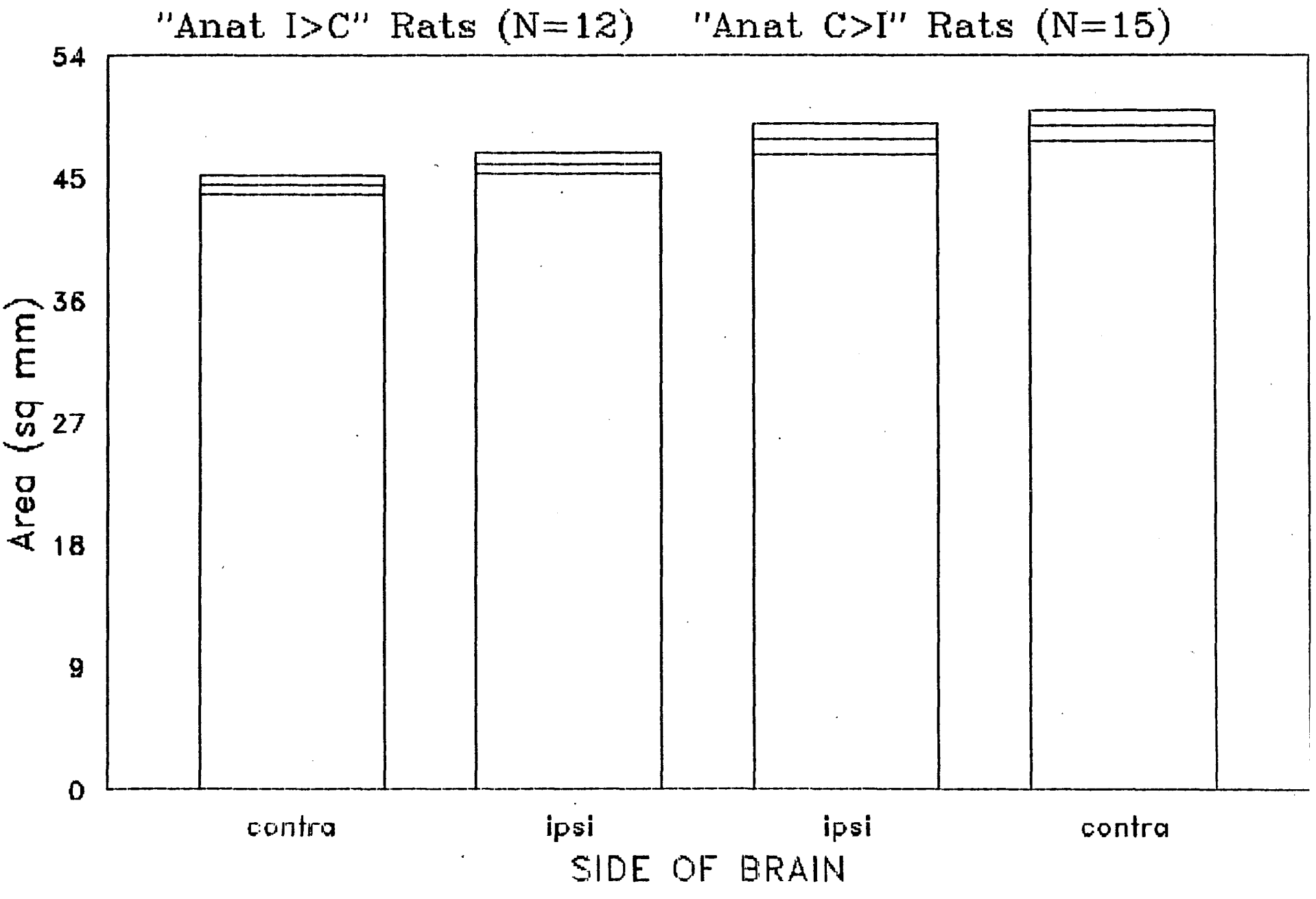
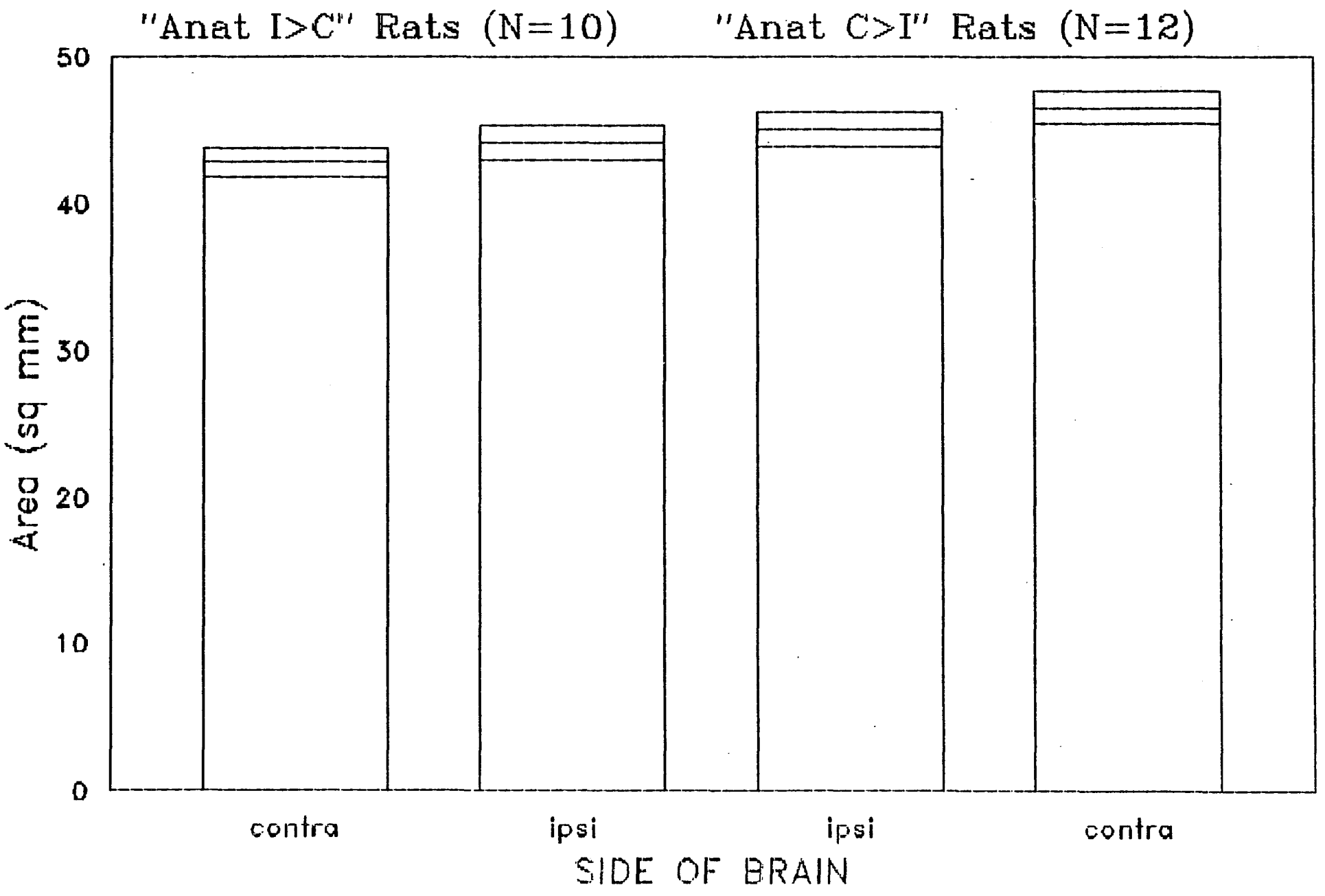
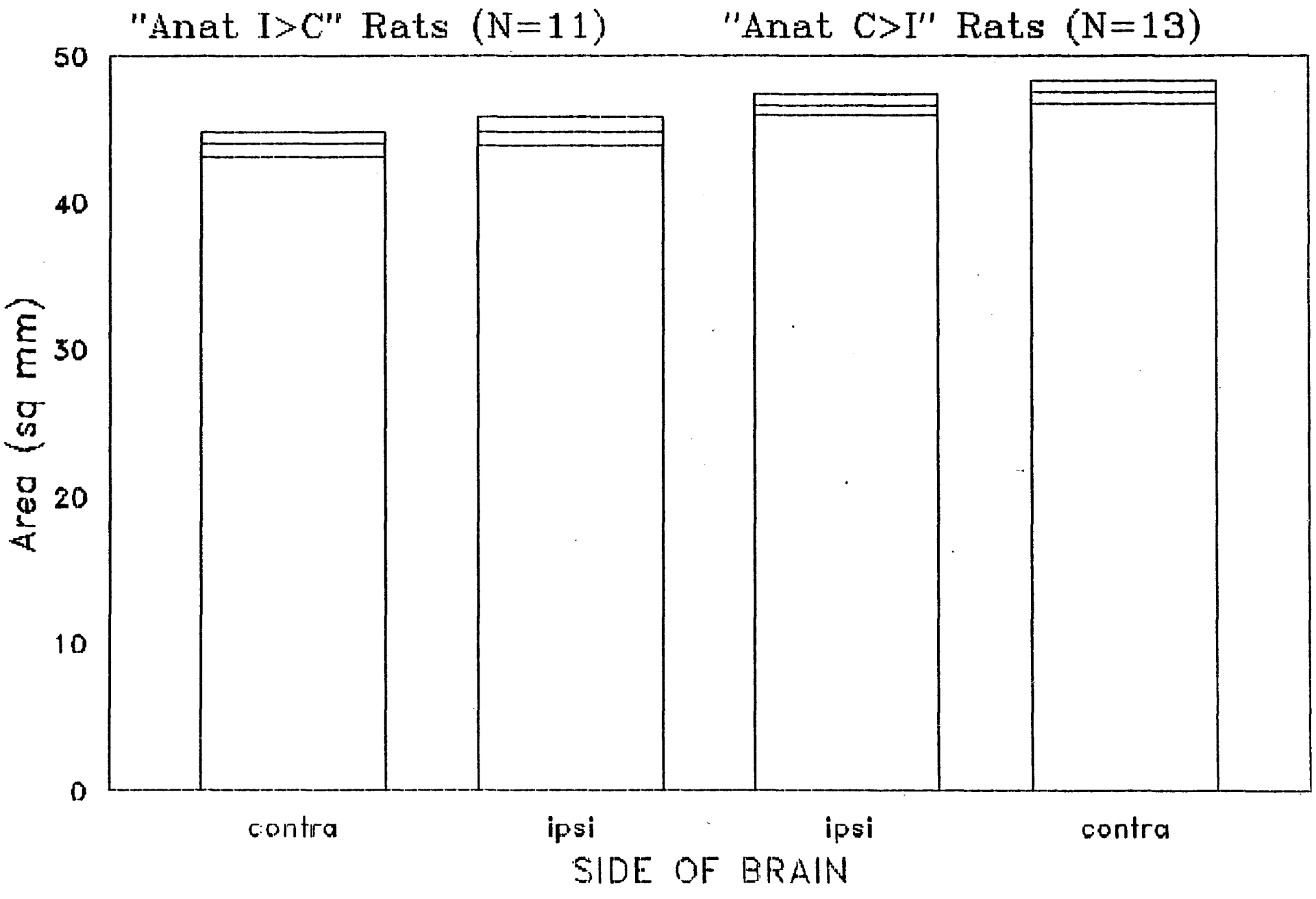


FIGURE 4.2. Mean (±SEM) areas of the contralateral and ipsilateral corpora striatum of rats belonging to either the "Anat C>I" or "Anat I>C" groups. The data in Figs. 4.2a and 4.2b (on the next page) correspond, respectively, to the A-P levels shown in Figs. 4.1a and 4.1b. At each level rats were defined as belonging to one or the other group based on which side had the larger area: the contralateral striatum has a larger area in the "Anat C>I" rats and the ipsilateral striatum has a larger area in the "Anat I>C." At both A-P levels the mean contralateral area of the "Anat C>I" group is significantly larger than that of the "Anat I>C" group ($p<0.05$ in both cases). In Fig. 4.2a the ipsilateral side of the "Anat C>I" group is also significantly larger than the contralateral side of the "Anat I>C" group ($p<0.05$). In Fig. 4.2b there are 5 fewer total rats because 2 were excluded for having equal areas on the two sides, and 3 had badly stained sections from which no tracings could be made. Of the 22 rats included in Fig. 4.2b, 11 are classified differently than they were in Fig. 4.2a.

(c) Mean (±SEM) areas of the contralateral and ipsilateral corpora striatum of rats assigned to either the "Anat C>I" or "Anat I>C" groups. Striatal areas from the two A-P levels were averaged and rats were assigned to one or the other group based on whether the contralateral or ipsilateral striatum had the larger mean area. The contralateral sides are significantly different ($p<0.05$) and the ipsilateral sides are not.


"Anat I>C" Rats (N=12)
"Anat C>I" Rats (N=15)
54
45
36
27
18
9
0
Area (sq mm)
contra
ipsi
ipsi
contra
SIDE OF BRAIN


"Anat I>C" Rats (N=10)
"Anat C>I" Rats (N=12)
50
40
30
20
10
0
Area (sq mm)
contra
ipsi
ipsi
contra
SIDE OF BRAIN


"Anat I>C" Rats (N=11)
"Anat C>I" Rats (N=13)
50
40
30
20
10
0
Area (sq mm)
contra
ipsi
ipsi
contra
SIDE OF BRAIN

For both levels studied the mean contralateral areas in the two groups of rats were significantly different ($p<0.05$ in both cases). The mean contralateral area was greater in the "Anat C>I" group by 9.9% and 8.6% at the first and second A-P levels, respectively. By contrast, the mean ipsilateral areas were 3.9% and 1.9% higher at the first and second A-P levels in the "Anat C>I" than in the "Anat I>C" group, respectively, and these differences were not significant. The correlation coefficients for the relationship between the contralateral area and the contralateral-ipsilateral difference were 0.44 ($p<0.03$) and 0.32 (ns) at the first and second A-P levels, respectively. For the relationship between the ipsilateral area and the contralateral-ipsilateral difference the respective correlation coefficients were 0.07 and -0.14 (both ns).

DISCUSSION

In the previous experiment (Chapter Three) the contralateral striatum of the "Ipsi>Contra" rats was found to be, on average, significantly heavier than the contralateral striatum of the "Contra>Ipsi" rats. The present analysis of previously collected data was done in order to determine whether the difference between the dissected striatal weights was real or whether it was a by-product of an unknown bias (e.g., poor dissection technique).

The present data agree nicely with the results of the dissections. These results indicate that (i) the contrala-

teral striatum of (DA-defined) "Ipsi>Contra" rats is, in fact, larger and heavier than the contralateral striatum of "Contra>Ipsi" rats, and (ii) the neurochemical results of the previous experiment are accurate.

The only questionable aspect about the present result stems from the fact that 50% of the rats switched groups at the two A-P levels. This suggests that these "switchers" had the smallest asymmetries at both levels, and that repeating the analysis using the average corpus striatum area should yield a result that is similar to those obtained in the analyses of the areas obtained at each individual A-P level. This expectation was confirmed. Figure 4.2c shows the results of using the mean areas to classify the rats: Again, the contralateral sides were different, the ipsilateral sides were not; the contralateral-ipsilateral difference was positively correlated with the contralateral side ($r=0.40$, $p=0.05$) and not with the ipsilateral side ($r=0.01$, ns).

In the following chapter the two population hypothesis will be tested further.

CHAPTER FIVE

EFFECTS OF UNILATERAL STRIATAL 6-HYDROXYDOPAMINE ON AMPHETAMINE-INDUCED ROTATION: FUNCTIONAL SUPPORT FOR THE TWO POPULATION MODEL

The present experiment employed the catecholaminergic neurotoxin, 6OHDA, in order to obtain more definitive evidence for or against the two population hypothesis. If the two population model has any validity then it would be predicted that the two groups of rats should respond differently to unilateral DA depletions in either the ipsilateral or the contralateral striatum. For example, in a Contra>Ipsi rat a lesion in the ipsilateral striatum would decrease the DA concentration in the ipsilateral striatum and therefore increase the endogenous contralateral>ipsilateral DA asymmetry. On the other hand the same exact lesion in the ipsilateral striatum of an Ipsi>Contra rat would, by reducing the DA concentration in the ipsilateral striatum, decrease or reverse the normal ipsilateral>contralateral striatal DA asymmetry. These neurochemical effects should, accordingly, have predictable behavioral correlates: The Contra>Ipsi rats should increase their turning in response to the ipsilateral lesions while the Ipsi>Contra rats should decrease -- or, possibly, even reverse the direction of -- their turning in response to the same lesions. Thus, the two population hypothesis predicts that in response to the same unilateral striatal lesion on the ipsilateral side (based on a preoperative test), some rats should increase their turning toward that side and some rats should decrease their turning towards that side. By contrast, based on current models of the relationship between striatal DA and circling behavior, it would be predicted that <u>all</u> rats with such lesions should

increase their turning toward the lesioned side.

The site to be lesioned in the present experiment was selected based on an experiment reported by Dunnett and Iversen (1982). The results of their experiment provide suggestive evidence for the notion that rats can decrease or reverse the direction of their circling in response to a unilateral striatal lesion. Dunnett and Iversen compared the effects on circling behavior of unilateral 6OHDA-induced lesions in six different regions of the rat striatum. They found no statistically significant differences between the number of net rotations made by each of the six groups in response to amphetamine ten days after lesioning. However, while five of the groups rotated predominantly toward the lesioned side, one group averaged more net rotations away from the lesioned side. Furthermore, while the behavioral effects of amphetamine are known to persist for more than 2-3 hours, Dunnett and Iversen tested their rats' rotational behavior for only twenty minutes following amphetamine administration. Since a rat's predominant direction of rotation rarely changes throughout the duration of a session, it was considered quite possible that statistically significant differences between group means would have emerged had the groups been allowed to continue to rotate for longer than twenty minutes. Even if significant differences between Dunnett and Iversen's groups had been found, though, explanations independent of the present two population hypothesis would have been possible (see Introduction, pg 21; TE Robinson et al., 1985); still, the site selected for lesioning in

the present study was the same dorso-medial region of the striatum that Dunnett and Iversen found resulted in net turning away from the lesioned side.

MATERIALS AND METHODS

The subjects were 106 male and 89 female Sprague-Dawley rats (Zivic-Miller) weighing 225-250 g at the start of the experiment. They were housed in groups of 2-4 in plastic cages with free access to food and water (lights on/off: 7:00 a.m./7:00 p.m.). Each rat was tested once for spontaneous nocturnal rotational behavior and net rotations, % preference, extra quarter turns, and % turning were calculated as described earlier.

One or two days following nocturnal testing, at approximately 10:00 a.m., the rats were tested for D-amphetamine-induced rotational behavior. Amphetamine was administered intraperitoneally after a fifteen minute habituation period and rotational behavior was recorded for two hours. Males (1.56 mg/kg) and females (1.25 mg/kg) were administered doses of amphetamine which Becker et al. (1982) showed result in approximately equal brain levels.

The next day rats were assigned to one of four treatments: no operation, vehicle only, or one of two doses of 6OHDA-HBr (1 or 6.2 ug, expressed as the salt, in 1 ul ice-cold 0.2 mg/ml ascorbate solution (Dunnett and Iversen, 1982), bubbled with N_2); the latter dose was the one used by Dunnett and Iversen. Injections were delivered with a 5 ul Hamilton syringe over a period of 6 min, with 3 additional

min allowed for diffusion of the solution before the syringe was slowly withdrawn (Dunnett and Iversen, 1982). The stereotaxic coordinates, A: 1.6, L: 2.4 V: -4.5 from dura (Pellegrino and Cushman, 1967), were chosen to match those of the "mid-dorsal CPU" lesions of Dunnett and Iversen (1982), and were confirmed in pilot work by localizing 1 ul injections of gentian violet. All intra-striatal injections were made on the same side towards which the rat made most of its turns in response to amphetamine the day before.

Rats were retested for amphetamine-induced rotation 7-8 days following surgery, and 7-12 days later striatal concentrations of DA, DOPAC, HVA, 5HT, and 5HIAA were measured as described earlier.

RESULTS

Neurochemical Effects of Unilateral 6OHDA Treatment

The effects of the unilateral intrastriatal administration of 6OHDA on the left and right striatal concentrations of DA, DOPAC, HVA, 5HT, and 5HIAA, and on the ratios DOPAC: DA, HVA:DA, and 5HIAA:5HT are presented in Table 5.1. Table 5.2 summarizes the side differences in these measures. Since there were no significant vehicle effects the unoperated and vehicle-only controls were pooled to form a single control group for both sexes.

Effects on DA, DOPAC, and HVA. A four factor ANOVA on striatal DA concentrations revealed that 6OHDA produced a

(text continues on pg 121)

TABLE 5.1. Effect of unilateral striatal injection of 6OHDA on mean (±SEM) striatal monoamine and metabolite concentrations (ug/gm); group N's in parentheses.

	Females				Males			
Direction of Rotation[1]	Left		Right		Left		Right	
Side[2]	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
Dose of 6OHDA (ug)	DA[3]							
Controls	10.489 ±.387	10.710 ±.348	10.743 ±.221	10.482 ±.237	9.781 ±.176	10.078 ±.196	9.695 ±.249	9.539 ±.229
	(17)		(26)		(35)		(24)	
1.00	10.738 ±.394	8.799 ±.396	10.356 ±.444	9.044 ±.452	9.468 ±.425	8.878 ±.393	10.118 ±.318	8.568 ±.390
	(9)		(8)		(11)		(12)	
6.24	10.557 ±.385	6.872 ±.414	10.775 ±.343	5.544 ±.454	10.113 ±.355	6.983 ±.299	9.892 ±.213	5.087 ±.392
	(14)		(14)		(10)		(14)	

[1]Based on first (i.e., pre-operative) test in response to amphetamine. On the following day operated rats received intra-striatal injections of 6OHDA or vehicle on the same side towards which they made most of their turns during this test.

[2]Based on first (i.e., pre-operative) test in response to amphetamine. The ipsilateral (Ipsi) side is the side towards which the rat made most of its turns, and the contralateral (Contra) side is the side away from which the rat made most of its turns; thus, all operated rats received intra-striatal 6OHDA or vehicle-only injections on the ipsilateral side.

[3]Significant main effects of sex ($p<0.001$), 6OHDA ($p<0.001$), and side ($p<0.001$); significant interactions: 6OHDA X side ($p<0.001$), direction of rotation X side ($p<0.001$), and 6OHDA X direction of rotation X side ($p<0.009$).

TABLE 5.1 (cont'd).

Direction of Rotation	Females				Males			
	Left		Right		Left		Right	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
Dose of 6OHDA (ug)	DOPAC[4]							
Controls	1.386 ±.066	1.454 ±.071	1.370 ±.044	1.338 ±.033	1.353 ±.039	1.388 ±.037	1.265 ±.053	1.243 ±.053
	(17)		(26)		(35)		(24)	
1.00	1.210 ±.061	1.040 ±.078	1.281 ±.034	1.135 ±.063	1.330 ±.077	1.259 ±.076	1.341 ±.046	1.122 ±.048
	(9)		(8)		(11)		(12)	
6.24	1.310 ±.046	0.873 ±.061	1.320 ±.077	0.756 ±.057	1.194 ±.065	0.822 ±.054	1.214 ±.045	0.689 ±.042
	(14)		(14)		(10)		(14)	
	DOPAC/DA[5]							
Controls	0.132 ±.004	0.136 ±.006	0.128 ±.003	0.129 ±.004	0.139 ±.004	0.139 ±.004	0.131 ±.005	0.130 ±.005
	(17)		(26)		(35)		(24)	
1.00	0.114 ±.006	0.118 ±.006	0.125 ±.004	0.126 ±.004	0.140 ±.004	0.142 ±.007	0.133 ±.004	0.132 ±.004
	(9)		(8)		(11)		(12)	
6.24	0.127 ±.007	0.129 ±.009	0.122 ±.005	0.141 ±.007	0.118 ±.005	0.118 ±.005	0.122 ±.002	0.140 ±.006
	(14)		(14)		(10)		(14)	

[4]Significant main effects of 6OHDA ($p<0.001$), and side ($p<0.001$); significant interactions: sex X 6OHDA ($p<0.035$), 6OHDA X side ($p<0.001$), and direction of rotation X side ($p<0.001$).

[5]Significant main effect of side ($p<0.001$); significant interactions: sex X 6OHDA ($p<0.02$), 6OHDA X side ($p<0.001$), and 6OHDA X direction of rotation X side ($p<0.001$); the direction of rotation X side interaction was marginally significant ($p<0.055$).

TABLE 5.1 (cont'd).

Direction of Rotation	Females				Males			
	Left		Right		Left		Right	
Side:	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
Dose of 6OHDA (ug)				HVA[6]				
Controls	0.884 ±.052 (16)	0.919 ±.050	0.887 ±.038 (26)	0.867 ±.035	0.833 ±.032 (35)	0.857 ±.030	0.794 ±.034 (24)	0.790 ±.037
1.00	0.880 ±.055 (9)	0.858 ±.056	0.901 ±.031 (8)	0.817 ±.039	0.822 ±.064 (11)	0.816 ±.058	0.819 ±.028 (11)	0.717 ±.029
6.24	0.953 ±.098 (14)	0.660 ±.064	0.953 ±.053 (14)	0.654 ±.050	0.871 ±.055 (10)	0.654 ±.039	0.787 ±.053 (14)	0.588 ±.082
				HVA/DA[7]				
Controls	0.085 ±.005 (16)	0.087 ±.004	0.083 ±.003 (26)	0.083 ±.003	0.085 ±.003 (35)	0.085 ±.002	0.082 ±.003 (24)	0.083 ±.004
1.00	0.082 ±.005 (9)	0.100 ±.009	0.088 ±.004 (8)	0.091 ±.003	0.087 ±.005 (11)	0.093 ±.006	0.081 ±.003 (11)	0.084 ±.003
6.24	0.090 ±.008 (14)	0.096 ±.007	0.089 ±.005 (14)	0.125 ±.010	0.086 ±.004 (10)	0.094 ±.005	0.079 ±.004 (14)	0.116 ±.009

[6]Significant main effects of sex ($p<0.009$), 6OHDA ($p<0.035$), and side ($p<0.001$); significant 6OHDA X sex interaction ($p<0.001$).

[7]Significant main effects of 6OHDA ($p<0.001$), and side ($p<0.001$); significant interactions: 6OHDA X side ($p<0.001$), direction of rotation X side ($p<0.008$), and 6OHDA X direction of rotation X side ($p<0.001$).

TABLE 5.1 (cont'd).

	Females				Males			
Direction of Rotation	Left		Right		Left		Right	
Side:	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
Dose of 6OHDA (ug)				5HT[8]				
Controls	0.728 ±.037	0.729 ±.036	0.676 ±.026	0.672 ±.028	0.718 ±.019	0.729 ±.020	0.758 ±.031	0.731 ±.025
	(16)		(26)		(33)		(24)	
1.00	0.678 ±.068	0.674 ±.045	0.751 ±.072	0.747 ±.055	0.651 ±.038	0.712 ±.046	0.768 ±.034	0.712 ±.029
	(8)		(8)		(11)		(11)	
6.24	0.620 ±.037	0.604 ±.034	0.637 ±.023	0.627 ±.024	0.616 ±.041	0.603 ±.058	0.587 ±.035	0.567 ±.019
	(14)		(15)		(10)		(12)	
				5HIAA[9]				
Controls	0.629 ±.027	0.648 ±.025	0.623 ±.022	0.612 ±.019	0.599 ±.019	0.611 ±.022	0.580 ±.024	0.566 ±.025
	(17)		(26)		(34)		(23)	
1.00	0.590 ±.033	0.577 ±.036	0.635 ±.039	0.648 ±.048	0.528 ±.031	0.547 ±.032	0.621 ±.025	0.594 ±.024
	(9)		(8)		(11)		(11)	
6.24	0.568 ±.023	0.534 ±.017	0.616 ±.023	0.617 ±.029	0.559 ±.021	0.569 ±.029	0.596 ±.025	0.544 ±.024
	(14)		(14)		(10)		(12)	

[8]Significant main effect of 6OHDA ($p<0.001$) with no interactions.

[9]Significant main effect of sex ($p<0.045$), and 6OHDA X direction of rotation X side interaction ($p<0.005$).

TABLE 5.1 (cont'd).

	Females				Males			
Direction of Rotation	Left		Right		Left		Right	
Side:	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
Dose of 6OHDA (ug)	5HIAA/5HT[10]							
Controls	0.886	0.913	0.946	0.950	0.839	0.841	0.764	0.773
	±.046	±.046	±.038	±.047	±.026	±.025	±.022	±.026
	(16)		(26)		(33)		(23)	
1.00	0.871	0.843	0.869	0.876	0.819	0.785	0.822	0.824
	±.041	±.041	±.041	±.039	±.040	±.045	±.030	±.039
	(8)		(8)		(11)		(11)	
6.24	0.941	0.909	0.976	0.977	0.936	1.007	1.055	1.003
	±.040	±.040	±.048	±.044	±.057	±.084	±.067	±.038
	(14)		(14)		(10)		(12)	

[10]Significant main effect of 6OHDA ($p<0.001$) and sex X 6OHDA interaction ($p<0.025$).

TABLE 5.2. Effect of unilateral 6OHDA injection into the ipsilateral striatum on the difference (mean±SEM) between contralateral and ipsilateral striatal monoamine and metabolite concentrations (ug/gm); group N's in parentheses.

	Females		Males	
Direction of Rotation[1]	Left	Right	Left	Right
Dose of 6OHDA (ug)	DA[2]			
Controls	-0.221	0.261	-0.297	0.156
	±.370	±.101	±.173	±.220
	(17)	(26)	(35)	(24)
1.00	1.940	1.312	0.590	1.549
	±.525	±.371	±.304	±.383
	(9)	(8)	(11)	(12)
6.24	3.685	5.231	3.130	4.804
	±.401	±.429	±.503	±.480
	(14)	(14)	(10)	(14)

[1]Based on first (i.e., pre-operative) test in response to amphetamine. On the following day operated rats received 6OHDA or vehicle-only injections on the same side towards which they made most of their turns during this test.

[2]Significant main effects of 6OHDA ($p<0.001$), and direction of rotation ($p<0.001$); significant 6OHDA X direction of rotation interaction ($p<0.008$); also, marginally significant main effect of sex ($p<0.055$).

TABLE 5.2 (cont'd).

	Females		Males	
Direction of Rotation	Left	Right	Left	Right
Dose of 6OHDA (ug)		DOPAC[3]		
Controls	-0.069	0.032	-0.035	0.022
	±.045	±.027	±.026	±.034
	(17)	(26)	(35)	(24)
1.00	0.170	0.146	0.071	0.219
	±.053	±.045	±.048	±.050
	(9)	(8)	(11)	(12)
6.24	0.437	0.564	0.371	0.525
	±.063	±.065	±.048	±.056
	(14)	(14)	(10)	(14)
		DOPAC/DA[4]		
Controls	-0.004	-0.001	0.003	0.008
	±.004	±.002	±.002	±.002
	(17)	(26)	(35)	(24)
1.00	-0.004	-0.001	-0.002	0.001
	±.003	±.003	±.005	±.002
	(9)	(8)	(11)	(12)
6.24	-0.003	-0.019	0.000	-0.018
	±.006	±.005	±.003	±.006
	(14)	(14)	(10)	(14)

[3]Significant main effects of 6OHDA ($p<0.001$), and direction of rotation ($p<0.001$), with no interactions.

[4]Significant main effect of 6OHDA ($p<0.001$), and significant 6OHDA X direction of rotation interaction ($p<0.001$). The effects of direction of rotation was marginally significant ($p<0.06$).

TABLE 5.2 (cont'd).

	Females		Males	
Direction of Rotation	Left	Right	Left	Right
Dose of 6OHDA (ug)		HVA[5]		
Controls	-0.035	0.020	-0.024	0.004
	±.024	±.014	±.013	±.014
	(16)	(26)	(35)	(24)
1.00	0.022	0.084	0.005	0.102
	±.050	±.033	±.022	±.029
	(9)	(8)	(11)	(11)
6.24	0.293	0.299	0.217	0.199
	±.073	±.052	±.070	±.092
	(14)	(14)	(10)	(14)
		HVA/DA[6]		
Controls	-0.002	-0.001	0.000	-0.001
	±.002	±.001	±.001	±.001
	(16)	(26)	(35)	(24)
1.00	-0.018	-0.003	-0.006	-0.003
	±.008	±.001	±.002	±.002
	(9)	(8)	(11)	(11)
6.24	-0.006	-0.036	-0.008	-0.037
	±.004	±.008	±.005	±.010
	(14)	(14)	(10)	(14)

[5]Significant main effect of 6OHDA ($p<0.001$); no interactions.
[6]Significant main effects of 6OHDA ($p<0.001$), and direction of rotation ($p<0.006$); significant 6OHDA X direction of rotation interaction ($p<0.001$).

TABLE 5.2 (cont'd).

	Females		Males	
Direction of Rotation	Left	Right	Left	Right
Dose of 6OHDA (ug)		5HT		
Controls	-0.002	0.004	-0.012	0.027
	±.019	±.014	±.017	±.026
	(16)	(26)	(33)	(24)
1.00	0.004	0.004	-0.061	0.056
	±.046	±.035	±.032	±.033
	(8)	(8)	(11)	(11)
6.24	0.016	0.010	0.013	0.020
	±.024	±.022	±.029	±.029
	(14)	(15)	(10)	(12)
		5HIAA[7]		
Controls	-0.019	0.011	-0.012	0.015
	±.012	±.010	±.012	±.011
	(17)	(26)	(34)	(23)
1.00	0.013	-0.013	-0.019	0.027
	±.023	±.029	±.011	±.011
	(9)	(8)	(11)	(11)
6.24	0.034	-0.001	-0.011	0.052
	±.014	±.027	±.015	±.020
	(14)	(14)	(10)	(12)

[7]Significant sex X direction of rotation interaction ($p<0.005$).

TABLE 5.2 (cont'd).

	Females		Males	
Direction of Rotation	Left	Right	Left	Right
Dose of 6OHDA (ug)	5HIAA/5HT			
Controls	-0.026	-0.004	-0.001	-0.009
	±.014	±.024	±.017	±.025
	(16)	(26)	(33)	(23)
1.00	0.028	-0.007	0.034	-0.003
	±.019	±.028	±.028	±.024
	(8)	(8)	(11)	(11)
6.24	0.032	-0.001	-0.071	0.052
	±.034	±.039	±.057	±.051
	(14)	(14)	(10)	(12)

dose dependent depletion of DA on the injected side without altering the concentration of DA on the uninjected side. In addition, and unexpectedly, there was a significant main effect of sex, and a significant interaction between the direction of rotation and side: Females had higher levels of DA than males, and the right rotators had larger DA depletions than the left rotators. The sex difference in DA levels was due largely to the difference between the controls since a post-hoc ANOVA comparing only the male and female control rats was still highly significant ($p<0.001$). Two factor ANOVA's were done comparing DA concentrations in the left and right rotators of each sex at each 6OHDA dose level: The results indicated that while the side effect was significant in all four cases, the direction of rotation X side interaction was significant only for the rats treated with the higher dose of 6OHDA. Thus, it may be concluded that in the four way ANOVA, the significant interaction between direction of rotation and side was due mainly to the larger DA depletions in right rotators at the higher dose of 6OHDA for both males and females.

The results of the analysis on DOPAC levels were similar, the main difference being that there was no sex difference in DOPAC levels; still, as with DA, DOPAC levels were reduced unilaterally on the injected side and right rotators had larger depletions than left rotators. This difference between left and right rotators in DOPAC depletions was more evident in the males than in the females: Individual ANOVA's

did not result in a significant direction of rotation X side interaction at either dose of 6OHDA for the females ($F's<2$), but did for the males ($p<0.05$ at the lower dose, $p<0.06$ at the higher dose); still, each of these four ANOVA's resulted in highly significant side effects ($p's<0.001$).

The analysis of bilateral HVA levels showed a unilateral reduction on the treated side, and a significant main effect of sex (females>males), but no difference between the depletions in left and right rotators. This sex difference in HVA levels was due largely, but obviously not entirely, to the difference between the control rats since a post-hoc ANOVA comparing the controls alone was only marginally significant ($p<0.06$).

In summary, then, DA, DOPAC, and HVA were decreased on the 6OHDA injected side, more so for DA and DOPAC in the right rotators than in the left rotators; and DA and HVA levels were higher bilaterally in the females than in the males, mainly due to the differences between the control rats, especially for DA.

The results of the four factor ANOVA on DOPAC:DA ratios were more complex, primarily because qualitatively different effects on the two striata resulted from left and right sided lesions. Thus, in addition to a significant main effect of side, there were significant interactions between sex and 6OHDA dose, 6OHDA and side, and 6OHDA, direction of rotation and side; also the direction of rotation X side interaction was marginally significant ($p<0.055$). In order to determine where the major differences between groups were, post-hoc two

factor ANOVA's were done comparing the controls with one of the lesioned groups. The results of these analyses indicated that these effects were due to the following: In both the male and female right rotators receiving the higher dose of 6OHDA the contralateral DOPAC:DA ratio decreased while the ipsilateral ratio increased; by contrast, DOPAC:DA ratios were decreased in both striata of the left rotating males (high dose of 6OHDA) and females (low dose of 6OHDA).

The effects of unilateral intrastriatal 6OHDA on the ratio of HVA to DA were less complex but just as interesting because, again, left and right sided DA depletions resulted in significantly different effects: In both the males and the females receiving the higher dose of 6OHDA HVA:DA ratios increased on the lesioned side, but only in the right rotating rats; there was no increase at all in the HVA:DA ratio on the lesioned side of left rotators.

If the absolute values of the DOPAC:DA and HVA:DA ratios in the individual striata are ignored and only the differences between the contralateral and ipsilateral striata are considered (Table 5.2), then the effects of the unilateral 6OHDA injections can be summarized as follows: In both males and females, rats receiving the higher dose of 6OHDA had higher DOPAC:DA and HVA:DA ratios on the lesioned side if they were lesioned in the right striatum (i.e., if their preoperative amphetamine-induced direction of rotation was towards the right); on the other hand, there was no evidence of any asymmetry in either ratio among the male or female

left rotators (i.e., those rats lesioned on the left) receiving either dose of 6OHDA.

It was reasonable to expect that these differences between the DOPAC/DA and HVA/DA results in left and right lesioned rats (i.e., left and right rotators, respectively) might have been due to the fact that the right rotators had larger DA depletions than the left rotators. This possibility was tested by re-analyzing the DOPAC/DA and HVA/DA data in the following way. For the groups that received the higher dose of 6OHDA right rotators were excluded if they had a larger DA depletion (i.e., contralateral [DA] - ipsilateral [DA]) than the largest DA depletion found for any left rotator; and left rotators were excluded if they had a smaller DA depletion than the smallest DA depletion found for any right rotator. This was done for each sex individually and resulted in the removal of four males (2 left rotators and 2 right rotators) and seven females (1 left rotator and 6 right rotators). The remaining right rotators no longer had significantly larger DA depletions than the remaining left rotators. Still, when the DOPAC/DA and HVA/DA data were reanalyzed using these modified groups, the 6OHDA dose X direction of rotation X side interactions were still significant in both cases ($p<0.03$ and $p<0.001$, respectively). Thus, the difference in the DA depletions between the left and right rotators did not account for the higher DOPAC:DA and HVA:DA ratios in the lesioned striatum of the right rotators than of the left rotators.

Effects on 5HT and 5HIAA. The only significant result

of the four factor ANOVA on 5HT levels was a main effect of 6OHDA treatment, with no interactions; in other words, unilateral intrastriatal 6OHDA decreased 5HT concentrations in both striata across both sexes and both directions of rotation. Nevertheless, to get a better idea of whether the bilateral 5HT depletion was equally distributed across all four groups (i.e., male and female left and right rotators), two factor ANOVA's were done comparing each of the four 6OHDA (higher dose) treated groups with their respective controls. Three of these four ANOVA's revealed significant effects of 6OHDA without interactions; for the female right rotators the bilateral 5HT depletion was not significant ($p<0.3$) mainly because the control levels for this group were approximately 9% lower than in the other three groups.

The results of the four factor ANOVA on 5HIAA levels revealed no significant effects of 6OHDA treatment but did show: (i) a main effect of sex (females>males), which was not present when only the male and female controls were analyzed separately ($p<0.09$); and (ii) a significant three way interaction between sex, direction of rotation, and side, indicating that the mean left-right difference for all the males combined was significantly greater than for all the females combined, a difference that was not present when the controls were reanalyzed separately.

The results of the four way ANOVA on 5HIAA:5HT ratios revealed a significant effect of 6OHDA treatment as well as a significant sex X 6OHDA interaction. Two factor ANOVA's were

then done comparing the male and female left and right rotators treated with the higher dose of 6OHDA to their respective controls, and these analyses showed that the 6OHDA effect was present for both groups of males alone, but not for either group of females. Thus, unilateral intrastriatal 6OHDA treatment increased 5HIAA:5HT ratios bilaterally when both sexes are considered together, but more so in the males than in the females.

Defining "Classical" and "Paradoxical" Responders

In order to determine what effects the unilateral striatal lesions had on rotational behavior it was first necessary to establish criteria for estimating how much turning the 6OHDA treated rats would have done had they not been so treated. In order to establish such criteria the control rats' turning during the first (i.e., pre-operative) and second (i.e., post-operative) amphetamine tests were compared. Paired t tests revealed that both the males ($p<0.001$) and the females ($p<0.002$) increased their net rotations during the second test compared to the first. Unfortunately, however, while the correlation between the turning in the two tests was highly significant for both sexes (females: $r=0.44$, $p<0.003$; males: $r=0.51$, $p<0.001$), the ability of pre-operative turning to predict post-operative turning was clearly poor; thus, another means of predicting post-operative turning had to be devised.

Figures 5.1a and 5.1b show the relationship between net rotations during the first and second amphetamine tests that

was used to establish whether a lesioned rat increased or decreased its turning compared to the controls. As shown in the Figures both the males and females were divided into three groups based on their turning during the first and second tests, and a median "sensitization factor" (defined as the ratio between post-operative and pre-operative net rotations) was determined for each group. Using these sensitization factors the behavior of each of the lesioned rats was classified as either "classical" or "paradoxical." A rat's response was classified by first determining what the appropriate sensitization factor for comparison was, based on the number of net rotations the rat made during the pre-operative amphetamine test. Then, if the rat's ratio of post-operative to pre-operative net rotations was greater than the appropriate sensitization factor its response was considered classical; on the other hand, if the ratio of the rat's post-operative to pre-operative net rotations was less than the appropriate sensitization factor its response was considered paradoxical. Using these criteria 18 males and 26 females were classified as classical responders; 29 males and 20 females were classified as paradoxical responders.

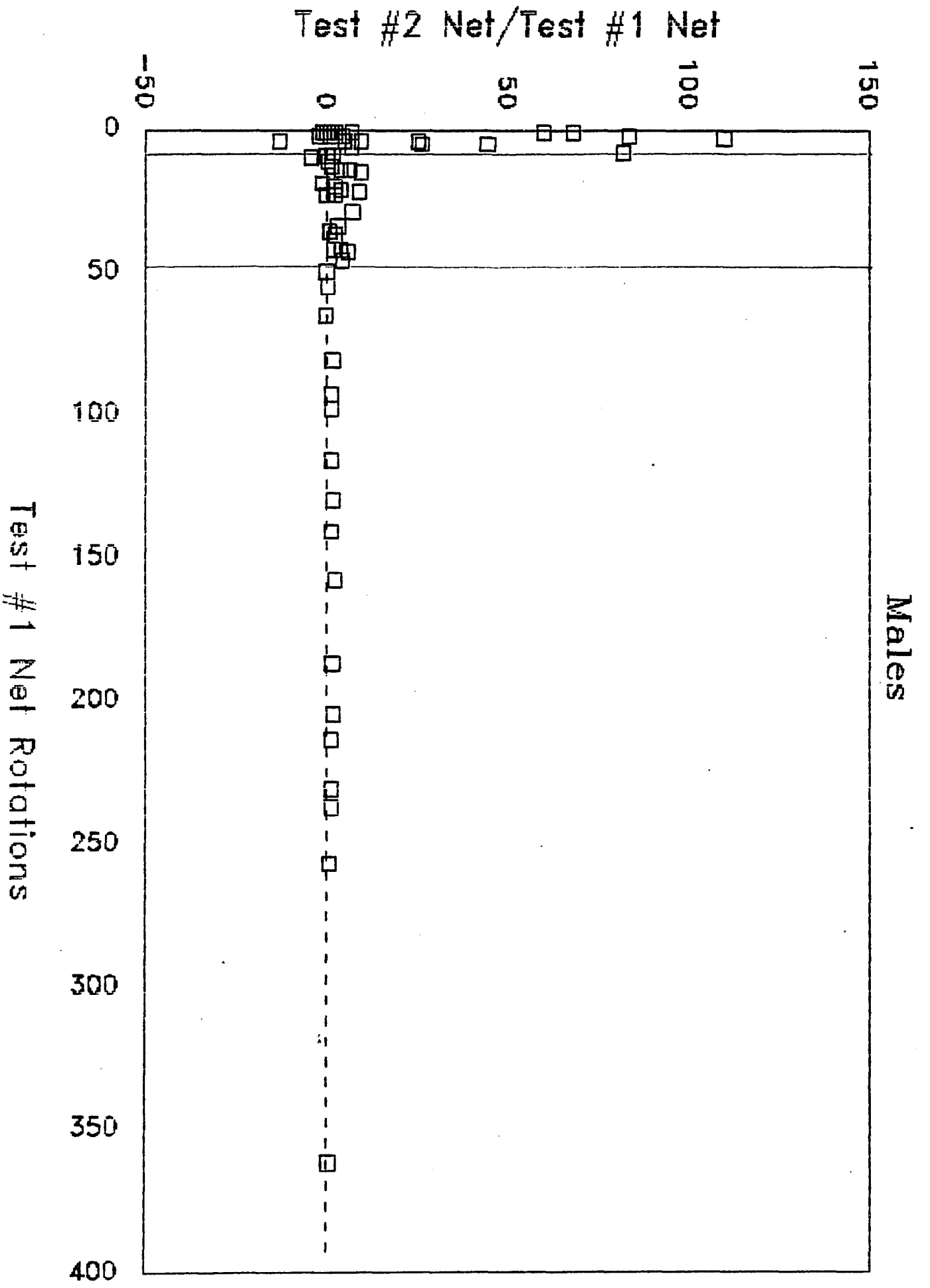
Comparison of Classical and Paradoxical Responders

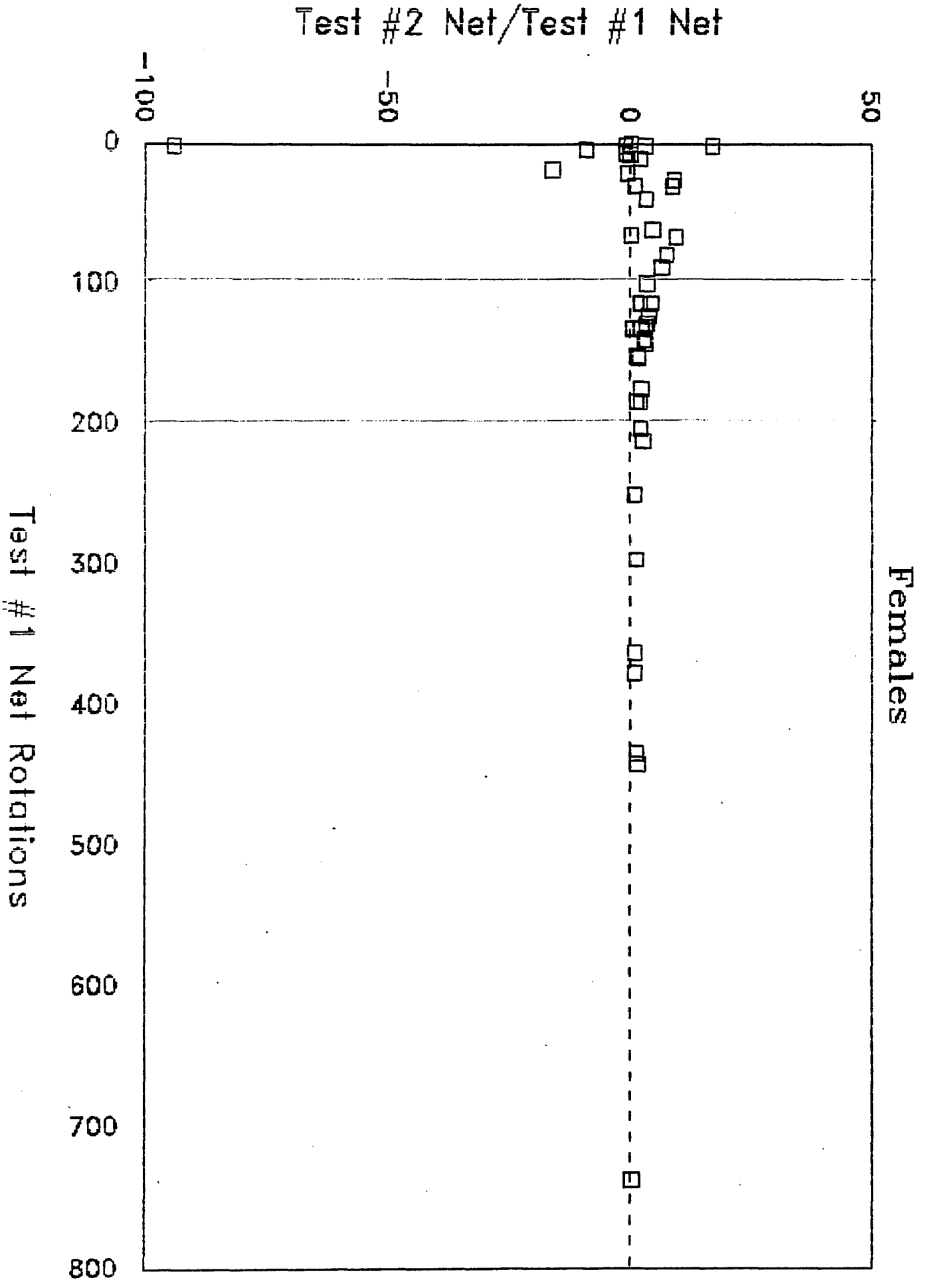
Behavior. As shown in Table 5.3 neither left nor right rotators were any more likely to be classified as having a classical or paradoxical behavioral response to the lesion. Furthermore, weak rotators were no more or less likely than strong rotators to be classified as classical or paradoxical

FIGURE 5.1. Relationship between net rotations by control rats during the first amphetamine test, and in the same direction during the second amphetamine test. A negative value was assigned to the second score if the rat did not rotate in the same direction during the two tests. A lesioned rat was classified as a "classical" or a "paradoxical" responder based on whether the ratio of its net rotations on the two tests was greater or less than the median ratio for the appropriate control group, respectively.

(a) Males. Median ratios used for classifying lesioned rats' responses were: for those that made less than 11 net rotations on the first test: 4.750; more than 10 but less than 51: 3.059; more than 50: 1.368.

(b) Females. Median ratios used for classifying lesioned rats' responses were: for those that made less than 101 net rotations on the first test: 2.550; more than 100 but less than 201: 2.186; more than 200: 1.240.


Test #2 Net/Test #1 Net
-50
0
50
100
150
0
50
100
150
200
250
300
350
400
Test #1 Net Rotations
Males


Test #2 Net/Test #1 Net
-100
-50
0
50
0
100
200
300
400
500
600
700
800
Test #1 Net Rotations
Females

responders. In Table 5.4 the classical and paradoxical responders are compared with respect to: (i) nocturnal % turning, (ii) nocturnal net rotations, (iii) pre-operative amphetamine-induced net rotations, (iv) post-operative net rotations, and (v) the increase in net rotations from the first to the second amphetamine test (i.e., the mean difference between net rotations made post- vs. pre-operatively). Group means were compared statistically with four factor ANOVA's, the four factors being sex, direction of pre-operative amphetamine-induced rotation, response to lesion (i.e., "classical" or "paradoxical"), and dose of 6OHDA.

Classical and paradoxical responders did not differ with respect to either nocturnal net rotations or pre-operative amphetamine-induced net rotations; however, in the latter analysis there was a significant main effect of sex indicating that the females rotated more in response to amphetamine pre-operatively than the males did. While the classical and paradoxical responders did not differ with respect to pre-operative amphetamine-induced turning, the results of the ANOVA's on both post-operative amphetamine-induced turning, and the increase in turning from the first to the second amphetamine tests both revealed highly significant response to lesion effects: the classical responders increased their amphetamine-induced turning significantly more than the paradoxical responders, and they (obviously) rotated significantly more than the paradoxical responders did after the lesion. In addition to these main response to lesion

TABLE 5.3. Number of left and right rotators classified as classical and paradoxical responders after treatment with one of the two doses of 6OHDA.

Dose of 6OHDA	Classical Responders		Paradoxical Responders	
	Left Rotators	Right Rotators	Left Rotators	Right Rotators
		Females		
1.00 ug	4	4	5	4
6.24 ug	10	8	4	7
		Males		
1.00 ug	4	3	7	9
6.24 ug	4	7	6	7

TABLE 5.4. Comparison of rotational behavior by "classical" and "paradoxical" responders; group N's in parentheses.

	Females				Males			
Direction of Rotation[1]	Left		Right		Left		Right	
Response to Lesion[2]	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical
Dose of 6OHDA (ug)	Nocturnal % Turning[3]							
1.00	41	62	41	51	43	69	45	42
	±5	±8	±8	±11	±5	±8	±4	±7
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	58	49	45	46	39	50	41	46
	±7	±5	±7	±6	±6	±8	±6	±5
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)
	Nocturnal Net Rotations[4]							
1.00	50	90	53	32	13	97	11	28
	±28	±33	±26	±8	±7	±46	±4	±22
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	107	50	31	34	26	39	24	41
	±84	±11	±14	±14	±13	±22	±11	±9
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)

[1]Based on first (i.e., pre-operative) test in response to amphetamine.
[2]Defined in text.
[3]Significant main effects of direction of rotation ($p<0.04$) and response to lesion ($p<0.025$).
[4]Significant main effect of direction of rotation ($p<0.02$), and significant interaction between 6OHDA, direction of rotation, and response to lesion ($p<0.04$).

TABLE 5.4 (cont'd).

	Females				Males			
Direction of Rotation	Left		Right		Left		Right	
Response to Lesion	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical
Dose of 6OHDA (ug)	Amphetamine Test #1 Net Rotations[5]							
1.00	222	92	159	95	67	94	49	55
	±87	±41	±44	±66	±36	±46	±26	±22
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	192	231	141	222	109	179	121	58
	±72	±54	±62	±85	±71	±99	±40	±20
	(4)	(10)	(7)	(8)	(6)	(4)	(7)	(7)
	Amphetamine Test #2 Net Rotations[6]							
1.00	156	396	61	300	53	286	11	152
	±56	±116	±177	±119	±38	±85	±29	±32
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	199	538	-21	703	70	357	163	215
	±44	±101	±40	±191	±101	±105	±51	±49
	(4)	(10)	(7)	(8)	(6)	(4)	(7)	(7)

[5]Significant main effect of sex ($p<0.02$).
[6]Significant main effects of sex ($p<0.02$) and response to lesion ($p<0.001$).

TABLE 5.4 (cont'd).

Direction of Rotation	Females				Males			
	Left		Right		Left		Right	
Response to Lesion	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical
Dose of 6OHDA (ug)	Increase in Amphetamine-Induced Net Rotations[7]							
1.00	-66	304	-98	206	-14	192	-39	97
	±45	±103	±160	±64	±14	±42	±30	±10
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	8	306	-163	481	-59	178	41	157
	±39	±74	±87	±143	±55	±17	±15	±48
	(4)	(10)	(7)	(8)	(6)	(4)	(7)	(7)

[7]Values represent the mean increase in the number of net rotations made during the second amphetamine test in the same direction as in the first amphetamine test, compared to the number of net rotations made during the first amphetamine test. Significant main effect of response to lesion ($p<0.001$), and significant interaction between sex and response to lesion ($p<0.008$).

effects, the ANOVA on post-operative turning revealed a significant main effect of sex indicating that the females rotated more than the males. These results are summarized in Table 5.5. Clearly, the two groups of rats did not respond to 6OHDA treatment in the same way.

The analysis of nocturnal % turning scores was the only ANOVA on pre-lesion behavioral measures to result in a significant main effect of response to lesion -- i.e., that resulted in a significant difference between classical and paradoxical responders. Grouped across both sexes, both directions of rotation, and both doses of 6OHDA, the classical responders had higher % turning scores than paradoxical responders. When the data were pooled across both directions of rotation and both 6OHDA treatments, subsequent post-hoc t tests revealed that the difference was significant only for the males (51±4 vs. 43±2; $p<0.05$), and not for the females (51±3 vs. 46±3).

Neurochemistry. Neurochemical measures in the contralateral and ipsilateral striata of the classical and paradoxical responders are summarized in Table 5.6; side differences are shown in Table 5.7. As was done for the behavioral measures above, ANOVA's were done for each neurochemical measure in order to detect differences between the two groups of responders. The five factors in the ANOVA were sex, direction of rotation, 6OHDA dose, behavioral response to lesion, and side, with the sides nested. The ANOVA on DA

(text continues on pg 150)

TABLE 5.5. Summary comparison of the rotational behavior of classical and paradoxical responders before and after 6OHDA treatment.

Amphetamine -Induced Net Rotations	Females		Males	
	Classical Responders (N=26)	Paradoxical Responders (N=20)	Classical Responders (N=18)	Paradoxical Responders (N=28[1])
Pre-6OHDA	186±37	175±34	93±27	86±21
Post-6OHDA	530±78	83±44	252±38	66±29
Net Increase	344±58	-92±46	159±21	-16±16

[1]Does not include a rat that made 7 net turns to the left before and 1077 net turns to the right after 6.24 ug 6OHDA.

TABLE 5.6. Comparison of mean (±SEM) striatal monoamine and metabolite concentrations (ug/gm) in "classical" and "paradoxical" responders; group N's in parentheses.

Direction of Rotation[1]	Left				Right			
Response to Lesion[2]	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side[3]	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				DA[4]				
Dose of 6OHDA (ug)				Females				
1.00	10.097	9.078	11.540	8.450	10.013	9.259	10.670	8.830
	±.182	±.392	±.700	±.783	±.305	±.447	±.865	±.849
	(5)		(4)		(4)		(4)	
6.24	11.472	6.634	10.191	6.967	11.037	6.218	10.514	4.870
	±.448	±.368	±.470	±.570	±.396	±.477	±.575	±.717
	(4)		(10)		(7)		(7)	
				Males				
1.00	9.362	8.652	9.653	9.273	9.925	8.529	10.697	8.686
	±.621	±.504	±.542	±.667	±.396	±.520	±.354	±.310
	(7)		(4)		(9)		(3)	
6.24	10.022	6.643	10.250	7.493	9.421	5.473	10.362	4.702
	±.602	±.405	±.164	±.339	±.236	±.520	±.260	±.587
	(6)		(4)		(7)		(7)	

[1]Based on first (i.e., pre-operative) test in response to amphetamine.
[2]Defined in text.
[3]Based on first (i.e., pre-operative) test in response to amphetamine. The ipsilateral (Ipsi) side is the side towards which the rat made most of its turns, and the contralateral (Contra) side is the side away from which the rat made most of its turns.
[4]Significant main effects of 6OHDA ($p<0.001$) and side ($p<0.001$); significant interactions: 6OHDA X direction of rotation ($p<0.05$), sex X side ($p<0.04$), 6OHDA X side ($p<0.001$), sex X direction of rotation X side ($p<0.05$), and 6OHDA X direction of rotation X side ($p<0.04$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				DOPAC[5]				
Dose of 6OHDA (ug)				Females				
1.00	1.177	1.087	1.252	0.982	1.233	1.103	1.329	1.168
	±.078	±.083	±.105	±.151	±.012	±.015	±.060	±.133
	(5)		(4)		(4)		(4)	
6.24	1.270	0.690	1.326	0.946	1.381	0.834	1.259	0.677
	±.087	±.028	±.057	±.073	±.095	±.066	±.124	±.088
	(4)		(10)		(7)		(7)	
				Males				
1.00	1.322	1.251	1.344	1.273	1.339	1.137	1.349	1.077
	±.090	±.077	±.159	±.181	±.049	±.064	±.135	±.026
	(7)		(4)		(9)		(3)	
6.24	1.143	0.784	1.270	0.880	1.131	0.711	1.297	0.667
	±.098	±.074	±.061	±.077	±.049	±.056	±.062	±.066
	(6)		(4)		(7)		(7)	

[5]Significant main effects of 6OHDA ($p<0.001$) and side ($p<0.001$); significant interactions: sex X 6OHDA ($p<0.05$), 6OHDA X side ($p<0.001$), and direction of rotation X side ($p<0.03$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				DOPAC/DA[6]				
Dose of 6OHDA (ug)				Females				
1.00	0.117	0.119	0.110	0.116	0.124	0.120	0.126	0.132
	±.007	±.005	±.011	±.014	±.005	±.004	±.080	±.004
	(5)		(4)		(4)		(4)	
6.24	0.111	0.104	0.133	0.139	0.125	0.136	0.119	0.146
	±.005	±.002	±.009	±.011	±.008	±.008	±.007	±.012
	(4)		(10)		(7)		(7)	
				Males				
1.00	0.141	0.146	0.138	0.135	0.135	0.134	0.126	0.124
	±.003	±.009	±.010	±.011	±.004	±.004	±.008	±.007
	(7)		(4)		(9)		(3)	
6.24	0.113	0.118	0.124	0.117	0.120	0.132	0.125	0.149
	±.005	±.008	±.008	±.005	±.004	±.007	±.003	±.010
	(6)		(4)		(7)		(7)	

[6]Significant main effect of side ($p<0.005$); significant interactions: sex X treatment ($p<0.04$), 6OHDA X side ($p<0.03$), direction of rotation X side ($p<0.02$), and 6OHDA X direction of rotation X side ($p<0.003$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				HVA[7]				
Dose of 6OHDA (ug)				Females				
1.00	0.814	0.824	0.963	0.901	0.840	0.809	0.962	0.826
	±.065	±.057	±.082	±.110	±.028	±.026	±.033	±.079
	(5)		(4)		(4)		(4)	
6.24	1.211	0.684	0.850	0.651	1.024	0.740	0.881	0.567
	±.246	±.081	±.085	±.086	±.085	±.068	±.059	±.061
	(4)		(10)		(7)		(7)	
				Males				
1.00	0.774	0.759	0.905	0.917	0.793	0.713	0.889	0.726
	±.069	±.057	±.132	±.116	±.031	±.039	±.047	±.032
	(7)		(4)		(9)		(3)	
6.24	0.911	0.640	0.812	0.675	0.749	0.651	0.825	0.525
	±.090	±.045	±.025	±.077	±.052	±.157	±.094	±.054
	(6)		(4)		(7)		(7)	

[7]Significant main effects of sex ($p<0.04$) and side ($p<0.001$); significant interactions: 6OHDA X response to lesion ($p<0.02$), 6OHDA X side ($p<0.001$), and direction of rotation X response to lesion X side ($p<0.03$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				HVA/DA[8]				
Dose of 6OHDA (ug)				Females				
1.00	0.081	0.091	0.084	0.110	0.084	0.088	0.091	0.094
	±.006	±.007	±.007	±.019	±.004	±.005	±.006	±.005
	(5)		(4)		(4)		(4)	
6.24	0.105	0.103	0.084	0.093	0.093	0.122	0.085	0.128
	±.020	±.010	±.009	±.009	±.008	±.012	±.006	±.018
	(4)		(10)		(7)		(7)	
				Males				
1.00	0.084	0.089	0.093	0.099	0.081	0.084	0.083	0.084
	±.007	±.009	±.008	±.009	±.004	±.004	±.003	±.007
	(7)		(4)		(9)		(3)	
6.24	0.090	0.097	0.080	0.090	0.079	0.114	0.079	0.117
	±.006	±.005	±.003	±.009	±.005	±.018	±.008	±.008
	(6)		(4)		(7)		(7)	

[8]Significant main effect of side ($p<0.001$); significant interactions: 6OHDA X side ($p<0.01$), direction of rotation X side ($p<0.04$), 6OHDA X direction of rotation X side ($p<0.001$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				5HT[9]				
Dose of 6OHDA (ug)				Females				
1.00	0.626	0.672	0.763	0.676	0.706	0.692	0.797	0.803
	±.034	±.049	±.183	±.103	±.132	±.108	±.074	±.018
	(5)		(4)		(4)		(4)	
6.24	0.626	0.697	0.618	0.567	0.606	0.610	0.664	0.642
	±.081	±.073	±.044	±.034	±.022	±.040	±.037	±.031
	(4)		(10)		(7)		(7)	
				Males				
1.00	0.634	0.728	0.681	0.684	0.805	0.744	0.670	0.626
	±.023	±.049	±.106	±.103	±.039	±.023	±.013	±.076
	(7)		(4)		(9)		(3)	
6.24	0.594	0.583	0.650	0.634	0.518	0.525	0.635	0.596
	±.058	±.088	±.061	±.069	±.035	±.031	±.047	±.019
	(6)		(4)		(7)		(7)	

[9]Significant main effect of 6OHDA ($p<0.001$); significant interactions: sex X 6OHDA X response to lesion ($p<0.03$), response to lesion X side ($p<0.03$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				5HIAA[10]				
Dose of 6OHDA (ug)				Females				
1.00	0.583	0.609	0.598	0.537	0.607	0.629	0.663	0.667
	±.025	±.038	±.074	±.068	±.078	±.092	±.026	±.044
	(5)		(4)		(4)		(4)	
6.24	0.627	0.562	0.545	0.524	0.646	0.663	0.664	0.642
	±.057	±.031	±.019	±.019	±.029	±.044	±.037	±.031
	(4)		(10)		(7)		(7)	
				Males				
1.00	0.495	0.518	0.585	0.598	0.633	0.613	0.587	0.541
	±.020	±.030	±.077	±.071	±.029	±.021	±.049	±.050
	(7)		(4)		(9)		(3)	
6.24	0.540	0.555	0.587	0.591	0.591	0.528	0.586	0.559
	±.033	±.047	±.009	±.019	±.039	±.038	±.023	±.018
	(6)		(4)		(7)		(7)	

[10]Significant main effects of sex ($p<0.03$) and direction of rotation ($p<0.015$); significant interactions: sex X direction of rotation X response to lesion ($p<0.045$), sex X direction of rotation X side ($p<0.002$).

TABLE 5.6 (cont'd).

Direction of Rotation	Left				Right			
Response to Lesion	"Paradoxical"		"Classical"		"Paradoxical"		"Classical"	
Side	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi
				5HIAA/5HT[11]				
Dose of 6OHDA (ug)				Females				
1.00	0.935	0.912	0.765	0.729	0.891	0.920	0.847	0.832
	±.020	±.033	±.074	±.036	±.064	±.045	±.058	±.061
	(5)		(4)		(4)		(4)	
6.24	1.020	0.822	0.909	0.944	1.071	1.062	0.906	0.913
	±.062	±.054	±.049	±.049	±.070	±.071	±.058	±.046
	(4)		(10)		(7)		(7)	
				Males				
1.00	0.785	0.723	0.879	0.893	0.801	0.798	0.877	0.892
	±.036	±.043	±.092	±.075	±.031	±.027	±.077	±.136
	(7)		(4)		(9)		(3)	
6.24	0.944	1.033	0.925	0.969	1.206	1.081	0.947	0.947
	±.085	±.126	±.079	±.114	±.103	±.036	±.067	±.051
	(6)		(4)		(7)		(7)	

[11]Significant main effect of 6OHDA ($p<0.001$), and significant sex X 6OHDA X response to lesion interaction ($p<0.03$).

TABLE 5.7. Comparison of the mean (±SEM) contralateral-ipsilateral side differences in striatal monoamine and metabolite concentrations (ug/gm) in "classical" and "paradoxical" responders; group N's in parentheses.

	Females				Males			
Direction of Rotation[1]	Left		Right		Left		Right	
Response to Lesion[2]	Paradoxical	Classical	Paradoxical	Classical	Paradoxical	Classical	Paradoxical	Classical
Dose of 6OHDA (ug)				DA[3]				
1.00	1.019	3.091	0.754	1.870	0.710	0.380	1.395	2.012
	±.328	±.824	±.173	±.637	±.326	±.671	±.469	±.664
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	4.838	3.224	4.818	5.644	3.379	2.757	3.949	5.660
	±.801	±.397	±.634	±.582	±.792	±.501	±.509	±.704
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)
				DOPAC[4]				
1.00	0.090	0.271	0.130	0.161	0.071	0.072	0.202	0.272
	±.045	±.088	±.027	±.092	±.067	±.075	±.058	±.109
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	0.580	0.380	0.547	0.582	0.359	0.391	0.421	0.630
	±.110	±.072	±.110	±.077	±.081	±.026	±.036	±.094
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)

[1]Based on first (i.e., pre-operative) test in response to amphetamine.
[2]Defined in text.
[3]Significant main effects sex ($p<0.045$), 6OHDA ($p<0.001$), and direction of rotation ($p<0.008$); significant interactions: sex X direction of rotation ($p<0.05$), and 6OHDA X direction of rotation ($p<0.04$).
[4]Significant main effects of 6OHDA ($p<0.001$) and direction of rotation ($p<0.03$).

TABLE 5.7 (cont'd).

Direction of Rotation	Females				Males			
	Left		Right		Left		Right	
Response to Lesion	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical
Dose of 6OHDA (ug)	DOPAC/DA[5]							
1.00	-0.002	-0.007	0.004	-0.006	-0.004	0.003	0.001	0.001
	±.004	±.004	±.002	±.006	±.007	±.003	±.002	±.001
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	0.006	-0.006	-0.010	-0.027	-0.005	0.008	-0.012	-0.024
	±.006	±.008	±.002	±.009	±.003	±.003	±.005	±.010
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)
	HVA[6]							
1.00	-0.010	0.062	0.031	0.137	0.015	-0.012	0.080	0.162
	±.024	±.114	±.018	±.052	±.028	±.039	±.037	±.027
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	0.527	0.199	0.283	0.314	0.271	0.137	0.098	0.030
	±.201	±.045	±.103	±.029	±.097	±.098	±.163	±.082
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)

[5]Significant effects of 6OHDA ($p<0.03$) and direction of rotation ($p<0.02$), and significant 6OHDA X direction of rotation interaction ($p<0.003$).

[6]Significant effect of 6OHDA ($p<0.001$), and significant direction of rotation X response to lesion interaction ($p<0.03$).

TABLE 5.7 (cont'd).

Direction of Rotation	Females				Males			
	Left		Right		Left		Right	
Response to Lesion	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical
Dose of 6OHDA (ug)				HVA/DA[7]				
1.00	-0.011	-0.026	-0.004	-0.003	-0.006	-0.006	-0.003	-0.001
	±.004	±.017	±.003	±.001	±.002	±.004	±.002	±.007
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	0.003	-0.009	-0.028	-0.043	-0.007	-0.010	-0.035	-0.039
	±.012	±.002	±.005	±.015	±.004	±.011	±.019	±.011
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)
				5HT[8]				
1.00	-0.046	0.087	0.014	-0.006	-0.094	-0.004	0.060	0.044
	±.027	±.107	±.027	±.069	±.046	±.012	±.040	±.062
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	-0.071	0.051	-0.004	0.022	0.011	0.016	-0.007	0.039
	±.034	±.024	±.025	±.036	±.048	±.027	±.022	±.047
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)

[7]Significant main effects of 6OHDA ($p<0.008$) and direction of rotation ($p<0.04$); also, significant 6OHDA X direction of rotation interaction ($p<0.001$).
[8]Significant main effect of response to lesion ($p<0.03$).

TABLE 5.7 (cont'd).

	Females				Males			
Direction of Rotation	Left		Right		Left		Right	
Response to Lesion	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical	Para-doxical	Clas-sical
Dose of 6OHDA (ug)				5HIAA[9]				
1.00	-0.026	0.062	-0.023	-0.004	-0.023	-0.013	0.020	0.046
	±.017	±.035	±.032	±.054	±.014	±.019	±.013	±.013
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	0.065	0.022	-0.018	0.022	-0.015	-0.004	0.063	0.026
	±.029	±.014	±.033	±.036	±.024	±.017	±.035	±.010
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)
				5HIAA/5HT				
1.00	0.024	0.036	-0.029	0.015	0.062	-0.014	0.002	-0.015
	±.022	±.041	±.046	±.033	±.039	±.028	±.020	±.082
	(5)	(4)	(4)	(4)	(7)	(4)	(9)	(3)
6.24	0.199	-0.035	0.008	-0.007	-0.089	-0.044	0.124	0.000
	±.061	±.015	±.069	±.050	±.095	±.043	±.083	±.061
	(4)	(10)	(7)	(7)	(6)	(4)	(7)	(7)

[9]Significant sex X direction of rotation interaction ($p<0.002$).

levels revealed no significant response to lesion effects or interactions, indicating that the neurochemical effects of the 6OHDA treatments were no different in the classical and paradoxical responders. In other words, DA depletions were not different between the two groups. This was also the case for DOPAC levels as well as the DOPAC:DA and HVA:DA ratios; that is, there were no significant main effects or interactions involving the response to lesion.

There were five significant results of the ANOVA on HVA levels including two response to lesion interactions. These significant results included a main effect of sex (females>males), a main effect of side (contralateral>ipsilateral), and a 6OHDA dose X side interaction indicative of a dose dependent increase in the unilateral HVA depletion. In addition, the interaction between 6OHDA dose and response to lesion interaction was significant, indicating that the paradoxical responders had higher bilateral HVA levels at the lower 6OHDA treatment dose, but lower levels at the higher 6OHDA treatment dose. Lastly, there was a significant three way interaction between direction of rotation, behavioral response to lesion, and side. This effect was due to the fact that, on average, the left rotators that had a paradoxical behavioral response had larger contralateral-ipsilateral side differences than the left rotators that had a classical response; on the other hand, the right rotators that had a paradoxical behavioral response had smaller contralateral-ipsilateral side differences than the right rotators that had a classical behavioral response. None of

these differences between classical and paradoxical responders were significant in individual post-hoc comparisons.

The results of the ANOVA on 5HT levels showed, again, that 5HT was reduced bilaterally by the higher dose of 6OHDA (i.e., a significant main effect of 6OHDA). In addition, there was a significant sex X 6OHDA dose X response to lesion interaction and a significant response to lesion X side interaction. The former interaction was due to the fact that for the females the paradoxical responders had lower 5HT levels than the classical responders after being treated with the lower dose of 6OHDA, but they had higher 5HT levels than the classical responders after being treated with the higher dose of 6OHDA; on the other hand these relationships were reversed for the males. The latter interaction, between the behavioral response to the lesion and side, was due to the larger contralateral-ipsilateral 5HT differences in the classical responders than in the paradoxical responders. There was a significant three way interaction between sex, direction of rotation, and response to lesion in the ANOVA on 5HIAA concentrations: The left rotating females that increased their turning following the lesion had lower striatal 5HIAA concentrations than the left rotating females that decreased there turning; on the other hand, the right rotating females that increased their turning had higher 5HIAA concentrations than the right rotating females that decreased their turning following the lesion. For the males

the situation was reversed.

The ANOVA on 5HIAA:5HT ratios resulted in a significant three way interaction between sex, 6OHDA dose, and response to lesion. This was due to the fact that in response to the higher dose of 6OHDA the male paradoxical responders had higher 5HIAA:5HT ratios than the classical responders, while at the lower dose the converse was true; by contrast, at both doses of 6OHDA the female paradoxical responders had higher 5HIAA:5HT ratios.

Consistency of Turning Behavior in Unlesioned Rats

Nocturnal vs. Amphetamine-Induced Rotation. Correlation coefficients between the number of net rotations made nocturnally and the next day (in the same direction) in response to amphetamine were calculated separately for the 106 males and 89 females used in the present study -- males: 0.42, $p<0.001$; females: 0.40, $p<0.001$. These correlation coefficients are reminiscent of those, described earlier for the control rats, between the first and second amphetamine tests: statistically significant, but not very useful for quantitative predictions.

A subsequent analysis was done to determine whether nocturnal rotational behavior was useful as a predictor of the direction, irrespective of the magnitude, of subsequent amphetamine-induced rotation. Table 5.8 depicts the results: If a rat made 20 or more net rotations nocturnally with a preference of at least 70%, then there was an 86% chance that it would subsequently rotate in the same direction in

TABLE 5.8. Number of rats turning in the same direction both nocturnally and in response to amphetamine a day later.

	Females		Males	
	Same	Opposite	Same	Opposite
Nocturnal Test				
Net Rotations $\leq$ 20 or Preference < 70	27 (50%)	27	37 (52%)	34
Net Rotations > 20 & Preference $\geq$ 70	24 (96%)	1	28 (80%)	7

response to amphetamine -- males: 80% (28 of 35); females: 95% (24 of 25). If a rat made fewer than 20 net rotations at night or had a % preference of less than 70, the ability of nocturnal circling to predict which direction the rat would circle in response to amphetamine was no better than chance -- males: 56% (23 of 39); females: 50% (23 of 46).

First vs. Second Amphetamine Tests. A similar attempt was made to determine how well one test with amphetamine predicted the direction of rotation on a subsequent test. As shown in Table 5.9 if a rat made more than 20 net turns during the first amphetamine test, there was a 97% chance that it would rotate in the same direction a week later in response to another administration of amphetamine. For the rats that made less than 20 net turns on the first test there was a 69% chance that they would rotate in the same direction on the subsequent test.

DISCUSSION

The Two Population Model

The results of the present experiment demonstrate that two groups of rats can readily be identified on the basis of their behavioral responses to the unilateral depletion of striatal DA on the side towards which they normally rotate. Based on the two population model it was predicted that two such groups would be identified, and that the two groups would not differ in the magnitude of their DA depletions; thus, the present results are consistent with this model.

TABLE 5.9. Number of control rats (i.e., unoperated or vehicle-only) turning in the same direction in response to amphetamine a week apart.

	Females		Males	
	Same	Opposite	Same	Opposite
Amphetamine Test #1				
Net Rotations $\leq$ 20	3 (33%)	6	21 (81%)	5
Net Rotations > 20	33 (97%)	1	27 (96%)	1
Totals	36 (84%)	7	48 (89%)	6

Neurochemistry. As discussed earlier current thinking about the relationship between striatal DA and rotational behavior considers striatal DA to have only one effect on turning: to "push" the animal away from the side in which the DA acts. In the present experiment, therefore, it was crucial to demonstrate that the "classical" group did not have larger DA depletions than the "paradoxical" group. Otherwise it could have been argued that that was the neurochemical basis for the difference between the behavioral responses of the two groups.

DOPAC:DA and HVA:DA ratios are thought to reflect DA turnover rates, and have been demonstrated to increase in the striata on the same side in which 6OHDA has previously been administered either directly to the medial forebrain bundle (Hefti et al., 1985) or intraventricularly (Zigmond et al., 1984). Thus, in the present experiment it was also crucial to demonstrate that DOPAC/DA and HVA/DA side differences did not differ between the groups classified as classical and paradoxical responders. If the paradoxical responders had had smaller side differences in either measure it could have been argued that that was the reason why they did not increase their turning as much as the classical responders.

Despite the fact that magnitudes and side differences of striatal DA and DOPAC concentrations, and DOPAC:DA and HVA:DA ratios did not differ between the classical and paradoxical responders, there were two statistically significant HVA effects involving the two groups of rats (Tables 5.6 and 5.7). Both were interaction effects, one involving bilateral

striatal levels with the dose of 6OHDA; and one with pre-operative direction of rotation, indicating that HVA side differences were different in classical and paradoxical responders depending on which way the rats turned pre-operatively. In light of the small N's in each of the sub-groups these interaction effects, while suggestive, must be considered preliminary findings at this point.

The various statistically significant interaction effects that emerged from the ANOVA's on 5HT and 5HIAA concentrations, and 5HIAA:5HT ratios variously involving dose of 6OHDA, direction of rotation, and response to lesion must also be considered preliminary findings for now. There was one significant finding involving measures of 5HTergic function that might have been predicted on the basis of previous results. In the experiment described in Chapter Three it was found that Contra>Ipsi rats had greater contralateral-ipsilateral 5HT side differences. Thus, it was perhaps not surprising that in the present work the classical responders had significantly greater contralateral-ipsilateral 5HT differences than the paradoxical responders. Whether side differences in striatal 5HT play an important role in determining an animal's rotational behavior would appear to be an interesting question to address in the future.

Sex. The results of the experiments described in Chapters Two and Three showed that the sexes are not distributed equally in the Contra>Ipsi and Ipsi>Contra populations:

females are more likely to be members of the Contra>Ipsi group and males are more likely to be members of the Ipsi>Contra group (Table 3.7). Therefore, if the two population model is valid then it would be predicted that in the present experiment there should be a sex difference in the classification of rats as classical and paradoxical responders: females should have been more likely to be classified as classical responders, and males should have been more likely to be classified as paradoxical responders. In fact, while this expectation was borne out by the data (Table 5.5) the difference between the sexes was not nearly significant statistically (chi-squared = 2.39, p>0.10).

There is no obvious explanation for the lack of confirmation of this prediction; however, two possibilities may be considered. First, it may be related to the less than ideal criteria by which classical and paradoxical rats were classified. Perhaps if more exact predictions could have been made of rats' turning in response to the second administration of amphetamine, more females would have been classified as having classical responses and more males would have been classified as having paradoxical responses.

The second possibility -- a "physiological" explanation -- for why the proportions of males and females in the classical and paradoxical responding groups were not significantly different is that the proportions of male and female "Contra>Ipsi" and "Ipsi>Contra" rats themselves were markedly affected by the tests of amphetamine-induced

TABLE 5.10. Effect of amphetamine-induced rotational testing on the proportions of male and female control rats with DA concentrations higher in the contralateral or ipsilateral striata. Contralateral and ipsilateral striata defined with respect to nocturnal rotational direction (top), or first test with amphetamine (bottom).

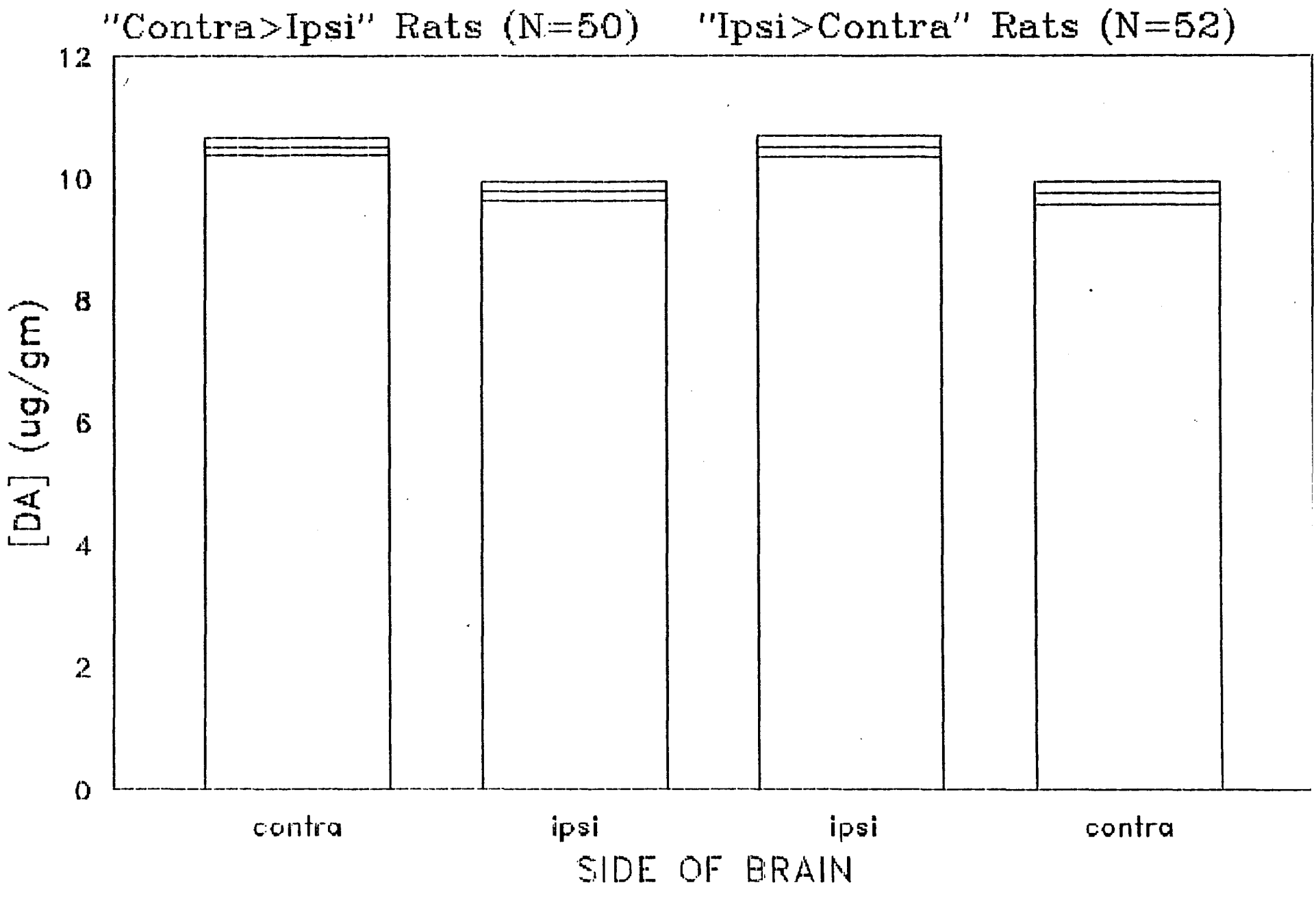
	Females		Males	
	Contra>Ipsi	Ipsi>Contra	Contra>Ipsi	Ipsi>Contra
Unoperated	10	16	21	17
Vehicle	10	7	9	12
Total	20	23	30	29

	Females		Males	
	Contra>Ipsi	Ipsi>Contra	Contra>Ipsi	Ipsi>Contra
Unoperated	14	12	18	20
Vehicle	11	6	12	9
Total	25	18	30	29

rotational behavior; in other words, amphetamine, or the combination of amphetamine and behavioral testing, altered the normal pattern of DA asymmetries (see Table 5.10; compare with Table 3.7). Other differences were detected between the amphetamine tested rats in the present experiment and the rats used in Chapter Three, which were not exposed to amphetamine. For example, a three factor (treatment X sex X side) ANOVA revealed increased striatal DA concentrations bilaterally in the amphetamine treated rats ($p<0.01$); and the contralateral>ipsilateral=ipsilateral>contralateral pattern of striatal DA concentrations (Figure 3.2) and Vmax values (Figure 3.1) found in the rats unexposed to amphetamine was not found in the present group of rats (Figure 5.2). Whether these differences were caused by the amphetamine treatments or by the behavioral testing (or both) cannot be determined from the present data. Nevertheless, the lack of a sex difference in the proportion of "Contra>Ipsi" and "Ipsi>Contra" male and female controls in the present experiment could certainly account for the lack of a sex difference in the proportion of 6OHDA treated males and females classified as paradoxical and classical responders.

Nocturnal % Turning. The results of the experiment described in Chapter Two suggested that nocturnal % turning scores may have some utility as a behavioral measure that differentiates between rats on the basis of whether the Vmax for DA uptake is higher in the ipsilateral (Ipsi>Contra rats) or in the contralateral (Contra>Ipsi rats) striatum. Ipsi>

FIGURE 5.2. Comparison of the mean (±SEM) contralateral and ipsilateral DA concentrations (ug/gm) in amphetamine-treated rats classified according to whether the contralateral or ipsilateral striatum had the higher DA concentration. Note that the contralateral>ipsilateral=ipsilateral>contralateral pattern present in Figure 3.2 is not evident in these amphetamine-treated rats.


"Contra>Ipsi" Rats (N=50)
"Ipsi>Contra" Rats (N=52)
12
10
8
6
4
2
0
[DA] (ug/gm)
contra
ipsi
ipsi
contra
SIDE OF BRAIN

Contra males had higher % turning scores than Contra>Ipsi males, and Ipsi>Contra females had lower % turning scores than Contra>Ipsi females (Table 2.3). Therefore, based on these behavioral data, in the present experiment it would be predicted that the male paradoxical responders would have higher % turning scores than the male classical responders; and the female paradoxical responders would have lower % turning scores than the female classical responders.

An entirely different prediction would be made based on the nocturnal % turning results obtained in the experiment described in Chapter Three, in which rats were assigned to the "Contra>Ipsi" and "Ipsi>Contra" groups based on striatal DA concentrations instead of Vmax's for DA uptake. In contrast to the % turning results obtained in Chapter Two, in Chapter Three nocturnal % turning scores were found to be higher in the "Ipsi>Contra" rats grouped across both sexes, though post-hoc tests revealed that the difference was significant only for the females and not for the males (Table 3.2). Thus, based on these behavioral data, in the present experiment it would be predicted that the male paradoxical responders might have non-significantly higher % turning scores than the male classical responders; and the female paradoxical responders would have higher % turning scores than the female classical responders.

The % turning results of the present experiment agree with neither set of predictions, either based on the behavioral results of Chapter Two or of Chapter Three. Grouped across both sexes the classical responders had higher

% turning scores than the paradoxical responders, and post-hoc testing revealed that this difference was significant only for the males and not for the females. Thus, while it may be considered somewhat interesting that statistically significant effects involving % turning scores were obtained in each of these three experiments, the biological meaning of this behavioral score remains obscure.

Unexpected Neurochemical Effects of 6OHDA

Three neurochemical results of the unilateral intrastriatal injections of 6OHDA were completely unexpected, and warrant further attention.

Asymmetric DA Depletions. The first unexpected neurochemical effect of the unilateral 6OHDA injections was the larger DA depletion that resulted from injections on the right compared to those made on the left. There are four possible explanations for this difference between right and left sided lesions. First, it may have been due to an unintended asymmetry in the actual injection sites. In pilot work prior to the start of this experiment, rats were injected unilaterally with 1 ul of gentian violet in order to find the stereotaxic coordinates that would match Dunnett and Iversen's (1982) "Mid-Dorsal CPU" lesions; then 5 additional rats were injected bilaterally to assure that injection sites in left- and right-biased rats would be symmetric. While no asymmetric pattern was obvious in the small number of rats examined, it is quite possible that if any small bias was present it could certainly become evident neurochemically in

the large series of rats used in the present study. For example, if left sided injections were placed more medially than right sided injections it could be argued that the rate of efflux of toxin from caudate parenchyma into the lateral ventricle would be faster on the right than the left; as a result the toxin would be able to act for longer on the right than on the left.

Assuming the 6OHDA injections were, in fact, symmetric then the three remaining explanations for the asymmetric depletions are: (i) right rotators are more sensitive to the neurotoxic effects of 6OHDA; (ii) equal numbers of DA neurons were affected in both striata, but the left striatum has a greater capacity to restore DA levels back towards normal; or (iii) the right striatum is more sensitive than the left striatum to the neurotoxic effects of 6OHDA -- i.e., more DA neurons on the right accumulated lethal concentrations of the toxin than on the left.

Asymmetric Metabolic Compensation. Previous investigators have found evidence that nigrostriatal DA turnover increases in response to an incomplete lesion (Agid et al., 1973; Westerink et al., 1978; Melamed et al., 1982; Hefti et al., 1984; Dravid et al., 1984). These increases have been interpreted as the neurochemical reflection of compensatory increases in firing rates of DAergic nigrostriatal neurons that survive the 6OHDA insult. Thus, the results of the present study suggest that nigrostriatal neurons in the right hemisphere -- or, right rotators -- have a greater capacitly

to compensate for the DA depleting effects of 6OHDA. The possibility that the asymmetries in DOPAC:DA and HVA:DA ratios were due to the larger DA depletions on the right was considered and rejected (pg 125).

As a result of this asymmetric result the previous reports of increased DA turnover were examined to determine which side of the brain was lesioned: In the four papers that reported which side was lesioned, all four experimental protocols employed right sided lesions only (Agid et al., 1973; Melamed et al., 1982; Hefti et al., 1984; Dravid et al., 1984). The one paper that employed electrolytic rather than 6OHDA lesions (Westerink et al., 1978) did not report which side was lesioned.

Bilateral 5HT Reduction. The third major unexpected finding was the bilaterally symmetric decrease in striatal 5HT content in response to the unilateral striatal injection of 6OHDA. Although this result was not anticipated it might have been, based on the work of Hery et al. (1979, 1980). In cats, using push-pull cannulae, continuously superfused with ^{3}H-tryptophan, these workers have found that unilateral treatments that facilitate DAergic neurotransmission in either the caudate or the substantia nigra result in bilateral decreases in ^{3}H-5HT recovered from the caudate nuclei. Since the results of Hery et al. imply that bilateral striatal 5HT activity is inversely related to unilateral DAergic transmission, in the present work, the unilateral decrease in striatal DAergic transmission by 6OHDA might have been expected to elicit bilateral increases in 5HT

activity. Consistent with this expectation, in the present work, were the bilaterally increased 5HIAA:5HT ratios and the bilaterally decreased 5HT concentrations. An understanding of the mechanisms underlying the present results would seem to have clinical relevance since striatal 5HT concentrations are known to be reduced in Parkinson's Disease (e.g., Scatton et al., 1983).

The above results notwithstanding, it should be noted that Stachowiak et al. (1984) have reported that bilateral intraventricular 6OHDA injections resulted in bilateral striatal DA depletions of more than 95% when the rats were sacrificed 5-8 months later, but had no effect on striatal concentrations of 5HT and 5HIAA. Whether the bilateral nature of their lesions or some other factor can account for the difference between the results of Stachowiak et al. and the results of the present experiment is an open question.

Nocturnal vs. Amphetamine-Induced Rotational Behavior

A valuable by-product of the design of the present experiment was that nocturnal and amphetamine-induced rotation data were obtained from the largest series of male and female rats ever tested. The present results indicate that the direction of nocturnal circling is a good predictor of the direction of amphetamine-induced rotation for those rats that make at least 20 net turns with a % preference of more than 70; for the other rats, the predominant direction of nocturnal circling has no predictive value. This result is in contrast with the previous report of Glick and Cox

(1978) that the direction of nocturnal circling predicts that of amphetamine-induced circling in some 90% of a large group of females. It is also in contrast with the conclusion of Robinson et al. (1984) that there is no way to predict the direction of amphetamine-induced rotation. The discrepancy in the conclusions reached in these three reports are probably due, at least in large measure, to differences between rats supplied by different breeders with respect to the amount of nocturnal circling they will characteristically do. This conclusion is based on our observations of 7-fold differences in the mean nocturnal circling behavior of rats supplied by eight different breeders (Glick, in preparation), including the Holtzman rats used in the experiments of Robinson and coworkers -- the Holtzmans that have been tested in this laboratory averaged less than 10 net rotations nocturnally.

Possible Clinical Implications of the Present Work

The results presented in this and the preceding chapters provide considerable support for the two population model. At this point it is natural to suggest the possibility that an analogous dichotomy might exist in humans as well; and, some tentative evidence for this notion was discussed in Chapter Three. If such a dichotomy does, in fact, exist in humans it might be expected that patients afflicted with Parkinson's Disease would also reflect this dichotomy. Recently, Zetusky et al. (1985) reported the results of their clinical work with patients afflicted with Parkinson's

Disease and reviewed the results of others. These results indicate that two sub-groups of Parkinson's Disease patients can indeed be identified on the basis of their symptomatology and, perhaps more importantly for the present discussion, on the basis of their clinical improvement in response to L-Dopa pharmacotherapy. One group is characterized by the predominance of postural instability, gait difficulty, bradykinesia, and a poor response to L-Dopa; the other group is characterized by the predominance of tremor, and a good response to L-Dopa. It may be premature to speculate about which of the Parkinsonian sub-groups might correspond to the groups identified in the present series of investigations ("Contra>Ipsi" vs. "Ipsi>Contra," or "Right>Left" vs. "Left> Right"), but the possibility that such a correspondence may in fact exist must be taken seriously: On the basis of the results of the experiment described in this chapter, as well as the results of Robinson and Becker (1983), it seems clear that when amphetamine-induced turning is used as a criterion for the inclusion of rats in studies designed to screen compounds for their potential usefulness in the treatment of Parkinson's Disease, only a sub-group of rats (i.e., only one of the populations) ultimately gets included in the experiment. It would therefore be quite logical to find two groups of responders to the drugs developed in this way. Thus, individual differences between Parkinson's patients may reflect individual differences between nigro-striatal asymmetry in normals.

SUMMARY

The relationship between nigro-striatal asymmetry and rotational behavior was investigated in rats. Striatal dopaminergic innervation was quantitated using the Vmax for dopamine uptake *in vitro*. It was found that rotational behavior was correlated with the magnitude of the Vmax asymmetry regardless of which striatum had the higher Vmax, and which direction a rat turned. These data were considered to be consistent with one of two models: either (i) the innervation asymmetry is related to the *amount* of rotation (i.e., the "gain") but not the *direction* of rotation, or (ii) there are two populations, or "kinds," of rats -- those that circle away from the side with the greater striatal dopaminergic innervation ("Contra>Ipsi" rats) and those that circle towards the side with the greater striatal dopaminergic innervation ("Ipsi>Contra" rats). The latter interpretation was favored by the fact that the two groups of rats were not mirror images of one another; that is, while the mean ipsilateral Vmax's in the two groups were similar, the contralateral Vmax's were significantly different. Furthermore, the contralateral-ipsilateral Vmax difference was correlated with the magnitude of the contralateral Vmax but not with that of the ipsilateral Vmax.

In a follow-up experiment the two population model was strengthened when the above findings were essentially replicated using striatal dopamine concentration instead of Vmax for dopamine uptake as the measure of striatal dopaminergic

innervation. Also, dopamine turnover in the two striata was found to be higher in the "Ipsi>Contra" rats than in the "Contra>Ipsi" rats. In this experiment, it was also found that the dissected caudate weights on the contralateral sides in the two populations were also different: While the ipsilateral caudate weights were virtually identical, the contralateral caudates were significantly lighter in the "Contra> Ipsi" rats than in the "Ipsi>Contra" rats; and, again, the contralateral-ipsilateral weight difference was correlated with the weight of the contralateral caudate but not with that of the ipsilateral caudate.

The relationships with caudate weight were confirmed in an experiment in which the areas of enlarged projections of coronal sections were measured.

Functional evidence supporting the two population model was obtained in an experiment in which the effects of 6-hydroxydopamine-induced dopamine depletions were made in the striatum on the side towards which the rats normally turned: Despite the fact that the lesions were quantitatively similar two groups of responders were clearly identifiable -- those that increased their turning in response to the lesion, and those whose turning was unchanged or was actually decreased.

These results indicate that the relationship between nigro-striatal asymmetry and rotational behavior are more complex than is currently believed. The proposed two population model may represent a closer approximation to reality than the generally accepted model for the relationship between nigro-striatal asymmetry and circling.

Additional findings from the present work, unrelated to the two population hypothesis, consist of the following:

(i) The K_m for striatal dopamine uptake *in vitro* is higher in females than in males.

(ii) The right striatum (or the striata of right rotators) is more sensitive to the dopamine depleting effects of 6-hydroxydopamine than is the left striatum (or the striata of left rotators).

(iii) Independent of (ii), the right striatum (or the striata of right rotators) undergoes a more radical metabolic compensation to the 6-hydroxydopamine-induced dopamine depletion than the left striatum (or the striata of left rotators).

(iv) In response to the unilateral depletion of striatal dopamine by 6-hydroxydopamine, serotonin levels decrease bilaterally, and serotonin turnover increases bilaterally.

ADDENDUM

Immediately following the completion of my experimental work Glick and Camarota repeated the 6OHDA experiment described in Chapter Five. Intra-nigral instead of intra-striatal injections were employed. Rats were classified as "classical" or "paradoxical" responders based on the same criteria employed in Chapter Five, and only males were used. As shown below, their findings provide additional support for the two population model. Despite the fact that their DA depletions averaged 77.9±3.6%, the results were very similar to those summarized in Table 5.5 (pg 137).

Amphetamine -Induced Net Rotations	Classical Responders (N=10)	Paradoxical Responders (N=10)
Pre-6OHDA	96±19	70±19
Post-6OHDA	363±55	57±23
Net Increase	267±61	-13±14
% DA Depletion	80.9±6.1	75.0±4.2

REFERENCES

Abrams, R, and Fink, M (1984) The present status of unilateral ECT: Some recommendations. J Affect Disorders 7:245-7

Agid, F, Javoy, F, and Glowinski, J (1973) Hyperactivity of remaining dopaminergic neurones after partial destruction of the nigro-striatal dopaminergic system in the rat. Nature New Biol 245:150-1

Anden, N-E, Carlsson, A, Dahlstrom, A, Fuxe, K, Hillarp, N-A, and Larsson, K (1964) Demonstration and mapping out of nigro-neostriatal dopamine neurons. Life Sci 3:523-30

Amaducci, L, Sorbi, S, Albanese, A, and Gainotti, G (1981) Choline acetyltransferase (ChAT) activity differs in right and left human temporal lobes. Neurol 31:799-805

Arnt, J, and Hyttel, J (1984) Differential inhibition by dopamine D-1 and D-2 antagonists of circling behaviour induced by dopamine agoniats in rats with unilateral 6-hydroxydopamine lesions. Eur J Pharmacol 102:349-54

Becker, JB, Robinson TE, and Lorenz, KA (1982) Sex differences and estrous cycle variations in amphetamine-elicited rotational behavior. Eur J Pharmacol 80:65-72

Besedovsky, HO, Sorkin, E, Felix, D, and Haas, H (1977) Hypothalamic changes during the immune response. Eur J Immunol 7:325-8

Birkmayer, W, and Hornykiewicz, O (1961) Der L-dioxyphenylalanin (=L-dopa)-effekt bei der Parkinson akinese. Klin-Ther Wschr 73:787

Bishop, DVM (1983) How sinister is sinistrality? J Royal Coll Phys Lond 17:161-72

Black, RS, and Robinson, RG (1985) Intracortical 5,7-dihydroxytryptamine depletes brain serotonin concentrations without affecting spontaneous activity. Pharmacol Biochem Behav 22:327-31

Blalok, JE, and Smith, EM (1985) A complete regulatory loop between the immune and neuroendocrine systems. Fed Proc 44:108-11

Bracco, L, Tiezzi, A, Ginanneschi, A, Campanella, C, and Amaducci, L (1984) Lateralization of choline acetyltransferase (ChAT) activity in fetus and adult human brain. Neurosci Lett 50:301-5

Bradshaw-McAnulty, G, Hicks, RE, and Kinsbourne, M (1984) Pathological left-handedness and familial sinistrality in relation to degree of mental retardation. Brain Cognition 3:349-56

Brass, CA, and Glick, SD (1981) Sex differences in drug-induced rotation in two strains of rats. Brain Res 223:229-234

Broca, P (1865) Sur le siège de la faculte du langage articule. Bull de la Societe d'Anthropologie 6:377-93

Bryden, MP (1982) Laterality: Functional asymmetry in the intact brain. Acad Press, New York

Camp, DM, Robinson, TE, and Becker, JB (1984) Sex differences in the effects of early experience on the development of behavioral and brain asymmetries in rats. Physiol Behav 33:433-9

Cohen, G (1984) Oxy-radical toxicity in catecholamine neurons. Neurotoxicol 5:77-82

Cools, AR (1977) Two functionally and pharmacologyically distinct dopamine receptors in the rat brain. Adv Biochem Psychopharmacol 16:215-25

Cools, AR, and van Rossum, JM (1980) Multiple receptors for brain dopamine in behavior regulation: Concept of dopamine-e and dopamine-i receptors. Life Sci 27:1237-53

Crespi, S, and Jouvet, M (1984) Has the rahe dorsalis nucleus an asymmetric function? Exp Brain Res 56:403-9

Cutler, NR, Post, RM, Rey, AC, and Bunney, WE, Jr (1981) Depression-dependent dyskinesias in two cases of manic-depressive illness. New Engl J Med 304:1088-9

Damasio, AR (1982) Reflecting on the work of R.W. Sperry. Trends Neurosci 5:222-4

Dark, KA, Ellman, G, Peeke, HVS, Galin, D, and Reus, VI (1984) Sex differences and asymmetries of catecholamines: Relation to turning preferences. Pharmacol Biochem Behav 20:327-30

Denenberg, VH (1984) Behavioral asymmetry. In: Geschwind, N, and Galaburda, AM (eds) Cerebral dominance: The biological foundations. Harvard, Cambridge, pp 114-33

Denenberg, VH, Garbanati, J, Sherman, G, Yutzey, DA, and Kaplan, R (1978) Infantile stimulation induces brain lateralization in rats. Science 201:1150-2

Dewson, JH (1977) Preliminary evidence off hemispheric asymmetry of auditory function in monkeys. In: Harnad, S, Doty, RW, Goldstein, L, Jaynes, J, and Krauthamer, G (eds) Lateralization in the nervous system. Acad press, New York, pp 63-74

Diamond, MC (1984) Age, sex, and environmental influences. In: Geschwind, N, and Galaburda, AM (eds) Cerebral dominance: The biological foundations. Harvard, Cambridge, pp 134-46

Diamond, MC, Murphy, GM, Akiyama, K, and Johnson, RE (1982) Morphologic hippocampal asymmetry in male and female rats. Exp Neurol 76:553-66

Direnfeld, LK, Albert, ML, Volicer, L, Langlais, PJ, Marquis, J, and Kaplan, E (1984) Parkinson's disease: The possible relationship of laterality to dementia and neurochemical findings. Arch Neurol 41:935-41

Dravid, A, Jaton, AL, Enz, A, and Frei, P (1984) Spontaneous recovery from motor asymmetry in adult rats with 6-hydroxydopamine-induce partial lesions of the substantia nigra. Brain Res 311:361-5

Drew, KD, Lyon, RA, Titeler, M, and Glick, SD (in press) Asymmetry in D-2 binding in female rat striata. Brain Res

Dunnett, SB, and Iversen, SD (1982) Spontaneous and drug-induced rotation following localized 6-hydroxydopamine and kainic acid-induced lesions of the neostriatum. Neuropharmacol 21:899-908

Eling, P (1984) Broca on the relation between handedness and cerebral speech dominance. Brain Lang 22:158-159

Ezerman, EB, and Kromer, LF (1985) Outbred Sprague-Dawley rats from two breeders exhibit different incidences of neuroanatomical abnormalities affecting the primary cerebellar fissure. Exp Brain Res 59:625-8

Falzi, G, Perrone, P, and Vignolo, LA (1982) Right-left asymmetry in the anterior speech region. Arch Neurol 39:239-40

Fleisher, LN, and Glick, SD (1979) Hallucinogen-induced rotational behavior in rats. Psychopharmacol 62:193-200

Freeman, RL, Galaburda, AM, Cabal, RD, and Geschwind, N (1985) The neurology of depression: Cognitive and behavioral deficits with focal findings in depression and resolution after electroconvulsive therapy. Arch Neurol 42:289-91

Friedland, RP, Budinger, TF, Koss, E, and Ober, BA (1985) Alzheimer's disease: Anterior-posterior and lateral hemispheric alteraltions in cortical glucose utilization. Neurosci Lett 53:235-40

Frigyesi, TL, and Purpura, DP (1967) Electrophysiological analysis of reciprocal caudato-nigral relations. Brain Res 6:440-56

Frumkin, LR, and Grim, P (1981) Is there pharmacological asymmetry in the human brain? An hypothesis for the differential hemispheric action of barbiturates. Intern J Neurosci 13:187-97

Gainotti, G (1983) Laterality of affect: The emotional behavior of right- and left-brain-damaged patients. In: Myslobodsky, MS (ed) Hemisyndromes: Psychobiology, neurology, psychiatry. Academic, New York, pp 175-92

Galaburda, AM (1984) Anatomical asymmetries. In: Geschwind, N, and Galaburda, AM (eds) Cerebral dominance: The biological foundations. Harvard, Cambridge, pp 11-26

Garbanati, JA, Sherman, GF, Rosen, GD, Hofmann, M, Yutzey, DA, and Denenberg, VH (1983) Handling in infancy, brain laterality and muricide in rats. Behav Brain Res 7:351-9

Gardner, EL, Zukin, RS, and Makman, MH (1980) Modulation of opiate receptor binding in striatum and amygdala by selective mesencephalic lesions. Brain Res 194:232-9

Gerendai, I (1984) Lateralization of neuroendocrine con-trol. In: Geschwind, N, and Galaburda, AM (eds) Cerebral dominance: The biological foundations. Harvard, Cambridge, pp 167-78

Gerendai, I, Rotsztejn, W, Marchetti, B, Kordon, C, and Scapagnini, U (1978) Unilateral ovariectomy-induced luteinizing hormone-releasing hormone content changes in the two halves of the mediobasal hypothalamus. Neurosci Lett 5:41-5

Geschwind, N (1985) Implications for genetics, evolution, and clinical syndromes. In: Glick, SD (ed) Cerebral lateralization in nonhuman species. Academic, Orlando, pp 247-278

Geschwind, N, and Behan, PO (1982) Left-handedness: Association with immune disease, migraine, and developmental learning disorders. Proc Natl Acad Sci USA 79:5097-100

Geschwind, N, and Levitsky, W (1968) Human brain: Left-right asymmetries in temporal speech region. Science 161: 186-7

Giambalvo, CT, and Snodgrass, SR (1978) Effect of parachloroamphetamine and 5,7-dihydroxytryptamine on rotation and dopamine turnover. Brain Res 149:453-67

Ginobili de Martinez, MS, Rodriguez de Turco, EB, and Barrantes, FJ (1985) Endogenous asymmetry of rat brain lipids and dominance of the right cerebral hemisphere in free fatty acid response to electroconvulsive shock. Brain Res 339:315-21

Glick, SD (1983) Cerebral lateralization in the rat and tentative extrapolations to man. In: Myslobodsky, MS (ed) Hemisyndromes: Psychobiology, neurology, psychiatry. Academic, New York, pp 7-26

Glick, SD, and Cox, RD (1976) Differential effects of unilateral and bilateral caudate lesions on side preferences and timing behavior in rats. J Comp Physiol Psychol 90:528-35

Glick, SD, and Cox, RD (1978) Nocturnal rotation in normal rats: Correlation with amphetamine-induced rotation and effects of nigrostriatal lesions. Brain Res 150:149-61

Glick, SD, and Cox, RD (1980) Striatal asymmetry and morphine reinforcement. Brain Res 197:253-5

Glick, SD, Cox, RD, Jerussi, TP, and Greenstein, S (1977) Normal and amphetamine-induced rotation of rats on a flat surface. J Pharm Pharmacol 29:51-2

Glick, SD, Hinds, PA, and Shapiro, RM (1983) Cocaine-induced rotation: Sex-dependent differences between left- and right-sided rats. Science 221:775-7

Glick, SD, Jerussi, TP, Waters, DH, and Green, JP (1974) Amphetamine-induced changes in striatal dopamine and acetylcholine levels and relationship to rotation (circling) in rats. Biochem Pharmacol 23:3223-5

Glick, SD, Meibach, RC, Cox, RD, and Maayani, S (1979) Multiple and interrelated functional asymmetries in rat brain. Life Sci 25:395-400

Glick, SD, and Morihisa, JM (1976) Changes in sensitivity of morphine-induced circling behavior ater chronic treatment and persistence after withdrawal in rats. Nature 260:159-61

Glick, SD, Meibach, RC, Cox, RD, and Maayani, S (1980) Phencyclidine-induced rotation and hippocampal modulation of nigrostriatal asymmetry. Brain Res 196:99-108

Glick, SD, Ross, DA, and Hough, LB (1982) Lateral asymmetry of neurotransmitters in human brain. Brain Res 234:53-63

Glick, SD, Weaver, LM, and Meibach, RC (1980) Lateralization of reward in rats: Differences in reingorcing thresholds. Science 207:1093-5

Glick, SD, Weaver, LM, and Meibach, RC (1981) Amphetamine enhancement of reward asymmetry. Psychopharmacol 73:323-7

Glick, SD, Weaver, LM, and Meibach, RC (1982) Asymmetrical effects of morphine and naloxone on reward mechanisms. Psychopharmacol 78:219-24

Glowinski, J, Tassin, JP, and Thierry, AM (1982) The meso-cortico-prefrontal dopaminergic neurons. Trends Neurosci 7:415-8

Goldschmidt, RC, Hough, LB, Glick, SD, and Padawer, J (1984) Mast cells in rat thalamus: Nuclear localization, sex difference and left-right asymmetry. Brain Res 323: 209-17

Gratton, A, and Wise, RA (1985) Mapping of contraversive and ipsiversive circling responses to ventral tegmental and substantia nigra electrical stimulation. Physiol Behav 35:61-5

Gray, EG, and Whittaker, VP (1962) The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. J Anat 96:79-88

Greenstein, S, and Glick, SD (1975) Improved automated apparatus for recording rotation (circling behavior) in rats or mice. Pharmacol Biochem Behav 3:507-10

Gregory, S, Shawcross, CR, and Gill, D (1985) The Nottingham ECT study: A double-blind comparison of bilateral, unilateral and simulated ECT in depressive illness. Brit J Psych 146:520-4

Groves, PM (1983) A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement. Brain Res Rev 5:109-32

Guillemin, R, Cohn, M, and Melnechuk, T (1985) Neural modulation of immunity. Raven, New York

Heffner HE, and Heffner RF (1984) Temporal lobe lesions and perception of species specific vocalizations by macaques. Science 226:75-6

Hefti, F, Enz, A, and Melamed, E (1985) Partial lesions of the nigrostriatal pathway in the rat: Acceleration of transmitter synthesis and release of surviving dopaminergic neurones by drugs. Neuropharmacol 24:19-23

Heikkila, RE, Shapiro, BS, and Duvoisin, RC (1981) The relationship between loss of dopamine nerve terminals, striatal [^{3}H]spiroperidol binding and rotational behavior in unilaterally 6-hydroxydopamine-lesioned rats. Brain Res 211:285-92

Herberg, LJ, and Franklin, KBJ (1973) A variable inclined plane for blocking the rat's brain. Physiol Behav 10:617-8

Herron, J (1980) Neuropsychology of left-handedness. Acad Press, New York

Hery, F, Bourgoin, S, Soubrie, P, Montastruc, JL, Artaud, F, and Glowinski, J (1979) In vivo release of newly synthesized ^{3}H-serotonin in the caudate nucleus and the substantia nigra of the cat: Possible control by dopaminergic mechanisms. Neurosci Lett Suppl 3:S238

Hery, F, Soubrie, P, Bourgoin, S, Montastruc, JL, Artaud, F, and Glowinski, J (1980) Dopamine released from dendrites in the substantia nigra controls the nigral and striatal release of serotonin. Brain Res 193:143-51

Hirschhorn, ID, Hittner, D, Gardner, EL, Cubella, J, and Makman, MH (1983) Evidence for a role of endogenous opioids in the nigrostriatal system: Influence of naloxone and morphine on nigrostriatal dopaminergic supersensitivity. Brain Res 270:109-17

Hodge, GK, and Butcher, LL (1979) Role of pars compacta of the substantia nigra in circling behavior. Pharmacol Biochem Behav 10:695-709

Hornykiewicz, O (1966) Dopamine (3-hydroxytryptamine) and brain function. Pharmacol Rev 18:925-64

Hyde, JF, and Jerussi, TP (1983) Sexual dimorphism in rats with respect to locomotor activity and circling behavior. Pharmacol Biochem Behav 18:725-9

Irwin, P (1985) Greater brain response of left-handers to drugs. Neuropsychologia 23:61-7

Jerussi, TP, and Glick, SD (1974) Amphetamine-induced rotation in rats without lesions. Neuropharmacol 13:283-6

Jerussi, TP, and Glick, SD (1975) Apomorphine-induced rotation in normal rats and interaction with unilateral caudate lesions. Psychopharmacol 40:329-34

Jerussi, TP, and Glick, SD (1976) Drug-induced rotation in rats without lesions: Behavioral and neurochemical indices of a normal asymmetry in nigrostriatal function. Psychopharmacol 47:259-60

Jerussi, TP, Glick, SD, and Johnson, CL (1977) Reciprocity of pre- and post-synaptic mechanisms involved in rotation as revealed by dopamine metabolism and adenylate cyclase stimulation. Brain Res 129:385-8

Jonsson, G (1976) Studies on the mechanisms of 6-hydroxydopamine neurotoxicity. Med Biol 54:406-20

Kemali, M (1984) Is brain asymmetry the rule or the exception? Morphological consideration on lower vertebrates. Adv Biol Psych 15:20-9

Koenig, JFR, and Klippel, RA (1963) The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore

Kolb, B, Sutherland, RJ, Nonneman, AJ, and Wishaw, IQ (1982) Asymmetry in the cerebral hemispheres of the rat, mouse, rabbit, and cat: The right hemisphere is larger. Exp Neurol 78:348-59

Kolb, B, and Whishaw, IQ (1980) Fundamentals of human neuropsychology. Freeman, San Fransisco

Kubos, KL, Pearlson, GD, and Robinson, RG (1982) Intracortical kainic acid induces an asymmetrical response in the rat. Brain Res 239:303-9

Kubos, KL, and Robinson, RG (1984a) Cortical undercuts in the rat produce asymmetrical behavioral response without altering catecholamine concentrations. Exp Neurol 83:646-53

Kubos, KL, and Robinson, RG (1984b) Asymmetrical effects of cortical island lesions in the rat. Behav Brain Res 11:89-93

Kuhar, MJ (1973) Neurotransmitter uptake: A tool in identifying neurotransmitter-specific pathways. Life Sci 13:1623-34

LeMay, M (1984) Radiological, developmental, and fossil asymmetries. In: Geschwind, N, and Galaburda, AM (eds) Cerebral dominance: The biological foundations. Harvard, Cambridge, pp 26-42

Levi, G, and Raiteri, M (1973) GABA and glutamate uptake by subcellular fractions enriched in synaptosomes: Critical evaluation of some methodological aspects. Brain Res 57:165-85

Lewinski, A, Gerendai, I, Pawlikowski, M, and Halasz, B (1982) Unilateral posterior deafferentation of the hypothalamus and mitotic activity of thyroid follicular cells under normal conditions and after hemithyroidectomy. Endocrinol Exp 16:75-80

Lowry, OH, Rosebrough, NJ, Farr, AL, and Randall, RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:263-75

Luria, AR (1970) Traumatic aphasia. Mouton, The Hague

Melamed, E, Hefti, F, and Wurtman, RJ (1982) Compensatory mechanisms in the nigrostriatal dopaminergic system in Parkinson's Disease: Studies in an animal model. Isr J Med Sci 18:159-63

Miller, JC (1983) Sex differences in dopaminergic and cholinergic activity and function in the nigro-striatal system of the rat. Psychoneuroendocrinol 8:225-36

Mittleman, G, Fray, PJ, and Valenstein, ES (1985) Asymmetry in the effects of unilateral 6-OHDA lesions on eating and drinking evoked by hypothalamic stimulation. Behav Brain Res 15:263-7

Moran, TH, Kubos, KL, Sanberg, PR, and Robinson, RG (1984a) Marked behavioral and biochemical sensitivity to lesion size in the posterior cortex of the rat. Life Sci 35:1337-42

Moran, TH, Sanberg, PR, Kubos, KL, Goldrich, M, and Robinson, RG (1984b) Asymmetrical effects of unilateral cortical suction lesions: Behavioral characterization. Behav Neurosci 98:747-52

Nordeen, EJ, and Yahr, P (1982) Hemispheric asymmetries in the behavioral and hormonal effects of sexually differentiating mammalian brain. Science 218:391-4

Oke, A, Keller, R, Mefford, I, and Adams, RN (1978) Lateralization of norepinephrine in human thalamus. Science 200:1411-3

Oke, A, Lewis, R, and Adams, RN (1980) Hemispheric asymmetry of norepinephrine distribution in rat thalamus. Brain Res 188:269-72

Parker, RB, and Waud, DR (1971) Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. J Pharmacol Exp Ther 177:1-12

Parkinson, J (1817) An essay on the shaking palsy. Reprinted in: Arch Neurol Psych (1922) 7:681-710

Pearlson, GD, Kubos, KL, and Robinson, RG (1984) Effect of anterior-posterior lesion location on the asymmetrical behavioral biochemical response to cortical suction ablations in the rat. Brain Res 293:241-50

Pediconi, MF, and Rodriguez de Turco, EB (1984) Free fatty acid content and release kinetics as manifestations of cerebral lateralization in mouse brain. J Neurochem 43:1-7

Pellegrino, LJ, and Cushman, AJ (1967) A stereotaxic atlas of the rat brain. Appleton-Century-Crofts, New York

Pycock, CJ (1980) Turning behaviour in animals. Neurosci 5:461-514

Renoux, G, Biziere, K, Renoux, M, Guillaumin, J-M, and Degenne, D (1983) A balanced brain asymmetry modulates T cell-mediated events. J Neuroimmunol 5:227-38

Renoux, G, Renoux, M, Biziere, K, Guillaumin, JM, Bardos, P, and Degenne, D (1984) Involvement of brain neocortex and liver in the regulation of T cells: The mode of action of sodium diethyldithiocarbamate (Imuthiol). Immunopharmacol 7:89-100

Robinson, RG, and Coyle, JT (1980) The differential effect of right versus left hemispheric cerebral infarction on catecholamines and behavior in the rat. Brain Res 188:63-78

Robinson, RG, and Stitt, TG (1981) Intracortical 6-hydroxy-dopamine induces an asymmetrical behavioral response in the rat. Brain Res 213:387-95

Robinson, RG (1985) Lateralized behavioral and neurochemical consequences of unilateral brain injury in rats. In: Glick, SD (ed) Cerebral lateralization in nonhuman species. Academic, Orlando, pp 135-56

Robinson, RG, Starr, LB, Lipsey, JR, Rao, K, and Price, TR (1985) A two-year longitudinal study of poststroke mood disorders: In-hospital prognostic factors associated with six-month outcome. J Nerv Ment Dis 173:221-6

Robinson, TE, Becker, JB, and Ramirez, VD (1980) Sex differences in amphetamine-elicited rotational behavior and the lateralization of striatal dopamine in rats. Brain Res Bull 5:539-45

Robinson, TE, Becker, JB, and Presty, SK (1982) Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: Sex differences. Brain Res 253:231-41

Robinson, TE, and Becker, JB (1983) The rotational behavior model: asymmetry in the effects of unilateral 6-OHDA lesions of the substantia nigra in rats. Brain Res 264:127-31

Robinson, TE, Becker, JB, Camp, DM, and Mansour, A (1985) Variation in the pattern of behavioral and brain asymmetries due to sex differences. In: Glick, SD (ed) Cerebral lateralization in nonhuman species. Academic, Orlando

Ross, DA, and Glick, SD (1981) Lateralized effects of bilateral frontal cortex lesions in rats. Brain Res 210:379-82

Rosa, DA, Glick, SD, and Meibach, RC (1981) Sexually dimorphic brain and behavioral asymmetries in the neonatal rat. Proc Natl Acad Sci USA 78:1958-61

Rosa, DA, Glick, SD, and Meibach, RC (1982) Sexually dimorphic cerebral asymmetries in 2-deoxy-D-glucose uptake during postnatal development of the rat: Correlations with age and relative brain activity. Dev Brain Res 3:341-7

Rossor, M, Garett, N, and Iversen, L (1980) No evidence for lateral asymmetry of neurotransmitters in post-mortem human brain. J Neurochem 35:743-5

Roth, RH, Murrin, LC, and Walters, JR (1976) Central dopaminergic neurons: Effects of alterations in impulse flow on the accumulation of dihydroxyphenylacetic acid. Eur J Pharmacol 36:163-71

Rothman, AH, and Glick, SD (1976) Differential effects of unilateral and bilateral caudate lesions on side preference and passive avoidance behavior in rats. Brain Res 118:361-9

Sackeim, HA, Greenberg, MS, Weiman, AL, Gur, RC, Hungerbuhler, JP, and Geschwind, N (1982) Hemispheric asymmetry in the expression of positive and negative emotions: Neurologic evidence. Arch Neurol 39:210-8

Satz, P, Orsini, DL, Saslow, E, and Henry, R (1985) The pathological left-handedness syndrome. Brain Cognition 4:27-46

Scatton, B, Javoy-Agid, F, Rouquier, L, DuBois, B, and Agid, J (1983) Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's Disease. Brain Res 275:321-8

Schleifer, SJ, and Shapiro, RM (in press) Commentary on "Brain neocortex lateralized control of immune recognition" by G Renoux and K Biziere. Integrative Psych

Schneider, LH, Murphy, RB, and Coons, EE (1982) Lateralization of striatal dopamine (D2) receptors in normal rats. Neurosci Lett 33:281-4

Schoener, EP, and Elkins, DP (1984) Neuronal response to dopamine in rat neostriatum: A push-pull perfusion study. Neuropharmacol 23:611-6

Schultz, W (1982) Depletion of dopamine in the striatum as an experimental model of parkinsonism: Direct effects and adaptive mechanisms. Prog Neurobiol 18:121-66

Serafetinides, EA (1965) The significance of the temporal lobes and of hemispheric dominance in the production of LSD-25 symptomatology in man. Neuropsychol 3:69-79

Shapiro, RM, Badalamenti, JI, and Glick, SD (1983) A simple and rapid technique for preparing histological sections of brain. Pharmacol Biochem Behav 19:1049-50

Sherman, GF, and Galaburda, AM (1984) Neocortical asymmetry and open-field behavior in the rat. Exp Neurol 86:473-82

Sherman, GF, Galaburda, AM, and Geschwind, N (1982) Neuroanatomical asymmetries in non-human species. Trends Neurosci 5:429-31

Shoemaker, H, and Nickolson, VJ (1983) Dopamine uptake by rat striatal synaptosomes: Time- and temperature-dependent decay and protection by dithiothreitol and dopamine. J Neurochem 40:922-8

Slopsema, JS, van der Gugten, J, and de Bruin, JPC (1982) Regional concentrations of noradrenaline and dopamine in the frontal cortex of the rat: Dopaminergic innervation of the prefrontal subareas and lateralization of prefrontal dopamine. Brain Res 250:197-200

Snedecor, GW, and Cochran, WG (1967) Statistical methods, 6th edition. Iowa State Univ Press, Ames, Iowa

Sonsalla, PK, Gibb, JW, and Hanson, GR (1984) Opposite responses in the striato-nigral Substance P system to D1 and D2 receptor activation. Eur J Pharmacol 105:185-7

Sperry, RW, Gazzaniga, MS, and Bogen, JE (1969) Interhemispheric relationships: The neocortical commissures; syndromes of hemispheric disconnection. In: Vinken, PJ, and Bruyn, GW (eds) Handbook of clinical neurology, 4th edition, North Holland, Amsterdam

Stachowiak, MK, Bruno, JP, Snyder, M, Stricker, EM, and Zigmond, MJ (1984) Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. Brain Res 291:164-7

Starr, MS, and Kilpatrick, IC (1981) Bilateral asymmetry in brain GABA function? Neurosci Lett 25:167-72

Starr, MS, and Summerhayes, M (1985) Dysfunction of the midbrain angular complex can accentuate or attenuate circling behaviour in the rat. Exp Brain Res 58:45-55

Stoof, JC, and Kebabian, JW (1984) Two dopamine receptors: Biochemistry, physiology and pharmacology. Life Sci 35:2281-96

Subirana, A (1958) The prognosis in aphasia in relation to cerebral dominance and handedness. Brain 81:415-25

Tassin, JP, Cheramy, A, Blanc, G, Thierry, AM, and Glowinski, J (1976) Topographical distribution of dopaminergic innervation and of dopaminergic receptors in the rat striatum. I. Microestimation of ^{3}H-dopamine uptake and dopamine content in microdiscs. Brain Res 107:291-301

Terzian, H (1964) Behavioural and EEG effects of intracarotid sodium amytal injection. Acta Neurochir 12:230-9

Thiebot, M-H, and Soubrie, P (1979) Amphetamine-induced circling behavior in rats: Effects of unilateral microinjections of GABA and GABA-related drugs in substanta nigra. Brain Res 167:307-22

Tye, NC, Horaman, L, Wright, FC, Large, BT, and Pullar, IA (1977) Two dopamine receptors: Supportive evidence with the rat rotational model. Eur J Pharmacol 45:87-90

Ungerstedt, U (1971a) Adipsia and aphagia after 6-hydroxy-dopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol Scand Suppl 367:95-117

Ungerstedt, U (1971b) Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. Acta Physiol Scand Suppl 367:49-68

Ungerstedt, U (1971c) Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol Scand Suppl 367:69-93

Ungerstedt, U, and Arbuthnott, GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. Brain Res 24: 485-93

Vaccarino, FJ, and Franklin, KBJ, (1982a) Dopamine mediates ipsi- and contraversive circling elicited from the substantia nigra. Pharmacol Biochem Behav 17:431-4

Vaccarino, FJ, and Franklin, KBJ (1982b) Self-stimulation and circling reveal functional differences between medial and lateral substantia nigra. Behav Brain Res 5:281-95

Wada, JA, Clarke, R, and Hamm, A (1975) Cerebral hemispheric asymmetry in humans. Arch Neurol 32:239-46

Wernicke, C (1874) The aphasic symptom complex: A psychological study from an anatomic basis. Cohn and Wigert, Breslau (in German)

Westerink, BHC, van der Heyden, JAM, and Korf, J (1978) Enhanced dopamine metabolism after small lesions in the midbrain of the rat. Life Sci 22:749-56

Wheaton, JE, and McCann, SM (1976) Luteinizing hormone-releasing hormone in peripheral plasma and hypothalamus of normal and ovariectomized rats. Neuroendocrinol 20:296-310

Wheeler, SC, and Roth, RH (1980) Tolerance to fluphenazine and supersensitivity to apomorphine in central dopaminergic systems after chronic fluphenazine decanoate treatment. Naunyn-Schmiedeberg's Arch Pharmacol 312:151-9

Witelson, SF (1985) The brain connection: The corpus callosum is larger in left-handers. Science 229:665-8

Yamamoto, BK, and Freed, CR (1984) Reversal of amphetamine-induced circling preference in trained circling rats. Life Sci 34:675-82

Zetusky, WJ, Jankovic, J, and Pirozzolo, FJ (1985) The heterogeneity of Parkinson's Disease: Clinical and prognostic implications. Neurol 35:522-6

Zigmond, MJ, Acheson, AL, Stachowiak, MK, and Stricker, EM (1984) Neurochemical compensation after nigrostriatal bundle injury in an animal model of preclinical parkinsonism. Arch Neurol 41:856-61

Zimmerberg, B, Glick, SD, and Jerussi, TP (1974) Neurochemical correlate of a spatial preference in rats. Science 185: 623-5