**Neurochemistry, Cortical Morphology and Behaviour After Single and Combined
Neonatal Acetylcholine and Dopamine Lesions in Rats**

by

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ABSTRACT

The effects of single and combined neonatal acetylcholine and dopamine lesions on rat behaviour, neurochemistry and midline cortical pyramidal cell morphology were determined. On postnatal day 7, rats were pretreated with a noradrenergic reuptake inhibitor and administered intraventricular injections of either 600 ng of the cholinergic immunotoxin 192 IgG-saporin (192SAP), 100 µg of the catecholamine toxin 6-hydroxydopamine (6-OHDA), or a cocktail of both. 192SAP produced large reductions of choline acetyltransferase activity in the hippocampus and retrosplenial cortex (RSC), with lesser reductions in the medial prefrontal (mPFC) and anterior cingulate cortices (ACC). Layer V pyramidal cells from the mPFC and layer III pyramidal cells from the ACC exhibited dendritic regression in the apical tuft, and decreases in spine density from both apical and basal dendritic trees. The basal tree extent was reduced in layer III pyramidal cells of the RSC. Behaviorally, female lesioned rats were impaired on the first day of hidden platform water maze testing, suggesting mild visuospatial working memory impairment. 6-OHDA produced large depletions of dopamine and its metabolites in the caudate, mPFC and ACC. Surprisingly, pyramidal cell morphology was only moderately affected. Layer V mPFC pyramidal cells exhibited atrophy of oblique apical dendrites, and layer III pyramidal cells of the ACC and RSC exhibited reductions in higher order branching. Spine density was reduced from the apical and basal trees of ACC pyramidal cells only. However, these rats were profoundly impaired on a water maze task with either a hidden or cued platform. This was not due to hypersensitivity to swim stress, hypo- or hyperfunction at dopaminergic

synapses, or a deficit in sensorimotor gating. These rats may suffer a profound impairment of associative learning. Co-administration of the two toxins resulted in simple additive effects on neurochemistry and morphology. Behaviourally, dual lesioned rats were indistinguishable from rats with dopamine lesions. Thus, neonatal cholinergic and dopaminergic lesions produce long lasting effects on cortical pyramidal cell dendrites that may underlie behavioural impairments. These results further the understanding of human developmental disorders such as Rett and Down syndrome , which display cholinergic, and to a lesser extent, dopaminergic hypofunction, atrophy of cortical pyramidal neurons, and severe cognitive disability.

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LIST OF ABBREVIATIONS

ACC	anterior cingulate cortex
ACh	acetylcholine
AChE	acetylcholinesterase
ANOVA	analysis of variance
ChAT	choline acetyltransferase
CORT	corticosterone
DA	dopamine
DMI	desmethylinipramine
DOPAC	3,4-dihydroxyphenylacetic acid
E	embryonic day
5-HIAA	5-hydroxyindolacetic acid
HLDB	horizontal limb of the diagonal band of Broca
5-HT	5-hydroxytryptamine
HVA	homovanillic acid
i.c.v.	intracerebroventricular
LSD	Least Significant Difference
MHPG	3-methoxy,4-hydroxyphenethylene glycol
mPFC	medial prefrontal cortex
MS	medial septum
NBM	nucleus basalis magnocellularis

NGF	nerve growth factor
NS	not significant compared to control group mean
6-OHDA	6-hydroxydopamine
P	postnatal day
p75	low affinity nerve growth factor receptor
PPI	prepulse inhibition
r	Pearson's product-moment correlation coefficient
RSC	retrosplenial cortex
RSG	granular retrosplenial cortex
192SAP	192 IgG-saporin
SN	substantia nigra
VLDB	vertical limb of the diagonal band of Broca
VTA	ventral tegmental area

INTRODUCTION

Pyramidal neurons receive the vast majority of incoming synaptic contacts on their dendrites and spines. Altering the length, complexity or spine density of the dendritic tree changes the ability of the parent cell to sample axonal arrays. These changes can influence the function of the neuron and ultimately, behaviour. Aside from their synaptic signalling properties, substantial in vitro evidence indicates that neurotransmitters play an important role in shaping the structural features of their target cells. During development, both cholinergic and dopaminergic afferents arrive at their cortical targets during periods of synaptogenesis and synaptic sorting. This suggests that they are in a position to influence the dendritic architecture of pyramidal cells. The relationship between these neurotransmitters, neural structure, and function may be particularly relevant to the understanding of neurodevelopmental disorders such as Rett and Down syndrome. These disorders are associated with early cholinergic and dopaminergic dysfunction, as well as abnormalities in cortical pyramidal cell morphology. However, in vivo experimental studies have not yet investigated the long term effects of selective cholinergic or dopaminergic deafferentation on the morphological features of these cells. Thus the main purpose of the following study was to evaluate the effects of both single and combined neonatal cholinergic and dopaminergic lesions on behaviour and pyramidal cell morphology from midline cortical regions in rats. These findings provide new information about the role of neurotransmitters in the development of behaviour and appropriate dendritic morphology, as well as integral knowledge about the biological substrates underlying these neurodevelopmental disorders.

Structure-function relationships in the cortex

The idea that the postsynaptic structure of the cortex is correlated with functional ability has been derived mainly from experiments demonstrating that experience through training (Greenough et al., 1979, 1985; Leuner et al., 2003; Moser et al., 1994) or environmental enrichment (Diamond et al., 1976; Johansson & Belichenko, 2002) influences the dendritic architecture of cortical neurons. Experience generally increases the size of the dendritic field, and presumably the number of synapses received per cell (reviewed in Kolb et al., 1998). Furthermore, increases in synaptic density are associated with enhanced performance of learning and memory tasks in both normal aged (Geinisman et al., 1986) and brain injured rats (Kolb & Gibb, 1991, 1993; Kolb et al., 1997).

Most studies which have examined structure-function relationships in the cortex have restricted themselves to the analysis of pyramidal neurons. While generally considered to be a mammalian innovation, pyramidal cells are in fact evolutionarily conserved. Primitive pyramidals can be identified in reptilian cortex, as well as in phylogenetically older mammalian structures such as the hippocampus (Nieuwenhuys, 1994). Evolutionary conservation suggests that the rules which govern synaptic plasticity in these cells may also be conserved across species, which can increase the applicability of data obtained from other species to the clinical setting.

Pyramidal neurons are the most common cellular element of the cortex and are defined by a characteristic set of readily identifiable features. Each cell is distinguished by a conical body and a spiny dendritic tree. The apical tree consists of a long dendrite which extends up toward the pial surface. Before reaching the surface, the main shaft branches

into a tufted bouquet which terminates in layer I. Many smaller dendrites branch off the main shaft at varying points along its length, presumably to sample axonal arrays from each cortical layer. The basal dendrites extend outward from the cell body in a skirt-like formation. The long pyramidal axon extends downward and leaves the cortex via the corpus callosum to innervate subcortical structures, or to re-enter the cortex and innervate other cortical sites. These axons also give rise to several collaterals which extend outwards and upwards to innervate local cellular elements. Approximately 90% of pyramidal cells are immunopositive for the excitatory neurotransmitters l-glutamate or l-aspartate (Conti et al., 1987; Dori et al., 1989). As such, these cells form the main excitatory component of intrinsic cortical circuits, as well as the sole synaptic output, of the cortex.

The dendritic tree may constitute as much as 95% of the cell's receptive surface (Schade & Baxter, 1960). Expansion or shrinkage of the dendrites would therefore be expected to increase or decrease the number of synapses on the cell surface respectively. In support of this, the number of dendritic branches of a ciliary ganglion cell can be directly related to the number of synaptic inputs it receives (Purves & Hume, 1981). Furthermore, the fact that pyramidal cells receive the majority (70-95%) of their synaptic input on dendritic spines (Nieuwenhuys, 1994) makes it possible to infer changes in synaptic connectivity from dendritic structure in these cells without the use of ultrastructural techniques such as electron microscopy.

Anatomy and function of midline cortical structures

The fact that cortical volume increases dramatically across the evolutionary scale is interpreted as evidence that anatomically, this structure is the main repository of cognitive ability. Evidence from anatomical and lesion studies suggests that midline regions such as the medial prefrontal (mPFC), anterior cingulate (ACC) and retrosplenial cortices (RSC) may be integral to many types of learning and memory.

Anatomically, the mPFC receives a strong projection from the hippocampus (Carr & Sesack, 1996; Groenewegen et al., 1997; Thierry et al., 2000) and exhibits considerable reciprocal connectivity with the ACC (Fisk & Wyss, 1999). Pyramidal cells in the macaque mPFC have been shown to exhibit visuospatial “memory fields” (Goldman-Rakic, 1996). In other words, the maximal firing rates of certain pyramidal cells occur in response to visual stimuli located in a particular area of the visual field. These cells respond reliably to the same locations even upon multiple presentations of a visual stimulus. Different areas of the visual field are coded by a different sets of neurons. Like the visual cortex, these cells exhibit a columnar organization which is functionally dedicated to the same area of visual space. Goldman-Rakic has postulated that this phenomenon represents a cellular correlate of visuospatial working memory.

In the rat, mPFC lesions produce only mild (Hoh et al., 2003), or no spatial learning deficits (de Bruin et al., 2001; Dias & Aggleton, 2000; Ethier et al., 2001; Lacroix et al., 2002). This may be due to parallel processing of short term spatial memory by both this structure and the hippocampus (Lee & Kesner, 2003). However, navigation using idiothetic cues is impaired following mPFC lesions (de Bruin et al., 2001; Ethier et

al., 2001), as is prepulse inhibition (Yee, 2000). These deficits may also be related to attentional deficits or an impairment in response flexibility (Dias & Aggleton, 2000; Passetti et al., 2003).

The ACC projects indirectly via the entorhinal cortex to the hippocampus (Aggleton et al., 2000). The rostral portion of this region also exhibits considerable reciprocal connectivity with the mPFC (Fisk & Wyss, 1999). The ACC has a permissive but not necessary role in spatial learning, since lesions of this area increase the amount of training required to achieve a control level of performance in the water maze (Sutherland et al., 1988; Whishaw et al., 2001). Lesions of this region also produce associative deficits in Pavlovian conditioning tasks (Cardinal et al., 2003; Parkinson et al., 2000).

The RSC also projects indirectly to the hippocampus via the entorhinal cortex (Aggleton et al., 2000). This area receives corticocortical afferents from both the ACC (Fisk & Wyss, 1999) and visual cortex (van Groen & Wyss, 1992). Lesions of the RSC profoundly impair both idiothetic and allothetic (using visual cues) spatial navigation in rats (Harker & Whishaw, 2002; Sutherland et al., 1988; Whishaw et al., 2001).

The above data illustrate that there is considerable anatomical interconnectivity between midline cortical regions and the hippocampus, and within these regions themselves. Altering the postsynaptic structure of intrinsic neurons from these regions would change their synaptic connectivity, and presumably affects their ability to process information and respond appropriately to the environment. As the subsequent section illustrates, neurotransmitters may play a key role in shaping this connectivity during development.

Neurotransmitters as morphogens: in vitro experiments

The fact that some neurotransmitters can be detected embryonically, before the appearance of synaptic machinery, has suggested a role for these compounds in brain development (Lauder, 1988; Taylor et al., 1990). This idea was strengthened by the observation that some transmitters and their receptors are expressed only transiently during ontogeny (Daval et al., 1987; Lankford et al., 1987). Considerable in vitro evidence now indicates that the classical neurotransmitters acetylcholine (ACh) and dopamine (DA) can regulate many aspects of neural development.

In the chick sympathetic nervous system, ACh induces neurite retraction via a nicotinic (Pugh & Berg, 1994), as well as a muscarinic receptor mediated pathway (Small et al., 1995). Neurite outgrowth and growth cone motility is also inhibited by micromolar concentrations of ACh in cultured mouse spinal cord (Owen & Bird, 1995). In contrast, ACh promotes neurite outgrowth in the rat olfactory bulb via a nicotinic receptor mechanism (Coronas et al., 2000). In the chick dorsal root ganglion, ACh promotes neurite extension by both a muscarinic and nicotinic receptor mechanism (Tata et al., 2003). However, the ability of muscarinic agonists to increase neurofilament protein expression in these embryonic day 12 (E12) cultures, was absent in E18 cultures (Tata et al., 2003). This suggests that there may be a critical period for the morphogenic actions of ACh in some cell types.

Co-culture experiments have shown that rat basal forebrain cholinergic neurons induce soma enlargement, axonal and dendritic outgrowth, and expression of a pyramidal-specific neurofilament protein from a subset of embryonic cortical neurons (Ha et al.,

1998). Interestingly, evidence now indicates that ACh also exerts morphogenic effects in target tissues by regulating growth factor expression (da Penha Berzaghi et al., 1993; Zafra et al., 1992; reviewed in Dreyfus, 1998). Taken together, the above data illustrate that ACh has morphogenic properties that are mediated by different receptors and produce different effects depending on the developmental stage and type of tissue analyzed.

In the embryonic chick retina, DA application collapses growth cones and results in neurite retraction via a D1 receptor mechanism (Lankford et al., 1988). Conversely, DA results in neurite extension and arborization of cultured embryonic rat striatal neurons through a D1 mechanism (Schmidt et al., 1996, 1998). These results may be explained by the observation that DA release from a specific neuron in *lymnaea* (right pedal dorsal 1) not only increases the rate of growth cone advance from its target cells, but also collapses advancing growth cones from nontarget cells (Spencer et al., 2000). In cultured rat cortex, neurite outgrowth can either be stimulated or inhibited depending on the extracellular concentration of DA, and the type of receptor activated (Reinoso et al., 1996). Furthermore, co-culture experiments have shown that rat chromaffin cells grown in the presence of DA secreting striatal neurons show enhanced neurite extension which culminates in the formation of synaptic-like contacts between the two cell types (Zhang et al., 2000). Thus the morphogenic effects of DA exhibit a complex regulatory pattern which may depend on the type of tissue, the concentration of DA, and the receptor expressed by target cells.

The above data illustrate that both ACh and DA exert powerful morphogenic effects on many in vitro preparations. The fact that these afferents reach their cortical

targets during periods of synaptogenesis implies that they are in a position to influence the structural development of these cells. Furthermore, the handful of lesion studies available support the idea that morphogenic effects of these transmitters also occur in vivo (see below).

Origins and topographical organization of cortical cholinergic and dopaminergic afferents in the rodent

The ascending cholinergic basal forebrain system arises from discrete subcortical nuclei that project relatively ubiquitously to cortical and limbic areas. The basal forebrain consists of the medial septum (MS), the vertical and horizontal limbs of the diagonal band of Broca (VLDB, HLDB), and the nucleus basalis complex (comprising the ventral pallidum, globus pallidus and nucleus basalis magnocellularis (NBM)) (Mesulam et al., 1983). Neurogenesis in the basal forebrain is complete by E17 (Semba & Fibiger, 1988). Afferents from these nuclei which are immunopositive for the low affinity neurotrophin receptor (p75), and thus presumably cholinergic, invade the cortex at postnatal day (P) 1 and elaborate over the first postnatal week (Koh & Loy, 1989). However, acetylcholinesterase (AChE) positive fibres are not observed in the cortex until P5 (Kiss & Patel, 1992). Furthermore, both AChE and choline acetyltransferase (ChAT) positive fibres elaborate until at least two weeks post-partum (Kiss & Patel, 1992; Mechawar & Descarries, 2001).

The discrepancy between these two studies may relate to Koh and Loy's assumption that the p75 positive fibres emanating from the basal forebrain were

exclusively cholinergic. The fact that p75 is expressed relatively ubiquitously in the brain during development (Eckenstein, 1988; Friedman et al., 1991; Yan & Johnson, 1988) suggests that this may not have been the case. Indeed, p75 expression does not restrict itself to the cholinergic basal forebrain until the second week of life (Koh & Higgins, 1991). Alternatively, cholinergic fibres may reach their cortical targets perinatally, as suggested by Koh and Loy, but not release ACh until P5. Delayed release of ACh is supported by the observation that whole brain ChAT activity is low between E15 and P7 (8% of adult levels), but increases linearly thereafter (Coyle & Yamamura, 1976). The above studies demonstrate that cholinergic afferents reach their cortical targets sometime during the first postnatal week, but increased expression of cholinergic markers does not begin until the second week of life.

Four main sources of extrinsic cholinergic innervation have been identified in the rat neocortex. The first originates in the MS/VLDB. Fibres from these cells course forward to the genu of the corpus callosum, where they fasciculate and swing backward, travelling above the cingulate bundle to innervate the ACC and RSC (Eckenstein et al., 1988). The next originates in the HLDB. In the human, the HLDB primarily innervates the olfactory bulb (Mesulam et al., 1983). However in the rat, cholinergic cells from this nucleus also project strongly to the RSC (Gonzalo-Ruiz & Morte, 2000), piriform and entorhinal cortices (Eckenstein et al., 1988). Innervation to the rest of the cortex is derived mainly from the nucleus basalis complex. Fibres from this area fan out and pass through the internal and external capsule to innervate all remaining areas of cortex, including the mPFC (Eckenstein et al., 1998). Lastly, in addition to the innervation

provided by the basal forebrain, the tegmental area provides a weak innervation to medial frontal regions (Eckenstein et al., 1988).

Many cortical regions exhibit characteristic laminar patterns of innervation which are frequently associated with their nucleus of origin. To complicate matters, these patterns can overlap in areas of shared innervation. The sum total of these afferents, along with intrinsic cholinergic axons stemming from a subset of bipolar neurons (Eckenstein et al., 1988; Houser et al., 1985), produces the regionally-specific, laminar distribution of cholinergic fibres in the cortex. For example, the granular RSC (RSG) is innervated by the MS/VLDB and exhibits particularly dense bands of cholinergic terminals immediately above and below layers II/III. In contrast, the agranular RSC is innervated by the same nucleus, yet exhibits dense banding through layers I-III and V (Eckenstein et al., 1988; Houser et al., 1985).

Some rodent cortical areas do not exhibit any marked laminar input pattern. These areas include the mPFC, ACC, entorhinal, piriform, and motor cortices (Eckenstein et al., 1988; Houser et al., 1985; Mechawar et al., 2000). The mPFC is innervated primarily by the NBM, from which afferents often terminate in a banded formation which is densest in layers I-III and V (Eckenstein et al., 1988). The ACC is innervated primarily by the MS/VLDB, from which afferents exhibit varying patterns of lamination depending on the cortical region (Eckenstein et al., 1988). However, since inputs from the tegmentum also impinge upon these regions, it is not known whether the absence of lamination is characteristic of basal forebrain afferents in these regions or whether their pattern is merely disguised by overlapping inputs from other sources.

Forebrain dopaminergic innervation stems principally from two small nuclei in the ventral midbrain: the substantia nigra (SN) and the ventral tegmental area (VTA). Neurogenesis in these regions is complete by E14 (Voorn et al., 1988), and tyrosine hydroxylase positive fibres can be detected in the deep layers of the anterior cortical region by E16/17 (Kalsbeek et al., 1988; Verney et al., 1982). By E20, innervation has spread to the deep layers of the cingulate and rhinal cortices, and continues to extend laterally and caudally, albeit less densely, for several days after birth (Verney et al., 1982). Tyrosine hydroxylase positive fibres appear in the superficial layers of anterior cortical regions beginning at P2/3, after which they spread caudally and less densely to the occipital cortex (Berger et al., 1985b; Kalsbeek et al., 1988). These fibres elaborate profusely over the next three weeks, however adult levels of innervation are not achieved until about P60 (Berger et al., 1985b; Kalsbeek et al., 1988).

In the rat, there are two main classes of cortical dopaminergic afferents (Berger et al., 1991). The first arises from the VTA and reaches its cortical targets prenatally. Innervation from this nucleus is limited to deep cortical layers V and VI, and is detectable in most cortical regions (Berger et al., 1991; Descarries et al., 1987). These fibres are most prominent in the mPFC, and exhibit a rostrocaudal, mediolateral gradient of decreasing density.

The second class of dopaminergic afferent arises from the SN and reaches its targets during the first postnatal week (Berger et al., 1991). Innervation from nigral afferents is restricted to superficial layers I-III of the frontal, midline and rhinal cortices (Descarries et al., 1987). Innervation is heaviest in the superficial layers of the ACC,

particularly in layer III, and becomes considerably less dense in other midline and rhinal regions. The dopaminergic input to the RSC is relatively minor, however fibres can be detected in superficial layers, particularly in layer III (Berger et al., 1985a).

The above data illustrate that both cholinergic and dopaminergic afferents impinge upon midline areas of cortex while synaptogenesis is ongoing. These cortical areas have been implicated in cognitive functions such as spatial and working memory, associative learning, and attention. Changes to the microcircuitry of these regions could potentially disrupt these behaviours. In support of this, a handful of studies demonstrate that neonatal lesions of ascending cholinergic and dopaminergic afferents produce aberrant cortical morphology and behavioural impairments.

Consequences of neonatal cholinergic and dopaminergic deafferentation

Electrolytic lesions of the mouse NBM at P1, while producing only transient reductions in cortical ChAT activity and AChE fibre staining, are associated with persistent cytoarchitectural abnormalities in the somatosensory cortex (Hohmann et al., 1988). Nissl stain was used to identify a blurring of laminar boundaries between layers III, IV and V, a delay in the emergence of layer IV, and cortical thinning throughout this region. The lesioned mice displayed behavioural impairments which persisted into adulthood, namely deficits in water maze acquisition and in retention of a passive avoidance task, which these authors attributed to aberrant cortical morphology resulting from cholinergic deafferentation (Bachman et al., 1994; Hohmann et al., 1988). However, a later study from this group which examined dendritic changes using the Golgi technique

cast doubt on this hypothesis (Hohmann et al., 1991). A reduction in soma size and extent of both apical and basal dendritic trees in layer V somatosensory pyramidal cells was found one week following the lesion, but by two weeks post-lesion, many of these effects had normalized. No further time points were investigated. Despite this, these researchers maintain that the transient morphological changes associated with perinatal cholinergic deafferentation produce persistent deficits in behaviour (e.g., see Berger-Sweeney & Hohmann, 1997).

The above reports are confounded by the fact that electrolytic lesions are not specific for cholinergic neurons. However, about 10 years ago a new type of toxin was developed which could selectively lesion basal forebrain cholinergic cells (Wiley et al., 1991). This toxin, called 192 IgG-saporin (192SAP), selectively targets basal forebrain cholinergic cells using an antigen which is unique to these cells. 192 IgG is a monoclonal antibody to p75, the low-affinity nerve growth factor (NGF) receptor, which in adulthood is expressed almost exclusively on basal forebrain cholinergic cells (Woolf et al., 1989). The antibody is coupled to the plant toxin saporin, which is granted access to the cell via internalization of the 192 IgG-p75 receptor complex. Saporin then enzymatically disables ribosomes and arrests protein synthesis, resulting in cell death. Intracerebroventricular (i.c.v.) administration of 192SAP in adult rats results in selective destruction of p75-bearing cholinergic cells, while sparing the cells of other transmitter systems (Book et al., 1992; Heckers et al., 1994; Leanza et al., 1995; Roßner et al., 1995; Waite et al., 1994; Walsh et al., 1995; Wiley et al., 1991). Lesions of this type result in large depletions of cortical and hippocampal ChAT activity which exceed those produced by excitotoxic

lesions (Robinson et al., 1996).

In the neonate, 192SAP lesions made at P4 or P7 produce large depletions of cortical and hippocampal ChAT activity which persist for at least 8 months (Leanza et al., 1996; Pappas et al., 1996; Ricceri et al., 1997). However, lesions made at P1 do not significantly reduce ChAT activity (Ricceri et al., 1997). This suggests that perinatally, cholinergic cells do not express enough p75 receptors to be vulnerable to 192SAP toxicity. Furthermore, the fact that other cell types express p75 transiently during the first week of life, increases the likelihood of collateral damage to other systems by perinatal administration of 192SAP.

Neonatal lesion studies using 192SAP have thus far shed little light on the data provided by Hohmann and colleagues. For example, unlike electrolytic lesions, administration of as little as 400 ng of 192SAP at either P4 or P7 produces large and persistent depletions of cortical and hippocampal ChAT activity (Leanza et al., 1996; Pappas et al., 1996). However spatial memory deficits in the water maze are absent, even with higher doses (Sherren et al., 1999). Furthermore, cortical thinning in adulthood has not been observed (Leanza et al., 1996; Pappas et al., 1996). Still, this does not preclude the possibility that dendritic changes went undetected, since both studies used a Nissl stain to make their assessments.

One study has detected changes in dendritic morphology following a neonatal 192SAP lesion (Robertson et al., 1998). This group administered i.c.v. injections of 192SAP on P1 (100 ng) and P3 (200 ng), and reported a reduction in the number of first order apical dendrites and dendritic spine density in layer V pyramidal cells of the visual

cortex. However, the morphological assessments did not continue past the second week of life, nor were the rats assessed behaviourally. Thus no conclusions about the permanency of dendritic changes could be made, nor could they be related to behaviour.

Only one study has examined the effects of neonatal DA depletion on cortical morphology. Rats receiving bilateral thermal lesions of the VTA on P2 exhibited profound reductions in total dendritic length and branching of the basal tree of large layer V pyramidal cells in the mPFC at P36 (Kalsbeek et al., 1989c). However, this study was confounded by damage to serotonergic fibres of passage, which were transected in the course of creating the lesion. These authors noted no morphological effects of transecting these fibres alone, however the possibility that a synergistic effect between the two lesions occurred, cannot be discounted.

While the dendritic regression noted after the VTA lesion was substantial, the behavioural assessment of these rats was limited to activity and exploration in the open field (Kalsbeek et al., 1989a). Furthermore, these data were tainted by the confounding serotonergic lesion. In this regard, it is noteworthy that the hypoactivity these rats displayed was contrary to the reported effects of the selective dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA).

6-OHDA is similar in structure to the endogenous monoamines, and once injected into the brain it gains access to the cell through normal transmitter reuptake. Once inside, 6-OHDA forms free radicals and inhibits mitochondrial respiratory chain complexes I and IV (Glinka et al., 1997). When a selective DA lesion is desired, animals must be pretreated with a compound which blocks the reuptake of norepinephrine (NE) in order to prevent

damage to this system. Tricyclic antidepressants which selectively block NE reuptake, such as desmethylinipramine (DMI) or pargyline, are most commonly used for this purpose.

Numerous studies have investigated the effects of selective DA lesions made with 6-OHDA on the development of motor and cognitive behaviours. For example, neonatal 6-OHDA lesions have been used extensively to model attention deficit hyperactivity disorder, since large lesions at either P3 or P5 result in a transient period of hyperactivity during the first month of life (Archer et al., 1988; Davids et al., 2002; Luthman et al., 1997; Shaywitz et al., 1976, 1984; Takasuna & Iwasaki, 1996; Zhang et al., 2002). The hyperactivity is thought to result from receptor supersensitivity, since DA depleted rats are hyper-responsive to DA agonists (Kim et al., 2000; Neal-Beliveau & Joyce, 1999; Zhou & Palmiter, 1995).

Cognitive difficulties have also been reported following neonatal 6-OHDA lesions. Administration of 70 µg to neonatal rats (35 µg on P3 and a further 35 µg on P6) produces a lesion which is sufficiently mild that growth rate and locomotor activity remain unaffected (Heffner & Seiden, 1983). However, these rats are severely impaired at acquiring an operant conditioning response. Working memory is also reportedly impaired, regardless of the size of DA depletion. Specifically, intracisternal administration of either 50, 75 or 200 µg of 6-OHDA at P3 increases the number of errors and time taken to find all the food rewards in an eight arm radial maze (Luthman et al., 1997).

In contrast, spatial deficits in the water maze following neonatal 6-OHDA administration are reportedly dose-dependent, such that rats receiving 50 µg are

indistinguishable from controls but rats receiving doses of either 75 or 200 μg are impaired (Luthman et al., 1997). Rats in the 75 μg group were able to locate the platform before the trial ended, however they made only minimal improvements in platform location latency across days. Rats receiving 200 μg were never able to acquire the task. Striatal DA levels were reduced by 73%, 89% and 98% in the low, medium and high dose 6-OHDA groups respectively, suggesting that DA transmission must be reduced beyond a certain threshold before disruption of effective search behaviour in the water maze occurs.

The fact that lesions of similar size cause impairments in one task but not another may be related to the degree of stress engendered by each test. For example, footshock increases DA synthesis and utilization in control rats, but after thermal lesions of the VTA at P2, this response is either blunted or lost (Feenstra et al., 1992). Rats lesioned with 150 μg of 6-OHDA on P3 are not able to increase striatal DA efflux in response to an amphetamine challenge (Castañeda et al., 1991). Together, these data suggest that a sufficiently compromised DA system cannot synthesize enough DA to cope with the demand brought on by a stressful task.

In summary, these data suggest that neonatal cholinergic and dopaminergic lesions result in morphological changes in the cerebral cortex, which may in turn contribute to lasting behavioural impairments in these rats. However, in all but one study the data have been generated using non-selective lesion techniques. In addition, the permanence of these changes has not been addressed, which clouds the interpretation of the accompanying behaviour. Studies which examine dendritic arborization and spine density in cortical neurons in adulthood, as well as behaviour, are needed before any definitive conclusions

about the effects of these deafferenting lesions can be drawn.

Clinical implications

The relationship between early cholinergic and dopaminergic dysfunction, neuronal structure and behaviour may be particularly relevant to the understanding of neurodevelopmental disorders such as Rett and Down syndrome. Both disorders result from genetic defects with far-reaching consequences. In the case of Down syndrome, a full or partial trisomy can potentially affect hundreds of genes on chromosome 21. Rett syndrome is a gender-dependent disorder, the result of a single gene mutation occurring on the X chromosome (Amir et al., 1999). The afflicted gene, methyl-CpG-binding protein 2, codes for a global transcription repressor without which normal DNA silencing does not take place. Like Down syndrome, many hundreds or perhaps even thousands of genes are potentially affected.

Rett girls exhibit a normal pre- and perinatal period of development followed by a rapid decline in cognitive and psychomotor skills between six and eighteen months (Hagberg, 1995). The period of rapid regression usually stabilizes before 10 years of age. However, girls are left severely impaired in language and psychomotor ability.

Neurochemically, the Rett brain is characterized by decreases in ChAT activity in the cortex, hippocampus, thalamus and basal ganglia (Wenk et al., 1991). Not surprisingly, a decrease in the number of ChAT immunopositive cells in the basal forebrain has also been reported (Wenk & Hauss-Wegrzyniak, 1999). There are also significant reductions in NGF immunoreactivity in the frontal cortex (Lipani et al., 2000), which may be related to

the loss of cholinergic afferents, since NGF is trophic to these neurons (Gutiérrez et al., 1997; Ruberti et al., 2000). Evidence, albeit less robust, implicates DA hypofunction as well. Decreases in the number of melanin-containing cells in the SN, pars compacta, and in the number of DA reuptake sites in the caudate nucleus have been reported (Kitt & Wilcox, 1995; Wenk, 1997), as have decreases in the size of the caudate nucleus (Reiss et al., 1993; Subramaniam et al., 1997).

Down children are born with poor reflexes and muscle tone, and exhibit varying degrees of mental retardation. Developmental milestones such as sitting, standing and walking are achieved slowly. One study has noted decreased numbers of DA neurons in the VTA (Mann et al., 1987). In contrast, reduced cholinergic parameters are a well-established feature of the adult Down brain (Casanova et al., 1985; Coyle et al., 1986; Godridge et al., 1987). Cholinergic dysfunction is not yet apparent in second trimester fetal Down tissue (Lubec et al., 2001), suggesting that this system begins to develop normally, but becomes dysfunctional either late in gestation or sometime after birth. Furthermore, Alzheimer-like pathology in the form of neurofibrillary tangles and amyloid plaques invariably occurs as Down patients age (Mann et al., 1989), suggesting that this developmental disorder has a neurodegenerative component as well.

Abnormalities in cortical cytoarchitecture have been identified in the Rett brain, including reductions in arborization of basal pyramidal cell dendrites in the frontal and motor cortices and apical pyramidal dendrites in the motor cortex, as well as a reduction in dendritic spines (Armstrong et al., 1995, 1998; Belichenko et al., 1994). Widespread dendritic dysgenesis is also characteristic of Down syndrome, with reductions in length

and spine density of the basal tree emerging shortly after birth (Becker et al., 1986; Kaufmann & Moser, 2000; Takashima et al., 1981).

One aspect which is common to the clinical features of Rett syndrome and the neurochemical attributes of Down syndrome is a seemingly normal developmental period followed by a decline that occurs during a period of intense synaptogenesis. Because dendritic arborization and spine density may be modulated in part by signals received from cortically-projecting transmitter systems, the absence or hypofunction of these afferent fibres, particularly during critical periods of development, may contribute to the pathogenesis of secondary symptoms such as dendritic atrophy and ultimately, cognitive impairment.

RESEARCH PLAN

This study investigated the consequences of both single and combined neonatal cholinergic and dopaminergic lesions on neurochemistry, morphology and behaviour in adult male and female rats. No published record of a combined lesion exists, thus a novel surgical protocol was developed to create a dual ACh and DA lesion. The first step was to gather preliminary behavioural data from single and dual lesioned rats, and to establish the efficacy of these lesions through neurochemical assessments. These data also provided a basis for selecting cortical regions for the morphometric analysis. Next, further behavioural tests were conducted based on the behavioural performance of the previous groups of rats. Tissue from these rats was prepared for an analysis of dendritic

morphology. Third, the profound and intractable behavioural impairment observed in the DA rats was further investigated, by examining the sensitivity of these rats to swim stress, pharmacological manipulation of DA transmission, and sensorimotor gating via prepulse inhibition (PPI) of the acoustic startle response. The organization of these experiments is diagrammed in Figure 1. This study represents the first comprehensive assessment of dendritic morphology following early cholinergic and dopaminergic lesions. As such the data gathered herein provide new and definitive insights into the effects of these neurotransmitters on cortical development and their relationship with behaviour in adulthood.

Figure 1. Flow chart depicting the overall layout of experiments in this project. The first squad of single and combined ACh and DA lesioned rats was tested in the open field followed by the hidden platform water maze. These rats were sacrificed for neurochemistry. Because the rats with DA lesions exhibited such a profound performance impairment in the hidden platform test, the second squad of rats was tested in the open field followed by habituation to the water maze, and then cued platform testing. These rats were sacrificed for morphometry. A third squad of female control and DA lesioned rats was then created to further investigate the intactable behavioural impairment observed in the DA rats. The age of the rats at the time of testing is given in brackets.

METHODS

Animals

Thirty-nine female Sprague-Dawley rats were obtained 14 to 16 days pregnant from Charles River Laboratories (St-Constant, PQ) and were maintained on a 12 hour reversed light/dark schedule with free access to water and standard rat chow. The day of birth was designated P1, and the litters were culled to to 4 males and 4 females each on P2. In order to prevent maternal factors from influencing the results, each lesion type was assigned to one or more rats within each litter. The four groups of lesioned rats were thereby distributed across litters as evenly as possible. The lesions were administered on P7 (see below), after which the pups were monitored daily for general health. Beginning at P12-P14, each maternity cage was supplemented with rat chow moistened with water and a palatable protein supplement (chocolate flavoured Boost™). Supplementation was discontinued after weaning for most rats. However several cages of very small rats were given rat chow moistened with water for an additional two weeks.

Offspring were weaned between P23 and P28 and housed in pairs until sacrifice. The experimenter was blind to group membership following weaning, and remained so until all experimental procedures were complete. Body weights were monitored throughout the experiment, beginning on P28.

Surgery

On P7, the dam was removed from the home cage and was not returned until all the pups had fully regained consciousness. The home cage was placed on a heating pad to keep the pups warm in the interim. In order to block the uptake of 6-OHDA by NE terminals, subcutaneous injections of 10 mg/kg of DMI (Sigma-Aldrich) were administered in the nape of the neck. All pups received DMI one hour prior to surgery. The pup was then placed in a large nose cone and anaesthetized with a 3-4% halothane/oxygen mixture. Once a surgical plane of anaesthesia had been reached, the pup was placed in a plaster body mould mounted in a stereotaxic frame. The mould contained an opening in front of the nose through which a tube delivering 1.5-2.5% halothane/oxygen was passed to maintain anaesthesia throughout the procedure.

A midline incision was made to expose the skull, and two holes were drilled ± 1.8 mm lateral to Bregma. A Hamilton microsyringe fitted with a 30 gauge needle tip was slowly lowered 3.5 mm from the dura. 1.5 μ l of either the neurotoxin or vehicle solution was slowly infused into each lateral ventricle. The injections were made slowly over a period of 2 minutes and the needle was left in place for an additional 2 minutes to permit diffusion away from the injection site. The needle was then slowly retracted to prevent the toxin from being drawn into the needle tract. The incision was closed with tissue adhesive (3M) and an ear punch was used to designate group membership. The pups were warmed and returned to the home cage once they had regained consciousness.

The neurotoxins consisted of 1) a 0.2 μ g/ μ l solution of 192SAP (Advanced Targeting Systems Inc.) in vehicle to create the ACh lesion (300 ng/ventricle, or 600 ng of

192SAP total); 2) a 50 $\mu\text{g}/\mu\text{l}$ solution of 6-OHDA hydrobromide (Sigma-Aldrich) in vehicle to create the DA lesion (50 $\mu\text{g}/\text{ventricle}$, or 100 μg total, of 6-OHDA measured as free base); 3) 0.2 $\mu\text{g}/\mu\text{l}$ of 192SAP plus 50 $\mu\text{g}/\mu\text{l}$ solution of 6-OHDA hydrobromide in vehicle to create the dual lesion (600 ng of 192SAP plus 100 μg of 6-OHDA (free base) total). The vehicle solution was the same for each toxin, and consisted of phosphate buffered saline (PBS) plus 0.2 mg/ml ascorbic acid to prevent oxidation of the 6-OHDA. The 192SAP was diluted to the appropriate concentration and stored in aliquots at $-80\text{ }^{\circ}\text{C}$. It was thawed immediately before use, stored at $4\text{ }^{\circ}\text{C}$ between surgeries, and was not kept for more than 48 hours. The 6-OHDA solution was freshly made for each litter immediately before surgery, and was stored at $4\text{ }^{\circ}\text{C}$ in the dark between surgeries to minimize oxidation. Control rats received 1.5 μl per ventricle of the vehicle solution only.

In total, 14 male and 25 female pups received the vehicle solution, and are referred to as the control group; 15 male and 16 female pups received the 192SAP alone, and are referred to as the ACh group; 16 male and 65 female pups received the 6-OHDA alone, and are referred to as the DA group; and 24 male and 20 female pups received the 192SAP/6-OHDA cocktail, and are referred to as the dual group.

Open Field Test

Testing began between 3 and 3.5 months of age. All of the rats destined for water maze testing followed by neurochemistry or morphometry were initially tested in the open field. The groups consisted of 14 male and 14 female controls, 14 male and 14 female ACh, 12 male and 16 female DA, and 16 male and 16 female dual rats. The open field

apparatus consisted of a grey plexiglas box which was 80 cm long, 80 cm wide and 60 cm deep. The floor was demarcated by a 20 cm X 20 cm grid formation. Each rat was given one exposure to the apparatus per day over three consecutive days. An exposure consisted of placing the rat in a corner of the open field and allowing 5 minutes for exploration. Behaviour was video recorded and scored for 1) number of centre versus surround gridline crosses (all 4 paws cross a line), 2) number of wall rears (rat stands on hind legs with front paws touching the wall), 3) number of centre rears (rat stands on hind legs facing the centre field), 4) number of grooming episodes (licking, combing, scratching or biting any part of the body).

Hidden Platform Water Maze

Testing began 4 days after the completion of the open field test, at approximately 3.5 months of age. The groups consisted of 5 male and 5 female control, 6 male and 7 female ACh, 4 male and 6 female DA, and 5 male and 6 female dual rats. The water maze consisted of a polypropylene pool 1.6 m in diameter filled with tepid water ($21 \pm 1^\circ\text{C}$). The room contained ample visual cues by which the rats could spatially map the room (i.e., the door, the cage rack, the computer screen, the experimenter). To further facilitate spatial mapping, black cardboard was cut into various shapes and sizes and affixed to the walls.

The pool was divided into quadrants along two axes (north-south and east-west) to define four equidistant start points along the pool's perimeter. A clear, Plexiglas platform was submerged about 2 cm below the surface of the water in the centre of one of

the quadrants. Each daily session consisted of four swim trials, one from each start point along the perimeter (randomized daily). The rats were given 90 seconds in which to find the platform. If unsuccessful, they were guided to the platform by the experimenter. The rats remained on the platform for 15 seconds between trials. The latency to reach the platform was averaged across the four trials to yield a single latency score per session. Rats were given one session of hidden platform testing per day for five days. On the sixth and seventh day, cued navigation was assessed by suspending a black cardboard cube (11 cm³) directly above the hidden platform, about 20 cm from the surface of the water. The platform remained in the same quadrant throughout testing. These rats were subsequently sacrificed for neurochemistry.

Water Maze Habituation Procedure

Testing began 4 days after completion of the open field test, at approximately 3.5 months of age. The groups consisted of 9 male and 9 female control, 7 male and 7 female ACh, 8 male and 10 female DA, and 11 male and 10 female dual rats. The apparatus was the same as that described above, except that the temperature of the water was raised to 25 ± 1 °C, and was maintained at that temperature for all subsequent water maze testing. The habituation procedure consisted of gradually exposing the rats to a cued platform water maze task in four stages. Day 1 consisted of placing the rats in the pool on a raised platform for 60 seconds (rats stay dry). Day 2 consisted of placing the rats in the pool on a submerged platform for 60 seconds (paws get wet). On day 3, the rats were placed in the water next to the submerged platform and guided onto it (rats get wet). The last day

consisted of placing the rats in the pool about 30 cm away from the submerged platform and guiding them to it (rats swim). The black cube was suspended above the platform throughout habituation. These rats began cued platform testing the following day.

Cued Platform Water Maze

Testing began immediately after the completion of habituation and proceeded as for the hidden platform version. However in this version of the water maze, the visual cue was suspended above the platform throughout testing and the platform was moved to a new quadrant each day. Testing was performed for 10 consecutive days. These rats were subsequently sacrificed for morphometry.

Plasma Corticosterone Response to Forced Swim

Plasma corticosterone (CORT) was assessed after water maze exposure in a group of 11 female control and 35 female DA rats when they were approximately 3.5 months of age. Only female rats were prepared for this experiment in order to prevent attrition due to a change in housing conditions. Since social versus individual housing conditions can potentially influence the outcome of a stress study, the rats needed to remain socially housed throughout the duration testing in order to be included in the analysis. The female DA rats seemed less aggressive than the males, and thus less likely to require separation from their cagemate due to fighting. These rats had not previously experienced any behavioural testing.

Each rat was removed from the home room and quickly brought to another room for the baseline blood sample. The rat was then transported to the water maze room, where it was given one 90 second forced swim trial, similar to the normal water maze procedure but without any escape platform. It was then removed from this room and blood samples were taken immediately and at 10, 20, 40 and 60 minutes after the swim. Blood samples were collected between 0830 and 1300 hrs in order to minimize the influence of circadian variations.

To obtain the blood samples, each rat was quickly wrapped in a towel. The tail was warmed in hot tap water for a few seconds before removing the tip of the tail with a sterile razor blade. Approximately 200 μ l of blood was collected into iced eppendorf tubes containing 15 μ l of 10% EDTA to prevent clotting. The tail was then placed in a beaker of ice to provide analgesia and constrict the blood vessels. The tip of the tail was coated in styptik to prevent bleeding between collection intervals. Irritation of the wound was sufficient to promote bleeding for the collection of subsequent samples. After the last sample had been collected, the wound was closed securely with silver nitrate.

The blood samples were spun at 3600 rpm for 15 minutes at 4 °C. The plasma was aliquoted and stored at -80 °C until use. Plasma CORT levels were determined using a tritiated immunoassay kit from ICN Biochemicals. The standards were performed in triplicate and the samples in duplicate. Data was expressed as μ g per dL.

Effect of DA Agonist and Antagonist on Water Maze Performance

At approximately 6 months of age, 29 female DA rats who had participated in the stress study were randomly divided into three groups before receiving water maze testing. The first group (N = 10) received sterile saline, the second group (N = 9) received l-dopa (50 mg/kg, Sigma-Aldrich), and the third group (N = 10) received a combination of both the D1 antagonist SCH23390 (0.1 mg/kg, Sigma-Aldrich) and the D2 antagonist raclopride (2 mg/kg, Sigma-Aldrich). All drugs were dissolved in sterile saline and injected subcutaneously 60 minutes before testing began. All rats received four sessions of hidden platform water maze followed four days later by four sessions of cued platform water maze (see above for description).

Prepulse Inhibition

At approximately 7 months of age, sensorimotor gating was assessed using PPI of the acoustic startle reflex in 9 female control rats who had participated in the stress study and 10 female DA rats who had both participated in the stress study and who were injected with saline for the drug study. The apparatus consisted of startle chambers and transduction equipment, and the associated computer program (San Diego Instruments). The chambers were housed in sound attenuating enclosures with the background noise level set to 35 dB. The rats were given a habituation session of 20 trials using a 105 dB white noise pulse, followed by a testing session of 40 randomized trials using either the 105 dB pulse alone or in conjunction with one of 60 dB, 70 dB, 80 dB or 90 dB white noise prepulses. Baseline startle magnitude was calculated from the pulse alone trials

during the testing session. Prepulse startle data was expressed as a percentage of the baseline value.

Neurochemistry

Five male and 5 female control, 7 male and 7 female ACh, 5 male and 6 female DA and 5 male and 6 female dual rats who had been tested in the open field and hidden platform water maze were sacrificed for determination of brain monoamines and ChAT activity. The rats were briefly anaesthetized with CO₂ and decapitated two days following the completion of behavioural testing. The brains were quickly removed and placed on ice while the following areas were dissected out: hippocampus, septum, hypothalamus, midbrain (containing the SN, VTA and thalamus), hindbrain (containing the locus coeruleus and pons/medulla), and caudate. The cortical mantle was laid flat and dissected into three parts, as follows. A coronal cut was made immediately caudal to the last visible piece of lateral olfactory tract to separate the frontal region from the rest of the cortex (see top panel of Figure 2). This piece of cortex contained the mPFC and ACC, lateral frontal areas as well as some parietal cortex, and was designated the "anterior region". Next, 2 sagittal cuts were made in the remaining cortical mantle approximately 2 mm to either side of the midline (see bottom panel of Figure 2). This piece of cortex contained the RSC and some occipital areas, and was designated "RSC". The remaining piece of cortex contained temporal (and entorhinal), as well as some parietal and occipital areas, and was designated "temporal cortex". Samples were immediately frozen in isopentane and stored at -80 °C until processing. The left half of

Figure 2. Schematic depiction of the blunt dissection of the cortex. The top panel indicates the location of the coronal cut which separated the anterior cortex from the cortical mantle. The bottom panel depicts a brain section at the level of this coronal cut. The lines illustrate the location of the sagittal cuts which separated the RSC from the temporal region. Abbreviations: ACC, anterior cingulate cortex; Amyg, amygdala; CBL, cerebellum; cc, corpus callosum; Cg1, cingulate cortex, area 1; Cg2, cingulate cortex, area 2; CPu, caudate putamen; FL, somatosensory cortex, forelimb region; Fr1, motor cortex, area 1; Fr2, motor cortex, area 2; GP, globus pallidus; HL, somatosensory cortex, hindlimb region; HPC, hippocampus; Hyp, hypothalamus; IC, inferior colliculus; ic, internal capsule; Ins, insular cortex; OCC, occipital cortex; OLF, olfactory bulb; ot, optic tract; Par1, primary somatosensory cortex; Par2, secondary somatosensory cortex; Pir, piriform cortex; SC, superior colliculus; Thal, thalamus; vhc, ventral hippocampal commissure. Both figures were adapted from Paxinos & Watson (1998).

Schematic of the Cortical Dissection

each area was used for determination of monoamines and the right half was used for determination of ChAT activity, with the exception of the hypothalamus, brainstem and the septum, which were used in their entirety for either monoamines or ChAT respectively.

Levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), NE, 3-methoxy,4-hydroxyphenethylene glycol (MHPG), serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were determined using high performance liquid chromatography with electrochemical detection. The samples were processed using a modification of the method of Seegal et al. (1986). Levels were expressed as ng per mg of protein in the sample.

ChAT activity was determined using the method of Fonnum (1975). Briefly, samples were homogenized in 10 mM EDTA, 0.5% Triton X-100, and incubated for one hour at 37 °C in 20 mM EDTA, 300 mM NaCl, 50 mM NaPO₄, 8 mM choline bromide, 0.1 mM physostigmine, and 0.4 mM [³H]acetyl coenzyme A, pH 7.4. The radiolabelled ACh was extracted into BetaMax-ES scintillation cocktail (ICN) using Kalignost solution (10 mg sodium tetraphenylboron in 5 ml PB, 2 ml acetonitrile). Radioactivity in the sample was measured using a Hewlett Packard beta counter. Protein levels were determined by the method of Lowry (BioRad). Chat activity was expressed as nmol of ACh synthesized per hour, per mg of protein in the sample.

Morphometry

Nine male and 9 female control, 7 male and 7 female ACh, 8 male and 10 female DA, and 11 male and 10 female dual rats who received the cued platform water maze test were sacrificed for the morphological analyses. One month after completion of behavioural testing (about 5 - 5.5 months of age), the rats were deeply anaesthetized with sodium pentobarbitol and perfused with 200 ml of 0.9% saline. The brains were removed and placed in Golgi-Cox solution (Glaser & van der Loos, 1981) for 14 days in the dark. The Golgi-Cox solution was replaced with 30% sucrose, and the brains were kept for at least five days in the dark before sectioning. A vibratome was used to cut 200 μ m thick sections through the entire cortical mantle. The sections were mounted onto 2% gelatin coated slides and stained using the method of Gibb and Kolb (1998).

The analysis was restricted to the female rats, because they sustained the greatest behavioural impairment. The cortical areas analyzed were chosen based on existing anatomical literature, and the results of the neurochemical analyses. They consisted of 1) layer V pyramidal cells of the mPFC, corresponding to Zilles' (1985) area Cg3; 2) layer III pyramidal cells of the ACC, corresponding to Zilles' areas Cg1 and Cg2; and 3) layer III pyramidal cells of the RSG, corresponding to Zilles' area RSG. A camera lucida attached to an Olympus BX51 microscope was used to trace out the entire postsynaptic dendritic field of each cell, viewed with the 20X objective. Cells were selected from the optical planes located in the middle third of the tissue depth such that the apical and basal trees were contained entirely within the 200 μ m slice, and were not heavily obscured by blood vessels

or dense clusters of dendrites from neighbouring cells. Data was collected from the apical and basal dendritic trees and averaged for ten cells per cortical area per rat.

Figure 3 illustrates the morphometric analyses. A Sholl diagram, on which the concentric rings lie 20 μm apart, was used to estimate total dendritic length in both the apical and basal trees. The frequency of dendrites passing through each concentric ring of the Sholl diagram provided a measure of length as a function of distance from the cell body. Each branch segment was counted and assigned a branch order using the method of Coleman and Riesen (1968). Briefly, the main apical shaft was considered zero order, and apical branches arising directly from it were considered 1st order. Every bifurcation of the branch increased the order of the subsequent segment by one. Zero order was not assigned to branch segments in the basal dendritic field. Basal dendrites arising directly from the cell body were considered 1st order, with every bifurcation of the branch increasing the order of the subsequent segment by one. Spine density was determined from higher order branch segments (3rd or above) from both the apical tuft and basal tree using the 40X objective. A typical dendrite segment was 40-50 μm in length, on which the spines were drawn in and then counted. Spines were expressed as a density measure, or number per 10 μm length of dendrite. Spines located either above or beneath the dendrite are not visible with this method, so the values obtained likely underestimated the true spine density.

Figure 3. Diagram of a typical pyramidal cell. The concentric rings of the Sholl diagram are spaced 20 μm apart, and are used to estimate dendrite length. Each branch segment is numbered and counted to provide an index of complexity. Adapted from Kolb et al. (1998).

Data Analysis

All data were analyzed using SPSS 9.0 for Windows. The main analyses were performed with either a univariate or repeated measures analysis of variance (ANOVA). The statistical design of each analysis is described at the beginning of each subsection of the Results. ANOVA summary tables are contained in Appendix A. Post hoc tests were performed using Tukey's least significant difference (LSD), and correlations were determined using Pearson's product moment correlation coefficient (r).

RESULTS

General Health and Body Weight

Of the 44 pups who received dual lesions over the course of all experiments, 6 pups (5 male, 1 female) failed to thrive and died or were euthanized within three weeks of surgery. Of the 83 pups receiving DA lesions, 12 pups (1 male, 11 female) died or were euthanized within three weeks of surgery. Of the 31 ACh lesioned pups, two females died within one week of surgery. No mortality was observed in the control group (39 pups). Of the remaining rats, one male dual lesioned rat was euthanized at two months of age due to apparent kidney failure, and one female DA rat died at five months of unknown causes.

The surviving rats all appeared active and free of motility problems. However 3 male and 5 female DA rats, and 1 male and 8 female dual rats, exhibited oral motor dysfunctions such as compulsive licking and biting. This behaviour was not self-mutilatory as the rats always directed their energy toward external objects such as bedding, cage lids and lab coats. Of these rats, 1 male and 2 female DA, and 6 female dual rats repeatedly gnawed on the cage lid bars to the point of creating a calloused lesion on their snout. In fact, 2 of the female dual rats got their teeth caught in the lids while doing this, and were so severely injured from trying to extricate themselves that they were dropped from the study. Oral motor abnormalities did not emerge until the rats were at least 10 weeks of age, but were apparent in all affected rats by 14 weeks and persisted until sacrifice, at approximately 20 weeks. While this behaviour was not noted in either the controls or the ACh rats, 2 male ACh rats scratched compulsively under one ear to the point of creating

an open wound. These 2 rats were not tested behaviourally. Finally, four pairs of co-housed rats had to be separated and housed singly due to fighting (one male DA pair, one female DA pair, one male DA/dual pair and one female DA/dual pair). These rats were subsequently dropped from all further analyses.

Body weight was measured weekly from weaning age (4 weeks) to the time of sacrifice (19 weeks) in a cohort of 9 male and 9 female controls, 8 male and 7 female ACh, 11 male and 11 female DA and 13 male and 11 female dual rats who were destined for the morphological analyses. The data were analyzed using ANOVA with age as a repeated measure, and 192SAP, 6-OHDA and sex as the between subjects variables.

6-OHDA profoundly reduced body weight ($F(1,71)=30.32$, $p<0.001$). However the 192SAP X 6-OHDA interaction from the overall ANOVA ($F(1,71)=7.31$, $p<0.01$) indicated that 192SAP and 6-OHDA administered in conjunction had a different effect than when the toxins were administered alone. It can be seen from Figures 4 and 5 that despite 192SAP by itself reducing body weight, it also attenuated the weight reduction due to 6-OHDA. Furthermore, the significant age X 192SAP X 6-OHDA interaction ($F(12,852)=8.88$, $p<0.001$) indicated that this effect became more marked as the rats aged.

A significant age X 6-OHDA X sex interaction ($F(12,852)=6.30$, $p<0.001$) indicated that the 6-OHDA treatment affected the sexes differently depending on their age. Figures 4 and 5 show that the separation between 6-OHDA and non-6-OHDA rats was accelerated in females.

A significant effect of 192SAP was not detected in the overall ANOVA. However

Figure 4. Graph of male body weight. Both DA ($F(1,71)=30.32$, $p<0.001$) and ACh rats ($F(1,29)=12.14$, $p<0.001$) had lower body weights. However, weight loss due to 6-OHDA was attenuated by co-administration of the toxins ($F(1,71)=7.31$, $p<0.01$).

Male Body Weight

Figure 5. Graph of female body weight. Both DA ($F(1,71)=30.32$, $p<0.001$) and ACh rats ($F(1,29)=12.14$, $p<0.001$) had lower body weights. However, weight loss due to 6-OHDA was attenuated by co-administration of the toxins ($F(1,71)=7.31$, $p<0.01$).

Female Body Weight

the data clearly showed a difference between the control and ACh groups. When these groups were analyzed separately, 192SAP significantly reduced body weight in both males and females ($F(1,29)=12.14$, $p<0.001$).

Neurochemistry

Neurochemical analyses were performed in a group of 5 male and 5 female control, 7 male and 7 female ACh, 5 male and 6 female DA, and 5 male and 6 female dual rats. However one male and one female DA rat appeared to have incomplete DA lesions. They exhibited only a 25-30% decrease in striatal DA, compared to the 90% reduction seen in the remainder of the DA rats. These two rats were from the same litter and would therefore have received the same batch of 6-OHDA solution. It is possible that in these two cases the toxin was not prepared at the correct dose. Accordingly, these rats were removed from the analysis, bringing the number of DA rats to 4 male and 5 female. Since there were not enough subjects per group to analyze the data using multivariate statistics, each dependent measure was analyzed using a univariate ANOVA with 192SAP treatment, 6-OHDA treatment and sex as the between subjects variables. In order to control for type 1 errors, effects were considered statistically significant only at or below the 0.01 level. Post hoc tests between the control and lesion group means were performed with Tukey's LSD. NS denotes "not significantly different compared to control".

Anterior Cortex

ChAT activity was reduced by 33% in the ACh group ($p < 0.05$, LSD) and 31% in the dual group ($p < 0.05$, LSD) with respect to control levels ($F(1,36)=91.22$, $p < 0.001$) (Figure 6). No other significant effects on ChAT activity were detected. DA levels were reduced by 73% in the DA group ($p < 0.05$, LSD) and 79% in the dual group ($p < 0.05$, LSD) ($F(1,36)=70.78$, $p < 0.001$) (Figure 7). DOPAC and HVA were similarly reduced by 59% and 49% in the DA group ($p < 0.05$, LSD), and 70% and 77% in the dual group ($p < 0.05$, LSD) (DOPAC, $F(1,36)=129.23$, $p < 0.001$; HVA, $F(1,36)=10.11$, $p < 0.01$). NE levels were increased by the 6-OHDA treatment ($F(1,36)=13.01$, $p = 0.001$), by 17% in DA rats and 44% in dual rats (Figure 8). However only the dual rats were significantly elevated compared to control ($p < 0.05$, LSD). No effects on 5-HT were observed (Figure 9).

Retrosplenial Cortex

Figure 10 illustrates the 73% ($p < 0.05$, LSD) and 71% ($p < 0.05$, LSD) decrease in ChAT activity observed in the ACh and dual groups after the 192SAP treatment ($F(1,36)=880.37$, $p < 0.001$). DA levels were unaffected by either treatment (Figure 11). The ACh and dual groups also exhibited increases in NE levels of 33% (NS, LSD) and 63% ($p < 0.05$, LSD) respectively ($F(1,36)=16.93$, $p < 0.001$) (Figure 12). The effects on 5-HT are illustrated in Figure 13. A significant 6-OHDA X sex interaction was detected for 5-HT levels ($F(1,36)=7.32$, $p = 0.01$). Male DA and dual rats showed a 259% (NS, LSD) and 263% ($p < 0.05$, LSD) increase in 5-HT levels respectively, whereas female DA and

Figure 6. ChAT activity in the anterior cortex. ChAT activity was reduced by 33% in the ACh group ($p < 0.05$, LSD) and 31% in the dual group ($p < 0.05$, LSD) with respect to control levels ($F(1,36)=91.22$, $p < 0.001$).

Anterior Cortex

Figure 7. DA, DOPAC and HVA levels in the anterior cortex. DA levels were reduced by 73% in the DA group ($p < 0.05$, LSD) and 79% in the dual group ($p < 0.05$, LSD) ($F(1,36)=70.78$, $p < 0.001$). DOPAC and HVA were similarly reduced, by 59% and 49% in the DA group ($p < 0.05$, LSD), and 70% and 77% in the dual group ($p < 0.05$, LSD) (DOPAC, $F(1,36)=129.23$, $p < 0.001$; HVA, $F(1,36)=10.11$, $p < 0.01$).

Anterior Cortex

Figure 8. NE and MHPG levels in the anterior cortex. NE levels were increased by 17% in DA rats and 44% in dual rats ($F(1,36)=13.01$, $p=0.001$). However only the dual rats were significantly elevated compared to control ($p<0.05$, LSD).

Anterior Cortex

Figure 9. 5-HT and 5-HIAA levels in the anterior cortex. No significant effects of either toxin were observed.

Anterior Cortex

Figure 10. ChAT activity in the RSC. ChAT activity was reduced by 73% in the ACh group ($p < 0.05$, LSD) and 71% in the dual group ($p < 0.05$, LSD) after the 192SAP treatment ($F(1,36) = 880.37$, $p < 0.001$).

Retrosplenial Cortex

Figure 11. DA, DOPAC and HVA Levels in the RSC. No significant effects of either toxin were observed.

Retrosplenial Cortex

Figure 12. NE and MHPG levels in the RSC. The ACh and dual groups exhibited increases in NE levels of 33% (NS, LSD) and 63% ($p<0.05$, LSD) respectively ($F(1,36)=16.93$, $p<0.001$).

Retrosplenial Cortex

Figure 13. 5-HT and 5-HIAA levels in the RSC. Male DA and dual rats showed a 259% (NS, LSD) and 263% ($p < 0.05$, LSD) increase in 5-HT levels respectively, whereas female DA and dual rats did not differ from control (6-OHDA X sex interaction, ($F(1,36)=7.32$, $p=0.01$)). However, elevated levels of 5-HIAA were detected in 6-OHDA rats regardless of sex (140% increase in DA rats ($p < 0.05$, LSD) and 86% increase in dual rats (NS, LSD)), $F(1,36)=7.39$, $p=0.01$), indicating that 5-HT usage was increased in both male and female 6-OHDA rats.

Retrosplenial Cortex

dual rats did not differ from control. However, significantly elevated levels of 5-HIAA were detected in 6-OHDA rats regardless of sex (140% increase in DA rats ($p < 0.05$, LSD) and 86% increase in dual rats (NS, LSD)), $F(1,36)=7.39$, $p=0.01$), indicating that 5-HT usage was increased in both male and female 6-OHDA rats.

Temporal Cortex

ChAT activity was reduced after 192SAP by 41% in the ACh group ($p < 0.05$, LSD) and 35% in the dual group ($p < 0.05$, LSD) ($F(1,36)=118.12$, $p < 0.001$) (Figure 14). The levels of DA and its metabolites are illustrated in Figure 15. DA levels were reduced after 6-OHDA by 41% in the DA group (NS, LSD) and 46% in the dual group ($p < 0.05$, LSD) ($F(1,36)=24.94$, $p < 0.001$). DOPAC was also reduced by 48% (NS, LSD) and 32% (NS, LSD) in these groups respectively ($F(1,36)=11.24$, $p < 0.01$). While HVA levels exhibited similar reductions (30% and 41% in the DA and dual groups), this effect was not statistically significant. Interestingly, the ACh rats showed a 68% increase in DA levels and a 70% increase in DOPAC levels. While these effects did not achieve the 0.01 level of statistical significance (192SAP X 6-OHDA interaction, $F(1,36)=5.62$, $p=0.02$), they do suggest that increased utilization of DA in the temporal cortex is a consequence of neonatal 192SAP lesions.

No effects on NE or 5-HT levels were observed following either lesion. These data are illustrated in Figures 16 and 17 respectively.

Figure 14. ChAT activity in the temporal cortex. ChAT activity was reduced after 192SAP by 41% in the ACh group ($p < 0.05$, LSD) and 35% in the dual group ($p < 0.05$, LSD) ($F(1,36) = 118.12$, $p < 0.001$).

Temporal Cortex

Figure 15. DA, DOPAC and HVA Levels in the temporal cortex. DA levels were reduced by 41% in the DA group (NS, LSD) and 46% in the dual group ($p < 0.05$, LSD) ($F(1,36) = 24.94$, $p < 0.001$). DOPAC was also reduced by 48% (NS, LSD) and 32% (NS, LSD) in these groups respectively ($F(1,36) = 11.24$, $p < 0.01$). HVA levels exhibited similar reductions (30% and 41% in the DA and dual groups), but this effect was not statistically significant. ACh rats showed a 68% increase in DA levels and a 70% increase in DOPAC levels (192SAP X 6-OHDA interaction, $F(1,36) = 5.62$, $p = 0.02$), suggesting that increased utilization of DA in the temporal cortex is a consequence of neonatal 192SAP lesions.

Temporal Cortex

Figure 16. NE and MHPG levels in the temporal cortex. No significant effects of either toxin were observed.

Temporal Cortex

Figure 17. 5-HT and 5-HIAA levels in the temporal cortex. No significant effects of either toxin were observed.

Temporal Cortex

Hippocampus

As seen in Figure 18, ChAT activity after the 192SAP treatment was reduced in the hippocampus by 78% ($p < 0.05$, LSD) and 74% ($p < 0.05$, LSD) in the ACh and dual groups respectively ($F(1,36) = 662.07$, $p < 0.001$). No effects on DA levels were observed (Figure 19). NE levels were elevated by the 192SAP treatment ($F(1,36) = 8.50$, $p < 0.01$), but only the ACh group was statistically different from control (26% increase, $p < 0.05$, LSD) (Figure 20). No changes in 5-HT levels were observed in any group (Figure 21).

Medial Septum

The MS was assayed solely for ChAT activity, the data from which are illustrated in Figure 22. ChAT levels were reduced by 31% ($p < 0.05$, LSD) and 35% ($p < 0.05$, LSD) in the ACh and dual groups respectively ($F(1,36) = 59.97$, $p < 0.001$).

Caudate

ChAT activity was slightly but significantly reduced following the 192SAP treatment, by 12% in both the ACh and dual groups ($F(1,36) = 9.47$, $p < 0.01$; $p < 0.05$, LSD) (Figure 23). As shown in Figure 24, the 6-OHDA treatment produced a 90% loss of DA in both the DA and dual groups ($F(1,36) = 226.85$, $p < 0.001$; $p < 0.05$, LSD). DOPAC was reduced by 62% ($p < 0.05$, LSD) and 70% ($p < 0.05$, LSD) in these groups respectively ($F(1,36) = 71.28$, $p < 0.001$). HVA was also reduced, by 44% in DA rats (NS, LSD) and 48% in dual rats (NS, LSD), but this effect just missed the 0.01 level of significance

Figure 18. ChAT activity in the hippocampus. ChAT activity was dramatically reduced after the 192SAP treatment, by 78% ($p < 0.05$, LSD) and 74% ($p < 0.05$, LSD) in the ACh and dual groups respectively ($F(1,36)=662.07$, $p < 0.001$).

Hippocampus

Figure 19. DA, DOPAC and HVA levels in the hippocampus. No significant effects of either toxin were observed.

Hippocampus

Figure 20. NE and MHPG levels in the hippocampus. NE levels were elevated by the 192SAP treatment ($F(1,36)=8.50$, $p<0.01$), but only the ACh group was statistically different from control (26% increase, $p<0.05$, LSD). No effects on MHPG were observed.

Hippocampus

Figure 21. 5-HT and 5-HIAA levels in the hippocampus. No significant effects of either toxin were observed.

Hippocampus

Figure 22. ChAT activity in the medial septum. ChAT levels were reduced by 31% ($p < 0.05$, LSD) and 35% ($p < 0.05$, LSD) in the ACh and dual groups respectively ($F(1,36) = 59.97$, $p < 0.001$).

Medial Septum

Figure 23. ChAT activity in the caudate. ChAT activity was slightly but significantly reduced following the 192SAP treatment, by 12% in both the ACh and dual groups ($F(1,36)=9.47$, $p<0.01$; $p<0.05$, LSD).

Caudate

Figure 24. DA, DOPAC and HVA levels in the caudate. The 6-OHDA treatment produced a 90% loss of DA in both the DA and dual groups ($F(1,36)=226.85$, $p<0.001$; $p<0.05$, LSD). DOPAC was reduced by 62% ($p<0.05$, LSD) and 70% ($p<0.05$, LSD) in these groups respectively ($F(1,36)=71.28$, $p<0.001$). HVA was also reduced, by 44% in DA rats (NS, LSD) and 48% in dual rats (NS, LSD), but this effect just missed the 0.01 level of significance.

Caudate

Figure 25. NE levels in the caudate. No significant effects of either toxin were found. MHPG levels were undetectable in this nucleus.

Caudate

Figure 26. 5-HT and 5-HIAA levels in the caudate. No significant effects of either toxin were found.

Caudate

($F(1,36)=6.93$, $p=0.012$). No significant effects on NE (Figure 25) or 5-HT (Figure 26) levels were found.

Midbrain

6-OHDA reduced DA levels by 32% in the DA group ($p<0.05$, LSD) and by 20% (NS, LSD) in the dual group ($F(1,36)=8.10$, $p<0.01$) (Figure 28). No other significant effects were found. Graphs of ChAT activity, NE and 5-HT levels are located in Figures 27, 29 and 30, respectively.

Hypothalamus

The hypothalamus was assayed solely for catecholamines. No significant effects of the 6-OHDA or 192SAP treatment were observed in any of the transmitters or metabolites assayed. These data are illustrated in Figures 31 (DA levels), 32 (NE levels), and 33 (5-HT levels).

Brainstem

The brainstem was assayed solely for catecholamines. No significant effects of the 6-OHDA or 192SAP treatment were observed in any of the transmitters or metabolites assayed. These data are illustrated in Figures 34 (DA levels), 35 (NE levels), and 36 (5-HT levels).

Figure 27. ChAT activity in the midbrain. No significant effects of either toxin were found.

Midbrain

Figure 28. DA, DOPAC and HVA levels in the midbrain. 6-OHDA reduced DA levels by 32% in the DA group ($p < 0.05$, LSD) and by 20% (NS, LSD) in the dual group ($F(1,36)=8.10$, $p < 0.01$).

Midbrain

Figure 29. NE and MHPG levels in the midbrain. No significant effects of either toxin were found.

Midbrain

Figure 30. 5-HT and 5-HIAA levels in the midbrain. No significant effects of either toxin were found.

Midbrain

Figure 31. DA, DOPAC and HVA levels in the hypothalamus. No significant effects of either toxin were found.

Hypothalamus

Figure 32. NE and MHPG levels in the hypothalamus. No significant effects of either toxin were found.

Hypothalamus

Figure 33. 5-HT and 5-HIAA levels in the hypothalamus. No significant effects of either toxin were found.

Hypothalamus

Figure 34. DA, DOPAC and HVA levels in the brainstem. No significant effects of either toxin were found.

Brainstem

Figure 35. NE and MHPG levels in the brainstem. No significant effects of either toxin were found.

Brainstem

Figure 36. 5-HT and 5-HIAA levels in the brainstem. No significant effects of either toxin were found.

Brainstem

Pyramidal Cell Morphology

Morphological assessments were made on layer V pyramidal cells of the mPFC, layer III pyramidal cells of the ACC, and layer III pyramidal cells of the RSG, because these areas sustained the greatest loss of ChAT activity and DA transmission following the neonatal lesions. Three control, 1 ACh, 2 DA and 3 dual rats were not analyzed due to poor quality of the Golgi-Cox stain. Thus the groups consisted of 6 control, 6 ACh, 8 DA and 7 dual rats, for all measures except spine density, where the groups consisted of 5 control, 6 ACh, 5 DA and 5 dual female rats.

Data from the apical and basal dendritic trees were analyzed separately using a univariate ANOVA with 192SAP and 6-OHDA as the independent variables. Analyses of total dendrite length, total number of branches and spine density were considered significant at or below the 0.05 level. A priori tests were used to compare the control mean to each of the lesioned group means for each of these measures, regardless of whether or not the overall ANOVA was significant. In order to further characterize the total length and branching data, these variables were deconstructed into a measure of dendrite frequency as a function of distance from the cell body (number of dendrites crossing each concentric ring of the Sholl diagram), and branch frequency at each rank order. These variables were analyzed in the same way as the total length and branching data, without a priori testing. Lastly, measures of total length and number of branches were correlated with spine density within each group using Pearson's product moment correlation coefficient.

Layer V Pyramidal Cells in the mPFC

Sketches of representative layer V pyramidal cells from the mPFC are shown in Figure 37.

Apical Tree

Dendrite Length. The 192SAP treatment reduced the total length of the apical tree by 14% in ACh rats ($p < 0.05$, LSD) and 24% in dual rats ($p < 0.05$, LSD) ($F(1,23)=11.35$, $p < 0.01$). The total length of apical trees in the DA rats was reduced by 8%, but the main effect of 6-OHDA just missed significance ($F(1,23)=3.74$, $p=0.065$). These data are illustrated in Figure 38.

The concentric ring analyses revealed an interesting pattern of lesion-induced changes. The 6-OHDA treatment reduced apical dendrites from areas proximal to the cell body, specifically in the frequency of dendrites crossing through the first 14 rings (3rd ($F(1,23)=4.70$, $p < 0.05$), 4th ($F(1,23)=4.43$, $p < 0.05$), 6th ($F(1,23)=4.89$, $p < 0.04$), 8th ($F(1,23)=5.22$, $p < 0.04$), 9th ($F(1,23)=4.63$, $p < 0.05$), 10th ($F(1,23)=7.57$, $p < 0.02$), 12th ($F(1,23)=8.30$, $p < 0.01$), 13th ($F(1,23)=7.43$, $p < 0.02$), 14th ($F(1,23)=9.57$, $p < 0.01$)). Conversely, the 192SAP treatment reduced apical dendrites from areas distal to the cell body, beginning at the 9th concentric ring (9th ($F(1,23)=8.94$, $p < 0.01$), 10th ($F(1,23)=12.58$, $p < 0.01$), 14th ($F(1,23)=4.32$, $p < 0.05$), 15th ($F(1,23)=5.48$, $p < 0.03$), 19th ($F(1,23)=5.65$, $p < 0.03$), 21st ($F(1,23)=5.01$, $p < 0.04$), 26th ($F(1,23)=4.37$, $p < 0.05$), 27th ($F(1,23)=9.47$, $p < 0.01$), 28th ($F(1,23)=11.01$, $p < 0.01$), 29th ($F(1,23)=13.08$, $p=0.001$), 30th ($F(1,23)=7.40$,

Figure 37. Sketches of representative layer V pyramidal cells from the mPFC. The apical dendritic tree is represented in black, and the basal tree is in red. Note the selective regression in the apical tuft produced by the ACh lesion. The DA lesion predominantly produced regression of oblique, first order apical dendrites. The basal tree was slightly smaller in the lesioned cells, but these effects did not achieve statistical significance.

Layer V Pyramidals in the mPFC

Figure 38. Total apical dendrite length in the mPFC. The 192SAP treatment reduced the total length by 14% in ACh rats ($p < 0.05$, LSD) and 24% in dual rats ($p < 0.05$, LSD) ($F(1,23)=11.35$, $p < 0.01$). Total apical length was reduced by 8% in the DA rats, but the effect of 6-OHDA just missed significance ($F(1,23)=3.74$, $p=0.065$).

Total Apical Dendrite Length in Layer V Pyramidals of the mPFC

Figure 39. Sholl analysis of the mPFC apical tree. 6-OHDA reduced apical dendrites from areas proximal to the cell body, specifically in the frequency of dendrites crossing through the 3rd ($F(1,23)=4.70$, $p<0.05$), 4th ($F(1,23)=4.43$, $p<0.05$), 6th ($F(1,23)=4.89$, $p<0.04$), 8th ($F(1,23)=5.22$, $p<0.04$), 9th ($F(1,23)=4.63$, $p<0.05$), 10th ($F(1,23)=7.57$, $p<0.02$), 12th ($F(1,23)=8.30$, $p<0.01$), 13th ($F(1,23)=7.43$, $p<0.02$), and 14th rings ($F(1,23)=9.57$, $p<0.01$). Conversely, 192SAP reduced apical dendrites from areas distal to the cell body, specifically in dendrites crossing through the 9th ($F(1,23)=8.94$, $p<0.01$), 10th ($F(1,23)=12.58$, $p<0.01$), 14th ($F(1,23)=4.32$, $p<0.05$), 15th ($F(1,23)=5.48$, $p<0.03$), 19th ($F(1,23)=5.65$, $p<0.03$), 21st ($F(1,23)=5.01$, $p<0.04$), 26th ($F(1,23)=4.37$, $p<0.05$), 27th ($F(1,23)=9.47$, $p<0.01$), 28th ($F(1,23)=11.01$, $p<0.01$), 29th ($F(1,23)=13.08$, $p=0.001$), 30th ($F(1,23)=7.40$, $p<0.02$), and 31st rings ($F(1,23)=4.45$, $p<0.05$).

Sholl Analysis of Layer V Pyramidals in the mPFC: Apical Tree

$p < 0.02$), 31st ($F(1,23)=4.45$, $p < 0.05$)). These data are represented graphically in Figure 39.

Branch Number. The 192SAP treatment reduced the total number of apical branches by 12% in ACh rats (NS, LSD) and 24% in dual rats ($p < 0.05$, LSD) ($F(1,23)=8.39$, $p < 0.01$) (Figure 40). The branch order analyses revealed a similar pattern to that observed with the concentric rings. The 6-OHDA treatment reduced the number of 1st order branches ($F(1,23)=14.38$, $p=0.001$), which emanate from the main apical shaft between the soma and the tuft. Higher order branches were unaffected by 6-OHDA. In contrast, the effect of 192SAP was localized in higher order branches (3rd ($F(1,23)=4.78$, $p < 0.04$), 5th ($F(1,23)=5.73$, $p < 0.03$)), which are predominantly found in the distal tuft. These effects are illustrated graphically in Figure 41.

Spine Density. As shown in Figure 42, apical spine density was reduced by 26% in the ACh rats ($p < 0.05$, LSD) and by 30% in the duals ($p < 0.05$, LSD) ($F(1,17)=6.19$, $p < 0.03$). No effect of 6-OHDA was detected.

Correlations. Spine density was not correlated with either total length or number of branches within any of the groups. The associated r and p values are displayed in Table 1.

Figure 40. Total number of apical branches in the mPFC. 192SAP reduced the total number of apical branches by 12% in ACh rats (NS, LSD) and 24% in dual rats ($p < 0.05$, LSD) ($F(1,23) = 8.39$, $p < 0.01$).

**Total Apical Branches in
Layer V Pyramidals of the mPFC**

Figure 41. Apical branch order analysis in the mPFC. The 6-OHDA treatment reduced the number of 1st order branches ($F(1,23)=14.38$, $p=0.001$), which emanate from the main apical shaft between the soma and the tuft. In contrast, the effect of 192SAP was localized in higher order branches (3rd ($F(1,23)=4.78$, $p<0.04$), 5th ($F(1,23)=5.73$, $p<0.03$)), which are predominantly found in the distal tuft.

Apical Branch Order Analysis in Layer V Pyramidals of the mPFC

Figure 42. Apical spine density in the mPFC. Spine density was reduced by 26% in the ACh rats ($p < 0.05$, LSD) and by 30% in the duals ($p < 0.05$, LSD) ($F(1,17) = 6.19$, $p < 0.03$). No effect of 6-OHDA was detected.

**Apical Spine Density in
Layer V Pyramidals of the mPFC**

Spine Density	Total Length	Total Branching
Control	$r = -0.807$ $p = 0.099$	$r = -0.644$ $p = 0.241$
DA	$r = -0.414$ $p = 0.488$	$r = -0.479$ $p = 0.414$
ACh	$r = -0.242$ $p = 0.644$	$r = -0.288$ $p = 0.579$
Dual	$r = 0.741$ $p = 0.152$	$r = 0.629$ $p = 0.256$

Table 1. Correlations between apical mPFC variables. Spine density was correlated with total length and total branches in each of the four groups. No significant correlations were found.

Basal Tree

Dendrite Length. Neither the 192SAP nor the 6-OHDA treatments had a significant effect on the total length of the basal tree. However a priori tests showed that the 18% reduction observed in the dual rats was statistically different from control ($p < 0.05$, LSD) (Figure 43).

The concentric ring analyses revealed that 6-OHDA reduced the frequency of dendrites crossing through ring 1 only ($F(1,23)=5.04$, $p < 0.04$), whereas 192SAP had an effect only at the 2nd ring ($F(1,23)=4.63$, $p < 0.05$). However neither effect was marked enough to achieve significance in the overall ANOVA on total length. These data are shown in Figure 44.

Branch Number. Figure 45 illustrates the effect of 192SAP and 6-OHDA on total branching. The 192SAP treatment ($F(1,23)=10.73$, $p < 0.01$) reduced the total number of branches in the basal tree by 10% in the ACh rats (NS, LSD) and 16% in the duals ($p < 0.05$, LSD). No effect of 6-OHDA was detected.

The branch order analyses revealed that 192SAP reduced higher order branching, namely at the 4th ($F(1,23)=9.47$, $p < 0.01$) and 5th orders ($F(1,23)=6.43$, $p < 0.02$) (Figure 46).

Spine Density. Spine density in the basal tree was reduced by 25% in ACh ($p < 0.05$, LSD), 20% in DA (NS, LSD) and 34% in dual rats ($p < 0.05$, LSD), as illustrated in Figure 47. However, only the main effect of 192SAP achieved statistical significance ($F(1,17)=6.18$, $p < 0.03$).

Figure 43. Total basal dendrite length in the mPFC. Neither the 192SAP nor the 6-OHDA treatments had a significant effect on the total length of the basal tree. However a priori tests showed that the 18% reduction observed in the dual rats was statistically different from control ($p < 0.05$, LSD).

**Total Basal Dendrite Length in
Layer V Pyramidals of the mPFC**

Figure 44. Sholl analysis of the basal tree in the mPFC. 6-OHDA reduced the frequency of dendrites crossing through ring 1 only ($F(1,23)=5.04$, $p<0.04$), whereas 192SAP had an effect only at the 2nd ring ($F(1,23)=4.63$, $p<0.05$).

Sholl Analysis of Layer V Pyramidals in the mPFC: Basal Tree

Figure 45. Total number of basal branches in the mPFC. 192SAP reduced the total number of branches in the basal tree by 10% in the ACh rats (NS, LSD) and 16% in the duals ($p < 0.05$, LSD) ($F(1,23) = 10.73$, $p < 0.01$). No effect of 6-OHDA was detected.

**Total Basal Branches in
Layer V Pyramidals of the mPFC**

Figure 46. Basal branch order analysis in the mPFC. 192SAP reduced higher order branching, namely at the 4th ($F(1,23)=9.47$, $p<0.01$) and 5th orders ($F(1,23)=6.43$, $p<0.02$). No effect of 6-OHDA was detected.

Basal Branch Order Analysis in Layer V Pyramidals of the mPFC

Figure 47. Basal spine density in the mPFC. 192SAP reduced spine density by 25% in ACh ($p < 0.05$, LSD) and 34% in dual rats ($p < 0.05$, LSD) ($F(1,17) = 6.18$, $p < 0.03$). Spines were reduced by 20% in DA rats (NS, LSD), but the effect of 6-OHDA was not significant.

**Basal Spine Density in
Layer V Pyramidals of the mPFC**

Spine Density	Total Length	Total Branching
Control	$r = -0.264$ $p = 0.668$	$r = -0.393$ $p = 0.513$
DA	$r = 0.515$ $p = 0.375$	$r = 0.421$ $p = 0.480$
ACh	$r = 0.466$ $p = 0.351$	$r = -0.099$ $p = 0.852$
Dual	$r = 0.525$ $p = 0.364$	$r = 0.626$ $p = 0.258$

Table 2. Correlations between basal mPFC variables. Spine density was correlated with total length and total branches in each of the four groups. No significant correlations were found.

Correlations. Spine density was not correlated with either total length or number of branches within any of the groups. The associated r and p values are displayed in Table 2.

Layer III Pyramidals in the ACC

Sketches of representative layer III pyramidal cells from the ACC are shown in Figure 48.

Apical Tree

Dendrite Length. The 192SAP treatment reduced total dendrite length by 12% in ACh and 10% in dual rats ($F(1,23)=5.48$, $p<0.03$). However post hoc tests revealed that neither group differed significantly from control. Dendrite length was unaffected by 6-OHDA. A graph of total dendrite length is shown in Figure 49.

The concentric ring analyses (Figure 50) revealed that, like the trend observed in the mPFC, the 192SAP treatment reduced the frequency of dendrites crossing through rings distal to the cell body, namely in the area of the apical tuft (ring 11 ($F(1,23)=4.54$, $p<0.05$), ring 13 ($F(1,23)=4.84$, $p<0.04$)). While the overall effect of 6-OHDA on dendrite length was not significant, this toxin did reduce the frequency of dendrites crossing through ring 2 ($F(1,23)=7.51$, $p<0.02$) and ring 8 ($F(1,23)=4.71$, $p<0.05$).

Figure 48. Sketches of representative layer III pyramidal cells from the ACC. The apical dendritic tree is represented in black, and the basal tree is in red. Note the regression in the apical tuft of the ACh and dual cells. Both 192SAP and 6-OHDA produced mild atrophy of the basal tree.

Layer III Pyramidals in the ACC

Figure 49. Total apical dendrite length in the ACC. 192SAP treatment reduced total dendrite length by 12% in ACh (NS, LSD) and 10% in dual rats (NS, LSD) ($F(1,23)=5.48$, $p<0.03$). Dendrite length was unaffected by 6-OHDA.

**Total Apical Dendrite Length in
Layer III Pyramidals of the ACC**

Figure 50. Apical Sholl analysis in the ACC. 192SAP reduced the frequency of dendrites crossing through rings distal to the cell body, namely in the area of the apical tuft (ring 11 ($F(1,23)=4.54$, $p<0.05$), ring 13 ($F(1,23)=4.84$, $p<0.04$)). While the overall effect of 6-OHDA on dendrite length was not significant, the frequency of dendrites crossing through ring 2 ($F(1,23)=7.51$, $p<0.02$) and ring 8 ($F(1,23)=4.71$, $p<0.05$) were reduced.

Sholl Analysis of Layer III Pyramidals in the ACC: Apical Tree

Branch Number. Similar to the effects on dendrite length, the 192SAP treatment reduced branching by 11% in ACh and 16% in dual rats ($F(1,23)=6.73$, $p<0.02$). However once again, post hoc tests revealed that neither group was significantly different from control (Figure 51). No effect of 6-OHDA was detected.

The analyses of branch orders revealed that 192SAP reduced higher order branching only. Specifically, the number of 4th ($F(1,23)=8.13$, $p<0.01$) and 5th order branches ($F(1,23)=10.27$, $p<0.01$) were decreased. While the effect of 6-OHDA on total branching was not significant, this toxin did significantly decrease the number of 1st order branches ($F(1,23)=5.59$, $p<0.03$), which was similar to its effect in the apical tree of layer V pyramidal neurons in the mPFC. These data are illustrated in Figure 52.

Spine Density. Interestingly, there was a significant 192SAP X 6-OHDA interaction on spine density which indicated that the combined effect of these two lesions was not as severe as could be predicted from either of their effects alone ($F(1,23)=5.53$, $p<0.04$) (Figure 53). Spine density was reduced in both single lesioned groups, by 44% in ACh rats ($p<0.05$, LSD) and 32% in DA rats ($p<0.05$, LSD). However spine loss was not exacerbated in the dual group, which only exhibited a 39% reduction ($p<0.05$, LSD). In other words an additive effect of the lesions, commonly observed when both singly lesioned groups exhibited changes, was not seen here. Furthermore, only the main effect of 192SAP was statistically significant ($F(1,23)=10.60$, $p<0.01$).

Correlations. Spine density was not correlated with either total length or number of branches within any of the groups. See Table 3 for a list of the associated r and p values.

Figure 51. Total number of apical branches in the ACC. 192SAP reduced branching by 11% in ACh (NS, LSD) and 16% in dual rats (NS, LSD) ($F(1,23)=6.73$, $p<0.02$). No effect of 6-OHDA was detected.

**Total Apical Branches in
Layer III Pyramidals of the ACC**

Figure 52. Apical branch order analysis in the ACC. 192SAP reduced higher order branching, specifically in the 4th ($F(1,23)=8.13$, $p<0.01$) and 5th orders ($F(1,23)=10.27$, $p<0.01$). 6-OHDA reduced the number of 1st order branches ($F(1,23)=5.59$, $p<0.03$), which was similar to its effect in the apical tree of layer V pyramidal neurons in the mPFC.

Apical Branch Order Analysis in Layer III Pyramidals of the ACC

Figure 53. Apical spine density in the ACC. Spine density was reduced in both single lesioned groups, by 44% in ACh rats ($p < 0.05$, LSD) and 32% in DA rats ($p < 0.05$, LSD). However spine loss was not exacerbated in the dual group, which only exhibited a 39% reduction ($p < 0.05$, LSD) (192SAP X 6-OHDA interaction, $F(1,23)=5.53$, $p < 0.04$). Thus an additive effect of the lesions, commonly observed when both singly lesioned groups exhibited changes, was not seen here. Furthermore, only the main effect of 192SAP was statistically significant ($F(1,23)=10.60$, $p < 0.01$).

**Apical Spine Density in
Layer III Pyramidals of the ACC**

Spine Density	Total Length	Total Branching
Control	$r = 0.064$ $p = 0.919$	$r = 0.092$ $p = 0.883$
DA	$r = 0.821$ $p = 0.089$	$r = 0.659$ $p = 0.226$
ACh	$r = 0.076$ $p = 0.887$	$r = -0.402$ $p = 0.429$
Dual	$r = 0.299$ $p = 0.625$	$r = 0.636$ $p = 0.249$

Table 3. Correlations between apical ACC variables. Spine density was correlated with total length and total branches in each of the four groups. No significant correlations were found.

Basal Tree

Dendrite Length. As shown in Figure 54, the total length of the basal tree was slightly reduced in all three lesioned groups, by 10% in ACh (NS, LSD), 11% in DA (NS, LSD), and 16% (NS, LSD) in dual rats compared to controls, however no statistically significant effects of 192SAP or 6-OHDA were detected.

The concentric ring analyses (Figure 55) revealed that the 192SAP treatment tended to reduce proximal dendrites crossing through ring 1 ($F(1,23)=5.02$, $p<0.04$). Conversely the 6-OHDA treatment tended to reduce both proximal and distal dendrites crossing through ring 1 ($F(1,23)=7.27$, $p<0.02$) and ring 8 ($F(1,23)=4.74$, $p=0.04$).

Branch Number. Significant main effects of both the 192SAP treatment ($F(1,23)=5.05$, $p<0.04$) and 6-OHDA treatment ($F(1,23)=5.17$, $p<0.04$) were found in total branch number (Figure 56). Length was reduced by 6% in ACh (NS, LSD), 6% in DA (NS, LSD) and 17% in dual rats ($p<0.05$, LSD). The analyses of branch frequency at each order (Figure 57) revealed that only 2nd order branched were affected by 6-OHDA ($F(1,23)=6.76$, $p<0.02$). The effect of 192SAP was significant only at the 3rd order ($F(1,23)=8.46$, $p<0.01$).

Spine Density. As illustrated in Figure 58, spine density was decreased by 35% in ACh ($p<0.05$, LSD), 22% in DA ($p<0.05$, LSD) and 33% in dual rats ($p<0.05$, LSD), yet only the main effect of 192SAP was statistically significant ($F(1,23)=12.87$, $p<0.01$). However the presence of a 192SAP X 6-OHDA interaction which just missed significance ($F(1,23)=3.28$, $p=0.088$) suggested that in fact, spine density in the dual group was not as low as could be anticipated given the size of reductions seen in both

Figure 54. Total basal dendrite length in the ACC. Total length was slightly reduced in all three lesioned groups, by 10% in ACh (NS, LSD), 11% in DA (NS, LSD), and 16% (NS, LSD) in dual rats compared to controls, however no statistically significant effects of 192SAP or 6-OHDA were detected.

**Total Basal Dendrite Length in
Layer III Pyramidals of the ACC**

Figure 55. Basal Sholl analysis in the ACC. 192SAP treatment reduced dendrites crossing through ring 1 ($F(1,23)=5.02$, $p<0.04$). The 6-OHDA treatment reduced dendrites crossing through ring 1 ($F(1,23)=7.27$, $p<0.02$) and ring 8 ($F(1,23)=4.74$, $p=0.04$).

Sholl Analysis of Layer III Pyramidals in the ACC: Basal Tree

Figure 56. Total number of basal branches in the ACC. Significant main effects of both the 192SAP treatment ($F(1,23)=5.05$, $p<0.04$) and 6-OHDA treatment ($F(1,23)=5.17$, $p<0.04$) were found in total branch number. Length was reduced by 6% in ACh (NS, LSD), 6% in DA (NS, LSD) and 17% in dual rats ($p<0.05$, LSD).

**Total Basal Branches in
Layer III Pyramidals of the ACC**

Figure 57. Basal branch order analysis in the ACC. 6-OHDA reduced the number of 2nd order branches only ($F(1,23)=6.76$, $p<0.02$). The effect of 192SAP was significant only at the 3rd order ($F(1,23)=8.46$, $p<0.01$).

Basal Branch Order Analysis in Layer III Pyramidals of the ACC

Figure 58. Basal spine density in the ACC. Spine density was decreased by 35% in ACh ($p < 0.05$, LSD), 22% in DA ($p < 0.05$, LSD) and 33% in dual rats ($p < 0.05$, LSD), yet only the main effect of 192SAP was statistically significant ($F(1,23)=12.87$, $p < 0.01$). The presence of a 192SAP X 6-OHDA interaction which just missed significance ($F(1,23)=3.28$, $p=0.088$) suggested that in fact, spine density in the dual group was not as low as could be anticipated given the size of reductions seen in both singly lesioned groups.

**Basal Spine Density in
Layer III Pyramidals of the ACC**

Spine Density	Total Length	Total Branching
Control	$r = 0.567$ $p = 0.340$	$r = 0.860$ $p = 0.062$
DA	$r = 0.941$ $p = 0.017$	$r = 0.962$ $p = 0.009$
ACh	$r = 0.338$ $p = 0.513$	$r = 0.152$ $p = 0.774$
Dual	$r = 0.314$ $p = 0.606$	$r = 0.590$ $p = 0.295$

Table 4. Correlations between basal ACC variables. Spine density was significantly and positively correlated with total length and total branching in DA rats.

singly lesioned groups.

Correlations. Spine density was not correlated with either length or branching in control, ACh or dual rats. However in DA treated rats, spine density was significantly and positively correlated with total length and branching. This suggests that in DA rats, the cells which exhibited the smallest and simplest basal trees were also those with the fewest synapses. The associated r and p values are listed in Table 4.

Layer III Pyramidals in the RSG

Sketches of representative layer III pyramidal cells from the RSG are shown in Figure 59.

Apical Tree

Dendrite Length. As seen in Figure 60, neither 192SAP nor 6-OHDA had a significant effect on total dendrite length in the apical tree. The concentric ring analyses (Figure 61) indicated that 192SAP reduced the frequency of dendrites crossing through ring 9 ($F(1,23)=6.72$, $p<0.02$), however this effect was not marked enough to achieve significance in the overall ANOVA.

Branch Number. The total number of branches was unaffected by either 192SAP or 6-OHDA (Figure 62). However, 4th order branches were reduced by the 192SAP treatment ($F(1,23)=6.59$, $p<0.02$) (Figure 63).

Figure 59. Sketches of representative cells from the RSG. The apical tree is represented in black, and the basal tree is in red. A slight atrophy of the apical tuft can be seen in the ACh and dual cells. In contrast, 192SAP profoundly reduced the extent of the basal tree.

Layer III Pyramidals in the RSG

Figure 60. Total apical dendrite length in the RSG. Neither 192SAP nor 6-OHDA had a significant effect on total dendrite length in the apical tree.

**Total Apical Dendrite Length in
Layer III Pyramidals of the RSG**

Figure 61. Apical Sholl analysis in the RSG. 192SAP reduced the frequency of dendrites crossing through ring 9 ($F(1,23)=6.72$, $p<0.02$), however this effect was not marked enough to achieve significance in the overall ANOVA of dendrite length.

Sholl Analysis of Layer III Pyramidals in the RSG: Apical Tree

Figure 62. Total number of apical branches in the RSG. The total number of branches was unaffected by either 192SAP or 6-OHDA.

**Total Apical Branches in
Layer III Pyramidals of the RSG**

Figure 63. Apical branch order analysis in the RSG. 192SAP reduced the number of 4th order branches only ($F(1,23)=6.59$, $p<0.02$).

Apical Branch Order in Layer III Pyramidal of the RSG

Spine Density. As illustrated in Figure 64, spine density was reduced by 25% in the ACh group and 15% in the dual group, however the effect of 192SAP did not even approach significance.

Correlations. Spines were not correlated with either total length or branching in any of the groups. See Table 5 for the associated r and p values.

Basal Tree

Dendrite Length. The 192SAP treatment reduced total dendrite length by 23% in ACh rats ($p < 0.05$, LSD), and 20% in the dual rats (NS, LSD) ($F(1,23)=9.38$, $p < 0.01$). No effect of 6-OHDA was observed. Graphical representation of these data is found in Figure 65.

The concentric ring analyses (Figure 66) demonstrated that in 192SAP treated rats, thinning was apparent throughout the basal tree (ring 1 ($F(1,23)=4.66$, $p < 0.05$), ring 2 ($F(1,23)=9.32$, $p < 0.01$), ring 3 ($F(1,23)=9.02$, $p < 0.01$), ring 4 ($F(1,23)=7.45$, $p < 0.02$), ring 5 ($F(1,23)=7.68$, $p < 0.02$), ring 6 ($F(1,23)=4.28$, $p = 0.05$)).

Branch Number. Figure 67 illustrates that total branching was reduced in all three lesioned groups, by 16% in ACh ($p < 0.05$, LSD), 11% in DA ($p < 0.05$) and 17% in dual rats ($p < 0.05$). The effect of 192SAP was statistically significant ($F(1,23)=11.58$, $p < 0.01$), however the effect of 6-OHDA just missed significance ($F(1,23)=3.65$, $p = 0.069$).

The branch order analyses (Figure 68) revealed that 192SAP reduced the number of higher order branches (4th order ($F(1,23)=7.34$, $p < 0.02$), 5th order ($F(1,23)=8.49$,

Figure 64. Apical spine density in the RSG. Spine density was reduced by 25% (NS, LSD) in the ACh group and 15% (NS, LSD) in the dual group, however the effect of 192SAP did not even approach significance.

**Apical Spine Density in
Layer III Pyramidals of the RSG**

Spine Density	Total Length	Total Branching
Control	$r = -0.255$ $p = 0.678$	$r = 0.100$ $p = 0.873$
DA	$r = -0.641$ $p = 0.244$	$r = -0.690$ $p = 0.197$
ACh	$r = -0.174$ $p = 0.742$	$r = -0.357$ $p = 0.487$
Dual	$r = -0.413$ $p = 0.489$	$r = 0.151$ $p = 0.808$

Table 5. Correlations between apical RSG variables. Spine density was correlated with total length and total branches in each of the four groups. No significant correlations were found.

Figure 65. Total basal dendrite length in the RSG. 192SAP reduced total dendrite length by 23% in ACh rats ($p < 0.05$, LSD), and 20% in the dual rats (NS, LSD) ($F(1,23) = 9.38$, $p < 0.01$). No effect of 6-OHDA was observed.

**Total Basal Dendrite Length in
Layer III Pyramidals of the RSG**

Figure 66. Basal Sholl analysis in the RSG. 192SAP resulted in thinning throughout the basal tree (ring 1 ($F(1,23)=4.66$, $p<0.05$), ring 2 ($F(1,23)=9.32$, $p<0.01$), ring 3 ($F(1,23)=9.02$, $p<0.01$), ring 4 ($F(1,23)=7.45$, $p<0.02$), ring 5 ($F(1,23)=7.68$, $p<0.02$), ring 6 ($F(1,23)=4.28$, $p=0.05$)).

Sholl Analysis of Layer III Pyramidals in the RSG: Basal Tree

Figure 67. Total number of basal branches in the RSG. Branching was reduced in all three lesioned groups, by 16% in ACh ($p < 0.05$, LSD), 11% in DA ($p < 0.05$) and 17% in dual rats ($p < 0.05$). The effect of 192SAP was highly significant ($F(1,23)=11.58$, $p < 0.01$), however the effect of 6-OHDA just missed significance ($F(1,23)=3.65$, $p=0.069$).

**Total Basal Branches in
Layer III Pyramidals of the RSG**

Figure 68. Basal branch order analysis in the RSG. 192SAP reduced the number of 4th ($F(1,23)=7.34$, $p<0.02$), 5th ($F(1,23)=8.49$, $p<0.01$), and 6th order branches ($F(1,23)=6.50$, $p<0.02$). The 6-OHDA reduced 3rd order branches only ($F(1,23)=4.53$, $p<0.05$).

Basal Branch Order Analysis of Layer III Pyramidals in the RSG

$p < 0.01$), 6th order ($F(1,23)=6.50$, $p < 0.02$). The 6-OHDA only reduced 3rd order branches ($F(1,23)=4.53$, $p < 0.05$).

Spine Density. Spine density was reduced by 12% in ACh and 22% in dual rats, yet the effect of 192SAP did not achieve significance ($F(1,17)=3.44$, $p=0.088$). However post hoc tests showed that the dual group did significantly differ from control ($p < 0.05$, LSD). These data are illustrated in Figure 69.

Correlations. Spine density was not correlated with either total length or branching in any of the lesioned groups. See Table 6 for a list of r and p values.

Behaviour

Open Field

All rats destined for either neurochemistry or morphology were tested in the open field. The open field data were analyzed using a repeated measures ANOVA with test day as the within subjects variable and 192SAP treatment, 6-OHDA treatment and sex as between subjects variables.

Figure 69. Basal spine density in the RSG. Spine density was reduced by 12% in ACh (NS, LSD) and 22% in dual rats ($p < 0.05$, LSD), yet the effect of 192SAP did not achieve significance ($F(1,17)=3.44$, $p=0.088$).

**Basal Spine Density in
Layer III Pyramidals of the RSG**

Spine Density	Total Length	Total Branching
Control	$r = -0.728$ $p = 0.163$	$r = -0.221$ $p = 0.721$
DA	$r = -0.286$ $p = 0.641$	$r = -0.582$ $p = 0.303$
ACh	$r = -0.404$ $p = 0.427$	$r = 0.261$ $p = 0.617$
Dual	$r = 0.703$ $p = 0.186$	$r = 0.319$ $p = 0.601$

Table 6. Correlations between basal RSG variables. Spine density was correlated with total length and total branches in each of the four groups. No significant correlations were found.

Gridline Crossings. The analysis of gridline crossings (locomotor activity) included 14 male and 13 female control rats, 14 male and 12 female ACh rats, 15 male and 16 female DA rats, and 17 male and 15 female dual rats. The subject numbers listed here are slightly different from those in other open field measures (see Methods and below) due to occasional improper video recording which resulted in missing data for one female control, two female ACh, and one female dual rat.

There was a significant 6-OHDA X sex interaction indicating that the 6-OHDA treatment increased locomotor activity in female but not male rats ($F(1,109)=7.83$, $p<0.01$). 192SAP had no effect on locomotor activity. There was no main effect of test day suggesting that overall, activity levels were similar across days and that habituation to the open field did not occur. However the day X 6-OHDA interaction indicated that DA and dual rats had higher levels of activity on day 2 ($F(2,218)=3.42$, $p<0.04$). The data from males and females are illustrated separately, in Figures 70 and 71.

The gridline crosses were further subdivided into those made in the centre area of the maze, and those made in areas flanking the maze walls. The centre crossings were expressed as a percentage of total gridline crosses, and are illustrated in Figures 72 and 73. The day X 6-OHDA interaction ($F(2,218)=13.23$, $p<0.001$) indicated that the proportion of gridline crossings made in the centre area did not decrease across days in DA or dual rats, as it did in the other groups (main effect of test day, $F(2,218)=24.99$, $p<0.001$). The significant 192SAP X 6-OHDA interaction ($F(1,109)=16.35$, $p<0.001$) indicated that dual rats made more of their crossings in the centre of the maze than either the ACh or DA rats.

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Figure 70. Total gridline crosses in the open field in male rats. Locomotor activity in males from each of the three lesioned groups was similar to control. Activity levels did not habituate across test days.

Total Gridline Crosses in the Open Field: Males

Figure 71. Total gridline crosses in the open field in female rats. Locomotor activity was significantly increased in female DA and dual rats (6-OHDA X sex interaction, $F(1,109)=7.83$, $p<0.01$). 192SAP had no effect on locomotor activity. Activity levels did not habituate across test days.

Total Gridline Crosses in the Open Field: Females

Figure 72. Centre crossings in the open field in male rats. Centre crossings were expressed as a percentage of total crossings. Dual rats made more of their crossings in the centre area of the maze than either the ACh or DA rats (192SAP X 6-OHDA interaction, $F(1,109)=16.35$, $p<0.001$). The proportion of crossings made in the centre area did not decrease across days in DA or dual rats, as it did in the other groups (day X 6-OHDA interaction, $F(2,218)=13.23$, $p<0.001$).

**Percent of Crosses Made in the Centre of the Open Field:
Males**

Figure 73. Centre crossings in the open field in female rats. Centre crossings were expressed as a percentage of total crossings. Dual rats made more of their crossings in the centre of the maze than either the ACh or DA rats (192SAP X 6-OHDA interaction, $F(1,109)=16.35$, $p<0.001$). The proportion of crossings made in the centre area did not decrease across days in DA or dual rats, as it did in the other groups (day X 6-OHDA interaction, $F(2,218)=13.23$, $p<0.001$).

**Percent of Crosses Made in the Centre of the Open Field:
Females**

Rearing. The analysis of rearing (exploratory behaviour) included 14 male and 14 female control rats, 14 male and 14 female ACh rats, 15 male and 16 female DA rats, and 17 male and 16 female dual rats. These data are illustrated in Figures 74 and 75. The significant day X 192SAP ($F(2,224)=3.80$, $p<0.03$) and day X 6-OHDA ($F(2,224)=6.05$, $p<0.01$) interactions indicated that rearing decreased in control but not lesioned rats across days. Thus, habituation was attenuated in the lesioned rats. There were main effects of sex ($F(1,112)=14.00$, $p<0.001$), 192SAP ($F(1,112)=6.93$, $p=0.01$) and 6-OHDA ($F(1,112)=24.35$, $p<0.001$) indicating that females made more rears than males, and that both 192SAP and 6-OHDA reduced rearing behaviour. However the 192SAP X 6-OHDA interaction ($F(1,112)=6.59$, $p<0.02$) indicated that even though both toxins reduced rearing on their own, their co-administration did not exacerbate this reduction. Reductions in exploratory behaviour were similar across all lesioned groups.

Rears were subdivided into those made against the walls and those made toward the centre of the open field (exposing the abdomen), and expressed as a percentage of total rears. One female DA and one male dual rat were excluded from this analysis because on at least one test day they did not exhibit any rearing behaviour whatsoever. Similar to the data on total rears, the significant day X 192SAP ($F(2,220)=4.40$, $p<0.02$) and day X 6-OHDA ($F(2,220)=5.58$, $p<0.01$) interactions indicated that centre rears decreased in control but not lesioned rats across days. More specifically, the day X 6-OHDA X sex interaction ($F(2,220)=3.45$, $p<0.04$) indicated that centre rearing actually increased in male but not female 6-OHDA treated rats across days. Overall, 6-OHDA reduced the proportion of centre rears ($F(1,110)=20.61$, $p<0.001$). However the 192SAP

Figure 74. Total rears in the open field in male rats. Habituation of rearing behaviour was attenuated in the lesioned rats, since rearing decreased in control but not lesioned rats across days (day X 192SAP interaction, $F(2,224)=3.80$, $p<0.03$; day X 6-OHDA interaction, $F(2,224)=6.05$, $p<0.01$). Both 192SAP ($F(1,112)=6.93$, $p=0.01$) and 6-OHDA ($F(1,112)=24.35$, $p<0.001$) reduced rearing behaviour, but co-administration of these toxins did not exacerbate this effect (192SAP X 6-OHDA interaction, $F(1,112)=6.59$, $p<0.02$).

Total Rears in the Open Field: Males

Figure 75. Total rears in the open field in female rats. Habituation of rearing behaviour was attenuated in the lesioned rats, since rearing decreased in control but not lesioned rats across days (day X 192SAP interaction, $F(2,224)=3.80$, $p<0.03$; day X 6-OHDA interaction, $F(2,224)=6.05$, $p<0.01$). Both 192SAP ($F(1,112)=6.93$, $p=0.01$) and 6-OHDA ($F(1,112)=24.35$, $p<0.001$) reduced rearing behaviour, but co-administration of these toxins did not exacerbate this effect (192SAP X 6-OHDA interaction, $F(1,112)=6.59$, $p<0.02$).

Total Rears in the Open Field: Females

X 6-OHDA interaction ($F(1,110)=5.82$, $p<0.02$) indicated that centre rears were not as severely reduced in dual rats. Thus 192SAP appeared to attenuate the effects of 6-OHDA on this particular measure. These data are shown in Figures 76 and 77.

Grooming. Grooming data were available for 14 male and 14 female control rats, 15 male and 14 female ACh rats, 15 male and 16 female DA rats, and 17 male and 16 female dual rats. As can be seen in Figures 78 and 79, the 6-OHDA treatment significantly reduced grooming episodes ($F(1,113)=10.32$, $p<0.01$). There was no overall effect of test day however there was a significant day X 192SAP X 6-OHDA X sex interaction ($F(2,226)=3.4$, $p=0.03$). The frequency of grooming episodes across days decreased in female DA and ACh rats, increased in male ACh rats, but did not change amongst the other groups.

Hidden Platform Water Maze

The rats assigned to this task consisted of 5 male and 5 female controls, 6 male and 7 female ACh, 4 male and 5 female DA, and 5 male and 6 female dual rats destined for the neurochemical analysis. The hidden platform escape latencies were analyzed using a repeated measures ANOVA with 192SAP, 6-OHDA and sex as the between subjects variables and test day as the within subjects variable. The ACh rats had no difficulty with this task. Expectedly, the main effect of test day was highly significant, indicating the presence of a sharp learning curve ($F(4,104)=22.3$, $p<0.001$). However inspection of the data indicated that differences between the control and ACh groups may have been apparent on the first day of testing. Accordingly, the day 1 latencies from these two

Figure 76. Centre rears in the open field in male rats. Centre rears were expressed as a percentage of total rears. The proportion of rears made toward the centre of the maze decreased in control but not lesioned rats across test days (day X 192SAP, $F(2,220)=4.40$, $p<0.02$; day X 6-OHDA, $F(2,220)=5.58$, $p<0.01$). Centre rears actually increased in male but not female 6-OHDA treated rats (see Figure 77) across days (day X 6-OHDA X sex interaction, $F(2,220)=3.45$, $p<0.04$). Furthermore, co-administration of the two toxins in dual rats appeared to attenuate the decrease observed in DA rats on this particular measure (192SAP X 6-OHDA interaction ($F(1,110)=5.82$, $p<0.02$)).

**Percent of Rears Made in the Centre of the Open Field:
Males**

Figure 77. Centre rears in the open field in female rats. Centre rears were expressed as a percentage of total rears. The proportion of rears made toward the centre of the maze decreased in control but not lesioned rats across test days (day X 192SAP, $F(2,220)=4.40$, $p<0.02$; day X 6-OHDA, $F(2,220)=5.58$, $p<0.01$). Centre rears actually increased in male but not female 6-OHDA treated rats (see Figure 76) across days (day X 6-OHDA X sex interaction, $F(2,220)=3.45$, $p<0.04$). Furthermore, co-administration of the two toxins in dual rats appeared to attenuate the decrease observed in DA rats on this particular measure (192SAP X 6-OHDA interaction ($F(1,110)=5.82$, $p<0.02$)).

Percent of Rears Made in the Centre of the Open Field: Females

Figure 78. Grooming episodes in the open field in male rats. The frequency of grooming episodes across days decreased in female DA and ACh rats (compare with Figure 79), increased in male ACh rats, but did not change amongst the other groups (day X 192SAP X 6-OHDA X sex interaction, $F(2,226)=3.4$, $p=0.03$).

Grooming Episodes in the Open Field: Males

Figure 79. Grooming episodes in the open field in female rats. The frequency of grooming episodes across days decreased in female DA and ACh rats, increased in male ACh rats (compare with Figure 78), but did not change amongst the other groups (day X 192SAP X 6-OHDA X sex interaction, $F(2,226)=3.4$, $p=0.03$).

Grooming Episodes in the Open Field: Females

groups were reanalyzed separately using a univariate ANOVA, and a main effect of 192SAP was found ($F(1,19)=5.19$, $p<0.04$). Post hoc tests revealed that a difference existed only between female ACh and control rats ($p<0.05$, LSD).

As can be seen from Figures 80 and 81, spatial navigation was completely absent in the DA and dual rats (day X 6-OHDA interaction, $F(4,104)=17.78$, $p<0.001$; main effect of 6-OHDA, $F(1,26)=184.18$, $p<0.001$). Not only did these rats consistently fail to find the escape platform, they also failed to search the pool effectively, remaining along the pool's perimeter instead of venturing into the centre. Moreover, these rats were highly reactive, aggressive, and displayed behaviours suggestive of panic when placed in the water. The rats were capable of swimming, since all of them did swim occasionally, but spent most of each 90 second trial trying to climb the walls of the pool rather than search for the platform. Generally, they would not remain on the escape platform between trials, particularly during the first three days of testing. However, on the few occasions these rats did remain on the platform, they did not take the opportunity to look about the room. All of the 6-OHDA treated rats were smaller in stature compared to the controls, and several seemed so exhausted by their exertions in the pool that they had difficulty keeping afloat in the latter part of the test session. These rats (1 male, 1 female DA; 2 male, 5 female dual) were deemed physically incapable of performing the task and were dropped from the study. Thus, of the 6-OHDA treated rats initially assigned to this test, only 3 male and 4 female DA rats, and 3 male and 1 female dual rat, were able to complete it.

Figure 80. Hidden platform water maze in male rats. The ACh rats were indistinguishable from control, but spatial navigation was completely absent in the DA and dual rats (day X 6-OHDA interaction, $F(4,104)=17.78$, $p<0.001$; main effect of 6-OHDA, $F(1,26)=184.18$, $p<0.001$). These rats failed to search the pool effectively, remaining along the perimeter instead of venturing into the centre. Moreover, they displayed behaviours suggestive of panic when placed in the water. The rats were capable of swimming, since all of them did swim occasionally, but spent most of each 90 second trial trying to climb the walls of the pool rather than search for the platform. Generally, they would not remain on the escape platform between trials, but on the few occasions they did, they did not take the opportunity to look about the room. Even with the addition of a visual cue, performance remained at ceiling levels.

Hidden Platform Water Maze: Males

Figure 81. Hidden platform water maze in female rats. Female ACh rats were significantly different from control on the first day of testing ($F(1,19)=5.19$, $p<0.04$; $p<0.05$, LSD). Spatial navigation was completely absent in the DA and dual rats (day X 6-OHDA interaction, $F(4,104)=17.78$, $p<0.001$; main effect of 6-OHDA, $F(1,26)=184.18$, $p<0.001$). These rats failed to search the pool effectively, remaining along the perimeter instead of venturing into the centre. Moreover, they displayed behaviours suggestive of panic when placed in the water. The rats were capable of swimming, since all of them did swim occasionally, but spent most of each 90 second trial trying to climb the walls of the pool rather than search for the platform. Generally, they would not remain on the escape platform between trials, but on the few occasions they did, they did not take the opportunity to look about the room. Even with the addition of a visual cue, performance remained at ceiling levels.

Hidden Platform Water Maze: Females

Since the 6-OHDA treated rats were so hopelessly impaired in the hidden platform task, a visual cue was suspended above the platform on the 6th and 7th day to test their ability for cued navigation. The behaviours noted previously, such as wall climbing and inability to remain on the escape platform, persisted throughout this task as well. The significant day X 6-OHDA interaction ($F(1,26)=13.91$, $p<0.001$) and main effect of 6-OHDA ($F(1,26)=1674.93$, $p<0.001$) suggested that performance in 6-OHDA treated rats remained at ceiling levels, whereas the control and ACh rats improved slightly. Interestingly, there was also a main effect of sex indicating that males outperformed females in cued navigation ($F(1,26)=7.51$, $p<0.02$).

Since the DA and dual rats would not consistently remain on the escape platform, and did not obviously scan the room while there, it was not possible to determine whether they were spatially impaired. In other words, task performance in these rats was so hopelessly compromised that no conclusions about their abilities could be drawn.

In an attempt to improve the performance of DA and dual rats, the water temperature in the pool was raised to 25 ± 1 °C, and a habituation procedure was devised to gradually introduce them to the task over the course of four days. The rats were also started with the simpler, cued navigation task rather than the more complex spatial navigation task. It was reasoned that they could graduate to the hidden platform if they displayed proficiency with this version of the maze.

Cued Platform Water Maze After Habituation

The groups consisted of 9 male and 9 female controls, 7 male and 7 female ACh, 8 male and 10 female DA, and 11 male and 10 female dual water maze-naïve rats who were destined for the morphological analyses. Cued platform escape latencies were analyzed using a repeated measures ANOVA with 192SAP, 6-OHDA and sex as the between subjects variables and test day as the within subjects variable. The data from males and females are illustrated in Figures 82 and 83, respectively. The habituation procedure dramatically improved task performance in both DA and dual rats in that virtually all of the previous panic-like behaviours were abolished. The rats spent most of their time in the pool swimming and sat placidly on the platform between trials. Nevertheless, their swim pattern was still primarily thigmotaxic: the rats left the pool walls only briefly and rarely swam across the pool to the opposite side. Furthermore, unlike the control and ACh rats, they rarely scanned the room while on the platform. Even so, the DA and dual rats often found the platform within the 90 seconds allotted. However the main effect of 6-OHDA ($F(1,63)=70.65$, $p<0.001$) and the day X 6-OHDA interaction ($F(9,567)=9.51$, $p<0.001$) indicated that they never approached the level of proficiency exhibited by control and ACh rats. Interestingly, the significant day X 6-OHDA X sex interaction ($F(9,567)=2.71$, $p<0.01$) indicated that a slight improvement over time was detectable in male but not female 6-OHDA treated rats.

Figure 82. Cued platform water maze in male rats. The habituation procedure dramatically improved task performance in both DA and dual rats in that virtually all of the previous panic-like behaviours were abolished. The rats spent most of their time in the pool swimming and sat placidly on the platform between trials. Nevertheless, their swim pattern was still primarily thigmotaxic, and they rarely scanned the room while on the platform. While the DA and dual rats often found the platform within the 90 seconds allotted, they never approached a control level of performance ($F(1,63)=70.65$, $p<0.001$). Male 6-OHDA treated rats improved only slightly across test days (day X 6-OHDA X sex interaction ($F(9,567)=2.71$, $p<0.01$)).

Cued Platform Water Maze After Habituation: Males

Figure 83. Cued platform water maze in female rats. The habituation procedure dramatically improved task performance in both DA and dual rats in that virtually all of the previous panic-like behaviours were abolished. The rats spent most of their time in the pool swimming and sat placidly on the platform between trials. Nevertheless, their swim pattern was still primarily thigmotaxic, and they rarely scanned the room while on the platform. While the DA and dual rats often found the platform within the 90 seconds allotted, they never approached a control level of performance ($F(1,63)=70.65$, $p<0.001$). Furthermore, female 6-OHDA treated rats did not improve across test days (day X 6-OHDA X sex interaction ($F(9,567)=2.71$, $p<0.01$)).

Cued Platform Water Maze After Habituation: Females

Corticosterone Response to Mild Swim Stress in Female DA Rats

The striking performance improvement observed in the DA and dual rats following the habituation procedure suggested that rats with neonatal DA lesions might be particularly sensitive to stressful tasks. In order to test this hypothesis, baseline and evoked CORT levels were measured in response to a water maze-like stressor in a subset of female control and DA rats. The data from 11 control and 35 DA females were analyzed using a repeated measures ANOVA with 6-OHDA as the between subjects variable and post-swim interval as the within subjects variable.

Surprisingly, the only statistically significant effect detected in this analysis was of post-swim interval ($F(5,220)=63.66$, $p<0.001$). The 90 second swim produced an immediate threefold increase in CORT over baseline levels, which peaked about 40 minutes later. CORT levels remained elevated in both groups until at least 60 minutes post-stress. These data are depicted in Figure 84.

Effect of DA Agonist and Antagonists on Learning in the Water Maze

Since the CORT response to swim stress seemed normal in the DA rats, it was hypothesized that the abnormal reactivity to the water maze could be due to either hypo- or hyperfunction at DA synapses. Therefore the effects of l-dopa administration as well as of a D1/D2 antagonist cocktail were tested on the water maze performance of DA rats. The groups consisted of 29 female DA rats who had been used in the swim stress study. Ten of the rats received saline, 9 received l-dopa (50 mg/kg), and 10 received a cocktail of the D1 antagonist SCH23390 (0.1 mg/kg) and the D2 antagonist raclopride (2 mg/kg).

Figure 84. Plasma CORT levels in response to swim stress in DA rats. The only significant effect was of post-swim interval ($F(5,220)=63.66$, $p<0.001$). The 90 second swim produced an immediate threefold increase in CORT over baseline levels in both control and DA rats, which peaked about 40 minutes later. CORT levels remained elevated in both groups until at least 60 minutes post-stress.

Plasma CORT Levels After 90 Seconds of Swim Stress

The rats were first tested in the hidden platform task, followed by the cued platform task. The data from each task were analyzed using a repeated measures ANOVA with drug as the between subjects variable and test day as the within subjects variable.

Maladaptive behaviours such as persistent wall-climbing and unwillingness to remain on the escape platform were not observed in any of the rats, probably due to the fact that they had previously been exposed to the water maze during the swim stress study and were thus not completely naive to the task. Surprisingly, neither the agonist nor antagonists had any effect on performance of the hidden platform task, as can be seen from Figure 85. Furthermore, there was no effect of day or day X drug interaction, indicating that none of the rats improved their performance across test days.

After four days of hidden platform testing without noticeable improvement, the rats were switched to the cued platform task. Once again, neither blocking nor restoring DA function was of any benefit (Figure 86). In fact, the main effect of drug indicated that the saline group outperformed the other two groups in this task ($F(2,26)=9.51$, $p=0.001$; $p<0.05$, LSD). However the mean latencies of all three groups were very close to ceiling levels: 88.3 seconds, 88.7 seconds, and 84.0 seconds for the l-dopa, D1/D2 antagonist, and saline groups respectively. Thus these data do not suggest that either l-dopa or the antagonist cocktail worsened cued platform performance in the DA rats. Furthermore, the lack of effect of test day or a day X drug interaction indicated that performance did not improve over time in any of the rats.

Figure 85. Effects of dopaminergic drugs on hidden platform water maze performance in DA rats. L-dopa (50 mg/kg), and a D1 (SCH23390, 0.1 mg/kg)/D2 (raclopride, 2 mg/kg) antagonist cocktail were administered to determine if the water maze deficit was due to hypo- or hyperfunction at dopaminergic synapses. Neither the agonist nor antagonists had any effect on performance of the hidden platform task.

Hidden Platform Water Maze: Effects of Dopaminergic Drugs

Figure 86. Effects of dopaminergic drugs on cued platform water maze performance in DA rats. L-dopa (50 mg/kg), and a D1 (SCH23390, 0.1 mg/kg)/D2 (raclopride, 2 mg/kg) antagonist cocktail were administered to determine if the water maze deficit was due to hypo- or hyperfunction at dopaminergic synapses. Neither the agonist nor antagonists had any effect on performance of the hidden platform task.

Cued Platform Water Maze: Effects of Dopaminergic Drugs

Prepulse Inhibition

In order to further characterize the behavioural impairment in female DA rats, sensorimotor gating was assessed using prepulse inhibition of the acoustic startle reflex. The groups consisted of 9 female control rats who had participated in the swim stress study and the 10 female DA rats who had both participated in the swim stress study and received saline for the drug study. The data were analyzed using a repeated measures ANOVA with 6-OHDA as the between subjects variable and prepulse intensity as the within subjects variable.

The main effect of prepulse intensity indicated that all of the rats showed an intensity-dependent inhibition of the acoustic startle reflex ($F(4,68)=87.65$, $p<0.001$). No effects of the 6-OHDA treatment were detected (Figure 87).

Figure 87. Sensorimotor gating in DA rats. PPI was tested in response to a 105 dB white noise pulse preceded by one of either 60 dB, 70 dB, 80 dB or 90 dB white noise prepulses. The mean prepulse startle data was expressed as a percentage of the baseline (pulse alone) value. The DA rats did not differ from controls. Both groups showed an intensity-dependent inhibition of the acoustic startle reflex ($F(4,68)=87.65$, $p<0.001$).

Prepulse Inhibition

DISCUSSION

General Health and Body Weight

192SAP Lesions

Similar to previous studies from this laboratory (Pappas et al., 1996; Pappas et al., 2000; Sherren et al., 1999), the ACh lesion resulted in few post-surgical deaths (6% in the ACh group versus none for controls). Considering that p75 receptor expression does not restrict itself to the basal forebrain until the second week postnatal (Koh & Higgins, 1991), these deaths could be related to a non-specific effect of a high dose of toxin. This is not likely though, since administration of 840 ng of 192SAP to rats at a time when p75 is expressed relatively ubiquitously throughout the brain (P1), does not result in significant mortality, nor does it significantly reduce ChAT activity (Ricceri et al., 1997). Hence the mortality associated with 192SAP administration appears to be predicated on its anti-cholinergic, rather than non-specific, effects. Indeed, doses of 800 ng administered at later time points such as P4 or P7 produce significant mortality (Leanza et al., 1995; Pappas et al., unpublished observations).

Several studies have reported that neonatal 192SAP lesions reduce body weight during infancy and around the weanling age (Ricceri et al., 1997; Robertson et al., 1998; Sherren et al., 1999). It is not known whether this results from malnutrition, for example through maternal neglect or an impaired ability to obtain food resources (i.e., mother's milk), or from a more general effect on growth control. However weight gain in these rats

increases dramatically following weaning, suggesting that malnutrition during the preweaning period is a significant factor in determining the effects of this lesion on body weight (Sherren et al., 1999).

In contrast to these earlier reports, the present study noted no differences in body weight between control and ACh rats around the weanling age (P28). This may have been due to the palatable food supplementation which was instituted at P12 for all pups, and lends credence to the idea that ACh pups are not as adept at obtaining food from the mother as their littermate controls. While weight gain was normal in the weeks immediately following weaning, it slowed between 7 and 8 weeks of age. By the last time point analyzed (19 weeks), both male and female ACh rats were approximately 14% lighter than controls. An outcome such as this would not be expected if differences in body weight were simply the result of malnutrition early in life. These data suggest that neonatal 192SAP lesions produce long-lasting changes in metabolic function or appetite control, or perhaps a dysfunction which emerges as the rats mature.

6-OHDA Lesions

The mortality rate in DA rats was 14%, more than twice that found in ACh rats. Primiparous mothers are reportedly more attentive to 6-OHDA lesioned rat pups than littermate controls (Piccirillo et al., 1979), which suggests that maternal neglect does not occur following this type of lesion. Nevertheless, the profoundly reduced body weights of the surviving rats indicates that mortality likely resulted from severe malnutrition.

Similar to other reports (Bruno et al., 1987; Castañeda et al., 1990; Luthman et al.,

1997; Takasuna & Iwasaki, 1996), the DA rats were significantly lighter than their littermate controls at every time point analyzed despite the palatable food supplement given in plentiful amounts to each maternity cage. This may be reminiscent of the terminal syndrome of aphagia and adipsia which occurs following adult 6-OHDA lesions (Breese & Traylor, 1971; Ungerstedt, 1971). Lesioned adults must be maintained through tube feeding for several weeks before the gradual recovery of ingestive behaviours occurs (Zigmond & Stricker, 1973). Furthermore, recovery is associated with increases in synthesis and release of DA, and upregulation of postsynaptic receptors (Zigmond & Stricker, 1989). Thus ingestive behaviours are at least partially under dopaminergic control in adulthood.

DA begins to regulate ingestion sometime during the second week of life, since feeding is sensitive to haloperidol administration at P12 and in adulthood, but not at P6 (Potter & Bruno, 1989, 1991). Consistent with this, mice with a selective inactivation of the tyrosine hydroxylase gene from dopaminergic but not noradrenergic cells, succumb to a syndrome of hypoactivity, aphagia and adipsia beginning around P10-P15 (Zhou & Palmiter, 1995). Interestingly, both haloperidol and the catecholaminergic synthesis inhibitor α -methyl-p-tyrosine can suppress feeding in 6-OHDA lesioned rats at either P12 or in adulthood (Potter & Bruno, 1989, 1991). This suggests that ingestive behaviours remain sensitive to dopaminergic manipulations throughout the lifespan even in rats sustaining large DA lesions early in life. Rats who survive these lesions may either retain enough residual dopaminergic activity following the lesion to support this behaviour, or

experience an upregulation in residual dopaminergic fibres soon after the lesion, which then sustains feeding behaviour.

Dual Lesions

The mortality rate following the dual lesion was similar to that observed following the DA lesion. This was surprising given that each lesion alone resulted in several deaths. In fact, food supplementation was instituted mainly because of the expectation that the combined effects of two large forebrain lesions would result in higher mortality than either lesion alone. Given that the mortality rates following the dual and DA lesion were similar, it is likely that the post-surgical deaths in the dual rats occurred mainly from malnutrition.

Curiously however, the surviving dual rats also gained more weight over time than the DA rats. This was unexpected, since each lesion alone suppressed weight gain. This effect could be explained if there were differences in lesion size between the dual rats and their singly lesioned counterparts, but this was not the case. However the dual lesion may have produced an undetected, compensatory neurochemical change in brain circuits which are involved in ingestive behaviours. It is also possible that this lesion produces a unique effect on growth or metabolic functioning.

Neurochemistry

192SAP Lesions

192SAP resulted in significant reductions in ChAT activity in both male and female rats from all three cortical regions, as well as from the hippocampus, medial septum and caudate. ChAT activity was reduced only in target fields or originating nuclei of basal forebrain cholinergic afferents. Furthermore, the largest reductions were observed in areas innervated primarily by the MS/VLBD, namely the hippocampus and RSC. In these two regions, ChAT activity was reduced by more than 70%.

Basal forebrain cholinergic cells and cerebellar Purkinje cells are the only two cell types which express the p75 receptor in adulthood (Woolf et al., 1989). The cortical cholinergic projection from the tegmental area does not express p75 (Woolf et al., 1989), and as such is not likely to be susceptible to 192SAP toxicity at P7. Consistent with this, ChAT activity was only moderately reduced in the anterior cortical region, where tegmental, NBM and MS/VLDB afferents overlap.

The present results are generally consistent with previous work from this and other laboratories. However it is difficult to compare the results of the cortical ChAT assay to other studies, since most have reported data from either whole cortex (Pappas et al., 1996), or from a single, large, anterior chunk (Ricceri et al., 1997, 2002). The only other study to examine several discrete cortical areas found depletions similar to those reported here, ranging from 40% in the prefrontal cortex, to 82% in the parietal region (Leanza et al., 1996).

A small but significant reduction in striatal ChAT activity was noted after the 192SAP lesion. Only one other study, also from this laboratory, has examined striatal ChAT activity following neonatal 192SAP administration, and reported no difference from control levels (Pappas et al., 1996). This discrepancy may be explained by differences in the ChAT assay technique which were required in order to comply with recent changes in Atomic Energy Control Board regulations. In the course of modifying the assay protocol, the relative amounts of some compounds were altered to conform more closely to the method of Fonnum (1975), on which the original assay was based. These modifications not only increased the number of nanomoles of ACh made per milligram of tissue per hour (by increasing the amount of substrate), they also appeared to improve phase extraction of the radiolabelled ACh. In fact, the small reduction in striatal ChAT activity noted here does agree with the only other existing report on striatal cholinergic markers following a P7 192SAP lesion. In that study, a 22% reduction in ChAT immunolabelled cells from the lateral portions of the nucleus was found (Sherren et al., 1999).

The present study is the first to report a comprehensive and regionally specific assessment of ChAT activity following P7 192SAP lesions, and it again demonstrates the efficacy of 192SAP as a cholinotoxin in the neonate. Nevertheless, the ChAT depletions obtained here were by no means complete. However, increasing the dose to effect a greater lesion is not a viable alternative, since 800 ng administered at either P4 or P7 produces significant mortality (Leanza et al., 1996; Pappas et al., unpublished observations).

It should be acknowledged that there are limits to the size of lesion obtainable with 192SAP. This toxin works by gaining access to cholinergic cells via the p75 receptor, yet only about 90% of the cholinergic cells in the adult basal forebrain express p75 (Batchelor et al., 1989). Accordingly, i.c.v. doses of as much as 8 or 10 μ g in adults, which produce a near complete ablation of p75 immunopositive cells in the basal forebrain, fail to reduce cortical or hippocampal ChAT activity beyond about 85% (Book et al., 1992; Leanza et al., 1995). While the percentage of cholinergic neurons which co-express p75 during development is not known, cell counts in the basal forebrain following P7 lesions indicate that ChAT immunopositive cells are less severely affected than p75 immunopositive cells (Sherren et al., 1999). This suggests that like in the adult, not all cholinergic cells are expressing the p75 receptor at P7. Furthermore, incomplete lesions may also result from upregulation of ACh synthesis and storage in residual cholinergic terminals, as has been demonstrated after partial fimbria-fornix lesions in adult rats (Lapchak et al., 1991). Thus while 192SAP is currently the most effective and selective cholinotoxin available, complete cholinergic lesions have remained out of reach. It would be worthwhile to systematically explore factors such as booster injections of 192SAP at varying intervals to determine if a greater lesion could be achieved.

Other neurochemical systems were also affected by neonatal 192SAP administration. NE was moderately increased in the hippocampus and RSC of both male and female ACh rats. Previous studies have also reported increased NE in whole cortex (Pappas et al., 1996) as well as in prefrontal, parietal and occipital regions (Leanza et al., 1996), suggesting that this amine might functionally compensate for neonatal ACh

hypofunction. However no changes in MHPG levels, which have not been measured in previous studies, were detected. This indicates that the excess NE is not in fact being utilized, as might be expected from a compensating system. This data is consistent with a report showing that neonatal NE lesions do not exacerbate the behavioural consequences of neonatal ACh lesions (Pappas et al, 2000).

The increased NE is more likely derived from p75-expressing sympathetic axons arising from the superior cervical ganglion. Cholinergic cells are dependent on NGF, which their axons take up from secreting cells in cholinergic projection fields (Gutiérrez et al., 1997; Ruberti et al., 2000). Cholinergic deafferentation results in an excess of NGF in these projection fields (Yu et al., 1996). Sympathetic axons presumably grow into deafferented sites along a chemoaffinity gradient of this NGF, and present as thickened, varicose ingrowths which are immunopositive for both p75 and dopamine β -hydroxylase. This phenomenon has been well-documented in the hippocampus following fimbria-fornix transection (reviewed in Crutcher, 1987), as well as in the hippocampus and RSC following neonatal 192SAP lesions (Pappas et al., 1996, 2000; Sherren et al., 1999).

In addition to increased NE, 70% increases in DA and DOPAC were observed in the temporal cortex of both male and female ACh rats. These effects just missed significance due to the strict alpha level. It is unlikely that these findings were spurious though, since the increase in DA was substantial and was mirrored by a similar rise in DOPAC. Non-significant DA increases of about 50% have been observed in the prefrontal, parietal and occipital cortices (Leanza et al., 1996). However another study has reported no changes in whole cortex DA levels (Pappas et al., 1996). Overall, these data

suggest that compensatory upregulation of the dopaminergic system might occur following neonatal ACh lesions. It would be instructive to investigate this possibility in future studies.

6-OHDA Lesions

6-OHDA administration caused a profound reduction in striatal and anterior cortex DA levels, with somewhat lesser reductions in temporal cortex and midbrain. DA depletions were paralleled by reductions in the metabolites DOPAC and HVA, except in the midbrain. Large depletions were observed only in brain areas from which nigrostriatal and mesocorticolimbic afferents arise and terminate.

These data are generally consistent with numerous other studies (Archer et al., 1988; Breese et al., 1984; Castañeda et al., 1990; Heffner & Seiden, 1983; Luthman et al., 1997; Pappas et al., 1992; Raskin et al., 1983; Takasuna & Iwasaki, 1996; Whishaw et al., 1994). However, previous studies have only reported levels of DA and its metabolites from whole cortex or the prefrontal region. The present study is the first to demonstrate that DA loss occurs in both the anterior and temporal cortices, but not in the RSC. These results are consistent with topographical studies showing that the mPFC, ACC and entorhinal areas receive the heaviest cortical dopaminergic innervation (Berger et al., 1991; Descarries et al., 1987).

The caudate, which is the main terminus of the nigrostriatal pathway, displayed the largest DA lesion of any brain area examined. This finding is typical of i.c.v. administration of 6-OHDA (Archer et al., 1988; Breese et al., 1984; Castañeda et al., 1990; Heffner &

Seiden, 1983; Luthman et al., 1997; Pappas et al., 1992; Raskin et al., 1983; Takasuna & Iwasaki, 1996; Whishaw et al., 1994). However the striatal lesion, while extremely large, was not quite complete. Virtually complete striatal lesions have been achieved using 150 μ g of 6-OHDA at P3 (Castañeda et al., 1991; Whishaw et al., 1994) or 200 μ g at P3 and P6 (Archer et al., 1988; Luthman et al., 1997). Yet given the 14% mortality rate observed here, these higher doses might have caused substantial mortality if administered at P7.

DA levels in the midbrain were reduced only moderately and DA metabolites not at all. Tyrosine hydroxylase activity has been shown to increase in residual terminals following adult 6-OHDA lesions (Acheson & Zigmond, 1981; Acheson et al., 1980; Wolf et al., 1989), and sprouting of residual nigral cells has been documented following neonatal i.c.v. (Fernandes Xavier et al., 1994) or intrastriatal injections of 6-OHDA (Penit-Soria et al., 1997). It is thus likely that a compensatory response which increased DA function, such as synthesis upregulation or sprouting from residual cells, occurred in the midbrain region.

Curiously, the hippocampus did not sustain a detectable DA lesion. This is contrary to the only other study assaying this area, which reported a 50% reduction in both hippocampal DA and DOPAC (Takasuna & Iwasaki, 1996). The dose used in that study (70 μ g) was lower than that used here. However the toxin was administered in two injections on P2 and P4. It is possible that the dopaminergic projection to the hippocampus is more susceptible to damage at this time, or that earlier lesions do not result in the same degree of hippocampal recovery as P7 lesions.

Moderate increases in NE were noted in the anterior cortical region of DA rats. This may reflect a compensatory upregulation of NE synthesis or release from forebrain terminals. In support of this, males exhibited moderate increases in NE and females moderate increases in MHPG in the brainstem, whereas both sexes exhibited moderate increases in midbrain MHPG. These subcortical effects did not achieve the 0.01 level of significance, but fit an overall pattern consistent with an upregulation of NE function. However as mentioned above, partial noradrenergic lesions made with 6-OHDA in adults increase tyrosine hydroxylase activity in residual hippocampal terminals and locus coeruleus cell bodies (Acheson & Zigmond, 1981; Acheson et al., 1980). Thus the upregulation of noradrenergic transmission observed here may have been caused by partial damage to noradrenergic cells, rather than a compensatory response to the DA lesion per se.

The caudate of DA rats exhibited a moderate but non-significant increase in 5-HIAA. Striatal serotonergic hyperinnervation is a well-documented consequence of neonatal 6-OHDA lesions (Breese et al., 1984; Bruno et al., 1987; Castañeda et al., 1991; Mrini et al., 1995; Penit-Soria et al., 1997; Whishaw et al., 1994; Zhang et al., 2002; reviewed in Kostrzewa et al., 1998). This phenomenon usually manifests as a substantial increase in both striatal 5-HT and 5-HIAA (Breese et al., 1984; Castañeda et al., 1991; Penit-Soria et al., 1997; Whishaw et al., 1994), and requires an excess of 80% loss of striatal DA to emerge (Gong et al., 1993). An increase in 5-HT was not observed here, nor was the increase in 5-HIAA as robust as that noted in other studies. Differences in lesion size are not likely to account for the discrepancy, since the aforementioned

threshold level of striatal denervation was certainly achieved here. The principal difference between this study and others is in the timing of the lesion. Hyperinnervation has been noted following 6-OHDA lesions made at P2 (Penit-Soria et al., 1997), P3 (Castañeda et al., 1991; Mrini et al., 1995; Whishaw et al., 1994) and P5 (Breese et al., 1984; Zhang et al., 2002), but not at P15 or P27 (Bruno et al., 1987). Thus there appears to be a critical window for serotonergic hyperinnervation which closes early in the second week of life.

Interestingly, a considerable and significant increase in 5-HT and 5-HIAA was observed in the RSC of male DA rats. Female DA rats had comparable increases in 5-HIAA in this area, but 5-HT was unaffected. Similar effects have not been reported in other studies (Breese et al., 1984; Castañeda et al., 1991). However these changes may have gone undetected since the cortex has not previously been dissected as it was here. The source of the 5-HT increase is unknown, however it appears to be selective to dopaminergic denervation, since it was not observed in the ACh rats. It is possible that, in a fashion similar to striatal serotonergic hyperinnervation, existing terminals in the RSC sprout in response to DA denervation. Yet this area does not receive heavy dopaminergic innervation, a fact which was reflected in the absence of detectable DA changes following the lesion. The underlying mechanism of serotonergic upregulation in this area and its functional implications merit further examination.

Dual Lesions

The dual lesioned rats mirrored their singly lesioned counterparts on virtually all neurochemical measures. When administered in conjunction, 6-OHDA and 192SAP had simple, additive effects which did not interact in any way. The changes associated with the single lesions were almost always present and of equal magnitude in dual lesioned rats.

However, there were a few notable exceptions, which are outlined below.

Despite the fact that ACh rats exhibited a dramatic increase in temporal cortex DA and DOPAC, the levels of DA and its metabolites in dual rats were similar to those of DA rats. Thus dopaminergic upregulation in the temporal cortex following a neonatal ACh lesion may depend upon an intact DA system.

Post hoc tests showed that cortical NE levels were significantly elevated only in the dual rats. This is consistent with the notion that cortical NE increases via two different mechanisms following each lesion. However, the moderate hippocampal NE increase in ACh rats was not observed in the dual group. This may be explained if the moderate but non-significant 28% reduction in hippocampal NE observed in the DA rats is considered. Any NE increases due to sympathetic sprouting in the dual rats may have been mitigated by decreases caused by the 6-OHDA treatment.

Cell Morphology

192SAP Lesions

In layer V pyramidal cells of the mPFC, the ACh lesion reduced total length and branching in the apical dendritic tree. These effects were found in higher order branches in areas distal to the cell body, suggesting that they occurred primarily in the apical tuft. Spine density in the tuft was also reduced, by 26%. In the basal dendritic tree, only the number of higher order branches was reduced. Spine density in this area was 25% less than control.

In layer III pyramidal cells of the ACC, total length and branching were reduced in the apical tree. Once again these effects were observed only in higher order branches distal to the cell body, in the apical tuft. Spine density was markedly reduced in this region, by 44%. The length of the basal dendritic tree was not significantly affected by this lesion. However higher order branches were slightly reduced, and spine density was greatly reduced, by 35%.

In layer III pyramidal cells of the RSG, the effect of the ACh lesion on total length and total branching of the apical tree did not achieve statistical significance. However the frequency of distal dendrites was reduced, as were higher order dendrites, suggesting once again that a small amount of regression occurred in the apical tuft. Spine density in the tuft was not statistically different from control. The total length of the basal tree was markedly reduced by more than 20%, as was higher order branching. Once again though, basal spine density was not significantly different from control.

The ACh lesion produced similar morphological effects in the apical dendritic trees of pyramidal cells, which were apparent in each cortical area examined. Dendritic regression occurred in the apical tuft, regardless of the region or whether the cells were located in superficial or deep cortical layers. Oblique apical dendrites which arose close to the cell body were not affected. Curiously, these effects did not correlate with the degree of cholinergic denervation since in the severely lesioned RSG, reductions in apical dendrite length and higher order branching were not pronounced enough to reach statistical significance, whereas in the moderately denervated mPFC and ACC these effects were highly significant.

In contrast, the effect of the ACh lesion on the extent of the basal dendritic trees in each cortical area was regionally-specific and correlated with lesion size. In the heavily denervated RSG, both total length and higher order branching were reduced. However in the moderately denervated mPFC and ACC, higher order branching was reduced but total length was unaffected.

There is only one published report on the effect of neonatal 192SAP lesions on cortical cell morphology to which the current results can be compared (Robertson et al., 1998). This group examined layer V pyramidal cells of the visual cortex after administration of two unilateral injections of 192SAP at P1 (100 ng) and P3 (200 ng). At both P7 and P9, a reduction in soma size was apparent, as well as a slightly shorter apical shaft (as measured by distance from the soma to the pial surface), fewer 1st order apical branches, and reduced spine density on both the main apical shaft and the tuft. No effects on numbers of 1st or 2nd order dendrites, or spine density, were observed in the basal tree.

The regression of oblique apical dendrites seen on layer V visual cortex pyramidal contrasts with the selective effect on apical tufts observed in the present study. However, the visual cortex contains a particularly dense area of cholinergic innervation centering around layer IV (Eckenstein et al., 1988). In the Roberston et al. study, the area along the apical shaft from which most dendrites were missing corresponded to areas which would normally course through this layer. Since layer IV receives the heaviest innervation, it may also suffer the greatest deafferentation following an ACh lesion. These data suggest that a threshold of cholinergic loss may be required to produce dendritic regression.

In support of this hypothesis, the pattern of dendritic atrophy observed in layer III pyramidal of the RSG is consistent with the laminar distribution of cholinergic afferents in this area, where the densest bands of cholinergic terminals occur above and below layers II/III (Eckenstein et al., 1988). In the RSG, reduced length and branching were observed in the basal dendritic tree which would normally lie in lower layer III and layer IV, and to a lesser extent in the apical tuft, which would normally lie in layers I and II.

Unlike the RSG, dense banding of cholinergic terminals is not characteristic of the mPFC and ACC. In these areas, cholinergic terminals appear evenly distributed throughout the cortical layers (Eckenstein et al., 1988; Mechawar et al., 2000). Despite this, dendritic atrophy in these cells was confined to the apical tufts and higher order branches of the basal tree. In neither area were apical branches lying proximal to the cell body affected, even though presumably the toxin would have eliminated cholinergic terminals from all layers equally.

It should be noted however, that the pattern of atrophy in the mPFC and ACC may

not be inconsistent with a threshold of loss hypothesis. The laminar distribution of afferents in these regions which can be solely attributed to either the NBM, MS/VLDB, or the tegmentum, is not known. It is thus quite possible that the affected dendrites were those which primarily receive basal forebrain rather than tegmental innervation.

It should also be noted that the laminar distribution of cortical cholinergic inputs has so far only been investigated using ChAT immunoreactivity. Currently, no information exists on the laminar distribution of cortical afferents from p75-expressing versus non-p75-expressing cholinergic cells in the MS/VLDB or the NBM. Thus the pattern of regression observed in each cortical region could also reflect lamina-specific innervation patterns of p75-expressing cells. This issue could be clarified in future studies by comparing the density of ChAT versus p75 immunopositive terminal varicosities across cortical laminae.

On a cautionary note, it should not be assumed that the dendritic dysgenesis occurred solely as a result of cholinergic deafferentation of pyramidal cells. Cholinergic afferents have been observed to contact both pyramidal and non-pyramidal cortical elements (Houser et al., 1985; Mrzljak et al., 1995). Thus removal of cholinergic input may affect pyramidal cells directly, or indirectly through deafferentation of interneurons which in turn impinge upon these cells. Indeed, the differences between the effects of 192SAP in mPFC, ACC and RSG could reflect differing degrees of interneuron denervation in each of these areas.

One striking finding of the present study was the regionally specific effects the ACh lesion produced on spine density. Since pyramidal neurons receive the majority of

their synaptic contacts on spines, it can be inferred that decreases in spine density reflect reduced synaptic, and presumably cholinergic, input to these cells. Curiously however, spines were most affected in the mPFC and ACC, which sustained only moderate (i.e., 30% reduction in ChAT activity) damage. They were not affected in the heavily lesioned RSG (i.e., 70% reduction in ChAT activity). Furthermore, basal forebrain afferents comprise only a small part of the synaptic input to pyramidal cells, and only about 25% of cholinergic contacts are made on spines (Houser et al., 1985; Mrzljak et al., 1995). This suggests that the spine loss observed in the mPFC and ACC may not simply reflect the loss of cholinergic afferents *per se*.

By far the largest source of pyramidal cell input stems from cortico-cortical afferents from local or distant pyramidal cells. Since the vast majority of pyramidal cells are excitatory, and since spines are almost always associated with at least one asymmetric synapse (Nieuwenhuys, 1994), it is possible that the dramatic decreases in mPFC and ACC spine density signify a decrease in cortico-cortical connections to these cells. This implies that cholinergic afferents are involved in either promoting or maintaining cortico-cortical contacts in the mPFC and ACC, but not the RSG. However, since pyramidal axons are also known to innervate the dendrites of their parent cell (Lübke et al., 1996), the loss of spines could also reflect a loss of intrinsic pyramidal innervation.

Synaptic reorganization of other afferent contacts may also have contributed to the morphological changes, or lack thereof, observed in each cortical region. This phenomenon has been well documented in the deafferented septum and hippocampus of the adult rat (reviewed in Cotman & Nadler, 1978, and Raisman, 1985). These authors

suggest that postsynaptic sites survive deafferentation in the adult nervous system, and that their presence has a stimulating effect on synapse formation such that adjacent axons are induced to reinnervate denuded sites. Furthermore, where possible these sites exhibit a preference for homotypic rather than heterotypic reinnervation, which may act to reduce the degree of abnormality amongst new synaptic connections.

Synaptogenesis is ongoing during the first and second week of life, particularly amongst cholinergic afferents. It is quite possible that at the time the lesions were performed, many cholinergic synapses had not yet been firmly established and were eliminated rather than replaced. This would explain the dendritic atrophy and reduced spine density observed in the mPFC and ACC. Alternatively, while the RSG also showed dendritic atrophy, spine density was not reduced. This suggests that either cholinergic afferents are not involved in promoting and maintaining synaptic connections in the RSG, or synaptic reorganization occurred, which may have preserved spines on these cells.

Synaptic reorganization is an appealing explanation for the preserved spine density in the RSG for at least one important reason. Cholinergic basal forebrain neurons express the p75 receptor throughout adulthood because they depend upon NGF for their survival and maintenance (Gutiérrez et al., 1997; Ruberti et al., 2000). Deafferentation allows target-derived NGF to accumulate in cholinergic terminal fields (Yu et al., 1996). The fact that ectopic sympathetic axons have been documented in the hippocampus and RSG of neonatally lesioned rats (Pappas et al., 1996; Sherren et al., 1999), but not in the ACC (Pappas et al., unpublished observations), suggests that NGF levels in the RSG are elevated following the lesion. Since exogenous NGF can reverse the lesion-induced

dendritic atrophy and spine reductions observed in rats with unilateral cortical lesions (Kolb et al., 1997), it is possible that target-derived NGF initiates synaptic reorganization in the RSG. Future studies should examine the identity of synaptic inputs to cortical cells in rats with neonatal ACh lesions, to determine if reorganization has occurred.

Robertson et al. (1998) provided the first in vivo evidence that selective neonatal cholinergic denervation alters cortical dendritic morphology, a hypothesis which has been advocated with questionable support for over a decade (Bachman et al., 1994; Hohmann et al., 1988; Hohmann et al., 1991; Hohmann & Berger-Sweeney, 1997). However the brief survival times of the rats in that study precluded any conclusions about the permanency of these effects. The present results show for the first time that there are long term consequences of neonatal ACh lesions on pyramidal cell morphology, both in severely and moderately denervated areas of cortex. Neonatal cholinergic denervation produces regionally specific changes in cortical pyramidal cells that are detectable well into adulthood, and are thus likely permanent.

6-OHDA Lesions

In contrast to the 192SAP lesion, the 6-OHDA lesion had considerably less effect on pyramidal cell morphology. In the mPFC, the number of oblique, 1st order apical dendrites was reduced in layer V pyramidal cells. The basal tree of these cells was generally not affected, nor were apical or basal spine densities different from control. The apical tree of layer III pyramidal cells in the ACC was unaffected. However higher order branching was reduced in the basal tree, and both apical and basal spine densities were reduced by more

than 20% compared to control. In layer III pyramidal cells of the RSG, the only detectable effect of the DA lesion was a slight reduction in higher order branching of the basal tree.

Thus far, only one other study has assessed the effects of neonatal DA lesions on pyramidal cell morphology (Kalsbeek et al., 1989c). Thermal lesions of the VTA at P1 produced a sizable reduction in total length and branch frequency of layer V pyramidal cells of the mPFC of rats at P36. Unfortunately this study was confounded by the fact that the lesion also destroyed serotonergic fibres of passage. While Kalsbeek et al. did not observe any morphological effects of transecting these fibres alone, the possibility that the dendritic regression was produced by a synergistic effect of combined DA and 5-HT denervation cannot be ruled out. Furthermore, no aspects of the apical tree were examined, nor were spines assessed by this group. Thus the present study provides the first comprehensive and regionally specific assessment of pyramidal cell morphology following selective neonatal DA lesions.

Contrary to the findings of Kalsbeek et al., the P7 DA lesion did not produce a dramatic effect on the basal tree of layer V cells in the mPFC. This discrepancy is not likely due to differences in lesion size, since the neurochemical analysis revealed a substantial reduction in DA and its metabolites in this region. In fact, this group reported similar reductions in mPFC DA levels following their thermal lesion (Kalsbeek et al., 1989b).

It is possible that differences in lesion timing account for the discrepancy between this study and that of Kalsbeek et al. The dopaminergic innervation to deep layers of the mPFC arises mainly from the VTA and arrives prenatally (Verney et al., 1982). In the

Kalsbeek study, these afferents were lesioned just after they had reached their cortical targets. By the time the DA lesions were created in the present study, synaptic contacts from tegmental afferents would most likely have been well-established. However synaptogenesis amongst nigrostriatal afferents, which arrive postnatally and impinge upon supragranular cortical layers (Verney et al., 1982), would likely still be ongoing. Thus dopaminergic afferents may be necessary for the establishment but not the maintenance of pyramidal dendrites. This hypothesis could be readily tested by examining the morphological consequences of earlier 6-OHDA lesions.

It was quite surprising that the DA lesion had such modest effects on cortical cytoarchitecture given the findings of Kalsbeek et al., and the dramatic morphogenic effects of chronic upregulation of DA function by drugs of abuse. For example, prenatal exposure to cocaine results in long-lasting hypertrophy of layer III and V pyramidal neurons in the ACC (Jones et al., 1996, 2000; Murphy et al., 1997). In the adult, chronic exposure to cocaine results in hypertrophy of both apical and basal dendrites of layer V pyramidal cells from the mPFC (Robinson & Kolb, 1999). These studies demonstrate a consistent effect of cocaine on apical and basal cell morphology in the mPFC and ACC, regardless of whether or not the drug is given pre- or postnatally. However unlike cocaine, chronic exposure to amphetamine in adulthood only affects the apical trees of layer V mPFC cells (Robinson & Kolb, 1999). The reason for the differences between cocaine and amphetamine in this regard has not yet been elucidated. Yet given the basal atrophy produced by combined DA and 5-HT denervation (Kalsbeek et al., 1989c), and the mild apical regression observed in the present study, it is interesting to speculate on its relation

to cocaine's ability to release many amines, including serotonin.

The effects of the present study were regionally-specific, in that different aspects of pyramidal cells were affected in each of the three cortical areas. However it is interesting to note that for the most part, these changes occurred in dendrites which normally course through layers III/IV. For example in the mPFC, first order apical dendrites which normally arise from the main apical shaft and orient predominantly in layers III and IV were reduced. In the ACC and RSG, only the basal trees of the layer III pyramidals, which would also normally lie in layers III and IV, were affected. Cortical layers I-III normally receive afferents from the substantia nigra (Berger et al., 1991). Since the largest DA depletions were detected in the caudate, the main terminus of the nigrostriatal pathway, it seems plausible that cortical layers receiving nigral afferents also received the greatest dopaminergic denervation, and hence, dendritic regression.

Despite the fact that nigral afferents terminate in each of layers I through III, it was interesting to note that no effects were observed in the apical tufts of cells in any region. This may be related to a threshold of loss effect on cell morphology. For example, the dopaminergic innervation to the ACC and RSG is concentrated mainly in layer III (Berger et al., 1985; Descarries et al., 1987; Van Eden et al., 1987). Furthermore, the ACC receives a substantially greater innervation than the RSG. Thus it is not surprising that dendritic changes were observed only in the basal trees of the layer III cells. Nor is it surprising that the changes seen in the heavily innervated ACC were more pronounced (i.e., effects on both length and branching) than in the lesser innervated RSG.

The DA lesion did not significantly affect spine density in either the mPFC or the

RSG. This is consistent with the fact that only a small minority (about 15%) of cortical dopaminergic terminals contact spines (Erickson et al., 2000). In contrast, the ACC did show a substantial decrease in spines. The one study which examined the laminar distribution of DA terminals rather than axons in the rat reported that the highest terminal density occurred in layers I-III of the ACC (Descarries et al., 1987). While it is not known whether a larger proportion of contacts are made on spines in this region, it is plausible that the heavier innervation would result in more deafferented spines following the lesion, explaining why changes in spine density were detectable here but not in other areas. However as discussed previously, it is also possible that neonatal DA lesions result in significant synaptic reorganization which may have preserved the spines as well as other morphological features of cells from each region.

Dual Lesions

The morphological changes produced by the dual lesion appeared to be a simple, additive combination of the changes produced by either lesion alone. In no instance was there evidence of a synergistic effect of the lesions. For example, in layer V cells of the mPFC, where 192SAP produced atrophy in the apical tuft and 6-OHDA produced atrophy of proximal 1st order dendrites, the dual lesion produced apical trees with both stunted tufts and fewer proximal dendrites. In a similar fashion, neither lesion alone produced significant effects on the length of the basal tree of these cells, however length was significantly reduced by the dual lesion.

There was one apparent exception to this rule. In the ACC, post hoc tests showed

that both apical and basal spine densities were decreased by either lesion alone, yet the effect of the dual lesion on spine density was no greater than that noted following the ACh lesion. This could occur if cholinergic and dopaminergic afferents impinge on the same dendritic spines or areas of the shaft of ACC pyramidal cells, but it is not known if this is indeed the case. Alternatively, spines in the ACC may be more sensitive to deafferentation, such that the lesioning of a single diffuse subcortical input system causes a maximal spine loss. This is highly speculative however, particularly given the present state of knowledge of cortical microcircuitry. Further information about the effects of single versus combined lesions is warranted before explanations can be considered.

Behaviour

192SAP Lesions

The behavioural effects of the ACh lesion were limited. Exploration but not locomotion was reduced in both male and female rats in the open field. Female rats were impaired on the first day of hidden platform water maze testing, but not on any subsequent days or on the cued platform task. Male rats did not exhibit any spatial impairments.

The ACh lesion had no effect on locomotor activity in the open field at three months of age. Other groups have also failed to detect an effect of this lesion on activity levels in either preweanling (Ricceri et al., 1997, 2002) or adult rats (Leanza et al., 1996). However rearing was significantly reduced in the ACh rats. This is consistent with reports

of decreased open field rearing in preweanling rats following either P7 192SAP lesions or scopolamine (Ricceri et al., 1997, 2002).

Female ACh rats had longer escape latencies on the first day of hidden platform water maze testing compared to female controls. This effect has previously been reported in both male and female ACh rats tested in the water maze at 45 or 90 days of age (Sherren et al., 1999). The fact that task performance was only affected on the first day of testing suggests that these rats may have suffered from a mild working memory impairment. For example, on the first test day, the rat must construct and hold in memory a spatial map of the room in order to find the platform quickly. Because the platform remains in the same position throughout testing, this task acquires an additional, reference memory component on the second day. Thus the first day of water maze testing relies most heavily on visually based working memory skills.

Goldman-Rakic (1996) has postulated that the visuospatial “memory fields” exhibited by pyramidal cells in the macaque mPFC constitute a cellular correlate of visuospatial working memory. As such, compromised signalling between these cells could lead to “noisy” circuits and blurred boundaries of the visuospatial memory field. Whether memory fields also occur in the mPFC of the rat is unknown. However, the ACh lesion caused a significant reduction in spine density and regression of the apical tuft in pyramidal cells from both the mPFC and ACC, as well as a reduction in the extent of the basal tree in the RSG. Any one or more of these changes could potentially have contributed to the mild deficit observed in these rats. Indeed, given the magnitude of dendritic changes observed, it is perhaps more surprising that larger deficits were not observed. However, it

is possible that the water maze is too simple a task to detect subtle impairments in working memory. In support of this, working memory deficits have repeatedly been noted in male ACh rats on both a complex land and water-based radial arm maze (Pappas et al., 2000; Pappas et al., unpublished observations).

First day water maze latency differences in male ACh rats were not observed here, nor in one other study (Pappas et al., 1996). Working memory deficits in males are detectable on more complex tasks such as the radial arm maze (Pappas et al., 2000), which suggests that the behavioural consequences of neonatal ACh lesions are more pronounced in female than in male rats. This is consistent with another report indicating that passive avoidance impairments following neonatal 192SAP lesions are more severe in females than in males (Ricceri et al., 2002). Taken together, these data suggest that neonatal ACh lesions produce subtle impairments in visuospatial working memory which are more pronounced in females than in males. Future studies should determine if sex differences are also detectable in the morphological consequences of these lesions, and in the performance of more complex working memory tasks.

6-OHDA Lesions

In contrast to the ACh lesion, the DA lesion produced dramatic effects on behaviour. Female but not male rats were hyperactive in the open field. Rearing was reduced in both sexes. Both females and males were completely incapable of spatial navigation in the hidden platform water maze, and persistently made ineffective responses such as wall climbing and leaping off the platform between trials. The gradual habituation

procedure dramatically improved task performance by abolishing these behaviours. Yet despite the addition of a visual cue, the DA rats continued to perform extremely poorly.

CORT levels in the DA rats were no different from control after the forced swim, suggesting that these rats were not differentially sensitive to swim stress. Remarkably, neither hidden nor cued platform water maze performance were improved by restoring or blocking DA function pharmacologically. Moreover, PPI was not impaired in these rats, suggesting that they were processing sensory information normally.

The open field hyperactivity displayed by female DA rats was unusual. An extensive body of work has shown that neonatal DA lesions result in a transient period of hyperkinesia, between about P12 and P35 (Archer et al., 1988; Davids et al., 2002; Luthman et al., 1997; Raskin et al., 1983; Shaywitz et al., 1976; Zhang et al., 2001; Zhang et al., 2002). Hyperactivity has been detected as late as P77 (Takasuna & Iwasaki, 1996). However by this time the effect is considerably reduced (Luthman et al., 1997). In the present study, the rats were tested in the open field between 3 and 3.5 months of age. Based on these previous reports, it was surprising to find such a substantial effect of the DA lesion on open field activity.

It is unlikely that the hyperactivity observed here resulted from differences in lesion size between this and other studies, since both the cortical and striatal DA depletions were similar to those obtained elsewhere. However, one potentially significant difference between this and the previously cited studies is the time of day in which the locomotor measurements were taken. The present experiment assessed locomotor activity during the nocturnal period, when rats are normally awake, whereas virtually all other research has

examined locomotor activity during the diurnal period. The one study which assessed spontaneous activity in DA rats of both sexes over 24 hours reported increased nocturnal, but not diurnal, activity at six months of age (Castañeda et al., 1991). Thus while diurnal hyperactivity may wane as DA rats age, nocturnal hyperactivity persists into adulthood. Future studies could determine the time course of nocturnal hyperactivity, and whether it is sensitive to methylphenidate or amphetamine, as is the case for juvenile rats who have sustained a neonatal DA lesion (Davids et al., 2002; Luthman et al., 1989; Shaywitz et al., 1978).

It is noteworthy that hyperactivity was observed only in the female rats. Only one other study has reported activity data from each sex separately (Takasuna & Iwasaki, 1996). Interestingly, these authors observed that male 6-OHDA lesioned rats showed a moderate increase in activity levels over controls at P77, whereas in females this increase was more than twice as large. Taken together these data suggest that hyperactivity may be particularly pronounced in female rats. The reason for this merits further exploration, particularly since attention deficit hyperactivity disorder is predominantly diagnosed in male children (Barkley, 1997). These data also underscore the necessity of examining both males and females following neonatal lesions.

The DA lesion reduced exploratory rearing by both male and female DA rats. This is consistent with other reports of reduced rearing in the open field (Archer et al., 1988; Kalsbeek et al., 1989a; Luthman et al., 1997), as well as reduced head-dipping in the holeboard test (Luthman et al., 1997).

Similar to other published reports (Archer et al., 1988; Luthman et al., 1997; Pappas et al., 1992), the DA lesion caused a striking impairment in water maze performance, particularly during the initial hidden platform experiment when the rats were completely naïve to the maze. Not a single lesioned rat managed to consistently find the platform, even after seven consecutive days of testing (two of which involved a cued platform). Despite being able to swim, these rats persistently made ineffective responses such as trying to climb the walls of the maze, rather than searching for the hidden platform. Unlike the controls, they had little opportunity to spatially map the test room since they would not remain on the platform between trials. While some rats started sitting on the platform by the fourth day of training, they all persisted in wall climbing for the full seven days. Thus they were so hopelessly impaired in performing the task that no conclusions about their spatial abilities could be drawn.

Significant improvements in task performance were gained by gradually introducing these rats to the water. Once habituated to the pool, they rarely attempted wall climbing, occasionally swam away from the side to explore the interior of the pool, and almost always sat on the platform between trials. Once task performance had improved, many of the rats were able to find the cued platform within the 90 second time limit. However they still did not learn the task, since platform location latencies remained near the maximum. This was surprising given that the rats received ten consecutive days of training and that the task consisted of simply swimming toward the visual cue suspended directly over the platform.

Rather than a spatial deficit, the inability of these rats to learn the cued platform task could reflect a severe associative learning deficit. For example, in the first water maze study most of these rats would not stay on the platform between trials until the fourth day of testing, suggesting that they were very slow to associate the platform with escape. Moreover, while the rats who were gradually habituated to the maze were able to associate the platform with escape immediately, they never learned to associate the suspended cube with the platform. Arguably, the platform was a proximal, contiguous cue that could be successfully, albeit slowly, linked with escape, whereas the suspended cube was too distant or abstract to be associated with escape.

Interestingly, one other group has reported differences in performance (as opposed to learning) between control and neonatal 6-OHDA lesioned rats. In that study, the DA rats required significantly more time to escape shock in an active avoidance task (Takasuna & Iwasaki, 1996). However instead of running or avoiding like the controls, these rats exhibited behaviours such as rearing in response to the conditioned and unconditioned stimulus, despite the presence of similar shock intensity thresholds between the groups. These results could also be explained by a deficit in associative learning, in that the lesioned rats may have had difficulty associating the distal cue (buzzer) with its outcome (footshock).

In support of this hypothesis, recent research has suggested a role for DA in stimulus association. For example, the association of otherwise neutral stimuli elicits DA release in the nucleus accumbens (Young et al., 1998). Furthermore, DA mediation in the amygdala is necessary to reinstate extinguished lever pressing for cocaine by a conditional

cue (See et al., 2003). Thus it would be instructive to further characterize associative learning in DA rats.

Another explanation for the poor water maze acquisition in the DA rats is that they suffered an extreme stress response which grossly impeded their performance. The mPFC is rich in glucocorticoid receptors and lesions of this area in both neonates and adults have been shown to enhance the CORT response to stress (Brake et al., 2000; Diorio et al., 1993). Given the substantial reductions of DA in the frontal cortex of the DA rats, it was thought that they also might show an enhanced response to swim stress. However, no differences in CORT levels were detected between control and lesioned rats after the forced swim: both groups showed a robust increase over baseline. This indicates that at least in terms of CORT secretion, the forced swim was equally stressful for both the control and the DA rats. However, it remains possible that some other aspect of the stress response was affected, or that the swim procedure produced a severe anxiogenic response in these rats. It would thus be worthwhile to assess the effect of anxiolytic drugs on the ability of these rats to learn spatial navigation and other tasks.

Surprisingly, l-dopa supplementation had no effect on water maze learning in DA rats, in either the spatial or the cued version. This contrasts with two previous studies showing that increasing DA function with methylphenidate or amphetamine improves escape performance and conditioned odour learning respectively in lesioned pups (Shaywitz et al., 1978; Wool et al., 1987). One possible reason for this discrepancy is the time at which the assessments were made. The aforementioned studies tested the rats as pups and as weanlings, but not during adulthood. It is possible that later occurring

consequences of the lesion may not have had time to manifest. Alternatively, the lesioned rats in this study may have lost some compensatory stimulant-triggered process as their brains matured. While it is also possible that 6-OHDA lesioned rats are less sensitive to DA agonists such as l-dopa than to indirect agonists such as amphetamine, this seems unlikely, since doses of as little as 10 mg/kg of l-dopa can induce self-mutilatory behaviour (Breese et al., 1984).

Striatal lesions on P7 heighten sensitivity to both D1 and D2 agonists (Neal-Beliveau & Joyce, 1999; Thomas et al., 1998), suggesting that denervation supersensitivity of these receptors may have been responsible for some of the behavioural impairments observed in the DA rats. However, combined blockade of D1 and D2 receptors had no effect on the abysmal water maze performance of the rats in this study. Thus denervation supersensitivity does not adequately account for their deficits.

Sensorimotor gating was also intact in the DA rats. This was surprising given their profound and intractable water maze impairment. However, it is consistent with one other study of rats with neonatal excitotoxic lesions of the mPFC, wherein no disruption of PPI was found (Brake et al., 2000). Conversely, 6-OHDA lesions of the mPFC in adult rats do disrupt PPI (Bubser & Koch, 1994). Perhaps after neonatal DA lesions, there is a unique reorganization of the neural circuits subserving PPI which does not occur following adult lesions.

The fact that the behaviour of the DA rats was not obviously associated with either sensitivity to stress, DA hypofunction, denervation supersensitivity, or abnormal sensorimotor gating, suggests that structural changes in brain circuitry may have been

responsible for their impairment. While dramatic structural changes were not observed in either the mPFC, the ACC or the RSG in these rats, this does not signify that these cells were normal. As discussed previously, a near-normal dendritic structure may have been supported by significant synaptic reorganization following the lesion. Moreover, reinnervation of denervated DA synaptic sites by heterotypic afferents may be functionally devastating to these rats. The result of the lesion may not simply have been to remove the modulatory effect of DA on cortical cells, but to subject them to undue modulation from other systems. This could produce significant behavioural disruptions which in turn are resistant to pharmacological manipulations of the dopaminergic system.

Dual Lesions

The behavioural outcome of the dual lesion was generally similar to that of the DA lesion. Female but not male dual rats were hyperactive in the open field and rearing was reduced. While both the ACh and DA lesions by themselves reduced rearing, this effect was not exacerbated by co-administration of the toxins.

These rats also performed very poorly on the hidden platform water maze, precluding an assessment of their spatial learning abilities. After a gradual habituation to the cued platform task their performance improved, but platform location latencies remained near ceiling levels. All in all, the behavioral effects of the two lesions together were not different from the DA lesion alone.

The fact that the neurochemical and morphological consequences of the dual lesion were essentially additive effects of the single lesions suggests that an additive effect could

be anticipated for the behaviour. This seemed to be the case. For example, locomotor activity was unaffected by the ACh lesion and activity levels were not different between the DA and dual rats. While the ACh lesion had minimal effects on water maze performance, both the DA and dual rats performed near ceiling levels. It would be instructive to examine the effects of dual lesions on a learning task with which DA rats displayed some competence, or perhaps by re-examining the effects of combined lesions on water maze performance in rats with a less severe DA lesion.

The only behaviour which did not display additive effects was rearing. Rearing was reduced by either lesion alone, but was not further reduced by the dual lesion. These data strongly suggest that reduced exploration is a nonspecific consequence of neonatal injury. This effect is not likely related to neonatal surgery or anaesthesia, since 192SAP lesions at P1 do not affect rearing, nor do they cause a significant ACh lesion (Ricceri et al., 1997).

Given that reduced exploration was not specific to one type of lesion, it is possible that external factors contributed to this deficit. For example, differences in the frequency of maternal licking and grooming can affect later responses to novelty and stress in offspring (Caldji et al., 1998). While no assessments have been made of mother-pup interactions in ACh lesioned rats, primiparous dams are reportedly more attentive to 6-OHDA lesioned pups than to littermate controls (Piccirillo et al., 1979). Thus the dynamics of mother-pup interaction between lesioned and non-lesioned pups and its effect on subsequent exploratory behaviour merit future study.

Summary and Conclusions

Administration of 192SAP at P7 resulted in large, persistent depletions of ChAT activity in the hippocampus and RSC, as well as lesser reductions in anterior and temporal cortical regions. Layer V pyramidal cells in the mPFC and layer III pyramidal cells in the ACC exhibited atrophy of the apical dendritic tree, primarily in the tuft, as well as a marked reduction in spine density on both apical and basal dendrites. Layer III pyramidal cells of the RSG had substantially reduced basal dendritic trees. These effects were apparent well into adulthood and are thus likely permanent.

Despite the prominent neurochemical and anatomical effects of this lesion, these rats performed similarly to controls on the behavioural tests, with two modest exceptions. Exploration of an open field was reduced and female rats showed a slight impairment on the first day of hidden platform water maze testing. Together with existing data, this suggests that rats with neonatal ACh lesions may suffer from an impairment of visuospatial working memory, which is more pronounced in females than in males. Future studies should examine the performance of lesioned females on more complex tasks such as the radial arm maze. Furthermore, the present study demonstrates that pyramidal cell morphology in midline cortical areas does not need to be intact for the normal expression of many behaviours in the rat. This implies that either dendritic regression must surpass a certain threshold before behavioural effects appear, or that spatial information is processed in parallel, and other brain areas are able to compensate for reduced cortical connectivity.

Administration of 6-OHDA at P7 resulted in large depletions of DA and its metabolites from the caudate and anterior cortical region, with lesser reductions in the

temporal cortex and midbrain. Dendritic morphology from midline cortical areas was surprisingly intact. The DA lesion caused a regression of first order, oblique, apical dendrites from layer V pyramidal cells of the mPFC, and a slight thinning of the basal tree (although total length was not significantly different from control). Total branching of the basal tree of layer III pyramidal cells from the RSG was also slightly reduced. The lesion did not affect spine density in either area. Layer III pyramidal cells of the ACC also exhibited a slight reduction in branching of the basal tree. In contrast though, a substantial reduction in spines was observed from both the apical and basal dendrites of these cells. These effects were apparent in adulthood and are thus likely permanent.

Unlike the ACh lesion, the neonatal DA lesion was behaviourally devastating. Female rats were hyperactive in the open field, but exploration was reduced in both sexes. Navigation in the hidden platform water maze was absent in these rats, however task performance was so hopelessly compromised that no conclusions as to their spatial learning abilities could be drawn. Habituation to the cued platform water maze improved performance, but these rats still could not learn the task. This and other studies suggest that rats with neonatal DA lesions may suffer from a severe impairment in associative learning. Future research should examine the performance of these rats in simple conditioning tasks. Alternatively, behaviour could be investigated in rats with smaller DA lesions, such that task performance is not confounded with learning ability.

Further testing revealed that female DA rats were not differentially sensitive to a mild forced swim stress, were resistant to pharmacological up- or downregulation of DA transmission during hidden or cued platform navigation, and did not display deficient

sensorimotor gating. As discussed previously, reorganized afferents may have produced a behavioural phenotype which was resistant to manipulation of DA transmission. It would thus be interesting to determine whether learning is sensitive to pharmacological manipulation of other systems in these rats.

Co-administration of 192SAP and 6-OHDA resulted in large, persistent reductions in forebrain ACh and DA transmission. Surprisingly, the dual lesion had simple, additive effects on neurochemistry and cortical morphology, with the exception of spine density in the layer III ACC pyramidal cells. This measure was not reduced beyond the level observed in ACh rats. Behaviourally, these rats were indistinguishable from the DA rats. However, the fact that exploratory behaviour was reduced by all three lesions suggests that this may be a general consequence of neonatal brain damage, possibly related to external factors such as maternal care.

Interestingly, the DA lesion was associated with the least dendritic changes, but produced a profound and intractable behavioural impairment. Furthermore, the fact that an associative learning deficit could be responsible for this impairment might have far reaching implications for behaviour. Simple associations underlie many aspects of cognition, and greatly influence how we respond to the environment. Thus the DA system may be particularly important for the expression of many aspects of behaviour. This implies that DA hypofunction might play a more significant role in many of the behavioural features of disorders such as Rett and Down syndrome than previously thought.

In contrast, the ACh lesion was associated with the greatest dendritic changes, but produced the least effect on behaviour. This was surprising given the association between dendritic dysgenesis and cognitive disability in humans (Huttenlocher, 1991; Purpura, 1974; Rakic et al., 1994). However these data do show that neonatal cholinergic hypofunction reduces synaptic connectivity in the cortex, and thus likely contributes to the dendritic dysgenesis observed in both the Rett and Down cortex. That this reduction is not associated with gross behavioural impairments in the rat may simply reflect the fact that rats do not rely as heavily on the cortex for cognition, as do humans.

The lack of significant cognitive impairments following the neonatal ACh lesion precludes its use as a model for the behavioural characteristics of disorders such as Rett and Down syndrome. However, the present study demonstrates the utility of this lesion in the study of morphological changes brought about by early cholinergic hypofunction. Specifically, it would be interesting to determine whether neonatal ACh lesions produce a global effect on dendritic morphology, or if atrophy is restricted to particular areas of cortex, or even particular aspects of pyramidal cells. Furthermore, it would be instructive to determine if factors such as environmental enrichment, which increase synaptic connectivity in the intact cortex, exert a similar influence following early cholinergic deafferentation. These data may provide new avenues in the design of treatment strategies for children with neurodevelopmental disorders.

REFERENCES

- Acheson, A.L. & Zigmond, M.J. (1981). Short and long term changes in tyrosine hydroxylase activity in rat brain after subtotal destruction of central noradrenergic neurons. *J. Neurosci.* 1: 493-504.
- Acheson, A.L., Zigmond, M.J. & Stricker, E.M. (1980). Compensatory increase in tyrosine hydroxylase activity in rat brain after intraventricular injection of 6-hydroxydopamine. *Science* 207: 537-534.
- Aggleton, J.P., Vann, S.D., Oswald, C.J.P. & Good, M. (2000). Identifying cortical inputs to the rat hippocampus that subserve allocentric spatial processes: a simple problem with a complex answer. *Hippocampus* 10: 466-474.
- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U. & Zoghbi, H.Y. (1999). Rett Syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* 23: 185-188.
- Archer, T., Danysz, W., Fredriksson, A., Jonsson, G., Luthman, J., Sundstrom, E. & Teiling, A. (1988). Neonatal 6-hydroxydopamine-induced dopamine depletions: motor activity and performance in maze learning. *Pharmacol. Biochem. Behav.* 31: 357-364.
- Armstrong, D.D., Dunn, J.K. & Antalffy, B. (1998). Decreased dendritic branching in frontal, motor and limbic cortex in Rett syndrome compared with trisomy 21. *J. Neuropathol. Exp. Neurol.* 57: 1013-1017.
- Armstrong, D.D., Dunn, J.K., Antalffy, B. & Trivedi, R. (1995). Selective dendritic alterations in the cortex of Rett syndrome. *J. Neuropathol. Exp. Neurol.* 54: 195-201.
- Bachman, E.S., Berger-Sweeney, J., Coyle, J.T. & Hohmann, C.F. (1994). Developmental regulation of adult cortical morphology and behavior: An animal model for mental retardation. *Int. J. Dev. Neurosci.* 12: 239-253.
- Barkley, R.A. (1997). Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol. Bull.* 121: 65-94.
- Batchelor, P.E., Armstrong, D.M., Blaker, S.N. & Gage, F.H. (1989). Nerve growth factor receptor and choline acetyltransferase colocalization in neurons within the rat forebrain: response to fimbria-fornix transection. *J. Comp. Neurol.* 284: 187-204.
- Becker, L.E., Armstrong, D.L. & Chan, F. (1986). Dendritic atrophy in children with Down's syndrome. *Ann. Neurol.* 20: 520-526.

- Belichenko, P.V., Oldfors, A., Hagberg, B. & Dahlstrom, A. (1994). Rett syndrome: 3-D confocal microscopy of cortical pyramidal dendrites and afferents. *Neuroreport* 5: 1509-1513.
- Berger, B., Gaspar, P. & Verney, C. (1991). Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. *Trends Neurosci.* 14: 21-28.
- Berger, B., Verney, C., Alvarez, C., Vigny, A. & Helle, K.B. (1985a). New dopaminergic terminal fields in the motor, visual (area 18b) and retrosplenial cortex in the young and adult rat. Immunocytochemical and catecholamine histochemical analyses. *Neuroscience* 15: 983-998.
- Berger, B., Verney, C., Febvret, A. & Helle, K.B. (1985b). Postnatal ontogenesis of the dopaminergic innervation in the rat anterior cingulate cortex (area 24). Immunocytochemical and catecholamine fluorescence histochemical analysis. *Brain Res.* 353: 31-47.
- Berger-Sweeney, J. & Hohmann, C.F. (1997). Behavioral consequences of abnormal cortical development: insights into developmental disabilities. *Behav. Brain Res.* 86: 121-142.
- Book, A.A., Wiley, R.G. & Schweitzer, J.B. (1992). Specificity of 192 IgG-saporin for NGF receptor-positive cholinergic basal forebrain neurons in the rat. *Brain Res.* 590: 350-355.
- Brake, W.G., Flores, G., Francis, D., Meaney, M., Srivastava, L.K. & Gratton, A. (2000). Enhanced nucleus accumbens dopamine and plasma corticosterone stress responses in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex. *Neuroscience* 96: 687-695.
- Breese, G.R. & Traylor, T.D. (1971). Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br. J. Pharmacol.* 42: 88-99.
- Breese, G.R., Baumeister, A.A., McCown, T.J., Emerick, S.G., Frye, G.D., Crotty, K. & Mueller, R.A. (1984). Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. *J. Pharmacol. Exp. Ther.* 231: 343-354.
- Bruno, J.P., Jackson, D., Zigmond, M.J. & Stricker, E.M. (1987). Effect of dopamine-depleting brain lesions in rat pups: role of striatal serotonergic neurons in behavior. *Behav. Neurosci.* 101: 806-811.

- Bubser, M. & Koch, M. (1994). Prepulse inhibition of the acoustic startle response of rats is reduced by 6-hydroxydopamine lesions of the medial prefrontal cortex. *Psychopharmacol. (Berl.)*. 113: 487-492.
- Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M. & Meaney, M.J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc. Natl. Acad. Sci. USA*. 95: 5335-5340.
- Cardinal, R.N., Parkinson, J.A., Marbini, H.D., Toner, A.J., Bussey, T.J., Robbins, T.W. & Everitt, B.J. (2003). Role of the anterior cingulate cortex in the control over behavior by Pavlovian conditioned stimuli in rats. *Behav. Neurosci.* 117:566-587.
- Carr, D.B. & Sesack, S.R. (1996). Hippocampal afferents to the rat prefrontal cortex: synaptic targets and relation to dopamine terminals. *J. Comp. Neurol.* 369: 1-15.
- Casanova, M.F., Walker, L.C., Whitehouse, P.J. & Price D. (1985). Abnormalities of the nucleus basalis in Down's syndrome. *Ann. Neurol.* 18: 310-313.
- Castañeda, E., Whishaw, I.Q., Lerner, L. & Robinson, T.E. (1990). Dopamine depletion in neonatal rats: effects on behavior and striatal dopamine release assessed by intracerebral microdialysis during adulthood. *Brain Res.* 508: 30-39.
- Coleman, P.D. & Riesen, A.H. (1968). Environmental effects on cortical dendritic fields: rearing in the dark. *J. Anat.* 102: 363-374.
- Conti, F., Rustioni, A., Petrusz, P. & Towle, A.C. (1987). Glutamate-positive neurons in the somatic sensory cortex of rats and monkeys. *J. Neurosci.* 7: 1887-1901.
- Coronas, V., Durand, M., Chabot, J.G., Jourdan, F. & Quirion, R. (2000). Acetylcholine induces neuritic outgrowth in rat primary olfactory bulb cultures. *Neuroscience*. 98: 213-219.
- Cotman, C.W. & Nadler, J.V. (1978). Reactive synaptogenesis in the hippocampus. In C.W. Cotman (Ed.), *Neuronal Plasticity* (pp. 227-271). New York: Raven Press.
- Coyle, J.T., Oster-Granite, M.L. & Gearhart, J.D. (1986). The neurobiologic consequences of Down Syndrome. *Brain Res. Bull.* 16: 773-687.
- Coyle, J.T. & Yamamura, H.I. (1976). Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain. *Brain Res.* 118: 429-440.

- Crutcher, K.A. (1987). Sympathetic sprouting in the central nervous system: a model for studies of axonal growth in the mature mammalian brain. *Brain Res. Rev.* 12: 203-233.
- da Penha Berzaghi, M., Cooper, J., Castren, E., Zafra, F., Sofroniew, M., Thoenen H. & Lindholm, D. (1993). Cholinergic regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) but not neurotrophin-3 (NT-3) mRNA levels in the developing rat hippocampus. *J. Neurosci.* 13: 3818-3826.
- Daval, G., Verge, D., Becerril, A., Gozlan, H., Spampinato, U. & Hamon, M. (1987). Transient expression of 5-HT_{1A} receptor binding sites in some areas of the rat CNS during postnatal development. *Int. J. Dev. Neurosci.* 5: 171-180.
- Davids, E., Zhang, K., Tarazi, F.I. & Baldessarini, R.J. (2002). Stereoselective effects of methylphenidate on motor hyperactivity in juvenile rats induced by neonatal 6-hydroxydopamine lesioning. *Psychopharmacol.* 160: 92-98.
- de Bruin, J.P., Moita, M.P., de Brabander, H.M. & Joosten, R.N. (2001). Place and response learning of rats in a Morris water maze: differential effects of fimbria fornix and medial prefrontal cortex lesions. *Neurobiol. Learn. Mem.* 75: 164-178.
- Descarries, L., Lemay, B., Doucet, G. & Berger, B. (1987). Regional and laminar density of the dopamine innervation in adult rat cerebral cortex. *Neuroscience* 21: 807-824.
- Diamond, M.C., Ingham, C.A., Johnson, R.E. Bennett, E.L. & Rosenzweig, M.R. (1976). Effects of environment on morphology of rat cerebral cortex and hippocampus. *J. Neurobiol.* 7: 75-85.
- Dias, R. & Aggleton, J.P. (2000). Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility. *Eur. J. Neurosci.* 12: 4457-4466.
- Diorio, D., Viau, V. & Meaney, M.J. (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.
- Dori, I., Petrou, M. & Parnavelas, J.G. (1989). Excitatory transmitter amino acid-containing neurons in the rat visual cortex: a light and electron microscopic immunocytochemical study. *J. Comp. Neurol.* 290: 169-184.
- Dreyfus, C. (1998). Neurotransmitters and neurotrophins collaborate to influence brain development. *Perspect. Dev. Neurobiol.* 5: 389-399.

- Eckenstein, F. (1988). Transient expression of NGF-receptor-like immunoreactivity in postnatal rat brain and spinal cord. *Brain Res.* 446: 149-154.
- Eckenstein, F.P., Baughman, R.W. & Quinn, J. (1988). An anatomical study of cholinergic innervation in rat cerebral cortex. *Neuroscience* 25: 457-474.
- Erickson, S.L., Sesack, S.R. & Lewis, D.R. (2000). Dopamine innervation of monkey entorhinal cortex: postsynaptic targets of tyrosine hydroxylase-immunoreactive terminals. *Synapse* 36: 47-56.
- Ethier, K., Le Marec, N., Rompre, P.P. & Godbout, R. (2001). Spatial strategy elaboration in egocentric and allocentric tasks following medial prefrontal cortex lesions in the rat. *Brain. Cogn.* 46: 134-135.
- Feenstra, M.G.P., Kalsbeek, A. & van Galen, H. (1992). Neonatal lesions of the ventral tegmental area affect monoaminergic responses to stress in the medial prefrontal cortex and other dopamine projection areas in adulthood. *Brain Res.* 596: 169-182.
- Fernandes Xavier, F.G., Doucet, G., Geffard, M. & Descarries, L. (1994). Dopamine neoinnervation in the substantia nigra and hyperinnervation in the interpeduncular nucleus of adult rat following neonatal cerebroventricular administration of 6-hydroxydopamine. *Neuroscience* 59: 77-87.
- Fisk, G.D. & Wyss, J.M. (1999). Associational projections of the anterior midline cortex in the rat: intracingulate and retrosplenial connections. *Brain Res.* 825: 1-13.
- Fonnum, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.* 24: 407-409.
- Friedman, W.J., Olson, L. & Persson, H. (1991). Temporal and spatial expression of NGF receptor mRNA during postnatal rat brain development analyzed by in situ hybridization. *Dev. Brain Res.* 63: 43-51.
- Geinisman, Y., de Toledo-Morrell, L. & Morrell, F. (1986). Aged rats need a preserved complement of perforated axospinous synapses per hippocampal neuron to maintain good spatial memory. *Brain Res.* 398: 266-275.
- Gibb, R. & Kolb, B. (1998). A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *J. Neurosci. Methods* 79: 1-4.
- Glaser, E.M. & van der Loos, H. (1981). Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. *J. Neurosci. Methods* 4: 117-125.

- Glinka, Y., Gassen, M. & Youdim, M.B. (1997). Mechanism of 6-hydroxydopamine neurotoxicity. *J. Neural Transm. Suppl.* 50: 55-66.
- Godridge, H., Reynolds, G.P., Czudek, C., Calcutt, N.A. & Benton, M. (1987). Alzheimer-like neurotransmitter deficits in adult Down's syndrome brain tissue. *J. Neurol. Neurosurg. Psychiatr.* 50: 775-778.
- Goldman-Rakic, P.S. (1996). Regional and cellular fractionation of working memory. *Proc. Natl. Acad. Sci. USA.* 93: 13473-13480.
- Gong, L., Kostrzewa, R.M., Perry, K.W. & Fuller, R.W. (1993). Dose-related effects of a neonatal 6-OHDA lesion on SKF-38393- and *m*-chlorophenylpiperazine-induced oral activity responses of rats. *Dev. Brain Res.* 76: 233-238.
- Gonzalo-Ruiz, A. & Morte, L. (2000). Localization of amino acids, neuropeptides and cholinergic markers in neurons of the septum-diagonal band complex projecting to the retrosplenial granular cortex of the rat. *Brain Res. Bull.* 52: 499-510.
- Greenough, W.T., Juraska, J.M. & Volkmar, F.R. (1979). Maze training effects on dendritic branching in occipital cortex of adult rats. *Behav. Neural. Biol.* 26: 287-297.
- Greenough, W.T., Larson, J.R. & Withers, G.S. (1985). Effects of unilateral and bilateral training in a reaching task on dendritic branching of neurons in the rat motor-sensory forelimb cortex. *Behav. Neural Biol.* 44: 301-314.
- Groenewegen, H.J., Wright, C.I. & Uylings, H.B.M. (1997). The anatomical relationships of the prefrontal cortex with limbic structures and the basal ganglia. *J. Psychopharmacol.* 11: 99-106.
- Gutiérrez, H., Miranda, M.I. & Bermúdez-Rattoni, F. (1997). Learning impairment and cholinergic deafferentation after cortical nerve growth factor deprivation. *J. Neurosci.* 17: 3796-3803.
- Ha, D.H., Roberston, R.T. & Weiss, J.H. (1998). Distinctive Morphological Features of a subset of cortical neurons grown in the presence of basal forebrain neurons in vitro. *J. Neurosci.* 18: 4201-4215.
- Hagberg, B. (1995). Rett syndrome: clinical peculiarities and biological mysteries. *Acta Paediatr.* 84: 971-976.
- Harker, K.T. & Whishaw, I.Q. (2002). Impaired spatial performance in rats with retrosplenial lesions: importance of the spatial problem and the rat strain in identifying lesion effects in a swimming pool. *J. Neurosci.* 22: 1155-1164.

- Heckers, S., Ohtake, T., Wiley, R.G., Lappi, D.A., Geula, C. & Mesulam, M.-M. (1994). Complete and selective cholinergic denervation of rat neocortex and hippocampus but not amygdala by an immunotoxin against the p75 NGF receptor. *J. Neurosci.* 14: 1271-1289.
- Heffner, T.G. & Seiden, L.S. (1983). Impaired acquisition of an operant response in young rats depleted of brain dopamine in neonatal life. *Psychopharmacol.* 79: 115-119.
- Hoh, T.E., Kolb, B., Eppel, A., Vanderwolf, C.H. & Cain, D.P. (2003). Role of the neocortex in the water maze task in the rat: a detailed behavioral and Golgi-Cox analysis. *Behav. Brain Res.* 138: 81-94.
- Hohmann, C.F., Brooks, A.R. & Coyle, J.T. (1988). Neonatal lesions of the basal forebrain cholinergic neurons result in abnormal cortical development. *Dev. Brain Res.* 42: 253-264.
- Hohmann, C.F., Kwiterovich, K.K., Oster-Granite, M.L. & Coyle, J.T. (1991). Newborn basal forebrain lesions disrupt cortical cytodifferentiation as visualized by rapid Golgi staining. *Cereb. Cortex* 1: 143-157.
- Houser, C.R., Crawford, G.D., Salvaterra, P.M. & Vaughn, J.E. (1985). Immunocytochemical localization of choline acetyltransferase in rat cerebral cortex: a study of cholinergic neurons and synapses. *J. Comp. Neurol.* 234: 17-34.
- Huttenlocher, P.R. (1991). Dendritic and synaptic pathology in mental retardation. *Pediatr. Neurol.* 7: 79-85.
- Johansson, B.B. & Belichenko, P.V. (2002). Neuronal plasticity and dendritic spines: effect of environmental enrichment on intact and postischemic rat brain. *J. Cereb. Blood Flow Metab.* 22: 89-96.
- Jones, L.B., Fischer, I. & Levin, P. (1996). Non-uniform alteration of dendritic development in the cerebral cortex following prenatal cocaine exposure. *Cereb. Cortex* 6: 431-445.
- Jones, L.B., Stanwood, G.D., Reinoso, B.S., Washington, R.A., Wang, H.-Y., Friedman, E. & Levitt, P. (2000). *In utero* cocaine-induced dysfunction of dopamine D1 receptor signaling and abnormal differentiation of cerebral cortical neurons. *J. Neurosci.* 20: 4606-4614.
- Kalsbeek, A., de Bruin, J.P.C., Matthijssen, M.A.H. & Uylings, H.B.M. (1989a). Ontogeny of open field activity in rats after neonatal lesioning of the mesocortical dopaminergic projection. *Behav. Brain Res.* 32: 115-127.

- Kalsbeek, A., Feenstra, M.G.P., van Galen, H. & Uylings, H.B.M. (1989b). Monoamine and metabolite levels in the prefrontal cortex and the mesolimbic forebrain following neonatal lesions of the ventral tegmental area. *Brain Res.* 479: 339-343.
- Kalsbeek, A., Matthijssen, M.A.H. & Uylings, H.B.M. (1989c). Morphometric analysis of prefrontal cortical development following neonatal lesioning of the dopaminergic mesocortical projection. *Exp. Brain Res.* 78: 279-289.
- Kalsbeek, A., Voorn, P., Buijs, R.M., Pool, C.W. & Uylings, H.B.M. (1988). Development of the dopaminergic innervation in the prefrontal cortex of the rat. *J. Comp. Neurol.* 269: 58-72.
- Kaufmann, W.E. & Moser, H.W. (2000) Dendritic anomalies in disorders associated with mental retardation. *Cereb. Cortex* 10: 981-991.
- Kim, D.S., Szczypka, M.S. & Palmiter, R.D. (2000). Dopamine-deficient mice are hypersensitive to dopamine receptor agonists. *J. Neurosci.* 20: 4405-4413.
- Kiss, J. & Patel, A.J. (1992). Development of the cholinergic fibres innervating the cerebral cortex of the rat. *Int. J. Dev. Neurosci.* 10: 153-170.
- Kitt, C.A. & Wilcox, B.J. (1995). Preliminary evidence for neurodegenerative changes in the substantia nigra of Rett syndrome. *Neuropediatrics* 26: 114-118.
- Koh, S. & Higgins, G.A. (1991). Differential regulation of the low-affinity nerve growth factor receptor during postnatal development of the rat brain. *J. Comp. Neurol.* 313: 494-508.
- Koh, S. & Loy, R. (1989). Localization and development of nerve growth factor-sensitive rat basal forebrain neurons and their afferent projections to hippocampus and neocortex. *J. Neurosci.* 9: 2999-3018.
- Kolb, B., Cote, S. Ribeiro-da-Silva, A. & Cuello, A.C. (1997). Nerve growth factor treatment prevents dendritic atrophy and promotes recovery of function after cortical injury. *Neurosci.* 76: 1139-1151.
- Kolb, B., Forgie, M., Gibb, R., Gorny, G. & Rowntree, S. (1998). Age, experience and the changing brain. *Neurosci. Biobehav. Rev.* 22: 143-159.
- Kolb, B. & Gibb, R. (1991). Sparing of function after neonatal frontal lesions correlates with increased cortical dendritic branching: a possible mechanism for the Kennard effect. *Behav. Brain Res.* 43: 51-56.

- Kolb, B. & Gibb, R. (1993). Possible anatomical basis of recovery of function after neonatal frontal lesions in rats. *Behav. Neurosci.* 107: 799-811.
- Kolb, B., Stewart, J. & Sutherland, R.J. (1997). Recovery of function is associated with increased spine density in cortical pyramidal cells after frontal lesions and/or noradrenaline depletion in neonatal rats. *Behav. Brain Res.* 89: 61-70.
- Kostrzewa, R.M., Reader, T.A. & Descarries, L. (1998). Serotonin neural adaptations to ontogenetic loss of dopamine neurons in rat brain. *J. Neurochem.* 70: 889-898.
- Lacroix, L., White, I. & Feldon, J. (2002). Effect of excitotoxic lesions of rat medial prefrontal cortex on spatial memory. *Behav. Brain Res.* 133: 69-81.
- Lankford, K.L., DeMello, F.G. & Klein, W.L. (1988). D1-type dopamine receptors inhibit growth cone motility in cultured retina neurons: evidence that neurotransmitters act as morphogenic growth regulators in the developing central nervous system. *Proc. Natl. Acad. Sci. USA.* 85:4567-4571.
- Lapchak, P.A., Jenden, D.J. & Hefti, F. (1991) Compensatory elevation of acetylcholine synthesis *in vivo* by cholinergic neurons surviving partial lesions of the septohippocampal pathway. *J. Neurosci.* 11: 2821-2828.
- Lauder, J.M. & Schambra, U.B. (1999). Morphogenetic roles of acetylcholine. *Environ. Health Perspect.* 107: 65-69.
- Leanza, G., Nilsson, O.G., Nikkhah, G., Wiley, R.G. & Bjorklund, A. (1996). Effects of neonatal lesions of the basal forebrain cholinergic system by 192 immunoglobulin G-saporin: biochemical, behavioural and morphological characterization. *Neuroscience* 74: 119-141.
- Leanza, G., Nilsson, O.G., Wiley, R.G. & Bjorklund, A. (1995). Selective lesioning of the basal forebrain cholinergic system by intraventricular 192 IgG-saporin: behavioural, biochemical and stereological studies in the rat. *Eur. J. Neurosci.* 7: 329-343.
- Lee, I. & Kesner, R.P. (2003). Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. *J. Neurosci.* 23: 1517-1523.
- Leuner, B., Falduto, J. & Shors, T.J. (2003). Associative memory formation increases the observation of dendritic spines in the hippocampus. *J. Neurosci.* 23: 659-665.
- Lipani, J.D., Meena, B., Bhattacharjee, M.D., Corey, D.M. & Lee, D.A. (2000). Reduced nerve growth factor in Rett Syndrome postmortem brain tissue. *J. Neuropathol. Exp. Neurol.* 59: 889-895.

- Lubec, B., Yoo, B.C., Dierssen, M., Balic, N. & Lubec, G. (2001). Down syndrome patients start early prenatal life with normal cholinergic, monoaminergic and serotonergic innervation. *J. Neural Transm. Suppl.* 61: 303-310.
- Lübke, J., Markram, H., Frotscher, M. & Sakmann, B. (1996). Frequency and dendritic distribution of autapses established by layer 5 pyramidal neurons in the developing rat neocortex: comparison with synaptic innervation of adjacent neurons of the same class. *J. Neurosci.* 16: 3209-3218.
- Luthman, J., Bassen, M., Fredriksson, A. & Archer, T. (1997). Functional changes induced by neonatal cerebral 6-hydroxydopamine treatment: effects of dose levels on behavioral parameters. *Behav. Brain Res.* 82: 213-221.
- Luthman, J., Fredriksson, A., Lewander, T., Jonsson, G. & Archer, T. (1989). Effects of d-amphetamine and methylphenidate on hyperactivity produced by neonatal 6-hydroxydopamine treatment. *Psychopharmacol.* 99: 550-557.
- Mann, D.M.A., Brown, A., Prinja, D., Davies, C.A., Landon, M, Masters, C.L. & Beyreuther, K. (1989). An analysis of the morphology of senile plaques in Down's syndrome patients of different ages using immunocytochemical and lectin histochemical techniques. *Neuropathol. Appl. Neurobiol.* 15: 317-329.
- Mann, D.M.A., Yates, P.O. & Marcyniuk, B. (1987). Dopaminergic neurotransmitter systems in Alzheimer's disease and in Down's syndrome at middle age. *J. Neurol. Neurosurg. Psychiatr.* 50: 341-344.
- Mechawar, N., Costantino, C. & Descarries, L. (2000). Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. *J. Comp. Neurol.* 428: 305-318.
- Mechawar, N. & Descarries, L. (2001). The cholinergic innervation develops early and rapidly in the rat cerebral cortex: a quantitative immunocytochemical study. *Neuroscience* 108: 555-567.
- Mesulam, M.-M., Mufson, E.J., Wainer, B.H. & Levey, A.I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience* 10: 1185-1201.
- Moser, M., Trommald, M. & Andersen, P. (1994). An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. *Proc. Natl. Acad. Sci. USA.* 91: 12673-12675.

- Mrini, A., Soucy, J., Lafaille, F., Lemoine, P. & Descarries, L. (1995). Quantification of the serotonin hyperinnervation in adult rat neostriatum after neonatal 6-hydroxydopamine lesion of nigral dopamine neurons. *Brain Res.* 669: 303-308.
- Mrzljak, L., Pappy, M., Leranth, C. & Goldman-Rakic, P.S. (1995). Cholinergic synaptic circuitry in the macaque prefrontal cortex. *J. Comp. Neurol.* 357: 603-617.
- Murphy, E.H., Fischer, I., Friedman, E., Grayson, D., Jones, L., Levitt, P., O'Brien-Jenkins, A., Wang, H.-Y. & Wang, X.-Y. (1997). Cocaine administration in pregnant rabbits alters cortical structure and function in their progeny in the absence of maternal seizures. *Exp. Brain Res.* 114: 433-441.
- Neal-Beliveau, B.S. & Joyce, J.N. (1999). Timing: a critical determinant of the functional consequences of neonatal 6-OHDA lesions. *Neurotoxicol. Teratol.* 21: 129-140.
- Nieuwenhuys, R. (1994). The neocortex. An overview of its evolutionary development, structural organization and synaptology. *Anat. Embryol.* 190: 307-337.
- Owen, A. & Bird, M. (1995). Acetylcholine as a regulator of neurite outgrowth and motility in cultured embryonic mouse spinal cord. *Neuroreport* 6: 2269-2272.
- Pappas, B.A., Davidson, C.M., Fortin, T., Nallathamby, S., Park, G.A.S., Mohr, E. & Wiley, R.G. (1996). 192 IgG-saporin lesion of basal forebrain cholinergic neurons in neonatal rats. *Dev. Brain Res.* 96: 52-61.
- Pappas, B.A., Murtha, S.J., Park, G.A., Condon, K.T., Szirites, R.M., Laventure, S.I. & Ally, A. (1992). Neonatal brain dopamine depletion and the cortical and behavioral consequences of enriched postweaning environment. *Pharmacol. Biochem. Behav.* 42: 741-748.
- Pappas, B.A., Nguyen, T., Brownlee, B., Tanasoiu, D., Fortin, T. & Sherren, N. (2000). Ectopic noradrenergic hyperinnervation does not functionally compensate for neonatal forebrain acetylcholine lesion. *Brain Res.* 867: 90-99.
- Parkinson, J.A., Willoughby, P.J., Robbins, T.W. & Everitt, B.J. (2000). Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. *Behav. Neurosci.* 114: 42-63.
- Passetti, F., Levita, L. & Robbins, T.W. (2003). Sulpiride alleviates the attentional impairments of rats with medial prefrontal cortex lesions. *Behav. Brain Res.* 138: 59-69.

- Paxinos G. & Watson, C. (1998). The rat brain in stereotaxic coordinates (4th Ed.). San Diego: Academic Press.
- Penit-Soria, J., Durand, D., Herve, D. & Besson, M.J. (1997). Morphological and biochemical adaptations to unilateral dopamine denervation of the neostriatum in newborn rats. *Neuroscience* 77: 753-766.
- Piccirillo, M., Cohen, D.J., Shaywitz, B.A., Alpert, J.E. & Marinelli, D. (1979). Maternal care received by rat pups treated with 6-hydroxydopamine. *Physiol. Behav.* 22: 69-75.
- Potter, B.M & Bruno, J.P. (1989). Food intake of rats depleted of dopamine as neonates by inhibition of catecholamine biosynthesis. *Neurosci. Lett.* 107: 295-300.
- Potter, B.M & Bruno, J.P. (1991). Infant rats depleted of brain dopamine as neonates exhibit normal independent ingestion. *Dev. Psychobiol.* 24: 65-79.
- Pugh, P.C. & Berg, D.K. (1994). Neuronal acetylcholine receptors that bind alpha-bungarotoxin mediate neurite retraction in a calcium-dependent manner. *J. Neurosci.* 14: 889-896.
- Purpura, D.P. (1974). Dendritic spine "dysgenesis" and mental retardation. *Science* 186: 1126-1128.
- Purves, D. & Hume, R.I. (1981). The relation of postsynaptic geometry to the number of presynaptic axons that innervate autonomic ganglion cells. *J. Neurosci.* 1: 441-452.
- Raisman, G. (1985). Synapse formation in the septal nuclei of adult rats. In C.W. Cotman (Ed.), *Synaptic Plasticity* (pp. 13-38). New York: Guilford Press.
- Raison, S., Weissmann, D., Rousset, C., Pujol, J.F. & Descarries, L. (1995). Changes in steady-state levels of tryptophan hydroxylase protein in adult rat brain after neonatal 6-hydroxydopamine lesion. *Neuroscience* 67: 463-475.
- Rakic, P., Bourgeois, J. & Goldman-Rakic, P.S. (1994). Synaptic development of the cerebral cortex: Implications for learning, memory, and mental illness. *Prog. Brain Res.* 102: 227- 243.
- Raskin, L.A., Shaywitz, B.A., Anderson, G.M., Cohen, D.J., Teicher, M.H. & Linakis, J. (1983). Differential effects of selective dopamine, norepinephrine or catecholamine depletion on activity and learning in the developing rat. *Pharmacol. Biochem. Behav.* 19: 743-749.

- Reinoso, B.S., Undie, A.S. & Levitt, P. (1996). Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. *J. Neurosci. Res.* 43: 439-453.
- Reiss, A.L., Faruque, F., Naidu, S., Abrams, M., Beaty, T., Bryan, R.N. & Moser, H. (1993). Neuroanatomy of Rett syndrome: a volumetric imaging study. *Ann. Neurol.* 34: 227-234.
- Ricceri, L., Calamandrei, G. & Berger-Sweeney, J. (1997). Different effects of postnatal day 1 versus 7 192 immunoglobulin G-saporin lesions on learning, exploratory behaviors, and neurochemistry in juvenile rats. *Behav. Neurosci.* 111: 1292-1302.
- Ricceri, L., Hohmann, C. & Berger-Sweeney, J. (2002). Early neonatal 192 IgG saporin induces learning impairments and disrupts cortical morphogenesis in rats. *Brain Res.* 954: 160-172.
- Robertson, R.T., Gallardo, K.A., Claytor, K.J., Ha, D.H., Ku, K.-H., Yu, B.P., Lauterborn, J.C., Wiley, R.G., Yu, J., Gall, C.M. & Leslie, F.M. (1998). Neonatal treatment with 192 IgG-saporin produces long-term forebrain cholinergic deficits and reduces dendritic branching and spine density of neocortical pyramidal neurons. *Cereb. Cortex* 8: 142-155.
- Robinson, T.E. & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur. J. Neurosci.* 11: 1598-1604.
- Robinson, J.K., Wenk, G.L., Wiley, R.G., Lappi, D.A. & Crawley, J.N. (1996). 192IgG-saporin immunotoxin and ibotenic acid lesions of nucleus basalis and medial septum produce comparable deficits on delayed nonmatching to position in rats. *Psychobiology* 24: 179-186.
- Roßner, S., Schliebs, R., Härtig, W. & Bigl, V. (1995). 192IgG-saporin-induced selective lesion of cholinergic basal forebrain system: neurochemical effects on cholinergic neurotransmission in rat cerebral cortex and hippocampus. *Brain Res. Bull.* 38: 371-381.
- Ruberti, F., Capsoni, S., Comparini, A., Di Daniel, E., Franzot, J., Gonfloni, S., Rossi, G., Berardi, N. & Cattaneo, A. (2000). Phenotypic knockout of nerve growth factor in adult transgenic mice reveals severe deficits in basal forebrain cholinergic neurons, cell death in the spleen, and skeletal muscle dystrophy. *J. Neurosci.* 20: 2589-2601.
- Schade, J.B. & Baxter, C.F. (1960). Changes during growth in the volume and surface area of cortical neurons in the rabbit. *Exp. Neurol.* 2: 158-178.

- Schmidt, U., Beyer, C., Oestreicher, A.B., Schilling, K. & Pilgrim, C. (1996). Activation of dopaminergic D1 receptors promotes morphogenesis of developing striatal neurons. *Neuroscience* 74: 453-460.
- Schmidt, U., Pilgrim, C. & Beyer, C. (1998). Differentiative effects of dopamine on striatal neurons involve stimulation of the cAMP/PKA pathway. *Mol. Cell Neurosci.* 11: 9-18.
- See, R.E., Fuchs, R.A., Ledford, C.C. & McLaughlin, J. (2003). Drug addiction, relapse, and the amygdala. *Ann. N.Y. Acad. Sci.* 985: 294-307.
- Seegal, R.F., Brosch, K.O. & Bush, B. (1986). High-performance liquid chromatography of biogenic amines and metabolites in brain, cerebrospinal fluid, urine and plasma. *J. Chromatogr.* 377: 131-144.
- Semba, K. & Fibiger, H.C. (1988). Time of origin of cholinergic neurons in the rat basal forebrain. *J. Comp. Neurol.* 269: 87-95.
- Shaywitz, B.A., Klopfer, J.H. & Gordon, J.W. (1978). Methylphenidate in 6-hydroxydopamine-treated developing rat pups. *Arch. Neurol.* 35: 463-469.
- Shaywitz, B.A., Teicher, M.H., Cohen, D.J., Anderson, G.M., Young, J.G. & Levitt, P. (1984). Dopaminergic but not noradrenergic mediation of hyperactivity and performance deficits in the developing rat pup. *Psychopharmacol.* 82: 73-77.
- Shaywitz, B.A., Yager, R.D. & Klopfer, J.H. (1976). Selective brain dopamine depletion in developing rats: an experimental model of minimal brain dysfunction. *Science* 191: 305-308.
- Sherren, N., Pappas, B.A. & Fortin, T. (1999). Neural and behavioral effects of intracranial 192 IgG-saporin in neonatal rats: sexually dimorphic effects? *Dev. Brain Res.* 114: 49-62.
- Small, D.H., Reed, G., Whitefield, B. & Nurcombe, V. (1995). Cholinergic regulation of neurite outgrowth from isolated chick sympathetic neurons in culture. *J. Neurosci.* 15: 144-151.
- Spencer, G.E., Lukowiak, K. & Syed, N.I. (2000). Transmitter-receptor interactions between growth cones of identified Lymnaea neurons determine target cell selection in vitro. *J. Neurosci.* 20: 8077-8086.
- Subramaniam, B., Naidu, S. & Reiss, A.L. (1997). Neuroanatomy in Rett syndrome: cerebral cortex and posterior fossa. *Neurology* 48: 399-407.

- Sutherland, R.J., Whishaw, I.Q. & Kolb, B. (1988). Contributions of cingulate cortex to two forms of spatial learning and memory. *J. Neurosci.* 8: 1863-1872.
- Takashima, S., Becker, L.E., Armstrong, D.L. & Chan, F. (1981). Abnormal neuronal development in the visual cortex of the human fetus and infant with Down's Syndrome. A quantitative and qualitative golgi study. *Brain Res.* 225: 1-21.
- Takasuna, M. & Iwasaki, T. (1996). Active and passive avoidance learning in rats neonatally treated with intraventricular 6-hydroxydopamine. *Behav. Brain Res.* 74: 119-126.
- Tata, A.M., Cursi, S., Biagioni, S. & August-Tocco, G. (2003). Cholinergic modulation of neurofilament expression and neurite outgrowth in chick sensory neurons. *J. Neurosci. Res.* 73: 227-234.
- Taylor, J., Docherty, M. & Gordon-Weeks, P.R. (1990). GABAergic growth cones: release of endogenous γ -aminobutyric acid precedes the expression of synaptic vesicle antigens. *J. Neurochem.* 54: 1689-1699.
- Thierry, A.-M., Gioanni, Y., Degenetais, E. & Glowinski, J. (2000). Hippocampo-prefrontal cortex pathway: anatomical and electrophysiological characteristics. *Hippocampus* 10: 411-419.
- Thomas, W.S., Neal-Beliveau, B.S. & Joyce, J.N. (1998). There is a limited critical period for dopamine's effects on D1 receptor expression in the developing rat neostriatum. *Dev. Brain Res.* 111: 99-106.
- Ungerstedt, U. (1971). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol. Scand. Suppl.* 367: 95-122.
- Van Eden, C.G., Hoorneman, E.M.D., Buijs, R.M., Matthijssen, M.A.H., Geffard, M. & Uylings, H.B.M. (1987). Immunocytochemical localization of dopamine in the prefrontal cortex of the rat at the light and electron microscopical level. *Neuroscience* 22: 849-862.
- van Groen, T. & Wyss, J.M. (1992). Connections of the retrosplenial dysgranular cortex in rat. *J. Comp. Neurol.* 315: 200-216.
- Verney, C., Berger, B., Adrien, J., Vigny, A. & Gay, M. (1982). Development of the dopaminergic innervation of the rat cerebral cortex. A light microscopic immunocytochemical study using anti-tyrosine hydroxylase antibodies. *Brain Res.* 281: 41-52.

- Voorn, P., Kalsbeek, A., Jorritsma-Byham, B. & Groenewegen, H.J. (1988). The pre- and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat. *Neuroscience* 25: 857-887.
- Waite, J.J., Wardlow, M.L., Chen, A.C., Lappi, D.A., Wiley, R.G. & Thal, L.J. (1994). Time course of cholinergic and monoaminergic changes in rat brain after immunolesioning with 192 IgG-saporin. *Neurosci. Lett.* 169: 154-158.
- Walsh, T.J., Kelly, R.M., Dougherty, K.D., Stackman, R.W., Wiley, R.G. & Kutscher, C.L. (1995). Behavioral and neurobiological alterations induced by the immunotoxin 192-IgG-saporin: cholinergic and non-cholinergic effects following i.c.v. injection. *Brain Res.* 702: 233-245.
- Wenk, G.L. (1997). Rett syndrome: neurobiological changes underlying specific symptoms. *Prog. Neurobiol.* 51: 383-391.
- Wenk, G.L. & Hauss-Wegrzyniak, B. (1999). Altered cholinergic function in the basal forebrain of girls with Rett syndrome. *Neuropediatrics* 30: 125-129.
- Wenk, G.L., Naidu, S., Casanova, M.F., Kitt, C.A. & Moser, H. (1991). Altered neurochemical markers in Rett's syndrome. *Neurology* 41: 1753-1756.
- Whishaw, I.Q., Gorny, B., Tran-Nguyen, L.T.L., Castañeda, E., Miklyaeva, E.I. & Pellis, S.M. (1994). Making two movements at once: impairments of movement, posture, and their integration underlie the adult skilled reaching deficit of neonatally dopamine-depleted rats. *Behav. Brain Res.* 61: 65-77.
- Whishaw, I.Q., Maaswinkel, H., Gonzalez, C.L.R. & Kolb, B. (2001). Deficits in allothetic and idiothetic spatial behavior in rats with posterior cingulate cortex lesions. *Behav. Brain Res.* 118: 67-76.
- Wiley, R.G., Oeltmann, T.N. & Lappi, D.A. (1991). Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Res.* 562: 149-153.
- Wolf, M.E., Zigmond, M.J. & Kapatos, G. (1989). Tyrosine hydroxylase content of residual striatal dopamine nerve terminals following 6-hydroxydopamine administration: a flow cytometric study. *J. Neurochem.* 53: 879-885.
- Wool, R.S., Weldon, D.A., Shaywitz, B.A., Anderson, G.M., Cohen, D.J. & Teicher, M.H. (1987). Amphetamine reverses learning deficits in 6-hydroxydopamine-treated rat pups. *Dev. Psychobiol.* 20: 219-232.

- Woolf, N.J., Gould, E. & Butcher, L.L. (1989). Nerve growth factor receptor is associated with cholinergic neurons of the basal forebrain but not the pontomesencephalon. *Neuroscience* 30: 143-152.
- Yan, Q. & Johnson, E.M. (1988). An immunohistochemical study of the nerve growth factor receptor in developing rats. *J. Neurosci.* 8: 3481-3498.
- Yee, B.K. (2000). Cytotoxic lesion of the medial prefrontal cortex abolishes the partial reinforcement extinction effect, attenuates prepulse inhibition of the acoustic startle reflex and induces transient hyperlocomotion, while sparing spontaneous object recognition memory in the rat. *Neuroscience* 95: 675-689.
- Young, A.M., Ahier, R.G., Upton, R.L., Joseph, M.H. & Gray, J.A. (1998). Increased extracellular dopamine in the nucleus accumbens of the rat during associative learning of neutral stimuli. *Neuroscience* 83: 1175-1183.
- Yu, J., Wiley, R.G. & Perez-Polo, R.J. (1996). Altered NGF levels in different brain areas after immunolesion. *J. Neurosci. Res.* 43: 213-223.
- Zafra, F., Lindholm, D., Castren, E., Hartikka, J. & Thoenen, H. (1992). Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J. Neurosci.* 12: 4793-4799.
- Zhang, K., Davids, E., Tarazi, F.I. & Baldessarini, R.J. (2002). Effects of dopamine D₄ receptor-selective antagonists on motor hyperactivity in rats with neonatal 6-hydroxydopamine lesions. *Psychopharmacol.* 161: 100-106.
- Zhang, K., Davids, E., Tarazi, F.I. & Baldessarini, R.J. (2002). Serotonin transporter binding increases in caudate-putamen and nucleus accumbens after neonatal 6-hydroxydopamine lesions in rats: implications for motor hyperactivity. *Dev. Brain Res.* 137: 135-138.
- Zhang, K., Tarazi, F.I. & Baldessarini, R.J. (2001). Role of dopamine D₄ receptors in motor hyperactivity induced by neonatal 6-hydroxydopamine lesions in rats. *Neuropsychopharmacol.* 25: 624-632.
- Zhang, L., Castell, A., Avila, E., Drucker-Colin, R. & Escobar, A. (2000). Immunocytochemical, ultrastructural and neurochemical evidences on synaptogenesis and dopamine release of rat chromaffin cells co-cultured with striatal neurons. *J. Neuropathol. Exp. Neurol.* 59: 170-174.
- Zhou, Q.-Y. & Palmiter, R.D. (1995). Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. *Cell* 83: 1197-1209.

Zigmond, M.J. & Stricker, E.M. (1973). Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions. *Science* 182: 717-720.

Zigmond, M.J. & Stricker, E.M. (1989). Animal models of parkinsonism using selective neurotoxins: clinical and basic implications. *Int. Rev. Neurosci.* 31: 1-79.

Zilles, K. (1985). The cortex of the rat. Berlin: Springer-Verlag.

APPENDIX A: ANOVA SUMMARY TABLES**Appendix A1. ANOVA Summary Tables for Body Weight****Body Weight: All Groups**

Source	df	MS	F	p
192SAP	1	1114.793	0.029	0.866
6-OHDA	1	1183978.7	30.324	0.000
Sex	1	4539464.8	116.263	0.000
192SAP X 6-OHDA	1	285292.84	7.307	0.009
192SAP X Sex	1	653.872	0.017	0.897
6-OHDA X Sex	1	102826.45	2.634	0.109
192SAP X 6-OHDA X Sex	1	41922.931	1.074	0.304
Error	71	39044.829		
Age	12	577626.98	1021.927	0.000
Age X 192SAP	12	383.472	0.678	0.773
Age X 6-OHDA	12	21422.146	37.9	0.000
Age X Sex	12	80973.324	143.257	0.000
Age X 192SAP X 6-OHDA	12	5017.823	8.877	0.000
Age X 192SAP X Sex	12	157.445	0.277	0.992
Age X 6-OHDA X Sex	12	3561.509	6.301	0.000
Age X 192SAP X 6-OHDA X Sex	12	404.053	0.715	0.738
Error	852	565.233		

Body Weight: Control vs. ACh Group

Source	df	MS	F	p
192SAP	1	137965.43	12.139	0.002
Sex	1	2573917.2	226.464	0.000
192SAP X Sex	1	22723.92	1.999	0.168
Error	29	11365.696		
Age	12	350631.57	1186.541	0.000
Age X 192SAP	12	3257.059	11.022	0.000
Age X Sex	12	50537.220	171.018	0.000
Age X 192SAP X Sex	12	301.579	1.021	0.429
Error	348	295.507		

Appendix A2. ANOVA Summary Tables for Neurochemistry**Anterior Cortex: ChAT Activity**

Source	df	MS	F	p
192SAP	1	1121.530	91.222	0.000
6-OHDA	1	2.843	0.231	0.633
Sex	1	11.420	0.929	0.342
192SAP X 6-OHDA	1	0.078	0.006	0.937
192SAP X Sex	1	0.208	0.017	0.897
6-OHDA X Sex	1	4.891	0.398	0.532
192SAP X 6-OHDA X Sex	1	17.742	1.443	0.237
Error	36	12.295		
Total	44			

Anterior Cortex: DA Levels

Source	df	MS	F	p
192SAP	1	0.210	0.674	0.417
6-OHDA	1	22.079	70.776	0.000
Sex	1	0.001	0.005	0.943
192SAP X 6-OHDA	1	0.005	0.018	0.895
192SAP X Sex	1	0.045	0.145	0.705
6-OHDA X Sex	1	0.021	0.068	0.795
192SAP X 6-OHDA X Sex	1	0.084	0.272	0.605
Error	36	0.312		
Total	44			

Anterior Cortex: DOPAC Levels

Source	df	MS	F	p
192SAP	1	0.027	2.485	0.124
6-OHDA	1	1.443	129.233	0.000
Sex	1	0.000	0.001	0.979
192SAP X 6-OHDA	1	0.002	0.235	0.631
192SAP X Sex	1	0.048	4.328	0.045
6-OHDA X Sex	1	0.006	0.558	0.460
192SAP X 6-OHDA X Sex	1	0.011	1.037	0.315
Error	36	0.011		
Total	44			

Anterior Cortex: HVA Levels

Source	df	MS	F	p
192SAP	1	1.211	3.255	0.080
6-OHDA	1	3.761	10.108	0.003
Sex	1	0.218	0.587	0.449
192SAP X 6-OHDA	1	0.000	0.000	0.992
192SAP X Sex	1	2.357	6.335	0.016
6-OHDA X Sex	1	0.305	0.820	0.371
192SAP X 6-OHDA X Sex	1	2.073	5.571	0.024
Error	36	0.372		
Total	44			

Anterior Cortex: NE Levels

Source	df	MS	F	p
192SAP	1	4.943	5.069	0.031
6-OHDA	1	12.690	13.014	0.001
Sex	1	0.180	0.184	0.670
192SAP X 6-OHDA	1	1.833	1.880	0.179
192SAP X Sex	1	2.250	2.308	0.137
6-OHDA X Sex	1	1.935	1.984	0.168
192SAP X 6-OHDA X Sex	1	0.180	0.184	0.670
Error	36	0.975		
Total	44			

Anterior Cortex: MHPG Levels

Source	df	MS	F	p
192SAP	1	0.093	2.577	0.117
6-OHDA	1	0.083	2.284	0.139
Sex	1	0.002	0.079	0.780
192SAP X 6-OHDA	1	0.001	0.043	0.838
192SAP X Sex	1	0.129	3.547	0.068
6-OHDA X Sex	1	0.094	2.607	0.115
192SAP X 6-OHDA X Sex	1	0.000	0.001	0.970
Error	36	0.036		
Total	44			

Anterior Cortex: 5-HT Levels

Source	df	MS	F	p
192SAP	1	19.854	0.673	0.417
6-OHDA	1	1.563	0.053	0.819
Sex	1	72.666	2.463	0.125
192SAP X 6-OHDA	1	0.265	0.009	0.925
192SAP X Sex	1	95.005	3.220	0.081
6-OHDA X Sex	1	26.935	0.913	0.346
192SAP X 6-OHDA X Sex	1	0.072	0.002	0.961
Error	36	29.503		
Total	44			

Anterior Cortex: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	7.153	0.481	0.492
6-OHDA	1	13.131	0.883	0.354
Sex	1	0.060	0.004	0.950
192SAP X 6-OHDA	1	3.222	0.217	0.644
192SAP X Sex	1	0.184	0.012	0.912
6-OHDA X Sex	1	7.857	0.529	0.472
192SAP X 6-OHDA X Sex	1	0.020	0.001	0.971
Error	36	14.863		
Total	44			

Retrosplenial Cortex: ChAT Activity

Source	df	MS	F	p
192SAP	1	3005.790	880.372	0.000
6-OHDA	1	0.695	0.204	0.655
Sex	1	3.342	0.979	0.329
192SAP X 6-OHDA	1	0.343	0.100	0.753
192SAP X Sex	1	1.425	0.417	0.522
6-OHDA X Sex	1	0.000	0.000	0.992
192SAP X 6-OHDA X Sex	1	3.230	0.946	0.337
Error	36	3.414		
Total	44			

Retrosplenial Cortex: DA Levels

Source	df	MS	F	p
192SAP	1	0.751	0.097	0.757
6-OHDA	1	1.201	0.155	0.696
Sex	1	2.646	0.342	0.562
192SAP X 6-OHDA	1	3.362	0.435	0.514
192SAP X Sex	1	1.885	0.244	0.625
6-OHDA X Sex	1	3.312	0.428	0.517
192SAP X 6-OHDA X Sex	1	1.469	0.190	0.666
Error	36	7.737		
Total	44			

Retrosplenial Cortex: DOPAC Levels

Source	df	MS	F	p
192SAP	1	5.016	0.690	0.412
6-OHDA	1	0.501	0.069	0.794
Sex	1	3.297	0.454	0.505
192SAP X 6-OHDA	1	0.947	0.130	0.720
192SAP X Sex	1	0.600	0.083	0.775
6-OHDA X Sex	1	0.216	0.030	0.864
192SAP X 6-OHDA X Sex	1	9.035	1.243	0.272
Error	36	7.268		
Total	44			

Retrosplenial Cortex: HVA Levels

Source	df	MS	F	p
192SAP	1	0.074	0.033	0.857
6-OHDA	1	0.924	0.407	0.528
Sex	1	4.482	2.132	0.153
192SAP X 6-OHDA	1	4.103	1.806	0.187
192SAP X Sex	1	1.809	0.796	0.378
6-OHDA X Sex	1	0.046	0.020	0.888
192SAP X 6-OHDA X Sex	1	10.104	4.448	0.042
Error	36	2.271		
Total	44			

Retrosplenial Cortex: NE Levels

Source	df	MS	F	p
192SAP	1	100.090	16.926	0.000
6-OHDA	1	2.707	0.458	0.503
Sex	1	2.327	0.394	0.534
192SAP X 6-OHDA	1	15.403	2.605	0.115
192SAP X Sex	1	2.093	0.354	0.556
6-OHDA X Sex	1	0.531	0.090	0.766
192SAP X 6-OHDA X Sex	1	0.091	0.015	0.902
Error	36	5.913		
Total	44			

Retrosplenial Cortex: MHPG Levels

Source	df	MS	F	p
192SAP	1	0.199	1.139	0.293
6-OHDA	1	0.704	4.038	0.052
Sex	1	0.086	0.495	0.486
192SAP X 6-OHDA	1	0.540	3.098	0.087
192SAP X Sex	1	0.055	0.318	0.576
6-OHDA X Sex	1	0.396	2.270	0.141
192SAP X 6-OHDA X Sex	1	0.286	1.640	0.209
Error	36	0.174		
Total	44			

Retrosplenial Cortex: 5-HT Levels

Source	df	MS	F	p
192SAP	1	22.766	0.557	0.460
6-OHDA	1	117.391	2.873	0.099
Sex	1	0.031	0.001	0.978
192SAP X 6-OHDA	1	1.881	0.046	0.831
192SAP X Sex	1	18.730	0.458	0.503
6-OHDA X Sex	1	298.959	7.317	0.010
192SAP X 6-OHDA X Sex	1	1.726	0.042	0.838
Error	36	40.858		
Total	44			

Retrosplenial Cortex: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	27.528	0.865	0.359
6-OHDA	1	235.293	7.393	0.010
Sex	1	24.808	0.780	0.383
192SAP X 6-OHDA	1	1.409	0.044	0.835
192SAP X Sex	1	2.101	0.066	0.799
6-OHDA X Sex	1	2.971	0.093	0.762
192SAP X 6-OHDA X Sex	1	35.201	1.106	0.300
Error	36	31.825		
Total	44			

Temporal Cortex: ChAT Levels

Source	df	MS	F	p
192SAP	1	1039.643	118.116	0.000
6-OHDA	1	5.003	0.568	0.456
Sex	1	2.112	0.240	0.627
192SAP X 6-OHDA	1	5.167	0.587	0.449
192SAP X Sex	1	0.550	0.062	0.804
6-OHDA X Sex	1	11.774	1.338	0.255
192SAP X 6-OHDA X Sex	1	0.665	0.076	0.785
Error	36	8.802		
Total	44			

Temporal Cortex: DA Levels

Source	df	MS	F	p
192SAP	1	16.274	4.072	0.051
6-OHDA	1	102.413	24.939	0.000
Sex	1	8.672	2.112	0.155
192SAP X 6-OHDA	1	23.066	5.617	0.023
192SAP X Sex	1	2.983	0.726	0.400
6-OHDA X Sex	1	10.717	2.610	0.115
192SAP X 6-OHDA X Sex	1	6.665	1.623	0.211
Error	36	4.107		
Total	44			

Temporal Cortex: DOPAC Levels

Source	df	MS	F	p
192SAP	1	5.469	3.699	0.062
6-OHDA	1	16.615	11.237	0.002
Sex	1	2.217	1.499	0.229
192SAP X 6-OHDA	1	2.123	1.436	0.239
192SAP X Sex	1	2.950	1.955	0.166
6-OHDA X Sex	1	1.715	1.160	0.289
192SAP X 6-OHDA X Sex	1	0.001	0.001	0.971
Error	36	1.479		
Total	44			

Temporal Cortex: HVA Levels

Source	df	MS	F	p
192SAP	1	0.059	0.199	0.658
6-OHDA	1	0.503	1.680	0.203
Sex	1	0.806	2.694	0.109
192SAP X 6-OHDA	1	0.000	0.003	0.960
192SAP X Sex	1	0.018	0.061	0.807
6-OHDA X Sex	1	0.089	0.300	0.588
192SAP X 6-OHDA X Sex	1	0.138	0.461	0.502
Error	36	0.299		
Total	44			

Temporal Cortex: NE Levels

Source	df	MS	F	p
192SAP	1	7.173	5.933	0.020
6-OHDA	1	3.695	3.056	0.089
Sex	1	0.002	0.002	0.968
192SAP X 6-OHDA	1	0.536	0.443	0.510
192SAP X Sex	1	3.280	2.713	0.108
6-OHDA X Sex	1	0.568	0.470	0.497
192SAP X 6-OHDA X Sex	1	1.347	1.114	0.298
Error	36	1.209		
Total	44			

Temporal Cortex: MHPG Levels

Source	df	MS	F	p
192SAP	1	0.010	0.334	0.567
6-OHDA	1	0.002	0.074	0.788
Sex	1	0.001	0.046	0.831
192SAP X 6-OHDA	1	0.004	0.145	0.706
192SAP X Sex	1	0.086	2.895	0.097
6-OHDA X Sex	1	0.000	0.000	0.983
192SAP X 6-OHDA X Sex	1	0.024	0.799	0.377
Error	36	0.030		
Total	44			

Temporal Cortex: 5-HT Levels

Source	df	MS	F	p
192SAP	1	96.238	1.570	0.218
6-OHDA	1	11.638	0.190	0.666
Sex	1	15.168	0.248	0.622
192SAP X 6-OHDA	1	6.731	0.110	0.742
192SAP X Sex	1	8.112	0.132	0.718
6-OHDA X Sex	1	141.171	2.304	0.138
192SAP X 6-OHDA X Sex	1	8.863	0.145	0.706
Error	36	61.283		
Total	44			

Temporal Cortex: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	7.843	1.410	0.243
6-OHDA	1	0.366	0.066	0.799
Sex	1	0.675	0.121	0.730
192SAP X 6-OHDA	1	4.825	0.868	0.358
192SAP X Sex	1	26.629	4.788	0.035
6-OHDA X Sex	1	1.153	0.207	0.652
192SAP X 6-OHDA X Sex	1	0.344	0.062	0.805
Error	36	5.562		
Total	44			

Medial Septum: ChAT Activity

Source	df	MS	F	p
192SAP	1	2154.496	59.974	0.000
6-OHDA	1	13.008	0.362	0.551
Sex	1	222.947	6.206	0.017
192SAP X 6-OHDA	1	1.008	0.028	0.868
192SAP X Sex	1	11.719	0.326	0.571
6-OHDA X Sex	1	101.948	2.838	0.101
192SAP X 6-OHDA X Sex	1	157.022	1.587	0.216
Error	36	35.924		
Total	44			

Hippocampus: ChAT Activity

Source	df	MS	F	p
192SAP	1	5130.958	662.071	0.000
6-OHDA	1	8.664	1.118	0.297
Sex	1	2.696	0.348	0.559
192SAP X 6-OHDA	1	0.406	0.052	0.820
192SAP X Sex	1	10.651	1.374	0.249
6-OHDA X Sex	1	47.256	6.098	0.018
192SAP X 6-OHDA X Sex	1	24.365	3.144	0.085
Error	36	7.750		
Total	44			

Hippocampus: DA Levels

Source	df	MS	F	p
192SAP	1	3.265	0.926	0.342
6-OHDA	1	6.121	1.736	0.196
Sex	1	0.243	0.069	0.794
192SAP X 6-OHDA	1	0.120	0.034	0.854
192SAP X Sex	1	1.185	0.336	0.566
6-OHDA X Sex	1	13.914	3.947	0.055
192SAP X 6-OHDA X Sex	1	0.003	0.001	0.974
Error	36	3.526		
Total	44			

Hippocampus: DOPAC Levels

Source	df	MS	F	p
192SAP	1	0.780	0.289	0.594
6-OHDA	1	0.107	0.040	0.843
Sex	1	0.530	0.197	0.660
192SAP X 6-OHDA	1	3.635	1.348	0.253
192SAP X Sex	1	7.961	2.953	0.094
6-OHDA X Sex	1	4.036	1.497	0.229
192SAP X 6-OHDA X Sex	1	0.985	0.365	0.549
Error	36	2.696		
Total	44			

Hippocampus: HVA Levels

Source	df	MS	F	p
192SAP	1	3.456	0.840	0.366
6-OHDA	1	0.463	0.112	0.739
Sex	1	0.069	0.017	0.898
192SAP X 6-OHDA	1	1.976	0.480	0.493
192SAP X Sex	1	3.522	0.856	0.361
6-OHDA X Sex	1	0.380	0.092	0.763
192SAP X 6-OHDA X Sex	1	5.405	1.313	0.259
Error	36	4.116		
Total	44			

Hippocampus: NE Levels

Source	df	MS	F	p
192SAP	1	27.452	8.498	0.006
6-OHDA	1	16.125	4.992	0.032
Sex	1	0.067	0.021	0.885
192SAP X 6-OHDA	1	0.569	0.176	0.677
192SAP X Sex	1	0.180	0.056	0.815
6-OHDA X Sex	1	1.183	0.366	0.549
192SAP X 6-OHDA X Sex	1	4.322	1.338	0.255
Error	36	3.231		
Total	44			

Hippocampus: MHPG Levels

Source	df	MS	F	p
192SAP	1	0.341	0.596	0.445
6-OHDA	1	0.464	0.811	0.374
Sex	1	0.004	0.007	0.932
192SAP X 6-OHDA	1	0.545	0.954	0.335
192SAP X Sex	1	0.037	0.065	0.800
6-OHDA X Sex	1	0.916	1.602	0.214
192SAP X 6-OHDA X Sex	1	1.175	2.054	0.160
Error	36	0.572		
Total	44			

Hippocampus: 5-HT Levels

Source	df	MS	F	p
192SAP	1	0.072	0.002	0.963
6-OHDA	1	51.078	1.581	0.217
Sex	1	18.701	0.579	0.452
192SAP X 6-OHDA	1	38.830	1.202	0.280
192SAP X Sex	1	40.883	1.266	0.268
6-OHDA X Sex	1	1.751	0.054	0.817
192SAP X 6-OHDA X Sex	1	37.472	1.160	0.289
Error	36	32.299		
Total	44			

Hippocampus: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	1.459	0.049	0.825
6-OHDA	1	173.616	5.875	0.021
Sex	1	13.457	0.455	0.504
192SAP X 6-OHDA	1	14.676	0.497	0.486
192SAP X Sex	1	27.386	0.927	0.342
6-OHDA X Sex	1	144.917	4.904	0.033
192SAP X 6-OHDA X Sex	1	10.490	0.355	0.555
Error	36	29.552		
Total	44			

Caudate: ChAT Levels

Source	df	MS	F	p
192SAP	1	161.528	9.472	0.004
6-OHDA	1	10.507	0.616	0.438
Sex	1	24.971	1.464	0.234
192SAP X 6-OHDA	1	1.415	0.083	0.775
192SAP X Sex	1	12.899	0.756	0.390
6-OHDA X Sex	1	0.148	0.009	0.926
192SAP X 6-OHDA X Sex	1	53.090	3.113	0.086
Error	36	17.054		
Total	44			

Caudate: DA Levels

Source	df	MS	F	p
192SAP	1	0.278	0.000	0.982
6-OHDA	1	128877.31	226.854	0.000
Sex	1	1874.404	3.299	0.078
192SAP X 6-OHDA	1	15.909	0.028	0.868
192SAP X Sex	1	930.812	1.638	0.209
6-OHDA X Sex	1	645.095	1.136	0.294
192SAP X 6-OHDA X Sex	1	1595.173	2.808	0.102
Error	36	568.108		
Total	44			

Caudate: DOPAC Levels

Source	df	MS	F	p
192SAP	1	10.449	0.384	0.539
6-OHDA	1	1937.698	71.280	0.000
Sex	1	34.542	1.271	0.267
192SAP X 6-OHDA	1	6.112	0.225	0.638
192SAP X Sex	1	164.299	6.044	0.019
6-OHDA X Sex	1	0.002	0.000	0.993
192SAP X 6-OHDA X Sex	1	14.272	0.525	0.473
Error	36	27.184		
Total	44			

Caudate: HVA Levels

Source	df	MS	F	p
192SAP	1	2.825	0.152	0.699
6-OHDA	1	128.432	6.928	0.012
Sex	1	29.310	1.581	0.217
192SAP X 6-OHDA	1	5.470	0.295	0.590
192SAP X Sex	1	2.716	0.147	0.704
6-OHDA X Sex	1	1.994	0.108	0.745
192SAP X 6-OHDA X Sex	1	11.681	0.630	0.433
Error	36	18.539		
Total	44			

Caudate: NE Levels

Source	df	MS	F	p
192SAP	1	4.424	2.410	0.129
6-OHDA	1	0.839	0.457	0.503
Sex	1	1.667	0.908	0.347
192SAP X 6-OHDA	1	0.194	0.106	0.747
192SAP X Sex	1	0.268	0.146	0.705
6-OHDA X Sex	1	1.491	0.812	0.373
192SAP X 6-OHDA X Sex	1	0.165	0.090	0.766
Error	36	1.836		
Total	44			

Caudate: 5-HT Levels

Source	df	MS	F	p
192SAP	1	95.850	4.553	0.040
6-OHDA	1	0.985	0.047	0.830
Sex	1	1.987	0.094	0.760
192SAP X 6-OHDA	1	7.824	0.372	0.546
192SAP X Sex	1	0.262	0.012	0.912
6-OHDA X Sex	1	0.693	0.033	0.857
192SAP X 6-OHDA X Sex	1	10.604	0.504	0.482
Error	36	21.054		
Total	44			

Caudate: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	0.047	0.003	0.954
6-OHDA	1	62.923	4.413	0.043
Sex	1	3.282	0.230	0.634
192SAP X 6-OHDA	1	5.796	0.406	0.528
192SAP X Sex	1	28.509	1.999	0.166
6-OHDA X Sex	1	6.347	0.445	0.509
192SAP X 6-OHDA X Sex	1	2.505	0.176	0.678
Error	36	14.259		
Total	44			

Midbrain: ChAT Activity

Source	df	MS	F	p
192SAP	1	27.260	3.156	0.084
6-OHDA	1	2.532	0.293	0.592
Sex	1	0.102	0.012	0.914
192SAP X 6-OHDA	1	1.258	0.146	0.705
192SAP X Sex	1	0.680	0.079	0.781
6-OHDA X Sex	1	3.959	0.458	0.503
192SAP X 6-OHDA X Sex	1	28.746	3.328	0.076
Error	36	8.638		
Total	44			

Midbrain: DA Levels

Source	df	MS	F	p
192SAP	1	0.813	0.238	0.628
6-OHDA	1	27.551	8.076	0.007
Sex	1	0.083	0.025	0.876
192SAP X 6-OHDA	1	2.835	0.831	0.368
192SAP X Sex	1	2.300	0.674	0.417
6-OHDA X Sex	1	0.032	0.010	0.923
192SAP X 6-OHDA X Sex	1	0.133	0.039	0.844
Error	36	3.411		
Total	44			

Midbrain: DOPAC Levels

Source	df	MS	F	p
192SAP	1	2.345	2.830	0.101
6-OHDA	1	2.250	2.715	0.108
Sex	1	1.258	1.518	0.226
192SAP X 6-OHDA	1	0.519	0.626	0.434
192SAP X Sex	1	1.387	1.674	0.204
6-OHDA X Sex	1	0.086	0.104	0.749
192SAP X 6-OHDA X Sex	1	0.013	0.016	0.899
Error	36	0.829		
Total	44			

Midbrain: HVA Levels

Source	df	MS	F	p
192SAP	1	0.291	0.183	0.672
6-OHDA	1	0.347	0.218	0.644
Sex	1	0.043	0.027	0.870
192SAP X 6-OHDA	1	0.906	0.568	0.456
192SAP X Sex	1	6.655	4.172	0.048
6-OHDA X Sex	1	0.658	0.413	0.525
192SAP X 6-OHDA X Sex	1	0.662	0.415	0.523
Error	36	1.595		
Total	44			

Midbrain: NE Levels

Source	df	MS	F	p
192SAP	1	1.719	0.526	0.473
6-OHDA	1	4.369	1.338	0.255
Sex	1	1.006	0.308	0.582
192SAP X 6-OHDA	1	0.745	0.228	0.636
192SAP X Sex	1	0.019	0.006	0.939
6-OHDA X Sex	1	4.903	1.501	0.228
192SAP X 6-OHDA X Sex	1	0.162	0.050	0.825
Error	36	3.265		
Total	44			

Midbrain: MHPG Levels

Source	df	MS	F	p
192SAP	1	0.032	0.076	0.784
6-OHDA	1	1.740	4.131	0.050
Sex	1	0.101	0.241	0.627
192SAP X 6-OHDA	1	0.001	0.004	0.953
192SAP X Sex	1	1.586	3.766	0.060
6-OHDA X Sex	1	0.101	0.240	0.627
192SAP X 6-OHDA X Sex	1	0.899	2.135	0.153
Error	36	0.421		
Total	44			

Midbrain: 5-HT Levels

Source	df	MS	F	p
192SAP	1	0.044	0.001	0.971
6-OHDA	1	1.750	0.053	0.819
Sex	1	38.248	1.168	0.287
192SAP X 6-OHDA	1	51.132	1.561	0.220
192SAP X Sex	1	35.986	1.099	0.302
6-OHDA X Sex	1	10.722	0.327	0.571
192SAP X 6-OHDA X Sex	1	21.273	0.650	0.426
Error	36	32.748		
Total	44			

Midbrain: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	228.345	2.481	0.124
6-OHDA	1	8.588	0.093	0.762
Sex	1	32.007	0.348	0.559
192SAP X 6-OHDA	1	112.802	1.225	0.276
192SAP X Sex	1	345.975	3.759	0.060
6-OHDA X Sex	1	3.409	0.037	0.848
192SAP X 6-OHDA X Sex	1	56.792	0.617	0.437
Error	36	92.048		
Total	44			

Hypothalamus: DA Levels

Source	df	MS	F	p
192SAP	1	0.866	0.081	0.778
6-OHDA	1	19.126	1.788	0.190
Sex	1	0.475	0.044	0.834
192SAP X 6-OHDA	1	2.427	0.227	0.637
192SAP X Sex	1	0.456	0.043	0.838
6-OHDA X Sex	1	10.754	1.005	0.323
192SAP X 6-OHDA X Sex	1	0.413	0.039	0.845
Error	36	10.698		
Total	44			

Hypothalamus: DOPAC Levels

Source	df	MS	F	p
192SAP	1	14.475	0.913	0.346
6-OHDA	1	18.339	1.157	0.289
Sex	1	9.368	0.591	0.447
192SAP X 6-OHDA	1	37.773	2.382	0.131
192SAP X Sex	1	4.935	0.311	0.580
6-OHDA X Sex	1	45.157	2.848	0.100
192SAP X 6-OHDA X Sex	1	18.670	1.178	0.285
Error	36	15.855		
Total	44			

Hypothalamus: HVA Levels

Source	df	MS	F	p
192SAP	1	0.664	0.103	0.750
6-OHDA	1	8.286	1.284	0.265
Sex	1	0.243	0.038	0.847
192SAP X 6-OHDA	1	2.944	0.456	0.504
192SAP X Sex	1	1.017	0.158	0.694
6-OHDA X Sex	1	10.926	1.694	0.201
192SAP X 6-OHDA X Sex	1	6.631	1.028	0.317
Error	36	6.450		
Total	44			

Hypothalamus: NE Levels

Source	df	MS	F	p
192SAP	1	10.525	0.393	0.535
6-OHDA	1	8.732	0.326	0.571
Sex	1	148.457	5.545	0.024
192SAP X 6-OHDA	1	0.476	0.018	0.895
192SAP X Sex	1	4.986	0.186	0.669
6-OHDA X Sex	1	1.433	0.054	0.818
192SAP X 6-OHDA X Sex	1	17.519	0.654	0.424
Error	36	26.775		
Total	44			

Hypothalamus: MHPG Levels

Source	df	MS	F	p
192SAP	1	5.272	1.953	0.171
6-OHDA	1	0.646	0.239	0.628
Sex	1	0.081	0.030	0.863
192SAP X 6-OHDA	1	3.841	1.423	0.241
192SAP X Sex	1	0.036	0.013	0.908
6-OHDA X Sex	1	4.176	1.547	0.222
192SAP X 6-OHDA X Sex	1	4.538	1.681	0.203
Error	36	2.700		
Total	44			

Hypothalamus: 5-HT Levels

Source	df	MS	F	p
192SAP	1	38.985	1.095	0.302
6-OHDA	1	41.466	0.836	0.367
Sex	1	19.535	1.622	0.211
192SAP X 6-OHDA	1	380.786	1.930	0.173
192SAP X Sex	1	119.947	1.032	0.316
6-OHDA X Sex	1	104.396	3.906	0.056
192SAP X 6-OHDA X Sex	1	235.927	0.393	0.535
Error	36	91.733		
Total	44			

Hypothalamus: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	199.515	1.095	0.302
6-OHDA	1	152.269	0.836	0.367
Sex	1	295.491	1.622	0.211
192SAP X 6-OHDA	1	351.508	1.930	0.173
192SAP X Sex	1	187.998	1.032	0.316
6-OHDA X Sex	1	711.370	3.906	0.056
192SAP X 6-OHDA X Sex	1	71.593	0.393	0.535
Error	36	182.131		
Total	44			

Brainstem: DA Levels

Source	df	MS	F	p
192SAP	1	39.021	3.579	0.067
6-OHDA	1	1.851	0.170	0.683
Sex	1	26.893	2.466	0.125
192SAP X 6-OHDA	1	7.439	0.682	0.414
192SAP X Sex	1	50.520	4.633	0.038
6-OHDA X Sex	1	0.004	0.000	0.984
192SAP X 6-OHDA X Sex	1	46.691	4.282	0.046
Error	36	10.904		
Total	44			

Brainstem: DOPAC Levels

Source	df	MS	F	p
192SAP	1	0.323	0.072	0.790
6-OHDA	1	0.402	0.089	0.767
Sex	1	0.462	0.103	0.750
192SAP X 6-OHDA	1	0.206	0.046	0.832
192SAP X Sex	1	5.272	1.172	0.286
6-OHDA X Sex	1	4.792	1.066	0.309
192SAP X 6-OHDA X Sex	1	0.350	0.078	0.782
Error	36	4.496		
Total	44			

Brainstem: HVA Levels

Source	df	MS	F	p
192SAP	1	0.277	0.259	0.614
6-OHDA	1	4.346	4.054	0.052
Sex	1	1.295	1.208	0.279
192SAP X 6-OHDA	1	1.639	1.528	0.224
192SAP X Sex	1	2.153	2.008	0.165
6-OHDA X Sex	1	0.319	0.298	0.589
192SAP X 6-OHDA X Sex	1	0.808	0.753	0.391
Error	36	1.072		
Total	44			

Brainstem: NE Levels

Source	df	MS	F	p
192SAP	1	46.148	2.633	0.113
6-OHDA	1	2.946	0.168	0.684
Sex	1	9.772	0.558	0.460
192SAP X 6-OHDA	1	3.091	0.176	0.677
192SAP X Sex	1	1.070	0.061	0.806
6-OHDA X Sex	1	9.740	0.556	0.461
192SAP X 6-OHDA X Sex	1	105.859	6.040	0.019
Error	36	17.525		
Total	44			

Brainstem: MHPG Levels

Source	df	MS	F	p
192SAP	1	0.342	0.251	0.619
6-OHDA	1	5.112	3.758	0.060
Sex	1	0.041	0.031	0.862
192SAP X 6-OHDA	1	1.229	0.903	0.348
192SAP X Sex	1	1.477	1.086	0.304
6-OHDA X Sex	1	7.803	5.737	0.022
192SAP X 6-OHDA X Sex	1	0.843	0.619	0.436
Error	36	1.360		
Total	44			

Brainstem: 5-HT Levels

Source	df	MS	F	p
192SAP	1	0.379	0.007	0.935
6-OHDA	1	67.821	1.211	0.278
Sex	1	3.769	0.067	0.797
192SAP X 6-OHDA	1	0.866	0.015	0.902
192SAP X Sex	1	32.799	0.586	0.449
6-OHDA X Sex	1	4.780	0.085	0.772
192SAP X 6-OHDA X Sex	1	1.979	0.035	0.852
Error	36	55.992		
Total	44			

Brainstem: 5-HIAA Levels

Source	df	MS	F	p
192SAP	1	48.120	1.286	0.264
6-OHDA	1	189.114	5.055	0.031
Sex	1	82.418	2.230	0.146
192SAP X 6-OHDA	1	43.299	1.157	0.289
192SAP X Sex	1	80.763	2.159	0.150
6-OHDA X Sex	1	41.844	1.118	0.297
192SAP X 6-OHDA X Sex	1	23.507	0.628	0.433
Error	36	37.412		
Total	44			

Appendix A3. ANOVA Summary Tables for Pyramidal Cell Morphology**mPFC, Layer V, Apical Tree: Total Dendrite Length**

Source	df	MS	F	p
192SAP	1	410309.610	11.350	0.003
6-OHDA	1	135245.800	3.741	0.065
192SAP X 6-OHDA	1	821.973	0.023	0.881
Error	23	36151.858		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 1

Source	df	MS	F	p
192SAP	1	0.000	0.000	0.991
6-OHDA	1	1.333	4.131	0.054
192SAP X 6-OHDA	1	0.043	0.135	0.717
Error	23	0.323		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 2

Source	df	MS	F	p
192SAP	1	0.002	0.006	0.940
6-OHDA	1	1.455	3.177	0.088
192SAP X 6-OHDA	1	0.014	0.032	0.860
Error	23	0.458		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 3

Source	df	MS	F	p
192SAP	1	0.026	0.037	0.849
6-OHDA	1	3.341	4.704	0.041
192SAP X 6-OHDA	1	0.256	0.360	0.554
Error	23	0.710		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 4

Source	df	MS	F	p
192SAP	1	0.132	0.185	0.671
6-OHDA	1	3.153	4.429	0.046
192SAP X 6-OHDA	1	0.057	0.080	0.780
Error	23	0.712		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 5

Source	df	MS	F	p
192SAP	1	0.396	0.527	0.475
6-OHDA	1	3.161	4.205	0.052
192SAP X 6-OHDA	1	0.330	0.439	0.514
Error	23	0.752		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 6

Source	df	MS	F	p
192SAP	1	0.046	0.076	0.786
6-OHDA	1	3.026	4.890	0.037
192SAP X 6-OHDA	1	0.029	0.047	0.830
Error	23	0.619		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 7

Source	df	MS	F	p
192SAP	1	0.645	1.339	0.259
6-OHDA	1	1.914	3.974	0.058
192SAP X 6-OHDA	1	0.211	0.438	0.515
Error	23	0.482		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 8

Source	df	MS	F	p
192SAP	1	0.634	2.020	0.169
6-OHDA	1	1.638	5.220	0.032
192SAP X 6-OHDA	1	0.204	0.652	0.428
Error	23	0.314		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 9

Source	df	MS	F	p
192SAP	1	1.501	8.941	0.007
6-OHDA	1	0.777	4.626	0.042
192SAP X 6-OHDA	1	0.037	0.223	0.641
Error	23	0.168		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 10

Source	df	MS	F	p
192SAP	1	2.814	12.579	0.002
6-OHDA	1	1.693	7.570	0.011
192SAP X 6-OHDA	1	0.472	2.110	0.160
Error	23	0.224		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 11

Source	df	MS	F	p
192SAP	1	0.783	3.755	0.065
6-OHDA	1	0.881	4.225	0.051
192SAP X 6-OHDA	1	0.332	1.593	0.220
Error	23	0.209		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 12

Source	df	MS	F	p
192SAP	1	0.749	4.032	0.057
6-OHDA	1	1.541	8.299	0.008
192SAP X 6-OHDA	1	0.146	0.784	0.385
Error	23	0.186		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 13

Source	df	MS	F	p
192SAP	1	0.039	0.354	0.558
6-OHDA	1	0.823	7.429	0.012
192SAP X 6-OHDA	1	0.003	0.032	0.859
Error	23	0.111		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 14

Source	df	MS	F	p
192SAP	1	0.663	4.322	0.049
6-OHDA	1	1.469	9.572	0.005
192SAP X 6-OHDA	1	0.091	0.599	0.447
Error	23	0.154		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 15

Source	df	MS	F	p
192SAP	1	0.972	5.484	0.028
6-OHDA	1	0.594	3.353	0.080
192SAP X 6-OHDA	1	0.044	0.253	0.619
Error	23	0.177		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 16

Source	df	MS	F	p
192SAP	1	0.630	2.911	0.101
6-OHDA	1	0.311	1.435	0.243
192SAP X 6-OHDA	1	0.202	0.935	0.344
Error	23	0.261		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 17

Source	df	MS	F	p
192SAP	1	0.762	2.670	0.116
6-OHDA	1	0.057	0.200	0.659
192SAP X 6-OHDA	1	0.009	0.034	0.855
Error	23	0.285		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 18

Source	df	MS	F	p
192SAP	1	0.712	1.790	0.194
6-OHDA	1	0.081	0.204	0.656
192SAP X 6-OHDA	1	0.081	0.204	0.656
Error	23	0.398		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 19

Source	df	MS	F	p
192SAP	1	3.310	5.646	0.026
6-OHDA	1	0.274	0.468	0.501
192SAP X 6-OHDA	1	0.139	0.237	0.631
Error	23	0.586		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 20

Source	df	MS	F	p
192SAP	1	2.908	3.222	0.086
6-OHDA	1	0.012	0.013	0.909
192SAP X 6-OHDA	1	0.190	0.210	0.651
Error	23	0.902		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 21

Source	df	MS	F	p
192SAP	1	4.537	5.007	0.035
6-OHDA	1	0.066	0.073	0.790
192SAP X 6-OHDA	1	0.129	0.142	0.710
Error	23	0.906		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 22

Source	df	MS	F	p
192SAP	1	3.748	4.090	0.055
6-OHDA	1	0.041	0.045	0.834
192SAP X 6-OHDA	1	0.065	0.072	0.791
Error	23	0.916		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 24

Source	df	MS	F	p
192SAP	1	3.761	3.284	0.083
6-OHDA	1	0.363	0.317	0.579
192SAP X 6-OHDA	1	0.050	0.044	0.836
Error	23	1.145		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 25

Source	df	MS	F	p
192SAP	1	2.554	2.960	0.099
6-OHDA	1	0.556	0.644	0.430
192SAP X 6-OHDA	1	0.420	0.487	0.492
Error	23	0.863		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 26

Source	df	MS	F	p
192SAP	1	2.470	4.367	0.048
6-OHDA	1	0.048	0.086	0.773
192SAP X 6-OHDA	1	0.392	0.693	0.414
Error	23	0.566		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 27

Source	df	MS	F	p
192SAP	1	2.746	9.468	0.005
6-OHDA	1	0.081	0.279	0.602
192SAP X 6-OHDA	1	0.072	0.249	0.623
Error	23	0.290		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 28

Source	df	MS	F	p
192SAP	1	1.660	11.014	0.003
6-OHDA	1	0.008	0.054	0.818
192SAP X 6-OHDA	1	0.036	0.245	0.625
Error	23	3.467		
Total	27	11.215		

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 29

Source	df	MS	F	p
192SAP	1	1.020	13.076	0.001
6-OHDA	1	0.000	0.006	0.937
192SAP X 6-OHDA	1	0.018	0.242	0.628
Error	23	0.078		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 30

Source	df	MS	F	p
192SAP	1	0.484	7.397	0.012
6-OHDA	1	0.002	0.040	0.844
192SAP X 6-OHDA	1	0.004	0.074	0.789
Error	23	0.065		
Total	27			

mPFC, Layer V, Apical Tree: Sholl Analysis, Ring 31

Source	df	MS	F	p
192SAP	1	0.131	4.450	0.046
6-OHDA	1	0.001	0.042	0.839
192SAP X 6-OHDA	1	0.001	0.050	0.824
Error	23	0.029		
Total	27			

mPFC, Layer V, Apical Tree: Total Number of Branches

Source	df	MS	F	p
192SAP	1	133.368	8.391	0.008
6-OHDA	1	38.560	2.426	0.133
192SAP X 6-OHDA	1	5.022	0.316	0.579
Error	23	15.894		
Total	27			

mPFC, Layer V, Apical Tree: Number of 1st Order Branches

Source	df	MS	F	p
192SAP	1	5.591	3.836	0.062
6-OHDA	1	20.963	14.381	0.001
192SAP X 6-OHDA	1	0.078	0.054	0.819
Error	23	1.458		
Total	27			

mPFC, Layer V, Apical Tree: Number of 2nd Order Branches

Source	df	MS	F	p
192SAP	1	7.721	3.774	0.064
6-OHDA	1	0.740	0.362	0.553
192SAP X 6-OHDA	1	0.000	0.000	0.997
Error	23	2.046		
Total	27			

mPFC, Layer V, Apical Tree: Number of 3rd Order Branches

Source	df	MS	F	p
192SAP	1	5.595	4.778	0.039
6-OHDA	1	0.802	0.685	0.416
192SAP X 6-OHDA	1	0.067	0.057	0.813
Error	23	1.171		
Total	27			

mPFC, Layer V, Apical Tree: Number of 4th Order Branches

Source	df	MS	F	p
192SAP	1	2.310	3.661	0.068
6-OHDA	1	0.108	0.171	0.683
192SAP X 6-OHDA	1	1.284	2.034	0.167
Error	23	0.631		
Total	27			

mPFC, Layer V, Apical Tree: Number of 5th Order Branches

Source	df	MS	F	p
192SAP	1	3.327	5.730	0.025
6-OHDA	1	0.007	0.014	0.908
192SAP X 6-OHDA	1	0.116	0.200	0.659
Error	23	0.581		
Total	27			

mPFC, Layer V, Apical Tree: Spine Density

Source	df	MS	F	p
192SAP	1	3.925	6.186	0.024
6-OHDA	1	1.226	1.931	0.183
192SAP X 6-OHDA	1	0.493	0.777	0.390
Error	17	0.635		
Total	21			

mPFC, Layer V, Basal Tree: Total Dendrite Length

Source	df	MS	F	p
192SAP	1	114086.750	1.989	0.172
6-OHDA	1	208320.260	3.631	0.069
192SAP X 6-OHDA	1	989.722	0.017	0.897
Error	23	57365.727		
Total	27			

mPFC, Layer V, Basal Tree: Sholl Analysis, Ring 1

Source	df	MS	F	p
192SAP	1	10.366	3.968	0.058
6-OHDA	1	13.163	5.038	0.035
192SAP X 6-OHDA	1	1.652	0.632	0.435
Error	23	2.613		
Total	27			

mPFC, Layer V, Basal Tree: Sholl Analysis, Ring 2

Source	df	MS	F	p
192SAP	1	14.243	4.628	0.042
6-OHDA	1	7.055	2.293	0.144
192SAP X 6-OHDA	1	0.351	0.114	0.739
Error	23	3.077		
Total	27			

mPFC, Layer V, Basal Tree: Sholl Analysis, Ring 3

Source	df	MS	F	p
192SAP	1	10.540	2.109	0.160
6-OHDA	1	18.396	3.682	0.068
192SAP X 6-OHDA	1	0.037	0.008	0.932
Error	23	4.997		
Total	27			

mPFC, Layer V, Basal Tree: Sholl Analysis, Ring 6

Source	df	MS	F	p
192SAP	1	1.239	0.716	0.406
6-OHDA	1	6.519	3.768	0.065
192SAP X 6-OHDA	1	0.087	0.051	0.824
Error	23	1.730		
Total	27			

mPFC, Layer V, Basal Tree: Sholl Analysis, Ring 9

Source	df	MS	F	p
192SAP	1	0.227	1.063	0.313
6-OHDA	1	0.789	3.690	0.067
192SAP X 6-OHDA	1	0.468	2.188	0.153
Error	23	0.214		
Total	27			

mPFC, Layer V, Basal Tree: Total Number of Branches

Source	df	MS	F	p
192SAP	1	156.583	10.728	0.003
6-OHDA	1	25.548	1.750	0.199
192SAP X 6-OHDA	1	0.860	0.059	0.810
Error	23	14.596		
Total	27			

mPFC, Layer V, Basal Tree: Number of 1st Order Branches

Source	df	MS	F	p
192SAP	1	0.427	0.647	0.429
6-OHDA	1	2.059	3.123	0.090
192SAP X 6-OHDA	1	0.049	0.076	0.786
Error	23	0.659		
Total	27			

mPFC, Layer V, Basal Tree: Number of 2nd Order Branches

Source	df	MS	F	p
192SAP	1	2.613	2.240	0.148
6-OHDA	1	1.505	1.290	0.268
192SAP X 6-OHDA	1	0.058	0.050	0.826
Error	23	1.167		
Total	27			

mPFC, Layer V, Basal Tree: Number of 3rd Order Branches

Source	df	MS	F	p
192SAP	1	4.192	2.493	0.128
6-OHDA	1	0.024	0.014	0.905
192SAP X 6-OHDA	1	0.012	0.008	0.931
Error	23	1.682		
Total	27			

mPFC, Layer V, Basal Tree: Number of 4th Order Branches

Source	df	MS	F	p
192SAP	1	15.911	9.466	0.005
6-OHDA	1	0.585	0.348	0.561
192SAP X 6-OHDA	1	0.143	0.085	0.773
Error	23	1.681		
Total	27			

mPFC, Layer V, Basal Tree: Number of 5th Order Branches

Source	df	MS	F	p
192SAP	1	6.833	6.433	0.018
6-OHDA	1	0.743	0.700	0.411
192SAP X 6-OHDA	1	0.462	0.435	0.516
Error	23	1.062		
Total	27			

mPFC, Layer V, Basal Tree: Number of 6th Order Branches

Source	df	MS	F	p
192SAP	1	0.825	2.519	0.126
6-OHDA	1	0.011	0.036	0.852
192SAP X 6-OHDA	1	0.100	0.306	0.585
Error	23	0.328		
Total	27			

mPFC, Layer V, Basal Tree: Number of 7th Order Branches

Source	df	MS	F	p
192SAP	1	0.215	2.499	0.128
6-OHDA	1	0.257	2.984	0.098
192SAP X 6-OHDA	1	0.009	0.105	0.748
Error	23	0.086		
Total	27			

mPFC, Layer V, Basal Tree: Number of 8th Order Branches

Source	df	MS	F	p
192SAP	1	0.008	0.971	0.335
6-OHDA	1	0.031	3.631	0.069
192SAP X 6-OHDA	1	0.027	3.147	0.089
Error	23	0.087		
Total	27			

mPFC, Layer V, Basal Tree: Spine Density

Source	df	MS	F	p
192SAP	1	5.575	6.177	0.024
6-OHDA	1	3.177	3.520	0.078
192SAP X 6-OHDA	1	0.507	0.562	0.464
Error	17	0.903		
Total	21			

ACC Layer III, Apical Tree: Total Dendrite Length

Source	df	MS	F	p
192SAP	1	92829.876	5.479	0.028
6-OHDA	1	4456.649	0.263	0.613
192SAP X 6-OHDA	1	277.005	0.016	0.899
Error	23	16941.959		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 1

Source	df	MS	F	p
192SAP	1	0.008	0.043	0.837
6-OHDA	1	0.591	3.075	0.093
192SAP X 6-OHDA	1	0.423	2.202	0.151
Error	23	0.192		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 2

Source	df	MS	F	p
192SAP	1	0.010	0.040	0.843
6-OHDA	1	1.882	7.509	0.012
192SAP X 6-OHDA	1	0.397	1.585	0.221
Error	23	0.251		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 3

Source	df	MS	F	p
192SAP	1	0.100	0.231	0.636
6-OHDA	1	0.381	0.878	0.358
192SAP X 6-OHDA	1	0.074	0.172	0.682
Error	23	0.434		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 4

Source	df	MS	F	p
192SAP	1	0.058	0.149	0.703
6-OHDA	1	0.479	1.228	0.279
192SAP X 6-OHDA	1	0.000	0.002	0.967
Error	23	0.390		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 5

Source	df	MS	F	p
192SAP	1	0.837	1.767	0.197
6-OHDA	1	0.212	0.448	0.510
192SAP X 6-OHDA	1	0.009	0.020	0.888
Error	23	0.474		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 6

Source	df	MS	F	p
192SAP	1	0.382	0.522	0.477
6-OHDA	1	1.705	2.330	0.141
192SAP X 6-OHDA	1	0.004	0.007	0.936
Error	23	0.732		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 7

Source	df	MS	F	p
192SAP	1	0.624	0.617	0.440
6-OHDA	1	1.819	1.797	0.193
192SAP X 6-OHDA	1	0.162	0.160	0.692
Error	23	1.012		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 8

Source	df	MS	F	p
192SAP	1	0.716	0.766	0.391
6-OHDA	1	4.405	4.713	0.040
192SAP X 6-OHDA	1	0.173	0.185	0.671
Error	23	0.935		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 9

Source	df	MS	F	p
192SAP	1	1.748	0.747	0.199
6-OHDA	1	2.232	2.232	0.149
192SAP X 6-OHDA	1	0.050	0.051	0.823
Error	23	1.000		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 10

Source	df	MS	F	p
192SAP	1	3.778	4.144	0.053
6-OHDA	1	1.987	2.179	0.153
192SAP X 6-OHDA	1	0.257	0.282	0.601
Error	23	0.912		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 11

Source	df	MS	F	p
192SAP	1	3.790	4.544	0.044
6-OHDA	1	0.025	0.031	0.863
192SAP X 6-OHDA	1	2.101	2.518	0.126
Error	23	0.834		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 12

Source	df	MS	F	p
192SAP	1	2.767	3.541	0.073
6-OHDA	1	0.500	0.640	0.432
192SAP X 6-OHDA	1	0.327	0.419	0.524
Error	23	0.419		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 13

Source	df	MS	F	p
192SAP	1	3.911	4.839	0.038
6-OHDA	1	0.118	0.146	0.706
192SAP X 6-OHDA	1	0.001	0.002	0.962
Error	23	0.808		
Total	27			

ACC Layer III, Apical Tree: Sholl Analysis, Ring 14

Source	df	MS	F	p
192SAP	1	2.604	3.675	0.068
6-OHDA	1	0.225	0.318	0.578
192SAP X 6-OHDA	1	0.000	0.001	0.981
Error	23	0.709		
Total	27			

ACC Layer III, Apical Tree: Total Number of Branches

Source	df	MS	F	p
192SAP	1	64.839	6.730	0.016
6-OHDA	1	0.340	0.035	0.853
192SAP X 6-OHDA	1	3.793	0.394	0.537
Error	23	9.635		
Total	27			

ACC Layer III, Apical Tree: Number of 1st Order Branches

Source	df	MS	F	p
192SAP	1	0.325	0.903	0.352
6-OHDA	1	2.015	5.593	0.027
192SAP X 6-OHDA	1	0.551	1.530	0.229
Error	23	0.360		
Total	27			

ACC Layer III, Apical Tree: Number of 2nd Order Branches

Source	df	MS	F	p
192SAP	1	0.030	0.053	0.821
6-OHDA	1	0.420	0.721	0.405
192SAP X 6-OHDA	1	0.030	0.053	0.821
Error	23	0.582		
Total	27			

ACC Layer III, Apical Tree: Number of 3rd Order Branches

Source	df	MS	F	p
192SAP	1	2.271	2.426	0.133
6-OHDA	1	0.485	0.518	0.479
192SAP X 6-OHDA	1	0.002	0.002	0.963
Error	23	0.936		
Total	27			

ACC Layer III, Apical Tree: Number of 4th Order Branches

Source	df	MS	F	p
192SAP	1	8.964	8.132	0.009
6-OHDA	1	0.557	0.505	0.484
192SAP X 6-OHDA	1	0.024	0.022	0.883
Error	23	1.102		
Total	27			

ACC Layer III, Apical Tree: Number of 5th Order Branches

Source	df	MS	F	p
192SAP	1	6.039	10.271	0.004
6-OHDA	1	0.021	0.037	0.850
192SAP X 6-OHDA	1	0.155	0.264	0.612
Error	23	0.588		
Total	27			

ACC Layer III, Apical Tree: Number of 6th Order Branches

Source	df	MS	F	p
192SAP	1	0.074	1.155	0.294
6-OHDA	1	0.012	0.200	0.659
192SAP X 6-OHDA	1	0.035	0.542	0.469
Error	23	0.064		
Total	27			

ACC Layer III, Apical Tree: Spine Density

Source	df	MS	F	p
192SAP	1	13.712	10.596	0.005
6-OHDA	1	3.636	2.810	0.112
192SAP X 6-OHDA	1	7.156	5.530	0.031
Error	17	1.294		
Total	21			

ACC Layer III, Basal Tree: Total Dendrite Length

Source	df	MS	F	p
192SAP	1	75187.148	1.632	0.214
6-OHDA	1	106560.61	2.313	0.142
192SAP X 6-OHDA	1	7112.930	0.154	0.698
Error	23	46069.407		
Total	27			

ACC Layer III, Basal Tree: Sholl Analysis, Ring 1

Source	df	MS	F	p
192SAP	1	9.382	5.022	0.035
6-OHDA	1	13.590	7.274	0.013
192SAP X 6-OHDA	1	0.001	0.001	0.981
Error	23	1.868		
Total	27			

ACC Layer III, Basal Tree: Sholl Analysis, Ring 2

Source	df	MS	F	p
192SAP	1	13.449	4.246	0.051
6-OHDA	1	8.434	2.663	0.116
192SAP X 6-OHDA	1	0.013	0.004	0.949
Error	23	3.168		
Total	27			

ACC Layer III, Basal Tree: Sholl Analysis, Ring 3

Source	df	MS	F	p
192SAP	1	9.261	1.885	0.183
6-OHDA	1	5.547	1.129	0.299
192SAP X 6-OHDA	1	0.373	0.076	0.785
Error	23	4.913		
Total	27			

ACC Layer III, Basal Tree: Sholl Analysis, Ring 4

Source	df	MS	F	p
192SAP	1	4.810	0.872	0.360
6-OHDA	1	4.858	0.880	0.358
192SAP X 6-OHDA	1	0.893	0.162	0.691
Error	23	5.519		
Total	27			

ACC Layer III, Basal Tree: Sholl Analysis, Ring 8

Source	df	MS	F	p
192SAP	1	0.015	0.093	0.763
6-OHDA	1	0.810	4.735	0.040
192SAP X 6-OHDA	1	0.001	0.009	0.924
Error	23	0.171		
Total	27			

ACC Layer III, Basal Tree: Sholl Analysis, Ring 9

Source	df	MS	F	p
192SAP	1	0.000	0.005	0.945
6-OHDA	1	0.181	3.099	0.092
192SAP X 6-OHDA	1	0.010	0.181	0.674
Error	23	0.058		
Total	27			

ACC Layer III, Basal Tree: Total Number of Branches

Source	df	MS	F	p
192SAP	1	81.311	5.051	0.035
6-OHDA	1	83.261	5.172	0.033
192SAP X 6-OHDA	1	6.379	0.396	0.535
Error	23	16.098		
Total	27			

ACC Layer III, Basal Tree: Number of 1st Order Branches

Source	df	MS	F	p
192SAP	1	0.204	0.342	0.565
6-OHDA	1	2.234	3.734	0.066
192SAP X 6-OHDA	1	1.721	2.877	0.103
Error	23	0.598		
Total	27			

ACC Layer III, Basal Tree: Number of 2nd Order Branches

Source	df	MS	F	p
192SAP	1	0.790	1.022	0.322
6-OHDA	1	5.227	6.761	0.016
192SAP X 6-OHDA	1	1.974	2.553	0.124
Error	23	0.773		
Total	27			

ACC Layer III, Basal Tree: Number of 3rd Order Branches

Source	df	MS	F	p
192SAP	1	9.088	8.462	0.008
6-OHDA	1	3.558	3.312	0.082
192SAP X 6-OHDA	1	0.043	0.041	0.842
Error	23	1.074		
Total	27			

ACC Layer III, Basal Tree: Number of 4th Order Branches

Source	df	MS	F	p
192SAP	1	4.984	2.263	0.146
6-OHDA	1	3.096	1.406	0.248
192SAP X 6-OHDA	1	1.071	0.486	0.493
Error	23	2.202		
Total	27			

ACC Layer III, Basal Tree: Number of 5th Order Branches

Source	df	MS	F	p
192SAP	1	2.535	1.509	0.232
6-OHDA	1	1.723	1.026	0.322
192SAP X 6-OHDA	1	0.065	0.039	0.845
Error	23	1.680		
Total	27			

ACC Layer III, Basal Tree: Spine Density

Source	df	MS	F	p
192SAP	1	10.335	12.867	0.002
6-OHDA	1	1.970	2.453	0.136
192SAP X 6-OHDA	1	2.637	3.282	0.088
Error	17	0.803		
Total	21			

RSG, Layer III, Apical Tree: Total Dendrite Length

Source	df	MS	F	p
192SAP	1	59243.580	3.416	0.077
6-OHDA	1	3762.005	0.217	0.646
192SAP X 6-OHDA	1	5308.091	0.306	0.585
Error	23	17341.156		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 1

Source	df	MS	F	p
192SAP	1	0.889	1.210	0.283
6-OHDA	1	1.655	2.254	0.147
192SAP X 6-OHDA	1	0.269	0.367	0.551
Error	23	0.734		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 2

Source	df	MS	F	p
192SAP	1	0.889	1.210	0.283
6-OHDA	1	1.655	2.254	0.147
192SAP X 6-OHDA	1	0.269	0.367	0.551
Error	23	0.734		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 3

Source	df	MS	F	p
192SAP	1	1.693	2.535	0.125
6-OHDA	1	0.741	1.109	0.303
192SAP X 6-OHDA	1	0.141	0.211	0.650
Error	23	0.668		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 7

Source	df	MS	F	p
192SAP	1	1.147	2.624	0.119
6-OHDA	1	0.005	0.012	0.915
192SAP X 6-OHDA	1	0.028	0.065	0.802
Error	23	0.437		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 8

Source	df	MS	F	p
192SAP	1	1.939	2.064	0.164
6-OHDA	1	0.017	0.019	0.892
192SAP X 6-OHDA	1	0.163	0.174	0.681
Error	23	0.940		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 9

Source	df	MS	F	p
192SAP	1	8.112	6.720	0.016
6-OHDA	1	0.019	0.016	0.900
192SAP X 6-OHDA	1	0.097	0.080	0.779
Error	23	1.207		
Total	27			

RSG, Layer III, Apical Tree: Sholl Analysis, Ring 10

Source	df	MS	F	p
192SAP	1	5.686	4.110	0.054
6-OHDA	1	0.151	0.109	0.744
192SAP X 6-OHDA	1	1.106	0.800	0.380
Error	23	1.383		
Total	27			

RSG, Layer III, Apical Tree: Total Number of Branches

Source	df	MS	F	p
192SAP	1	42.170	3.088	0.092
6-OHDA	1	12.980	0.951	0.340
192SAP X 6-OHDA	1	2.271	0.166	0.687
Error	23	13.655		
Total	27			

RSG, Layer III, Apical Tree: Number of 1st Order Branches

Source	df	MS	F	p
192SAP	1	0.598	1.614	0.217
6-OHDA	1	0.551	1.488	0.235
192SAP X 6-OHDA	1	0.007	0.020	0.888
Error	23	0.370		
Total	27			

RSG, Layer III, Apical Tree: Number of 2nd Order Branches

Source	df	MS	F	p
192SAP	1	0.506	0.540	0.470
6-OHDA	1	1.697	1.811	0.192
192SAP X 6-OHDA	1	0.500	0.533	0.473
Error	23	0.937		
Total	27			

RSG, Layer III, Apical Tree: Number of 3rd Order Branches

Source	df	MS	F	p
192SAP	1	4.777	2.856	0.105
6-OHDA	1	3.197	1.911	0.180
192SAP X 6-OHDA	1	0.155	0.093	0.763
Error	23	1.673		
Total	27			

RSG, Layer III, Apical Tree: Number of 4th Order Branches

Source	df	MS	F	p
192SAP	1	7.886	6.593	0.017
6-OHDA	1	0.014	0.012	0.913
192SAP X 6-OHDA	1	0.005	0.004	0.948
Error	23	1.196		
Total	27			

RSG, Layer III, Apical Tree: Number of 5th Order Branches

Source	df	MS	F	p
192SAP	1	1.365	1.873	0.184
6-OHDA	1	0.020	0.028	0.869
192SAP X 6-OHDA	1	0.753	1.032	0.320
Error	23	0.729		
Total	27			

RSG, Layer III, Apical Tree: Number of 7th Order Branches

Source	df	MS	F	p
192SAP	1	0.007	0.040	0.844
6-OHDA	1	0.002	0.011	0.919
192SAP X 6-OHDA	1	1.058	5.274	0.031
Error	23	0.201		
Total	27			

RSG, Layer III, Apical Tree: Number of 7th Order Branches

Source	df	MS	F	p
192SAP	1	0.007	0.040	0.844
6-OHDA	1	0.002	0.011	0.919
192SAP X 6-OHDA	1	1.058	5.274	0.031
Error	23	0.201		
Total	27			

RSG, Layer III, Apical Tree: Spine Density

Source	df	MS	F	p
192SAP	1	1.754	1.621	0.220
6-OHDA	1	0.022	0.021	0.886
192SAP X 6-OHDA	1	1.262	1.166	0.295
Error	17	1.082		
Total	21			

RSG, Layer III, Basal Tree: Total Dendrite Length

Source	df	MS	F	p
192SAP	1	728179.240	9.378	0.006
6-OHDA	1	1617.952	0.021	0.886
192SAP X 6-OHDA	1	2974.570	0.038	0.847
Error	23	77647.863		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 1

Source	df	MS	F	p
192SAP	1	12.525	4.656	0.042
6-OHDA	1	3.080	1.145	0.296
192SAP X 6-OHDA	1	4.936	1.835	0.189
Error	23	2.690		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 2

Source	df	MS	F	p
192SAP	1	44.372	9.324	0.006
6-OHDA	1	0.275	0.058	0.812
192SAP X 6-OHDA	1	2.676	0.562	0.461
Error	23	4.759		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 3

Source	df	MS	F	p
192SAP	1	93.327	9.024	0.006
6-OHDA	1	0.552	0.053	0.819
192SAP X 6-OHDA	1	0.235	0.023	0.881
Error	23	10.342		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 4

Source	df	MS	F	p
192SAP	1	93.921	7.447	0.012
6-OHDA	1	4.009	0.318	0.578
192SAP X 6-OHDA	1	0.571	0.045	0.833
Error	23	12.612		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 5

Source	df	MS	F	p
192SAP	1	48.365	7.679	0.011
6-OHDA	1	2.597	0.412	0.527
192SAP X 6-OHDA	1	0.026	0.004	0.949
Error	23	6.298		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 6

Source	df	MS	F	p
192SAP	1	9.121	4.276	0.050
6-OHDA	1	0.324	0.152	0.700
192SAP X 6-OHDA	1	1.087	0.510	0.483
Error	23	2.133		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 7

Source	df	MS	F	p
192SAP	1	1.752	3.855	0.062
6-OHDA	1	0.009	0.020	0.889
192SAP X 6-OHDA	1	0.215	0.473	0.498
Error	23	0.454		
Total	27			

RSG, Layer III, Basal Tree: Sholl Analysis, Ring 8

Source	df	MS	F	p
192SAP	1	0.389	3.507	0.074
6-OHDA	1	0.055	0.505	0.485
192SAP X 6-OHDA	1	0.022	0.204	0.655
Error	23	0.111		
Total	27			

RSG, Layer III, Basal Tree: Total Number of Branches

Source	df	MS	F	p
192SAP	1	234.114	11.582	0.002
6-OHDA	1	73.677	3.645	0.069
192SAP X 6-OHDA	1	50.371	2.492	0.128
Error	23	20.214		
Total	27			

RSG, Layer III, Basal Tree: Number of 1st Order Branches

Source	df	MS	F	p
192SAP	1	0.976	1.502	0.233
6-OHDA	1	1.224	1.883	0.183
192SAP X 6-OHDA	1	0.738	1.136	0.298
Error	23	0.650		
Total	27			

RSG, Layer III, Basal Tree: Number of 2nd Order Branches

Source	df	MS	F	p
192SAP	1	1.367	0.918	0.348
6-OHDA	1	2.661	1.787	0.194
192SAP X 6-OHDA	1	1.173	0.788	0.384
Error	23	1.489		
Total	27			

RSG, Layer III, Basal Tree: Number of 3rd Order Branches

Source	df	MS	F	p
192SAP	1	1.490	1.355	0.256
6-OHDA	1	4.977	4.526	0.044
192SAP X 6-OHDA	1	0.945	0.859	0.364
Error	23	1.100		
Total	27			

RSG, Layer III, Basal Tree: Number of 4th Order Branches

Source	df	MS	F	p
192SAP	1	17.867	7.343	0.012
6-OHDA	1	0.523	0.215	0.647
192SAP X 6-OHDA	1	6.364	2.615	0.119
Error	23	2.433		
Total	27			

RSG, Layer III, Basal Tree: Number of 5th Order Branches

Source	df	MS	F	p
192SAP	1	15.048	8.487	0.008
6-OHDA	1	2.597	1.464	0.239
192SAP X 6-OHDA	1	5.544	3.127	0.090
Error	23	1.773		
Total	27			

RSG, Layer III, Basal Tree: Number of 6th Order Branches

Source	df	MS	F	p
192SAP	1	7.178	6.497	0.018
6-OHDA	1	2.372	2.146	0.156
192SAP X 6-OHDA	1	4.027	3.645	0.069
Error	23	1.105		
Total	27			

RSG, Layer III, Basal Tree: Number of 7th Order Branches

Source	df	MS	F	p
192SAP	1	1.277	3.792	0.064
6-OHDA	1	0.025	0.076	0.786
192SAP X 6-OHDA	1	0.793	2.355	0.139
Error	23	0.337		
Total	27			

RSG, Layer III, Basal Tree: Spine Density

Source	df	MS	F	p
192SAP	1	2.221	3.440	0.081
6-OHDA	1	1.008	1.561	0.228
192SAP X 6-OHDA	1	0.007	0.11	0.916
Error	17	0.646		
Total	21			

Appendix A4. ANOVA Summary Tables for Behaviour**Open Field: Total Gridline Crosses**

Source	df	MS	F	p
192SAP	1	9535.062	1.604	0.208
6-OHDA	1	79521.959	13.381	0.000
Sex	1	126047.71	21.209	0.000
192SAP X 6-OHDA	1	44.504	0.007	0.931
192SAP X Sex	1	1386.501	0.233	0.630
6-OHDA X Sex	1	46537.891	7.831	0.006
192SAP X 6-OHDA X Sex	1	0.640	0.641	0.425
Error	109	5943.098		
Day	2	1735.789	2.286	0.104
Day X 192SAP	2	678.700	0.894	0.411
Day X 6-OHDA	2	2597.695	3.422	0.034
Day X Sex	2	724.603	0.954	0.387
Day X 192SAP X 6-OHDA	2	915.386	1.206	0.301
Day X 192SAP X Sex	2	2414.776	3.181	0.043
Day X 6-OHDA X Sex	2	1362.838	1.795	0.169
Day X 192SAP X 6-OHDA X Sex	2	834.5	1.099	0.335
Error	218	759.207		

Open Field: Percent of Total Crosses Made in the Centre

Source	df	MS	F	p
192SAP	1	11.561	0.143	0.706
6-OHDA	1	13.124	0.162	0.688
Sex	1	0.409	0.005	0.943
192SAP X 6-OHDA	1	1324.296	16.353	0.000
192SAP X Sex	1	111.772	1.380	0.243
6-OHDA X Sex	1	5.545	0.068	0.794
192SAP X 6-OHDA X Sex	1	2.755	0.034	0.854
Error	109	80.983		
Day	2	420.127	24.994	0.000
Day X 192SAP	2	53.688	3.194	0.043
Day X 6-OHDA	2	222.353	13.228	0.000
Day X Sex	2	0.294	0.017	0.983
Day X 192SAP X 6-OHDA	2	2.591	0.154	0.857
Day X 192SAP X Sex	2	13.805	0.821	0.441
Day X 6-OHDA X Sex	2	9.483	0.564	0.570
Day X 192SAP X 6-OHDA X Sex	2	10.596	0.630	0.533
Error	218	16.809		

Open Field: Total Rears

Source	df	MS	F	p
192SAP	1	1552.491	6.929	0.010
6-OHDA	1	5455.815	24.352	0.000
Sex	1	3135.625	13.996	0.000
192SAP X 6-OHDA	1	1476.836	6.592	0.012
192SAP X Sex	1	61.175	0.273	0.602
6-OHDA X Sex	1	142.150	0.634	0.427
192SAP X 6-OHDA X Sex	1	90.458	0.404	0.526
Error	112	224.042		
Day	2	509.437	11.979	0.000
Day X 192SAP	2	161.702	3.802	0.024
Day X 6-OHDA	2	257.162	6.047	0.003
Day X Sex	2	6.533	0.154	0.858
Day X 192SAP X 6-OHDA	2	64.744	1.522	0.220
Day X 192SAP X Sex	2	18.786	0.442	0.643
Day X 6-OHDA X Sex	2	37.836	0.890	0.412
Day X 192SAP X 6-OHDA X Sex	2	11.734	0.276	0.759
Error	224	42.526		

Open Field: Percent of Total Rears Made Toward the Centre

Source	df	MS	F	p
192SAP	1	1.754	0.012	0.914
6-OHDA	1	3064.874	20.605	0.000
Sex	1	22.749	0.153	0.697
192SAP X 6-OHDA	1	865.356	5.818	0.018
192SAP X Sex	1	246.185	1.655	0.201
6-OHDA X Sex	1	2.670	0.018	0.894
192SAP X 6-OHDA X Sex	1	166.769	1.121	0.292
Error	110	148.747		
Day	2	152.723	4.009	0.019
Day X 192SAP	2	167.621	4.440	0.013
Day X 6-OHDA	2	212.487	5.578	0.004
Day X Sex	2	9.980	0.262	0.770
Day X 192SAP X 6-OHDA	2	54.390	1.428	0.242
Day X 192SAP X Sex	2	48.279	1.267	0.284
Day X 6-OHDA X Sex	2	131.486	3.452	0.033
Day X 192SAP X 6-OHDA X Sex	2	76.928	2.019	0.135
Error	220	38.095		

Open Field: Grooming Episodes

Source	df	MS	F	p
192SAP	1	0.962	0.482	0.489
6-OHDA	1	20.586	10.318	0.002
Sex	1	3.560	1.785	0.184
192SAP X 6-OHDA	1	0.256	0.128	0.721
192SAP X Sex	1	4.127	2.069	0.153
6-OHDA X Sex	1	0.920	0.461	0.498
192SAP X 6-OHDA X Sex	1	1.796	0.900	0.345
Error	113	1.995		
Day	2	1.007	1.165	0.314
Day X 192SAP	2	0.208	0.241	0.786
Day X 6-OHDA	2	0.903	1.045	0.354
Day X Sex	2	2.429	2.810	0.062
Day X 192SAP X 6-OHDA	2	0.330	0.382	0.683
Day X 192SAP X Sex	2	3.102	3.589	0.029
Day X 6-OHDA X Sex	2	1.187	1.373	0.255
Day X 192SAP X 6-OHDA X Sex	2	2.975	3.442	0.034
Error	226	0.864		

Hidden Platform Water Maze: All Groups

Source	df	MS	F	p
192SAP	1	39.103	0.080	0.780
6-OHDA	1	90045.396	184.184	0.000
Sex	1	877.233	1.794	0.192
192SAP X 6-OHDA	1	5.088	0.010	0.920
192SAP X Sex	1	27.859	0.057	0.813
6-OHDA X Sex	1	176.631	0.361	0.553
192SAP X 6-OHDA X Sex	1	190.082	0.389	0.538
Error	26	488.889		
Day	4	2584.039	22.255	0.000
Day X 192SAP	4	68.612	0.591	0.670
Day X 6-OHDA	4	2064.381	17.779	0.000
Day X Sex	4	147.397	1.269	0.287
Day X 192SAP X 6-OHDA	4	149.723	1.289	0.279
Day X 192SAP X Sex	4	76.761	0.661	0.620
Day X 6-OHDA X Sex	4	33.421	0.288	0.885
Day X 192SAP X 6-OHDA X Sex	4	21.401	0.184	0.946
Error	104	116.111		

Hidden Platform Water Maze: Cued Trials

Source	df	MS	F	p
192SAP	1	4.664	0.125	0.727
6-OHDA	1	62587.094	1674.927	0.000
Sex	1	280.650	7.511	0.011
192SAP X 6-OHDA	1	48.034	1.285	0.267
192SAP X Sex	1	0.09128	0.002	0.961
6-OHDA X Sex	1	0.683	0.018	0.894
192SAP X 6-OHDA X Sex	1	77.233	2.067	0.162
Error	26	37.367		
Day	1	5.790	0.240	0.629
Day X 192SAP	1	0.09869	0.004	0.950
Day X 6-OHDA	1	336.083	13.913	0.001
Day X Sex	1	73.564	3.045	0.093
Day X 192SAP X 6-OHDA	1	77.022	3.189	0.086
Day X 192SAP X Sex	1	66.516	2.754	0.109
Day X 6-OHDA X Sex	1	54.013	2.236	0.147
Day X 192SAP X 6-OHDA X Sex	1	0.874	0.036	0.851
Error	26	24.156		

Hidden Platform Water Maze: Control vs. ACh Group on Day 1

Source	df	MS	F	p
192SAP	1	845.579	5.194	0.034
Sex	1	50.356	0.309	0.585
192SAP X Sex	1	244.732	1.503	0.235
Error	19	162.800		
Total	23			

Cued Platform Water Maze After Habituation

Source	df	MS	F	p
192SAP	1	129.153	0.022	0.883
6-OHDA	1	416935.08	70.654	0.000
Sex	1	16286.828	2.760	0.102
192SAP X 6-OHDA	1	1563.976	0.265	0.608
192SAP X Sex	1	36.613	0.006	0.937
6-OHDA X Sex	1	4584.356	0.777	0.381
192SAP X 6-OHDA X Sex	1	48.044	0.008	0.928
Error	63	5901.123		
Day	9	2484.183	36.366	0.000
Day X 192SAP	9	132.979	1.947	0.043
Day X 6-OHDA	9	649.288	9.505	0.000
Day X Sex	9	56.912	0.833	0.586
Day X 192SAP X 6-OHDA	9	65.585	0.960	0.472
Day X 192SAP X Sex	9	45.760	0.670	0.737
Day X 6-OHDA X Sex	9	185.191	2.711	0.004
Day X 192SAP X 6-OHDA X Sex	9	42.812	0.627	0.775
Error	567	68.311		

Plasma CORT Levels After Mild Swim Stress in Female DA Rats

Source	df	MS	F	p
6-OHDA	1	1796.190	2.020	0.162
Error	44	889.020		
Time	5	12679.305	63.662	0.000
Time X 6-OHDA	5	141.105	0.708	0.618
Error	220	199.166		

Hidden Platform Water Maze: Effects of Dopaminergic Drugs in Female DA Rats

Source	df	MS	F	p
Drug	2	173.980	0.879	0.427
Error	26	197.994		
Day	3	101.987	1.469	0.229
Day X Drug	3	62.723	0.904	0.497
Error	78	69.404		

Cued Platform Water Maze: Effects of Dopaminergic Drugs in Female DA Rats

Source	df	MS	F	p
Drug	2	268.412	9.510	0.001
Error	26	28.224		
Day	3	94.836	1.857	0.144
Day X Drug	3	55.185	1.081	0.381
Error	78	51.056		

Prepulse Inhibition in Female DA Rats

Source	df	MS	F	p
6-OHDA	1	2765.255	2.151	0.161
Error	17	1285.676		
Intensity	4	21816.052	87.648	0.000
Intensity X 6-OHDA	4	200.176	0.804	0.527
Error	68	248.906		