CEREBRAL LATERALIZATION OF DOPAMINE-MEDIATED FUNCTIONS IN THE RAT

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ABSTRACT

There is evidence that a number of psychiatric disorders, particularly depression and schizophrenia, are frequently associated with lateralized disturbances of brain function, or alterations in *normal* patterns of lateralized function. It is also known that many such disturbances, especially in schizophrenia, involve central dopamine (DA) systems. Several studies in recent years have established that lower animals often exhibit lateralization of brain function, both on the neurochemical and behavioral level. Central DA systems appear to be particularly asymmetrical. Left/right hemispheric asymmetries in rats have been reported at the population level for a variety of DA-modulated behaviors which may variously reflect motor, sensory, spatial, or stress/arousal processes. However, the directions of reported population asymmetries can vary across studies, depending in part upon the particular processes predominantly reflected in the measured behavior, and consequently leading to difficulties in interpretation. Given the potential relevance to both normal and abnormal brain function in humans, it is of much interest to determine what paraliels exist between lateralization in humans and lower animals.

The objectives of the present thesis were twofold. First, we sought to determine which DA-mediated behaviors exhibit left/right hemispheric asymmetries at the population level in rats, by employing specific paradigms to measure motor, sensorimotor, spatial and stress-related processes. The basic approach was to compare the effects of unilateral DA-depleting lesions (with 6-hydroxydopamine), in left or right brain structures of male rats. Behavioral and neurochemical asymmetries were also examined in nonlesioned controls. A second objective was to study the role of interhemispheric connections in the expression of

behavioral asymmetries characteristic of rats with unilateral lesion-induced DA depletion. Specifically, we describe the effects of sectioning the corpus callosum in unilaterally lesioned (6-OHDA) rats, on motor and sensorimotor asymmetries.

Regarding the first objective, hemispheric population asymmetries were not found for any of three measures of motor activation, in rats with left or right lesions of the substantia nigra. These measures included ipsiversive turning behavior in response to amphetamine, contraversive turning in response to apomorphine and spontaneous locomotor measures in activity monitors. Similarly, groups did not differ in a measure of sensory/spatial bias, namely the orientation to edges during exploration of a large openfield. The same animals did differ however, in the performance of the Morris water maze task for spatial localization, suggesting that right brain mechanisms may be preferentially involved in successful task performance. A follow-up study with the water maze paradigm, using nonlesioned rats distinguished by the preferred direction of amphetamine-induced turning (and by inference the hemisphere of greater DA activity), further supported a preferential role for right brain DAergic mechanisms in this task. An additional test of population hemispheric asymmetry which focused on stress mechanisms, compared the effects of mesocortical DA depletion (left, right or bilateral) on the development of restraint stress-induced gastric pathology. Rats depleted of DA in the right anterior midline cortex, developed significantly more severe stress pathology than did nonlesioned controls. In contrast, left or bilateral cortical DA depletion resulted in nonsignificant trends for increased pathology. All three lesion types resulted in significant and unique effects on DAergic systems in subcortical brain structures, which may have in part contributed to the

asymmetric effects on development of stress pathology.

Regarding the second objective of the study, it was found that corpus callosum section eliminated the asymmetrical orientation to openfield edges in unilaterally lesioned rats. Conversely, there was no effect of callosotomy on asymmetries in direction of turning behavior, either drug-induced or externally cued in the behavioral competition for food. Taken together with the report that callosal section potentiates lateralization of emotional expression, the findings emphasize the anatomical dissociability of these functional asymmetries, despite their mediation by DAergic systems at various levels.

Based on these and other literature reports, it is proposed that the most fundamental processes exhibiting consistent left/right hemispheric population biases in rats, are those related to stress. The greatest degree of functional asymmetry is found in the cortex, which modulates subcortical structures in a highly asymmetrical manner. The data extend recent suggestions that the right cortex is preferentially involved in the mediation of high arousal states (such as uncontrollable stress). Other studies have shown that activation of mesocortical DA by stress initially favors the left brain, and later predominates in the right brain as stress is prolonged. Given the evidence that cortical DA facilitates coping ability, and based on a variety of neurochemical and behavioral reports of DAergic asymmetries, it is suggested that a *normal* left brain DAergic dominance may exist at the population level, for both rats and humans. Such an asymmetry is proposed to confer an adaptive advantage in the rapid execution of responses to minor stressors. Finally, it is proposed that disturbances in patterns of cortical activity may lead to (psycho)pathological states which are associated with vulnerability to stress.

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DEDICATION

This thesis is dedicated to my family in Nova Scotia, without whom none of this would have been possible. For their endless support and encouragement, and help along the way, especially when needed the most, I am eternally grateful. I love you all.

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CHAPTER 1: INTRODUCTION

1.1 Review of central dopamine (DA) systems

1.1.1 *Anatomical review of central DA systems*

The two largest and most studied central DA systems are the nigrostriatal and the mesocorticolimbic DA systems, which originate in the A9 and A10 cell groups of the mesencephalon, respectively. As the majority of studies of DA function have been directed to these two systems, the present examination of asymmetric DA function will also focus on these systems. It should be pointed out however, that there are a number of smaller DAergic cell groups throughout the central nervous system. Such groups include the tuberoinfundibular system which regulates neuroendocrine function, along with a retinal DA system and a periglomerular (olfactory bulb) system. For an excellent anatomical overview of all of these DA systems, the reader is referred to Lindvall and Bjorklund (1987).

The nigrostriatal DA system was the first DAergic system to be characterized in detail, and a review of these early studies is provided by Hattori (1993). The A9 cell bodies are located in the dorsal substantia nigra (pars compacta), and give rise to a massive projection system to the caudate and putamen of the dorsal striatum (Anden et al., 1964). This cell group also sends minor projections to the globus pallidus, nucleus accumbens and subthalamic nucleus (Lindvall and Bjorklund, 1987), as well as the amygdala (Leviel et al., 1986).

The A10 cell body group is located in the ventral tegmental area or VTA, and innervates a wide variety of cortical and limbic structures (see Simon et al., 1979; Oades and

Halliday, 1987; Lindvall and Bjorklund, 1987). The most prominent projections of this system are to the nucleus accumbens, olfactory tubercle and bed nucleus of the stria terminalis, collectively referred to as the ventral striatum. Other limbic structures innervated by the A10 system include the amygdala, lateral septum, lateral habenula and ventral hippocampus. The mesocortical portion of this system innervates primarily medial prefrontal and anterior cingulate cortex, but also pyriform and entorhinal cortex. Finally, an additional subcortical projection of VTA DA neurons has recently been characterized in the ventral pallidum (Klitenick et al., 1992). It should also be stated that there is a slight overlap in the projection areas of the A9 and A10 DA neurons, in that there is some innervation of the nucleus accumbens by A9 (substantia nigra) neurons, and also innervation of the ventromedial caudate/putamen by VTA neurons (Lindvall and Bjorklund, 1987). As well, both DA cell groups supply inputs to the amygdala.

In terms of the density of DA terminals, as revealed by immunohistochemical methods, the most dense DAergic innervation of the brain is found in the caudate/putamen. The second most dense region(s) of DAergic innervation include the nucleus accumbens and olfactory tubercle. The bed nucleus of the stria terminalis, the amygdala (particularly central, lateral and basolateral) and parts of the septum receive dense DAergic inputs as well, although not to the extent of nucleus accumbens. Finally, the additional limbic and cortical regions mentioned above, as well as hypothalamus, contain a distribution of DA terminals ranging from sparse to moderate (Lindvall and Bjorklund, 1987).

Throughout this thesis, four major DA terminal regions are chosen for biochemical study. These regions are the striatum (caudate/putamen), nucleus accumbens, amygdala and

cortex (medial prefrontal/anterior cingulate). Not only do these regions mediate the functions chosen for study in the present thesis, but importantly, these terminal fields are themselves heavily interconnected with each other and with the DA cell body regions. For example, the striatum provides feedback to the substantia nigra (Parent, 1990) and the amygdala projects to both substantia nigra and VTA (Wallace et al., 1992). The medial prefrontal cortex innervates the striatum (McGeorge and Faull, 1989) as well as the amygdala, nucleus accumbens and VTA (Christie et al., 1985; Sesack et al., 1989; Hurley et al., 1991). Moreover, the amygdala projects heavily to nucleus accumbens, striatum and medial prefrontal cortex (McDonald, 1991a,b).

An additional point of anatomical interest is that while the majority of the projections just described are ipsilateral, many projections between these structures have been shown to be either contralateral, or bilateral, thus regulating both hemispheres (Altar et al., 1983; McGeorge and Faull, 1989; Granato et al., 1991).

While this is by no means an exhaustive review of the relevant anatomical systems to be studied, the above description makes evident the basis for the extensive functional interrelationships between DA systems in various brain regions. The notion of a functional DA network has been presented in detail elsewhere (Le Moal and Simon, 1991), and is a theme which will be revisited throughout this thesis.

In any study of DAergic function, it is important to realize that several DA receptor subtypes exist, with distinct anatomical distributions and pharmacological actions. The two major classes of DA receptors are 'D_1-like' and 'D_2-like', named after the original two subtypes. Activation of D_1-like receptors (D_1 and D_5) and D_2-like receptors (D_2, D_3 and D_4)

has opposite effects on the adenylate cyclase second messenger system. For a recent review of DA receptors, see Seeman and Van Tol (1994). While no attempt is made in this thesis to dissociate DAergic functions on the receptor subtype level, it may be helpful to consider that DA actions in any given brain region may vary with the combination of receptor subtypes characteristic of that region.

Finally, it should be stated that the predominant electrophysiological effect of DA on its target neurons is to inhibit firing (eg. Thierry et al., 1990; Rolls et al., 1984), although as just noted, it is possible that the nature of this effect may vary somewhat between regions. Perhaps a more useful way to interpret DAergic effects on its target structures, rather than simply inhibition, is as a 'gating' function or as what is frequently described as increasing the 'signal to noise ratio' (Rolls et al., 1984; Lindvall and Bjorklund, 1987; Daniel et al., 1991). As such, DA could modulate its target structures in a way which maintains neural activity within an optimal range, by setting the 'gain' or level of responsiveness on one hand, and preventing excessive excitation on the other. Simply stated, DAergic projections may optimize the neural outputs, and subsequently the associated functions, of the target structures which they innervate.

1.1.2 *Role of DA in motor function*

The first behavioral function associated with central DA was that of motor activation. Much of the early research was stimulated by the suggestion that a disturbance of the DA-containing nigrostriatal pathway was the primary cause of the pathology of Parkinson's disease, characterized by severe motor impairments (Hornykiewicz, 1966).

It was then demonstrated in rats that unilateral destruction of the ascending

nigrostriatal DA neurons with the neurotoxin 6-hydroxydopamine (6-OHDA), resulted in pronounced, asymmetrical motor activity. Rats with this treatment turned spontaneously toward the side of the lesion (ipsiversive), and when injected with amphetamine which releases and blocks the reuptake of DA from the intact terminals, the ipsiversive turning behavior was greatly increased (Ungerstedt, 1971a). Conversely, the same treatment was found to induce postsynaptic (striatal) receptor supersensitivity on the side of the lesion, such that treatment with apomorphine, which directly stimulates DA receptors, induced turning in the contraversive direction or away from the lesion (Ungerstedt, 1971b). These findings strongly demonstrated that stimulation of striatal DA sets the direction of locomotor activity, and that imbalances in striatal DA, lead to locomotor activity (turning) directed away from the side of greater DA activity. This unilateral (6-OHDA) lesion model has since been used extensively to study DAergic mechanisms, and will also be employed extensively in the present thesis.

Since the early work of Ungerstedt, hundreds of animal studies have elaborated on the role of DA in motor function. It is now well recognized that spontaneous activity is reduced by treatments which interfere with DA transmission, such as bilateral 6-OHDA or electrolytic lesions of ascending DA systems, DA-depleting drugs or DA receptor blockers. On the other hand, a wide variety of drugs which facilitate DA transmission, either directly or indirectly, increase locomotor activity and may produce stereotyped or repetitive motor behaviors depending on the dose (for review, see Beninger, 1983). The large majority of anatomical studies (mainly in rats) demonstrating a facilitatory role of DA on motor activity, have focused on either the (dorsal) striatum or the nucleus accumbens (eg. Joyce et al., 1981;

Beninger, 1983; Clarke et al., 1988), although a similar stimulatory role has been described for the medial prefrontal cortex (Morency et al., 1987). Additionally, studies with selective DA agonists and antagonists have revealed a particularly important role for D_2 receptor stimulation in motor activation, with concurrent D_1 activation resulting in varying degrees of synergism or opposition, depending on the particular motor response (Barone et al., 1986; Eilam et al., 1992).

Electrophysiological studies have also supported the role of DA in motor activation, as substantia nigra DA neurons have been shown to increase their activity (bilaterally) during circling behavior in rats (Diana et al., 1989). A similar study in monkeys found that nigral (pars compacta) DA neurons increase their activity during, and in some cases before, the execution of reaching movements of the contralateral arm (Schultz et al., 1983). As neuronal activity was increased for either trained or untrained movements, it was suggested that nigral DA neurons do not encode detailed movement parameters, but play a more general role in motor activation.

1.1.3 *Role of DA in sensorimotor integration*

It has been known for some time that unilateral electrolytic lesions of the lateral hypothalamic area of rats, result in contralateral sensory neglect as reflected in the impaired orientation to a wide variety of sensory stimuli (Turner, 1973; Marshall et al., 1974). This impairment in sensory responsiveness has been shown to be due to disruption of the ascending nigrostriatal DA projections (Ljungberg and Ungerstedt, 1976; Dunnett et al., 1985). On the other hand, electrophysiological studies in cats (Strecker and Jacobs, 1985) and monkeys (Ljungberg et al., 1992) have found that DA neurons respond to salient sensory

stimuli.

A number of other studies have established that apparent sensory impairments following DA lesions, are not due to disturbed perception *per se*, but are sensorimotor in nature. In behavioral paradigms in which rats with unilateral nigrostriatal lesions were required to respond in either direction (with respect to lesion), to laterally presented stimuli, it was determined that lesioned animals had no difficulty in perceiving and responding to stimuli from either side of space, as long as they were not required to respond in the direction contralateral to their lesion (Hoyman et al., 1979; Carli et al., 1985; Brown and Robbins, 1989). These results show that sensory perception (whether ipsilateral or contralateral) was intact, but the ability to *initiate* responses to contralateral space was impaired. Interestingly, once contralateral responses were initiated, their completion times were quite normal (Carli et al., 1985). Another study reported that depletion of striatal (but not nucleus accumbens) DA, impaired reaction time performance in an operant task, suggesting the importance of striatal DA in the initiation of complex, goal-oriented responses (Amalric and Koob, 1987). Finally, rats with unilateral 6-OHDA lesions which had recovered the ability to respond to contralateral stimuli, were found to have a permanent inability to do so, if at the time of stimulus presentation they were already engaged in another behavior such as eating (Schallert and Hall, 1988). These findings all stress the importance of striatal DA in the ability to integrate or convert sensory inputs into appropriate behavioral (ie. motor) responses.

Another example of the relationship between DA and sensory responsiveness is the finding that intact rats which are bandaged on one side of the head and given apomorphine, will circle in the direction of the sensory intact side (Szechtman, 1983), suggesting that the

increased activity following DA stimulation is guided by sensory stimuli. A dissociation of motor effects from sensorimotor functions is suggested in rats with unilateral 6-OHDA lesions of the substantia nigra, by the manner in which these animals explore their surroundings. In the center of a testing environment, the rats will move predominantly in an ipsiversive direction, following their lesion-induced motor bias. However, when at the edge (a highly salient stimulus), they will reverse or override this bias such that they orient with the intact hemisphere contralateral to the edge (Steiner et al., 1988; Ziegler and Szechtman, 1988b; Fornaguera-Trias et al., 1993). This spontaneous measure of directional responsiveness will be examined in detail in the present thesis. An additional paradigm designed to study mechanisms of sensorimotor integration, is the 'dodging' paradigm of Whishaw (1988), which will be described in detail in chapter 5.

While there is substantial evidence to indicate that DAergic mechanisms are involved in orienting to sensory stimuli, the following section will demonstrate that there can be a great deal of specificity in the type of stimuli and conditions which elicit DAergic responses, and that such responses are associated with learning and adaptation.

1.1.4 *Role of DA in learning and memory*

There are many studies which suggest that DA plays an important facilitatory role in a variety of learning and memory processes, or that disruption of DA systems impairs performance in learning tasks (for review see Beninger, 1983).

Indirect support for this statement is suggested by the fact that most, if not all, of the brain structures commonly associated with memory functions, are modulated by DAergic inputs, including basal ganglia, frontal cortex, amygdala and hippocampus. While it is well

beyond the scope of the present thesis to review the extensive literature on memory systems in animals, studies have described specific learning and/or memory deficits in rats following lesions of the caudate (Dunnett and Iversen, 1981; Packard and White, 1990), nucleus accumbens (Annett et al., 1989), medial frontal cortex (Kolb, Pittman et al., 1982), and amygdala and hippocampus (eg. McDonald and White, 1993).

Many studies have shown a more direct involvement of DAergic mechanisms in learning and memory, although in several instances it is difficult to dissociate effects on motor performance of a response, or incentive/motivational factors, from effects on learning and memory *per se*. For example, a study in rats using *in vivo* microdialysis, found that prefrontal DA release increased during performance of an operant discrimination task for food (Yamamuro et al., 1994), but this does not specifically address effects on learning. Another study in rats found a significant inverse correlation between active avoidance performance (to avoid shock) and hippocampal D_2 receptor binding (Pogun et al., 1992), although again it is difficult to determine the role of activity and arousal factors in this effect.

However, other studies have more successfully dissociated such effects. Using microdialysis in the basolateral amygdala of rats, significant increases in DA and metabolite levels were seen during an operant discrimination task for food reward, but no changes were seen in a group with the same motor performance demands and motivational state, which were not required to discriminate between testing conditions for their food reward (Hori et al., 1993). Another study employing systemic injection of DAergic drugs in mice, showed that latent learning performance was predominantly affected by D_2 receptor manipulation, while locomotor activity involved both D_1 and D_2 receptors (Ichihara et al., 1993). In rats

subjected to bilateral depletion of striatal DA, the ability to learn the location of a hidden platform in the Morris water maze was substantially impaired, and while such treatments result in a number of motor disturbances, swimming behavior in this paradigm was described as quite normal (Hagan et al., 1983; Whishaw and Dunnett, 1985). In monkeys trained for spatial delayed alternation performance, depletion of DA in the prefrontal cortex results in impairments which a) were comparable to those caused by surgical ablation of that area and b) could be restored to normal by DA agonists (Brozoski et al., 1979). Such tasks as the latter, do not involve deprivational states, and these lesions do not affect motor ability. Furthermore, in a human study of schizophrenic patients given amphetamine, performance on a cognitive task (Wisconsin Card Sort Test) as well as mean conceptual level were significantly improved, and correct performance was correlated to blood flow increases in prefrontal cortex (Daniel et al., 1991). Finally, memory enhancing effects of amphetamine have also been reported in animals, and found to involve (at least) the nigrostriatal DA system (White, 1988).

From the above examples, it can be seen that there is a diversity of findings across species to suggest that DA plays a role in facilitating at least certain aspects of learning and memory functions in a number of brain regions. Probably the most direct evidence however, of the role of DA in learning, comes from electrophysiological recording studies of DA neurons in monkeys. In classical conditioning paradigms, it was found that while DA neurons initially responded to a primary food reward, the same neurons later transferred this response to conditioned stimuli which predicted reward, while ceasing to respond to the primary reward itself (Ljungberg et al., 1992). The same neurons failed to respond to similar

stimuli not associated with successful task performance. Interestingly, DA neurons of all three major cell body groups (A8, A9 and A10) responded with the same pattern during all phases of experimentation, thus showing a regional homogeneity of function. Such findings provide further support for previous suggestions that DA is importantly involved in the establishment and maintenance of incentive motivational learning (Beninger, 1983).

It was concluded from the electrophysiological experiments of Ljungberg, that DA neurons respond to novel stimuli eliciting orienting responses, primary unconditioned rewards and conditioned incentive stimuli, thus suggesting a prominent role for DA neurons in the acquisition of behavioral tasks necessary for the adaptation to changing environments. As will be described in the following section, DA neurons not only respond to appetitive stimuli, but are importantly activated by a wide variety of aversive situations, leading to adaptive behavioral and physiological changes necessary for homeostasis and survival.

1.1.5 *Central DA activation and stress*

As will be shown in this section, one of the functions of central DA is to mediate reactions to aversive or stressful situations and to prepare the individual for adaptive behavioral and physiological responses.

It has been known for some time that a physical stressor such as tail pinch induces behaviors in rats, indicative of DAergic activation (Antelman and Szechtman, 1975). As well, it has been noted that amphetamine, which releases and prolongs DA activity, produces behavioral and biochemical changes characteristic of a stressor (Antelman et al., 1980,1983).

Interest in the role of DA in stress increased rapidly with the report that the mesocortical DA system is selectively activated by mild stressors (Thierry et al., 1976). This

finding has since been replicated and further characterized many times. The stressor most commonly employed in these animal studies has been mild electric footshock, and the latter has been found to increase tyrosine hydroxylase activity in the VTA, and to increase the levels of DA metabolites in the prefrontal cortex (Deutch and Roth, 1990). Downregulation of D2 receptors (suggestive of increased DA release) in prefrontal cortex, but not striatum or nucleus accumbens, has also been observed following footshock or tailshock stress (MacLennan et al., 1989). Stress-induced activation of the mesocortical DA system has also been shown to be prevented by anxiolytic agents such as benzodiazepine agonists, and potentiated by the anxiogenic inverse agonists (Claustre et al., 1986; Deutch and Roth, 1990).

While the prefrontal cortical DA projections appear especially responsive to stressful inputs, other DA systems are also activated to varying degrees by stress (eg. Puglisi-Allegra et al., 1991; Doherty and Gratton, 1992). In general, activation of DA neurons in the prefrontal cortex is more pronounced than that in the nucleus accumbens, which in turn is greater than the activation of nigrostriatal DA neurons. A typical example of this is a report showing that following intermittent tail shock, extracellular DA levels increased 25% over baseline in striatum, 39% in accumbens and 95% in medial prefrontal cortex (Abercrombie et al., 1989). Although less frequently examined, stress also elicits DAergic activation in the amygdala (Herman et al., 1982), particularly the central, lateral and basolateral nuclei, as well as in the septum (Coco et al., 1992).

Regional differences in the DA stress response may depend on the nature of the particular stressor used, as stressors of different sensory modalities most likely activate different neural circuits, which in turn activate the various DA projections to different

degrees (Deutch and Roth, 1990; Louilot et al., 1986). For instance, although the prefrontal DA response to stress is frequently more pronounced than other regions, one study found that tail pinch induced DA activation in nucleus accumbens but not in prefrontal cortex, while immobilization stress increased DA metabolism in both areas (Scatton et al., 1988).

Activation of DAergic systems has also been seen following exposure to a previously neutral stimulus which had been paired with a stressor. Such DAergic activation in response to "conditioned fear" was initially thought to be a peculiar feature of the mesocortical DA system (eg. Herman et al., 1982; Claustre et al., 1986), but this effect has since been observed in DA projections to the amygdala and the dorsal septum as well (Coco et al., 1992). These findings parallel the electrophysiological experiments of Ljungberg et al. (1992) showing conditioned responses of DA neurons to behaviorally significant stimuli.

Another factor influencing the DAergic stress response is the degree of controllability over the stressor. It has been reported that uncontrollable footshock produces large increases in prefrontal DA activity (turnover), while identical escapable shock does not induce such increases (Bertolucci-D'Angio et al., 1990; Carlson et al., 1993; Deutch and Roth, 1990).

Individual or strain differences in the stress-induced activation of prefrontal DA systems have led to insights into the functional role of this biochemical response. Studies with Roman low avoidance (RLA) and Roman high avoidance (RHA) rat strains have been particularly informative. Based on many characteristics, such as excessive defecation and freezing behavior, exaggerated corticosterone responses to novelty and so on, RLA rats are considered much more "emotional" or anxious than RHA rats, and as such do not cope well with stress. In response to various stressors including novelty, loud noise and

immobilization (tested separately), prefrontal DA metabolism was increased only in the RHA strain, ie. in the animals which deal effectively with stress (Scatton et al., 1988). Only following a more severe stressor, namely an aggressive social interaction with a male intruder, were the RLA rats able to show any increase in cortical DA activity. These findings have led to the hypothesis that DAergic activation, particularly of the mesocortical system, serves to heighten attention or vigilance and is associated with effective coping strategies (Claustre et al., 1986; Scatton et al., 1988; Bertolucci-D'Angio et al., 1990).

The above interpretation is consistent with the hypothesis that physio/pathological consequences of stressor exposure (in humans or lower animals), follow not so much from the aversive nature of the stressor *per se*, but from the inability to deal with it (Vogel, 1985). In an animal model of stress-induced pathology, further support for an adaptive or protective role for DA has been demonstrated, as central DA mechanisms inhibit the development of restraint stress-induced gastric ulcers in rats. This protective role for DA has been documented in the amygdala (Henke et al., 1991; Glavin, 1992; Ray and Henke, 1991; Ray et al., 1988a), nucleus accumbens (Xing et al., 1991), as well as in the caudate (Glavin, 1992). Such a role for DA has not been specifically investigated in the prefrontal cortex with this model of pathology, although electrolytic lesions of the VTA potentiate restraint-induced gastric pathology (Ray et al., 1988b).

In summary, central DA systems play a crucial role in the adaptation to ever changing situations. Not only are DA neurons activated by novel and potentially important stimuli, but they aid in orienting and directing attention. They also facilitate cognitive functions associated with assessing relevant sensory inputs, which will form the basis for an

appropriate behavioral and/or physiological response. In addition, DA activation directly modulates the motor and sensorimotor functions necessary for the execution of a response, and regulates internal homeostatic mechanisms associated with stress and arousal, which may provide a form of coping feedback for the individual.

Given such an extensive range of functions, it is hardly surprising that abnormalities of central DAergic systems are associated with a variety of pathological conditions in humans, although in some cases it is difficult to dissociate a causal relationship from secondary or compensatory changes in DA systems.

1.1.6 *Role of DA in neuropsychiatric disorders*

As stated above, abnormalities of central DA function are linked to a variety of pathological states. The implications of the present thesis however, will be more concerned with the area of psychopathology, rather than neurology, and therefore the motor disturbances with which DA is clearly associated will only be briefly mentioned.

It has been recognized for some time that selective degeneration of the DAergic cell bodies of the substantia nigra, is the primary pathological feature of Parkinson's disease (Hornykiewicz, 1966), which is characterized by tremors, bradykinesia or slowing of movements, and by impaired initiation of movement. The actual cause of this degenerative disorder however, is still the focus of much speculation (Langston, 1989). DAergic mechanisms are also involved in the pathogenesis of a variety of dyskinetic syndromes (eg. tardive dyskinesia). Such syndromes can develop as side effects of treatment with antipsychotic agents (DA blockers or neuroleptics), and are related to the upregulation of DA receptors associated with such treatments (for review, see Baldessarini, 1980).

A childhood disorder with both motor and behavioral components, also appears to be modulated by DAergic mechanisms. Attention deficit-hyperactivity disorder is reportedly associated with reduced striatal blood flow (particularly the right striatum), and amphetamine-like drugs such as methylphenidate apparently reverse this abnormality along with the behavioral disturbances (Lou et al., 1989).

Another area of interest concerns the potential involvement of DA mechanisms in drug addiction (for theories on this topic, see Wise and Bozarth, 1987; Robinson and Berridge, 1993). It is well known that most drugs of abuse are readily self-administered by animals, and activate central DA systems, particularly mesolimbic projections to the nucleus accumbens. In fact, vulnerability to amphetamine self-administration in rats can be predicted on the basis of a number of factors associated with DAergic activation (Piazza et al., 1989). These studies suggest that both the craving and the positive reinforcement aspects of drug-taking, may have a DAergic basis. In a potentially related theme, a substantial number of Parkinson's patients suffer from major depression (which often predates motor symptoms). These patients show a significant lack of sensitivity to the euphoriant effects of methylphenidate, and this 'anhedonic' response is suggested to result from the additional degeneration of DAergic neurons in the VTA (Cantello et al., 1989).

The suggestion has also been made that DA disturbances may be involved in obsessive compulsive disorder (Goodman et al., 1990). Although this disorder is normally associated with serotonin systems, it was suggested that many of the compulsive and perseverative features of this condition, parallel the behaviors of rats with 'sensitized' DA systems (eg. Eilam et al., 1989).

However, the psychopathological condition in which DA has been most frequently implicated (by far), is schizophrenia. Support for a role of DA in psychosis was suggested by the fact that amphetamine addicts can develop a paranoid psychosis virtually identical to schizophrenia (Connell, 1958; Ellinwood, 1967). Stress is also a well known precipitating factor for triggering psychotic episodes in predisposed individuals (Nicholson and Neufeld, 1989). Conversely, it was found that drugs which were effective as antipsychotic agents, shared the ability to block DA receptors, and that the extent of these two properties was positively correlated (for review, see Davis et al., 1991; Seeman, 1993).

Changes in the (*post mortem*) levels of DA in the brains of schizophrenics have not been consistently observed, but increases in DA have been reported in nucleus accumbens (Bird et al., 1979; Davis et al., 1991), caudate (Davis et al., 1991) and amygdala (Reynolds, 1983). Studies of DA receptor density changes in schizophrenia have almost universally reported increases in striatal D_2 receptor binding (see Davis et al., 1991; Seeman, 1993). While in many cases the increased binding may be due to long term medication with DA blockers (eg. Hess et al., 1987), the same finding has been reported in at least one case (using *in vivo* positron emission tomography) in patients who never received medication (Wong et al., 1986).

The initial hypothesis that schizophrenia is related to a general hyperactivity of DAergic systems has fallen out of favour due to inconsistent findings across studies, and is being replaced by theories based on a) cortical DAergic deficit and b) subcortical DAergic hyperfunction (Davis et al., 1991; Deutch, 1992; Grace, 1991). The cortical deficit is suggested to account for negative symptoms such as flat affect and withdrawal, while

contributing to subcortical dysregulation which would account for positive symptoms like hallucinations and paranoid delusions. While there is much merit to these theories, particularly regarding cortical deficits in schizophrenia (see Weinberger, 1987; Weinberger et al., 1989; Daniel et al., 1991), it is still difficult to reconcile some of the subcortical findings with this view. For example, a study using synaptosomal preparations from *post mortem* schizophrenic brains, showed that K^+-stimulated [^{3}H]DA release was decreased, and DA autoreceptor function was increased, in both nucleus accumbens and caudate, but not cortex (Hetey et al., 1991). These findings were not dependent on medication history, and are more characteristic of a hypofunctional, rather than hyperfunctional DA system.

A more complete understanding of the role of DA in schizophrenia may emerge by considering the role of laterality in this disorder. Some have proposed that schizophrenia is in part the result of impaired processes of communication between the hemispheres (Coger and Serafetinides, 1990; Doty, 1989; Jaynes, 1976). Others have suggested, based on a wide variety of findings, that schizophrenia is predominantly a disorder of left hemisphere pathology (Crow et al.,1989; Flor-Henry, 1989). Schizophrenics also exhibit several abnormalities in autonomic functions (Zahn et al., 1981; Zahn, 1988) more characteristic of subjects with left brain damage than right brain damage (Meadows and Kaplan, 1994). It also appears that many of the lateralized effects reported in schizophrenics have a DAergic basis (see Seeman, 1993). For example, the increase in DA levels in the amygdala of schizophrenics is restricted to the left brain (Reynolds, 1983). Left/right imbalances in striatal D_2 binding (favoring the right) are exaggerated in schizophrenics, in comparison to controls (Reynolds et al., 1987). Still others have suggested that much symptomatology of

schizophrenia parallels features of animals with lesions of the left nigrostriatal DA system (Early et al., 1989a,b). For instance, schizophrenics (unmedicated) have been shown to be significantly biased to turn or circle towards the left (Bracha, 1987), and also to show a relative neglect of the right visual field (Posner et al., 1988). Based on similar findings, it has been suggested that psychotic symptoms (at least in subgroups of schizophrenics) may be due to hyperfunctioning of right brain DAergic systems (Bracha, 1989). Drug-induced psychosis (by mescaline) has also been suggested to be primarily due to right brain striato-limbic hyperactivity, as revealed by brain imaging (Oepen, 1989). Interestingly, the previously cited report of subcortical reductions in DA release and increases in autoreceptor function in schizophrenics, and suggestive of DAergic *hypo*function (Hetey, 1991), used tissue exclusively from the left hemisphere.

These findings not only reveal that DAergic systems are significantly altered in schizophrenia, but suggest that the lateralized nature of the abnormalities may in some way be related to the symptomatology of this disorder. It is largely because of this implication, that the present thesis focuses on lateralized specialization, and in particular, the possibility that DAergic actions may not be identical in the left and right hemispheres.

1.2 Cerebral asymmetries in humans and animals

1.2.1 *Overview of brain asymmetries in humans*

The present overview of cerebral asymmetries in humans, is by necessity far from exhaustive and will focus primarily on those areas most relevant to the present thesis. As well, the emphasis throughout this thesis will be on hemispheric asymmetries at the

population level, rather than the individual level. Volumes have been written about cerebral asymmetry in humans, and the reader is referred to some of these works (eg. Geschwind and Galaburda, 1987; Ottoson, 1987; Hellige, 1990, 1993).

Probably the most obvious asymmetry in humans is that of handedness, and the predominance of right-handedness in the population has given rise to the concept of the left brain being 'motor dominant'. Early studies with brain damaged patients also showed the left hemisphere to be dominant in language ability (at least in consistent right handers), although later studies with split-brain patients would reveal that the right hemisphere also possessed substantial capacity for language comprehension and cognitive ability (Sperry, 1982).

Throughout the literature on asymmetry, many adjectives have been used to distinguish the major roles of the two hemispheres. Examples of left brain descriptives include cognitive, neutral, unconcerned, analytical, sequential, intentional, central and reflective. Right brain descriptives have included emotional, concerned, vigilant, spatial, holistic, peripheral and impulsive (eg. Bear, 1983; Hellige, 1993). While such distinctions have theoretical and functional merit, they have frequently been overstated (even in everyday usage), as any higher functions involve the well integrated activity of both hemispheres.

Among the demonstrated hemispheric asymmetries in humans, are anatomical differences between the left and right brain. It has been reported in a number of studies that the right frontal cortex is more frequently larger than the left, than is the converse situation. On the other hand, the occipital cortex is generally reported to be larger on the left than the right (for review see Geschwind and Galaburda, 1987). The most pronounced anatomical asymmetry in the human brain is that of the planum temporale (Witelson and Pallie, 1973),

a region adjacent to the Sylvian fissure and associated with language functions. This structure is most often larger in the left hemisphere (from birth), and as with the other anatomical asymmetries, right handers exhibit greater asymmetry than left handers (Geschwind and Galaburda, 1987). Another asymmetry in humans has been reported for the globus pallidus, as sixteen of eighteen brains examined revealed a larger volume for the left brain structure (Kooistra and Heilman, 1988). Since this asymmetry was also present in infants, it is unlikely to be a consequence of greater right limb usage. Given the role of this structure as the effector system of the neostriatum, it was suggested that such an asymmetry may be the neurological basis of the handedness distribution of the population.

Population hemispheric asymmetries in regional neurochemical measures have also been reported in *post mortem* human brain, although systematic studies of this nature have not been frequently undertaken. In one study, the distribution of norepinephrine was shown to be strongly lateralized in the thalamus, with posterior regions showing a left bias and anterior regions being right biased (Oke et al , 1978). Another study reported right biased asymmetries in serotonin (5HT) systems of the mediofrontal cortex (Arato et al., 1991). Levels of the 5HT metabolite (5HIAA), as well as reuptake binding sites, were significantly right biased while the levels of 5HT itself showed a nonsignificant trend in the same direction. Hemispheric asymmetries have also been described in the globus pallidus. In this structure, both DA and choline acetyltransferase (synthesizing enzyme for acetylcholine) exhibit a significant left hemisphere bias (Glick et al., 1982). This study also reported that low overall levels of DA in either caudate, putamen or globus pallidus, were positively correlated with left-sided bias. DA was the only one of five neurotransmitter systems

analysed, in which absolute level was related to hemispheric asymmetry. Finally, it has been reported that DA (D_2) receptor binding density is significantly greater in the right putamen than the left (Reynolds et al., 1987). Interpretation of the functional significance of the above findings is very difficult however, given the lack of behavioral data (eg. handedness or other rating scales) to correlate with *post mortem* findings. The rapidly expanding field of *in vivo* brain imaging, with increasingly selective ligands and finer anatomical resolution, will most likely provide much insight in the area of functional brain asymmetries, for both normal and pathological states.

Functional hemispheric asymmetries have been described for the processing of spatial information. Such descriptions are based in large part on the differential effects of left and right brain damage on contralateral neglect. While damage to either hemisphere, particularly parietal cortex, may produce neglect of contralateral hemispace, this effect is typically much more severe following right brain injury (see Weintraub and Mesulam, 1987, 1989). These authors concluded, based on this and other observations, that while the left brain directs attention only to contralateral hemispace, the right brain modulates the distribution of attention across both hemispaces, and is thus the dominant hemisphere for spatial attention. Similar indications that the left brain in primarily responsive to contralateral inputs, while the right is responsive to bilateral inputs, have been described in normal subjects in metabolic studies (eg. Reivich et al., 1984) and EEG studies (Heilman and Van Den Abell, 1980). While there is little argument that there is *something* asymmetrical in the expression of neglect or spatial attention, it has been wisely pointed out that such measures or processes involve several components (ie. exploratory-motor, sensory-representational, motivational

and arousal), any of which may be of particular importance in the asymmetrical expression of 'spatial attention' (Weintraub and Mesulam, 1989).

Processes related to emotionality have also been shown to exhibit hemispheric specialization. The right hemisphere has been said to be superior to the left in both the perception and production of emotional aspects of speech and facial expression (Bear, 1983). Unilateral brain damaged patients vary in the nature of emotional responses depending on side of damage. Brain damage (or cerebral inactivation with sodium amytal) of the left hemisphere frequently results in a 'depressive-catastrophic' reaction, while damage or inactivation of the right hemisphere predominantly results in an indifference or even euphoric reaction (Gainotti, 1983, 1987). Voluntary contraction of unilateral facial muscles in normal subjects has even been found to differentially affect emotional perceptions. Contraction of left facial muscles (right brain stimulation) increased feelings of sadness, while right facial contraction resulted in generally positive but difficult to characterize experiences (Schiff and Lamon, 1989). In a study employing a novel technique for lateralized viewing of films, normal subjects reported higher subjective emotional ratings when films of either 'positive' or 'negative' emotional content, were viewed by the right brain, than when viewed by the left brain (Wittling and Roschmann, 1993). A number of studies of EEG activation in various emotional situations have strongly supported other findings of asymmetrical emotional processing, with a particularly important role attributed to the frontal cortical region (for reviews see Davidson and Tomarken, 1989; Davidson, 1992). There is also evidence to suggest that asymmetries in emotional processes are based on hemispheric differences in the regulation of autonomic nervous system function (Gainotti, 1987; Meadows and Kaplan,

1994). Finally, it has been reported that asymmetries in emotional expression are reduced in normal volunteers by treatment with the DA blocker chlorpromazine (Hartley et al., 1989), thus suggesting a modulatory role for DAergic systems in asymmetrical emotion-related functions.

As will be seen in the following section, population hemispheric asymmetries are not unique to humans, as had long been thought, but are widespread in lower animals as well.

1.2.2 *Review of animal studies of cerebral asymmetry*

Cerebral asymmetry has long been assumed to be unique to the human domain, largely due to the presence of handedness and language. As pointed out in a recent review however, asymmetry throughout nature is more the rule than the exception, from the behavior of atoms to the highest life forms (Hegstrom and Kondepudi, 1990).

Much of the early work on asymmetries in animals was stimulated by Nottebohm (1977), who reported that song production in some species of songbirds was predominantly controlled by the left hemisphere, suggesting that the left hemisphere may be predisposed for subserving communicative functions across species. Handedness however, does not appear to exhibit population asymmetries in lower animals. In mice for instance, paw preference shows a strong bias at the individual level (as it does with other species), but the direction is equally distributed in the population (Collins, 1977).

A number of anatomical cerebral asymmetries have been described in animals. In terms of total hemisphere size, the right hemisphere has been found to be larger than the left in the rat, mouse, rabbit and cat (Kolb, Sutherland et al., 1982; Kolb et al., 1984). Among the great apes, parallels with human asymmetries have been observed. In gorillas, the

occipital lobe is larger on the left than the right, while in chimpanzees and orangutans, the 'language' areas of the temporal lobe are larger on the left than the right. Conversely, the frontal lobe was found to be larger on the right than the left in baboons (for review, see Geschwind and Galaburda, 1987).

Neurochemical asymmetries have also been described, and virtually all such studies have been done in rats. One finding which parallels that in humans, is that norepinephrine is strongly lateralized in the thalamus, but again no functional correlates have been associated with this finding (Oke et al., 1980). Cholinergic asymmetries have also been reported, as the density of muscarinic binding sites, as well as their rate of down-regulation, is greater in the right than the left cerebral cortex (Pediconi et al., 1993). However, the huge majority of studies of hemispheric asymmetry have focused on DAergic systems, and the behaviors associated with them.

Since the work of Ungerstedt (1971a,b) with unilateral 6-OHDA rats, it was known that animals turn away from the side of greater net DA activity. It was then observed that normal rats given amphetamine, or monitored undrugged overnight, exhibited consistent directional preferences in turning behavior, suggesting the existence of endogenous asymmetries in DAergic systems (Glick et al., 1976, 1977; Jerrussi and Glick, 1976). Since then, a number of population hemispheric asymmetries have been found for striatal DA receptor binding (Drew et al., 1986; Schneider et al., 1982; Glick et al., 1988), as well as DA content in frontal cortex (Rosen et al., 1984; Slopsema et al., 1982) and mesencephalon (Afonso et al., 1993). The direction of these asymmetries however, varies from study to study.

Population asymmetries have been reported for the direction of rotation in response to amphetamine, and for turning direction in other situations such as choice of arms in a T-maze (Glick and Ross, 1981a; Fitzgerald et al., 1990; Castellano et al., 1987, 1989). In all these studies, the population bias was to turn to the right, implying a left brain dominance for motor activation.

A series of experiments employing a variety of lesion techniques in the lateral frontal cortex, or ligation of the middle cerebral artery, have consistently reported that right brain manipulations result in hyperactivity, while comparable left brain lesions do not (Robinson, 1979; Robinson and Stitt, 1981; Pearlson and Robinson, 1981; Kubos et al., 1982; Starkstein et al., 1988). It was suggested from these studies that the asymmetrical mechanism of this effect was in the output fibres of the right cortex. A similar asymmetry in the induction of hyperactivity has been observed by this group in the nucleus accumbens as well. In this case, hyperactivity was induced by either unilateral electrolytic lesions (Kubos et al., 1987; Starkstein et al., 1988), DA agonists (Belcheva et al., 1990), or cholecystokinin (Belcheva et al., 1994) in the right, but not left, nucleus accumbens. This suggested that the output neurons from the nucleus accumbens are functionally asymmetric in the modulation of motor activity.

Other studies examining the direction of initial turning upon entering an openfield, found a left turning bias (Sherman et al., 1980; LaHoste et al., 1988), which was interpreted as the result of a spatially dominant right hemisphere (Sherman et al., 1980). While it is difficult to distinguish whether this is predominantly a reflection of motor or spatial processes, another study has reported a right brain dominance for a more purely spatial

function. In the latter case, rats with lesions of the right parietal cortex were found to be more impaired in localization of an escape platform in the Morris water maze, than rats with a comparable lesion in the left brain (Crowne et al., 1992). Also related to spatial processes, was a study of contralateral neglect with unilateral lesions of dorsomedial prefrontal cortex (Vargo et al., 1988). Unlike the human situation, left brain damage was found to cause severe multimodal contralateral neglect, while right side lesions were more variable.

Finally, a number of studies have revealed population cerebral asymmetries in the expression of emotional or stress-related behaviors. Animal studies in this area were begun by Denenberg and colleagues, who performed a series of experiments using rats with unilateral neocortical ablations. These studies showed that the right hemisphere is preferentially involved in the expression of emotion-based behaviors such as openfield exploration, mouse killing and conditioned taste aversions (see Denenberg, 1981, 1983). Later studies with unilateral lesions restricted to parietal cortex, also suggested that the right brain was dominant in mediating 'emotionality', as reflected in the greater release of motor activity following right brain lesions (Crowne et al., 1987; Maier and Crowne, 1993). Recently, studies have shown more specific hemispheric asymmetries in the medial prefrontal cortex, which are directly linked to stress and mediated by DAergic projections (Carlson et al., 1988, 1991, 1993). The latter findings will be examined in detail in chapter 4 of the present thesis.

It is now apparent that a number of cerebral hemispheric asymmetries have been observed at the population level in animals, many of which may be directly related to asymmetrical activity of DAergic systems. For this reason, the present series of experiments

systematically focuses on the behavioral and neurochemical consequences of DAergic manipulations of the right vs. left hemisphere. Since central DAergic systems modulate such a variety of functions, and DA systems appear to be especially lateralized in the rat, any such lateralization may reflect adaptive advantages of inherent patterns of asymmetry. By experimentally inducing comparable imbalances in left or right DAergic systems, specific brain processes may be revealed which are functionally asymmetrical in the rat.

1.3 Aims of the present studies

The primary aim of the present experiments is to investigate the existence of left/right hemispheric asymmetries at the population level in rats (chapters 2-4). The behavioral and/or physiological processes chosen for study are known to be modulated by central DAergic systems. The basic approach involves comparing the effects of unilateral DA-depleting lesions of left and right brain structures in paradigms intended to measure motor, sensorimotor, spatial and stress-related processes. As well, the occurrence of population left/right asymmetries in nonlesioned animals is examined.

Chapter 2 compares the effects of left vs. right nigrostriatal DA depletion on i) drug-induced turning ii) spontaneous motor activity and iii) the 'sensory/spatial' orientation to edges of an openfield. While it was hypothesized that the left and right hemispheres were specialized for motor vs. spatial processes respectively, left/right population hemispheric asymmetries were not observed for these behaviors under the present study conditions. In nonlesioned rats however, a left biased cortical DA asymmetry was observed at the population level.

Chapter 3 examines nigrostriatal DA asymmetries in relation to spatial navigation, assessed with the Morris water maze paradigm. In this case, the hypothesized right hemisphere specialization for spatial function was observed as right side lesions were more disruptive of task performance than were left lesions. It is proposed that the emergence of hemispheric asymmetries in the water maze paradigm may be related to the relatively high degree of arousal associated with this task.

Chapter 4 focuses on asymmetrical regulation of response to stress, based on a variety of studies suggestive of a right hemisphere dominance in emotion and stress-related processes. The development of restraint stress-induced gastric pathology (ulcers) was studied in rats depleted of DA in either the left, right or bilateral medial frontal cortex. DA depletion of the right cortex was found to result in particularly severe stress pathology relative to restrained shams. Left or bilateral DA depletion resulted in nonsignificant increases in stress pathology compared to shams. In addition, striking differences were seen in the effects of left, right and bilateral cortical lesions on subcortical DA systems. It is suggested from these and other data, that the medial frontal cortex, under DAergic modulation, is asymmetrically specialized for coping with prolonged, uncontrollable stress. These functional imbalances may involve the asymmetrical influence of the cortex on the functioning of subcortical DA systems.

It is concluded from these studies that DA-mediated motor and sensory processes do not show evidence of population left/right asymmetries to the same extent as those processes more related to stress and coping, in which the mesocortical DA system plays an important role.

A secondary aim of the present work was to study the anatomical substrates of interhemispheric integration required for the expression of behavioral asymmetries which are characteristic of rats with unilateral 6-OHDA lesions of the substantia nigra (chapter 5). In this experiment, transection of the corpus callosum was found to clearly alter the expression of some, but not all, of the asymmetries associated with such animals. Asymmetrical orientation to edges in unilaterally lesioned rats was completely abolished in rats with additional callosal section. Regarding motor bias, the direction of turning behavior, either drug-induced or externally cued, was unaffected by callosotomy although the magnitude of these behaviors was reduced. The results provide insight into the interhemispheric regulation of motor and sensorimotor processes, and reveal dissociations of several DA-mediated asymmetries. The results also support a role for the corpus callosum in the bilateral coordination of sensory information needed for behavioral responses, and in maintaining asymmetries in sensory/spatial processes which may be adaptive where brain function is compromised unilaterally.

CHAPTER 2: STUDY OF HEMISPHERIC ASYMMETRIES OF MOTOR AND SENSORIMOTOR FUNCTION IN RATS WITH LEFT OR RIGHT LESIONS OF SUBSTANTIA NIGRA

2.1 Introduction

As reviewed in section 1.2.2, there have been a number of reports of behavioral left/right population asymmetries related to central DA systems in rats. For example, amphetamine-induced turning has been shown to exhibit a slight but significant right-sided population bias (Glick and Ross, 1981a). The same study also found that right-turning rats were generally more active than left-turners. Other studies have shown a similar right-sided population bias for a variety of measures of side preference, either spontaneous or in escaping from shock (Castellano et al., 1987, 1989; Fitzgerald et al., 1990). Still other studies have described left-sided population asymmetries in side preferences in T-maze or in openfield (Sherman et al., 1980; Camp et al., 1984; LaHoste et al., 1988b). These results have variously been interpreted as asymmetries in motor, sensorimotor, or spatial functions, and while it may be difficult to dissociate among these possibilities, the general consensus in the literature is that the left brain predominates where motor activation is concerned, and the right brain predominates in spatial processing or the directing of attention.

The extensive series of studies by Robinson and colleagues (reviewed in section 1.2.2) also supports the idea that the left hemisphere facilitates motor activation to a greater extent than does the right, both at cortical and subcortical levels.

The primary purpose of the present experiment was to systematically examine the

existence of DA-mediated left/right population asymmetries for a variety of behavioral measures in the same animals. For this we employed rats with either left, right or sham 6-OHDA lesions of the substantia nigra.

Tests of asymmetrical motor function included recording the magnitude of drug-induced turning behavior in rotometers, and the measurement of spontaneous, undrugged motor activity in automated activity monitors. A separate test designed to reflect differences in sensory attentiveness or the spatial direction of attention, measured the orientation to edges of a large openfield (also undrugged). As described earlier (section 1.1.3), rats with unilateral striatal DA depletion prefer to orient to edges with the intact hemisphere contralateral to the edge (Steiner et al., 1988; Ziegler and Szechtman, 1988a,b; Fornaguera-Trias et al., 1993). From the studies cited, it was predicted that motor activation, either drug-induced turning or spontaneous activity, would be more pronounced following preferential activation of the left hemisphere. Conversely, the sensory/spatial bias of edge orientation was predicted be more pronounced when the right hemisphere was intact, than when the left hemisphere was intact.

A secondary aim of the present experiment, was to determine if the expression of motor asymmetry, either at the population or individual level, could predict the direction or magnitude of asymmetry in edge orientation, not only within unilaterally lesioned groups, but within the sham group as well. While it is known that DA mechanisms mediate not only turning behavior, but edge behavior as well (see section 1.1.3), the relationship between these measures of asymmetry is not clear. One study reported a relationship between amphetamine-induced turning and asymmetrical edge behavior (Schwarting, Steiner et al.,

1991), but these measures were taken in the same test and environment, where one measure can confound the other. Therefore, we measured turning behavior in spherical rotometers, where edges are not present to interfere with the measure(s) of motor asymmetry, and edge behavior in a large openfield.

The results suggest that in the present animals and conditions, no left/right population asymmetries exist, either for the motor or sensory/spatial functions examined. As well, the data from both lesioned and nonlesioned groups suggest that these two types of behavioral asymmetries are essentially independent in nature.

2.2 Methods

2.2.1 *Animals and Group Assignment*

Fifty-eight male Sprague-Dawley rats weighing 250-300 gm at the start of testing were individually housed in a temperature controlled colony room on a 12 hr light/dark cycle (lights on at 7:00). Food and water were available ad lib and all behavioral testing described in this thesis was conducted during the light portion of the diurnal cycle.

Following daily handling for a week, rats were assessed for endogenous directional bias in an undrugged swim test (three 2 min. trials spaced 10 min. apart, in a 2 m diameter pool, water temp. 25° C). The time spent swimming clockwise (CW) and counterclockwise (CCW) was recorded and averaged across trials, and a net bias computed for each rat. The animals were ranked on a continuum from strong CW bias to strong CCW bias and assigned alternately to one of five groups, such that each group contained the representative range of biases in the population. The groups thus did not differ in terms of mean prelesion directional bias, and as a population, these biases did not differ significantly from zero. We

have previously found this test to be a fast and simple method of assessing directional bias with minimal stress and no drugs, as this measure in individual rats is highly consistent from trial to trial, and correlates positively with subsequent directional measures of amphetamine rotation (unpublished observations).

Twenty-two rats received left-sided lesions of the substantia nigra; 22 received right-sided lesions. Within each side condition, half the rats (n=11) were lesioned using 4 μg of 6-OHDA and half with 8 μg of 6-OHDA. In addition to the four lesion groups, 14 rats received sham lesions in the left (n=7) or right (n=7) substantia nigra. Two doses of 6-OHDA were used in an attempt to provide more variation in the range of striatal DA depletion, so that left and right lesion groups could later be equated for extent of DA depletion if necessary. (Pilot studies had indicated that the extent of DA depletion itself may be asymmetrical, thus confounding interpretation of results).

2.2.2 *Surgical procedures*

Thirty minutes prior to anesthesia with 40 mg/kg Somnotol (i.p.), rats were injected with 15 mg/kg (i.p.) desipramine to protect noradrenergic neurons. A 30-guage stainless steel cannula was stereotaxically directed to the left or right substantia nigra pars compacta (4.8 mm posterior to Bregma, ±1.6 mm lateral to midline and 7.5 mm ventral to skull, with skull horizontal according to Paxinos and Watson, 1982). Injections of 6-OHDA hydrobromide (Sigma Chemical Co., 4 or 8 μg/4 μl, dissolved in 0.1% ascorbate saline) were made over 4 min., with the cannula left in place an additional 4 min. Control rats received an equivalent volume of vehicle, in the left or right substantia nigra.

2.2.3 *Drug-induced turning behavior*

Turning behavior in response to DA agonists was recorded in automated rotometers which were transparent Plexiglas hemispheric bowls (44 cm diameter), and modified from that originally described by Greenstein and Glick (1975). Briefly, the rat is fitted with a velcro harness, connected to an overhead position sensing device, consisting of four photocells. Activation of photocells signals an IBM microcomputer as to the occurrence and direction of quarterturns by the rat. The number of fullturns, defined as four sequential quartertuns in the same direction, are then computed for both CW and CCW turns, and used to determine net directional bias.

Drug-induced turning in rotometers, rather than spontaneous, undrugged (or nocturnal) rotation in rotometers, was used to measure motor bias, as the former is much less time consuming, and these measures of motor bias correlate strongly (Glick, Jerussi and Zimmerberg, 1977). Turning behavior was monitored in response to apomorphine (APO, Sigma, 0.25 mg/kg, s.c.) at 2 weeks post-lesion. The net number of contraversive fullturns was recorded for APO from 5-20 min. post-injection. Ipsiversive turning behavior was measured following d-amphetamine sulfate (AMPH, Sigma, 2.0 mg/kg, s.c.) at 11 weeks post-lesion. AMPH-induced turning was recorded from 30-45 min. post-injection. A second AMPH test was conducted during week 16.

2.2.4 *Spontaneous orientation to edges of a large openfield*

Eight weeks post-lesion, all animals were videotaped for spontaneous (undrugged) behavior for 10 min. after being placed in the center of a large (1.6 x 1.6 m), elevated openfield with no walls in a quiet room. The openfield consisted of a clear glass surface (60

cm height) above a slightly angled mirror, and was positioned equidistant (approximately 80 cms) from three walls and a curtain. A colour video camera (Sony) was located above the curtain rod angled downward at roughly a 45° angle to cover the entire openfield. The camera operator was out of view of the test animals.

Tapes were analysed by an observer blind to the purpose of the experiment, to determine the time spent by each rat within a body width of the edge while clearly aligned in either a CW or CCW direction (ie. rat's torso at less than a 45° angle with respect to the edge). This frequently but not necessarily involved locomotion. Edge orientation asymmetry was computed as (CW-CCW)/(CW+CCW), such that positive values reflect a net CW bias, or left side of body predominantly adjacent to the edge. Tapes were randomly reanalysed by the primary investigator to ensure consistency in scoring. This test was repeated for control (sham-lesioned) rats at 21 weeks post-lesion.

2.2.5 *Spontaneous (undrugged) motor activity*

In addition to the above measures of behavioral asymmetry (turning behavior for motor bias and edge orientation for sensorimotor bias), rats were tested for undrugged general motor activity, independent of direction. Animals were placed into Omnitech activity monitors (60 x 60 cm) for 50 min. during week 21. Several parameters of general activity were monitored by computer at five minute intervals, with the primary measures of interest being total distance travelled and total number of discrete locomotor bouts.

2.2.6 *Brain dissections and biochemical analysis*

At the completion of testing (week 22), rats were sacrificed by decapitation. Brains were rapidly dissected on ice and four DA terminal regions which show extensive anatomical

interconnections were sampled (Hurley, Herbert, Moga and Saper, 1991; McDonald, 1991a,b; Sesack, Deutch, Roth and Bunney, 1989). Two mm coronal slices were obtained using an adult rat brain mold (Zivic-Miller), with the guidance of the atlas of Paxinos and Watson (1982). In lesioned animals, the full extent of the striatum was removed bilaterally with a 3 mm diameter tissue punch. In control rats, additional regions dissected included the entire nucleus accumbens and amygdala, using a 2 mm tissue punch in each case. As well, anterior midline cortex was dissected, which encompassed both medial prefrontal and anterior cingulate cortex. The cortical region extended from approximately -0.5 mm to 3.5 mm anterior to Bregma, and from the medial cortical wall to the corpus callosum. No attempt was made to further subdivide any of these four DA terminal regions. Samples were immediately frozen on dry ice, and stored at -80° C for subsequent analysis by high performance liquid chromatography with electrochemical detection (HPLC-EC), for levels of DA and a major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC). Left and right brain structures were always analysed separately.

Brain samples were weighed upon thawing, homogenized in 1.5 mls of 0.25 N acetic acid, containing 5 µg EGTA (ethyleneglycol-bis-tetraacetic acid) and 10 µg glutathione per mg tissue. Following centrifugation at 3000 g for 30 min. at 4° C, samples were refrozen an additional week. At this time, they were recentrifuged at 12,000 g for 5 min. at 4° C. A volume of supernatant equivalent to 2 mg tissue was combined with 2000 pg of 3,4-dihydroxy benzylamine (DHBA) as internal standard, and made to a final volume of 400 µl by addition of 0.1 N acetic acid. To this, 20 mg of Al_2O_3 was added, along with 400 µl of 2M Tris buffer. The samples were shaken, recentrifuged and supernatant discarded. The

remainder was washed three times with 2M Tris before extraction with 200 µl 0.1 N perchloric acid. Samples of 20 µl were injected into a Waters HPLC-EC system. The Waters eluent was modified with an additional 20 mg/100 mls of octane sulfonate.

2.2.7 *Statistical analysis*

Each dependent measure was analysed using a Side (of injection) x Dose (of 6-OHDA) analysis of variance (ANOVA). For edge orientation bias, sham-lesioned rats (considered as Dose zero) were subdivided according to side of vehicle injection for analysis. Planned comparisons between left and right subgroups at individual doses were performed with independent t-tests. For striatal DA depletion and drug-induced turning behaviors, only rats receiving 6-OHDA were included in the ANOVA. For spontaneous undrugged activity in activity monitors, ANOVA was performed with Side x Dose (including shams), and time factor with repeated measures on Time samples. Endogenous asymmetries in DA content or turnover in shams were assesed by comparing the left and right brain values of individual structures using paired t-tests. As well, a measure of association between variables was computed using Pearson correlation coefficient (r). A criterion of $p < 0.05$ was used for statistical significance and all reported values are two-tailed.

All statistical procedures described throughout this thesis were performed with the statistical package SPSS/PC+.

2.3 Results

In examining striatal DA depletion among lesion groups, a main effect for Dose was found ($F_{1,43}= 7.33$, $p = 0.010$), as the high dose of 6-OHDA produced greater depletion than the low dose (97.3% ± 0.5 vs. 85.1% ± 4.4, respectively). However, the effect of Side of

lesion, or Side x Dose interaction was not significant ($p > 0.05$), suggesting that mean striatal DA depletion was not different between left and right lesioned rats (90.5% ± 3.6 vs. 92.0% ± 3.1, respectively).

2.3.1 *Turning behavior in rotometers*

Fig. 2.1 summarizes the effects of left or right unilateral 6-OHDA lesions on drug-induced turning behavior in rotometers. A main effect of Dose was observed for APO (but not AMPH) turning ($F_{1,43}$= 5.88, p= 0.020), as the high dose (6-OHDA) groups rotated the most under APO. No significant Side of lesion effects or Side x Dose interactions were found on any test. Thus, while both left and right lesions induce a very strong motor bias, the extent of this asymmetrical motor behavior reveals no L/R hemispheric asymmetry.

In nonlesioned shams, the drug-induced turning showed a nonsignificant trend to be directed away from the more active striatum, using (L-R)/(L+R) as an index of striatal DA turnover asymmetry (APO, r= .50, p= 0.083; AMPH #1, r= .33, p= 0.276; AMPH #2, r= .53, p= .065). Drug-induced turning biases showed highly significant correlations with asymmetrical cortical DA turnover (APO, r= .75, p= 0.003; AMPH #1, r= .67, p= 0.012; AMPH #2, r= .68, p= .010), such that turning behavior was again directed away from the hemisphere of greater DAergic activity. As a group, mean drug-induced turning bias in shams, did not differ significantly from zero ($p > 0.05$, t-test), suggesting no L/R hemispheric asymmetry in this relatively small sample of rats.

2.3.2 *Orientation to edges of an openfield*

In total, rats spent a mean of 53.5 (± 2.2) % of the 10 min. test clearly aligned with the edge, during which time rats were primarily engaged in active thigmotactic scanning. Fig.

2.2A shows the edge orientation bias for each group. Lesioned rats aligned with the edge such that the intact striatum was predominantly contralateral to the edge. This orientation bias differed significantly across groups (Main Effect for Side, $F_{1,52} = 54.37$, $p < 0.001$). A significant Side x Dose interaction ($F_{2,52} = 4.26$, $p < 0.02$) was also found. Left and right subgroups differed significantly from each other at the 4 and 8 µg dose of 6-OHDA ($p<0.05$), whereas in vehicle-injected rats, left and right subgroups did not differ in edge orientation bias. Each group except shams had a bias which was significantly different from zero ($p < 0.05$) and all lesion groups except Left(4 µg) differed significantly from shams ($p < 0.05$, t-tests in each case). In terms of edge orientation bias in the *expected* direction, that is, with the intact hemisphere contralateral to the edge, mean edge bias for left-lesioned rats across dose was 0.37 ± 0.05 vs. 0.36 ± 0.08 for right-lesioned rats (Fig. 2.2B). The virtually identical *magnitudes* of these asymmetries suggest that denervation of either the left or right striatum are equally effective in inducing this sensorimotor bias.

Further evidence of the role of striatal DA in the edge behavior is shown in Fig. 2.3. In non-lesioned animals, side of higher DA turnover in striatum (but not n. accumbens, amygdala or cingulate and prefrontal cortex) was related to edge orientation such that rats tended to align with the more active striatum contralateral to the edge. While asymmetrical striatal DA activity is reflected in the asymmetrical investigation of one's environment in individual animals, mean edge bias did not differ significantly from zero ($p > 0.05$, t-test), indicating no population bias for edge orientation in nonlesioned rats.

2.3.3 *Relationship between turning biases and edge bias*

These two types of behavioral bias were poorly correlated in both lesioned and non-

lesioned rats. In lesioned rats, whose direction of bias in both behaviors is essentially determined by the lesion, the magnitude of edge bias in the expected direction was not significantly correlated with the magnitude of either APO (r= -.17, n= 44, p= .266) or AMPH (r= -.08, n= 44, p= .606) turning behavior.

In nonlesioned rats, where behavioral asymmetries of each type are modulated by endogenous DAergic asymmetries, the direction of edge bias was not correlated with that of turning bias: openfield test #1 (APO, r= -.28, p= .317; AMPH, r= .04, p= .889); openfield test #2 (APO, r= -.12, p= .700; AMPH, r= -.23, p= .452).

Thus the two types of behavioral asymmetries appear to reflect largely independent and dissociable processes. Moreover, the present results provide no evidence to indicate a L/R hemispheric asymmetry at the population level, in the DAergic modulation of these processes.

2.3.4 *Spontaneous (undrugged) motor activity*

The spontaneous motor activity of left, right or sham lesioned rats, measured in activity monitors, did not differ significantly across time samples, as seen in Fig. 2.4. Analysis of distance travelled (Fig. 2.4A and B) revealed significant effects for Time sample ($F_{9,396}$= 10.16, $p < 0.001$) and for Dose ($F_{2,44}$= 3.41, $p = 0.042$), as rats lesioned with 4 µg of 6-OHDA showed the greatest spontaneous activity. However, no significant effect of Side was observed ($F_{1,44}$= .30, n.s.), nor any interactions. If shams were excluded from this analysis, the same pattern of results emerges with significant main effects for Time sample ($F_{9,315}$= 11.22, $p < 0.001$) and Dose ($F_{1,35}$= 6.59, $p = 0.015$), but not Side of lesion ($F_{1,35}$= .63, n.s.). No significant interactions were found between Side, Dose and Time sample.

Analysis of the number of discrete locomotor bouts or movements (see Fig. 2.4C and D), again showed no significant effects for Side (either with the shams included in analysis, or not), and no significant interactions. The only significant factor was Time sample ($F_{9,396}$= 7.27, $p < 0.001$).

Thus while Dose (of 6-OHDA) appears to be involved in the expression of one measure of spontaneous activity, Side (of lesion) was not associated with any significant effects on observed motor activity.

Within the nonlesioned group, spontaneous motor activity was unrelated to the side of vehicle injection, as well as to all measures of regional DA function or regional asymmetry measures ($p > 0.05$ in all cases, Pearson's correlations).

2.3.5 *Endogenous neurochemical asymmetries in shams*

Regional DA content and turnover (DOPAC/DA) in non-lesioned animals are shown in Fig. 2.5. While DA content did not exhibit L/R hemispheric asymmetry in the subcortical regions examined, left cortical DA was approximately double that of the right ($p < 0.05$, paired t-test). No significant L/R asymmetries were found for DA turnover in any structure.

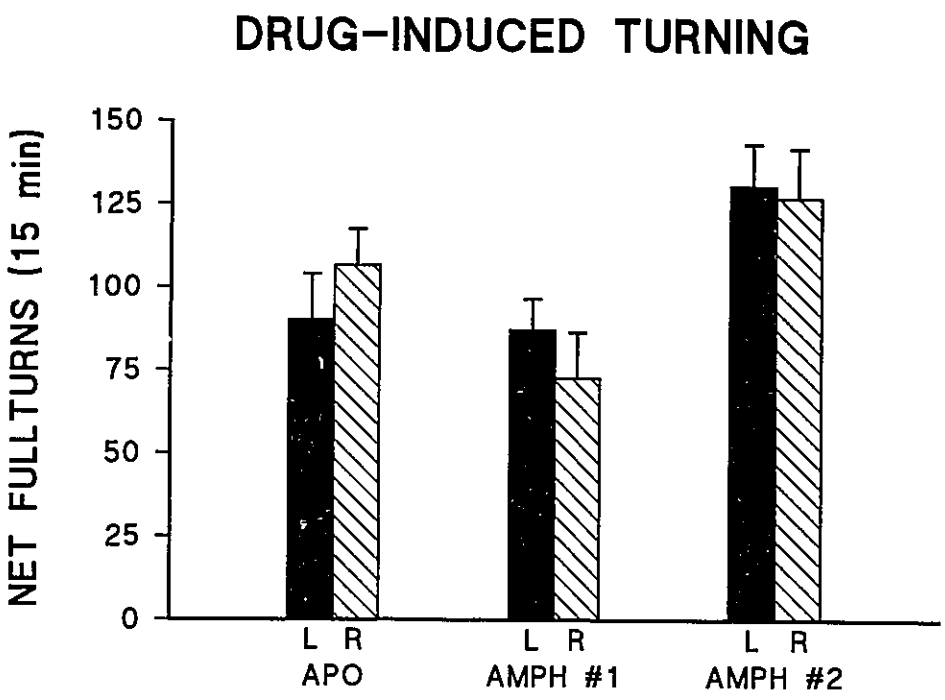
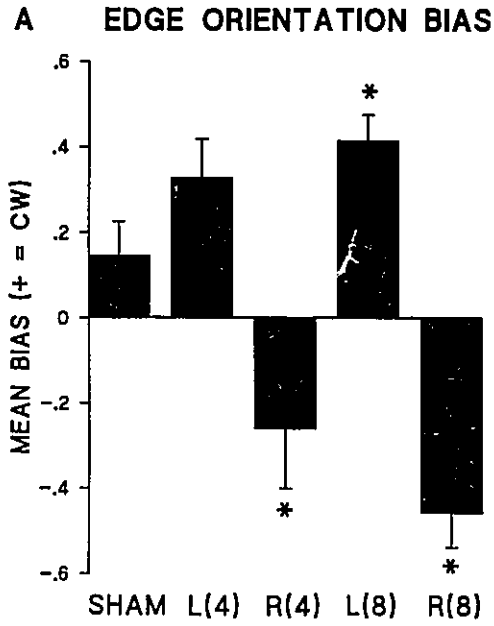


Fig. 2.1 Drug-induced turning behavior in rats with unilateral 6-OHDA lesions of the substantia nigra. Within each side of lesion condition, doses of 6-OHDA are combined (n = 22/side). Net contraversive fullturns in response to apomorphine (APO), and net ipsiversive fullturns in response to amphetamine (AMPH), did not differ between left (L) and right (R) lesioned rats on any test ($p > 0.05$). This suggests that (at least with this sample size), no evidence of L/R nigrostriatal asymmetry is apparent, as reflected in the extent of both contraversive and ipsiversive turning behavior measured in rotometers.



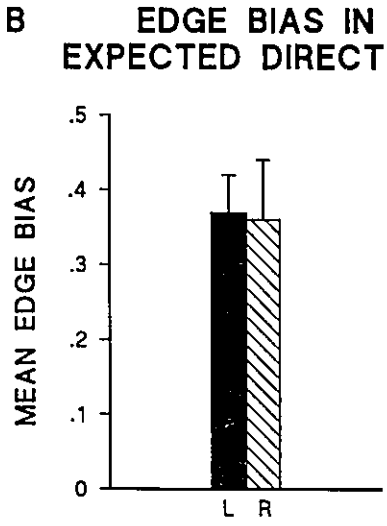


Fig. 2.2 Edge orientation bias in shams and unilaterally lesioned rats, as measured at the edges of a large openfield. In Fig. **2.2A**, L and R refer to left and right lesions respectively, and the numbers in brackets represent the dose of 6-OHDA (μg). Left and right vehicle-injected shams did not differ significantly and are combined for presentation. * $p < 0.05$ with respect to shams. All lesion groups (but not shams) differed significantly from zero in this measure of sensorimotor bias, such that the intact striatum was predominantly contralateral to the edge. In Fig. **2.2B**, doses are combined to demonstrate that in terms of this lesion-induced behavioral bias in the "expected" direction, L and R lesioned animals are biased to a virtually identical extent, thus suggesting no L/R hemispheric asymmetry in the striatal DAergic modulation of orientation to edges.

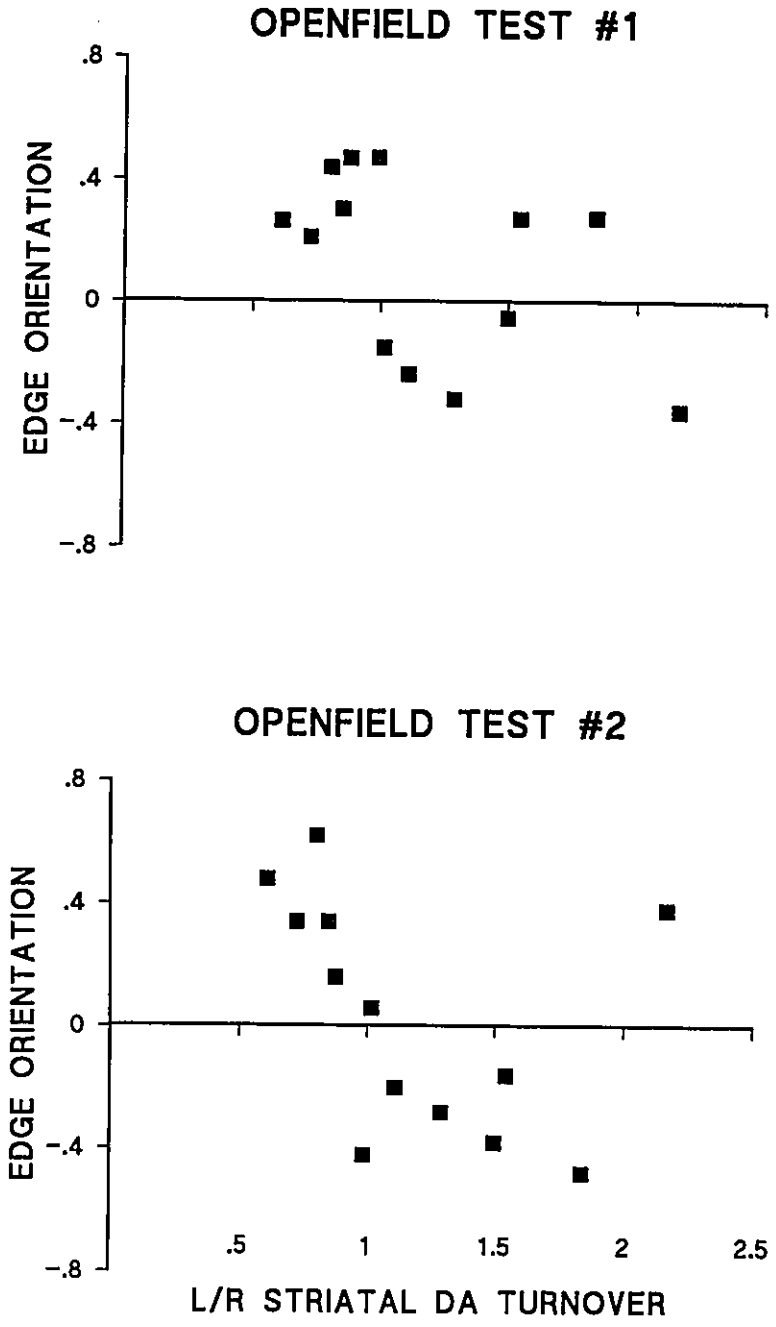


Fig. 2.3 Endogenous asymmetries in striatal DA turnover (DOPAC/DA) and edge orientation bias in non-lesioned shams. These neurochemical and behavioral asymmetries were directionally related in sham-lesioned rats (test #1, r= -.49, p= 0.046; test #2, r= -.42, p= 0.074). Excluding one outlier on test 2 (conducted much closer to the time of sacrifice than the first test) resulted in a correlation of r= -.81 and $p < 0.001$. These results suggest that while individual rats tend to orient with the more active striatum contralateral to the edge, there is no L/R side preference for edge bias as a group, as the mean of individual biases does not differ significantly from zero.

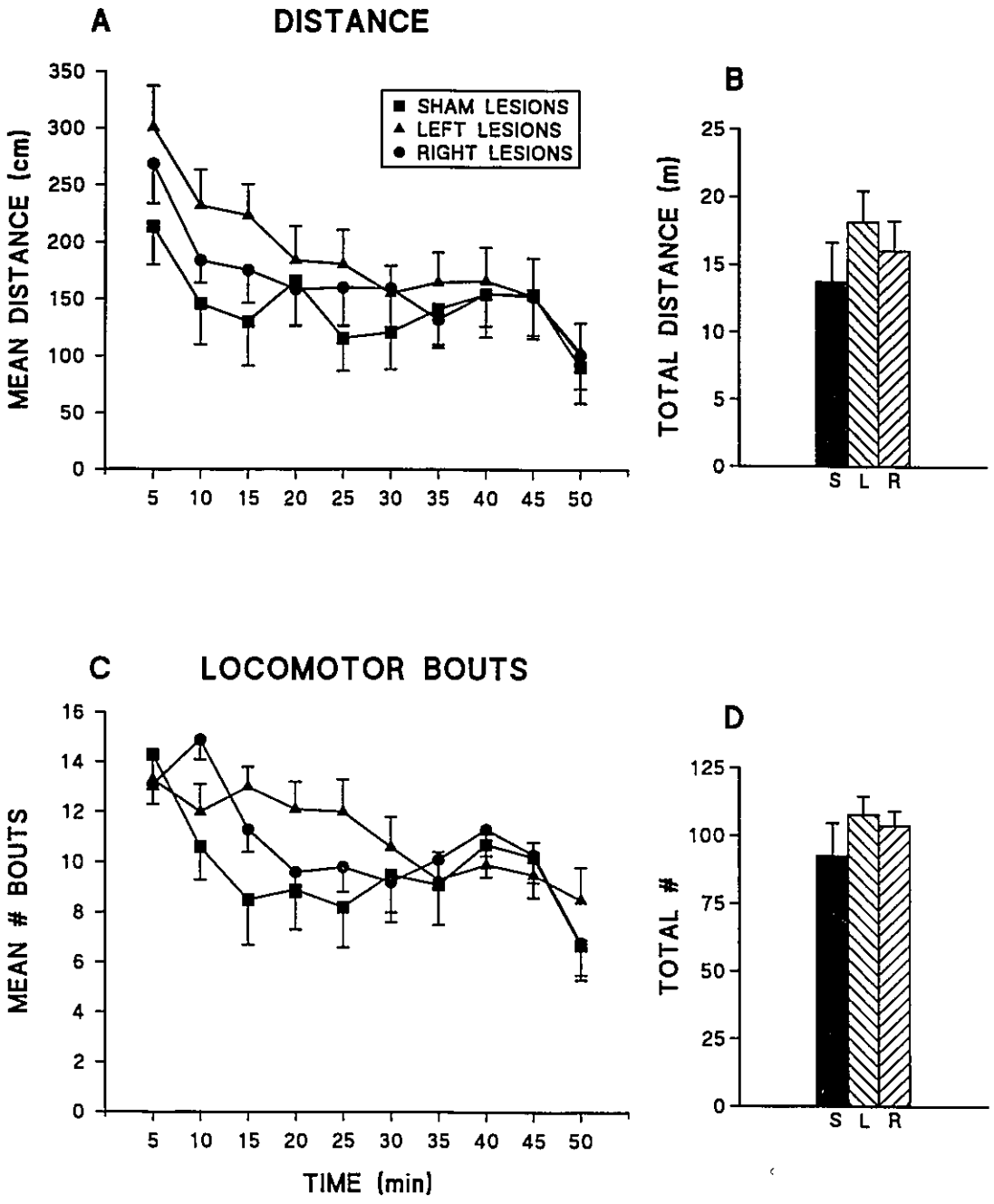
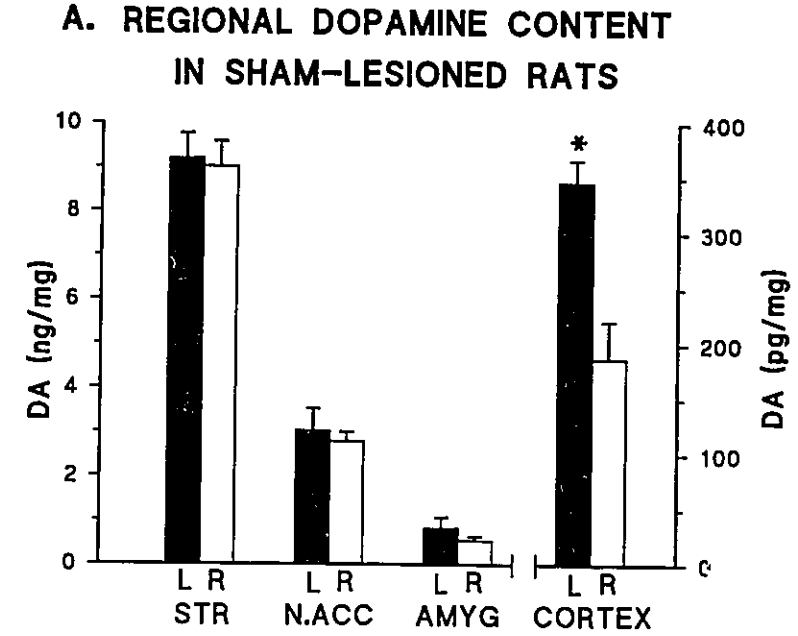


Fig. 2.4 Spontaneous (undrugged) motor activity in rats with unilateral 6-OHDA lesions of the substantia nigra. Low and high dose groups are combined for presentation within each Side condition. Figs. **A** and **C** show the time course of activity measured in automated activity monitors, while **B** and **D** summarize the total distance travelled and the total number of movements, respectively. Left (L) and right (R) lesioned rats did not differ significantly from shams (S) across samples or on total activity measures, nor did L and R groups differ from each other. These results fail to show an asymmetrical lesion effect on spontaneous motor activity. See text for statistical details.



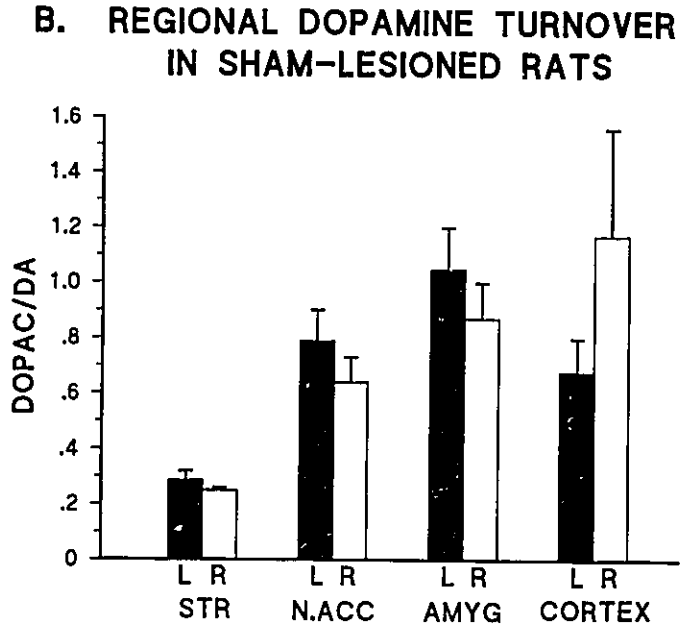


Fig. 2.5 Regional DA content (A) and turnover (B) in non-lesioned rats. While no significant L/R differences were found for either measure in the striatum (STR), nucleus accumbens (N.ACC), or amygdala (AMYG), a significant left-biased asymmetry was found for cortical DA content. The tendency for an endogenous right-biased imbalance in cortical DA turnover, was not significant and largely reflects the lesser amount of DA in the right cortex. * $p < 0.05$, paired t-test.

2.4 Summary and Discussion

The present results do not support the hypothesis that DA-dependent population asymmetries exist for either motor activity (drug-induced turning or spontaneous activity) or sensory/spatial functions (ie. the neglect of contralateral space in edge orientation). While this may be in contrast to a number of studies described in the introduction, several points need to be considered before drawing any conclusions.

First, it must be noted that in the case of amphetamine rotation, the most cited report of a right-sided population bias in nonlesioned rats (Glick and Ross, 1981a), required hundreds of animals to demonstrate this effect. In studies describing similar right-sided population biases in motor responses using much smaller samples, the behaviors in question either involved a substantial stress or arousal component, as in the preferred direction to escape shock, or else the population biases emerged strongly only after repeated task performance (Castellano et al., 1987, 1989; Fitzgerald et al., 1990). From the latter studies, it would appear that the behavioral tasks eliciting the greatest population laterality appear to be specifically those which are the most stressful or arousing.

The direction of amphetamine-induced turning preferences has been shown to be dramatically altered following uncontrollable stress (Carlson et al., 1987), with biases being potentiated, reduced or even reversed, depending on the initial direction of turning and the sex of the rat. A number of studies have recognized that the direction of asymmetries of this sort (ie. turning behavior, side preferences, etc.) are sex-dependent (Camp et al., 1984; Robinson et al., 1985; LaHoste et al., 1988b), which would account for some of the variability in the direction of previously reported population asymmetries. Early experience

is also very important in the expression of asymmetrical behavior and most likely accounts for much of the variability in the asymmetry literature. Early handling (removal from the litter for ten minutes a day during preweaning) has pronounced long term effects on expression of asymmetries, generally facilitating pre-existing biases, or allowing latent biases to emerge (Denenberg et al., 1978; Denenberg, 1981; Denenberg et al., 1985; Sherman et al., 1980; Maier and Crowne, 1993).

It is therefore quite possible that in the present study, the relative lack of such early treatments or highly stressful testing, is an important factor in the reported absence of population asymmetries.

The numerous reports of the asymmetrical induction of hyperactivity following right hemisphere lesions by Robinson and coworkers (reviewed in section 1.2.2), describe an asymmetry much more consistent in direction than those for turning behavior or side preferences. This hyperactivity asymmetry following cortical lesions however, is probably not dependent upon catecholaminergic systems (Kubos and Robinson, 1984). This same group however, has reported DA-mediated asymmetrical effects on motor activity in the nucleus accumbens (Belcheva et al., 1990). The present 6-OHDA lesions of the substantia nigra may have had an insufficient effect on nucleus accumbens DA systems for any such asymmetries to emerge.

In spite of the lack of left/right hemispheric population asymmetries we report, a number of points can be made regarding the mechanisms of both turning and edge asymmetries.

In the unilaterally lesioned animal, the direction of behavior, either turning or edge

exploration, is essentially determined by the side of the 6-OHDA lesion. The intact striatum preferentially directs (amphetamine-induced) turning and spontaneous edge exploration. However, the extent of drug-induced turning was not correlated with edge investigation bias in lesioned animals, suggesting a dissociation of these behaviors. The results in sham-lesioned rats further support this contention. In this case, the animals exhibit endogenous asymmetries in turning and edge behavior, but their directions are uncorrelated, suggesting the separation, or possibly competition of motor and sensory/spatial processes, both under DAergic modulation.

Asymmetrical edge behavior (in addition to turning behavior) has been shown to be modulated by DAergic mechanisms (Pisa and Szechtman, 1986; Schwarting, Bonatz et al., 1991; Schwarting, Steiner et al., 1991). It has also been suggested that asymmetrical edge behavior is a more sensitive behavioral index of (partial) unilateral striatal DA depletion than is turning behavior (Steiner et al., 1988; Fornaguera-Trias et al., 1993). The present study extends these findings by showing that endogenous imbalances in striatal DA activity in *non*lesioned rats are associated with asymmetrical edge behavior (Fig 2.3).

The independence of turning and edge asymmetries supports earlier findings which suggested the dissociation of these variables (Pisa and Szechtman, 1986; Wise and Holmes, 1986). A similar dissociation has been made on anatomical grounds, in studies of functional recovery from unilateral 6-OHDA lesions using embryonic grafts. Regional specificity was reported within the striatum, which localized rotation effects to the dorsal striatum, and sensorimotor responsiveness to the ventrolateral striatum (Dunnett et al., 1981a,b). It is therefore possible that in intact rats, either a motor or a sensorimotor asymmetry will

predominantly guide behavior, depending on the striatal subregion of greater DAergic asymmetry. Alternatively, or additionally, a hierarchy of processes may exist in the normal animal, such that in the absence of salient lateralized stimuli as in the rotometer, the animal will guide its movements in the direction of its motor asymmetry, an asymmetry independent of external cues. During spontaneous investigation of the environment (edges of an openfield), a sensorimotor or perhaps spatial bias can emerge, which is cue-dependent and not predicted by the direction of endogenous motor bias. It is proposed that the sensory/spatial bias is of "higher" origin (presumably cortical), and that only in cases of exceptionally strong motor bias, as in the unilaterally lesioned animal, can the direction of motor bias predict that of sensorimotor bias.

In summary, neither motor nor sensory/spatial asymmetries, as reflected in turning and edge behavior respectively, revealed a left/right hemispheric asymmetry at the population level. In unilaterally lesioned rats the direction of these behavioral asymmetries is relatively invariant, in contrast to controls. Thus while DA activity may modulate both behaviors at the level of the striatum, these asymmetries may involve distinct corticostriatal circuits of the nature described by Alexander et al. (1986).

CHAPTER 3: STUDIES OF LEFT/RIGHT HEMISPHERIC ASYMMETRIES IN ABILITY TO PERFORM THE MORRIS WATER MAZE TASK

3.1 Introduction

As reviewed in Section 1.1.4, there is a variety of evidence to suggest that DA is involved in learning behavioral tasks and adaptive responses (eg. Beninger, 1983; Ljungberg et al., 1992).

There have also been indications that some degree of asymmetry in central DA systems in intact animals (usually assessed by amphetamine-induced turning behavior) is associated with superior performance in some learning paradigms. Rats which showed consistent turning preferences ('rotators') were found to exhibit significant retention of a spatial discrimination response in an active avoidance paradigm, while 'nonrotators' did not (Zimmerberg et al., 1978). A second study replicated these findings, showing that rotating rats both learned and retained a spatial discrimination response to escape from shock, better than nonrotators (Willar and Crowne, 1989). Beneficial effects of nigrostriatal asymmetry have also been described in relation to delayed spatial alternation in a water T-maze (Crowne, Tokrud et al., 1992) as well as in certain aspects of spatial learing ability in the Morris water maze (Camp et al., 1981). It has been suggested that a moderate degree of striatal asymmetry may be optimal for overall learning ability and strategic exploration of one's environment (Glick et al., 1977).

While individual asymmetries of DA systems may be advantageous for some forms

of learning (particularly those with a strong spatial component), population left/right hemispheric asymmetries have either not been examined or reported in such studies. One study found that right, rather than left, parietal cortex lesions cause significant impairments in performance of the Morris water maze task (Crowne, Novotny et al., 1992) but it is not known if this effect is in any way mediated by effects on DAergic systems.

The purpose of the present study is to examine whether a population left/right hemispheric asymmetry contributes to the performance of a spatial learning task. The learning paradigm chosen is the Morris water maze task, which does not involve the use of electric shock or deprivational states. In this task, rats are required to use spatial cues to locate a submerged escape platform in a circular pool of water from a variety of starting points, and later to relearn the platform location when it is moved to a new position (reversal testing). It has been shown that performance in this task is sensitive to central DAergic manipulations as bilateral depletion of striatal DA eliminates the ability to perform this task, despite the ability to swim vigorously (Hagan et al., 1983; Whishaw and Dunnett, 1985). This impairment was suggested to result from a disrupted ability to use distal cues (Whishaw and Dunnett, 1985). The same study found that unilateral striatal DA depletion impairs the rate of acquisition of this task, although these animals eventually perform at control levels. This latter fact served as a starting point for the hypothesis of a role of side of lesion in the performance of this task. Given that right brain mechanisms have been shown to be asymmetrically specialized in spatial functions in rats (Crowne, Novotny et al., 1992), and that DAergic systems facilitate performance in this task (Hagan et al., 1983; Whishaw and Dunnett, 1985), we hypothesized that DAergic mechanisms in the right brain would be of

greater importance in performance of this learning task than DAergic mechanisms in the left brain.

In the first experiment of this study, rats with left vs. right 6-OHDA lesions of the substantia nigra (same animals as in chapter 2) were tested for performance in the water maze. In the second experiment of this study, nonlesioned animals were examined for water maze performance, and subsequently assessed for the direction of amphetamine-induced turning behavior so that rats could be grouped according to endogenous asymmetries of DA function. The results suggest that certain aspects of water maze performance are facilitated when imbalances in central DA function favor the right, rather than the left brain, both in lesioned and nonlesioned animals.

3.2 Experiment 1 - Water maze performance in rats with unilateral substantia nigra lesions.

3.2.1 Methods

As stated above, the animals used in the present study are the same as those from the experiment described in chapter 2, although this portion of the experiment will be described seperately. One group therefore consisted of sham lesions (n=14), with other groups of rats receiving unilateral 6-OHDA lesions of the left or right substantia nigra (n = 22/group). Behavioral testing in the water maze was conducted at four weeks post-lesion, and as such, the only other testing between the time of lesioning and water maze testing, was apomorphine-induced turning behavior at two weeks post-lesion.

3.2.1.1 *Water maze testing*

Testing in the water maze took place in a 2 m circular pool with water temperature of 25°C. An escape platform (9 cm diameter, made of clear Plexiglas) was located 1 cm

below the water surface, and the water was covered with a layer of styrofoam chips, to visually obscure the platform location. Animals received four consecutive days of acquisition training, in which the escape platform remained in a fixed location in one of the four quadrants. Reversal testing was conducted on the fifth day, in which the platform location was switched to the opposite quadrant. On each of the five test days, rats received four trials lasting up to 2 min. and spaced 10 min apart. Rats finding the platform successfully on any trial remained there for 30 sec, and those not finding it within 2 min. were placed on the platform for 30 sec. The starting position on the first trial each day was varied, and starting positions differed on each of the four daily trials, although the order of starting positions did not vary from day to day. All trials were videotaped from directly overhead, and the latency to locate the platform (up to 120 sec) was recorded for each trial from the video records.

3.2.1.2 *Statistical analysis*

A two-way ANOVA was performed to measure mean daily escape latencies across the four days of acquisition, using a three level factor of Group (sham, left or right lesion), with repeated measures on days. A separate oneway ANOVA was performed for mean escape latencies on the day of reversal testing. The first trial of Day 1 acquisition and the first trial of reversal testing were omitted from analysis, as rats had no previous knowledge of platform location on these trials. Following significant group effects or interactions, Duncan's multiple range tests were performed for post hoc comparisons among individual groups.

3.2.2 Results

As reported in the results of Chapter 2, the extent of striatal DA depletion (same rats as present) was equivalent between left and right lesion groups.

The effects of unilateral 6-OHDA lesions of the substantia nigra on escape performance in the water maze are shown in Fig. 3.1. Across the four days of acquisition, groups failed to differ significantly in performance ($F_{2,55}$= 1.85, p = 0.167). A main effect for Days was highly significant ($F_{3,153}$= 25.93, p < 0.001), as performance improved over days, while no significant Group x Days interaction was observed ($F_{3,153}$= .70, p = .649).

In reversal testing, there was a significant difference among groups ($F_{2,55}$= 5.18, p = 0.009). Right lesioned rats took significantly longer to locate the escape platform than both shams (p < 0.01) and left lesioned animals (p < 0.05). Left lesioned rats and shams did not differ significantly in performance in either phase of water maze testing.

3.3 Experiment 2 - Water maze performance in nonlesioned lateralized rats

3.3.1 Methods

3.3.1.1 *Animals and water maze testing*

Twenty-four male Sprague-Dawley rats weighing 250-300 gms at the start of the experiment, were handled daily for a week prior to testing. On the last day of handling, rats were given an undrugged test of directional bias, based on the net direction of swimming behavior, as described in Section 2.2.1. Water maze testing commenced the following week and proceeded as described for Experiment 1 of the present chapter, with four days of acquisition followed by one day of reversal testing. One difference in testing procedures was that greater care was taken to randomize the ordering of starting positions.

3.3.1.2 *Amphetamine-induced rotation*

Three weeks following testing in the water maze, animals received an injection of d-amphetamine sulfate (2.0 mg/kg, s.c.), and were monitored for endogenous turning biases in rotometers, as previously described (Section 2.2.3). On this basis, rats performing five or more net fullturns in either direction were characterized as CW turners or CCW turners, allowing for comparisons of escape performance between subgroups. A second identical AMPH test was administered 10 days after the first. Brain regions did not undergo biochemical analysis at the completion of this study.

3.3.1.3 *Statistical analysis*

ANOVAs were performed across the four days of acquisition testing as described in Section 3.1.2.2, for mean daily escape latencies, with repeated measures on days. The grouping factor in this case was CW (n = 9) versus CCW (n = 11) turners. Four nonrotating rats (less than five net fullturns) were omitted from analysis. Again, a separate oneway ANOVA was performed for mean latencies on the day of reversal testing, and trial 1 of acquisition (Day 1) and reversal were omitted.

3.3.2 Results

The escape performance of CCW and CW turning rats, in locating the hidden platform, is summarized in Fig. 3.2. Across the four days of acquisition, a significant main effect was found for Group, as CCW turning rats had faster escape latencies than CW turners ($F_{1,18}$= 7.67, p = 0.013). There was also a highly significant effect for Days, as performance improved for both groups ($F_{3,54}$= 17.11, p < 0.001); however, there was not a significant Group x Days interaction ($F_{3,54}$= 2.37, n.s.), suggesting that while the level of performance

differed between the groups, the rate of improvement or learning, did not differ significantly.

No significant differences were found between CCW and CW turning rats on the day of reversal testing ($p > 0.05$).

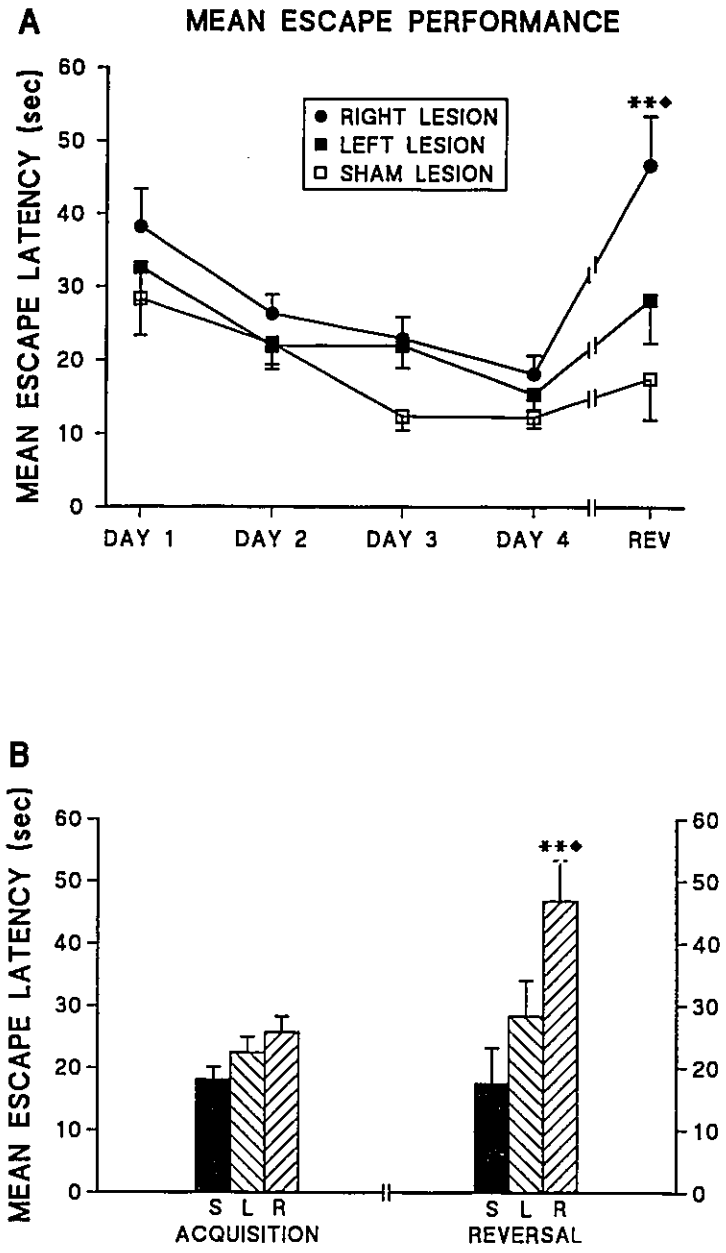


Fig. 3.1 Water maze performance in rats with unilateral 6-OHDA or sham lesions of the substantia nigra. Data are represented as mean (± S.E.M.) daily escape latencies to locate the hidden platform across the four days of acquisition training and one day of reversal training (A). Fig. B summarizes the data as mean escape latencies collapsed across all trials of acquisition and reversal. * $p < 0.05$, ** $p < 0.01$ relative to shams, ♦ $p < 0.05$ relative to left lesions. S, L and R represent sham, left and right lesion groups respectively.

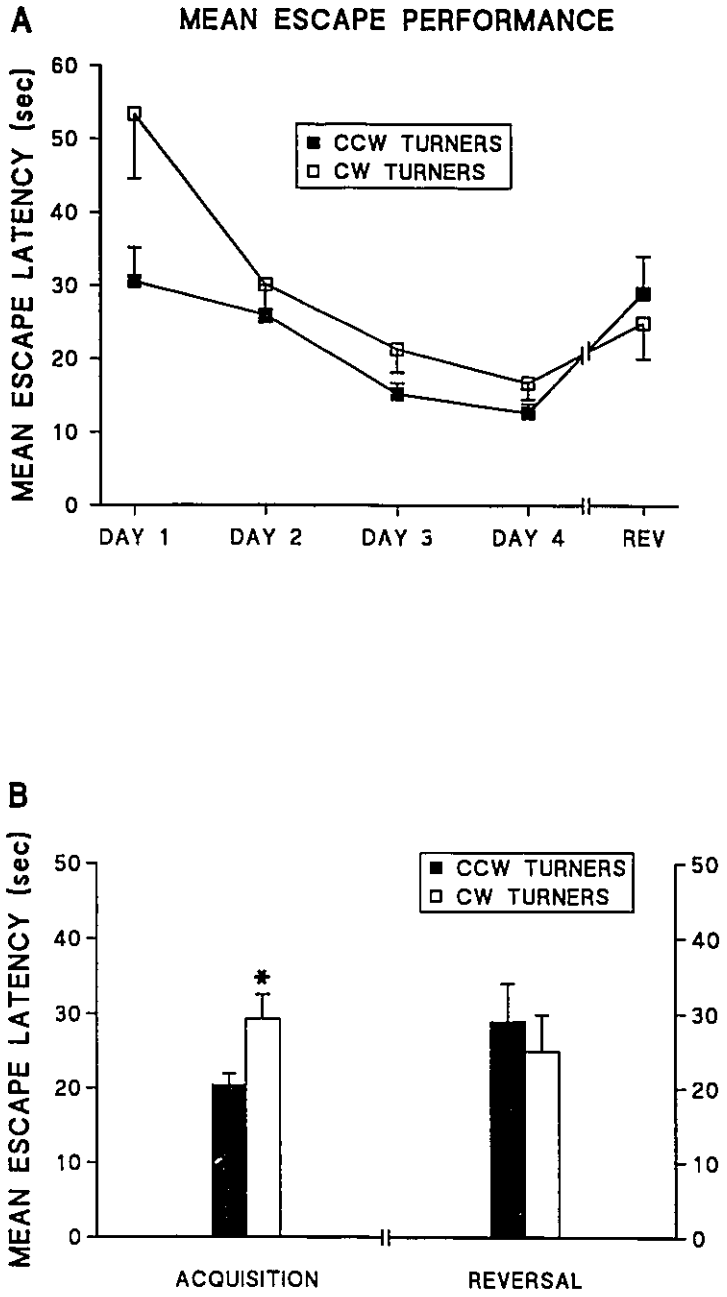


Fig. 3.2 Water maze performance in nonlesioned rats grouped according to the direction of amphetamine-induced turning behavior. Data are expressed as mean daily escape latencies (**A**) and as mean latencies collapsed across all acquisition and reversal trials (**B**) for counterclockwise turners (CCW, n=11) or clockwise turners (CW, n=9). CCW turners, suggestive of right brain DAergic dominance, performed significantly better (low escape latencies) than CW animals across acquisition, but not reversal. * $p < 0.05$.

3.4 Summary and discussion

From Exp. 1, it can be seen that 6-OHDA lesions of the right substantia nigra result in greater impairments in locating the hidden platform, than comparable lesions of the left substantia nigra. Specifically, in reversal testing, right-lesioned rats performed significantly worse than both controls and left-lesioned rats, suggesting that right brain DA mechanisms are relatively more important than those in the left in this aspect of performance. In Exp. 2, CCW turning rats performed better across acquisition than did CW turners, suggesting that right brain DA mechanisms facilitate aspects of water maze performance relatively more than those in the left, in nonlesioned as well as lesioned animals.

It may appear unusual that significant left/right differences were found during reversal in the lesion study, and during acquisition in the nonlesion study. However, it is not logically sound to assume that a unilateral DA-depleting lesion is identical in kind, and simply an exaggerated version of an endogenous asymmetry in DA systems of nonlesioned rats, as reflected in the direction of turning behavior. Specific brain processes mediating different aspects of task performance may be differently influenced in the lesion and nonlesion conditions. Substantial functional reorganization takes place following virtually any brain lesion, and such reorganization may itself be asymmetrical. A good example of this will be seen in the results of chapter 4 of the present thesis. Regardless of the specific task parameters affected in the present experiments, right brain DA activity appears to play a specialized role in at least some aspects of task performance.

Regarding Exp. 1, a number of points can be made considering the data obtained with these same animals from chapter 2. First, it is unlikely that the present effect on escape

performance can be attributed to differences in striatal DA depletion, as this index of lesion size was not significantly different between left and right lesion groups. Similarly, it is unlikely that the water maze results could be explained by differences in motor capacity between the two lesion groups. Neither spontaneous motor activity (in activity monitors) nor drug-induced turning behavior following apomorphine or amphetamine showed a significant side of lesion effect.

Perhaps the most obvious interpretation of these results is that the left and right lesion groups exhibit subtle differences in spatial learning ability. In neither Exp. 1 nor 2 however, was a Group x Days interaction seen during task acquisition, suggesting that the rate of improvement or learning did not differ. In reversal testing of Exp. 1, the notable impairment in right-lesioned animals would appear to be a more specific learning deficit. Even here however, caution must be exercised. Despite the high escape latencies of right-lesioned rats in this condition, it was clear from casual observation that these animals remained longer in the vicinity of the previous platform location and did not readily abandon the former search strategy. Such perseverative tendencies in right-lesioned rats could indicate a failure of executive function. Alternately, such perseveration may reflect a sense of desperation or panic rather than specific learning deficits, although admittedly this is speculative.

Given the nature of the task, it is also tempting to interpret the results in terms of a more general dominance of right hemispheric mechanisms in the processing of spatial information. Such an interpretation would have parallels in the human literature (eg. Weintraub and Mesulam, 1987, 1989; Hellige, 1993). Even in rats, such assertions of left/right population asymmetries have been made in regard to contralateral neglect following

prefrontal cortical lesions (Vargo et al., 1988), as well as water maze performance following parietal cortex lesions (Crowne, Novotny et al., 1992). While asymmetrical spatial function may play some role in the present results, it is far from a complete explanation, particularly in the case of Exp.1. It may be recalled from chapter 2, that these same animals did not differ in the magnitude of edge orientation bias in the openfield. In other words, in spontaneous exploration of a novel environment, these left and right lesion groups showed an identical directional distribution in orienting to the single most salient feature of the environment (ie. the edge). If the right hemisphere, under DAergic modulation, is somehow specialized for representation of the spatial surround, the water maze results would suggest that such asymmetries only emerge in situations of high arousal with specific behavioral demands and are probably more than simply 'spatial' in nature.

A moderate degree of arousal is well known to be associated with optimal performance (Selye, 1974). Since performance is all that can truly be said to differ in the above experiments, it is conceivable that if a fundamental functional asymmetry mediates the described effects, it may be more closely related to general arousal mechanisms rather than specific processes of motor capacity, learning ability or spatial functions. In support of this hypothesis, are recent findings of lateralized influences of DAergic systems in the ability to deal effectively with highly arousing situations such as shock avoidance (Carlson and Glick, 1989, 1991; Carlson et al., 1993). Specifically, DA activity in the right medial prefrontal cortex, but not the left, has been correlated with successful escape performance in shock avoidance (Carlson et al., 1993). The issue of cerebral DA asymmetries in stress and arousal will be explored in depth in chapter 4.

CHAPTER 4: LEFT/RIGHT HEMISPHERIC ASYMMETRY OF STRESS RESPONSES: ASYMMETRICAL EFFECTS OF MESOCORTICAL DOPAMINE DEPLETION ON STRESS ULCER PATHOLOGY AND SUBCORTICAL DOPAMINE SYSTEMS

4.1 Introduction

Studies investigating left/right hemispheric asymmetries at the population level of rodents, particularly those related to emotionality, were motivated largely by the work of Denenberg and colleagues (for reviews, see Denenberg, 1981; Denenberg and Yutzey, 1985). In a series of studies it was found that handling in infancy is sufficient to reveal lateralized specialization for a variety of behaviors. In one study, it was found that handled rats with right neocortical ablation were significantly more active when tested in an openfield than were handled rats with left neocortical ablation (Denenberg et al., 1978). Such increases in exploratory activity are interpreted as a reduction of emotionality (eg. freezing behavior). As well, increased openfield activity has been reported following right, but not left, lesions restricted to parietal cortex (Crowne et al., 1987).

Other studies have shown hemispheric asymmetries for behaviors which may be more directly related to emotional or affective function. Again using handled rats with left or right neocortical ablation, Denenberg (1983) reported that rats with the right hemisphere intact show significantly more emotional behavior than with the left hemisphere intact. In one case, this was found for a learned emotional behavior (retention of a conditioned taste aversion), and in another case for a spontaneous emotional behavior (muricide or mouse killing).

More recently, stress-related hemispheric asymmetries have been linked to central DAergic systems. It has been shown that rats which are left turners in response to amphetamine (reflecting relatively greater right brain DAergic activation) show a stronger sensitization effect to repeated amphetamine treatment than right turners, and also have significantly higher levels of plasma adrenocorticotropin (ACTH) and norepinephrine following undrugged exposure to a novel environment (LaHoste et al., 1988a,b).

As reviewed in section 1.1.5, the mesocortical DA system is particularly responsive to stressful inputs. Moreover, detailed investigations by Carlson and coworkers have uncovered lateralized differences in the activation of this system, which are dependent on the nature (controllability) and duration of the stressor (Carlson et al., 1988, 1991, 1993). This suggests not only a functional role for such activation, but also the asymmetrical regulation of stress responses.

The purpose of the present experiment is to test functional significance of lateralized manipulations of the mesocortical DA system, on the ability to cope with stress. Groups of rats received either left, right, bilateral or sham DA-depleting lesions of the prefrontal cortical DA terminal fields, and were then subjected to a cold restraint stress procedure. This stress paradigm allows for the measurement of a physiological consequence of coping ability, ie. gastric stress ulcer pathology, without the necessity of learned responses, motor activity or inflicting of physical pain like shock. As such, it is considered as much a psychological stressor as a physical stressor. This pathology measure is reduced by treatments facilitating central DA activity and increased by those interfering with DA transmission, although such effects in cortex have not yet been demonstrated (see Glavin et al., 1991).

There is also a substantial body of evidence showing that manipulations of the prefrontal cortex produce significant alterations in the functional state of subcortical DA systems (Deutch, 1992; Deutch et al., 1990; Rosin et al., 1992; Glick and Greenstein, 1973; Jaskiw et al., 1990, 1991; Mitchell and Gratton, 1992; Pycock et al., 1980a,b; Ross and Glick, 1981). In addition to examining effects of asymmetrical cortical manipulations on stress pathology, we also took this opportunity to study the effects of these treatments on *post mortem* neurochemical measures in subcortical DA terminal regions.

The results not only suggest a prominent role for the right prefrontal cortex in the development of stress pathology, but also demonstrate that each type of cortical lesion, produces distinct changes in subcortical DAergic function.

4.2 Methods

4.2.1 *Prelesion test and group assignment*

Seventy-eight male Sprague-Dawley rats (Charles River, Canada) weighing 300-350 gms at the start of the experiment were individually housed in a temperature controlled colony room on a 12 hr light/dark schedule (lights on at 08:00 hr). Food and water were available ad lib unless otherwise specified.

Following a week of daily handling, animals were assessed for endogenous directional bias (reflective of cerebral DAergic asymmetry, Jerussi and Glick (1976)) by recording turning behavior as previously described, in response to d-amphetamine sulfate (2.0 mg/kg, s.c., Sigma). A net directional bias was computed for each rat from the total number of quarterturns in the clockwise (CW) and counterclockwise (CCW) directions; bias = (CW-CCW)/(CW+CCW). Rats were then ranked on a continuum from strong CW bias

to strong CCW bias, and distributed evenly among five treatment conditions such that groups did not differ with respect to mean endogenous directional asymmetries.

4.2.2 *Surgical procedures*

All rats underwent stereotaxic surgery, receiving either 0.1% ascorbate saline vehicle (n=33) or 6-hydroxydopamine hydrobromide (6-OHDA, Sigma) in the left, right or bilateral cortex (n=15/group). Vehicle-injected rats were subdivided into groups of nonrestrained (n=12) and restrained (n=21) shams, based on subsequent stress procedures.

Following a twenty minute pretreatment with desipramine (20 mg/kg, i.p., Sigma), rats were anesthetized with sodium pentobarbital (Somnotol, 30 mg/kg, i.p.), with a supplementary dose of a ketamine/xylazine mixture (5:1) as necessary. Three injection sites per hemisphere were chosen (see Fig. 4.1), targeting the regions of most dense DAergic innervation, based on mapping studies by Lindvall and Bjorklund (1987). Thus, targets of DA denervation included not only medial prefrontal cortex, but also extended posteriorly to the deep layers of the anterior cingulate cortex, to maximally deplete the entire anterior midline cortex DA innervation. Injection coordinates were a) 3.0 mm anterior to Bregma, 0.8 mm lateral to midline, and 4.1 mm ventral to dura b) 1.7 A, 0.8 L, 2.7 V; and c) 0.2 A, 0.6 L, 2.4 V. Surgery was performed with skull horizontal according to Paxinos and Watson (1982). Each injection site received a 4 µl infusion of vehicle or 6-OHDA over a 4 min period via a 30 gauge cannula, which remained in place an additional 4 minutes. In total, lesioned rats received 12 µgs 6-OHDA per lesioned hemisphere.

4.2.3 *Cold-restraint stress procedures*

Fourteen days post-operatively, and following overnight food deprivation, animals

were restrained in Plexiglas rodent restrainers (Fisher Scientific Co.) for 3 hr at 4° C in a large, well ventilated refrigerator. Immediately after restraint, rats were sacrificed by decapitation. The nonrestrained shams received an equal period of food deprivation prior to sacrifice, but no cold-restraint procedure. Central DA terminal regions were rapidly dissected on ice (see below) and were stored at -80° C for future biochemical analysis by HPLC-EC. Stomachs were dissected out, cut along the greater curvature and washed in cold water. They were then examined under a dissecting microscope (10x) with an ocular micrometer, for the number and total cumulative length of gastric mucosal haemorrhagic lesions (ulcers), measured to the nearest 0.1 mm.

4.2.4 *Brain dissection and biochemical analysis*

Four DA terminal regions were dissected out, as described in section 2.2.6 and included anterior midline cortex, striatum, nucleus accumbens and amygdala. Left and right brain structures were sampled separately. The deliberately large cortical region dissected (see Fig. 4.1), encompassed the three injection sites, included the medial prefrontal cortex and extended posteriorly to the anterior cingulate. The subcortical structures were dissected in their entirety.

Following storage at -80°C, samples were thawed, weighed and homogenized in .25N acetic acid, with 5 µg EGTA (ethyleneglycol-bis- tetraacete) and 10 µg glutathione per mg tissue. Samples were centrifuged at 3500 rpm for 30 min. Homogenate supernatants were then filtered and microcentrifuged using 0.22 µm nylon filters (Costar). Varying volumes of filtered supernatant (depending on the tissue region analysed) were combined with 3,4-dihydroxybenzylamine (DHBA) as internal standard, and injected onto a silica/C18 column

(particle size 10 μm) using a Waters HPLC (WISP 710B) and a Coulochem II electrochemical detector with high sensitivity analytical cell. Mobile phase consisted of 6.9 gm of sodium phosphate monobasic, 125 mg heptane sulfonate, 80 mg ethylenediamine tetraacetate (EDTA) and 30 ml methanol per liter of double distilled water (pH 3.50).

Compounds analysed included DA, the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and norepinephrine (NE).

4.2.5 *Statistical analysis*

A two-factor analysis of variance (ANOVA) was used to analyse the gastric stress ulcer data, with lesion condition (Group) and prelesion directional bias (Bias) as between factors. The latter was a three level factor representing strong CW rotators, nonrotators, and strong CCW rotators. The nonrestrained sham group was excluded from this analysis. Duncan's multiple range test was used to make posthoc between group comparisons. The same two-factor ANOVA was performed for DA content and DA turnover (DOPAC/DA), individually for each of the eight brain regions examined. Nonrestrained shams were included in these analyses. For the biochemical measures, planned comparisons were performed between individual groups and the restrained sham group for any given structure, using independent Student's t-tests (two-tailed). Correlations between variables were assessed using Pearson's correlation coefficient (r). The chosen level of significance for all comparisons was $p < 0.05$.

Additionally, in order to determine if the various treatment conditions differentially altered the functional relationships among brain structures, a factor analysis was performed separately for each group. In these analyses, the eight measures of regional DA turnover

were used as variables to be correlated. Factors were then extracted from the correlational matrices, and following a varimax rotation procedure, the variables which loaded most significantly on each factor (ie. were most closely correlated) were grouped together, as depicted in Fig. 4.5 (see also Fig. 4.5 legend).

4.3 Results

4.3.1 *Gastric stress pathology*

From Fig. 4.2, it can be seen that animals depleted of DA in the right cortex exhibited the most severe stress-induced gastric pathology of any group. Statistical analysis provides the following support for this observation. The main effect for Group was significant ($F_{3,65} = 3.12$, $p = 0.033$); posthoc comparisons showed that the only between group difference was between the right lesioned group and (restrained) shams ($p < 0.01$). Due to the relatively large number of animals employed, the study was performed in two stages or replicates, and although one replicate exhibited greater overall pathology than the other, the pattern of group results was virtually identical in each case (ie. right lesions producing the greatest pathology).

All ulcers were found in the glandular (acid-secreting) portion of the stomach. The number of ulcers and their total cumulative length were highly correlated across restrained animals ($r = .76$, $n = 66$, $p < 0.001$), subsequently "pathology" refers only to total ulcer length unless otherwise specified.

4.3.2 *Lesion-induced cortical DA depletion*

Inspection of Fig. 4.3A reveals that each side of cortex injected with 6-OHDA was markedly depleted of DA. Interestingly, the nonlesioned cortex showed partial depletion in right but not left lesioned rats. This was supported by statistical analysis: main effects for

Group were found for DA content in both the left ($F_{4,76} = 22.7$, $p < 0.001$) and right ($F_{4,77} = 34.9$, $p < 0.001$) cortex. Individual comparisons showed that compared to the corresponding structure of the restrained shams, each lesioned cortex contained significantly less DA ($p < 0.001$ in all cases); moreover, DA content of the left cortex in right lesioned rats was significantly reduced relative to restrained shams ($p < 0.05$). The extent of depletion (DA content of injected cortex) did not differ between left and right lesioned groups ($p > 0.05$).

As the precise location of (6-OHDA) injection sites could not be determined since cortical tissue was dissected for biochemical analysis, it should be mentioned that the tips of cannula tracts could frequently be discerned during the dissection procedure. In all such cases, the tips were positioned as intended, both in terms of depth and in left/right displacement from the medial cortical wall, suggesting that surgical bias is not likely to account for any reported asymmetries in pathology or neurochemistry.

4.3.3 *Lesion-induced changes in subcortical DA content*

Figs. 4.3B-D reveal the effects of cortical lesions on DA content in subcortical structures. Most notably, right cortical lesions were associated with reductions of striatal DA in both hemispheres (Fig. 4.3B, $p < 0.001$), while bilateral lesions reduced right n. accumbens DA (Fig. 4.3C, $p < 0.05$) and elevated left amygdala DA (Fig. 4.3D, $p < 0.05$). The significant main effects of Group in each subcortical region were: left striatum, $F_{4,75} = 8.82$, $p < 0.001$; right striatum, $F_{4,75} = 4.12$, $p = 0.005$; right n. accumbens, $F_{4,77} = 3.93$, $p = 0.006$; left amygdala, $F_{4,77} = 5.97$, $p < 0.001$; right amygdala, $F_{4,77} = 2.72$, $p = 0.037$; left n. accumbens, n.s.

4.3.4 *Lesion-induced changes in regional DA turnover*

Figs. 4.4A-D show the changes in regional DA turnover (DOPAC/DA) associated with the various treatment conditions. None of the lesions significantly affected cortical DA turnover (Fig. 4.4A). However, subcortical DA turnover was altered as follows: left lesions increased DA turnover in both the left and right amygdala (Fig. 4.4D, $p < 0.05$), right lesions elevated striatal turnover (Fig. 4.4B; left, $p < 0.001$; right, $p < 0.01$), and bilateral cortical lesions increased right n. accumbens turnover (Fig. 4.4C, $p < 0.05$), while decreasing right amygdala turnover (Fig. 4.4D, $p < 0.05$). The increased amygdala turnover in left lesioned rats was associated with increased levels of DOPAC ($p < 0.05$), while the enhanced striatal turnover in right lesioned animals was not, ie. the latter apparent turnover increase was due to reductions in striatal DA content in this group. Significant main effects of Group on DA turnover were as follows: right cortex, $F_{4,77} = 3.17$, $p = 0.019$; left striatum, $F_{4,75} = 8.81$, $p < 0.001$; right striatum, $F_{4,75} = 3.49$, $p = 0.012$; right n. accumbens, $F_{4,77} = 3.52$, $p = 0.012$; left amygdala, $F_{4,77} = 4.71$, $p = 0.002$; right amygdala, $F_{4,77} = 5.68$, $p = 0.001$; left cortex and left n. accumbens, n.s.

4.3.5 *Stress-induced biochemical changes in shams*

As seen in Figs. 4.3 and 4.4, there were no significant differences in either DA content or DA turnover (DOPAC/DA) between restrained and nonrestrained shams for any of the eight brain regions examined. While this may seem surprising (particularly for DA turnover), some changes were observed for DOPAC alone. In amygdala, DOPAC was higher in stressed vs. nonstressed shams on the left side, (440 ± 34 vs. 330 ± 26 pg/mg tissue, $p = 0.039$), but failed to reach significance on the right side (454 ± 31 vs. 361 ± 30, $p = 0.054$,

two-tailed t-tests). DOPAC levels showed (nonsignificant) trends for elevations in cortex of restrained relative to nonrestrained shams (left cortex, 201 ± 16 vs. 170 ± 10 pg/mg tissue, p = .105; right cortex, 206 ± 12 vs. 173 ± 12, p = .078). Trends for increased striatal or n. accumbens DOPAC levels were not evident as a function of stress in shams.

4.3.6 *Relationship between amphetamine response and stress pathology*

Prelesion directional bias (based on amphetemine-induced turning in rotometers) was not a significant factor with regard to stress pathology, or any regional measures of DA content or turnover (no effects of Bias factor in two-way ANOVAs).

4.3.7 *Relationships between regional DA turnover and pathology*

When considering all four groups of restrained animals together, gastric stress pathology was more closely related to DA turnover (DOPAC/DA) in the left striatum than in any other structure (r = .32, n = 66, p = 0.01). In the restrained sham group alone, the left striatum was again most closely correlated with pathology (r = .63, n = 21, p = 0.002). Pathology did not correlate significantly with DA turnover in any region in the lesion groups. In terms of DOPAC alone, levels of this metabolite in the right cortex (and no other structure) were significantly correlated with pathology across groups of stressed rats (r = -.39, n = 66, p = 0.011), ie., the more DOPAC in the right cortex, the less pathology was seen. This correlation was not significant in any single group however.

4.3.8 *Regional interactions of DA activity: Effect of treatment condition*

In addition to the asymmetric and highly specific regulation by the cortex, of the regional DA systems described above, the various treatment conditions also resulted in disticntive patterns in the manner in which regional DA activity is correlated among

structures. Based on factor analyses of regional DA activity, Fig. 4.5 demonstrates that in nonrestrained shams, three factors (indicated by distinctive line types) emerged revealing an asymmetrical pattern of functionally linked DAergic circuits. In restrained shams however, tight coupling of DA activity is seen among subcortical structures except the right n. accumbens which loaded on an independent factor. Cortical activity (both left and right) loaded on a separate factor. A unique pattern of functional relationships emerges in the reorganization occurring after each type of cortical lesion (in conjunction with stress). Notably, in both unilateral l on groups, cortical turnover becomes closely (abnormally?) linked with subcortical DA systems. In contrast, bilateral lesions result in a reversion of cortical DA activity to an independent factor as in restrained shams, with extensive coupling of subcortical function.

While the permanence or generality of these activity patterns can only be speculative, these models are intended to visually represent another way in which cortical DA mechanisms asymmetrically alter brain function.

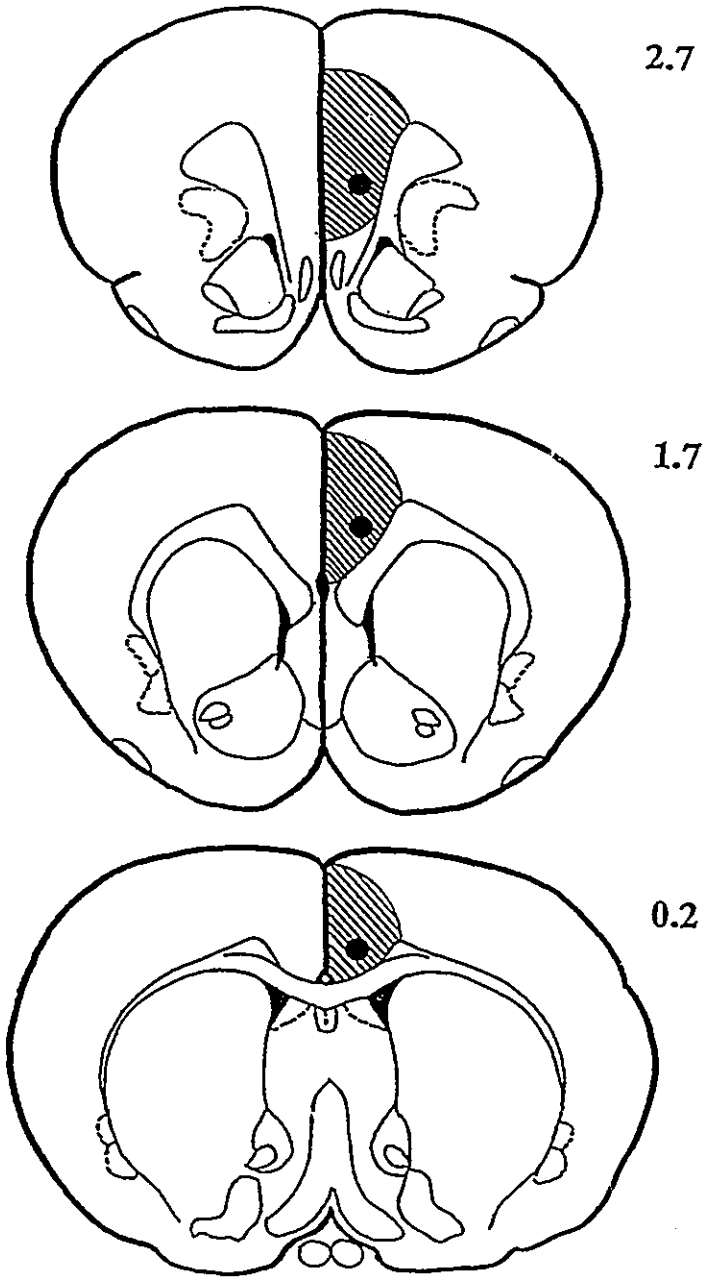


Fig. 4.1. Extent of cortical region of study. Closed circles represent the targeted sites of injection of 6-OHDA, while the shaded areas correspond to the region of cortex sampled for biochemical analysis. The numbers refer to the distance (in mm) anterior to bregma for each section, as reproduced from Paxinos and Watson (1982). While the bottom two figures correspond exactly to the injection coordinates used, the top figure is a close approximation (0.3 mm posterior) to the actual coordinates.

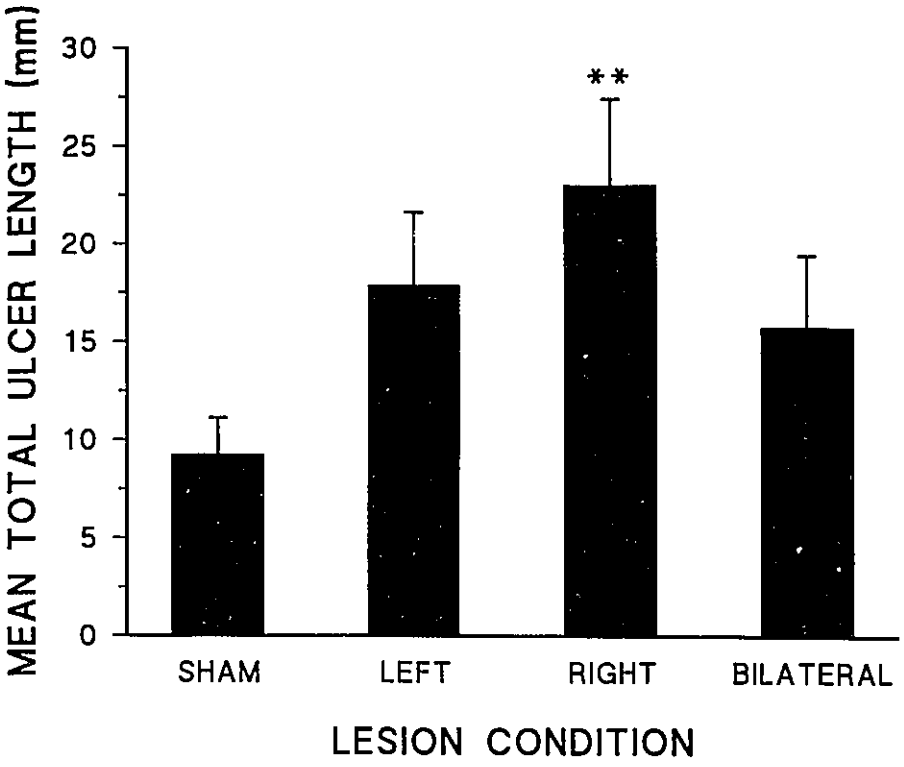


Fig. 4.2. Mean total gastric stress pathology (± S.E.M) in rats with left, right or bilateral cortical DA depletion and sham controls. The nonrestrained sham group is not shown as none of the rats in this group exhibited any gastric pathology. **$p < 0.01$ compared to restrained shams; no other comparisons significant (Duncan's multiple range test).

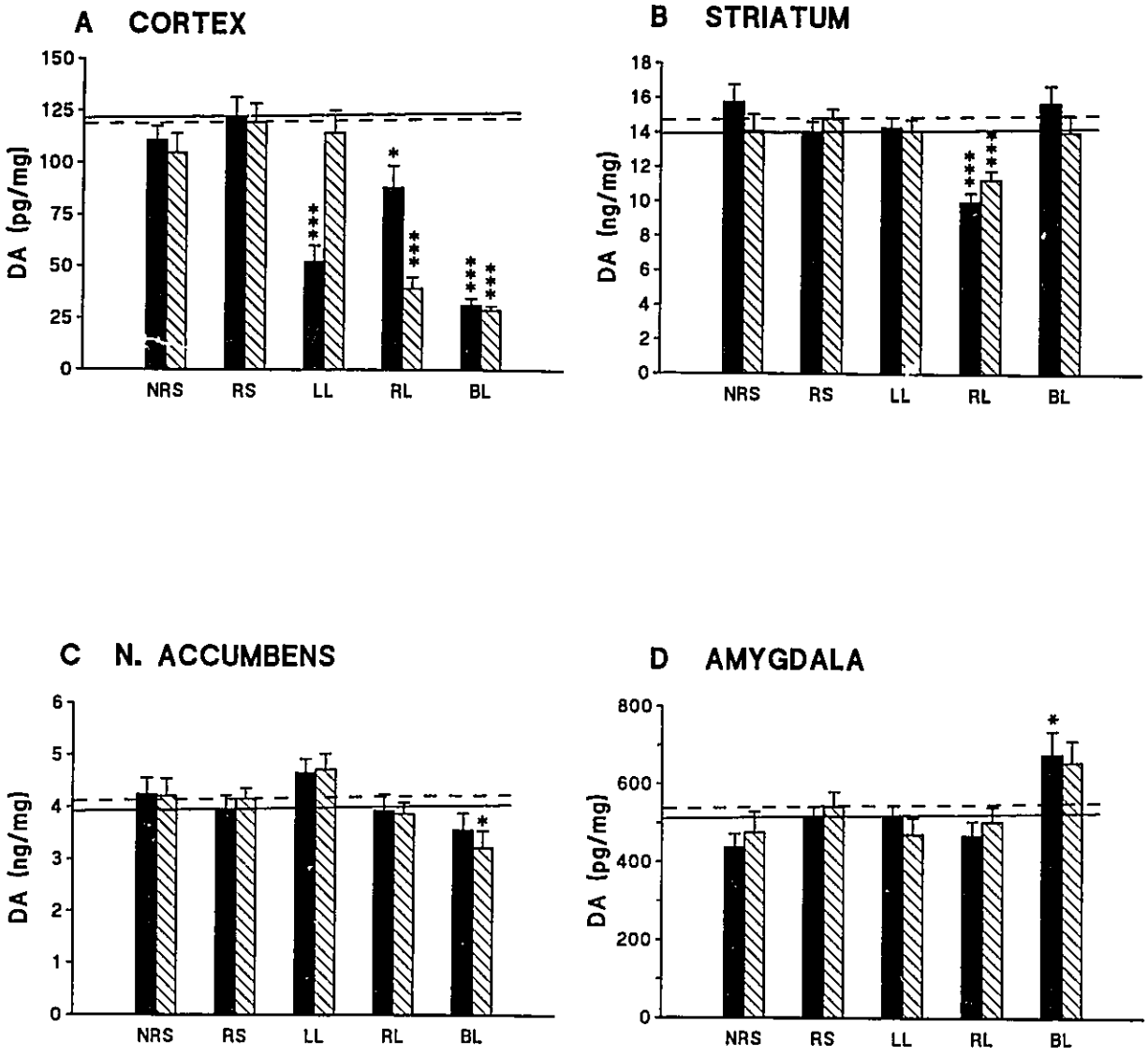


Fig. 4.3. Regional dopamine content across groups (NRS, nonrestrained shams; RS, restrained shams; LL, left cortical lesions; RL, right cortical lesions; BL, bilateral cortical lesions). Solid bars represent left brain structures while striped bars represent right brain structures. Solid and dashed lines respectively, represent values for left and right brain structures of restrained shams. In addition to highly significant depletion of cortical DA by 6-OHDA, lesions also affected subcortical DA content as follows: right cortex lesions resulted in bilateral reductions of striatal DA, while bilateral cortex lesions reduced right n. accumbens DA and increased left amygdala DA (compared to corresponding structures of restrained shams). * $p < 0.05$, *** $p < 0.001$ (Student's t-tests).

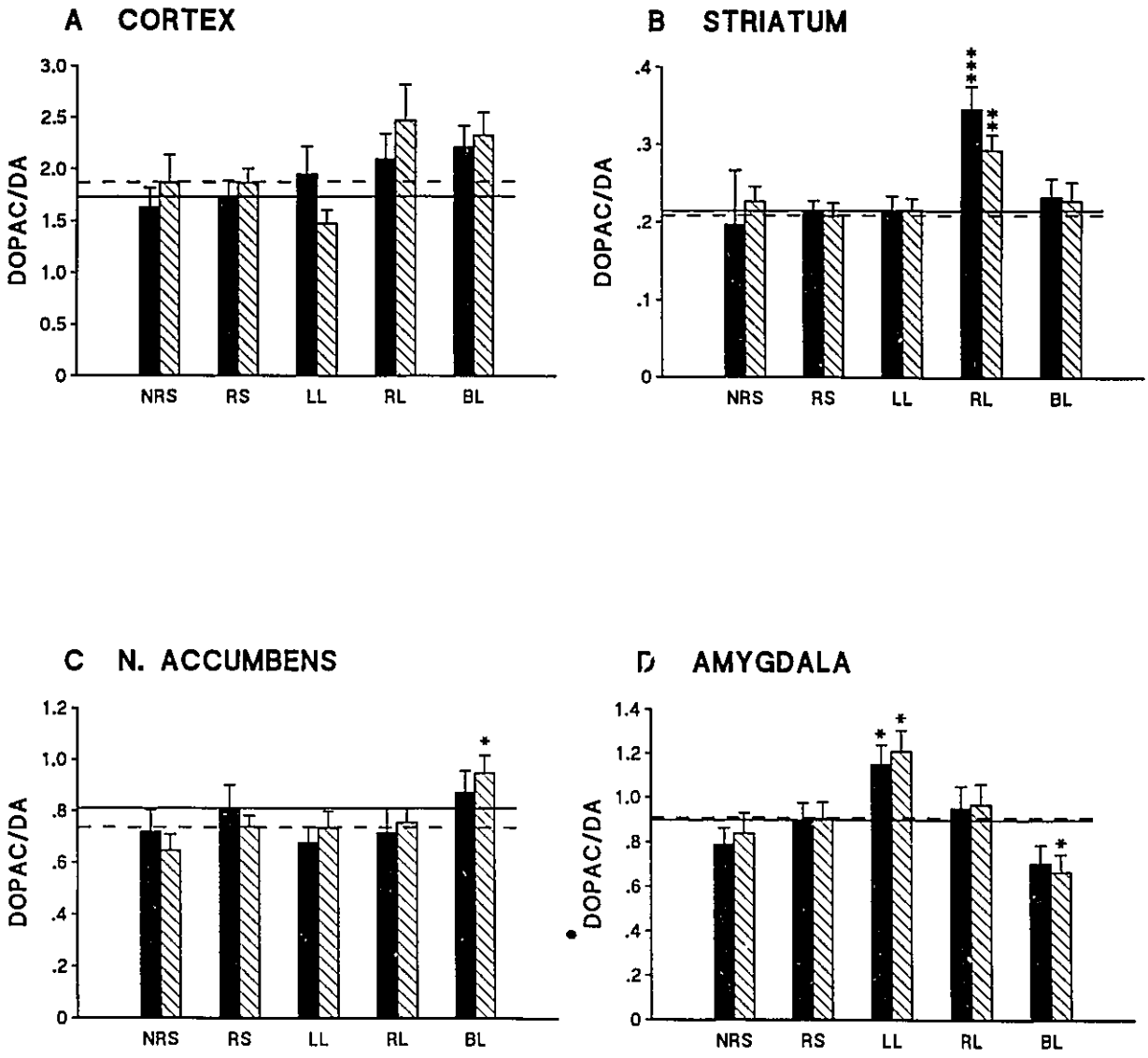


Fig. 4.4. Regional dopamine turnover across groups (NRS, nonrestrained shams; RS, restrained shams; LL, left cortical lesions; RL, right cortical lesions; BL, bilateral cortical lesions). Solid bars represent left brain structures while striped bars represent right brain structures. Solid and dashed lines respectively, represent values for left and right brain structures of restrained shams. Cortical DA depletion did not result in significant changes in cortical DA turnover (DOPAC/DA). Lesion effects on subcortical DA turnover were as follows: left lesioned rats showed significant bilateral elevations in amygdala DA turnover, right lesioned animals demonstrated bilateral increases in striatal DA turnover, while bilateral cortex lesions increased right n. accumbens turnover and reduced right amygdala turnover. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared to restrained shams).

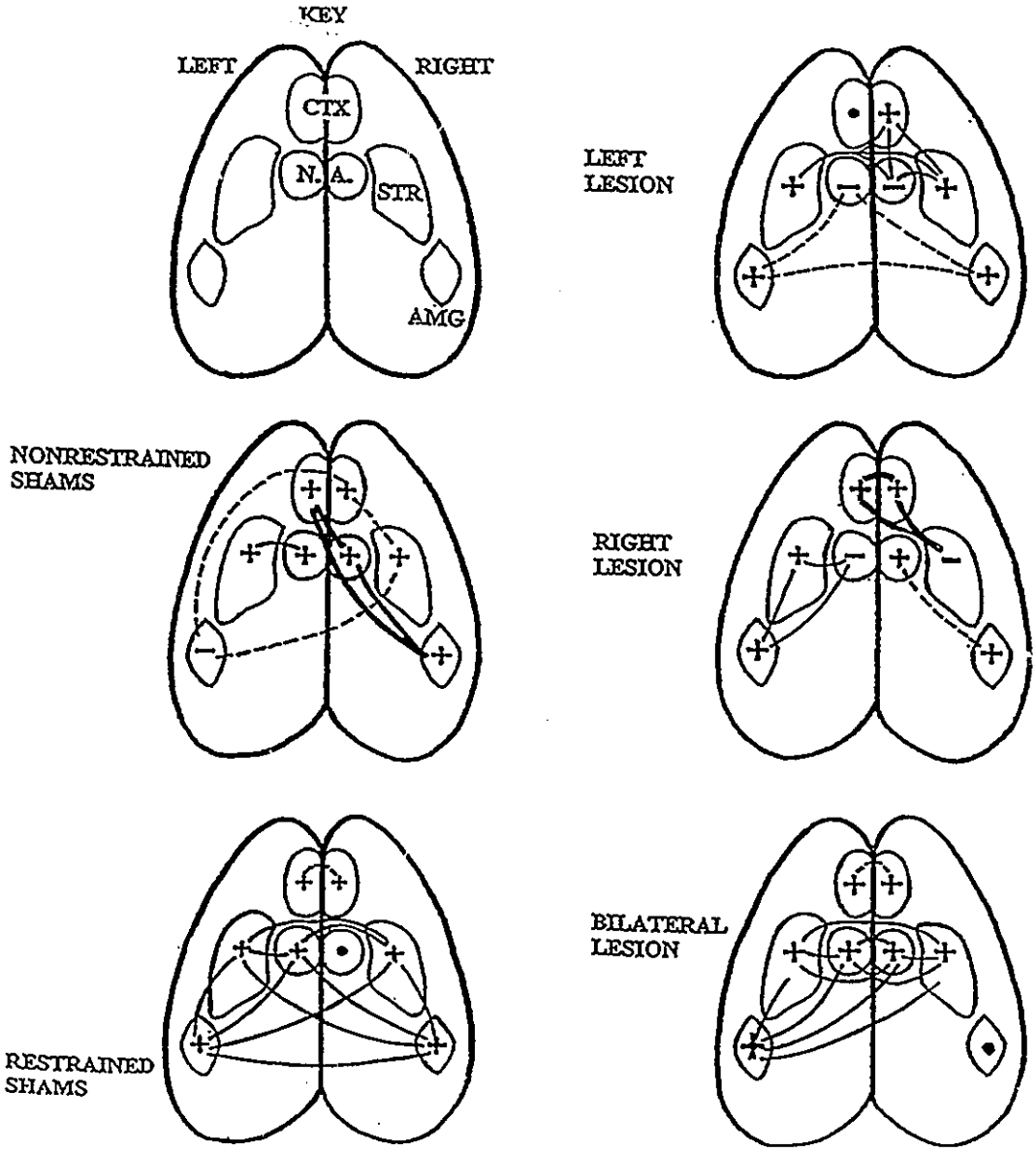


Fig. 4.5. Functional interrelationships of regional DA activity by group. These schematic models are intended as visual representations of how patterns of regional DA activity, change across treatment condition. The models are based on factor analyses performed for each group using the eight variables of regional DA turnover as variables to be correlated. Each type of line (broken, thick or thin) represents separate factors of related variables, and each variable or region is grouped according to its strongest factor loading, ie. strongest correlations. The models demonstrate the manner in which the various treatment conditions differentially alter the functional interrelationships of the DAergic network. It can be seen that the tight coupling of DA activity among structures is not only asymmetrical, but varies distinctly with treatment condition. ● indicates when a single brain region loads on a separate factor; + and - denote the direction of correlations. Abbreviations used are CTX - cortex, N.A. - nucleus accumbens, STR - striatum, AMG - amygdala.

4.4 Summary and discussion

The major finding of the present study is the highly significant increase in stress ulcer pathology resulting from the depletion of right cortical DA. Furthermore, each type of cortical lesion is associated with unique changes in subcortical DA terminal fields. These and other findings suggest that the right prefrontal cortex in at the top of a neural hierarchy for dealing with highly stressful situations. Before discussing this interpretation of the findings in detail however, five points regarding the results deserve comment.

Firstly, it may seem surprising given the large body of literature on stress and DAergic activation, that stress did not significantly affect DA content or turnover in nonlesioned rats, although some elevations of DOPAC were described in amygdala and cortex. A reason for this apparent discrepancy may be a consequence of the duration of restraint (3 hr) chosen for the purpose of pathology development. It has been shown that restraint-induced changes in extracellular mesolimbic DA and metabolites are biphasic (as measured by microdialysis), being increased for up to ninety minutes, but suppressed thereafter for up to four hours (Puglisi-Allegra et al., 1991). It is in keeping with this biphasic time course that postmortem tissue analysis (reflecting integrated activity over a three hour period) would reveal no net changes between stressed and unstressed rats as observed.

Secondly, it is unlikely that the greater pathology seen in right lesioned rats, is an artifact of the additional (partial) depletion of DA in the intact left cortex. Specifically, if total depletion were the critical factor in producing pathology, then the bilateral lesion group should have had the most severe pathology, but this was not the case. As well, the level of

left cortical DA in right lesioned rats did not correlate with pathology (data not shown). The finding that right but not left cortical lesions affect contralateral cortex, may itself be a reflection of an inherently and functionally asymmetric cortex, rather than a determinant of the asymmetrical effects on pathology and subcortical DA systems.

Thirdly, a caution in interpretation of the results which should be pointed out, regards the possible role of norepinephrine (NE) in the observed effects. Despite pretreatment with desipramine, significant depletions of cortical NE were seen which paralleled those of DA virtually identically, although not to the same extent (data not shown). While this would not negate the asymmetrical effects we report, it cannot be ruled out that NE or NE/DA interactions could play a role either in pathology development or in cortical influences on subcortical DA systems.

Fourthly, it is improbable that diffusion away from the injection sites (to other DA terminal regions) could account for the reported asymmetrical effects on subcortical DA systems. If diffusion were a relevant factor, then it seems unlikely that only right cortex injections would deplete striatal DA (bilaterally at that) and left or bilateral injections would not (Fig. 4.3B). If there was diffusion it would be difficult to explain how bilateral injections reduced DA in the right n. accumbens, but right cortex injections alone did not, and left cortex injections even tended to increase n. accumbens DA bilaterally. Furthermore, other lesion techniques such as ligation of the middle cerebral artery (Robinson, 1979) and suction lesions of frontal cortex (Pearlson and Robinson, 1981), have produced similar asymmetrical neurochemical effects. In particular, infusion of haloperidol into the right, but not left, carotid artery, produced bilateral reductions in striatal DA content (Hyde and Jerussi, 1992).

This is a striking parallel to the effect reported here for right, but not left, cortical DA depletion. Taken together, these findings strengthen the case for inherent asymmetries in the organization of the rodent brain, which involve DAergic systems and may be more consistent across studies than is currently appreciated.

Finally, although the differential effects of lesion condition on subcortical DA systems were pronounced and highly specific, the question arises as to the role of stress in producing these changes. Unstressed lesion groups were not included in the study, as the primary aim was to investigate asymmetries in the development of stress pathology. However, a review of the effects of bilateral prefrontal cortical DA depletion on subcortical DA systems (Deutch, 1992) has concluded that such lesions do not cause changes in *basal* function of subcortical DA systems; rather they cause enhanced responsiveness in these systems to pharmacological or environmental (stress) challenges. It is thus likely that the subcortical changes presently reported, reflect the interaction of lesions and stress.

Regarding the stress ulcer pathology data, the tendency for all three lesion groups to show a potentiation of pathology suggests a protective role for the mesocortical DA innervation. As stated in section 1.1.5, the same role for DA in preventing stress pathology has been documented in the amygdala, caudate and accumbens. In fact, even peripheral DA (D_1 receptor) mechanisms at the level of the gastric mucosa have been shown to reduce ulcer formation (Glavin, 1991; Glavin et al., 1991).

There are a number of means by which the mesocortical DA system could interact with physiological systems associated with stress. DAergic neurons in the VTA are known to contain glucocorticoid receptors (Harfstrand et al., 1986), and adrenalectomy has been

shown to reduce the stress-induced mesocortical DA response (Imperato et al., 1989). Conversely, lesions of the VTA decrease both basal and stress-induced corticosterone secretion (Casolini et al., 1993). As well, the medial prefrontal cortex has been shown to exert negative feedback control over the hypothalamic-pituitary-adrenal (HPA) axis (Diorio et al., 1993). From this data, it could be suggested that the mesocortical DA system is part of a feed-forward loop activating HPA function.

In addition to neuroendocrine influences, the medial prefrontal cortex (particularly the infralimbic area) is known to send direct projections to brainstem autonomic nuclei such as the dorsal motor nucleus of the vagus, the nucleus of the solitary tract and the parabrachial nucleus, as well as to limbic regions which in turn project to these nuclei (Cechetto and Saper, 1990; Hurley et al., 1991; Neafsey, 1990). Excitotoxic lesions of medial frontal cortex significantly disrupt a number of behavioral and autonomic reactions typical of conditioned emotional responses (Frysztak and Neafsey, 1991, 1993). Moreover, single-units in the anterior cingulate (a region also included in the present cortical analysis) respond to restraint stress; electrical stimulation and electrolytic lesions of this region, respectively facilitate and reduce gastric pathology (Henke, 1984; Sullivan and Henke, 1986). The fact that the effect of electrolytic lesions is opposite in direction to that of 6-OHDA lesions, suggests that endogenous DA has an inhibitory modulatory action on those cortical neurons whose (over)stimulation could otherwise facilitate pathology development. The mesocortical DA innervation is thus positioned to play a key modulatory role both in neuroendocrine and autonomic aspects of stress responses.

The finding of a highly significant ulcer-enhancing effect of right cortical lesions is

consistent with numerous reports of hemispheric asymmetries of stress-related functions in rats (Carlson et al., 1988, 1991, 1993; Carlson and Glick, 1989, 1991; Crowne et al.,1987; Denenberg, 1981; LaHoste et al., 1988a,b; Sullivan and Szechtman, 1990). In humans as well, it is frequently found that the right hemisphere plays a specialized role in the processing of emotional information, or the normal expression of emotion (Bear, 1983; Gainotti, 1983, 1987; Hartley et al., 1989; Schiff and Lamon, 1989; Wittling and Roschmann, 1993; Wittling and Schweiger, 1993).

Broadly speaking, it has been said that the left hemisphere is associated with weak or mild emotions, while the right hemisphere is preferentially involved in experiencing strong emotions both in rats (Bianki, 1988, p.117) and humans (Denenberg, 1981, p.17). Specifically (in rats), lateralized activation of medial prefrontal DA systems occurs during uncontrollable stress (Carlson et al., 1988, 1991, 1993). During restraint stress this lateralized activation follows a particular temporal order: at 15 minutes, the left cortical DA system is more active, but following 60 minutes of restraint, the right side is more active (Carlson et al., 1991). It was suggested from this and other studies employing footshock, that the immediate response of the mesocortical DA system to stress favors the left cortex, and right cortical DA activation is associated with prolonged, uncontrollable stress (Carlson et al., 1988, 1991, 1993; Carlson and Glick, 1989, 1991).

The present results could also be interpreted in this context. In the proposed model of sequential left/right activation, left cortical DA activation in the intact animal, could be associated with stressor assessment or analysis, initiation of appropriate coping strategies and monitoring initial coping effectiveness. As stress is sufficiently prolonged and

uncontrollable, and coping attempts are perceived as ineffective, cortical DA activation would proceed to preferential right side involvement. Given these premises, the severe stress pathology seen in right lesioned rats following 3 hr of cold restraint could be a consequence of removing such an asymmetrical protective influence.

The somewhat puzzling finding that pathology in the bilateral lesion group did not reach the same extent as in right lesioned animals (but was more similar to left lesioned rats), may reflect the proposed role of left cortical DA in stressor assessment. In left or bilaterally lesioned rats, the compromised left cortical DA system may have rendered the animals less capable of accurately assessing either the nature of the stressor, or the effectiveness of its coping attempts. This may be supported by the fact that both left and bilateral (but not right) lesion groups showed significant changes in DA systems in the amygdala (Figs. 4.3 and 4.4), a structure whose role in attaching appropriate emotional and autonomic responses to stressful situations has been documented (for review, see Henke et al., 1991). In contrast, right lesioned rats may be relatively unimpaired in such processes, but lack the DAergic modulation needed to optimize coping with a fully perceived, severe stressor, leading to the greatest stress pathology. In summary, regarding the pattern of pathology results across lesion groups, it should be noted from the regional neurochemistry data that a) a right lesion is not a mirror image of a left lesion and b) a bilateral lesion is not the sum of a left and right lesion. Given the distinctiveness of the three lesion effects, it is therefore illogical to expect that pathology should be equal in left and right lesion groups, or most severe in the bilateral group.

The suggested specialization of the right cortex in conditions of prolonged or

uncontrollable stress, may derive in part from intrinsic asymmetries in cortical neuronal properties. Electrophysiological properties of prefrontal cortical neurons in rats show hemispheric differences in response to stimulation of contralateral prefrontal cortex, such that the right cortex appears more responsive to repeated stimulation of the left, than vice versa (Perez et al., 1990). Specifically, responses recorded in the right cortex exhibit greater amplitude increments and lower fatigability to repetitive stimulation, than those recorded in the left. These properties are consistent with the notion that the right cortex may be preferentially activated in situations of prolonged stimulation, as might be expected to occur during uncontrollable stress. Accordingly, the right cortex may be at the top of a hierarchy for the processing of prolonged (or brief but intense) stressful inputs. An implication of these asymmetrical neuronal properties is that the sequential (left to right) activation of mesocortical DA systems during stress (Carlson et al., 1988, 1991, 1993; Carlson and Glick, 1989, 1991) may follow from a primary left to right shift in intrinsic neural activity; ie. as the balance of neural activity shifts to the right, a compensatory increase in DAergic modulation follows. In keeping with this notion is the behavioral finding that indices of right (but not left) cortical DA function correlate with successful escape performance following uncontrollable shock (Carlson et al., 1993). Moreover, the present study demonstrated that across groups of restrained animals, levels of DOPAC in the right prefrontal cortex were significantly associated with less severe stress pathology.

Regarding the models of regional DA activity circuits depicted in Fig. 4.5, it is evident that the different treatment conditions are associated with unique patterns of correlated activity. While this approach to analyzing regional activity is not common, there

is much evidence that DA activity in the various terminal fields is functionally linked, either positively or negatively. Several studies have found changes in the activity of subcortical DA systems, either in nucleus accumbens or striatum, following (bilateral) DA denervation of medial prefrontal cortex (Deutch, 1992; Deutch et al., 1990; Jaskiw et al., 1991; Mitchell and Gratton, 1992; Pycock et al., 1980a,b). Similarly, manipulations in the amygdala cause changes in DAergic function, opposite in direction, in nucleus accumbens and prefrontal cortex (Simon et al., 1988; Rada and Hernandez, 1990). The importance of understanding DAergic function as part of an elaborate, integrated network has recently been emphasized in an extensive review (Le Moal and Simon, 1991). That regional DA activity is tightly coupled should not be surprising given the extensive anatomical interconnections of these four terminal fields, and the fact that many of these projections are not only contralateral, but bihemispheric (see section 1.1.1).

The present findings also suggest that the correlated DA activity within this network is highly asymmetrical, and a very recent report highlights this general principle. It has been found that DA blockade in either entorhinal cortex or hippocampus in rats causes significant enhancement of DA transmission in nucleus accumbens, but these effects are much more pronounced in the left hemisphere than in the right (Louilot and Le Moal, 1994).

Finally, in regard to the regional correlations in Fig. 4.5, it can be seen that between the two groups of nonlesioned rats, the stressed condition is associated with a more tightly coupled, interhemispheric pattern of activity between homotopic structures, than is the situation in unstressed shams. It has been reported that randomly applied prenatal stress increases the interhemispheric coupling of DA activity (but not that of serotonin) in

prefrontal cortex, nucleus accumbens and striatum, as measured in the adult offspring (Fride and Weinstock, 1987). The present results would suggest that an acute stress has similar effects on interhemispheric coupling in the intact animal. Interestingly, the same prenatal stress treatment has also been found to induce a permanent rightward shift in prefrontal cortical DA activity (Fride and Weinstock, 1988).

In summary, depletion of mesocortical DA renders rats more susceptible to stress-induced gastric pathology, particularly with right-sided depletion. Each type of cortical lesion produces distinct changes in the neurochemical status of subcortical DA systems in stressed animals. It is suggested from these and other findings, that the right (medial prefrontal and/or anterior cingulate) cortex is at the top of a neural hierarchy in situations of uncontrollable stress, and that endogenous cortical DA protects against the pathological effects of such stressors. Such influences of cortical DA on stress responses may either be direct, or act via effects on subcortical structures also known to influence stress-related processes.

CHAPTER 5: INTERHEMISPHERIC REGULATION OF BEHAVIORAL ASYMMETRIES IN RATS WITH UNILATERAL SUBSTANTIA NIGRA LESIONS: ROLE OF THE CORPUS CALLOSUM

5.1 Introduction

The role of interhemispheric communication in behavioral expression has long been of interest to scientists of several disciplines. In particular, the studies of Sperry and colleagues with split-brain patients have shed considerable insight into the role of the corpus callosum in the integration of neural processes, having implications from basic anatomy and behavior, to philosophical aspects of the nature of consciousness (for review, see Nobel prize acceptance speech of Sperry, 1982).

While many of the early animal studies of corpus callosum function employed monkeys or cats (for review, see Berlucchi, 1990), several recent studies using rats have shown that asymmetries in behavior, neurochemistry, and electrophysiology are altered by sectioning the corpus callosum (Crowne et al., 1988; Denenberg et al., 1985,1986; Glick et al., 1975, Bianki, 1988), suggesting that transcallosal communication is involved in their maintenance or expression.

As the present thesis is concerned with functional asymmetries of DAergic systems, it was of interest to determine the role played by the corpus callosum in the expression of behavioral asymmetries characteristic of rats with unilateral 6-OHDA lesions of the substantia nigra. Since the corpus callosum contains not only an intercaudate commissural system (Mensah and Deadwyler, 1974), but also abundant corticostriatal cross-projections

(McGeorge and Faull, 1989), it seemed reasonable that at least some asymmetries associated with unilateral striatal DA depletion, would be affected by callosotomy.

Three behavioral situations were chosen for study, all of which elicit predictable asymmetries in the unilateral 6-OHDA rat. The first was the "dodging" paradigm (detailed below), which involves performing a lateralized response to a lateralized stimulus, thus entailing the integration of both strong sensory and motor components. Second, drug-induced turning in response to both apomorphine and amphetamine was examined to assess more purely motor asymmetry. Thirdly, the spontaneous, undrugged investigation of edges of a large openfield (as characterized in chapter 2) was examined as a predominantly sensory/spatial asymmetry.

The dodging paradigm, intended for the study of sensorimotor integration (Whishaw, 1988; Whishaw and Tomie, 1988), is based on a naturally occurring and highly consistent rat behavior performed during competition for food. Briefly, when a feeding rat is approached from the side by another rat (the robber), it quickly moves or dodges away from the robber with its food. This is a 'normal' dodge. An alternative response, very rare in nonlesioned rats, is to turn into or jump over the approaching rat, this being an 'abnormal' dodge. Unilateral 6-OHDA lesions of the substantia nigra alter the normal behavioral pattern as follows: a) when a lesioned rat is approached contralateral to its lesion, it is impaired in its level of responding, but when it does dodge, it does so in the normal direction, following its lesion-induced motor bias b) when approached ipsilateral to its lesion, the animal responds at control levels, but mainly with abnormal dodges, thus still following its strong motor bias.

We proposed that for a normal dodge to occur in the test animal, a transfer of information takes place from the hemisphere opposite to the side of approach (which is primarily involved in perceiving the approach), back via the corpus callosum to the striatum adjacent to the side of approach. The crossed corticostriatal input would then instruct the animal to turn away from this striatum, and therefore away from the robber. We hypothesized that severing the callosum would restrict the corticostriatal inputs to the ipsilateral striatum, thus causing the animal to turn in the direction of the robber regardless of the side of approach (ie. increasing the percentage of abnormal dodges or decreasing the percentage of normal dodges) .

As edge behavior also involves orienting to contralateral sensory stimuli, we hypothesized that this behavioral asymmetry may also be disrupted by callosotomy. In contrast, it was hypothesized that drug-induced turning asymmetries would not be affected by callosotomy, as the behavior is not dependent on external cues, or such an interhemispheric transfer of information.

5.2 Methods

5.2.1 *Surgical and histological procedures*

Twenty male Long-Evans rats (Charles River, Quebec), weighing approximately 250 gm at the start of study were used and housed in pairs in a temperature-controlled colony room on a 12:12 hr light/dark cycle (lights on at 07:00). Following pretreatment with desipramine (15 mg/kg, i.p.) and anesthesia (sodium pentobarbital, 40 mg/kg), all rats received unilateral 6-OHDA HBr lesions (8 µg/4 µl) of the substantia nigra as described in section 2.2.2. Rats alternately received lesions in the left or right side. Immediately

afterwards (during the same operation), they received either callosal section (CC SECT) or sham procedure (SHAM CC), as described by Crowne and Richardson (1985). Briefly, a horizontal wire knife constructed from a 26-ga needle, was inserted at the dorsal cerebellar surface and advanced anteriorly along a predrilled longitudinal groove dorsal to the cerebellum. The assembly was then tilted 2° ventrally, the inner wire of the knife extended, and advanced for the extent of the corpus callosum, before retraction of the wire knife and removal. Sham procedures differed in that the knife was only advanced as far as the posterior tip of corpus callosum (wire never extended).

At the completion of the experiment, rats were given an overdose of sodium pentobarbital, perfused intracardially with physiological saline and 10% formalin. The brains were removed and stored in 10% formalin. They were later transferred to 30% sucrose, frozen and cut into 20 µm longitudinal sections, which were mounted and stained with thionin to determine the extent of callosal damage, as well as the cannula tip placements in the substantia nigra.

5.2.2 *"Dodging paradigm" testing*

Behavioral testing of dodging was based on the procedure of Whishaw (1988). Testing was conducted in Plexiglas cylinders (45 cm x 50 cm height) on a clear glass surface above an angled mirror. As tests were videotaped from approximately a 45° angle above the rats, behavior could be analysed from both above and below the animals. For a week prior to testing, rats were handled and habituated to the test apparatus on a daily basis. During this period, they were food-deprived for 23 hrs and allowed to free-feed for an hour in the test cylinder in the presence of a similarly fasted robber rat. Test days differed in that the two

rats competed for a single piece of food.

On test days, a test rat was first placed into the cylinder with a 3-4 gm food pellet. A trained robber rat (one of six non-operated male Sprague-Dawley rats used only for this purpose) was then introduced behind the feeding (test) rat, repeatedly as necessary, until ten approaches were made to each side of the test rat. If the food was stolen, it was returned to the test rat, and the robber rat was re-introduced to the test chamber. Three pre-lesion tests on consecutive days, and 7 post-lesion tests (on days 4, 5, 6, 7, 8, 14, 28) were conducted and videotaped for subsequent analysis. Behavioral measures of interest included i) attempts to dodge - the number of times the test rat attempted to dodge in response to the 10 approaches from each side; and, ii) the direction of attempted dodges (normal - away from the robber, or abnormal - into or over the robber). An attempt to dodge was defined as any observable movement by the test rat away from the initial feeding position, before the robber could touch the food. Any attempted dodge resulting in a lateral displacement of at least 90° from the initial feeding position (the great majority of all attempts), was scored as either normal or abnormal.

5.2.3 *Spontaneous orientation to edges of a large openfield*

Rats were placed individually in the center of a large, elevated openfield as described in section 2.2.4, and videotaped for 10 min between the 4th and 5th week post-lesion. The time spent with either side of the head aligned with the edge, ipsilateral (IPSI) or contralateral (CONTRA) with respect to side of lesion, was recorded during active investigation of edges (small amplitude head movements, sniffing and/or vibrissae movements directed specifically to the edge, with or without locomotion). Edge

investigation bias was calculated as (IPSI-CONTRA)/(IPSI+CONTRA), such that positive values indicate that the side ipsilateral to the lesion is predominantly adjacent to the edge. Positive values are thus expected of animals with unilateral 6-OHDA lesions, given the results of chapter 2.

5.2.4 *Drug-induced turning behavior*

Drug-induced turning was measured in automated rotometers as described in section 2.2.3. Contraversive turning following apomorphine (0.25 mg/kg, s.c.) was tested at 5 weeks post-lesion, and ipsiversive turning following d-amphetamine sulfate (2.0 mg/kg, s.c.) was tested from 8 to 10 weeks post-lesion.

5.2.5 *Spontaneous (undrugged) motor activity*

Measures of general motor activity were recorded for 60 min at 5 min. intervals in Omnitech activity monitors. These tests were conducted between 6 and 7 weeks post-lesion.

5.2.6 *Statistical analysis*

Data from the dodging experiment were analysed using an ANOVA with factors of Group (CC SECT or SHAM CC) by Test day, with repeated measures on the Test days factor. The three prelesion baseline tests were excluded from this analysis. Activity monitor data was analysed in the same manner, with a factor for Group, but with repeated measures for time samples. Data for drug-induced turning behavior and edge orientation bias were compared using independent Student's t-tests (two-tailed). Correlations of behavioral measures were performed using Pearson's correlations.

5.3 Results

5.3.1 *Histology results*

Fig. 5.1 represents the typical extent of callosal damage incurred by the knife-cut procedure. In all but one rat of the CC SECT group (excluded from analysis), the corpus callosum was severed, except for the extreme anterior, ventral tip (genu), leaving approximately 0.25 -0.5 mm^2 intact. In one animal, a comparable area of the extreme posterior tip (splenium) was spared. Extra-callosal damage included severing of the dorsal hippocampal commissure. As well, bilateral marginal damage was observed along the medial walls of the cerebral hemispheres, just dorsal and anterior to the corpus callosum. Cannula placements for all rats in the data analysis (n = 9 for SHAM CC, n = 8 for CC SECT), were less than 0.5 mm dorsal and/or anterior to the pars compacta, a placement we have previously observed to typically result in more than 90% striatal dopamine depletion.

5.3.2 *Effects of callosotomy on dodging behavior*

The results of the dodging experiments (which essentially represent externally cued turning behavior), are summarized in Figs. 5.2 and 5.3. It can be seen that callosal section significantly reduced the number of attempts to dodge approaches from the side ipsilateral (Fig. 5.2A) to the substantia nigra lesion (Group effect, $F_{1,15} = 5.64$, $p = 0.031$), as well as dodging attempts to contralateral approaches (Fig. 5.2C, Group effect, $F_{1,15} = 7.03$, $p = 0.018$). The impairment in dodging responses was consistent across post-lesion testing, as no significant interaction with days was found.

Unlike the magnitude of responding (attempts), the direction of attempted dodges was not significantly affected by callosotomy, either in response to ipsilateral approaches (Fig.

5.3A) or contralateral approaches (Fig. 5.3C, $p > 0.05$ in each case). In other words, unilaterally lesioned rats, with callosal section or not, predominantly follow their lesion-induced motor bias toward the side of lesion, when dodging approaches from either side. Thus dodging direction is predominantly 'normal' for contralateral approaches, but 'abnormal' for ipsilateral approaches.

To summarize, callosal section affects only the magnitude of attempts to respond to stimuli from either side of space, but has no effect on the direction of responses once initiated.

5.3.3 *Callosotomy effects on edge orientation behavior*

It can be seen from Fig. 5.4A that animals with unilateral 6-OHDA lesions alone (SHAM CC), demonstrated a bias in edge investigation significantly different from zero ($t_8 = 4.16$, $p < 0.01$). This behavioral bias was not evident in rats which also received callosal section, as this measure no longer differed from zero ($p > 0.05$). Consequently, the two study groups differed significantly in direct comparison ($t_{15} = 5.67$, $p = 0.006$).

Fig. 5.4B reveals that the total time or magnitude of lateralized edge investigation (ipsilateral + contralateral time) did not differ between groups ($p > 0.05$, t-test). Therefore, in contrast to dodging behavior where the magnitude, but not the direction of the motor response was reduced by callosal section, the more 'sensory' bias of spontaneous edge investigation was affected in direction, but not in magnitude by callosotomy.

5.3.4 *Callosal section and drug-induced turning behavior*

All fullturns induced by apomorphine or amphetamine were unidirectional, contraversive and ipsiversive respectively. The unidirectional nature of drug-induced turning

was not altered by callosal section. However, as seen in Fig. 5.5, the magnitude of apomorphine-induced turning behavior was reduced by almost half in callosal sectioned rats ($t_{15} = 2.7$, $p = 0.029$). In contrast, ipsiversive turning in response to amphetamine did not differ between groups ($p > 0.05$).

5.3.5 *Role of corpus callosum in spontaneous motor activity*

The effect of callosal section on spontaneous motor activity in response to a novel environment (activity monitors) is shown in Fig. 5.6. There was no significant main effect of Group across time samples for either distance travelled ($F_{1,15}$= .06, n.s.) or number of locomotor bouts ($F_{1,15}$= 1.77, n.s.). As expected, a highly significant effect of time sample was found for both distance ($F_{11,165}$= 78.6, $p < 0.001$) and locomotor bouts ($F_{11,165}$= 12.2, $p < 0.001$), as activity declined over the 1 hr. test period. As well, there was a significant Group x Sample interaction for distance travelled ($F_{11,165}$= 2.03, $p = 0.028$) but not number of locomotor bouts, as rats with callosal section tended to be hyperactive relative to sham sections between the 15 and 40 min. sample periods. This trend was only significant on the 25 min. time sample ($p < 0.05$, t-test).

It is apparent from the present results that in rats with unilateral 6-OHDA lesions of the substantia nigra, splitting of the corpus callosum does not impair either the extent of, or the initiation of, spontaneous motor activity; if anything, callosotomy tends to increase activity.

5.3.6 *Relationship between attempts to dodge and edge asymmetry*

It was found that the manner in which unilaterally lesioned rats align with openfield edges, is related to the ability to initiate responses in the dodging paradigm. Fig. 5.7 shows

that rats with the most dodging attempts (to contralateral approaches in this case) were those who showed the most asymmetrical edge behavior in the direction expected for unilaterally lesioned rats.

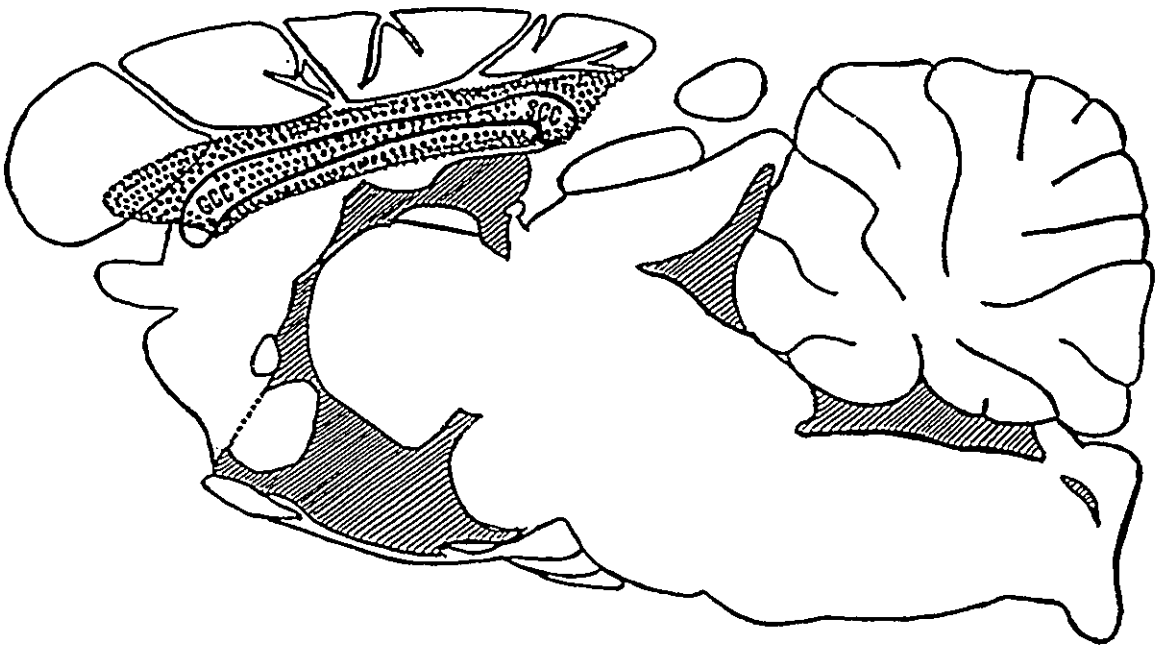


Fig. 5.1. Midsaggital (+ 0.1 mm) representation of a typical corpus callosum-sectioned rat. The extreme anterior, ventral tip of the callosum was spared in 7 of 8 rats included in the analysis. Adapted from the atlas of Paxinos and Watson (1982). GCC - genu corpus callosum, SCC - splenium corpus callosum.

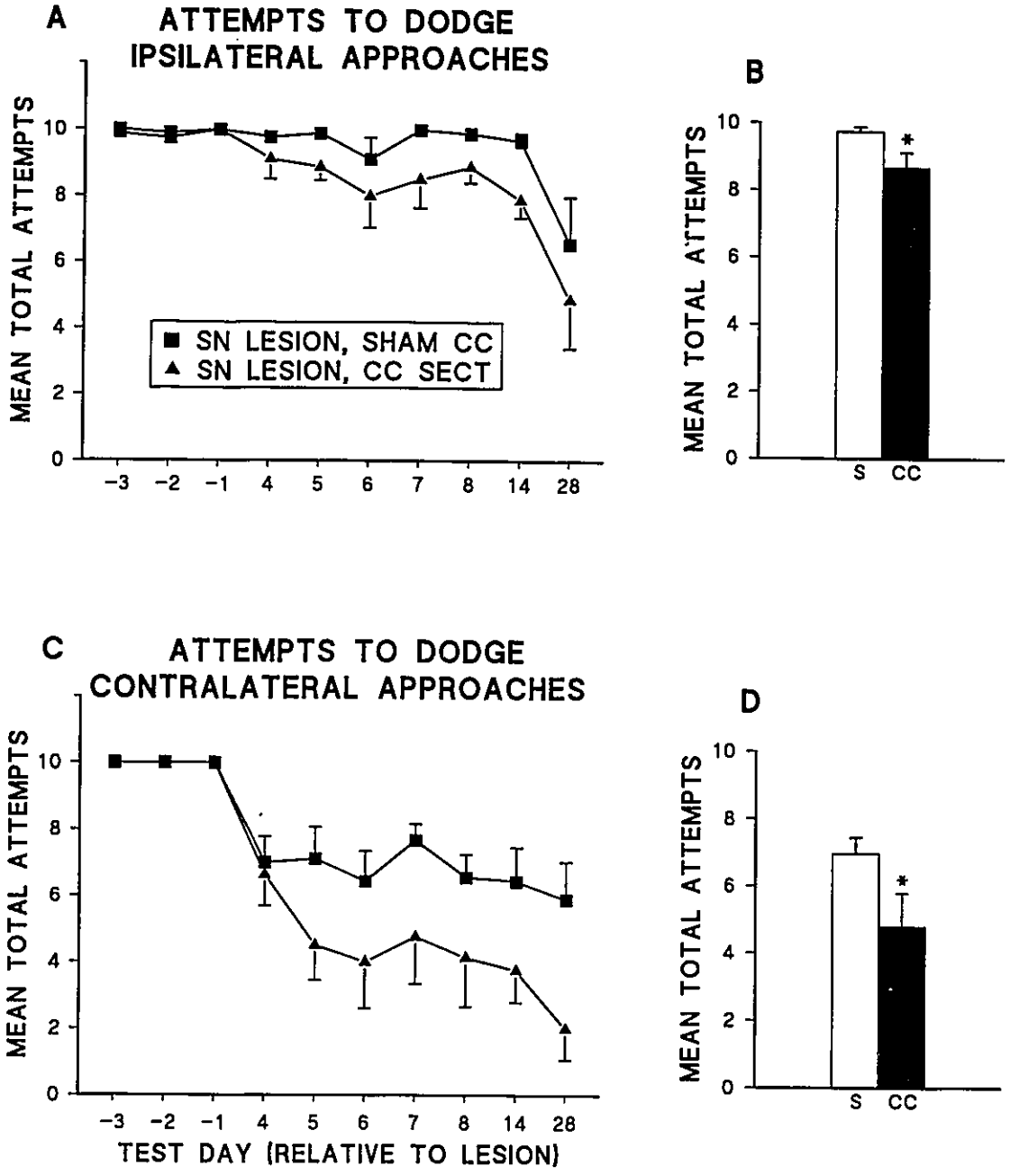


Fig. 5.2. Effect of corpus callosum section on attempts to dodge in rats with unilateral 6-OHDA lesions of the substantia nigra. Figs. **A** and **C** show the number of attempted dodges across test days, in response to ipsilateral and contralateral approaches, respectively. Figs. **B** and **D** depict the mean total dodging attempts collapsed across post-lesion test days, for ipsilateral and contralateral approaches, respectively. Data are expressed as group means (± S.E.M). Sectioning of the corpus callosum significantly reduced the ability of unilaterally lesioned rats to respond to dodges from either side of the lesion (see text for statistical details). The reduced responding to contralateral approaches by both groups (C), is typical of the unilaterally lesioned rat. Abbr. S - sham corpus callosum, CC - corpus callosum section, * $p < 0.05$ between groups.

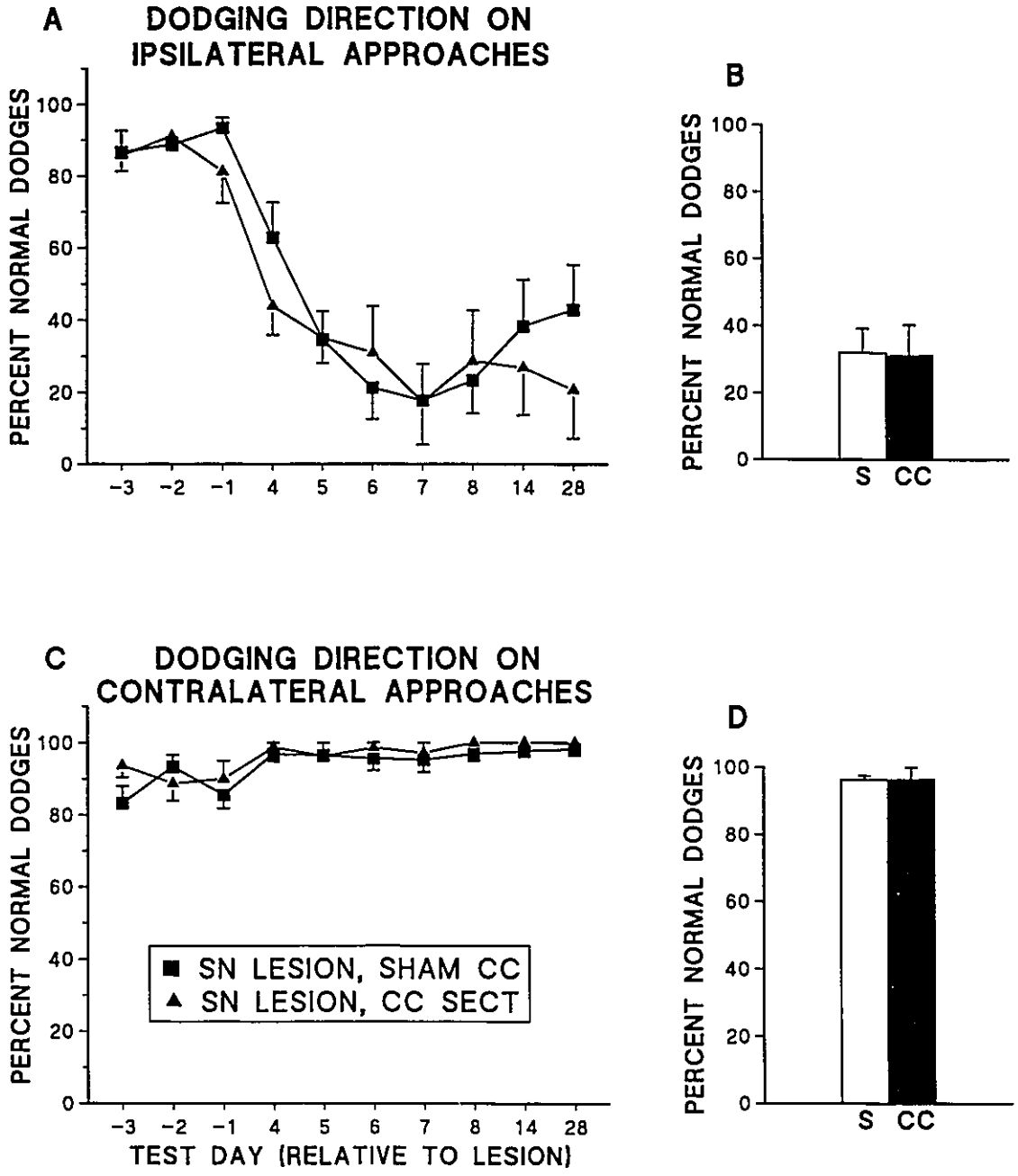


Fig. 5.3. Effect of corpus callosum section on direction of dodges in rats with unilateral 6-OHDA lesions of the substantia nigra. In contrast to attempts to dodge, the direction of attempted dodges was completely unaffected by callosotomy, on approaches from either side of the lesion. The direction of dodges is represented by the percentage of attempted dodges in the 'normal' direction, ie. away from the robber. Figs. **A** and **C** show the percentage of normal dodges across test days, in response to ipsilateral and contralateral approaches, respectively. Figs. **B** and **D** summarize the group data collapsed across post-lesion test days. The post-lesion decline in normal dodges to ipsilateral approaches by both groups (A), is typical of the unilaterally lesioned rat, as it shifts to abnormal dodges after lesioning, thus following its lesion-induced motor bias. Abbr. S - sham corpus callosum, CC - corpus callosum section, * $p < 0.05$ compared to SHAM CC.

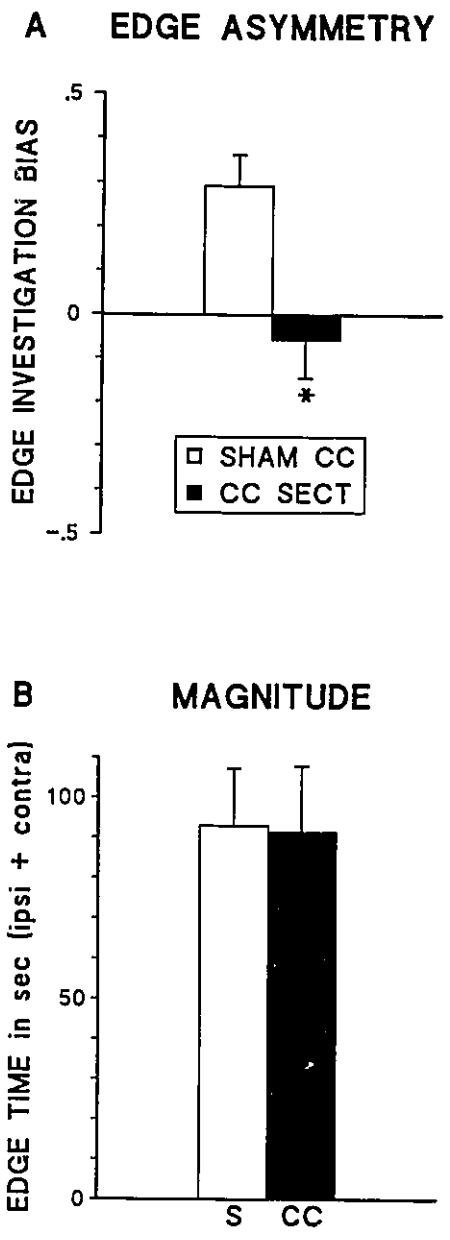


Fig. 5.4 Callosotomy effects on orientation to edges in unilaterally lesioned rats. Fig. **6.4A** demonstrates that while rats with only the unilateral SN lesion (SHAM CC) show a characteristic asymmetry in edge bias with the intact striatum contralateral to the edge (see Chp. 2), this asymmetrical behavior was completely abolished in the callosal section group (CC SECT). In Fig. **6.4B**, the total amount of lateralized edge investigation (ipsilateral + contralateral) is plotted, and demonstrates that the groups were indistinguishable on this measure. It is thus seen that while the total time of lateralized edge investigation is not influenced by the corpus callosum, the directional distribution of edge investigation is markedly affected by callosotomy. Abbr. S - sham corpus callosum, CC - corups callosum section, * $p < 0.05$, relative to shams.

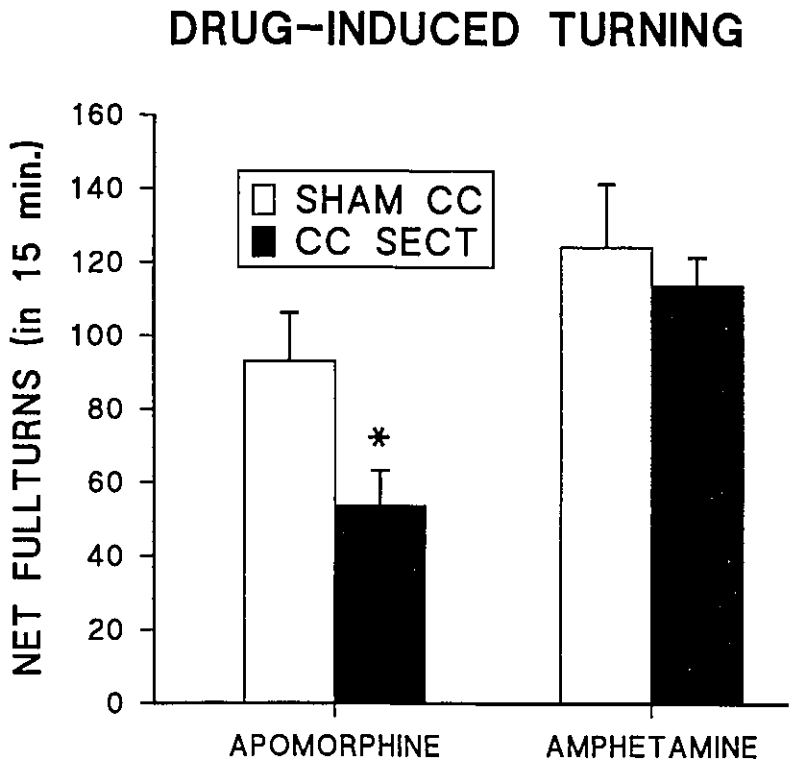


Fig. 5.5 Effects of callosal section on drug-induced turning. In rats with unilateral 6-OHDA lesions of the substantia nigra, collosal section was found to significantly reduce the magnitude of turning behavior in response to apomorphine, but not amphetamine. * $p < 0.05$ relative to shams.

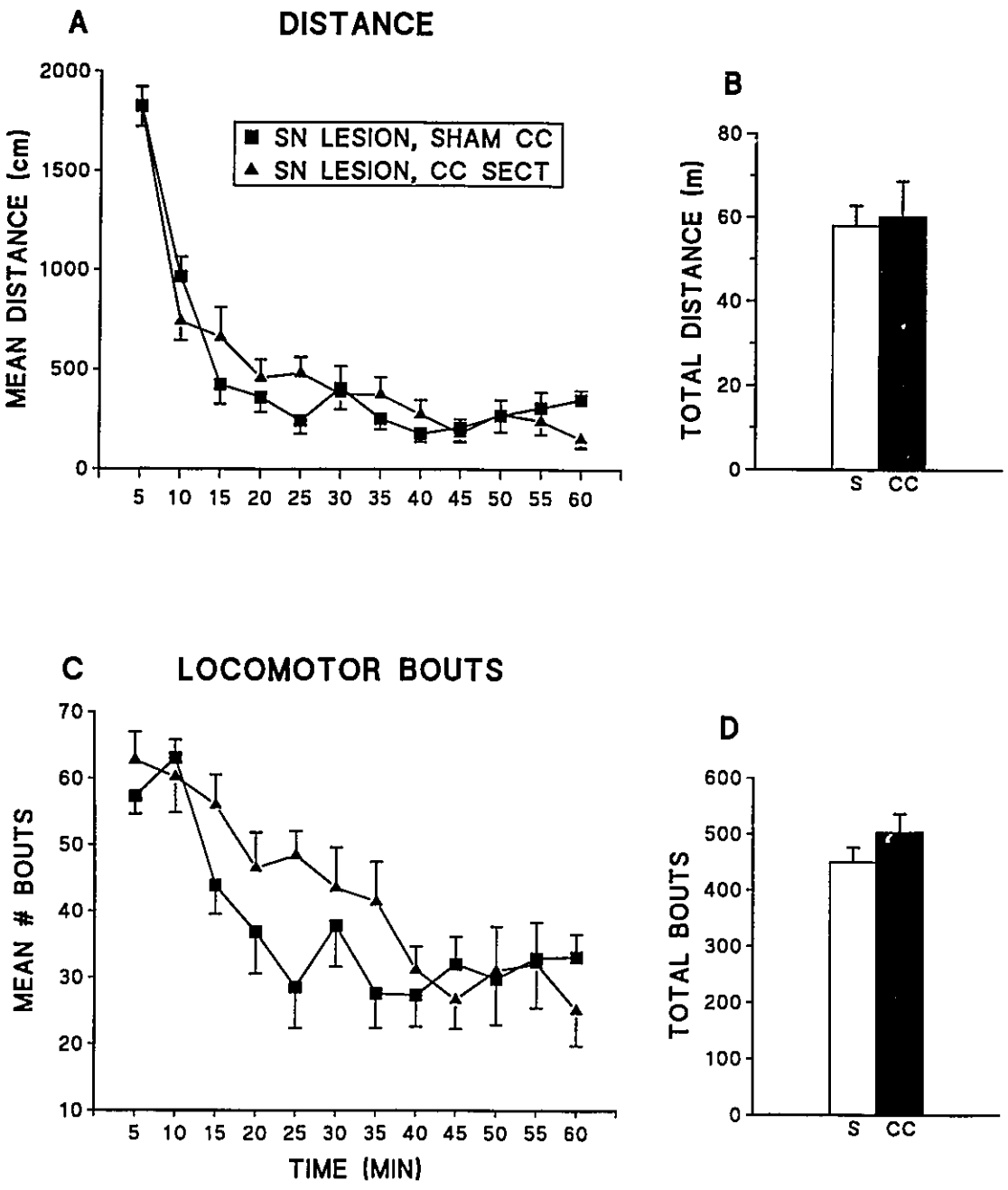


Fig. 5.6 Spontaneous motor activity and effect of callosotomy. Figs. **A** and **C** show the time course of activity across the 1 hr. test period in automated activity monitors, for both distance travelled (A) and number of distinct movements (C). Figs. **B** and **D** summarize the total group data collapsed across time samples. No significant main effect for group was observed across the sampling period for either dependent measure, despite a trend for hyperactivity by callosotomized rats between 15 and 40 minutes (see text for statistical details).

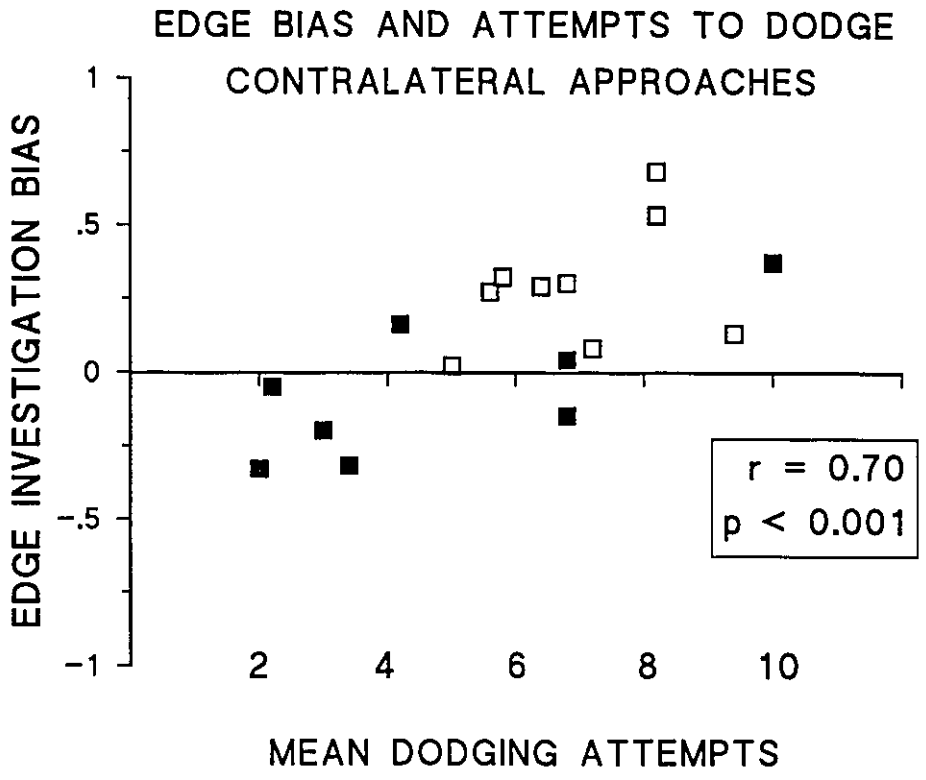


Fig. 5.7 Attempts to dodge and edge investigation bias in unilaterally lesioned rats. A significant relationship was observed between these measures of edge behavior and dodging behavior. Positive values for edge investigation bias indicate that the nonlesioned striatum is predominantly contralateral to the edge, which is the expected consequence of a unilateral 6-OHDA lesion of the substantia nigra (see Chapter 2 results). Open squares represent sham sectioned rats and closed squares represent corpus callosum sectioned animals. Mean dodging attempts are collapsed across the first week of post-lesion tests.

5.4 Summary and discussion

The present results suggest that transcallosal communication is necessary for the expression of the edge investigation bias induced by a unilateral DA lesion, but not for the other directional biases present (direction of dodging or drug-induced turning). However, the magnitudes of apomorphine-induced turning and dodging attempts are modulated by callosal pathways. The differential effects of callosotomy suggest that the behavioral asymmetries induced by a DAergic imbalance are dissociable, consistent with findings from previous studies (Glick, et al., 1979; Pisa and Szechtman, 1986; Ziegler and Szechtman, 1988b).

In regard to the dodging experiment, the prediction of a decreased percentage of normal dodges following callosotomy was clearly refuted, as the direction of dodging behavior was completely unaffected by callosal section. In other words, if the contralateral hemisphere (cortex) perceives the approaching rat and initiates the dodge, transcallosal pathways to the opposite striatum are not necessary for the animal to turn away from the robber. Nonetheless, the lack of effect on direction of dodging eliminates a potential route of information flow between stimulus perception and direction-specific turning, and supports the suggestion of Whishaw and Tomie (1988) that "each hemisphere functions to direct orienting movements contralaterally and dodging movements ipsilaterally". This also implies that even if the 'executive decision' to dodge originates in the cortex, the neural circuits mediating the choice of direction are either unilateral or subcallosal.

The finding of a bilateral impairment in initiation of a rapid response (dodging attempts) may reflect a specific effect of callosotomy. First, it is unlikely that the reduced

dodging attempts in CC sectioned rats were due to a generalized motor impairment. Sectioned animals were highly vigorous throughout testing, and in the activity monitors even tended toward hyperactivity (Fig. 5.6). This may not be surprising since callosal size is reported to be negatively correlated to openfield activity in intact rats (Zimmerberg and Michus, 1990). As well, children with attention deficit-hyperactivity disorder, have been shown to have a smaller corpus callosum than nondisabled controls, as measured with magnetic resonance imaging (Hynd et al., 1991).

Second, it is unlikely that the response impairment in callosal-sectioned rats is due to disruption of the necessary sensory input to perceive the robber. As reviewed in section 1.1.3, unilateral 6-OHDA lesions do not impair stimulus perception *per se* (Hoyman et al., 1979; Carli et al., 1985; Brown and Robbins, 1989). Callosal section should also not alter stimulus perception, as the dominant sensory modality required for dodging is via the vibrissae (Whishaw, 1988), and input from the vibrissae reaches its contralateral cortical receiving area subcallosally (Huston et al., 1990). The response impairment following callosotomy would then appear to derive from the reduced ability to use sensory information for bilaterally coordinated motor responses.

The finding that ability to dodge is related to the expression of sensory bias in openfield edge investigation, and that both are mediated by the callosum, suggests possible common mechanisms. Supporting this interpretation, both dodging attempts and edge asymmetry of the callosotomized rat which showed some sparing of the splenium (posterior callosum), both its dodging attempts and edge asymmetry were comparable to those of rats with an intact callosum (Fig. 5.7). In rats, the splenium may be sufficient to mediate

exchange of a variety of external sensory inputs required for a motor response, as suggested by studies of interocular transfer of visuomotor behavior (Mohn, 1984; Mohn and Russell, 1981).

The present results suggest that in rats with a 6-OHDA lesion of the substantia nigra, the corpus callosum maintains an asymmetry in sensory responsiveness to the openfield edge. This may be adaptive in the sense that the nonlesioned hemisphere, which is fully capable of responding to its sensory inputs, becomes dominant in guiding behavior and exploring the environment. Reduction of interhemispheric communication by callosotomy may lead to redundancy or inefficient coordination of function (as in ability to dodge). A similar adaptive role has been shown for the corpus callosum by Crowne et al. (1988), who demonstrated that the recovery from neglect following unilateral cortical lesions, is mediated by the callosum.

Callosotomy was not found to affect the direction of drug-induced turning, however it reduced the magnitude of apomorphine turning. One study found callosotomy to increase amphetamine turning behavior, but this study was not with unilaterally lesioned rats, and only the anterior portion of the callosum was severed (Glick et al., 1975). Another study found that callosotomy did not abolish contraversive turning following apomorphine in unilaterally lesioned (6-OHDA) rats, but did not compare the extent of turning of callosotomized rats with that of animals having unilateral 6-OHDA lesions but no callosotomy (Steiner et al., 1985). The present results suggest that callosotomy does not affect ipsiversive turning, in response to either amphetamine administration or approaching robbers in the dodging test. Instead, callosotomy altered apomorphine-induced contraversive turning which is dependent on DA receptor supersensivity. Striatal DA receptor

supersensivity is modulated by the prefrontal cortex (Herve et al., 1989), and given that many of the corticostriatal projections are crossed projections (McGeorge and Faull, 1989), callosotomy may have interfered with neural circuits which normally facilitate the compensatory upregulation of DA receptors following a unilateral 6-OHDA lesion.

An additional interpretation of the present results is also possible. That is, the selective effect of callosotomy on the direction of edge investigation, and not on the direction of turning, may reflect the ability of callosal section to affect asymmetrical behaviors primarily reflected in head movements (edge behavior), but not whole body postural adjustments as in turning behavior, at least in the unilateral 6-OHDA rat with a strong (postural) motor asymmetry. A similar distinction between neural circuits mediating these two types of movements has been made previously (Whishaw and Tomie, 1988; Mintz and Knowlton, 1993).

The present findings demonstrate clearly that the corpus callosum mediates some, but not all of the behavioral asymmetries resulting from unilateral DAergic depletion. As these tests focused primarily on motor and sensory asymmetries, it is of interest to review the role of the corpus callosum in other types of reported asymmetries, particularly those related to emotionality/stress, learning performance or hyperactivity.

Callosal sectioning has been found to release or potentiate the expression of lateralization of emotionality to the right parietal cortex, as reflected in openfield activity (Crowne et al., 1987; Maier and Crowne, 1993). It was previously described that the spontaneous emotional behavior of muricide in handled rats was lateralized to the right cortex, and this behavioral asymmetry is also potentiated by callosal section (Denenberg and

Yutzey, 1985; Denenberg et al., 1986). Such findings have led to suggestions that for processes relating to emotionality or arousal, the left cortex exerts interhemispheric inhibition over the right (Denenberg, 1981; Denenberg et al., 1986) via the corpus callosum. In a study showing an asymmetry in water maze performance, with right parietal lesions being more disruptive than left lesions, callosotomy was found to produce additive impairments in performance for either left or right lesions, actually reducing the left/right asymmetry (Crowne et al., 1992). Finally, the asymmetry in hyperlocomotion following a variety of unilateral frontal lesions, and specific to right brain lesions, has been reported to be completely unaffected by callosal section (Dewberry et al., 1986), suggesting that at least this behavioral asymmetry is not a function of interhemispheric inhibition or interaction, as proposed by Denenberg (1981).

This divergence of findings on the role of the callosum in behavioral asymmetries reflects the general principle that different asymmetries are organized along different dimensions in both rats and humans (Glick et al., 1979, Pisa and Szechtman, 1986; Camp et al., 1984). This is also consistent with the results of chapter 2, in that the asymmetries examined are essentially independent and dissociable.

CHAPTER 6: SUMMARY AND GENERAL DISCUSSION

6.1 Summary of findings

The present series of experiments revealed population left/right hemispheric asymmetries for some of the brain processes modulated by DAergic systems, but not for others.

Behavioral tests in rats with unilateral striatal DA depletion failed to demonstrate population hemispheric asymmetries for motor activation, either measured as spontaneous locomotor activity or drug-induced turning behavior. Similarly, sensory/spatial responsiveness, as measured by orientation to edges of an openfield, showed no evidence of left/right hemispheric asymmetry at the population level. However in the same animals, a hemispheric asymmetry emerged in spatial learning ability in the water maze, as right brain lesions caused greater impairments in reversal performance than did left brain lesions. A follow-up study with this paradigm using intact animals distinguished by the direction of turning behavior, further supported the suggestion that right brain DAergic mechanisms preferentially facilitate performance in this task.

A final test of population left/right hemispheric asymmetry focused on the role of the mesocortical DA system in response to stress. It was found that while left or bilateral cortical DA depletion tended to worsen stress-induced pathology relative to stressed shams, right cortex lesions alone resulted in pronounced increases in pathology compared to stressed shams. Each of the three lesion types was associated with unique changes in subcortical DA systems, with right cortex lesions affecting the striatum, while left and bilateral lesions

variously altered amygdala and nucleus accumbens DA function. The cortex lesions were also associated with unique disruptions in the patterns of correlated DA activity among DA terminal regions. These findings not only demonstrated that cortical mechanisms under DAergic modulation are functionally asymmetric in terms of coping with stress, but that the nature of cortical/subcortical interactions involving DA systems is also highly asymmetric.

The final study examined interhemispheric communication in the expression of behavioral asymmetries. Specifically, the effect of corpus callosum section was tested in rats with unilateral substantia nigra (6-OHDA) lesions, for the expression of three asymmetric behaviors characteristic of these rats. Employing the "dodging" paradigm of Whishaw (1988), it was found that callosotomy reduced the magnitude of response attempts (with no impairment of spontaneous motor activity) while having no effect on the directional distribution of attempted dodges. In contrast, the directional asymmetry in sensory/spatial investigation of edges described in chapter 2 was completely abolished by callosal section, with no effect on the magnitude (ie. total time) of lateralized edge investigation. Finally, the direction of drug-induced turning behavior following amphetamine or apomorphine was unaffected by callosal section, although the magnitude of apomorphine-induced turning was significantly reduced by callosotomy. These results further characterized and dissociated some of the behavioral asymmetries mediated by DAergic systems, and suggested that while the direction of turning behavior is not altered by callosotomy, asymmetries in edge behavior are maintained by the corpus callosum.

6.2 General discussion and theoretical implications

Population hemispheric asymmetries were not observed in the present experiments for measures of motor activity, turning behavior or sensory/spatial responsiveness to edges, indicating equivalent roles of left and right nigral DA systems in these processes. As discussed in chapter 2, numerous reports of population asymmetries have been described for turning behavior and spatial or side preferences in a variety of behavioral situations. However, the emergence of such biases, either in terms of direction or magnitude, may require the intervention of other factors which vary across studies, including strain or sex differences, prenatal experience, early handling or stress as adults (eg. Carlson and Glick, 1989; Fride and Weinstock, 1988; Denenberg, 1981; Carlson et al., 1987). One of the best such examples is an experiment in which rats were first tested for the direction of amphetamine rotation, subjected a week later to a session of uncontrollable footshock and retested for amphetamine rotation. Male rats which initially rotated to the left showed a potentiation of this behavior following stress, while those which initially rotated to the right reversed their endogenous turning preference to the left (Carlson et al., 1987). These findings suggest that the stress resulted in a rightward shift in cerebral DA function in all animals (at least in males), which translated behaviorally as increased turning toward the left. This implies that a) endogenous behavioral asymmetries are highly modifiable and dependent upon experiential history and b) asymmetries in stress/arousal processes may be more fundamentally consistent in direction, than the asymmetries which they modify.

The behavioral paradigms in the present studies which did reveal hemispheric asymmetries, were the Morris water maze and cold restraint stress, each of which

incorporates a substantial arousal component. In each case, the data suggested that DAergic mechanisms in the right brain are especially important in the adaptive processes associated with these situations.

Lesion studies in animals describing asymmetrical effects in the expression of emotionality or response to stress, have consistently implicated a dominant role for the right hemisphere (for reviews see Bianki, 1988; Denenberg, 1981). As discussed in section 4.4 however, it has been shown in intact animals that a sequential left to right shift in cortical DA activity is associated with stressful conditions, with mild or initial stress favoring left cortical activation, and intense or prolonged stress favoring right cortical activation (Carlson et al., 1988, 1991, 1993). From the present results, it appears that both the restraint stress procedure and the water maze task were sufficiently stressful in nature, so as to be preferentially affected by right brain DAergic depletion. This would seem more obvious for the 3 hr cold restraint procedure than for the water maze task. In retrospect however, the required response of locating a small submerged platform in a large pool of water was probably quite stressful, particularly on the first day of testing or reversal when the sense of control would be least (and when group differences tended to be most evident).

The above interpretation also implies that there should be behavioral situations in which *mild* stress or arousal is preferentially associated with left brain DAergic systems. In one study, rats were subjected to the mildest intensity of shock which would elicit an escape response, the latter being movement to either of the open arms of the T-maze. A rightward population bias (indicative of left brain DA dominance) was observed in the direction of escape (Fitzgerald et al., 1990). Another study with rats demonstrated the same result with

mild shock, as well as showing the same rightward bias in the direction of turning when suspended briefly by the tail (Castellano et al., 1989). Further support for the lateralized effects of stress on DAergic function, is the finding that 24 hr of food deprivation increases rightward turning behavior following amphetamine, but 48 hr of food deprivation increases leftward turning (Carlson et al., 1988). Such a functional shift parallels the finding with restraint where the initial response (15 min) of mesocortical DA activation favors the left brain, and later (1 hr) shifts to a right brain bias (Carlson et al., 1991). These findings also suggest that the more intense and/or uncontrollable the stressor, the faster such a directional activity shift takes place.

A broad implication of this view for normal behavior is that while the right brain may be dominant in perceiving or responding to highly arousing states, the left brain may functionally inhibit such right brain processes by dealing with small stressors before they become big stressors. Conceptually similar is the proposal by Denenberg (1981) that the (intact) left brain suppresses the emotional expression of the right brain by some form of interhemispheric inhibition. This conclusion was based on unilateral cortical ablation studies and later confirmed in split-brain rats which showed an exaggeration of lateralized emotional expression (Denenberg et al., 1986).

If the left cortex is preferentially involved in the inhibition of emotional expression as suggested by Denenberg, then it is possible that imbalances in DA function at the population level, could contribute to such lateralized specialization. In intact rats, the content of DA and/or DOPAC has been reported to be greater in the left than in the right medial prefrontal region (Carlson et al., 1988, 1993; Slopsema et al., 1982), although one study

reported higher DA content in the right medial prefrontal cortex (Rosen et al., 1984). In our lab, we have observed a significant left cortical bias in DA content of intact animals, not only in the chapter 2 results, but also in previous published (Sullivan and Szechtman, 1994) and unpublished experiments. (This finding was absent however in the chapter 4 results). Additionally, DA release in medial prefrontal cortex following systemic injection of amphetamine or cocaine has been shown to be more pronounced in the left hemisphere than in the right (Maisonneuve et al., 1990). A left brain DAergic dominance is also consistent with the reports of population biases in turning behavior, which have been predominantly rightward (Glick and Ross, 1981a; Fitzgerald et al., 1990; Castellano et al., 1987, 1989).

While findings suggestive of left brain DAergic dominance in intact animals are not universal, they do appear much more commonly than reports of right brain DAergic dominance, suggesting the possibility of an adaptive neurochemical asymmetry at the population level. Given the role of cortical DA in facilitating coping ability generally (Claustre et al., 1986; Scatton et al., 1988; and present results), and the preferential activity of left cortical DA systems upon initial exposure to stress (Carlson et al., 1988, 1991), such an asymmetry could conceivably reflect a lateralized specialization for the rapid management of minor stressors. Finally, and possibly related, is the finding that glucose utilization in normal rats is greater in the left than right frontal cortex (Glick et al., 1979).

In humans, an interesting interpretation of left/right specialization has been presented which is of relevance in this context. It has been elaborately proposed by Tucker and Williamson (1984) that the left brain is dominant for activation, and the right brain for arousal. In brief, the left brain activation system was said to maintain a state of motor

readiness and predominate in the ordered, sequential organization of behavior. This specialization was suggested to be the result of asymmetries of DA function favoring the left brain in normal humans. On the other hand, the arousal system of the right hemisphere was suggested to be preferentially related to noradrenergic function.

Asymmetries of arousal processes in humans have frequently been noted, particularly those processes involving the autonomic nervous system (eg. Gainotti, 1987; Meadows and Kaplan, 1994). One study of patients with comparable damage to the left or right hemisphere examined skin conductance levels as a measure of autonomic activity in response to neutral or unpleasant ('emotional') slides. Patients with right brain damage showed greatly *reduced* autonomic responses for either slide type relative to controls, while left brain damaged patients showed *increased* autonomic activity relative to controls, in the baseline or neutral condition (Meadows and Kaplan, 1994). These and other results lead the authors to suggest that although all patients could cognitively assess the emotional nature of stimuli, those with right brain damage can neither "generate nor receive the appropriate autonomic responses and feedback inherent in normal emotion" (Meadows and Kaplan, 1994). Such asymmetrical integration was suggested to be specific to arousal which was cortically mediated. Another interpretation of these findings (in addition to a right hemisphere dominance for autonomic activation) is that the *heightened* autonomic activity seen following left hemisphere damage, is due to disinhibition of right hemisphere mechanisms, in a manner similar to that proposed by Denenberg (1981) for rats.

A possible physiological basis for cerebral dominance in arousal/emotionality processes mediated by the autonomic nervous system (in humans or lower animals), was

suggested by Geschwind (1985). He pointed out the possibility that the striking asymmetries in peripheral innervation, especially by the vagus, may be paralleled by asymmetrical central representations. If left-biased visceral innervation, particularly for stomach and heart, is related to a right-biased cortical representation, then the most relevant cortical region in this regard may be the region sampled in the present thesis, which has been characterized as a vagal sensorimotor field (Cechetto and Saper, 1990).

While detailed studies of cerebral asymmetries in autonomic control in the rat are lacking, the present asymmetrical regulation of stress ulcer development (which is vagally mediated) suggests that parallels to the human asymmetry may exist in rats. Assuming such parallels, it may seem paradoxical to propose that the right cortex is preferentially associated with autonomic functions or highly stressful states, whereas the left cortex predominates in the initial response to stressful situations. However, any salient stimuli are likely to be initially perceived bilaterally through the relevant sensory pathways, and where appropriate, experience-based memories which would aid in coping responses may be stored or accessed bilaterally. Either hemisphere could thus have the *potential* to respond to the situation. It would then be possible for a left brain bias to emerge for initial responding, if that hemisphere is inherently predisposed for motor readiness as proposed by Tucker and Williamson (1984) for humans, or if left brain biases in (cortical) DAergic systems exist at the population level as suggested earlier for rats. Despite a left biased asymmetry in initiation of coping responses, the right brain could still predominate in *experiencing* the emotional impact of the situation as it progresses, thus providing helpful feedback for continued coping strategies. In cases where the stress is perceived as uncontrollable,

pathological outcomes may become manifest.

6.3 Implications for human pathology

The most direct implications of the present findings (and other animal data cited) for human pathology, may be for those conditions in which both stress and laterality appear to be involved. In terms of psychopathology, the two most notable examples may be depression and schizophrenia.

Regarding depression, which is generally associated with vulnerability to stress, electroencephalographic (EEG) studies have shown that while nondepressed subjects exhibit a left brain bias in frontal cortical activity, depressed subjects demonstrate the opposite pattern, ie. exaggerated EEG acivity in right frontal cortex (Schaffer et al., 1983; Davidson and Tomarken, 1989). Such findings parallel those found in infants where frontal EEG asymmetries favoring the left or right, are reliably associated with approach vs. withdrawal behaviors respectively (Davidson, 1992). Interestingly, and possibly related, is a recent finding in rats which showed that the levels of DA metabolites in the left prefrontal cortex are positively correlated with self-administration of the euphoriant cocaine, while levels in the right prefrontal cortex were negatively correlated with self-administration behavior (Glick et al., 1994). In the case of human depression, the right frontal activity bias could represent a hyperfunctional state of the intrinsic (right) cortical neurons or possibly reflect impaired inhibitory modulation of cortical neurons which may normally process stress-related inputs. In light of the particularly pathological effect of right cortical DA depletion (chapter 4), it is also of interest that depressives have been reported to exhibit a higher than

expected incidence of peptic ulcer disease compared to the general population or groups with other mental illnesses (Gosling, 1958).

As reviewed in section 1.1.6, schizophrenics exhibit a number of lateralized abnormalities. Current theories suggest that frontal cortical deficits in schizophrenics may give rise to subcortical dysregulation, particularly in relation to DA systems and thus contribute to symptomatology (Weinberger, 1987; Weinberger et al., 1992; Davis et al., 1991; Deutch, 1992; Grace, 1991; Seeman, 1993). The asymmetrical effects presently described for cortical/subcortical interactions in DA systems suggest that if cortical deficits are a feature of psychosis, then the lateralized nature of those defects may significantly influence a) which subcortical structures are additionally affected b) the resulting symptomatology and c) the extent to which symptoms may be exacerbated by stress.

There are also some specific points of interest regarding the potential role of the amygdala in psychosis. The present combination of bilateral cortical DA depletion and stress, reproduced a neurochemical feature of schizophrenic brains, namely a selective increase in DA content in left amygdala (Reynolds, 1983). It has also been noted that schizophrenic brain pathology is predominantly left-sided (Crow et al., 1989; Flor-Henry, 1989; Seeman, 1993). It may be of further interest that in both of the present groups receiving left brain lesions (left and bilateral groups), alterations in amygdala DA systems were observed. Given that the amygdala plays a key role in psychic/perceptual phenomena and emotional experience in humans (Gloor et al., 1981; Adolfs et al., 1994), it is possible that the amygdala may be a significant locus for the perceptual delusions and emotional inappropriateness so often typical of psychosis.

In addition to the above implications for psychopathology, the existence of cerebral asymmetries in rats has more general implications for health and (patho)physiology. It was previously mentioned that very brief daily handling during preweaning greatly facilitates the development of functional lateralization, particularly for emotion-related processes (Denenberg, 1981). This same manipulation, which was said to be more typical of natural development than that in artificial laboratory settings, produces significant longterm physiological effects. For instance, early handling has been shown to permanently upregulate hippocampal glucocorticoid receptors and prevent age-related loss of feedback regulation of stress hormones. This treatment also prevented both hippocampal neuronal cell loss and cognitive impairments associated with aging (Meaney et al., 1988). While these findings do not establish a causal link between lateralized emotional processing and a permanent facilitation in the regulation of neuroendocrine stress responses, the two are clearly associated and triggered by the same developmental stimulation.

These findings with early handling raise the possibility that if lateralization is related to more effective stress control, then disturbances of lateralized function may have consequences for a wide variety of pathologies in which stress (or stress hormones) plays a role. Such a possibility has recently been suggested in a human study (Wittling and Schweiger, 1993). Using a sophisticated technique for lateralized task presentation, subjects were assessed for the direction and magnitude of hemispheric control of cortisol secretion. While the most common pattern is for right brain control of cortisol secretion, it was found that subjects with strong left brain dominance for secretion control had a significantly higher degree of physical complaints derived from a wide variety of body systems.

It has been observed for some time that anomalous patterns of cerebral dominance in humans are associated with unusually high incidence of several abnormal conditions such as dyslexia, atypical drug responses and a variety of immune disorders (Geschwind, 1985). In regard to immune disorders, it has recently come to light that the cerebral cortex of rodents exerts highly asymmetrical effects on a number of immune parameters. Most of these studies which were performed with mice, suggest that the direction of such asymmetrical control depends on the particular immune measure employed, and frequently also depends on sex and paw preference (for review, see Neveu, 1992). In rats, it has been reported that the left neocortex is primarily associated with immunopotentiation, while the right neocortex is related to immunosuppression. The prefrontal cortex was said to be particularly associated with immune reactions (Vlajkovic et al., 1994).

Final comment

Animal studies have begun to have a major impact on the understanding of the biological bases of cerebral dominance. It is now clear that lateralization of function is not unique to the human realm as once thought, but is widespread in the animal world.

The present studies, along with several others with rats, suggest that those processes most closely related to stress may be among the most fundamental to exhibit hemispheric specialization. Considering the apparent parallels with human asymmetries of this nature, and the role of stress and laterality in numerous pathologies, it is suggested that the most fruitful future studies of cerebral lateralization in animals should concentrate on further defining the asymmetrical control of autonomic and neuroendocrine mechanisms. Similarly,

based on recent findings, there is much potential for gaining insight into neural-immune interactions, or the relationships between stress and immunity, by incorporating the study of hemispheric asymmetry into experimental designs. Additionally, the findings of asymmetrical regional interactions between brain structures, emphasize how little is really known of how the brain functions as an integrated whole. It will be necessary to investigate such interactions in detail, preferably with highly sensitive *in vivo* techniques, and in a variety of behavioral situations, so that a more accurate picture of integrated brain function may emerge.

It is hoped now, as it has been in the past (Glick and Ross, 1981b; Geschwind, 1985), that the careful examination of the nature and significance of asymmetries in animals, will lead to insights of much value in the understanding of both normal and abnormal brain function in humans.

REFERENCES

Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S. and M.J. Zigmond (1989). Differential effect of stress in *in vivo* dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J. Neurochem. 52:1655-1658.

Adolfs, R., Tranel, D., Damasio, H. and A. Damasio (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature 372:669-672.

Afonso, D., Santana, C. and M. Rodriquez (1993). Neonatal lateralization of behavior and brain dopaminergic asymmetry. Brain Res. Bull. 32:11-16.

Alexander, G.E., DeLong, M.R. and P.L. Strick (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex, Ann. Rev. Neurosci. 9:357-381.

Altar, A., Neve, K.A., Loughlin, S.E., Marshall, J.F. and J.H. Fallon (1983). The crossed mesostriatal projection: neurochemistry and developmental response to lesion. Brain Res. 279:1-8.

Amalric, M. and G.F. Koob (1987). Depletion of dopamine in the caudate nucleus but not in nucleus accumbens impairs reaction-time performance in rats. J. Neurosci. 7:2129-2134.

Anden, N.E., Carlsson, A., Dahlstrom, A., Fuxe, K., Hillarp, N.A. and K. Larsson (1964). Demonstration and mapping out of nigro-neostriatal dopamine neurons. Life Sci. 3:523-530.

Annett, L.E., McGregor, A. and T.W. Robbins (1989). The effects of ibotenic acid lesions of the nucleus accumbens on spatial learning and extinction in the rat. Behav. Brain Res. 31:231-242.

Antelman, S.M. and L.A. Chiodo (1983). Amphetamine as a stressor. In I. Creese (Ed.) Stimulants: neurochemical, behavioral and clinical perspectives. Raven Press, New York, pp. 269-299.

Antelman, S.M., Eichler, A.J., Black, C.A. and D. Kocan (1980). Interchangeability of stress and amphetamine in sensitization. Science 207:329-331.

Antelman, S.M. and H. Szechtman (1975). Tail pinch induces eating in sated rats which appears to depend on nigrostriatal dopamine. Science 189:731-733.

Arato, M., Frecska, E., MacCrimmon, D.J., Guscott, R., Saxena, B., Tekes, K. and L. Tothfaulusi (1991). Serotonergic interhemispheric asymmetry: Neurochemical and pharmaco-EEG evidence. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 15:759-764.

Baldessarini, R.J. (1980). Dopamine and the pathophysiology of dyskinesias induced by antipsychotic drugs. Ann. Rev. Neurosci. 3:23-41.

Barone, P., Davis, T.A., Braun, A.R. and T.N. Chase (1986). Dopaminergic mechanisms and motor function: characterization of D-1 and D-2 receptor interactions. Eur. J. Pharmacol. 123:109-114.

Bear, D.M. (1983). Hemispheric specialization and the neurology of emotion. Arch. Neurol. 40:195-202.

Belcheva, I., Belcheva, S., Petkov, V.V. and V.D. Petkov (1994). Asymmetry in behavioral responses to cholecystokinin microinjected into rat nucleus accumbens and amygdala. Neuropharmacology 33:995-1002.

Belcheva, I, Bryer, J.B., Starkstein, S.E., Honig, M., Moran, T.H. and R.G. Robinson (1990). Hemispheric asymmetry in behavioral response to D1 and D2 receptor agonists in the nucleus accumbens. Brain Res. 533:286-291.

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

Berlucchi, G. (1990). Commissurotomy studies in animals. In Boller, F. and J. Grafman (Eds.), Handbook of neuropsychology, Vol. 4, pp.9-48, Elsevier, New York.

Bertolucci-D'Angio, M., Serrano, A., Driscoll, P. and B. Scatton (1990). Involvement of mesocorticolimbic dopaminergic systems in emotional states. In Uylings, H.B.M., Van Eden, C.G., De Bruin, J.P.C., Corner, M.A. and M.G.P. Feenstra (Eds.), Progress in Brain Research, Vol. 85, The prefrontal cortex: Its structure, function and pathology, pp. 405-417, Elsevier, Amsterdam.

Bianki, V.L. (1988). The right and left hemispheres of the animal brain: Cerebral lateralization of function, Gordon and Breach Science Publishers, New York.

Bird, E.D., Spokes, E.G.S. and L.L. Iversen (1979). Increased dopamine concentration in limbic areas of brain from patients dying with schizophrenia. Brain 102:347-360.

Bracha, H.S. (1987). Asymmetric rotational (circling) behavior, a dopamine-related asymmetry: preliminary findings in unmedicated and never-medicated schizophrenic patients. Biol. Psychiat. 22:995-1003.

Bracha, H.S. (1989). Is there a right hemi-hyper-dopaminergic psychosis? Schizophr. Res. 2:317-324.

Brown, V.J. and T.W. Robbins (1989). Deficits in response space following unilateral striatal dopamine depletion in the rat. J. Neurosci. 9:983-989.

Brozoski, T.J., Brown, R.M., Rosvold, H.E. and P.S. Goldman (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkeys. Science 205:929-932.

Camp, D.M., Robinson, T.E. and J. Becker (1984). Sex differences in the effects of early experience on the development of behavioral and brain asymmetries in rats. Physiol. Behav. 33:433-439.

Camp, D.M., Therrien, B.A. and T.E. Robinson (1981). Spatial learning ability is related to an endogenous asymmetry in the nigrostriatal dopamine system in rats. Soc. Neurosci. Abs. 7:455.

Cantello, R., Aguggia, M., Gilli, M., Delsedime, M., Chiardocutin, I., Riccio, A. and R. Mutani (1989). Major depression in Parkinson's disease and the mood response to intravenous methylphenidate: possible role of the "hedonic" dopamine synapse. J. Neurol. Neurosurg. Psychiat. 52:724-731.

Carli, M., Evenden, J.L. and T.W. Robbins (1985). Depletion of unilateral striatal dopamine impairs initiation of contralateral actions and not sensory attention. Nature 313:679-682.

Carlson, J.N., Fitzgerald, L.W., Keller, R.W. and S.D. Glick (1991). Side and region dependent changes in dopamine activation with various durations of restraint stress. Brain Res. 550:313-318.

Carlson, J.N., Fitzgerald, L.W., Keller, R.W. and S.D. Glick (1993). Lateralized changes in prefrontal cortical dopamine activity induced by controllable and uncontrollable stress in the rat. Brain Research 630:178-187.

Carlson, J.N. and S.D. Glick (1989). Cerebral lateralization as a source of interindividual differences in behavior. Experientia 45:788-798.

Carlson, J.N. and S.D. Glick (1991). Brain laterality as a determinant of susceptibility to depression in an animal model. Brain Res. 550:324-328.

Carlson, J.N., Glick, S.D. and P.A. Hinds (1987). Changes in d-amphetamine elicited rotational behavior in rats exposed to uncontrollable footshock stress. Pharmacol. Biochem. Behav. 26:17-21.

Carlson, J.N., Glick, S.D., Hinds, P.A. and J.L. Baird (1988). Food deprivation alters dopamine utilization in the rat prefrontal cortex and asymmetrically alters amphetamine-

induced rotational behavior. Brain Res. 454:373-377.

Casolini, P., Kabbaj, M., Leprat, F., Piazza, P.V., Rouge-Pont, F., Angelucci, L., Simon, H., Le Moal, M. and S. Maccari (1993). Basal and stress-induced corticosterone secretion is decreased by lesion of mesencephalic dopaminergic neurons. Brain Res. 622:311-314.

Castellano, M.A., Diaz-Palarea, M.D., Rodriguez, M. and J. Barroso (1987). Lateralization in male rats and dopaminergic system: evidence of right-sided population bias. Physiol. Behav. 40:607-612.

Castellano, M.A., Diaz-Palarea, M.D., Rodriguez, M. and J. Barroso (1989). Behavioral lateralization in rats and dopaminergic system: individual and population laterality. Behav. Neurosci. 103:46-53.

Cechetto, D.F. and C.B. Saper (1990). Role of the cerebral cortex in autonomic function. In Central regulation of autonomic functions (eds. Loewy, A.D. and Spyer, K.M.), pp. 208-223, Oxford University Press, Oxford.

Christie, M.J., Bridge, S., James, L.B. and P.M. Beart (1985). Excitotoxin lesions suggest an aspartatergic projection from rat medial prefrontal cortex to ventral tegmental area. Brain Res. 333:169-172.

Clarke, P., Jakubovic, A. and H.C. Fibiger (1988). Anatomical analysis of the involvement of mesolimbocortical dopamine in the locomotor stimulant actions of d-amphetamine and apomorphine. Psychopharmacol. 96:511-520.

Claustre, Y., Rivy, J.P., Dennis, T. and B. Scatton (1986). Pharmacological studies on stress-induced increase in frontal cortical dopamine metabolism in the rat. J. Pharmacol. Exp. Ther. 238:693-700.

Coco, M.L., Kuhn, C.M., Ely, T.D. and C.D. Kilts (1992). Selective activation of mesoamygdaloid neurons by conditioned stress: attenuation by diazepam. Brain Res. 590:39-47.

Coger, R.W. and E.A. Serafetinides (1990). Schizophrenia, corpus callosum, and interhemispheric communication: a review. Psychiat. Res. 34:163-184.

Collins, R.L. (1977). Toward an admissible genetic model for the inheritance of the degree and direction of asymmetry. In S. Harnad, R.W. Doty, L. Goldstein, J. Jaynes and G. Krauthamer (Eds.), Lateralization in the nervous system. Academic, New York, pp. 137-150.

Connell, P.H. (1958). Amphetamine psychosis. Oxford University Press, London.

Crow, T.J., Ball, J., Bloom, S.R., Brown, R., Bruton, C.J., Colter, N., Frith, C.D., Johnstone, E.C., Owens, D.G.C. and G.W. Roberts (1989). Schizophrenia as an anomaly of development of cerebral asymmetry. Arch. Gen. Psychiat. 46:1145-1150.

Crowne, D.P., Adelstein, A., Dawson, K.A. and C. Richardson (1988). Some effects of commissurotomy on the reinstatement and potentiation of lesion deficits. Behav. Brain Res. 29:135-146.

Crowne, D.P., Novotny, M.F., Maier, S.E., and R. Vitols (1992). Effects of unilateral parietal lesions on spatial localization in the rat. Behav. Neurosci. 106:808-819.

Crowne, D.P. and C.M. Richardson (1985). A method to section the corpus callosum in the rat. Physiol. Behav. 34:847-850.

Crowne, D.P., Richardson, C.M. and K.A. Dawson (1987). Lateralization of emotionality in the right parietal cortex of the rat. Behav. Neurosci. 101:134-138.

Crowne, D.P., Tokrud, P.A. and P. Brown (1992). Relation of rotation to egocentric and allocentric spatial learning in the rat. Pharmacol. Biochem. Behav. 43:1151-1153.

Daniel, D.G., Weinberger, D.R., Jones, D.W., Zigun, J.R., Coppola, R., Handel, S., Bigelow, L.B., Goldberg, T.E., Berman, K.F. and J.E. Kleinman (1991). The effect of amphetamine on regional cerebral blood flow during cognitive activation in schizophrenia. J. Neurosci. 11:1907-1917.

Davidson, R.J. (1992). Anterior cerebral asymmetry and the nature of emotion. Brain & Cognition 20:125-151.

Davidson, R.J. and A.J. Tomarken (1989). Laterality and emotion: an electrophysiological approach. In F. Boller and J. Grafman (Eds.), Handbook of Neuropsychology, Vol. 3, Elsevier, New York, pp. 419-441.

Davis, K.L., Kahn, R.S., Ko, G. and M. Davidson (1991). Dopamine in schizophrenia: a review and reconceptualization. Am. J. Psychiat. 148:1474-1486.

Denenberg, V.H. (1981). Hemispheric laterality in animals and the effects of early experience. Behav. Brain Sci. 4:1-49.

Denenberg, V.H. (1983). Brain laterality and behavioral asymmetry in the rat. In P. Flor-Henry and J. Gruzelier (Eds.), Laterality and psychopathology, Elsevier, New York, pp. 29-38.

Denenberg, V.H., Gall, J.S., Berrebi, A. and D.A. Yutzey (1986). Callosal mediation of

cortical inhibition in the lateralized rat brain. Brain Res. 397:327-332.

Denenberg, V.H., Garbanati, J., Sherman, G., Yutzey, D.A. and R. Kaplan (1978). Infantile stimulation induces brain lateralization in rats. Science 201:1150-1152.

Denenberg, V.H. and D.A. Yutzey (1985). Hemispheric laterality, behavioral asymmetry, and the effects of early experience in rats. In S.D. Glick (Ed.), Cerebral lateralization in nonhuman species. Academic, New York, pp. 109-133.

Deutch, A.Y. (1992). The regulation of subcortical dopamine systems by the prefrontal cortex: interactions of central dopamine systems and the pathogenesis of schizophrenia. J. Neural Transm. [Suppl.] 36:61-89.

Deutch, A.Y., Clark, W.A. and R.H. Roth (1990). Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. Brain Res. 521:311-315.

Deutch, A.Y. and R.H. Roth (1990). The determinants of stress-induced activation of the prefrontal cortical dopamine system. In Uylings, H.B.M., Van Eden, C.G., De Bruin, J.P.C., Corner, M.A. and M.G.P. Feenstra (Eds.), Progress in Brain Research, Vol. 85, The prefrontal cortex: Its structure, function and pathology, Elsevier, Amsterdam, pp. 367-403.

Dewberry, R.G., Lipsey, J.R., Saad, K., Moran, T.H., and R.G. Robinson (1986). Lateralized response to cortical injury in the rat: interhemispheric interaction. Behav. Neurosci. 100:556-562.

Diana, M., Garcia-Munoz, M., Richards, J. and C.R. Freed (1989). Electrophysiological analysis of dopamine cells from the substantia nigra pars compacta of circling rats. Exp. Brain Res. 74:625-630.

Diorio, D., Viau, V. and M.J. Meaney (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J. Neurosci. 13:3839-3847.

Doherty, M.D. and A. Gratton (1992). High-speed chronoamperometric measurements of mesolimbic and nigrostriatal dopamine release associated with repeated daily stress. Brain Res. 586:295-302.

Doty, R.W. (1989). Schizophrenia: a disease of interhemispheric processes at forebrain and brainstem levels? Behav. Brain Res. 34:1-33.

Drew, K.L., Lyon, R.A., Titeler, M. and S.D. Glick (1986). Asymmetry in D-2 binding in female rat striata. Brain Res. 363:192-195.

Dunnett, S.B., Bjorklund, A., Stenevi, U. and S.D. Iversen (1981a). Behavioural recovery following transplantation of substantia nigra in rats subjected to 6-OHDA lesions of the nigrostriatal pathway. I. Unilateral lesions. Brain Res. 215:147-161.

Dunnett, S.B., Bjorklund, A., Stenevi, U. and S.D. Iversen (1981b). Grafts of embryonic substantia nigra reinnervating the ventrolateral striatum ameliorate sensorimotor impairments and akinesia in rats with 6-OHDA lesions of the nigrostriatal pathway. Brain Res. 229:209-217.

Dunnett, S.B. and S.D. Iversen (1981). Learning impairments following selective kainic acid-induced lesions within the neostriatum of rats. Behav. Brain Res. 2:189-209.

Dunnett, S.B., Lane, D.M. and P. Winn (1985). Ibotenic acid lesions of the lateral hypothalamus: comparison with 6-hydroxydopamine-induced sensorimotor deficits. Neuroscience 14:509-518.

Early, T.S., Posner, M.I., Reiman, E.M. and M.E. Raichle (1989a). Hyperactivity of the left striato-pallidal projection. Part I: Lower level theory. Psychiat. Dev. 2:85-108.

Early, T.S., Posner, M.I., Reiman, E.M. and M.E. Raichle (1989b). Left striato-pallidal hyperactivity in schizophrenia. Part II: Phenomenology and thought disorder. Psychiat. Dev. 2:109-121.

Eilam, D., Golani, I. and H. Szechtman (1989). D_2-agonist quinpirole induces perseveration of routes and hyperactivity but no perseveration of movements. Brain Res. 490:255-267.

Eilam, D., Talangbayan, H., Canaran, G. and H. Szechtman (1992). Dopaminergic control of locomotion, mouthing, snout contact, and grooming: opposing roles of D1 and D2 receptors. Psychopharmacol. 106:447-454.

Ellinwood, E.H. (1967). Amphetamine psychosis: I. Description of the individuals and process. J. Nerv. Ment. Dis. 144:273-283.

Fitzgerald, L.W., Glick, S.D. and J.N. Carlson (1990). Right-sided population bias in male rats: role of stress. Physiol. Behav. 48:475-477.

Flor-Henry, P. (1989). Psychopathology and hemispheric specialization: left hemisphere dysfunction in schizophrenia, psychopathy, hysteria and the obsessional syndrome. In F. Boller and J. Grafman (Eds.), Handbook of Neuropsychology, Vol. 3, Elsevier, New York, pp. 477-494..

Fornaguera-Trias, J., Schwarting, R.K.W., Boix, F. and J.P. Huston (1993). Behavioral indices of moderate nigro-striatal 6-hydroxydopamine lesion: a preclinical Parkinson's

model. Synapse 13:179-185.

Fride, E. and M. Weinstock (1987). Increased interhemispheric coupling of the dopamine systems induced by prenatal stress. Brain Res. Bull. 18:457-461.

Fride, E. and M. Weinstock (1988). Prenatal stress increases anxiety related behavior and alters cerebral lateralization of dopamine activity. Life Sci. 42:1059-1065.

Frysztak, R.J. and E.J. Neafsey (1991). The effect of medial frontal cortex lesions on respiration, "freezing," and ultrasonic vocalizations during conditioned emotional responses in rats. Cerebral Cortex 1:418-425.

Frysztak, R.J. and E.J. Neafsey (1993). The effect of medial prefrontal cortex lesions on cardiovascular conditioned emotional responses in the rat. Brain Res. 643:181-193.

Gainotti, G. (1983). Laterality of affect: the emotional behavior of right and left-brain-damaged patients. In M.S. Myslobodsky (Ed.), Hemisyndromes: psychopathology, neurology, and psychiatry. Academic, New York, pp. 175-192.

Gainotti, G. (1987). Disorders of emotional behaviour and of autonomic arousal resulting from unilateral brain damage. In D. Ottoson (Ed.), Duality and unity of the brain. Plenum, New York, pp. 161-179.

Geschwind, N. (1985). Implications for evolution, genetics, and clinical syndromes. In S.D. Glick (Ed.), Cerebral lateralization in nonhuman species. Academic, New York, pp. 247-278.

Geschwind, N. and A.M. Galaburda (1987). Cerebral lateralization: Biological mechanisms, associations, and pathology, MIT press, Cambridge, Mass.

Glavin, G. (1991). Dopamine and gastroprotection - The brain-gut axis. Dig. Dis. Sci. 36:1670-1672.

Glavin, G. (1992). Central dopamine involvement in experimental gastrointestinal injury. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 16:217-221.

Glavin, G., Murison, R., Overmier, J.B., Pare, W.P., Bakke, H.K., Henke, P.G. and D.E. Hernandez (1991). The neurobiology of stress ulcers. Brain Res. Rev. 16:301-343.

Glick, S.D., Crane, A.M., Jerussi, T.P., Fleisher, L.N. and J.P. Green (1975). Functional and neurochemical correlates of potentiation of striatal asymmetry by callosal section. Nature 254:616-617.

Glick, S.D. and S. Greenstein (1973). Possible modulating influence of frontal cortex on nigro-strital function. Br. J. Pharmacol. 49:316-321.

Glick, S.D., Jerussi, T.P. and L.N. Fleisher (1976). Turning in circles: the neuropharmacology of rotation. Life Sci. 18:889-896.

Glick, S.D., Jerussi, T.P. and B. Zimmerberg (1977). Behavioral and neuropharmacological correlates of nigrostriatal asymmetry in rats. In S. Harnad, R.W. Doty, L. Goldstein, J. Jaynes and G. Krauthamer (Eds.), Lateralization in the nervous system. Academic, New York, pp. 213-249.

Glick, S.D., Lyon, R.A., Hinds, P.A., Sowek, C. and M. Titeler (1988). Correlated asymmetries in striatal D1 and D2 binding: relationship to apomorphine-induced rotation. Brain Res. 455:43-48.

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

Glick, S.D., Raucci, J., Wang, S., Keller, R.W. and J.N. Carlson (1994). Neurochemical predisposition to self-administer cocaine in rats: individual differences in dopamine and its metabolites. Brain Res. 653:148-154.

Glick, S.D. and D.A. Ross (1981a). Right-sided population bias and lateralization of activity in normal rats. Brain Res. 205:222-225.

Glick, S.D. and D.A. Ross (1981b). Lateralization of function in the rat brain: basic mechanisms may be operative in humans. TINS (August):196-199.

Glick, S.D., Ross, D.A. and L.B. Hough (1982). Lateral asymmetry of neurotransmitters in human brain. Brain Res. 234:53-63.

Gloor, P., Olivier, A. and L.F. Quesney (1981). The role of the amygdala in the expression of psychic phenomena in temporal lobe seizures. In The amygdaloid complex, INSERM Symposium No. 20 (ed. Ben-Ari, Y.), pp.489-498, Elsevier/North-Holland Biomedical Press.

Goodman, W.K., McDougle, C.J., Price, L.H., Riddle, M.A., Pauls, D.L. and Leckman, J.F. (1990). Beyond the serotonin hypothesis: a role for dopamine in some forms of obsessive compulsive disorder? J. Clin. Psychiat. 51(Suppl):36-43.

Gosling, R.H. (1958). Peptic ulcer and mental disorder - II. J. Psychosomat. Res. 2:285-301.

Grace, A.A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41:1-24.

Granato, A., Santarelli, M. and D. Minciacchi (1991). Bihemispheric organization of amygdalo-cortical projections in the rat. Neurosci. Lett. 127:53-56.

Hagan, J.J., Alpert, J.E., Morris, R.G.M. and S.D. Iversen (1983). The effects of central catecholamine depletions on spatial learning in rats. Behav. Brain Res. 9:83-104.

Harfstrand, A., Fuxe, K., Cintra, A., Agnati, L.F., Zini, I., Wikstrom, A., Okret, S., Yu, Z., Goldstein, M., Steinbusch, H., Verhofstad, A. and J. Gustafsson (1986). Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc. Natl. Acad. Sci. USA 83:9779-9783.

Hartley, L.R., Strother, N., Arnold, P.K. and B. Mulligan (1989). Lateralization of emotional expression under a neuroleptic drug. Physiol. Behav. 45:917-921.

Hattori, T. (1993). Conceptual history of the nigrostriatal dopamine system. Neurosci. Res. 16:239-262.

Hegstrom, R.A. and D.K. Kondepudi (1990). The handedness of the universe. Sci. Amer. (Jan):108-115.

Heilman, K.M. and T. Van Den Abell (1980). Right hemispheric dominance for attention: the mechanism underlying hemispheric asymmetries of inattention. Neurology 30:327-330.

Hellige, J.B. (1990). Hemispheric asymmetry. Annu. Rev. Psychol. 41:55-80.

Hellige, J.B. (1993). Hemispheric asymmetry: What's right and what's left. Harvard University Press, Cambridge, Mass.

Henke, P.G. (1984). The anterior cingulate cortex and stress: Effects of chlordiazepoxide on unit-activity and stimulation-induced gastric pathology in rats. Int. J. Psychophysiol. 2:23-32.

Henke, P.G., Ray, A. and R.M. Sullivan (1991). The amygdala, emotions and gut function. Dig. Dis. Sci. 36:1633-1643.

Herman, J.P., Guillonneau, D., Dantzer, R., Scatton, B., Semerdjian-Rouquier, L. and M. Le Moal (1982). Differential effects of inescapable footshocks and of stimuli paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. Life Sci. 30:2207-2214.

Herve, D., Trovero, F., Blanc, G., Thierry, A.M., Glowinski, J. and J.P. Tassin (1989). Nondopaminergic prefrontocortical efferent fibers modulate D1 receptor denervation supersensitivity in specific regions of the rat striatum. J. Neurosci. 9:3699-3708.

Hess, E.J., Bracha, H.S., Kleinman, J.E. and I. Creese (1987). Dopamine receptor subtype imbalance in schizophrenia. Life Sci. 40:1487-1497.

Hetey, L., Schwitzkowsky, R., Ott, T. and H. Barz (1991). Diminished synaptosomal dopamine (DA) release and DA autoreceptor supersensitivity in schizophrenia. J. Neural Trans. [GenSect] 83:25-35.

Hori, K. Tanaka, J. and M. Nomura (1993). Effects of discrimination learning on the rat amygdala dopamine release: a microdialysis study. Brain Res. 621:296-300.

Hornykiewicz, O. (1966). Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Rev. 18:925-964.

Hoyman, L., Weese, G.D. and G.P. Frommer (1979). Tactile discrimination performance deficits following neglect-producing unilateral lateral hypothalamic lesions in the rat. Physiol. Behav. 22:139-147.

Hurley, K.M., Herbert, H., Moga, M.M. and C.B. Saper (1991). Efferent projections of the infralimbic cortex of the rat. J. Comp. Neurol. 308:249-276.

Hyde, J.F. and T.P. Jerussi (1992). Bilateral neurochemical changes induced by unilateral cerebral haloperidol administration: evidence for cerebral asymmetry in the rat. Pharmacol. Biochem. Behav. 42:457-464.

Hynd, G.W., Semrud-Clikeman, M., Lorys, A.R., Novey, E.S., Eliopulos, D. and H. Lyytinen (1991). Corpus callosum morphology in attention deficit-hyperactivity disorder: morphometric analysis of MRI. J. Learn. Disabil. 24:141-146.

Ichihara, K., Nabeshima, T. and T. Kameyama (1993). Mediation of dopamine D1 and D2 receptors in the effects of GBR 12909 on latent learning and locomotor activity in mice. Eur. J. Pharmacol. 234:155-163.

Imperato, A., Puglisi-Allegra, S., Casolini, P., Zocchi, A. and L. Angelucci (1989). Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: role of corticosterone. Eur. J. Pharmacol. 165:337-338.

Jaskiw, G.E., Karoum, F.K. and D.R. Weinberger (1990). Persistent elevations in dopamine and its metabolites in the nucleus accumbens after mild subchronic stress in rats with ibotenic acid lesions of the medial prefrontal cortex. Brain Res. 534:321-323.

Jaskiw, G.E., Weinberger, D.R. and J.N. Crawley (1991). Microinjection of apomorphine into the prefrontal cortex of the rat reduces dopamine metabolite concentrations in microdialysate from the caudate nucleus. Biol. Psychiat. 29:703-706.

Jaynes, J. (1976). The origin of consciousness in the breakdown of the bicameral mind, Houghton Mifflin Company, Boston.

Jerussi, T.P. and S.D. Glick (1976). Drug-induced rotation in rats without lesions: behavioral and neurochemical indices of a normal asymmetry in nigro-striatal function. Psychopharmacol. 47:249-260.

Joyce, J.N., Davis, R.E. and C. Van Hartesveldt (1981). Behavioral effects of unilateral dopamine injection into dorsal or ventral striatum. Eur. J. Pharmacol. 72:1-10.

Klitenick, M.A., Deutch, A.Y., Churchill, L. and P.W. Kalivas (1992). Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. Neuroscience 50:371-386.

Kolb, B., Mackintosh, A., Whishaw, I.Q. and R.J. Sutherland (1984). Evidence for anatomical but not functional asymmetry in the hemidecorticate rat. Behav. Neurosci. 98:44-58.

Kolb, B., Pittman, K., Sutherland, R.J. and I.Q. Whishaw (1982). Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic nucleus to spatially guided behavior in the rat. Behav. Brain Res. 6:365-378.

Kolb, B., Sutherland, R.J., Nonneman, A.J. and I.Q. Whishaw (1982). Asymmetry in the cerebral hemispheres of the rat, mouse, rabbit, and cat: The right hemisphere is larger. Exp. Neurol. 78:348-359.

Kooistra, C.A. and K.M. Heilman (1988). Motor dominance and lateral asymmetry of the globus pallidus. Neurology 38:388-390.

Kubos, K.L., Moran, T.H., and R.G. Robinson (1987). Differential and asymmetrical behavioral effects of electrolytic or 6-hydroxydopamine lesions in the nucleus accumbens. Brain Res. 401:147-151.

Kubos, K.L., Pearlson, G.D., and R.G. Robinson (1982). Intracortical kainic acid induces an asymmetrical behavioral response in the rat. Brain Res. 239:303-309.

Kubos, K.L. and R.G. Robinson (1984). Cortical undercuts in the rat produce asymmetrical behavioral response without altering catecholamine concentrations. Exp. Neurol. 83:646-653.

LaHoste, G.J., Mormede, P., Rivet, J.-M. and M. LeMoal (1988a). Differential sensitization to amphetamine and stress responsivity as a function of inherent laterality. Brain Res. 453:381-384.

LaHoste, G.J., Mormede, P., Rivet, J.-M. and M. LeMoal (1988b). New evidence for distinct patterns of brain organization in rats differentiated on the basis of inherent laterality. Brain Res. 474:296-384.

Langston, J.W. (1989). Current theories on the cause of Parkinson's disease. J. Neurol. Neurosurg. Psychiat. Special Supplement:13-17.

LeMoal, M. and H. Simon (1991). Mesocorticolimbic dopaminergic network: Functional and regulatory roles. Physiol. Rev. 71:155-233.

Leviel, V., Charriere, B., Fayada, C. and B. Guibert (1986). Nigroamygdaloid dopamine neurons: nigral modulation of their activity. Brain Res. 381:205-214.

Lindvall, O. and Bjorklund, A. (1987). Neuroanatomical localization of dopamine in the brain and spinal cord. In Handbook of schizophrenia, Vol.2: Neurochemistry and neuropharmacology of schizophrenia (eds. Henn, F.A and DeLisi, L.E.), pp. 49-99, Elsevier Science Publishers B.V.

Ljungberg, T., Apicella, P., and W. Schultz (1992). Responses of monkey dopamine neurons during learning of behavioral reactions. J. Neurophysiol. 67:145-163.

Ljungberg, T. and U. Ungerstedt (1976). Sensory inattention produced by 6-hydroxydopamine-induced degeneration of ascending dopamine neurons in the brain. Exp. Neurol. 53:585-600.

Lou, H.C., Henriksen, L., Bruhn, P., Borner, H. and J.B. Neilsen (1989). Striatal dysfunction in attention deficit and hyperkinetic disorder. Arch. Neurol. 46:48-52.

Louilot, A. and M. LeMoal (1994). Lateralized interdependence between limbicotemporal and ventrostriatal dopaminergic transmission. Neuroscience 59:495-500.

Louilot, A., LeMoal, M. and H. Simon (1986). Differential reactivity of dopaminergic neurons in the nucleus accumbens in response to different behavioral situations. An in vivo voltammetric study in free moving rats. Brain Res. 397:395-400.

MacLennan, A.J., Pelleymounter, M.A., Atmadja, S., Jakubovic, A., Maier, S.F. and H.C. Fibiger (1989). D2 dopamine receptors in the rat prefrontal cortex: characterization and alteration by stress. Brain Res. 477:300-307.

Maier, S.E. and D.P Crowne (1993). Early experience modifies the lateralization of emotionality in parietal lesioned rats. Behav. Brain Res. 56:31-42.

Maisonneuve, I.M., Keller, R.W. and S.D. Glick (1990). Similar effects of D-amphetamine

and cocaine on extracellular dopamine levels in medial prefrontal cortex of rats. Brain Res. 535:221-226.

Marshall, J.F., Richardson, J.S. and P. Teitelbaum (1974). Nigrostriatal bundle damage and the lateral hypothalamic syndrome. J. Comp. Physiol. Psych. 87:808-830.

McDonald, A.J. (1991). Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. Neuroscience 44:1-14.

McDonald, A.J. (1991). Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. Neuroscience 44:15-33.

McDonald, R.J. and N.R. White (1993). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav. Neurosci. 107:3-22.

McGeorge, A.J. and R.L.M. Faull (1989). The organization of the projection from the cerebral cortex to the striatum in the rat. Neuroscience 29:503-537.

Meadows, M.-E. and R.F. Kaplan (1994). Dissociation of autonomic and subjective responses to emotional slides in right hemisphere damaged patients. Neuropsychologia 32:847-856.

Meaney, M.J., Aitken, D.H., van Berkel, C., Bhatnagar, S. and R.M. Sapolsky (1988). Effect of neonatal handling on age-related impairments associated with the hippocampus. Science 239:766-768.

Mensah, P. and S. Deadwyler (1974). The caudate nucleus of the rat: cell types and the demonstration of a commissural system. J. Anat. 117:281-293.

Menzies, S.A., Hoff, J.T. and A.L. Betz (1992). Middle cerebral artery occlusion in rats: A neurological and pathological evaluation of a reproducible model. Neurosurgery 31:100-107.

Mintz, M. and B.J. Knowlton (1993). Dissociation of kainic acid lesion effects on the asymmetry of rotation and lateral head movements. Brain Res. Bull. 31:641-647.

Mitchell, J.B. and A, Gratton (1992). Partial dopamine depletion of the prefrontal cortex leads to enhanced mesolimbic dopamine release elicited by repeated exposure to naturally reinforcing stimuli. J. Neurosci. 12:3609-3618.

Mohn, G. (1984). Visuomotor behaviour of normal and corpus callosum-sectioned rats during interocular transfer. Behav. Brain Res. 11:95-101.

Mohn, G. and I.S. Russell (1981). The role of the corpus callosum and some subcortical commissures in interocular transfer in the hooded rat. Exp. Brain Res. 42:467-474.

Morency, M.A., Stewart, R.J. and R.J. Beninger (1987). Circling behavior following unilateral microinjections of cocaine into the medial prefrontal cortex: dopaminergic or local anesthetic effect? J. Neurosci. 7:812-818.

Neafsey, E.J. (1990). Prefrontal cortical control of the autonomic nervous system: Anatomical and physiological observations. In Uylings, H.B.M., Van Eden, C.G., De Bruin, J.P.C., Corner, M.A. and M.G.P. Feenstra (Eds.), Progress in Brain Research, Vol. 85, The prefrontal cortex: Its structure, function and pathology, pp. 147-166, Elsevier, Amsterdam.

Neveu, P.J. (1992). Asymmetrical brain modulation of the immune response. Brain Res. Rev. 17:101-107.

Nicholson, I.R. and R.W.J. Neufeld (1989). Forms and mechanisms of susceptibility to stress in schizophrenia. In Advances in the investigation of psychological stress (ed. Neufeld, R.J.W.), pp. 392-420, John Wiley & Sons, New York.

Nottebohm, F. (1977). Asymmetries in neural control of vocalization in the canary. In S. Harnad, R.W. Doty, L. Goldstein, J. Jaynes and G. Krauthamer (Eds.), Lateralization in the nervous system. Academic, New York, pp. 23-44.

Oades, R.D. and G.M. Halliday (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res. Rev. 12:117-165.

Oepen, G., Fuenfgeld, M., Harrington, A., Hermle, L. and H. Botsch (1989). Right hemisphere involvment in mescaline-induced psychosis. Psychiat. Res. 29:335-336.

Oke, A., Keller, R., Mefford, I. and R.N. Adams (1978). Lateralization of norepinephrine in human thalamus. Science 200:1411-1413.

Oke, A., Lewis, R. and R.N. Adams (1980). Hemispheric asymmetry of norepinephrine distribution in rat thalamus. Brain Res. 188:269-272.

Ottoson, D. (1987). Duality and unity of the brain: Unified functioning and specialisation of the hemispheres. Plenum press, New York.

Packard, M.G. and N.R. White (1990). Lesions of the caudate nucleus selectively impair "reference memory" acquisition in the radial maze. Behav. Neural Biol. 53:39-50.

Parent, A. (1990). Extrinsic connections of the basal ganglia. TINS 13:254-258.

Paxinos, G. and C. Watson (1982). The rat brain in stereotaxic coordinates. Academic Press, N.Y.

Pearlson, G.D. and R.G. Robinson (1981). Suction lesions of the frontal cerebral cortex in the rat induce asymmetrical behavioral and catecholaminergic responses. Brain Res. 218:233-242.

Pediconi, M.F., Roccamo de Fernandez, A.M. and F.J. Barrantes (1993). Asymmetric distribution and down-regulation of the muscarinic acetylcholine receptor in rat cerebral cortex. Neurochem. Res. 18:565-572.

Perez, H., Ruiz, S., Hernandez, A. and R. Soto-Moyano (1990). Asymmetry of interhemispheric responses evoked in the prefrontal cortex of the rat. J. Neurosci. Res. 25:139-142.

Piazza, P.V., Deminiere, J.- M., LeMoal, M. and H. Simon (1989). Factors that predict individual vulnerability to amphetamine self-administration. Science 245:1511-1513.

Pisa, M. and H. Szechtman (1986). Lateralized and compulsive exteroceptive orientation in rats treated with apomorphine. Neurosci. Lett. 64:41-46.

Pogun, S., Kanit, L., and B.E. Okur (1992). Learning-induced changes in D2 receptors of rat brain are sexually dimorphic. Pharmacol. Biochem. Behav. 43:71-75.

Posner, M.I., Early, T.S., Reiman, E., Pardo, P.J. and M. Dhawan (1988). Asymmetries in hemispheric control of attention in schizophrenia. Arch. Gen. Psychiat. 45:814-821.

Puglisi-Allegra, S., Imperato, A., Angelucci, L. and S. Cabib (1991). Acute stress induces time-dependent responses in dopamine mesolimbic system. Brain Res. 554:217-222.

Pycock, C.J., Carter, C.J. and R.W. Kerwin (1980a). Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J. Neurochem. 34:91-99.

Pycock, C.J., Carter, C.J. and R.W. Kerwin (1980b). Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats. Nature 286:74-77.

Rada, P. and L. Hernandez (1990). Opposite changes of dopamine turnover in prefrontal cortex and nucleus accumbens after amygdaloid kindling. Neurosci. Lett. 117:144-148.

Ray, A. and P.G. Henke (1991). The basolateral amygdala, dopamine and gastric stress ulcer formation in rats. Brain Res. 558:335-338.

Ray, A., Henke, P.G. and R.M. Sullivan (1988a). Effects of intra-amygdalar dopamine agonists and antagonists on gastric stress lesions in rats. Neurosci. Lett. 84:302-306.

Ray, A., Henke, P.G. and R.M. Sullivan (1988b). Central dopamine systems and gastric stress pathology in rats. Physiol. Behav. 42:359-364.

Reivich, M., Alavi, A. and R.C. Gur (1984). Positron emission tomographic studies of perceptual tasks. Ann. Neurol. 15(Suppl.):S61-S65.

Reynolds, G.P. (1983). Increased concentrations and lateral asymmetry of amygdala dopamine in schizophrenia. Nature 305:527-529.

Reynolds, G.P., Czudek, C., Bzowej, N. and P. Seeman (1987). Dopamine receptor asymmetry in schizophrenia. Lancet 1:979.

Robinson, R.G. (1979). Differential behavioral and biochemical effects of right and left hemispheric cerebral infarction in the rat. Science 205:707-710.

Robinson, R.G. and T.G. Stitt (1981). Intracortical 6-hydroxydopamine induced an asymmetrical behavioral response in the rat. Brain Res. 213:387-395.

Robinson, T.E. and J.B. Becker (1982). Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur. J. Pharmacol. 85:253-254.

Robinson, T.E., Becker, J.B., Camp, D.M. and A. Mansour (1985). Variation in the pattern of behavioral and brain asymmetries due to sex differences. In S.D. Glick (Ed.), Cerebral lateralization in nonhuman species. Academic, New York, pp. 185-231.

Robinson, T.E. and K.C. Berridge (1993). The neural basis of drug-craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18:247-291.

Rolls, E.T., Thorpe, S.J., Boytim, M., Szabo, I. and D.I. Perrett (1984). Responses of striatal neurons in the behaving monkey. 3. Effects of iontophoretically applied dopamine on normal responsiveness. Neuroscience 12:1201-1212.

Rosen, G.D., Finklestein, S., Stoll, A.L., Yutzey, A. and V.H. Denenberg (1984). Neurochemical asymmetries in the albino rat's cortex, striatum, and nucleus accumbens. Life Sci. 34:1143-1148.

Rosin, D.L., Clark, W.A., Goldstein, M., Roth, R.H. and A.Y. Deutch (1992). Effects of 6-hydroxydopamine lesions of the prefrontal cortex on tyrosine hydroxylase activity in mesolimbic and nigrostriatal dopamine systems. Neuroscience 48:831-839.

Ross, D.A. and S.D. Glick (1981). Lateralized effects of bilateral frontal cortex lesions in rats. Brain Res. 210:379-382.

Scatton, B., D'Angio, M., Driscoll, P. and A. Serrano (1988). An in vivo voltammetric study of the response of mesocortical and mesoaccumbens dopaminergic neurons to environmental stimuli in strains of rats with differing levels of emotionality. Ann. N. Y. Acad. Sci. 537:124-137.

Schaffer, C.E., Davidson, R.J. and C. Saron (1983). Frontal and parietal electroencephalogram asymmetry in depressed and nondepressed subjects. Biol. Psychiat. 18:753-762.

Schallert, T. and S. Hall (1988). 'Disengage' sensorimotor deficit following apparent recovery from unilateral dopamine depletion. Behav. Brain Res. 30:15-24.

Schiff, B.B. and M. Lamon (1989). Inducing emotion by unilateral contraction of facial muscles: A new look at hemispheric specialization and the experience of emotion. Neuropsychologia 27:923-935.

Schneider, L.H., Murphy, R.B. and E.E. Coons (1982). Lateralization of striatal dopamine (D2) receptors in normal rats. Neurosci. Lett. 33:281-284.

Schultz, W., Ruffieux, A. and P. Aebischer (1983). The activity of pars compacta neurons of the monkey substantia nigra in relation to motor activation. Exp. Brain Res. 51:377-387.

Schwarting, R.K.W., Bonatz, A.E., Carey, R.J. and J.P. Huston (1991). Relationship between indices of behavioral asymmetries and neurochemical changes following mesencephalic 6-hydroxydopamine injections. Brain Res. 554:46-55.

Schwarting, R.K.W., Steiner, H. and J.P. Huston (1991). Asymmetries in thigmotactic scanning: evidence for a role of dopaminergic mechanisms. Psychopharmacol. 103:19-27.

Seeman, P. (1993). Schizophrenia as a brain disease: the dopamine receptor story. Arch. Neurol. 50:1093-1095.

Seeman, P. and H.H.M. VanTol (1994). Dopamine receptor pharmacology. TiPS 15:264-270.

Selye, H. (1974). Stress without distress. W.B. Saunders, Philadelphia.

Sesack, S.R., Deutch, A.Y., Roth, R.H. and B.S. Bunney (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with *Phaseolus vulgaris* leucoagglutinin. J. Comp. Neurol. 290:213-242.

Sherman, G.F., Garbanati, J.S., Rosen, G.D., Yutzey, D.A. and V.H. Denenberg (1980). Brain and behavioral asymmetries for spatial preference in rats. Brain Res. 192:61-67.

Simon, H., Le Moal, M. and A. Calas (1979). Efferents and afferents of the ventral tegmental-A10 region studied after local injection of [^{3}H]leucine and horseradish peroxidase. Brain Res. 178:17-40.

Simon, H., Taghzouti, K., Gozlan, H., Studler, J.M., Louilot, A., Herve, D., Glowinski, J., Tassin, J.P. and M. Le Moal (1988). Lesion of dopaminergic terminals in the amygdala produces enhanced locomotor response to D-amphetamine and opposite changes in dopaminergic activity in prefrontal cortex and nucleus accumbens. Brain Res. 447:335-340.

Slopsema, J.S., Van Der Gugten, J. and J.P.C. De Bruin (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.

Sperry, R. (1982). Some effects of disconnecting the cerebral hemispheres. Science 217:1223-1226.

Starkstein, S.E., Moran, T.H., Bowersox, J.A. and R.G. Robinson (1988). Behavioral abnormalities induced by frontal cortical and nucleus accumbens lesions. Brain Res. 473:74-80.

Steiner, H., Bonatz, A.E., Huston, J.P. and R. Schwarting (1988). Lateralized wall-facing versus turning as measures of behavioral asymmetries and recovery of function after injection of 6-hydroxydopamine into the substantia nigra. Exp. Neurol. 99:556-566.

Steiner, H., Morgan, S. and J.P. Huston (1985). Effect of forebrain commissurotomy on recovery from unilateral 6-OHDA lesions of the substantia nigra and circling induced by apomorphine. Behav. Brain Res. 17:245-249.

Strecker, R.E. and B.L. Jacobs (1985). Substantia nigra dopaminergic unit activity in behaving cats: Effect of arousal on spontaneous discharge and sensory evoked activity. Brain Res. 361:339-350.

Sullivan, R.M. and P.G. Henke (1986). The anterior midline cortex and adaptation to stress ulcers in rats. Brain Res. Bull. 17:493-496.

Sullivan, R.M. and H. Szechtman (1990). Hemispheric asymmetry in development of amphetamine sensitization and its relation to emotionality in rats. FASEB Abs. 4:A678.

Sullivan, R.M. and H. Szechtman (1994). Left/right nigrostriatal asymmetry in susceptibility to neurotoxic dopamine depletion with 6-hydroxydopamine in rats. Neurosci. Lett. 170:83-

86.

Szechtman, H. (1983). Peripheral sensory input directs apomorphine-induced circling in rats. Brain Res. 264:332-335.

Thierry, A.M., Godbout, R., Mantz, J. and J. Glowinski (1990). Influence of the ascending monoaminergic systems on the activity of the rat prefrontal cortex. In Uylings, H.B.M., Van Eden, C.G., De Bruin, J.P.C., Corner, M.A. and M.G.P. Feenstra (Eds.), Progress in Brain Research, Vol. 85, The prefrontal cortex: Its structure, function and pathology, pp. 357-365, Elsevier, Amsterdam.

Thierry, A.M., Tassin, J.P., Blanc, G. and J. Glowinski (1976). Selective activation of mesocortical DA systems by stress. Nature 263:242-243.

Tucker, D.M. and P.A. Williamson (1984). Asymmetric neural control systems in human self-regulation. Psychol. Rev. 91:185-215.

Turner, B.H. (1973). Sensorimotor syndrome produced by lesions of the amygdala and lateral hypothalamus. J. Comp. Physiol. Psych. 82:37-47.

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

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

Vargo, J.M., Corwin, J.V., King, V. and R.L. Reep (1988). Hemispheric asymmetry in neglect produced by unilateral lesions of dorsomedial prefrontal cortex in rats. Exp. Neurol. 102:199-209.

Vlajkovic, S., Nikolic, V., Nikolic, A., Milanovic, S. and B.D. Jankovic (1994). Asymmetrical modulation of immune reactivity in left- and right-biased rats after ipsilateral ablation of the prefrontal, parietal and occipital brain neocortex. Int. J. Neurosci. (in press)

Vogel, W.H. (1985). Coping, stress, stressors and health consequences. Neuropsychobiol. 13:129-135.

Wallace, D.M., Magnuson, D.J. and T.S. Gray (1992). Organization of amygdaloid projections to brainstem dopaminergic, noradrenergic, and adrenergic cell groups in the rat. Brain Res. Bull. 28:447-454.

Weinberger, D.R. (1987). Implications of normal brain development for the pathogenesis

of schizophrenia. Arch. Gen. Psychiat. 44:660-669.

Weinberger, D.R., Berman, K.F., Suddath, R. and E.F. Torrey (1992). Evidence of dysfunction of a prefrontal-limbic network in schizophrenia: A magnetic resonance imaging and regional cerebral blood flow study of discordant monozygotic twins. Am. J. Psychiat. 149:890-897.

Weintraub, S. and M.-M. Mesulam (1987). Right cerebral dominance in spatial attention: Further evidence based on ipsilateral neglect. Arch. Neurol. 44:621-625.

Weintraub, S. and M.-M. Mesulam (1989). Neglect: hemispheric specialization, behavioral components and anatomical correlates. In F. Boller and J. Grafman (Eds.), Handbook of Neuropsychology, Vol. 2, Elsevier Science Publishers B.V., pp. 357-374.

Whishaw, I.Q. (1988). Food wrenching and dodging: use of action patterns for the analysis of sensorimotor and social behavior in the rat. J. Neurosci. Meth. 24:169-178.

Whishaw, I.Q. and S.B. Dunnett (1985). Dopamine depletion, stimulation or blockade in the rat disrupts spatial navigation and locomotion dependent upon beacon or distal cues. Behav. Brain Res. 18:11-29.

Whishaw, I.Q. and J. Tomie (1988). Food wrenching and dodging: a neuroethological test of cortical and dopaminergic contributions to sensorimotor behavior in the rat. Behav. Neurosci. 102:110-123.

White, N.R. (1988). Effect of nigrostriatal dopamine depletion on the post-training, memory improving action of amphetamine. Life Sci. 43:7-12.

Willar, C.D. and D.P. Crowne (1989). Circling, hemispheric asymmetry, and left-right discrimination. Brain Res. 500:405-407.

Wise, R.A. and M.A. Bozarth (1987). A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469-492.

Wise, R.A. and L.J. Holmes (1986). Circling from unilateral VTA morphine: direction is controlled by environmental stimuli. Brain Res. Bull. 16:267-269.

Witelson, S.F. and W. Pallie (1973). Left hemisphere specialization for language in the newborn: Neuroanatomical evidence of asymmetry. Brain 96:641-646.

Wittling, W. and R. Roschmann (1993). Emotion-related hemisphere asymmetry: subjective emotional responses to laterally presented films. Cortex 29:431-448.

Wittling, W. and E. Schweiger (1993). Alterations of neuroendocrine brain asymmetry: a neural risk factor affecting physical health. Neuropsychobiol. 28:25-29.

Wong, D.F., Wagner, H.N., Tune, L.E., Dannals, R.F., Pearlson, G.D., Links, J.M., Tamminga, C.A., Broussolle, E.P., Ravert, H.T., Wilson, A.A., Toung, J., Malat, J., Williams, J.A., O'Tuama, L.A., Snyder, L.J., Kuhar, M.J. and A. Gjedde (1986). Positron emision tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. Science 234:1558-1563.

Xing, L., Balaban, C., Seaton, J., Washington, J. and G. Kauffman (1991). Mesolimbic dopamine mediates gastric mucosal protection by central neurotensin. Am. J. Physiol. 260:G34-G38.

Yamamuro, Y., Hori, K., Iwano, H. and M. Nomura (1994). The relationship between learning performance and dopamine in the prefrontal cortex of the rat. Neurosci. Lett. 177:83-86.

Zahn, T.P. (1988). Studies of autonomic psychophysiology and attention in schizophrenia. Schizophr. Bull. 14:205-208.

Zahn, T.P., Carpenter, W.T. and T.H. McGlashan (1981). Autonomic nervous system activity in acute schizophrenia II. Relationships to short-term prognosis and clinical state. Arch. Gen. Psychiat. 38:260-266.

Ziegler, M.G. and H. Szechtman (1988a). Different behaviors induced by stimulation of the right and left striatum. Soc. Neurosci. Abs. 14:1140.

Ziegler, M.G.M. and H. Szechtman (1988b). Differences in the behavioral profile of circling under amphetamine and apomorphine in rats with unilateral lesions of the substantia nigra. Behav. Neurosci. 102:276-288.

Ziegler, M.G.M. and H. Szechtman (1990). Relation between motor asymmetry and direction of rotational behaviour under amphetamine and apomorphine in rats with unilateral degeneration of the nigrostriatal dopamine system. Behav. Brain Res. 39:123-133.

Zimmerberg, B. and L.W. Mickus (1990). Sex differences in corpus callosum: influence of prenatal alcohol exposure and maternal undernutrition. Brain Res. 537:115-122.

Zimmerberg, B., Strumpf, A.J. and S.D. Glick (1978). Cerebral asymmetry and left-right discrimination. Brain Res. 140:194-196.