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THE IMPORTANCE OF AGE TO THE MOTORIC EFFECTS OF STRIATAL DOPAMINE DEPLETION IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for

The Degree Doctor of Philosophy in the Graduate

School of the Ohio State University

By

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*****

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1998

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ABSTRACT

Two experiments explored the neurobiological basis of the different rates of recovery from motor deficits in adult and weanling rats following selective depletion of nigrostriatal dopamine (DA) with 6-hydroxydopamine (6-OHDA). First, adult rats that had been DA-depleted as weanlings and age-matched sham-treated controls received acute striatal infusions of the D2-like DA antagonist sulpiride (0, 3.0, and 10.0μg), resulting in dose-dependent akinesia and catalepsy in both groups. This demonstrated that at this age these DA-depleted rats continue to rely on striatal DA transmission for the sensorimotor behavior expressed following recovery of function. Also, 6-OHDA treated weanlings did not exhibit behavioral supersensitivity to this acute antagonist. Second, when sensorimotor recovery differed most dramatically between rats DA-depleted as adults or weanlings (5-8 days following the lesion), *in-vivo* microdialysis was used to assess both relative basal extracellular striatal DA concentrations and DA modulation of striatal acetylcholine (ACh) as indicated by the response to intrastriatal administration of the D2-like DA antagonist sulpiride (10 & 100 μM). DA-depleted adult rats exhibited significantly reduced basal extracellular striatal DA compared with sham-treated controls, while DA-depleted weanling rats exhibited DA concentrations similar to their age-matched sham-treated controls.

DA-depleted adult rats exhibited a trend toward the expected decrease in sulpiride-induced ACh efflux when compared to age-matched sham-treated controls. Interestingly, weanling rats exhibited reduced sulpiride-induced ACh efflux compared with adult rats, despite the higher basal extracellular ACh found in the weanlings. These results suggest that adult rats exhibit a reduction in striatal DA transmission at a time prior to recovery of motoric function following DA depletion. Weanling rats exhibited control-levels of extracellular striatal DA at a time when they show no signs of motoric deficit. However decreased sulpiride-induced striatal ACh

efflux rendered this test of postsynaptic DA transmission less useful, so this component of the correlation remains unclear. Weanling rats exhibit control levels of extracellular striatal DA which may underlie their failure to show behavioral supersensitivity to acute intrastriatal DA antagonist infusion. However the critical postsynaptic activity mediated by this increased striatal DA remains to be determined.

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This dissertation work suffered many false starts from which the techniques used were greatly improved, but no meaningful scientific advances could come. This extended the time which was originally allotted for the project on several occasions. I would also like to thank all the members of my dissertation committee: Drs. John Bruno, Jaqueline Bresnahan, and Norman Uretsky, and the mentor of my planned post-doctoral position Dr. George Rebec, for their patience during these extensions.

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FIELDS OF STUDY

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CHAPTER 1

GENERAL INTRODUCTION

The experiments in this document involve selective depletion of dopamine (DA) in the nigrostriatal DA system of the rat brain and its effects on sensorimotor behavior. The nigrostriatal DA system is comprised of dopaminergic neurons of the substantia nigra that innervate the striatum or caudate-putamen, that is part of a set of important, motor-related brain nuclei called the basal ganglia (see **Figure 1.1**). The nature and severity of DA depletion-induced sensorimotor deficits have been shown to decline dramatically the younger the rat is when it receives the DA depleting lesion (Weihmuller et al., 1989). These experiments are designed to explore this age-dependent compensation following depletion of DA by testing hypotheses about DA transmission in the rat striatum and its involvement in recovery of sensorimotor function. This introduction will be divided into three sections. First, I will discuss the involvement of the nigrostriatal DA system in rat sensorimotor behavior. Second, I will describe the consequences of selective depletion of DA in this system on sensorimotor behavior and what is known today about the mechanisms of compensation in the brain that seem to support recovery of function. Finally, I will contrast the sensorimotor deficits that result from depletion of nigrostriatal DA of adult rats versus young rats and elaborate on the hypotheses I tested in this work.

1.1 Sensorimotor behavior and the nigrostriatal dopamine system

In intact rodents, many motoric deficits can be elicited by the acute administration of DA antagonists. Typically these deficits involve impeded initiation of locomotion called akinesia (Svensson et al., 1993), decreased exploratory behavior (Schumacher et al., 1994; Carlsson, 1993; Kuribara, 1995), postural rigidity called catalepsy (Ossowska et al., 1990; Ögren et al.,

1988; Horikawa et al., 1997), and somatosensory neglect (Marshall, 1979; Johnson et al., 1992). Local striatal microinjection of DA antagonists can induce sensorimotor deficits in rats very similar to those resulting from their systemic administration (Costall et al., 1972; Ossowska et al., 1990; Meyer et al., 1993), indicating the striatum as a likely region where DA transmission is important in generating sensorimotor behavior. This evidence all implies that some level of DA transmission within the striatum is necessary for normal rat sensorimotor behavior, and that disruption of this transmission can lead to deviations from normal sensorimotor behavior. The experiments of this dissertation are designed to explore the responses of rats to decreases in striatal DA.

Parkinson's disease is based primarily on the deterioration of DA input to the caudate and putamen nuclei (referred to collectively as the neostriatum or striatum in this text, see **Figure 1.1**) (Sourkes, 1989; DeLong, 1990; Gerlach et al., 1996; Kish et al., 1988). Parkinson's disease includes motor symptoms such as a resting tremor, rigidity, postural abnormalities, and bradykinesia (Parkinson, 1955; Zigmond et al., 1984). The most direct association between loss of dopaminergic substantia nigra neurons and the symptoms of Parkinson's disease are the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Edwards, 1993). This toxic substance was derived from a poorly produced synthetic heroin, and selectively destroyed dopaminergic substantia nigra neurons in abusers of this substance, resulting in sensorimotor deficits similar to advanced stages of Parkinson's disease (Stein et al., 1995).
After this incident, selective application of the MPTP toxin into the primate brain was introduced as a useful animal model that closely approximates the clinical symptoms of Parkinson's disease as opposed to those expressed by rodents after destruction of the same system (Levy et al., 1997; Luquin et al., 1994; Pérez-Otaño et al., 1994).

1.2 The 6-hydroxydopamine-treated rat model

Another selective neurotoxin called 6-hydroxydopamine (6-OHDA) has proven to be most useful in exploring the neurobehavioral responses to depletion of DA in rats. In this experimental model, 6-OHDA is infused directly into specific brain regions to selectively deplete the

nigrostriatal dopamine input to the striatum (see Zigmond et al., 1989 for review). The effects of this treatment can be followed on any number of levels from changes in DA transmission to the resulting deficits in sensorimotor behavior. When the adult rat receives bilateral intracerebral infusions of 6-OHDA at a dose sufficient to destroy at least 80% of its nigrostriatal dopaminergic axons, it exhibits a well known syndrome of behavioral and ingestive deficits. These deficits include akinesia or decreased exploratory locomotion, catalepsy (Stricker et al., 1986), somatosensory neglect (Marshall, 1979), aphagia, and adipsia (Ungerstedt, 1971), at various degrees of intensity depending on the severity of the lesion (Sakai et al., 1994). The severity of these deficits are such that rats treated this way have a high mortality unless the animals are maintained via intragastric feedings. Severe lesions of this dopaminergic system (≥ 90% loss of postmortem striatal tissue DA content compared to vehicle-treated controls) result in continued expression of these deficits over approximately one month. However, when DA depletions are less severe (< 90% loss of post-mortem tissue dopamine content compared to vehicle-treated controls), rats typically recover from most of the deficits within a one week recovery period (Zigmond et al., 1990).

The rate of behavioral recovery from DA-depleting lesions seems to be related to compensatory changes that take place in the residual components of the DA system that survive the lesion (Stein et al., 1995). The most direct demonstration of this is the prompt return of sensorimotor deficits when DA antagonists are acutely administered following recovery from the deficits caused by the 6-OHDA treatment (Fletcher et al., 1987; Heffner et al., 1977). Striatal tissue DA remains significantly decreased when measured postmortem, as well as striatal tyrosine hydroxylase expression, long after sensorimotor recovery takes place, supporting the idea that recovery of function does not involve re-innervation of striatum by dopaminergic neurons (Rothblat et al., 1994). This, along with evidence obtained by directly measuring the presence of extracellular DA in the striatum after 6-OHDA-induced depletion of DA, support the proposal that volume transmission of DA from distal areas is important in supporting this compensation process (Bjelke et al., 1994; Schneider et al., 1994). It is generally believed that symptoms of Parkinson's disease remain hidden by these compensatory mechanisms until at least 80% of the substantia

nigra dopaminergic neurons have deteriorated (Zigmond et al., 1984; Zigmond et al., 1992), making early diagnosis difficult (Yahr, 1993).

In order to support volume transmission of DA into the striatum, one of the compensatory responses to depletion of nigrostriatal DA involves the dopaminergic neurons that survive the lesion (see **Figure 1.1**). With time the presence of DA within striata previously depleted of DA may reach near the extracellular concentrations expressed by intact rats (Robinson et al., 1988; Stachowiak et al., 1987; Schneider et al., 1994; Parsons et al., 1991; Zigmond et al., 1990). Presynaptic compensations such as increases in DA synthesis (Zigmond et al., 1984; Blanchard et al., 1995), decreases in metabolism, and increases in release seem to occur over a time course related to a rat's sensorimotor recovery following depletion of DA (Altar et al., 1987). There are also increases in dopaminergic nigrostriatal neuron firing rate, and decreases in high affinity DA uptake (due in part to loss of neuronal terminals) (Hollerman et al., 1990).

Postsynaptic compensatory changes also take place after depletion of nigrostriatal DA. Soon after the lesion there are increases in the firing rate of striatal neurons, resulting from disinhibition (due to the fact that the primary effect of DA on striatal neurons is inhibitory in nature, reduced DA would result in disinhibition), and indirect activation due to disinhibition of corticostriatal glutamate release (D2-like DA receptors are expressed on corticostriatal glutamatergic terminals, and serve to inhibit glutamate release, so decreased DA would result in disinhibition of corticostriatal glutamate release) (Calabresi et al., 1993). Following this initial increase in striatal neuron activity, this activity is gradually attenuated. This attenuation of activity is generally believed to result from the gradual restoration of inhibitory postsynaptic activity of endogenous extracellular DA (Orr et al., 1986; Nisenbaum et al., 1986; Orr et al., 1987). Striatal DA receptors have also been shown to increase in number (typically D2-like receptors) (Neve et al., 1991), or become supersensitive due to an amplification of second messenger signaling in response to DA binding that increases the potency of neuronal responses to DA (typically D1-like receptors) (Trugman et al., 1992; Hossain et al., 1993). These postsynaptic compensations after DA depletions do not arise unless the depletions are large, and many of them can be reversed by intrastriatal infusions of DA after the 6-OHDA treatment (Woiciechowsky et al., 1995). This

suggests a direct relationship between decreased striatal DA and the induction of postsynaptic compensatory responses.

The temporal correlation between the establishment of compensations after depletion of nigrostriatal DA and the recovery of sensorimotor function suggests that the compensations serve to support this behavioral recovery. The fact that all of the compensations seem to support a restoration of DA transmission within the striatum supports the conclusion that this DA transmission is crucial to the process of sensorimotor recovery. However, mere increased presence of DA within the DA-denervated striatum over time along with increases in postsynaptic sensitivity to DA only suggests the possibility of a modulatory role, but does not confirm this. One study demonstrated the partial restoration of the dopaminergic effects of systemic amphetamine in the denervated striatal hemisphere of unilaterally 6-OHDA-lesioned rats with microdialysis in combination with immunohistochemistry for the protein product of the immediate-early gene c-fos (Bjelke et al., 1994). This evidence more directly supports the proposed restoration of a modulatory role of DA with time following depletion of striatal DA, but the specificity of this modulation remains unclear.

1.3 The importance of age at the time of dopamine depletion

The description of the sensorimotor effects of dopamine depletions described above applies to rats depleted of striatal DA during adulthood. Depleting striatal DA in rats during development yields significantly different degrees of sensorimotor and ingestive deficit expression. The two phenomena most interesting to our laboratory are described broadly as *sparing* and *accelerated recovery*. These terms describe the sensorimotor effects of nigrostriatal DA depletions produced in rats as neonates (postnatal day 3) or as weanlings (postnatal day 27) respectively, in contrast to the severe and long-lasting deficits expressed by rats given these lesions as adults (postnatal day 60-90). When neonatal rats receive large (90-95%) depletions of nigrostriatal DA they are largely spared from the initial gross sensorimotor deficits (akinesia, catalepsy, and somatosensory neglect) seen after similar lesions in adult rats. When

weanling rats receive large (90-95%) depletions of nigrostriatal DA they typically express only modest degrees of gross sensorimotor deficits, and recover much more rapidly than in the case of rats lesioned as adults (Weihmuller et al., 1989). In fact, the adult-like degree and progression of sensorimotor deficit expression in response to DA depletion does not occur until lesions are produced in rats on postnatal day 35 or older ages, an age close to that of puberty in rats (Bruno et al., 1987). In this section the scope of the age dependence of nigrostriatal DA depletion related sensorimotor deficits, and the specific hypotheses addressed by the present work will be described.

The main focus of this dissertation is the different degrees of sensorimotor deficits expressed by rats depleted of striatal DA as weanlings versus as adults. The age-dependent sensorimotor deficits of rats depleted of nigrostriatal DA was initially characterized in the Bruno lab by Fredric Weihmuller (Weihmuller et al., 1989) and this dissertation was designed to explore those results in greater depth. Sensorimotor deficits were expressed by rats after nigrostriatal DA depletion postnatal day 27 were significant (compared with vehicle-treated controls) for approximately three days immediately following the lesion. This time course was designated *accelerated recovery*, as opposed to *sparing,* or lack of overt deficits seen after DA depletions administered on postnatal days 3, 15, or 20. These early deficits included akinesia, catalepsy, and somatosensory neglect, similar to the expressed deficits of rats depleted of DA as adults, but to a reduced degree by comparison. In contrast, sensorimotor deficits of increased magnitude were often expressed by rats depleted of DA as adults for several weeks.

There may be several possible neural mechanisms that serve to support *accelerated recovery* in rats after DA depletion as weanlings. The most immediate question, though, is whether the *accelerated recovery* is supported by the residual DA fibers surviving the lesion or not. Evidence has been obtained that residual DA fibers are necessary for recovery of function after striatal DA depletion in adult rats (Heffner et al., 1977; Zigmond et al., 1984). The most direct way to test for the necessity of striatal DA transmission for recovery of function after DA depletions of weanlings would be with an acute DA antagonist. This way, any deficits elicited by the antagonist would be reversible, and each rat serves as its own control. If the primary neural

mechanism supporting recovery of function after DA depletion is different from DA transmission from the residual striatal DA fibers surviving the lesion, then an acute DA antagonist would not be expected to induce any deficits. Of course any DA antagonist induced deficits in rats depleted of DA as weanlings would indicate that DA transmission remains necessary for recovery of function in these animals.

Our laboratory has recently done several experiments with acute DA antagonists and rats depleted of DA at various ages. Typically, these experiments have tested adult rats that had received DA depleting lesions on postnatal days 20, 27, and 35. At this age, lesioned rats have recovered from the initial gross deficits they express after DA depletion. Therefore, rats can be tested for sensorimotor deficits before and after DA antagonist administration, and increased deficit expression is interpreted as representing the necessity of DA transmission for recovery of function. Bonnie Johnson first demonstrated that rats depleted of DA on postnatal days 20 and 35 were sensitive to acute systemic DA antagonists (Johnson et al., 1992). This same experiment also demonstrated that rats lesioned on postnatal day 35 exhibit sensorimotor deficits in response to dramatically lower doses of DA antagonists than those required to induce deficits in their age-matched vehicle-treated controls. This phenomenon is called behavioral supersensitivity to antagonists, and suggests a reduced competitive capacity of DA in rats after DA depletion on postnatal day 35. My own work both replicated and extended this phenomenon, by demonstrating that adult rats that were depleted of DA on postnatal day 35 were behaviorally supersensitive to acute systemic DA antagonists, but adult rats that were lesioned on postnatal day 27 were not (Sandstrom et al., 1997). Therefore, sensorimotor deficits expressed by rats depleted of DA on postnatal day 35 are long-lasting, similar to the extent of deficit expression that follows DA depletion during adulthood, and after recovery these rats are behaviorally supersensitive to acute systemic DA antagonists. Sensorimotor deficits expressed by rats depleted of DA on postnatal day 27 disappear rapidly, and after recovery these rats are sensitive, but *not* supersensitive, to systemic DA antagonists. The point, then, is that doses of DA antagonists exist that induce sensorimotor deficits in adult rats that had been depleted of DA on postnatal day 35, but not those that had been depleted of DA on postnatal day 27. One possible

reason for this finding that deserves testing is that rats depleted of DA on postnatal day 27 produce more DA in areas where DA transmission remains necessary for their sensorimotor behavior. The most likely brain nucleus where DA transmission remains necessary for sensorimotor behavior expressed after recovery from DA depletion on postnatal day 27 would be the striatum, as described for intact rats in Section 1.1. Therefore, it would be even more informative to administer the DA antagonist directly into the striatum of adult rats that were depleted of DA on postnatal day 27. This would confirm the necessity of residual *striatal* DA transmission after recovery from the initial deficits expressed following DA depletion of these rats. This experiment is planned in this dissertation.

Does the DA necessary for the sensorimotor behavior of rats that have recovered from DA depletion on postnatal day 27 serve to modulate striatal information processing in the same way as it does in intact rats? One way to investigate this might be to examine the role of extracellular striatal DA in striatal processing while the process of recovery of function takes place. Correlating sensorimotor recovery with both the presence of increased concentrations of striatal DA and increased DA modulation of striatal neurons would paint a more complete picture of the role of striatal DA in supporting recovery of sensorimotor function following DA-depleting lesions. Among the many neurons within the striatum, the acetylcholine (ACh) interneurons have been used to assay the modulatory function of DA in the striatum. Soon after DA depletion of adult rats, the modulatory role of DA on striatal cholinergic neurons is diminished, and this function is restored and is temporally correlated with the recovery of sensorimotor function (Zigmond et al., 1990). DA modulates striatal cholinergic interneurons directly, through D2-like receptors expressed on these neurons (Stoof et al., 1992; LeMoine et al., 1990; Ikarashi et al., 1997; DeBoer et al., 1996; Stoof et al., 1992). The laboratory of Michael Zigmond demonstrated this DA modulation of striatal cholinergic interneurons with an in vitro preparation of striatal slices taken from 6-OHDA-treated rats. First, they showed that sulpiride, a D2-like DA antagonist known to cause increases in striatal ACh through disinhibition, was unable to promote [^{3}H]ACh efflux from electrically-stimulated striatal slices 3 days following depletion of nigrostriatal DA. In fact sulpiride-evoked [^{3}H]ACh efflux from these slices decreased in direct correlation with the

degree of tissue DA depletion. This sulpiride-evoked striatal [^{3}H]ACh efflux from depolarized striatal slices would be expected to depend upon the presence of endogenous DA inhibiting the cholinergic neurons, as the efflux would be a result of disinhibition. Therefore, shortly after the 6-OHDA treatment, the extracellular striatal DA concentration was insufficient to provide a basis for [^{3}H]ACh efflux. However, the second and more interesting finding was that similarly DA-denervated slices taken from rats 2 months after the lesion showed near intact levels of this sulpiride-evoked [^{3}H]ACh efflux (MacKenzie et al., 1989). At this time, the 6-OHDA treated rats had recovered from the sensorimotor deficits caused by this lesion. Therefore, DA modulation of striatal [^{3}H]ACh efflux correlated with recovery from the sensorimotor deficits associated with the 6-OHDA treatment, supporting the proposal that restored striatal DA transmission is involved in that behavioral recovery. DA was shown to have resumed a modulatory role within the striatum during the recovery process, rather than merely increasing extracellular concentration there. This is a much clearer and more specific demonstration of a physiological role of DA in the DA-depleted striatum than the previous studies, that depended on the assumption that increased striatal DA was involved in the gradual decrease in striatal neuronal activity (Orr et al., 1987; Nisenbaum et al., 1986; Orr et al., 1986). It would be interesting to test the relationship between recovery of sensorimotor function and DA modulation of striatal ACh following depletion of striatal DA in weanlings. This experiment is also planned in this dissertation.

The hypotheses of this dissertation project are as follows. **First, that striatal DA is *necessary* for maintenance of motoric behavior long after recovery from the initial modest motoric deficits expressed by weanling rats (postnatal day 27) after depletion of striatal DA with 6-OHDA. Second, that extracellular striatal DA will be available in greater quantity soon after depletion of striatal DA in weanling rats than after similar lesions of adult rats. Finally, that dopaminergic modulation of striatal ACh release will be demonstrable soon (within 5-8 days) following nigrostriatal DA depletion of weanling rats, but will not be seen within this interval after similar lesions of adult rats**, correlating with the different time courses of recovery of sensorimotor function seen after lesions at these two ages.

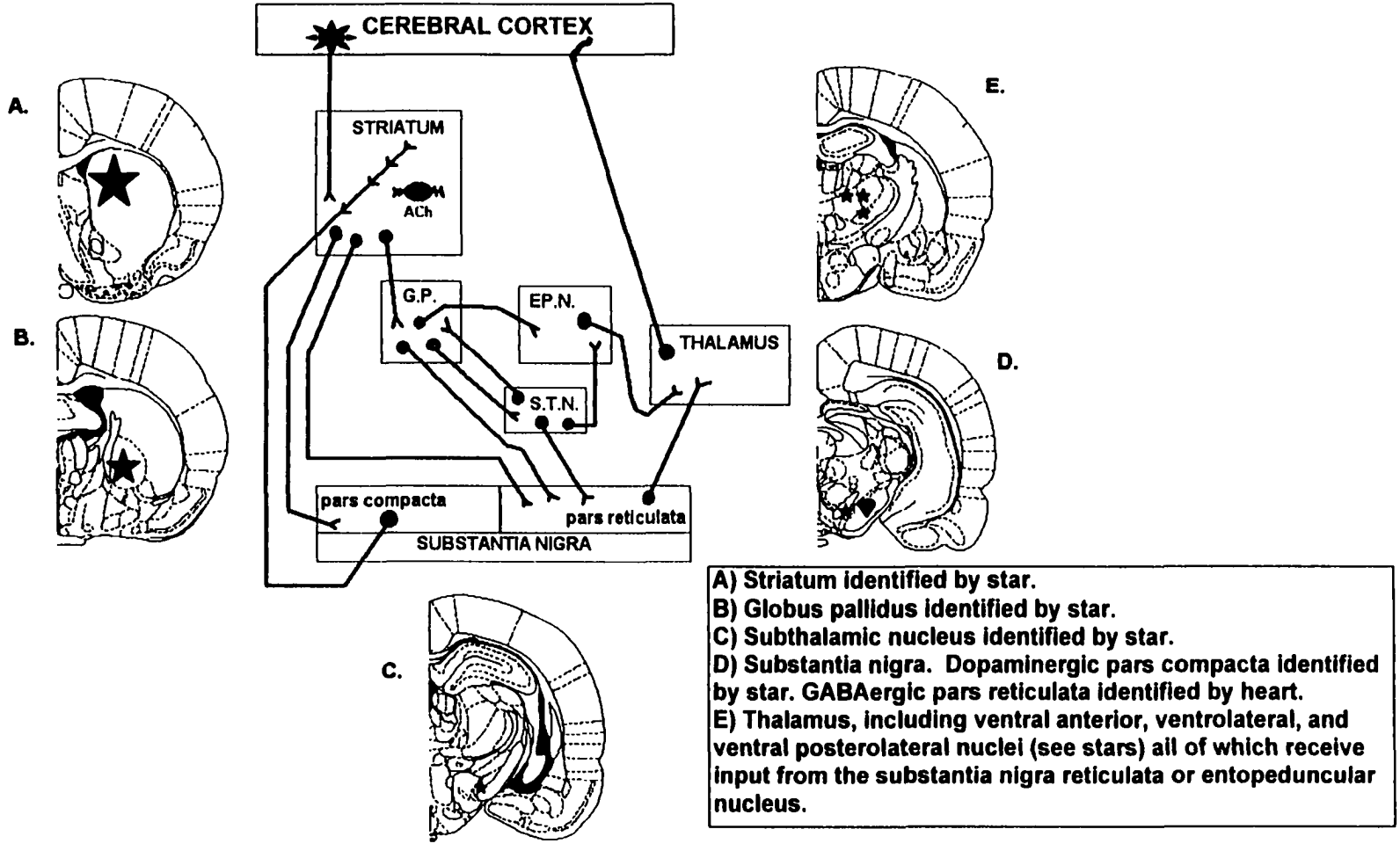

Figure 1.1

The basal ganglia motor loop. The circuitry of the basal ganglia as discussed in the text. Central diagram provides the main connections, surrounded by coronal sections (from Paxinos & Watson CDRom 1997) depicting the various structures. Abbreviations: G.P. = globus pallidus, S.T.N. = subthalamic nucleus, and EP.N. = entopeduncular nucleus.

CHAPTER 2

GENERAL METHODS

In the experiments described below, there were several common methods that will be described here. The unique aspects of each experiment will then be described in more detail within the subsequent chapters dedicated to each experiment. The common elements were the subjects used, housing, 6-hydroxydopamine (6-OHDA) infusion surgery for nigrostriatal dopamine (DA) depletion, supplemental feedings for rats with ingestive difficulties, the sensorimotor battery, and histological analysis for the two inclusion criteria: placement of intracerebral cannula, and striatal tissue DA content. These will be described in the sections to follow.

2.1 Subjects and Housing

Subjects used in these experiments were male Sprague-Dawley albino rats, either purchased as adults (225-250 grams) from Zivic-Miller Laboratories, or weanlings which were produced within our breeding facility. The adult males were immediately housed in groups on arrival in hanging wire cages (42 x 25 x 18 cm), with food and water available ad libitum, until they underwent DA depletion and cannulation surgery to prepare them for use in Experiment 2 (the microdialysis experiment). After their surgery, sham-treated rats were individually housed in plastic tubs (48 x 27 x 20 cm) on wood chip bedding. DA-depleted rats were housed in a modified infant incubation chamber maintained at 75-80 °F and 65% humidity until they overcame the marked initial ingestive difficulties common to such lesions, indicated by a weight gain beyond their pre-operative weight. Weights of all DA-depleted rats were monitored daily to track this progress.

Weanling rats were produced within our breeding colony. These rats were born into litters that were immediately culled to 8-10 pups (maximizing males), and the males were toe-clipped for identification purposes on postnatal day 2. Litters remained with the dam in hanging plastic tubs (48 x 27 x 20 cm) with food and water available ad libitum until postnatal day 21 when the males were weaned to the main vivarium. These rats underwent surgery on postnatal day 27, after which sham-treated rats destined for Experiment 1 (intrastriatal sulpiride) were multiply housed in hanging wire cages (42 x 25 x 18 cm), and those fitted with an intracerebral guide cannula for Experiment 2 (microdialysis) were housed individually in plastic tubs (48 x 27 x 20 cm). DA-depleted weanlings were also divided between Experiments, but they were initially housed in the modified infant incubation chamber until regular independent feeding returned. Weights of the DA-depleted rats were monitored daily to track this progress. Removal from the incubation chamber occurred when independent feeding resumed for at least three consecutive days. Rats to be used in Experiment 1 were then housed in groups in hanging wire cages, while those destined for Experiment 2 were separated into individual plastic tubs on wood-chip bedding.

2.2 Dopamine depletion

Rats in each experiment were treated with 6-OHDA or its vehicle solution (1.0 mg/ml ascorbic acid in 0.9% isotonic NaCl) prior to the experiment for which they were slated. Infusions stereotaxically targeted the medial forebrain bundle, the primary fiber bundle containing the dopaminergic nigrostriatal axons course. This route of 6-OHDA administration, rather than the intracerebroventricular infusions, reliably created large DA depletions necessary for the planned experiments after only a single infusion. Animals were anesthetized prior to surgery with either ketamine only (weanlings, 85.0 mg/kg), or combined ketamine and xylazine (adults, 85.0 mg/kg and 0.005 mg/kg, respectively). Rats were then placed into the stereotaxic apparatus, skull surface leveled anterior to posterior, and small holes were drilled for placement of the needles and infusion. Coordinates for adults were +5.0 mm anterior, and ±2.0 mm lateral from the inter-aural line, and -8.0 mm ventral from dural surface (Ervin et al., 1977). In the

weanlings, coordinates were +3.2 mm anterior, and ±1.7 mm lateral from the inter-aural line, and -8.0 mm ventral from dural surface (using rat developmental atlas (Sherwood et al., 1970)). Hamilton syringes (26 gauge, 10.0 μl) with 5.0 cm beveled needles were used to infuse 4.0 μl of 6-OHDA or its vehicle into the specific stereotaxic coordinates. Adult rats to be depleted of DA received 8.0 μg 6-OHDA dissolved in 4.0 μl of its vehicle. Weanling rats to be depleted of DA received 10.0 μg 6-OHDA dissolved in 4.0 μl of its vehicle. Vehicle or sham operated rats received 4 μl of the vehicle solution alone.

Because the adult rats receiving these infusions were always used for Experiment 2, their surgeries continued after the infusion to include fitting with an intracerebral guide cannula for the microdialysis probes used in that experiment (described in detail in Chapter 4, section 4.2). Weanling rats were either slated for Experiment 1 or Experiment 2. Therefore, either their wounds were closed with stainless steel clips (9.0 mm "Autoclip," Clay Adams – Becton Dickinson, MD) for Experiment 1 (intrastriatal sulpiride), or they were fitted with an intracerebral guide cannula immediately after the infusion for Experiment 2 (microdialysis).

2.3 Supplemental feedings

DA depleted rats kept in the modified infant incubation chamber were monitored daily for body weight changes. Wet mash of crushed rat chow pellets sweetened with a teaspoon of granular sucrose was provided daily for all lesioned rats in the incubation chamber. It is typical for adult rats depleted of striatal DA to exhibit ingestive deficits due to aphagia and adipsia (Stricker et al., 1986). It was therefore necessary to maintain the lesioned rats with supplemental feedings and lactated Ringers injections (2.0 ml i.p. for weanlings, 4.0 ml i.p. for adults) to maintain body weight and prevent dehydration. When a DA-depleted rat's body weight dropped below 80% of its preoperative weight, rats were given daily intragastric feedings of STAT nutritional supplement (PRN Pharmacal Inc., Pensacola FL) using either 8.0 cm (adults) or 5.0 cm (weanlings) curved "French catheter " style animal feeding tubes (Braintree Scientific Inc., Braintree MA). Adult rats received 8.0 ml, one or two times per day depending on the severity of the weight loss, while

weanlings received 4.0 ml once per day due to reduced stomach capacity. It was not as typical for weanling rats to require this supplemental feeding as it was for adults, although there were several weanlings which required feedings during the initial 3-4 days following 6-OHDA infusions. The body weights and quantity of supplemental intragastric feedings were recorded for later evaluation.

Pilot tests suggested that supplemental feedings would disrupt behavioral testing when given within 1 hour prior to testing. For this reason, feedings took place at early morning (between 0700 and 1000 hrs.) or late evening (between 1900 and 2200 hrs.) or both times, depending on the severity of their weight loss. Microdialysis experiments required removal of the rat from the vivarium and sensorimotor testing beginning early in the morning (between 0700 and 0800 hrs) of the testing day, so rats still requiring intragastric feedings would only be fed in the evenings of these days to prevent any stress from the feeding influencing the dialysis session.

2.4 Sensorimotor battery

All experiments utilized a battery of behavioral tests to monitor the impact of either the DA depletion alone or intrastriatal drug infusion on the rat's sensorimotor behavior. These tests were developed to monitor those behaviors (other than ingestive) which are typically disrupted by compromising nigrostriatal DA transmission either chronically with 6-OHDA or acutely with DA antagonist drugs. The range of tests administered was designed to capture the qualities and capacities for motor behavior exhibited by each rat, and include evaluations most appropriate for Parkinsonian sensorimotor deficits: A*kinesia*, was designed to measure the latency to initiate quadrapedal locomotion in a novel environment, reflecting difficulties in initiating locomotion often seen in Parkinsonian patients. *Open field activity,* as opposed to akinesia, was designed to measure exploratory motor behavior within a wide area over an extended period of time rather than simply its initiation, as reduced motion and reduced inclination towards the sides of the field or thigmotaxis would be expected from rats exhibiting Parkinsonian symptoms. *Catalepsy*, is designed to measure the latency to correct posture after it is forced into an awkward position, reflecting the muscular rigidity often seen in Parkinsonian patients. Finally, *somatosensory*

orientation was designed to measure the ability to recognize and respond to stimulation of the body surface, as somatosensory neglect is sometimes a part of Parkinsonian symptoms.

Akinesia, *catalepsy*, and *somatosensory orientation* measures were recorded separately in a 45 x 24 x 21 cm plastic testing tubs. *Open field activity* was recorded in a large, 1.20 x 1.20 x 0.46 m box open field divided into 36, 20 x 20 cm squares forming a grid on the bottom surface (see **Figure 2.1**). To measure akinesia, the rat was placed into the center of the testing tub, and the latency to move all four of its limbs was recorded in seconds (a 120-second ceiling was used to allow all tests to be finished within 5-8 minutes). To measure catalepsy, the rat's hind paws were placed on a Styrofoam platform (7.0 cm high for adults, 3.5 cm high for weanlings), and the latency to either fully ascend or descend was recorded in seconds (120 second ceiling). To measure somatosensory orientation, rats were probed in the hind-flank with a series of nine Von Frey hairs (Lafayette Instruments, IN) (see (Marshall, 1979), and also (Weihmuller et al., 1989)) attached to 14 cm wooden sticks ranging in local pressure when applied from 0.3 to 15.0 grams. Responses to each hair were categorized according to a standard scale: **1** = turns toward and bites at or sniffs at hair, **2** = turns in general direction of hair but does not specifically bite or sniff at the source of stimulation, **3** = exhibits generalized escape responses such as skin flinch, vocalization, or forward locomotion, or **4** = no response. From these categories, two values were extracted. **Plateau** response type was the best (i.e. most localized) orienting response expressed by the animal. **Force to plateau** was the lowest hair force necessary to reach the plateau response type. To measure open field activity, rats were placed into the center of the open field area (zone A, see **Figure 2.1**), and their locomotion was time-sampled during the first 10.0 seconds of each of six 30-second blocks over 3.0 minutes. The data recorded during each observation was the number of grid squares crossed and the specific region of the open field in which the animal spent the majority of the time (three concentric zones were delineated within the open field, see **Figure 2.1**).

These sensorimotor tests were all performed either following striatal infusion of a DA antagonist (Experiment 1), or daily (3 preoperative days and 8 postoperative days) to monitor the progress of sensorimotor recovery from 6-OHDA treatment prior to microdialysis (Experiment 2).

2.5 Tissue dopamine content and cannula placement verification

All rats were sacrificed by rapid decapitation at least two days following the last experiment performed. Brains were rapidly removed and frozen at –70°C until processing for both verification of cannula placement and quantification of tissue dopamine content.

Frozen brains were processed first by sectioning on a freezing microtome. Coronal sections were taken at 40 μm thickness. Sections containing tracts from either the bilateral intracranial infusion cannula (Experiment 1) or the microdialysis probe guide cannula (Experiment 2) were thaw-mounted onto gelatin-coated glass microscope slides and stained with cresyl violet to highlight and verify the placement of these cannula. Also, 1.0 mm^3 micropunches were taken with a stainless steel tube for subsequent analysis of tissue dopamine content and verification of the lesion. These micropunches were taken from both dorsolateral (just below the corpus callosum, aligned with the dorsal aspect of the lateral ventricle) and ventromedial (just above the border of the nucleus accumbens and aligned with the ventral aspect of the lateral ventricle) regions of the anterior striatum close to where the cannula were placed. These two regions were analyzed because the dorsal and ventral regions of the striatum have been found to be heterogeneous with respect to dopamine content and activity (Doucet et al., 1986; Marshall et al., 1990; Beal et al., 1985; Widmann et al., 1986; May et al., 1989; Garris et al., 1994), and the effects of medial forebrain bundle 6-OHDA treatments may also reflect this regional difference (Pehek et al., 1992; Okamura et al., 1995). Punches taken from both hemispheres were combined into separate plastic centrifuge tubes for each region, and immediately frozen on dry ice. The punches were then stored at –70°C until the time of processing.

Tissue micropunches were homogenized in 100 μl of 0.1 N $HClO_4$ containing 0.2mM sodium bisulphite and then centrifuged for 20 minutes at 12000 RPM to separate membranes and proteins. Ten μl of the supernatant from these centrifuged homogenates were then injected into a high performance liquid chromatography instrument over a reverse-phase microbore column (Sepstik MF8949 100x1mm C-18, Bioanalytical Systems, IN) with a mobile phase of

0.1M chloroacetic acid, 0.1 mM sodium EDTA, 1.2 mM sodium octyl sulfate, and 5% acetonitrile buffer. Quantification of tissue DA content was accomplished by electrochemical detection on a glassy carbon electrode at +550 mV. The centrifuge pellet was analyzed for protein content using the Pierce Protein Assay kit (Pierce, Rockford IL). Striatal tissue DA content was then reported as *p*mol DA per mg protein. **Among the 6-OHDA treated rats, only those with dorsal striatal tissue DA content depleted by greater than 90% of the average dorsal DA content of the vehicle-treated rats were included in subsequent analysis for either experiment.**

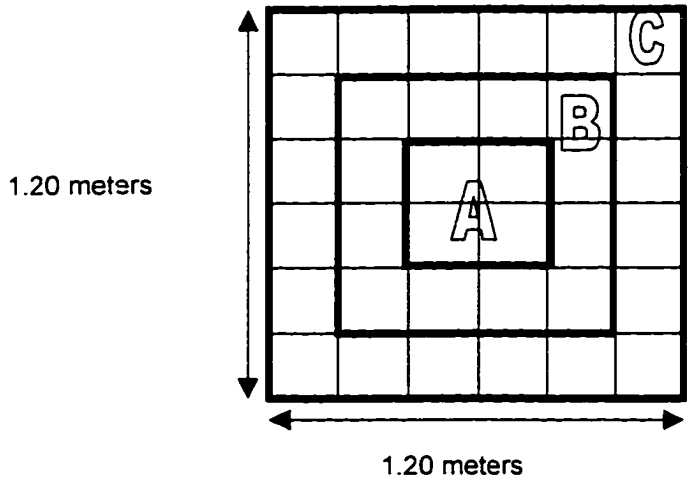

Figure 2.1

Open field zones shows a representation of the open field used in the sensorimotor battery. The zones referring to where the animal's "placement within the field" was recorded are shown. "**A**" represents the middle four squares where rat was initially placed, "**B**" represents the squares immediately surrounding A, and "**C**" represents the squares which border on the perimeter. These zones were chosen for their relationship to the intact animal's tendency for thigmotaxis, with little time spent in the center of the field.

CHAPTER 3

EXPERIMENT 1: SENSORIMOTOR EFFECTS OF INTRASTRIATAL SULPIRIDE

3.1 Introduction

This experiment addressed the following specific question: Does striatal DA transmission remain necessary for continued sensorimotor activity of rats depleted of DA as weanlings after their recovery from the initially expressed sensorimotor deficits? It has been demonstrated, as described in the General Introduction, that after recovery from the sensorimotor effects of DA depleting lesions administered during adulthood, rats continue to depend on striatal DA for their sensorimotor behavior. This has not been clearly demonstrated with rats after recovery from DA depleting lesions administered at the time of weaning. The fact that the nigrostriatal DA system may still be developing during this time (postnatal day 27) may lead to structural or neurochemical changes in the striata of weanling rats after DA depleting lesions that reduce the role of DA in their expressed sensorimotor behavior.

Part of my previous work involved a study I mentioned briefly in the General Introduction in which the sensorimotor dose-response to a mixed D1+D2 DA-receptor antagonist cis-flupentixol was tested in rats following DA-depletions at various ages. The rats in this study that were depleted of DA on postnatal day 27 were sensitive to the sensorimotor deficit-inducing effects of cis-flupentixol. More interestingly, the sensitivity of rats DA-depleted on day 27 to this DA antagonist was similar to that of the intact controls. This result suggests that these lesioned rats had similar levels of endogenous DA to compete with the antagonist, but the region where DA transmission continued to remain necessary for their sensorimotor behavior was unclear because the drug was administered systemically in this experiment.

The purpose of this study was to investigate this sensitivity of rats depleted of DA on postnatal day 27 to acute DA antagonists infused *locally* rather than systemically. Thus, the dependence of the rats on DA transmission *within the striatum* for the sensorimotor behavior expressed after recovery from DA depletion was tested. Also, this study used the selective D2-like DA receptor antagonist sulpiride rather than the mixed D1+D2 antagonist cis-flupentixol. Sulpiride was chosen because it was the most appropriate drug for Experiment 2. It was important to demonstrate that sensorimotor behavior could be disrupted after recovery from DA-depletion by blocking DA transmission with this drug in the striatum. As the previous study with cis-flupentixol demonstrated apparent normosensitivity to the sensorimotor effects of an acute systemic DA antagonist in rats depleted of DA as weanlings, it was informative to compare the sensitivity of DA-depleted rats with that of age-matched vehicle-treated controls to *striatal* DA antagonists to see if this similarity is maintained. To test this, weanling rats were depleted of DA, or treated with vehicle (as described above, Section 2.2), and then allowed to grow to adulthood. Then the sensorimotor effects of striatal administration of the DA antagonist sulpiride were tested according to the following procedures.

3.2 Procedures

During adulthood (postnatal day 60-80) a second intracranial surgery was performed on those rats that had been treated with 6-OHDA (n=8) or its vehicle (n=12) as weanlings (postnatal day 27). Bilateral infusion guide cannula (Plastics One, Roanoke VA) were stereotaxically fitted into the medial striata. All rats were anesthetized with ketamine and xylazine (85.0 mg/kg and 0.005 mg/kg, respectively) and fitted with these cannula in a stereotaxic apparatus. These bilateral cannulae were separated by a gap of 5.0 mm, and were lowered into the medial dorsal striatum at stereotaxic coordinates +0.7 mm anterior, and ±2.5 mm lateral from bregma, and -4.5mm ventral from the skull surface, and were secured to the skull with skull screws and dental cement (Duralay, Dental Mfg. Co. Worth IL). 6-OHDA treated rats recovered in the modified infant incubation chamber as these rats often had greater difficulty maintaining body temperature,

while sham-treated rats recovered in plastic tubs until they were transferred to their final housing (see above Section 2.1). After at least three days recovery from this surgery, testing began.

Rats were taken to the test room and allowed to sit in large 50 x 37 x 34 cm plastic bins for 30 minutes prior to the initiation of testing. Sensorimotor tests (details in Section 2.4) then began to determine the levels of *akinesia*, *catalepsy*, *somatosensory orientation*, and *open field activity* for each animal prior to administration of drug. Then each animal was infused with the first of three intrastriatally infused doses of the D2 DA antagonist sulpiride in volumes of 0.5 μl per hemisphere over 1.0 minute: 0 (vehicle isotonic NaCl, pH adjusted to 5.0 with hydrochloric acid), 3.0, or 10.0 μg per hemisphere. Each dose was tested in a within-subject design with dose order pseudo-randomized. Infusions were administered to awake rats by lowering and attaching the inner infusion cannula into the guide and placing them into a deep (50 x 37 x 34 cm) plastic tub during the infusion. Following infusion the rats remained attached to the apparatus for another minute before inner cannulae were removed and replaced by dummy cannulae. Then rats were individually placed into the deep plastic tubs for 15 minutes before the first sensorimotor test. Sensorimotor tests were repeated at 15, 30, and 60 minutes following drug infusion.

3.3 Statistical Analysis

Sensorimotor measures were each analyzed separately. The data from *akinesia*, *catalepsy*, and *open field* tests were continuous and parametric and were analyzed using a mixed two-way repeated measures ANOVA with condition (COND; vehicle, 6-OHDA) as the between-subjects, and DOSE (0, 3.0, and 10.0 μg) and TIME (baseline, 15, 30, and 60 min) as within-subject variables. Simple main effects and interactions with significance using an alpha of 0.05 were further examined using paired t-tests. An effort was made to minimize the number of planned comparisons and the Bonferroni method to correct alpha levels for repeated analysis was used to minimize the probability of type II errors (Keppel, 1991)

Von Frey plateau and force to plateau are non-parametric data. No statistical tests that I know of are available to analyze non-parametric data from mixed repeated measure and

between-subject designs (Gibbons, 1976). Therefore the analysis of these results were limited to either repeated measure testing of a single factor, or analysis of individual components selected from the repetition to explore effects of the between-subject factors. Because DOSE was the primary repeated measure of interest, Friedman tests were used to analyze each COND separately at two selected times: pre-drug baseline and 60 minutes following drug, because data from the other tests revealed maximal effects at 60 minutes. To address possible effects of condition, Kruskal-Wallis tests were used with COND as the grouping variable, and separate tests were performed at each of the three DOSES and at the same two TIMES (baseline, 60 minutes) analyzed above.

3.4 Results

The point of this experiment was to determine the necessity of striatal DA transmission for the sensorimotor behavior of rats that had recovered from DA depletions as weanlings. In this experiment striatal DA transmission was disrupted by infusions of sulpiride, so the primary result that would answer the question posed would be an effect of DOSE on the lesioned rats. Secondarily, one might expect that rats that had been depleted of striatal DA would be more sensitive to lower doses of sulpiride than their age-matched vehicle-treated controls. The effects of striatal sulpiride on each of the four sensorimotor measures will be discussed separately below, as some of the measures (akinesia and catalepsy) were more sensitive than others (somatosensory orientation and open field activity) in revealing these drug effects. The placements of intracerebral guide cannulae are shown in **Figure 3.1**. The average striatal DA content (± SEM) for rats in this experiment are shown in **Table 3.1**.

Figures 3.2 - 3.5 illustrate the effects of sulpiride on measures of akinesia taken at the various times prior to and following drug administration. There were significant main effects of DOSE, [$F(2,36) = 18.215$, $p < 0.001$], and [TIME $F(3,54) = 31.195$, $p < 0.001$], and TIME X DOSE interaction, [$F(6,108) = 6.937$, $p < 0.001$]. However there were no significant overall effects of lesion COND [$F(1,18) = 0.795$, $p = 0.384$], or COND X DOSE, [$F(2,36) = 0.591$, $p = 0.559$]. This

lack of a COND X DOSE effect of sulpiride on measures of akinesia indicates that there was no difference in sensitivity to the antagonist in the lesioned rats compared to the age-matched vehicle controls. The graphs shown in **Figures 3.2 - 3.5** demonstrate that the 30 and 60 minute tests following drug administration were similar, so the 30 minute time point was dropped from subsequent post hoc analyses. Because of the TIME X DOSE interaction, post-hoc comparisons were computed between select doses at select times, collapsing analysis across lesion condition. Five pairs were chosen for analysis: 0μg vs. 3.0μg at 15 min, 0μg vs. 10.0μg at 15 min, 0μg vs. 3.0μg at 60 min, 0μg vs. 10.0μg at 60 min, and 3.0μg vs. 10.0μg at 15 min. These tests were set against the Bonferroni corrected alpha of 0.01. Significant effects of both 3.0 and 10.0 μg were revealed only 15 minutes after the infusion [$t(0\mu g \text{ vs. } 3.0\mu g)_{19} = -2.864$, $p = 0.01$], [$t(0\mu g \text{ vs. } 10.0\mu g)_{19} = -3.714$, $p = 0.001$], and this significant effect remained 60 minutes after the infusion $t(0\mu g \text{ vs. } 3.0\mu g)_{19} = -2.991$, $p = 0.008$], [$t(0\mu g \text{ vs. } 10.0\mu g)_{19} = -6.251$, $p < 0.001$]. The 3.0μg and 10.0μg doses were not significantly different from each other at 15 minutes according to the corrected alpha [$t(3.0\mu g \text{ vs. } 10.0\mu g)_{19} = -2.570$, $p = 0.019$], but there was certainly a strong trend towards a difference here.

Figures 3.6 – 3.9 illustrate the effects of sulpiride on measures of catalepsy. There were significant main effects of DOSE, [$F(2,36) = 10.54$, $p < 0.001$], of TIME, [$F(3,54) = 17.222$, $p < 0.001$], and TIME X DOSE interaction, [$F(6,108) = 7.242$, $p < 0.001$]. Once again, there was no significant effect of lesion COND, [$F(1,18) = 2.098$, $p = 0.165$], nor was there a COND X DOSE interaction, [$F(2,36) = 0.215$, $p = 0.807$]. This lack of a COND X DOSE interaction indicates that there was no difference in sensitivity to the antagonist in the lesioned rats compared with the age-matched vehicle controls. The striatal sulpiride-induced catalepsy also seemed to be similar between 30 and 60 minutes following drug administration, so the 30 minute time point was again dropped from subsequent post hoc analyses. Because of the TIME X DOSE interaction, post-hoc comparisons were computed for the same five pairs as explored in akinesia, collapsing analysis across lesion condition (Bonferroni alpha = 0.01). These comparisons revealed significant effects of both 3.0μg and 10μg sulpiride at 60 minutes following infusion, [$t(0\mu g \text{ vs. } 3.0\mu g)_{19} = -3.837$, $p = 0.001$], [$t(0\mu g \text{ vs. } 10.0\mu g)_{19} = -4.178$, $p = 0.001$]. Only

10.0μg sulpiride reached significance by 15 minutes, [$t(0\mu g$ vs. $10.0\mu g)_{19} = -3.313$, $p = 0.004$], while 3.0μg did not reach significance at this time according to the Bonferroni alpha, [$t(0\mu g$ vs. $3.0\mu g)_{19} = -1.939$, $p = 0.067$]. Also similar to the akinesia results, the 3.0μg and 10.0μg doses were not significantly different from each other at 15 minutes according to the corrected alpha [$t(3.0\mu g$ vs. $10.0\mu g)_{19} = -2.570$, $p = 0.022$], but there was a strong trend towards a difference here.

Open field activity scores are illustrated in **Figures 3.10 – 3.13**. In this measure, sulpiride was expected to decrease line crossings and therefore decrease the scores after drug infusion compared with those measured before. There was *no* main effect of DOSE obtained from this measure, [$F(2,36) = 2.005$, $p = 0.149$]. The main effect of TIME was significant, [$F(3,54) = 58.193$, $p < 0.001$]. There was a trend towards a TIME X DOSE interaction, [$F(6,108) = 2.119$, $p = 0.057$], suggesting that when the two conditions are collapsed, whatever significance of TIME X DOSE that may be present in either condition separately is lost. Finally, there was a significant 3-way interaction of COND X TIME X DOSE, that suggested that there may be either DOSE or TIME effects that were unique to either lesion condition. To explore this possibility, two-way repeated measure ANOVAs were performed for each condition separately, analyzing for the within subject variables DOSE and TIME. Within vehicle condition there were DOSE, [$F(2,22) = 4.917$, $p = 0.017$], and TIME, [$F(3,33) = 55.434$, $p < 0.001$] simple main effects, as well as a TIME X DOSE interaction, [$F(6,66) = 2.432$, $p = 0.035$]. However within the DA-depleted condition, there was *no* DOSE effect, [$F(2,14) = 0.046$, $p = 0.956$], and only TIME, [$F(3,21) = 15.665$, $p < 0.001$], and the TIME X DOSE interaction, [$F(6,42) = 2.336$, $p = 0.049$] were significant. Post hoc analysis was restricted to the vehicle condition with this measure as this was the only condition that had a DOSE effect. The same five pairs were analyzed for this measure as with akinesia and catalepsy (Bonferroni alpha = 0.01). Significant effects of 3.0μg sulpiride were revealed at 60 minutes, [$t(0\mu g$ vs. $3.0\mu g)_{11} = 3.715$, $p = 0.003$]. There was a strong trend for an effect of 10.0μg sulpiride at this time, [$t(0\mu g$ vs. $10.0\mu g$, $11)_{11} = 2.715$, $p = 0.02$], but this did not reach the significance required by the stringent Bonferroni corrected alpha. See conclusions for further elaboration.

Figures 3.14 and 3.15 illustrate the effects of intrastriatal sulpiride on somatosensory orientation. To summarize these effects, the pre-drug and 60 minutes post-drug scores are shown for the vehicle (0 μg) and 10 μg doses for both Von Frey **plateau** and **force to plateau**. There were no obvious effects of DOSE or lesion (COND) revealed from these tests. Friedman tests for DOSE effects were performed separately for the vehicle and 6-OHDA treated rats at the two times mentioned in Section 3.3 (baseline and 60 minutes), and none of these tests reached significance (all p's > 0.09). The individual Kruskal-Wallis tests of COND did reveal some significant differences. The significant effects of COND for Von Frey **plateau** were obtained at the baseline before 0 μg, [Chi(baseline, 0 μg)$_1$ = 5.0, p = 0.025], at 60 minutes following 0 μg, [Chi(60 min, 0 μg)$_1$ = 5.029, p = 0.025], and 60 minutes following 10.0 μg [Chi(60 min, 10.0 μg)$_1$ = 5.727, p = 0.017]. The significant effects of COND for Von Frey **force to plateau** were obtained at the baseline before 0 μg, [Chi(baseline, 0 μg)$_1$ = 3.872, p = 0.049], and at the baseline before 3.0 μg, [Chi(baseline, 3.0 μg)$_1$ = 4.012, p = 0.045]. None of the other Kruskal-Wallis tests of COND were significant (all p's > 0.07).

3.5 Conclusions from Experiment 1

The goal of this experiment was to determine the necessity of striatal DA transmission in rats after recovery from DA depletions sustained while they were weanlings. At least two of the sensorimotor measures (akinesia and catalepsy) provided insights into residual sensitivity to DA antagonists in this experiment. These results suggest that even long after DA depleting lesions, stimulation of striatal DA receptors remains necessary for the sensorimotor behavior expressed by these rats. Further, it can be posited that stimulation of D2-like DA receptors remains necessary for the sensorimotor behavior expressed by rats after DA-depleting lesions, as sulpiride is a selective D2-like receptor antagonist. These results extend the results of the previous study with cis-flupentixol (Sandstrom et al., 1997), suggesting that striatal DA receptors may have been involved in the sensorimotor effects obtained in that experiment.

Another interesting result of this experiment was the apparent normosensitivity of rats depleted of DA as weanlings to DA antagonists during adulthood. This normosensitivity is supported by the tests which revealed DOSE effects (akinesia and catalepsy), as neither of them revealed COND X DOSE interactions. Interpretation of these effects require caution, or course, as only two doses were used resulting in a small dose-response range. Differences in sensitivity may have been present at doses lower than those used in this experiment. However the apparent normosensitivity of lesioned rats obtained with these results are similar to the sensitivities expressed by rats which were lesioned at the same age in the previous study using cis-flupentixol (Sandstrom et al., 1997). In that study, a different dose range was used with systemic administration. Behavioral supersensitivity is highly dependent upon the dose range used in the study, as it may be that higher doses result in ceiling effects, leaving little room to reveal increased sensitivity in the suspect condition. Therefore, if a dose is used which does demonstrate supersensitivity in one population of subjects but not in another, the lack of supersensitivity in the unaffected group would be a more reliable finding. In the cis-flupentixol study, supersensitivity to the akinesia-inducing effects of the lower dose (0.0625 mg/kg) was demonstrated in adult rats that were depleted of DA on postnatal day 35. Therefore, the response of rats lesioned on day 35 to this dose represents a positive control for supersensitivity: the dose of cis-flupentixol where the lesioned rats exhibited significant akinesia while the age-matched vehicle-treated controls did not. In this same study, adult rats that had been DA-depleted on postnatal day 27 exhibited similar akinesia to the age-matched vehicle-treated controls following 0.0625 mg/kg cis-flupentixol. With the positive control population in this study, the lack of supersensitivity found in rats DA depleted on postnatal day 27 seems more reliable. The supersensitivity expressed by rats depleted of DA on day 35 to systemic DA antagonists is likely a result of the same underlying pharmacology exhibited by rats depleted of DA as adults in response to acute DA blockade (Heffner et al., 1977).

Somatosensory orientation tests did not reveal a dose effect, nor were there any interesting differences in effects between conditions. Therefore the rats of either condition were insensitive to striatal sulpiride as measured by this test, and it was uninformative. Problems with

the orientation data came, in part, from the fact that no mixed designs are available for statistical analysis of non-parametric data, and tests had to divide data taken from a repeated measures design, reducing power. Therefore there was a decreased probability of rejecting the null hypothesis (DA is not necessary for sensorimotor behavior after recovery from DA depletions administered to weanling rats) even though it may be false (Gibbons, 1976). Also, the expressed range of **plateau** values was reduced in this study from 4 to 3, as maximal deficits in orientation reached plateau at a 3-type response at higher pressures, despite the expression of some 4-type responses at lower pressures. Therefore, the range of plateaus may not have been great enough, suggesting that the rats may have required higher sulpiride doses to induce greater degrees of neglect by comparison to the vehicle dose.

Sensitivity of lesioned rats failed to differ from that of the age-matched vehicle controls in all but one measure, open field activity, and this was only revealed after deeper scrutiny of the data. Open field activity did seem to reveal a difference between vehicle controls and lesioned rats, with a DOSE effect only significant in the vehicle rats. Yet those results themselves revealed a significant effect of the 3.0μg dose at 60 minutes following infusion, but only a trend at the 10.0μg dose. It is my contention that the nature of the time-sampling, coupled with low basal activity, left little room for the resolution of drug effects. It may have been that some residual deficits in exploratory behavior contributed to reductions in the basal activity of the lesioned rats, but this would require further study. There is some indication that these rats rapidly habituated to the open field environment, reducing the likelihood of exploratory activity. This can be seen by comparing the effects of the vehicle (0μg) dose seen in **Figure 3.10** with that seen in **Figure 3.11**. Given this reduced basal behavior, some sort of activating stimuli or conditions may have been useful, such as testing during the nocturnal phase. Under these conditions, there might have been more resolving power due to the higher basal activity, and greater sulpiride-induced decrements might be expected. These results illustrate the value of multiple sensorimotor tests to reveal the effects of the striatal sulpiride rather than relying on a single measure. If only akinesia or catalepsy were used, there would be no question that both rats depleted of DA and vehicle controls were equally sensitive to striatal sulpiride (given the doses used). Using only

open field activity to measure the effects of local sulpiride may well have led to a different conclusion.

Many possibilities might be suggested to account for the apparent normosensitivity expressed by rats that were depleted of DA as weanlings to the DA antagonist used in this experiment. Among these, one particularly intriguing possibility is that the DA released from residual nigrostriatal dopaminergic terminals results in a close approximation of the levels of extracellular DA present in the age-matched vehicle controls. These conditions would provide the basis for a comparable degree of competition between striatal DA and the antagonist in lesioned and vehicle rats, and lead to the results obtained in this experiment. This possibility, coupled with the observed differences in the rate of sensorimotor recovery following depletion of DA as weanlings versus as adults ((Weihmuller et al., 1989), and this dissertation), suggests that the striatal DA transmission supporting sensorimotor behavior expressed after rats are lesioned as weanlings begins to modulate striatal target cell activity more rapidly and efficaciously than following similar lesions of adult rats. The next experiment was designed to explore this possibility.

STRIATAL TISSUE DOPAMINE DEPLETION

Condition	DORSAL DA	VENTRAL DA	% DEPLETION	
VEHICLES	36.83 ±3.77	40.39 ±4.02	DORSAL	VENTRAL
6-OHDA	0.66 ±0.22	5.31 ±1.99	-98.21±0.61	-86.86±4.94

Table 3.1

Tissue DA content (± SEM) expressed as (pmol DA) / (mg protein) as described in text (Section 2.5). Values given for dorsal and ventral micropunches, and the percent DA depletions calculated from the dorsal and ventral micropunches and compared against the average DA content of vehicle controls.

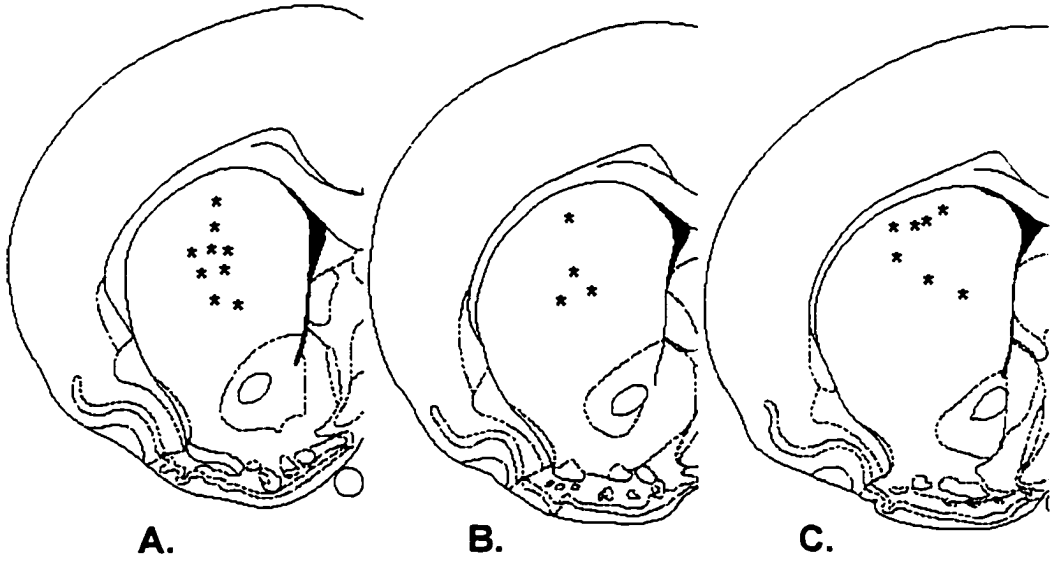

Figure 3.1

Placements of intrastriatal infusion cannulae in all rats from Experiment 1. Frontal sections, taken from (Paxinos and Watson, 1997 CDRom), represent the following coordinates in mm anterior to Bregma: A. 1.00 mm, B. 0.70 mm, C. 0.48 mm.

Figure 3.2 Akinesia at baseline.

Figure 3.2 depicts the mean (±S.E.M.) basal duration of akinesia (sec.) exhibited by all rats included in Experiment 1. This measure was taken prior to infusion of sulpiride as described in Section 3.2. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg, but only to indicate the distinction between the basal akinesia expressed prior to each dose as no drug had been infused at this time. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

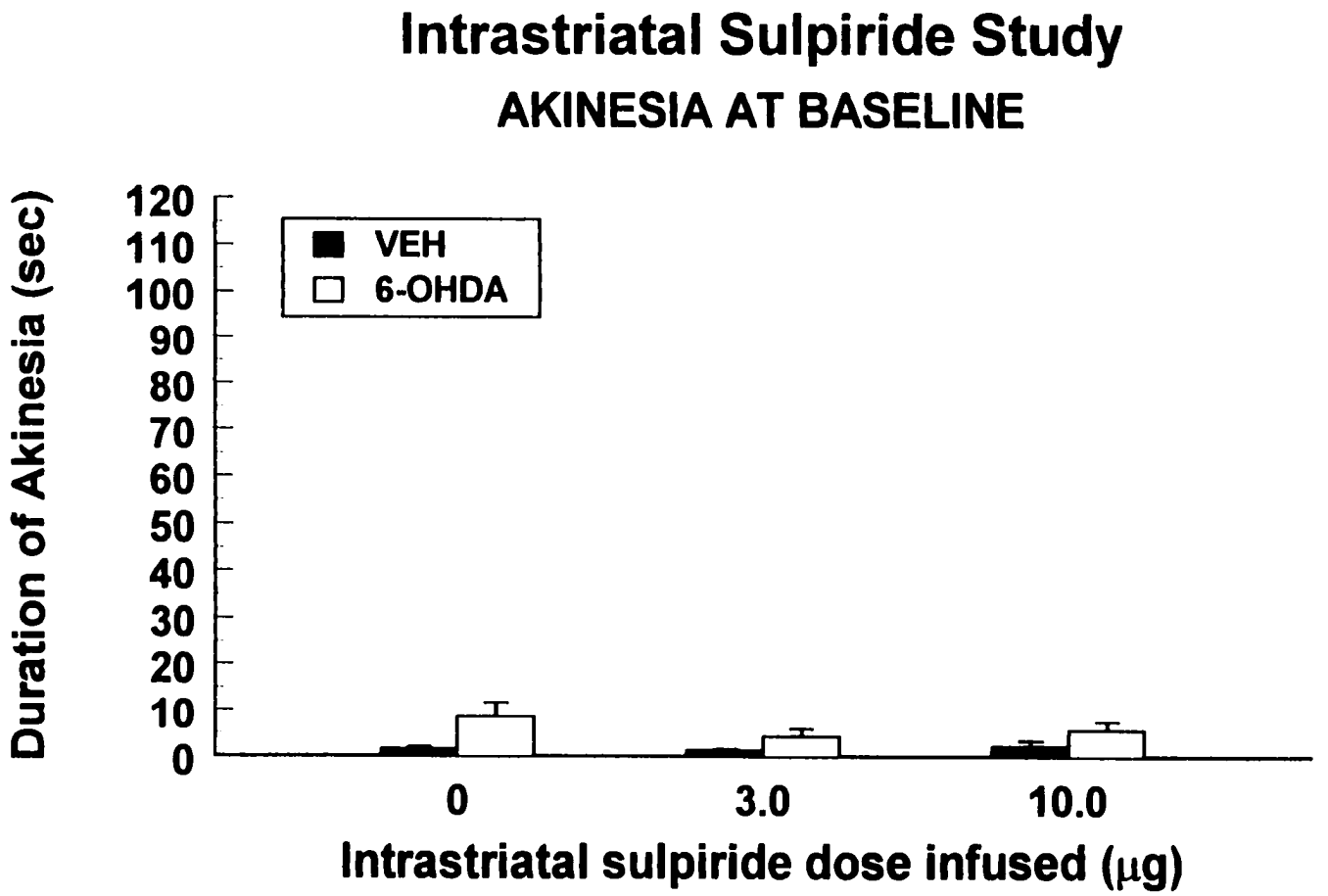
Intrastriatal Sulpiride Study
AKINESIA AT BASELINE
Duration of Akinesia (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.2

Figure 3.3: Akinesia at 15 minutes following sulpiride infusion.

Figure 3.3 depicts the mean (±S.E.M.) duration of akinesia (sec.) at 15 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

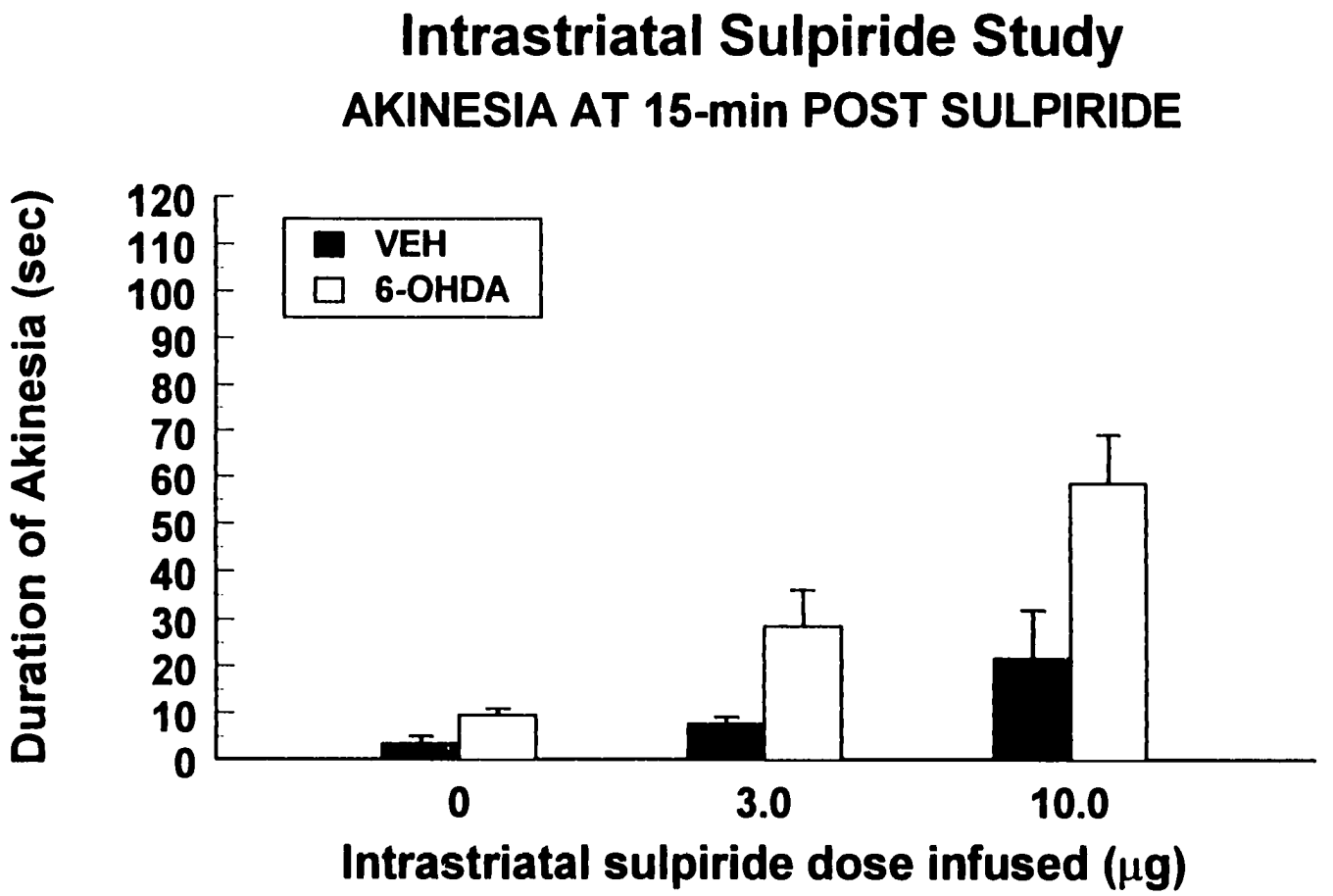
Intrastriatal Sulpiride Study
AKINESIA AT 15-min POST SULPIRIDE
Duration of Akinesia (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.3

Figure 3.4: Akinesia at 30 minutes following sulpiride infusion.

Figure 3.4 depicts the mean (±S.E.M.) duration of akinesia (sec.) at 30 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

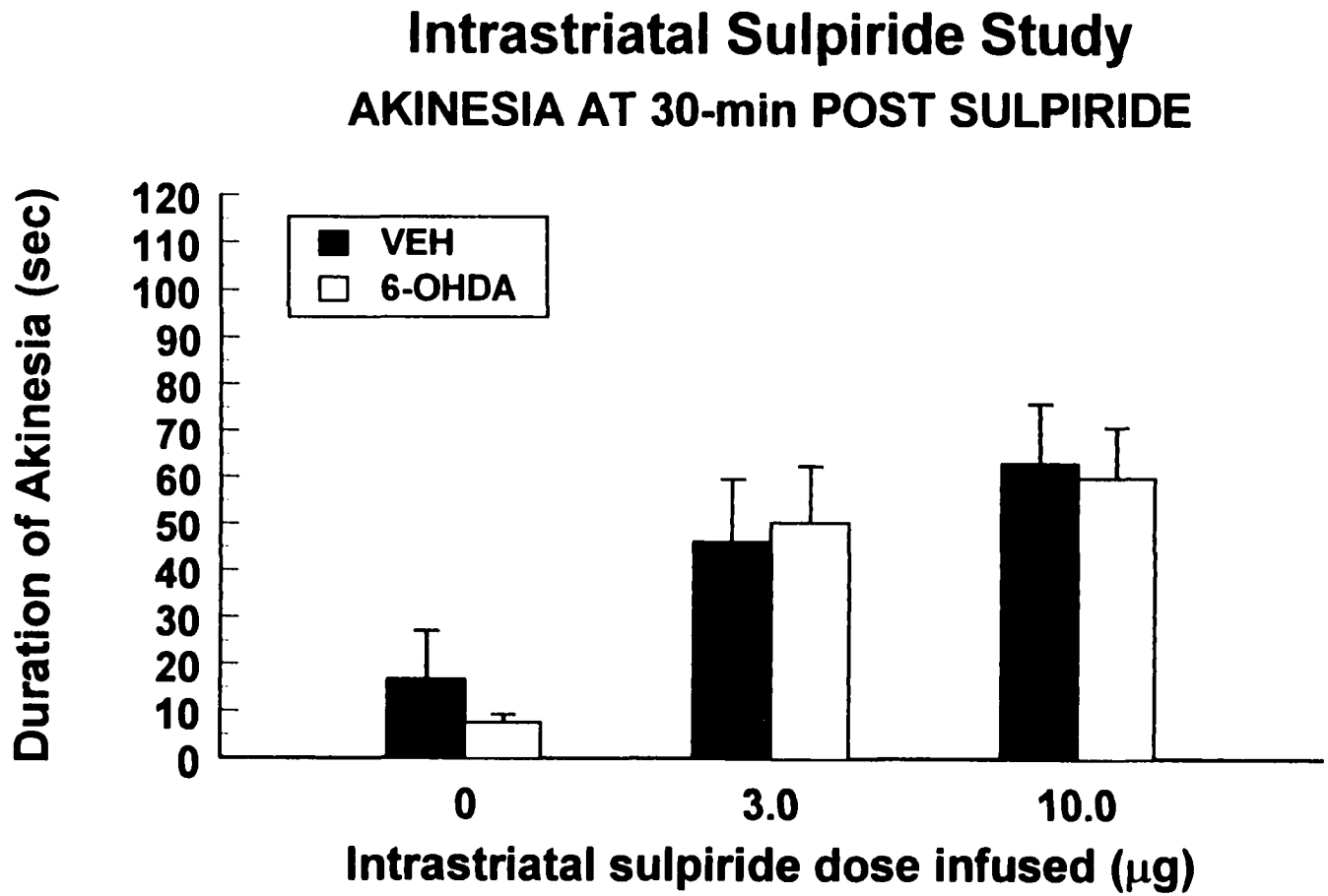
Intrastriatal Sulpiride Study
AKINESIA AT 30-min POST SULPIRIDE
Duration of Akinesia (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.4

Figure 3.5: Akinesia at 60 minutes following sulpiride infusion.

Figure 3.5 depicts the mean (±S.E.M.) duration of akinesia (sec.) at 60 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

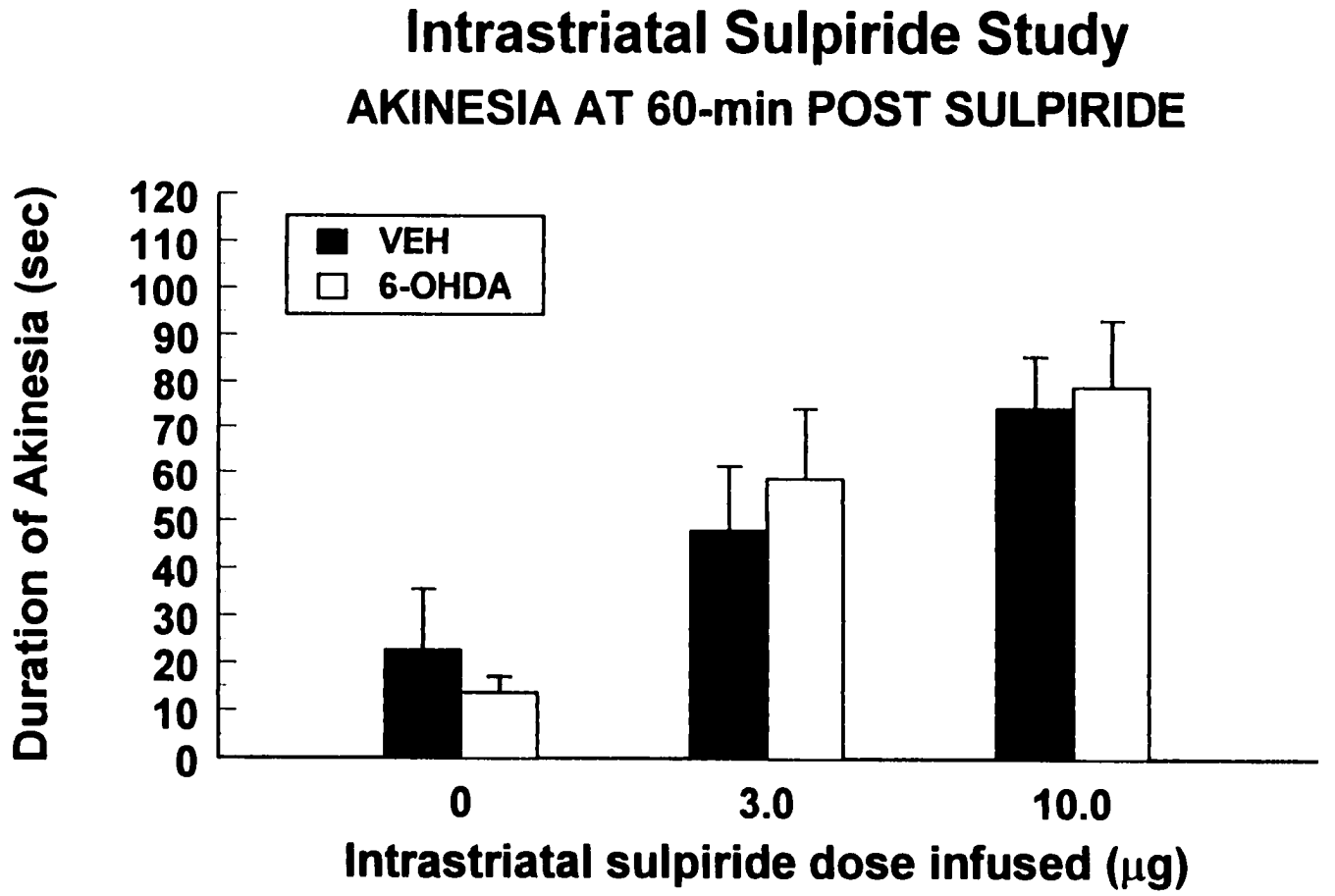
Intrastriatal Sulpiride Study
AKINESIA AT 60-min POST SULPIRIDE
Duration of Akinesia (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.5

Figure 3.6: Catalepsy at baseline.

Figure 3.6 depicts the mean (±S.E.M.) basal duration of catalepsy (sec.) exhibited by all rats included in Experiment 1. This measure was taken prior to infusion of sulpiride as described in Section 3.2. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere, but only to indicate the distinction between the basal catalepsy expressed prior to each dose as no drug had been infused at this time. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

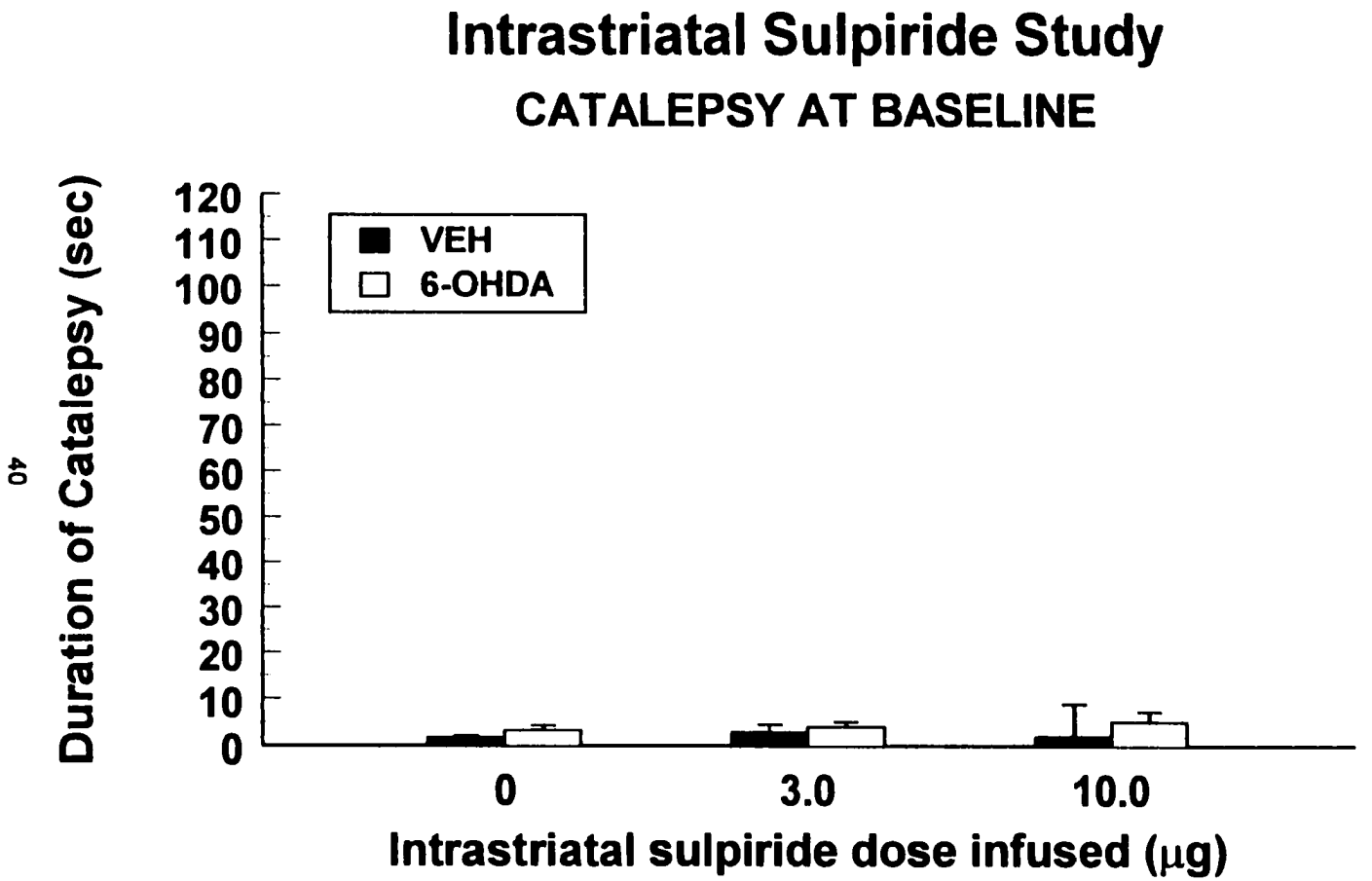
Intrastriatal Sulpiride Study
CATALEPSY AT BASELINE
Duration of Catalepsy (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.6

Figure 3.7: Catalepsy at 15 minutes following sulpiride infusion.

Figure 3.7 depicts the mean (±S.E.M.) duration of catalepsy (sec.) at 15 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

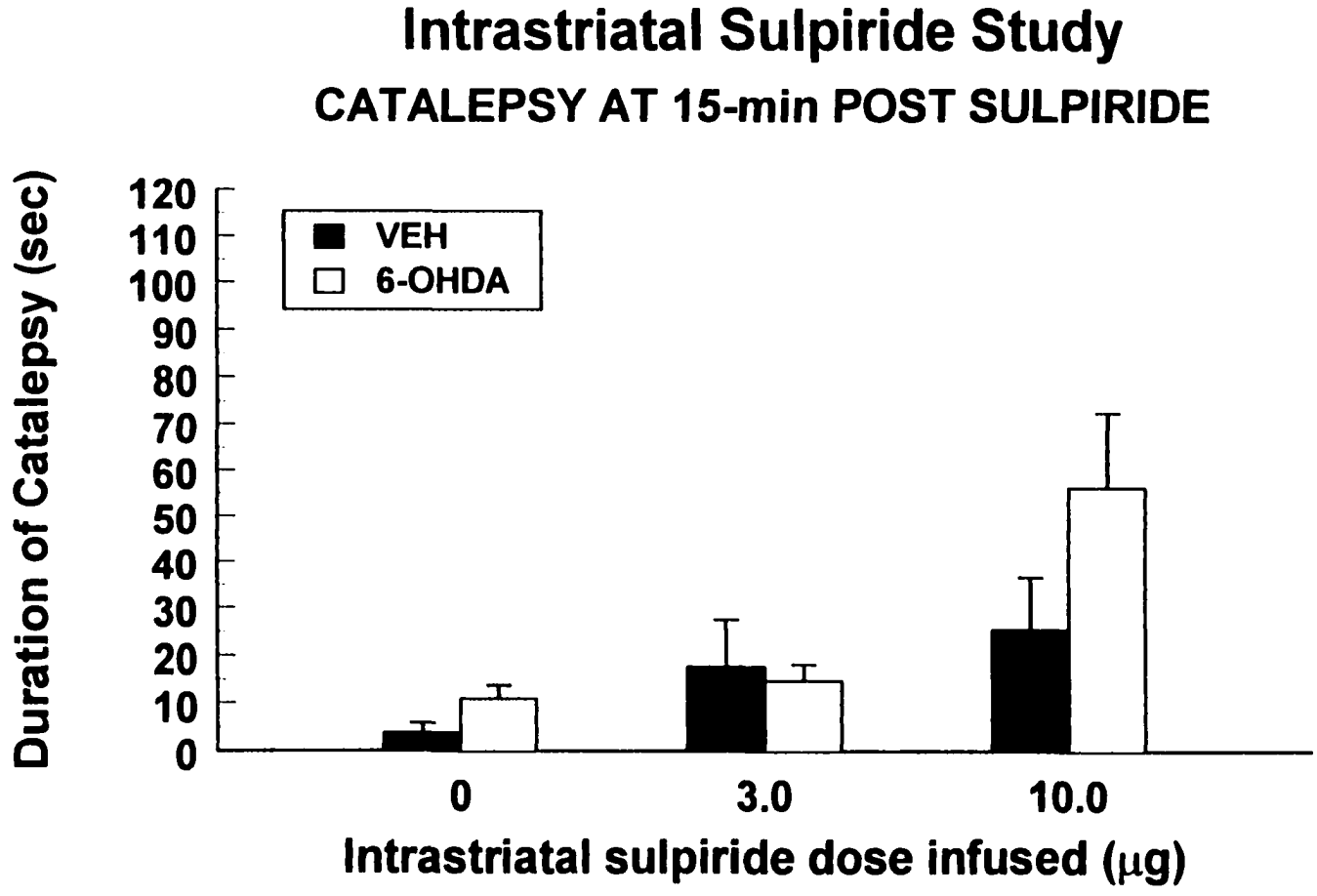
Intrastriatal Sulpiride Study
CATALEPSY AT 15-min POST SULPIRIDE
Duration of Catalepsy (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.7

Figure 3.8: Catalepsy at 30 minutes following sulpiride infusion.

Figure 3.8 depicts the mean (±S.E.M.) duration of catalepsy (sec.) at 30 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

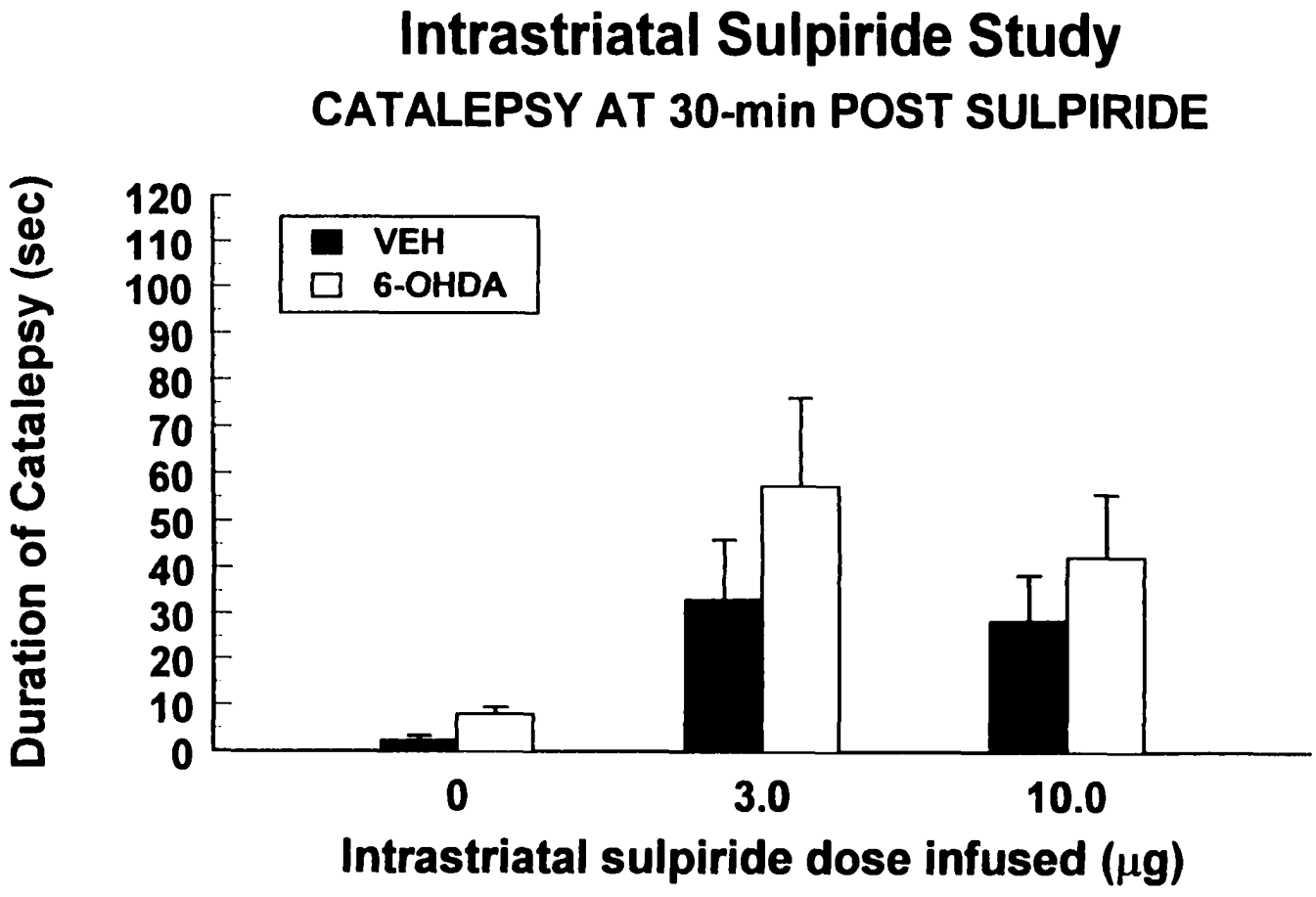
Intrastriatal Sulpiride Study
CATALEPSY AT 30-min POST SULPIRIDE
Duration of Catalepsy (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.8

Figure 3.9: Catalepsy at 60 minutes following sulpiride infusion.

Figure 3.9 depicts the mean (±S.E.M.) duration of catalepsy (sec.) at 60 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

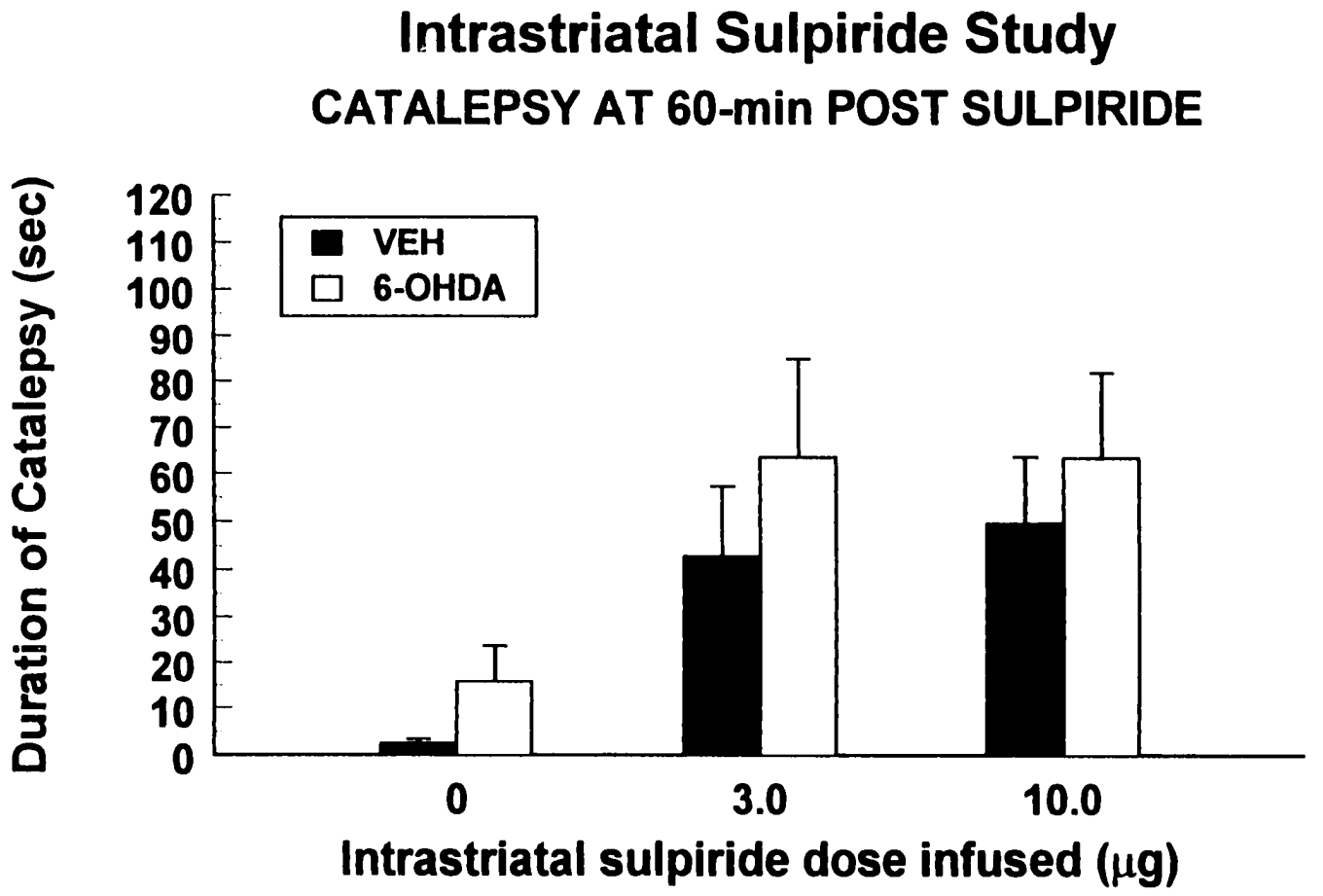
Intrastriatal Sulpiride Study
CATALEPSY AT 60-min POST SULPIRIDE
Duration of Catalepsy (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.9

Figure 3.10: Open field activity at baseline.

Figure 3.10 depicts the mean (±S.E.M.) basal open field activity (lines crossed) exhibited in Experiment 1. This measure was taken prior to infusion of sulpiride as described in Section 3.2. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere, but only to indicate the distinction between the basal open field activity expressed prior to each dose, as no drug had been infused at this time. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

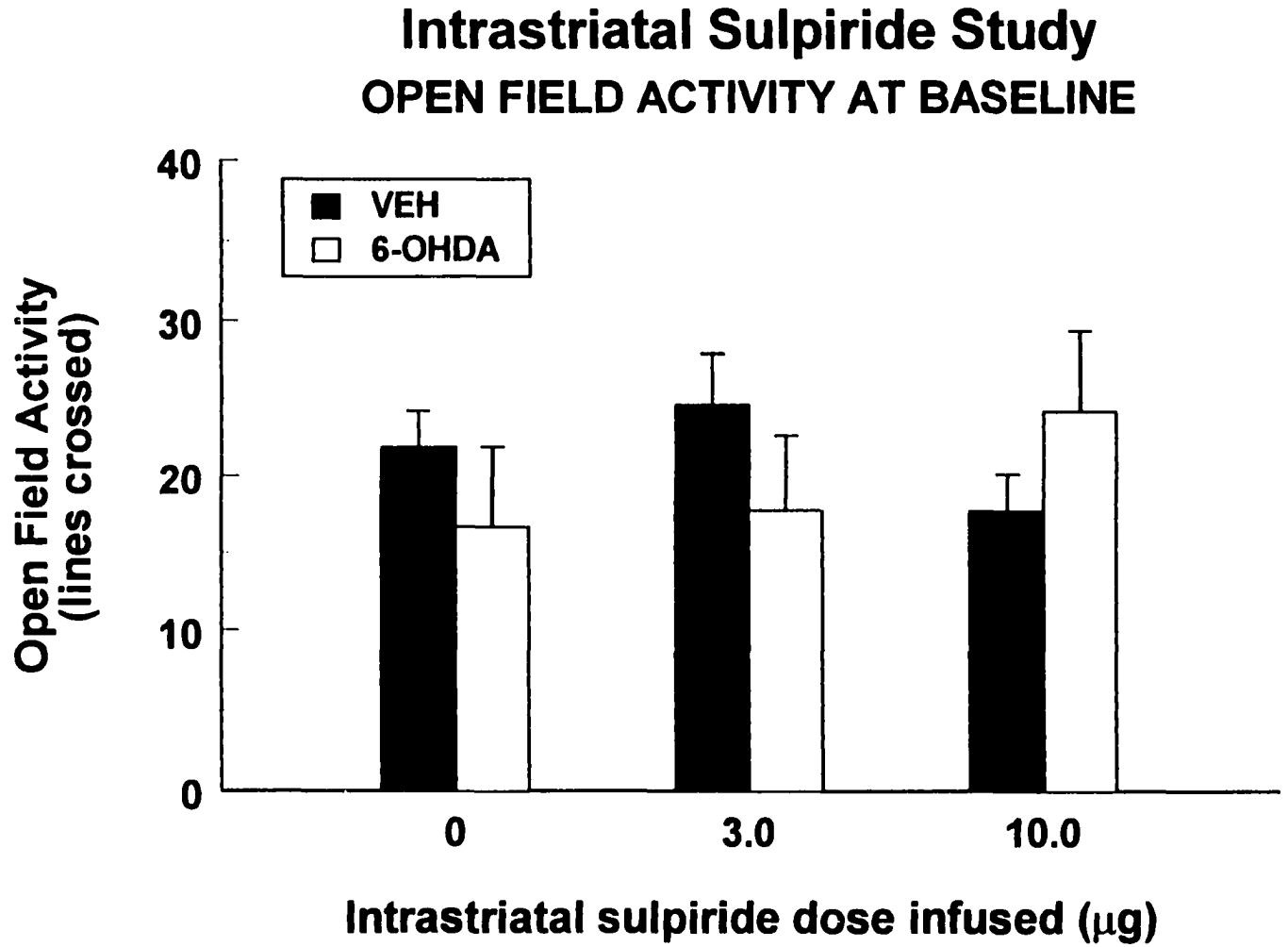
Intrastriatal Sulpiride Study
OPEN FIELD ACTIVITY AT BASELINE
VEH
6-OHDA
Open Field Activity
(lines crossed)
40
30
20
10
0
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.10

Figure 3.11: Open field activity at 15 minutes following sulpiride infusion.

Figure 3.11 depicts the mean (±S.E.M.) open field activity (lines crossed) at 15 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

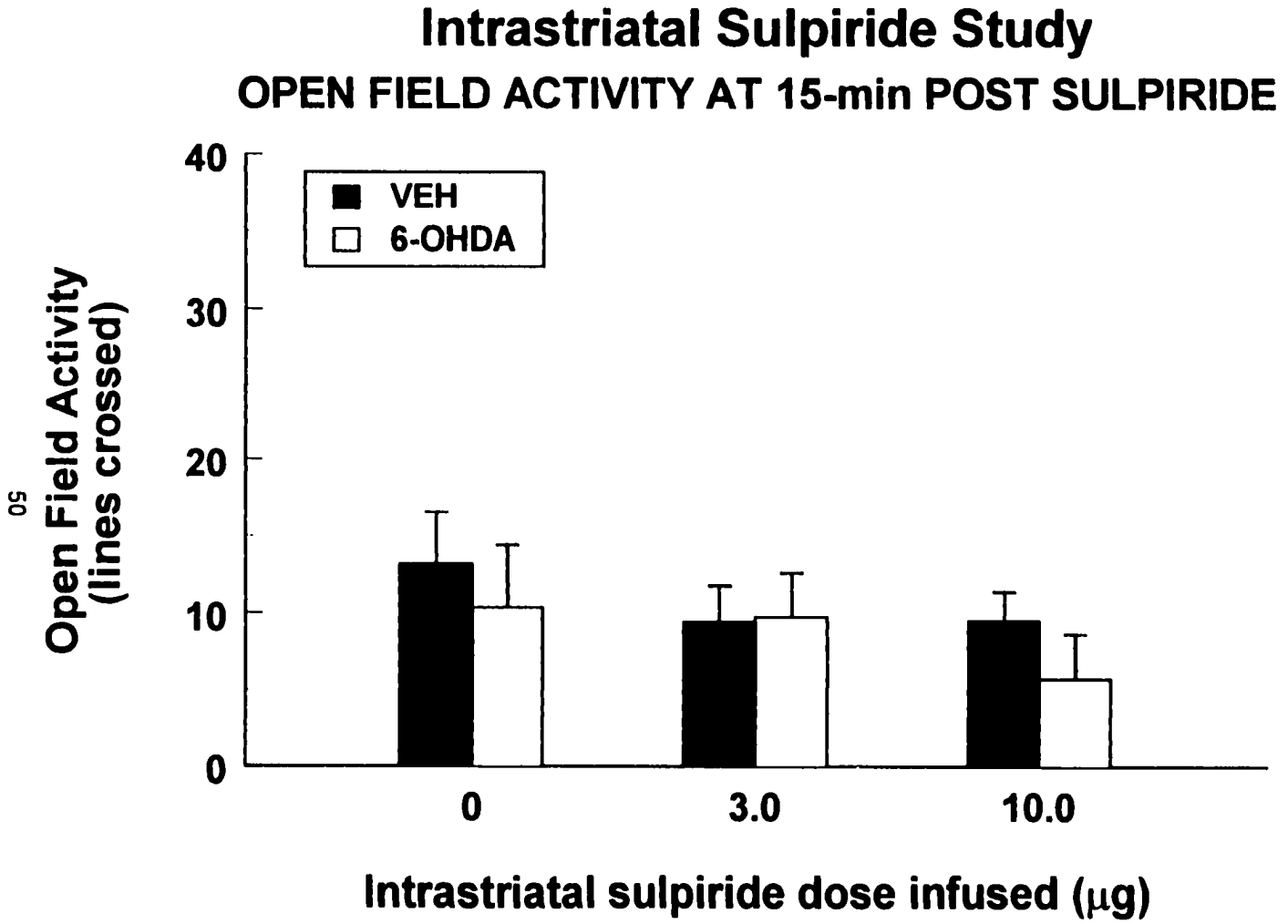
Intrastriatal Sulpiride Study
OPEN FIELD ACTIVITY AT 15-min POST SULPIRIDE
VEH
6-OHDA
Open Field Activity
(lines crossed)
50
40
30
20
10
0
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.11

Figure 3.12: Open field activity at 30 minutes following sulpiride infusion.

Figure 3.12 depicts the mean (±S.E.M.) open field activity (lines crossed) at 30 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

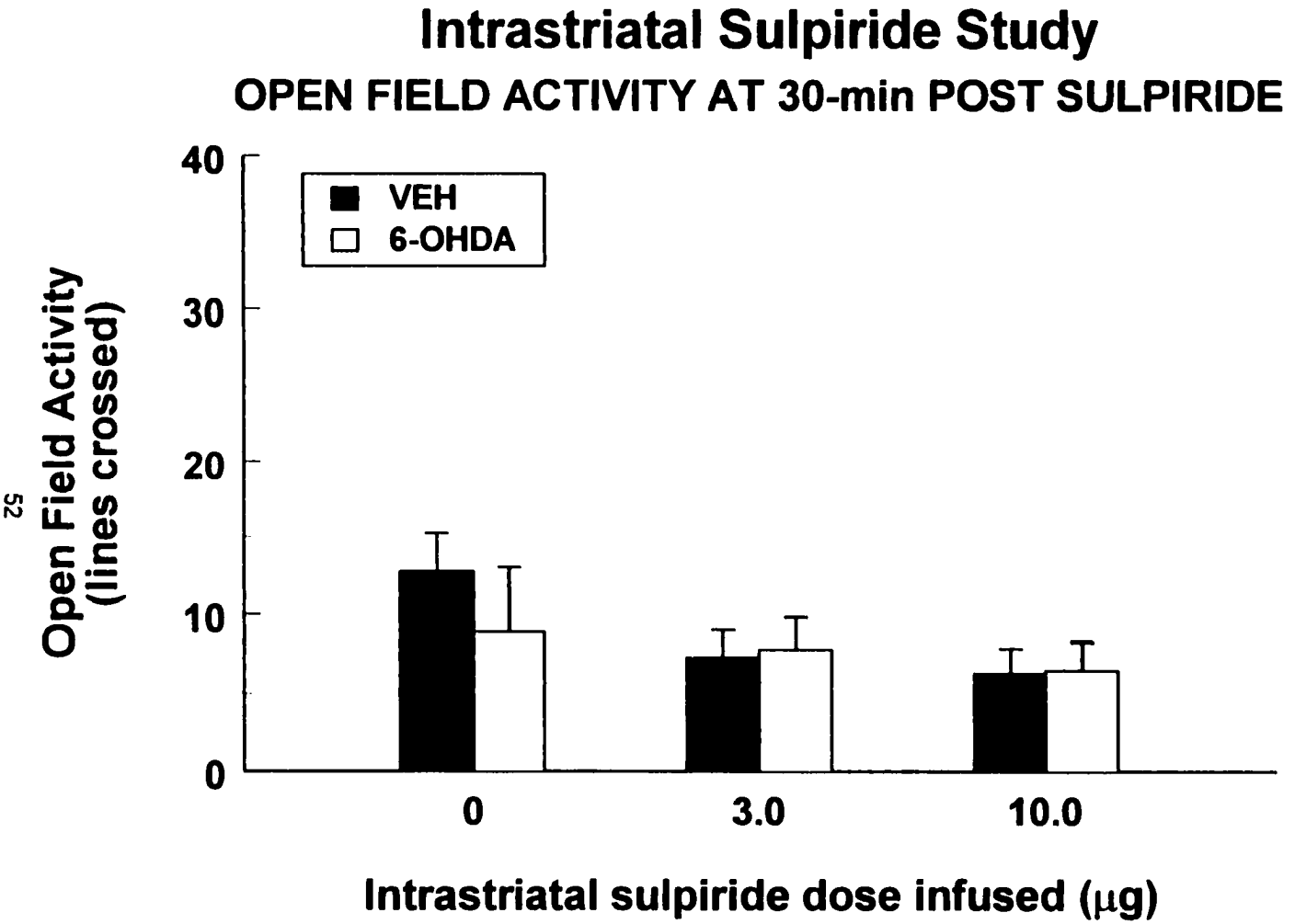
Intrastriatal Sulpiride Study
OPEN FIELD ACTIVITY AT 30-min POST SULPIRIDE
VEH
6-OHDA
Open Field Activity
(lines crossed)
40
30
20
10
0
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.12

Figure 3.13: Open field activity at 60 minutes following sulpiride infusion.

Figure 3.13 depicts the mean (±S.E.M.) open field activity (lines crossed) at 60 minutes following the sulpiride infusion in Experiment 1. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). Doses depicted on the abscissa include 0 μg (vehicle saline pH = 5.0), 3.0 μg, and 10.0 μg per hemisphere. See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

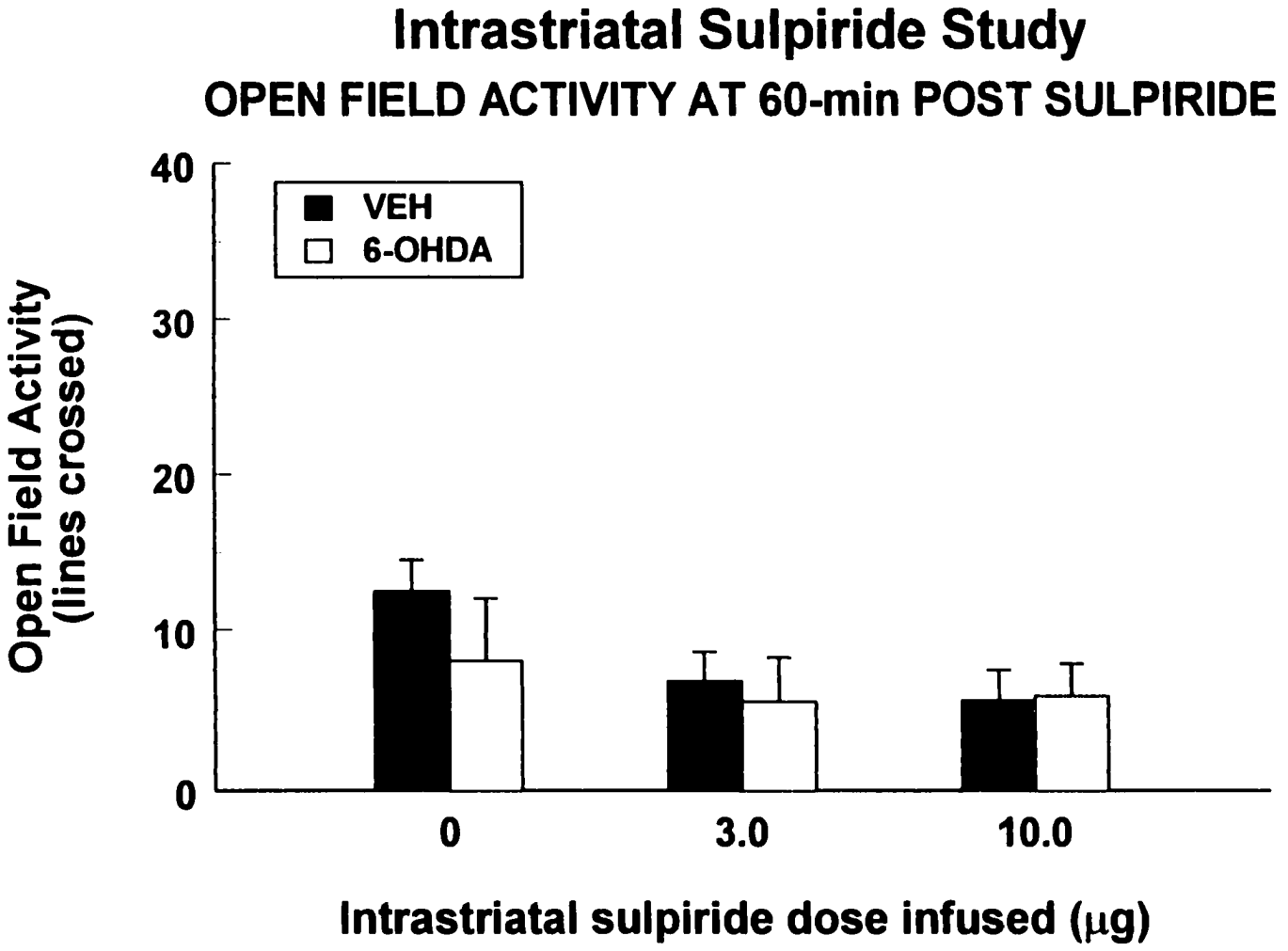
Intrastriatal Sulpiride Study
OPEN FIELD ACTIVITY AT 60-min POST SULPIRIDE
Open Field Activity (lines crossed)
40
30
20
10
0
VEH
6-OHDA
0
3.0
10.0
Intrastriatal sulpiride dose infused (μg)

Figure 3.13

Figure 3.14: Somatosensory Orientation plateau response type.

Figure 3.14 depicts the mean (± S.E.M.) plateau somatosensory response type (1-4), described in Section 3.2. These responses were obtained at forces of stimulation shown in Figure 3.15. In an effort to summarize the large amount of somatosensory data, specific times and doses were selected where maximal responses were exhibited in the other dependent measures. The abscissa presents both sulpiride dose {**0** μg (vehicle saline pH = 5.0), and **10.0** μg}, and time {before (**pre**) and 60 minutes following (**post**)} in relation to the infusion. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

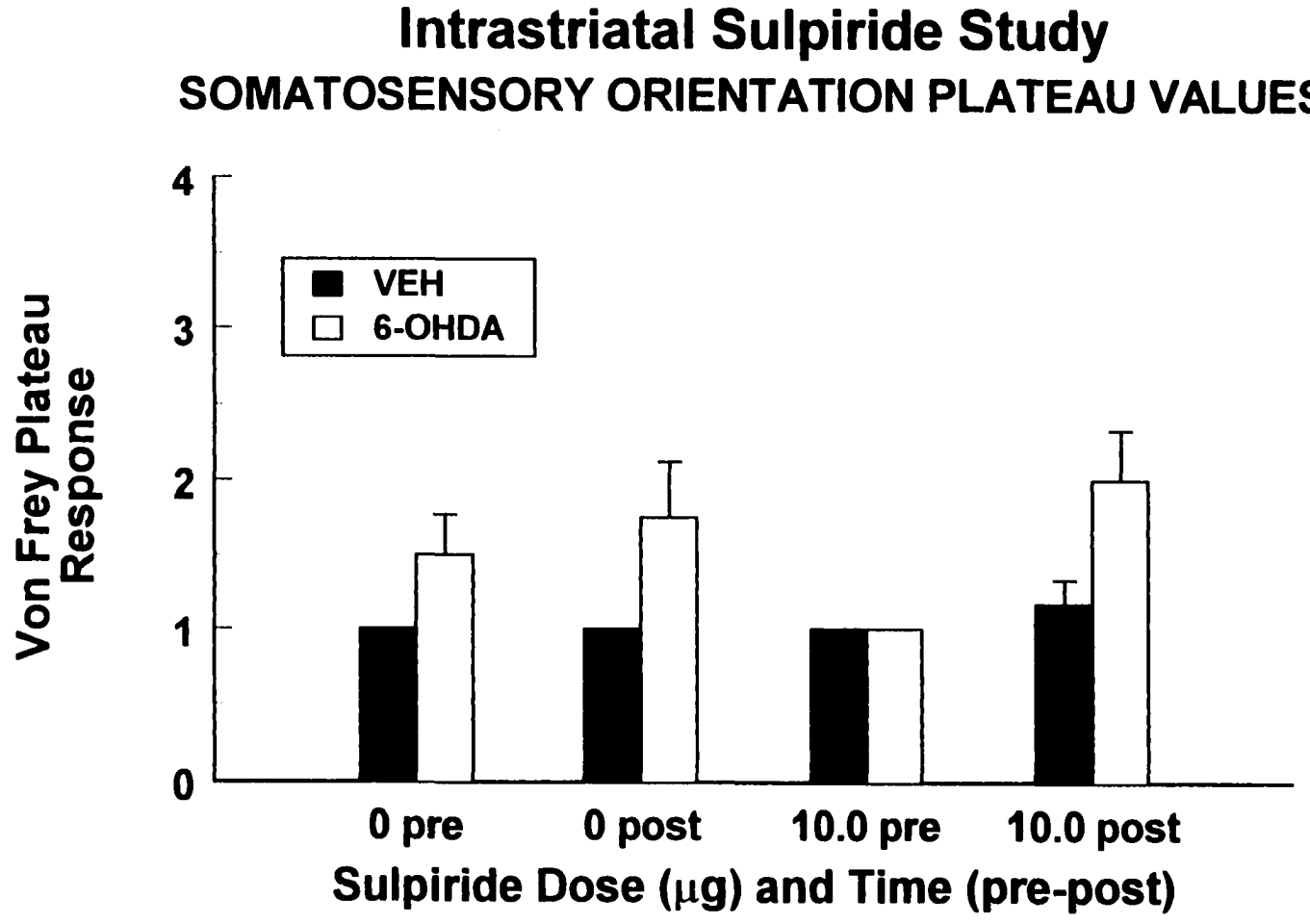
Intrastriatal Sulpiride Study
SOMATOSENSORY ORIENTATION PLATEAU VALUES
Von Frey Plateau Response
4
3
2
1
0
VEH
6-OHDA
0 pre
0 post
10.0 pre
10.0 post
Sulpiride Dose (μg) and Time (pre-post)

Figure 3.14

Figure 3.15: Somatosensory Orientation force to plateau.

Figure 3.15 depicts the mean (± S.E.M.) force (grams) of stimulation necessary to reach the plateau response types depicted in Figure 3.14. In an effort to summarize the large amount of somatosensory data, specific times and doses were selected where maximal responses were exhibited in the other dependent measures. The abscissa presents both sulpiride dose {**0** μg (vehicle saline pH = 5.0), and **10.0** μg}, and time {before (**pre**) and 60 minutes following (**post**)} in relation to the infusion. The open bars represent the 6-OHDA-treated rats (n=8), and the closed bars represent the vehicle-treated controls (n=12). See Table 3.1 for striatal tissue DA content and percent DA-depletion information.

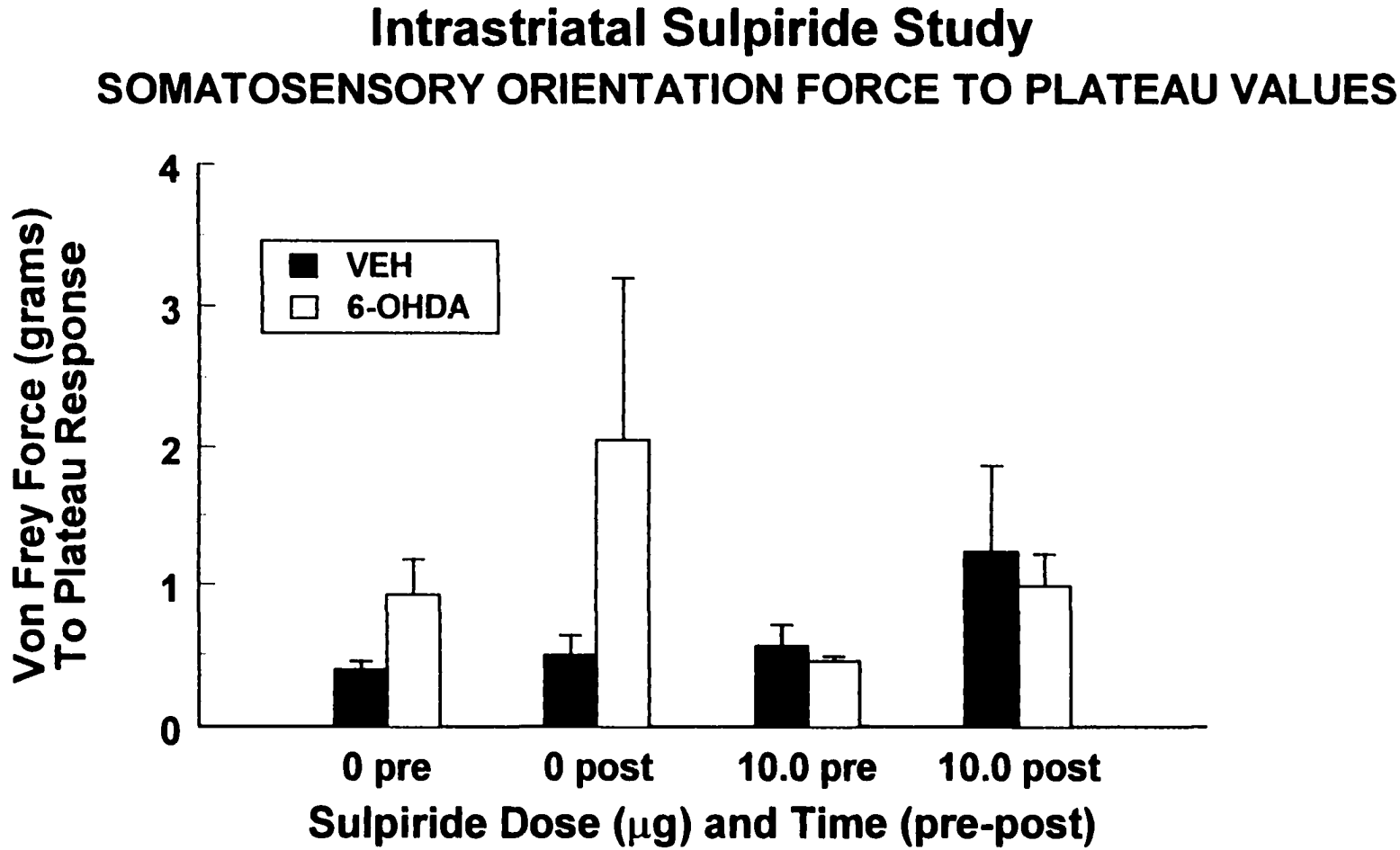
Intrastriatal Sulpiride Study
SOMATOSENSORY ORIENTATION FORCE TO PLATEAU VALUES
Von Frey Force (grams)
To Plateau Response
4
3
2
1
0
VEH
6-OHDA
0 pre
0 post
10.0 pre
10.0 post
Sulpiride Dose (μg) and Time (pre-post)

Figure 3.15

CHAPTER 4

EXPERIMENT 2: CHANGES IN EXTRACELLULAR STRIATAL DA AND ITS MODULATION OF STRIATAL PROCESSING FOLLOWING DA DEPLETION

4.1 Introduction

Experiment 1 provided two important conclusions. The first was that rats depleted of DA as weanlings remain sensitive to an acute DA antagonist applied locally into the striatum, long after the lesion was administered. Because the rats were tested as adults, this finding must be qualified, as it may have nothing to do with the *accelerated recovery* of sensorimotor behavior seen after DA-depletion in weanlings. Further study is needed to determine whether this sensitivity exists sooner after the DA depletion in weanling rats. The second conclusion was that the sensitivity expressed by rats DA-depleted as weanlings to a striatal DA antagonist was similar to the sensitivity of the age-matched vehicle-treated controls, and no supersensitivity was detected. The absence of supersensitivity may represent a complex set of neural compensations, but one explanation could be that extracellular striatal DA concentrations in the lesioned rats of Experiment 1 were similar to the levels of the intact controls at the time they were tested. It is also conceivable that the residual extracellular striatal DA that was competing with the DA antagonist of Experiment 1 is present in higher concentrations much sooner after the lesion in rats depleted of DA as weanlings than after similar lesions of adult rats. Among the two hypotheses this experiment was designed to address, the first applies to this possibility: **extracellular striatal DA will be available in greater quantity soon after depletion of striatal DA in weanling rats than after similar lesions of adult rats.**

Extracellular changes in neurotransmitter concentrations can be measured with great sensitivity and precision using in-vivo microdialysis, avoiding the ambiguity of measurement often

encountered using other in-vivo techniques such as voltametry (Kawagoe et al., 1993). The microdialysis technique has been used in the past to assess the rate of striatal DA restoration following unilateral DA depleting lesions of adult rat striatum (Robinson et al., 1988; Robinson et al., 1994). The goal of this experiment was to determine whether the rate of striatal DA restoration following bilateral DA depleting lesions of weanling or adult rats correlated with their different rates of sensorimotor recovery. To accomplish this, striatal DA efflux was measured using microdialysis within 8 days following DA depletions of weanlings or adults, and compared with DA efflux obtained from age-matched vehicle controls.

It may be that the postulated increase in extracellular striatal DA efflux is not only present in greater quantities sooner after DA depleting lesions of weanling rats than after similar lesions of adult rats, but that it also begins modulating striatal target cell activity by this time as well. Among the striatal neurons which are modulated by DA, the cholinergic neurons represent an ideal choice for several reasons. First, that striatal ACh comes exclusively from the cholinergic interneurons, and is modulated through the D2-like DA receptors expressed on these neurons (Stoof et al., 1992; LeMoine et al., 1990; Ikarashi et al., 1997; DeBoer et al., 1996; Stoof et al., 1992). Therefore, DA modulation of striatal ACh efflux is by definition a local phenomenon, and the effects of the D2-like antagonist sulpiride could be used as a basis for this assay. Second, a correlation between recovery of sensorimotor function following DA-depleting lesions of adult rats and DA modulation of striatal ACh has already been demonstrated in-vitro (MacKenzie et al., 1989), such that early after the lesions this modulation is significantly reduced compared with after sensorimotor recovery is achieved. Finally, the technique of microdialysis from the striatum of freely-moving rats, and the assay used to quantify DA modulation of striatal ACh, had been previously used in the Bruno lab (Johnson et al., 1995), so many of the technical concerns had already been worked out. The second hypothesis this experiment was designed to address was the following: **dopaminergic modulation of striatal ACh release will be demonstrable soon (within 5-8 days) following nigrostriatal DA depletion of weanling rats, but will not be seen within this interval after similar lesions of adult rats**, correlating with the different time courses of recovery of sensorimotor function seen after lesions at these two ages.

The two hypotheses of this Experiment were dependent upon a common experimental protocol which was designed to obtain microdialysis efflux samples from rats soon after they had been treated with 6-OHDA or its vehicle as weanlings or adults. Although the hypotheses were distinct, they were related, as there were constraints introduced because experiments needed to be performed within a restricted time following the DA-depletion surgery. So these experiments involved analyzing a common pool of microdialysis efflux samples for DA and for ACh.

It is well known that changes in neurotransmitter efflux measured with microdialysis may originate from damage caused by the introduction of the probe, rather than from neuronal impulses. Several procedural components will be introduced to ensure that the dialysis samples are representative of the physiological striatal response to the DA antagonist. It has been demonstrated that the introduction of tetrodotoxin (TTX) to the mobile phase in microdialysis experiment reduces the efflux of neurotransmitters which originate from physiological, impulse dependent, neuronal release (Damsma et al., 1987; Westerink et al., 1988). It was be important to demonstrate the neuronal impulse-dependency of the ACh measured by the protocol used in our laboratory, so I performed a final experiment with TTX in representative animals to accomplish this.

4.2 Procedures

This experiment compared rats depleted of nigrostriatal DA as weanlings with adults soon after the lesion when their sensorimotor recovery status are most disparate. Rats in this experiment received a medial forebrain bundle infusion of 6-OHDA or its vehicle (see General Methods), and an intracerebral guide cannula for microdialysis (with locking stylet, standard 10 mm length, Bioanalytical Systems) was implanted into mid-dorsal striatum. Coordinates for the guide cannula implantation in weanlings were (based on the intra-aural line) +7.0-7.5mm anterior, ±2.6mm lateral, and –4.0mm ventral from skull surface. Coordinates for the adults were (based on bregma) +0.5mm anterior, ±2.6 mm lateral, and –3.6 mm ventral from skull surface. Guide

cannulae were affixed to the skull with skull screws and Durelon cement (ESPE, Germany) after the medial forebrain bundle infusion was completed.

Sensorimotor tests were performed as described above (General Methods and in Experiment 1) for akinesia, catalepsy, somatosensory orientation, and open field activity daily beginning two days prior to surgery to establish a baseline level of behavior for each animal. During the five to eight days following the above-described surgery, sensorimotor tests were administered daily. Rats were also habituated to the microdialysis environment (circular bins, 36cm high and 40cm diameter) for 30 minutes per day during the initial 5 days following the surgery.

At 5 or 6 days following the surgery, microdialysis testing began (see **Figure 4.1** for time line). Two dialysis sessions were performed on each rat, with one intervening day between each session. Dialysis probes (0.5 mm OD, Bioanalytical Systems, IN) consisted of different lengths of semipermeable membrane tips that were used for either adult rats (4.0 mm) or weanling rats (3.0 mm) due to the different dorsal-ventral size of striatum at these two ages. These probes were inserted into the guide cannula so that the semipermeable tip of the probe extended into the striatum (see **Table 4.1** for session layout). The basic environment and set up for microdialysis is shown in **Figure 4.2**. Two syringes of artificial cerebrospinal fluid (aCSF) (pH = 6.8-7.0, 155 mM Na^+, 2.9 mM K^+, 1.1 mM Ca^{2+}, 0.83 mM Mg^{2+}, 132.8 mM Cl^-, 5.9 mM glucose, and 0.1 μM of the acetylcholinesterase inhibitor neostigmine bromide) perfused the dialysis lines at 2.0 μl/min. One syringe contained vehicle (drug free) artificial CSF and the other contained either 10 or 100 μM of the D2-like DA antagonist sulpiride. After insertion of the dialysis probe, a four hour *discard period* began during which no efflux collections were taken. This period increases the likelihood that the efflux represents Na^+-gated impulse flow (Westerink, 1995). Subsequently, eight 15 minute baseline collections were taken into small centrifuge tubes (35 mm long, 6 mm diameter), while the drug-free CSF was perfused through the striatal probe, and immediately frozen upon removal. Every other baseline collection tube contained 10 μl of an antioxidant compound for protection of DA in the dialysate (Robinson et al., 1988). This antioxidant was composed of 250μl 50mM EDTA, and 2.5μl of 1M sodium bisulphite in 25 ml of 0.05M perchloric acid. After baseline

collections were completed, the lines were switched to the drug-containing syringe and a 15 minute interval elapsed to allow the sulpiride to be perfused into the striatum. Four "drug" collections were taken, only to be analyzed for ACh. Then the lines were switched back to the drug-free syringe and 15 minutes elapsed to allow the drug to be cleared from the lines, followed by two "post-drug" collections for ACh (see **Table 4.1** for dialysis session collections).

The four baseline dialysates containing the antioxidant were later analyzed for DA with high performance liquid chromatography (HPLC) using electrochemical detection on a glassy carbon electrode. Methods for dopamine analysis are described in detail above (see General Methods). Twenty microliters of all other dialysates were analyzed for ACh levels with (HPLC), separated by a reverse-phase microbore column (530x1mm ACh/Ch MF8904 Bioanalytical Systems, IN). Analysis utilized enzymatic conversion of ACh to hydrogen peroxide via acetylcholinesterase and choline oxidase and electrochemical detection on a platinum electrode at +500 mV.

4.3 Impulse-dependency of dialysates

The possibility that ACh efflux measured with microdialysis may originate from causes other than the physiological effects of the DA antagonist used was mentioned above in the introduction. To explore the possibility that the acetylcholine measured during basal efflux and during the sulpiride perfusion was due to damage or was otherwise disassociated from axonal depolarization of striatal cholinergic neurons, representative animals were tested in a third dialysis session prior to sacrifice. This session began two days following the final dialysis session of the experiments described above. Tetrodotoxin (TTX) was perfused locally through reverse dialysis during this experiment, to block the voltage-gated sodium channels of striatal neurons during dialysis (Damsma et al., 1987). The protocol for this session followed a similar timeline to the above described experimental sessions, with the same *discard period* after insertion of the probe, aCSF, and flow rate (2.0 µl/minute). After the discard period, four fifteen minute baseline collections were taken. Following these collections, the input lines were switched to aCSF

containing 1.0 μM TTX, for two more 15-minute collections. Then, the input lines were switched to aCSF containing 1.0 μM TTX and 100 μM sulpiride, for four 15 minute collections. Finally, input lines were switched back to aCSF containing 1.0 μM TTX, but without sulpiride, for two final collections. It was expected that the introduction of TTX would minimize the ACh efflux collected to the point of the detection limits of the HPLC apparatus used to measure it (approximately 10 femptomoles), and block the ACh increase typically associated with sulpiride in the striatum (at least in the intact control rats).

4.4 Statistical Analysis

Statistical analysis of the parametric sensorimotor measures (akinesia, catalepsy, and open field activity) consisted of mixed repeated-measure ANOVAs with lesion condition (COND; vehicle, 6-OHDA) and AGE (adults or weanlings) as between-subject variables and DAY (last pre-surgery versus 8 post-surgery days) as within-subject. Each of these sensorimotor measures were analyzed for effects separately. Simple main effects were further explored using pairwise t-tests, and an attempt was made to minimize the number of comparisons made. The Bonferroni corrected alpha was used to assess significance in these pairwise tests to reduce the probability of Type I errors (Keppel, 1991).

The somatosensory orientation measure was analyzed using the non-parametric tests described for Experiment 1, except that COND and AGE were analyzed separately for the effects of the repeated measure DAY using the Friedman test for related samples. Kruskal-Wallis tests were then used to analyze the effect of COND within each AGE on selected DAYs that were representative of the progress of sensorimotor recovery during the experiment: the last pre-surgery day, and post surgery days 1, 5, and 8.

DA obtained from the basal dialysates were analyzed with mixed repeated-measure and between subject ANOVAs for AGE (adult, weanling), COND (vehicle, 6-OHDA), and INTERVAL (0, 30, 60, and 90 minutes following insertion of the probe). DA values were processed in two ways. First, the *basal DA efflux* (pmols DA per 10 μl dialysate) from the dialysate was analyzed

after first correcting this value for the recovery of the dialysis probe. Second, *fractional DA efflux* was calculated by taking the *basal DA efflux* and dividing it by the average striatal tissue DA content measured from the dorsal and ventral micropunches taken from that particular rat (see **General Methods**). When indicated, the overall ANOVA was broken down to determine if there were COND effects within either AGE.

The ACh microdialysis data were analyzed by first obtaining median baseline values of striatal ACh (pmol/20μl dialysate) from each experimental dialysis session. The first level of analysis addressed the possibility that basal ACh efflux would differ between rats of different AGES (adult, weanling) or lesion COND (vehicle, 6-OHDA). Two-way ANOVAs (COND X AGE) were performed on the median basal ACh values corrected for the recovery percentage obtained from the in-vitro tests, thus normalizing against the diffusion parameters of different dialysis probes. Next, the effects of sulpiride (DOSE) were analyzed by first calculating percent change from the median baseline for the last baseline and each of the four "post drug" collections following sulpiride (see **Table 4.1**). Because the responses of the intact rats in the two AGES to sulpiride differed dramatically at the main dose (100 μM), analysis of these data was divided into four parts. First, the percentage of the last baseline as compared with the median baseline was compared in an ANOVA with COND (vehicle, 6-OHDA), and AGE (adult , weanling) as factors. This was necessary to establish that the basis for comparison (last baseline) remained stable across each of these populations. Second, a mixed repeated measure ANOVA was performed on data *from the adult rats* using collection INTERVAL (last baseline, 15, 30, 45, and 60 minutes post sulpiride) and DOSE (10 or 100 μM sulpiride) as within-subject, and COND (vehicle, 6-OHDA) as the between-subject variable. Third, the age-dependency of the drug response (INTERVAL) *from 100 μM sulpiride response* was analyzed with a mixed repeated-measure ANOVA with COND (vehicle, 6-OHDA) and AGE (adult, weanling) as between-subject effects and INTERVAL (last baseline, 15, 30, 45, and 60 minutes post sulpiride) as within-subject effects. Finally, to address the possibility of return to baseline ACh levels after the mobile phase was returned to sulpiride-free aCSF, four T-tests were performed between the percentages of the last baseline and the final "*post vehicle*" collection (see **Table 4.1**). Simple main effects and their

interactions were then analyzed using either one-way ANOVAs for COND or AGE at the 100μM DOSE, or individual pairwise t-tests. An effort was made to reduce the number of pairwise t-tests, and the Bonferroni corrected alpha was used to minimize the probability of Type 1 errors (Keppel, 1991).

4.5 Results

The DA depletions of rats in this experiment are shown in **Table 4.2**. The results of this experiment will be divided between the **sensorimotor** and the **neurochemical**, as the overall point of this experiment was to demonstrate a correlation between these two measures. Placement of the microdialysis probes for all rats are shown in **Figure 4.3**.

SENSORIMOTOR RESULTS

The sensorimotor battery was administered to each rat from two days prior to surgery through eight days following surgery. Previous experiments in our laboratory (Weihmuller et al., 1989) demonstrated significant differences in the rate of sensorimotor recovery between the two ages tested in this experiment. It was therefore expected that there would be significant differences in deficit expression between the CONDITIONS (vehicle, 6-OHDA) of the adult rats for most if not all of the days tested, but not the weanling rats. The following are the results of each sensorimotor test performed.

Figure 4.4 shows the akinesia expressed by the rats of this experiment. There were significant overall main effects of AGE [$F(1,27) = 47.36$, $p < 0.001$], COND [$F(1,27) = 47.23$, $p < 0.001$], DAY [$F(8,216) = 4.878$, $p < 0.001$], and interactions AGE X COND [$F(1,27) = 37.12$, $p < 0.001$], DAY X AGE [$F(8,216) = 3.795$, $p < 0.001$], DAY X COND [$F(8,216) = 5.179$, $p < 0.001$], and DAY X AGE X COND [$F(8,216) = 4.092$, $p < 0.001$]. Because all the interactions were significant in the overall ANOVA, I divided the analysis between the AGES and analyzed each separately. The akinesia results from the adult rats revealed significant effects of COND

[F(1,14) = 61.96, p < 0.001], DAY [F(8, 112) = 4.761, p < 0.001], and the interaction DAY X COND [F(8,112) = 5.099, p < 0.001]. Interestingly, the akinesia results from the weanling rats only revealed a trend in COND [F(1,13) = 4.19, p = 0.061], and there was *no* significance of DAY [F(8,104) = 1.57, p = 0.14], or the interaction DAY X COND [F(8,104) = 1.26, p = 0.27]. The lack of significance of COND among the weanling rats was expected given the obviously similar akinesia results between the conditions of this AGE shown in **Figure 4.4**. Post hoc comparisons of COND in the adult rats revealed that the deficits remained significant from Day 1 [$t(\text{Day 1})_{14}$ = -6.975, p < 0.001] through Day 8 [$t(\text{Day 8})_{14}$ = -2.532, p = 0.024]. Recovery from the sensorimotor deficit akinesia was exhibited by the weanling rats during this week period following surgery, but not by the adult rats.

Figure 4.5 shows the catalepsy expressed by the rats of this experiment. There were significant overall main effects of AGE [F(1,27) = 50.62, p < 0.001], COND [F(1,27) = 63.15, p < 0.001], DAY [F(8,216) = 8.50, p < 0.001], and interactions AGE X COND [F(1,27) = 45.95, p < 0.001], DAY X AGE [F(8,216) = 5.76, p < 0.001], DAY X COND [F(8,216) = 7.13, p < 0.001], and DAY X AGE X COND [F(8,216) = 4.93, p < 0.001]. Once again, all interactions were significant in the overall ANOVA, so I divided the analysis between the AGES and analyzed each separately. The catalepsy results from the adult rats revealed significant effects of COND [F(1,14) = 70.65, p < 0.001], DAY [F(8, 112) = 8.24, p < 0.001], and the interaction DAY X COND [F(8,112) = 6.95, p < 0.001]. Catalepsy results from the weanling rats were not significant in either of the parameters, COND [F(1,13) = 1.88, p = 0.193], DAY [F(8,104) = 1.68, p = 0.112], or the interaction DAY X COND [F(8,104) = 1.64, p = 0.123]. Once again, the lack of significance of COND among the weanling rats was expected given the obviously similar catalepsy results between the conditions of this AGE shown in **Figure 4.5**. Post hoc comparisons of COND in the adult rats revealed that the deficits remained significant from Day 1 [$t(\text{Day 1})_{14}$ = -7.268, p < 0.001] through Day 8 [$t(\text{Day 8})_{14}$ = -3.123, p = 0.007]. Recovery from the sensorimotor deficit catalepsy was exhibited by the weanling rats during this week period following surgery, but not by the adult rats

Figure 4.6 shows the open field activity expressed by the rats of this experiment. There were significant overall main effects of AGE [$F(1,27) = 29.70$, $p < 0.001$], COND [$F(1,27) = 7.831$, $p < 0.001$], and DAY [$F(8,216) = 2.11$, $p = 0.036$] and the interaction DAY X COND [$F(8,216) = 3.28$, $p = 0.001$] . However the interactions AGE X COND [$F(1,27) = 0.588$, $p = 0.45$], DAY X AGE [$F(8,216) = 1.26$, $p = 0.27$], and DAY X AGE X COND [$F(8,216) = 0.276$, $p = 0.973$] were not significant. The open field activity results did not exhibit any significant interactions which included the AGE factor. The fact that these interactions with AGE are not significant reduces the justification for separate analysis of each age. However the complete set of open field activity shown in **Figure 4.6** suggested that the source of the AGE effect may have been the generally greater activity exhibited by the weanling rats. Also, one of the likely factors reducing the significance of the intrastriatal sulpiride infusion of Experiment 1 was the fact that the baseline exploratory activity was reduced in both conditions. It may be that the adult rats had significant condition effects, but the lower baseline activity of the intact rats reduced the significance of the AGE X COND interaction ($p = 0.45$). So despite the lack of main effects supporting such analysis, I divided the analysis between the AGES to determine if the results would demonstrate a significant effect of COND in the adults but not the weanlings. This difference was revealed, again, as it was for akinesia and catalepsy. The open field results from the adult rats revealed significant effects of COND [$F(1,14) = 20.03$, $p = 0.001$], and the interaction DAY X COND [$F(8,112) = 2.65$, $p = 0.011$], but not DAY [$F(8, 112) = 1.55$, $p = 0.148$]. Open field results from the weanling rats were not significant in any of the parameters, COND [$F(1,13) = 1.15$, $p = 0.303$], DAY [$F(8,104) = 1.67$, $p = 0.115$], or the interaction DAY X COND [$F(8,104) = 1.34$, $p = 0.234$]. Once again, the lack of significance of COND among the weanling rats was expected given the similarities between the open field activity exhibited by the two conditions of this AGE shown in **Figure 4.6**. Post hoc comparisons of COND in the adult rats revealed that the deficits remained significant from Day 1 [$t(\text{Day } 1)_{14} = 3.431$, $p = 0.004$] through Day 8 [$t(\text{Day } 8)_{14} = 3.638$, $p = 0.003$]. Recovery from deficits in open field exploratory activity was exhibited by the weanling rats during this week period following surgery, but not by the adult rats.

Figures 4.7 and **4.8** show the results from the somatosensory orientation tests on representative days following surgery. As described in the procedures, only select DAYs (pre-, and 1, 5, and 8 days following surgery) were analyzed for the effects of treatment on the somatosensory responses. The results for DAY were analyzed separately with Friedman tests for each AGE and COND factor. There was a significant DAY effects of both *plateau response* and *force to plateau* in the 6-OHDA treated adults [*plateau* Chi-Square (3) = 7.976, p = 0.47], [*force* Chi-Square (3) = 14.308, p = 0.003]. No other AGE or COND factor revealed significant effects of DAY in either *plateau response* or *force to plateau*. Kruskal-Wallis tests revealed significant COND effects in both the *plateau response* and *force to plateau* of the *adults* on Day 1 [*plateau* Chi-Square (1) = 8.571, p = 0.003], [*force* Chi-Square (1) = 12.21, p < 0.001], Day 5 [*plateau* Chi-Square (1) = 4.451, p = 0.035], [*force* Chi-Square (1) = 6.708, p = 0.010], and on Day 8 [*plateau* Chi-Square (1) = 6.429, p = 0.011], [*force* Chi-Square (1) = 7.622, p = 0.006]. Interestingly, the *weanling rats* only showed significant COND effects of *force to plateau* on Day 1 [*force* Chi-Square (1) = 5.192, p = 0.023], but none of the other tests of COND were significant. This COND effect of the weanlings can be seen on **Figure 4.8**. Kruskal-Wallis tests revealed a significant AGE effect of *force to plateau* between both the 6-OHDA and the vehicle-treated rats on the Pre-surgery test [*6-OHDA force* Chi-Square (1) = 4.457, p = 0.035], [*vehicle force* Chi-Square (1) = 4.772, p = 0.029]. Interestingly, the 6-OHDA-treated rat's *force to plateau* demonstrated significant AGE effects on every Day. There were significant AGE effects in the lesioned rats from *force to plateau* on Day 1 [*6-OHDA force* Chi-Square (1) = 9.254, p = 0.002] (which was presumably based on the same source as the COND effect on this Day, see **Figure 4.8**), Day 5 [*6-OHDA force* Chi-Square (1) = 6.751, p = 0.009], and on Day 8 [*6-OHDA force* Chi-Square (1) = 8.303, p = 0.004]. The AGE effect only revealed significance for the *plateau response* in the 6-OHDA treated rats, and only on post surgery Day 1 [*6-OHDA plateau* Chi-Square (1) = 4.558, p = 0.033], and Day 8 [*6-OHDA plateau* Chi-Square (1) = 4.571, p = 0.033], with only a trend on Day 5 [*6-OHDA plateau* Chi-Square (1) = 3.086, p = 0.079].

The body weights of the 6-OHDA treated rats were monitored throughout this experiment and occasionally these rats required intragastric feedings. **Figure 4.9** presents the difference

between the weanling and adult 6-OHDA treated rats on recovery of body weight which is an indication of feeding behavior. Two-way ANOVAs were performed to compare the 6-OHDA treated rats for effects of AGE (adult, weanling) and DAY (pre-, and post-surgery 1-8). There were significant effects of DAY [$F(8,88) = 3.559$, $p = 0.001$], AGE [$F(1,11) = 6.18$, $p = 0.03$], and the interaction DAY X AGE [$F(8,88) = 7.642$, $p < 0.001$]. Post hoc comparisons were done between pre-surgery weight and post surgery days 1, 3, and 5. All of these comparisons were significant for the adult 6-OHDA treated rats [$t(\text{pre vs. } 1)_6 = -13.203$, $p < 0.001$; $t(\text{pre vs. } 3)_6 = -13.919$, $p < 0.001$; $t(\text{pre vs. } 5)_6 = -10.084$, $p < 0.001$]. None of these comparisons were significant for the weanling 6-OHDA treated rats [$t(\text{pre vs. } 1)_5 = -2.13$, $p = 0.086$; $t(\text{pre vs. } 3)_5 = -0.279$, $p = 0.791$; $t(\text{pre vs. } 5)_5 = 0.693$, $p = 0.519$], although there was a slight trend toward significance between pre-surgery and the first post surgery day ($p = 0.086$). The reference weights of the age-matched intact controls suggest that the weanling rats, having started with lower body weights, may have been gaining weight at a faster more steady rate than the adults as part of their maturation. As shown in the insert, the rats lesioned as weanlings occasionally required intragastric feedings. However this was not as common as in the case of the lesioned adult rats.

NEUROCHEMICAL RESULTS

The DA efflux results are shown in **Figures 4.10** and **4.11**. The DA efflux data was analyzed for the values *basal DA efflux* and *fractional DA efflux* as described in Section 4.4. The overall *basal DA efflux* analysis revealed only a trend of COND [$F(1,28) = 3.508$, $p = 0.072$], and no main effects of AGE [$F(1,28) = 0.278$, $p = 0.602$], or INTERVAL [$F(3,84) = 0.382$, $p = 0.767$]. The interactions COND X INTERVAL [$F(3,84) = 0.005$, $p = 1.000$], AGE X INTERVAL [$F(3,84) = 0.580$, $p = 0.630$], COND X AGE [$F(1,28) = 2.218$, $p = 0.148$], and COND X AGE X INTERVAL [$F(3,84) = 0.496$, $p = 0.686$] were equally less revealing for the overall analysis. The overall *fractional DA efflux* analysis, however, did result in a significant effect of COND [$F(1,28) = 11.975$, $p = 0.002$], but also had no significant main effects of

AGE [F(1,28) = 0.342, p = 0.564], or INTERVAL [F(3,84) = 1.875, p = 0.140]. The interactions COND X INTERVAL [F(3,84) = 1.869, p = 0.141], COND X AGE [F(1,28) = 0.408, p = 0.528] were equally less revealing. However, there were trends revealed in the interactions AGE X INTERVAL [F(3,84) = 2.324, p = 0.081]and COND X AGE X INTERVAL [F(3,84) = 2.264, p = 0.087] which were worthy of note.

Upon inspection of the data shown in **Figures 4.10** and **4.11**, it seemed clear that the lack of an AGE X COND interaction within the *fractional DA efflux* was probably meaningful because both adult and weanling 6-OHDA treated rats had similar increases in this measure compared with the vehicle controls. However similar inspection of the *basal DA efflux* did not lead to this conclusion. This measure seemed to be revealing three of the four populations of rats with a similar DA efflux, and only one with potentially significant decreases. Despite the lack of an AGE X COND interaction in the *basal DA efflux* measure, it seemed that performing separate analysis within each age may yet reveal important information regarding how the extracellular DA of the 6-OHDA treated rats compared with the age-matched vehicle treated controls. It may be, for example, that the AGE X COND effects present were skewed by the fact that although the adult vehicle, weanling vehicle, and weanling 6-OHDA treated rats all seem to have similar *basal DA efflux* (see **Figure 4.10**), the variability of these data coupled with the lone population of adult 6-OHDA treated rats being compared to the other three populations washed out the interaction. Therefore, separate analysis was performed for the COND effect within adult and weanling rats. Interestingly, the *basal DA efflux* showed a significant effect of COND in the adult rats [F(1,15) = 5.139, p = 0.039], but not in the weanling rats [F(1,13) = 0.087, p = 0.773]. The *fractional DA efflux*, however, showed a significant effect COND in both adults [F(1,15) = 5.401, p = 0.035] and weanlings [F(1,13) = 16.222, p = 0.001]. The difference between *basal DA efflux* and *fractional DA efflux* are apparent in **Figures 4.10** and **4.11**.

One possible effect of reduced DA in the striatum is an increased release of ACh from the cholinergic neurons due to disinhibition (see discussion below). Therefore, the first level of analysis on the striatal ACh data (as described in Section 4.4) was a 2-way ANOVA on the median basal ACh efflux measured from the dialysates. This analysis only revealed a significant

effect of AGE (adult, weanling) [F(1,28) = 4.871, p = 0.036], and *not* of COND (vehicle, 6-OHDA) [F(1,28) = 0.013, p = 0.911] or the interaction AGE X COND [F(1,28) = 0.415, p = 0.525]. This interesting result suggests an increased ACh efflux in the weanling rats compared with the adult rats, also shown in **Figure 4.12**.

The second analysis was done to compare the percent change from the median basal ACh efflux measured in the last baseline collection, which was used for the pre-drug score in all the sulpiride experiments. There were no significant effects of any factor (AGE, COND, or their interactions) this value (all p's > 0.45).

An inspection of the data shown in **Figures 4.13** and **4.14** suggested that only the adult rats had any chance of a significant DOSE (10, 100 μM sulpiride) effect. However, the only significant effect revealed was of collection INTERVAL (baseline, 15, 30, 45, and 60 minutes post sulpiride) [F(4,32) = 21.43, p < 0.001]. There were a trends toward significance of COND [F(1,8) = 3.846, p = 0.086], and the interaction INTERVAL X COND [F(4,32) = 2.553. p = 0.058]. But there was no overall main effect of DOSE [F(1,8) = 0.735, p = 0.416], or a DOSE X COND interaction [F(1,8) = 0.643, p = 0.446]. The trend toward a COND effect suggested that, given the dramatic differences shown for the 100 μM dose in the adult rats (**Figures 4.13** and **4.15**), a COND effect may exist only at this dose. The ANOVA for the 100 μM dose in the adults, however, revealed only a strong trend for COND [F(1,14) = 4.381, p = 0.055]. This is, however, a *strong* trend and worthy of interest.

The effect of AGE was incorporated into the analysis at the 100 μM dose. These data are shown in **Figure 4.15**. Once again, the only significant effect revealed was of collection INTERVAL [F(4,108) = 12.996, p < 0.001]. However there were trends for COND [F(1,27) = 3.61, p = 0.068], and the interactions INTERVAL X AGE [F(4,108) = 2.37, p = 0.057], COND X AGE [F(1,27) = 3.184, p = 0.086], and INTERVAL X COND X AGE [F(4,108) = 2.072, p = 0.089]. Because I had done a separate analysis of the adult rats at this dose, I performed a separate analysis of the weanling rats only. This analysis revealed significance only of INTERVAL [F(4,52) = 4.188, p = 0.005]. There was not even a trend toward significance in the weanling COND [F(1,13) = 0.019, p = 0.891].

To determine whether the influence of sulpiride on striatal ACh began to return to basal levels after removal of the drug from the perfusion medium, separate t-tests were performed between the pre-drug baseline and the last collection of the session (post-vehicle, see Procedures). These values were significantly different in the vehicle treated adults [$t(8) = 2.896$, $p = 0.020$], and the 6-OHDA treated weanlings [$t(5) = -3.091$, $p = 0.027$]. The other rat populations tested did *not* exhibit significant differences between these two values (all p's > 0.49).

The TTX results are shown in **Figure 4.16**. These results are essentially self explanatory and can be understood from this Figure. Statistics were not performed on these data as only two animals are represented in each group (COND X AGE), but these results were similar to the results of other laboratories (Damsma et al., 1987). Both the basal ACh efflux and the effect of sulpiride were blocked by perfusion of TTX in the aCSF. This suggests that the ACh efflux measured in the other sessions originated from voltage-gated neuronal impulses TTX is known to block (Westerink et al., 1988; Damsma et al., 1987).

4.6 Conclusions from Experiment 2

The hypotheses of this experiment were two-fold. First, that **extracellular striatal DA will be available in greater quantity soon after depletion of striatal DA in weanling rats than after similar lesions of adult rats.** Second, that **dopaminergic modulation of striatal ACh release will be demonstrable soon (within 5-8 days) following nigrostriatal DA depletion of weanling rats, but will not be seen within this interval after similar lesions of adult rats**, correlating with the different time courses of recovery of sensorimotor function seen after lesions at these two ages. Several meaningful conclusions can be drawn from the results of this experiment

It is clear from all the tests of the sensorimotor battery that the 6-OHDA treated weanling rats recovered from any modest deficits much more quickly than the 6-OHDA treated adult rats. Interestingly, the open field activity scores were useful in demonstrating recovery of function following 6-OHDA treatment, even when they were not so useful in demonstrating the

sensorimotor effects of striatal sulpiride infusion in Experiment 1. It is my sense that this is due in part to the general tendency of the weanling rats to exhibit a greater amount of exploratory activity than the adult rats. As was suggested in the conclusions of Experiment 1, it is a decrease in open field activity that is necessary to suggest a deficit, so if rats are not very active to begin with, there may be difficulty in achieving a significant enough decrease, even when rats may be exhibiting deficits. In this experiment, the differences between the vehicle and 6-OHDA treated adult rat open field behavior were dramatic enough to be significant for the duration of the time they were tested.

Another interesting sensorimotor effect was the somatosensory data. Once again these data became useful in demonstrating recovery from 6-OHDA-induced deficits, but were not as useful in demonstrating intrastriatal sulpiride-induced deficits. Interestingly, a significant condition effect of the Von Frey *force to plateau* was found on the first day following 6-OHDA/sham treatment of the weanlings. This was the only indication of sensorimotor deficit revealed in weanlings treated with 6-OHDA, apart from the body weight data which is indirect. It is interesting the way useful information can be gained from sensorimotor tests in this experiment that did not reveal interesting patterns in Experiment 1.

The sensorimotor effects of 6-OHDA demonstrated in rats lesioned on postnatal day 27 by Weihmuller (Weihmuller et al., 1989), although also modest, were more dramatic (greater than 60 seconds of akinesia and catalepsy), and lasted longer (up to three days following 6-OHDA), than was the case with the rats of this experiment. Differences in procedures may account for this, such as different technique when administering the various sensorimotor tests. However I believe the most likely, and most interesting possible explanation for this difference is the difference in 6-OHDA infusion coordinates. Weihmuller infused 6-OHDA into the lateral ventricles, while I infused it into the medial forebrain bundle. Although the extent of the striatal DA depletion resulting from these different infusion routes were comparable, it is possible that the effects of the route used by Weihmuller left a different distribution of residual DA fibers than the route I used. The techniques we each used to assess the post-mortem striatal tissue DA content may not have been sensitive to this potential difference.

With respect to the first hypothesis, the results demonstrated an interesting difference between the *basal DA efflux*, and the *fractional DA efflux* values obtained from the dialysates. Although there was no effect of AGE revealed in the analysis, when the two ages (adults and weanlings) were analyzed separately, the effects of COND within these ages differed depending on whether *basal DA efflux*, or *fractional DA efflux* was addressed. Both adults and weanlings showed significantly different *fractional DA efflux* between the age-matched vehicle and 6-OHDA treated conditions. Here, the *fractional DA efflux* was significantly increased in the 6-OHDA treated rats, as expected, but in both ages to a similar degree. However the *basal DA efflux* revealed an interesting difference between weanling and adult 6-OHDA treated rats. Only the adults showed significantly different *basal DA efflux* between conditions (when comparison was restricted to the age-matched populations), where values were dramatically reduced in the 6-OHDA treated population. The fact that only the adult 6-OHDA treated rats showed significantly reduced *basal DA efflux*, but both adult and weanling rats showed significantly increased *fractional DA efflux* compared to the age-matched vehicle-treated controls, suggests that the different factors *fractional DA efflux* is calculated to normalize (reuptake of DA into residual terminals, residual terminal DA concentration, possible differences in the DA lesion) ultimately serve to decrease differences in extracellular DA that may be present between 6-OHDA treated rats of either age. It has been demonstrated that DA reuptake is the primary neuronal process effecting the DA measured with microdialysis (Smith et al., 1994). Therefore, if the calculation of *fractional DA efflux* normalizes the DA reuptake by approximating the reuptake sites with the post-mortem striatal tissue DA content, then this measure may be insensitive to differences that exist in the striatal extracellular DA between rats lesioned as weanlings or adults that depends on differences in reuptake. Alternatively, the way striatal tissue was obtained and analyzed for DA content, providing the value that became the denominator of the *fractional DA efflux* measure, may have implied the wrong conclusion about the density of residual DA fibers in the weanling 6-OHDA treated striata. **Table 4.2** shows values for percent DA depletion for comparison between the adult and weanling 6-OHDA treated rats, which seem very similar regardless of age. However it may be that the 1 millimeter diameter micropunches taken from

dorsal and ventral striata of each rat were too small a proportion of the overall volume of the greater nucleus to adequately quantify the extent of the lesion. If there were more residual fibers along the diagonal patch from dorsomedial to ventrolateral in the weanling rats, these may not have been accounted for with the current procedures. The dorsolateral region of the striatum, however, has been well associated with sensorimotor behavior, so decreased DA in this region might be expected to result in sensorimotor deficits (Pehek et al., 1992). This was shown not to occur in the weanling 6-OHDA treated rats of this experiment.

The origin of the DA in the striatal extracellular space is possibly not as important to recovery from sensorimotor deficits as the neurophysiological role this DA is performing in the striatum. There were no differences demonstrated between the *basal DA efflux* of intact and 6-OHDA treated weanlings (when comparison was restricted to age-matched populations -- given significance of COND in the adult, but not the weanling rats). This suggests that extracellular DA is decreased to a greater extent in the 6-OHDA treated adult rats (by comparison to their age-matched controls) than is the case in the 6-OHDA treated weanling rats (by comparison to their age matched controls), supporting the first hypothesis.

With respect to the second hypothesis, the statements regarding the neurophysiological role of DA within the striatum in the context of recovery are admittedly weaker. The confirmation of the second hypothesis is based on the existence of INTERVAL effects, and trends that are stronger in the adult rats than in the weanling rats. In fact, there was a decreased sensitivity, or extent of effect, following the higher dose of sulpiride (100 μM) in the intact weanling rats that can be seen in **Figure 4.15**, so the lack of a trend toward significance at this dose in the weanling rats may represent insensitivity or decreased output capacity, rather than similar sensitivity of both conditions. Another interesting phenomenon is that the ACh efflux from rats lesioned as weanlings rises slightly (but not significantly) above that of the age-matched vehicle controls after 100 μM sulpiride (see **Figure 4.14 & 4.15**). This would never be expected to happen in the adults, where the fact that there was not a clearly significant COND effect at 100 μM is perplexing ($p = 0.055$).

It seems that neither of the rat populations tested were very sensitive to 10 μM sulpiride. However this dose remains useful in demonstrating that increases in striatal ACh are not generated simply as a function of time following probe insertion per se. Of course the clearest way to demonstrate this would be to introduce a completely drug-free dialysis session. But it remains clear from the effects of 10 μM in both ages and conditions that the increases in ACh efflux measured after 100 μM sulpiride were generated by sulpiride, and not necessarily due to probe insertion damage or some other non-physiological effect. The TTX effect shown in **Figure 4.16** also supports this conclusion.

Unfortunately it seems that the second hypothesis was not clearly proven true by this experiment. Although sulpiride did seem to have an effect, the variance of this effect was such that this experiment did not yield clear and irrefutable evidence supporting the hypothesis. However I am not willing to reject the hypothesis outright on the basis of these data. The existence of trends in the adult rats, and not in the weanling rats, are suggestive that further experiments that increase the power of the observations by increasing the number of subjects may yet yield significance. Unfortunately I have done all I can with this experiment. I originally intended to test adult rats after they had recovered from the sensorimotor deficits, but this proved to be unworkable due to practical and procedural limitations. If I had more time and resources, I would first increase the number of adult rats tested with 100 μM sulpiride. Then, I would move to a higher dose of sulpiride with both the adults and weanlings. The decreased sensitivity, or extent of effect, demonstrated in the weanlings is such that 100 μM sulpiride may be adequate as the low dose for these rats, but a higher dose may be necessary to demonstrate a clear sensitivity. It is important that the lack of a COND effect in these weanling rats be demonstrated with a dose that at least the intact rats are clearly sensitive to, as this may be the result of insensitivity in the present experiment.

It was interesting that the effects of 100 μM sulpiride were reduced in the weanling rats. It does not seem, from the data of the present study, that there is less basal DA in the striata of weanling rats. So this might be eliminated from the possible sources of the decreased response to sulpiride. DA modulation of striatal ACh release has been demonstrated far earlier than the age

tested in this experiment (Coyle et al., 1976). Also the ability of the muscarinic ACh antagonist scopolamine to block the expression of catalepsy following DA receptor blockade is present prior to this age (Fitzgerald et al., 1989), suggesting that DA modulation of ACh is relevant to sensorimotor behavior by this age. There was no reason to believe that intact weanling rats would not be equally sensitive, or exhibit an equal extent of response to similar doses of sulpiride that were effective in adult rats. In fact, the first two weanling rats pilot tested with 100 μM sulpiride exhibited similar percent increases from basal ACh efflux in response to this dose to that of the adult intact rats. For some unknown reason this effect did not generalize to the rest of this rat population (7 additional intact weanling rats). This was not based on the time they were tested, the placement of the dialysis probe, or any obvious deformations of the brains. I am frankly at a loss to explain this.

NEOSTIGMINE CONSIDERATIONS

Recent experiments of other laboratories have demonstrated that the effect of systemic amphetamine on striatal ACh efflux can be dramatically altered by increased concentrations of the acetylcholinesterase inhibitor neostigmine (Acquas et al., 1998; De Boer et al., 1996). The basis of this alteration has been suggested to be the interaction between ACh effects on muscarinic receptors and DA effects on D1 or D2 like receptors. When neostigmine is used, it blocks the primary mechanism whereby ACh is removed from the extracellular space and its post-synaptic effects terminated. So higher concentrations of neostigmine used in microdialysis experiments can potentially disrupt the neurophysiology of the striatum. In the present experiment, the neostigmine was used to establish a baseline ACh efflux that was detectable with the sensitivity of the HPLC used in our laboratory. Although experiments to determine the lowest possible concentration of neostigmine were not done, there are several reasons why the results of this experiment are not particularly subject to the sorts of concerns raised by these recent experiments. First, the TTX experiment demonstrated that even the basal ACh efflux measured with the concentration used in the present experiment (0.1 μM) was reduced to the detection

limits of the HPLC assay. Second, the purpose of this experiment was to demonstrate differences in the capacity of the DA antagonist to induce increases in ACh efflux between rats of various conditions. If one condition (vehicle control) is sensitive to sulpiride and an increase in ACh efflux results, this condition becomes the *positive control* for the age group. If the other condition (6-OHDA) is insensitive to sulpiride at the same neostigmine concentration, then the most likely factor supporting this difference would be the effects of the DA depletion. There may be many neurophysiological effects of DA depletion other than decreases in the sensitivity of ACh efflux to a D2-like DA antagonist, but this was the assay chosen for the present experiment. The expectation, based on the results of Fibiger (Acquas et al., 1998), would be that higher neostigmine would decrease the striatal ACh efflux in response to a D2 DA antagonist. Therefore, if anything the neostigmine would be expected to decrease the sensitivity of this assay and make it less likely that the sensitivity needed to support the hypothesis will be obtained. Anything that is expected to run contrary to the hypothesis only strengthens the results once they are obtained.

COLLECTION	ANALYSIS FOR	EXPERIMENT
Baseline 1	DOPAMINE	Basal dopamine efflux
Baseline 2	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Baseline 3	DOPAMINE	Basal dopamine efflux
Baseline 4	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Baseline 5	DOPAMINE	Basal dopamine efflux
Baseline 6	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Baseline 7	DOPAMINE	Basal dopamine efflux
Baseline 8	ACETYLCHOLINE	Striatal sulpiride ACh modulation
*** 15 minutes elapsed ***		
Post drug 1	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Post drug 2	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Post drug 3	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Post drug 4	ACETYLCHOLINE	Striatal sulpiride ACh modulation
*** 15 minutes elapsed ***		
Post vehicle 1	ACETYLCHOLINE	Striatal sulpiride ACh modulation
Post vehicle 2	ACETYLCHOLINE	Striatal sulpiride ACh modulation
*** 15 minutes elapsed ***		
In Vitro ACh*	ACETYLCHOLINE	Dialysis probe ACh recovery
In Vitro DA*	DOPAMINE	Dialysis probe DA recovery

* Experiment performed in-vitro, with dialysis probe placed in a small centrifuge tube containing known concentration of ACh (3.0 pmol/20µl) or DA (4.0 pmol/20µl).

Table 4.1

Microdialysis collections taken during a dialysis experiment. Each collection was 15 minutes in duration and at a flow rate of 2.0 µl per minute. Vials for dopamine analysis contained antioxidant described in the text.

STRIATAL TISSUE DOPAMINE DEPLETION

ADULT RATS

Condition	DORSAL DA	VENTRAL DA	% DEPLETION	
VEHICLES	76.27±6.64	56.89 ±5.96	DORSAL	VENTRAL
6-OHDA	1.18 ±0.47	1.97 ±1.01	-98.45 ±0.62	-96.53 ±1.79

WEANLING RATS

Condition	DORSAL DA	VENTRAL DA	% DEPLETION	
VEHICLES	32.23±1.80	35.63 ±3.71	DORSAL	VENTRAL
6-OHDA	0.94 ±0.31	1.63 ±0.30	-97.08 ±0.95	-95.43 ±0.85

Table 4.2

Tissue DA content (± SEM) expressed as (pmol DA) / (mg protein) as described in text (Section 2.5). Values given for dorsal and ventral micropunches, and the percent DA depletions calculated from the dorsal and ventral micropunches and compared against the average DA content of vehicle controls.

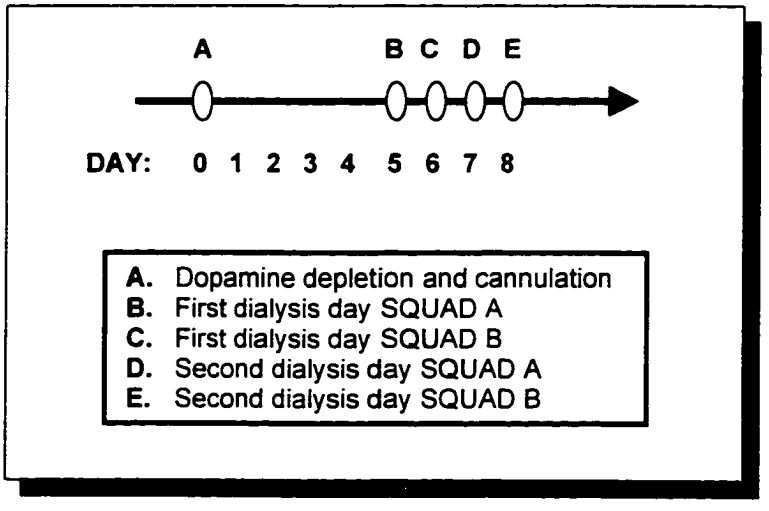

Figure 4.1

Time line shows the timing of the microdialysis study over days, in relation to the day of the dopamine-depletion and cannulation surgery. Rats were dialyzed between 5 and 8 days post surgery. Up to four rats (2 per "SQUAD") could undergo surgery on any given session to maintain this schedule.

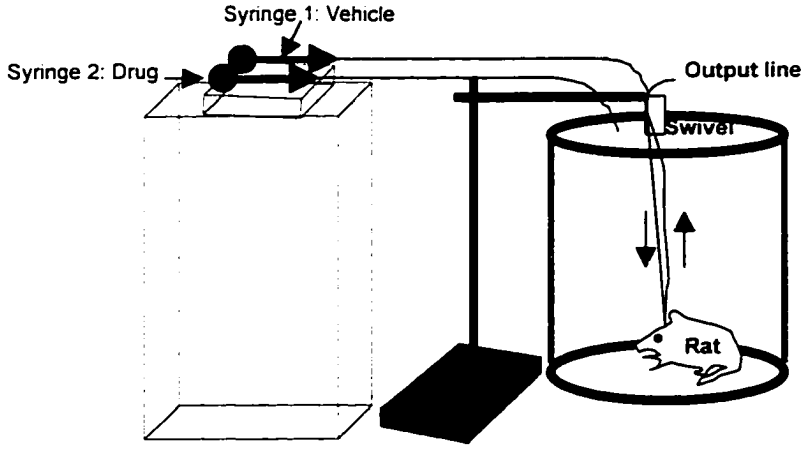

Figure 4.2

Microdialysis set up. Two 2.5 ml syringes pumped artificial CSF through microdialysis tubing. Connections at a fluid swivel allowed the rat subject full range of motion within the dialysis bowl. Collections were taken from the output line indicated on the diagram. Baseline collections (15 minutes each) were taken while the vehicle CSF line was connected to the swivel. Drug collections started 15 minutes following the switch to connect the drug-containing CSF line to the swivel. Final collections were obtained 15 minutes after switch back to vehicle CSF. See **Table 4.1** for details.

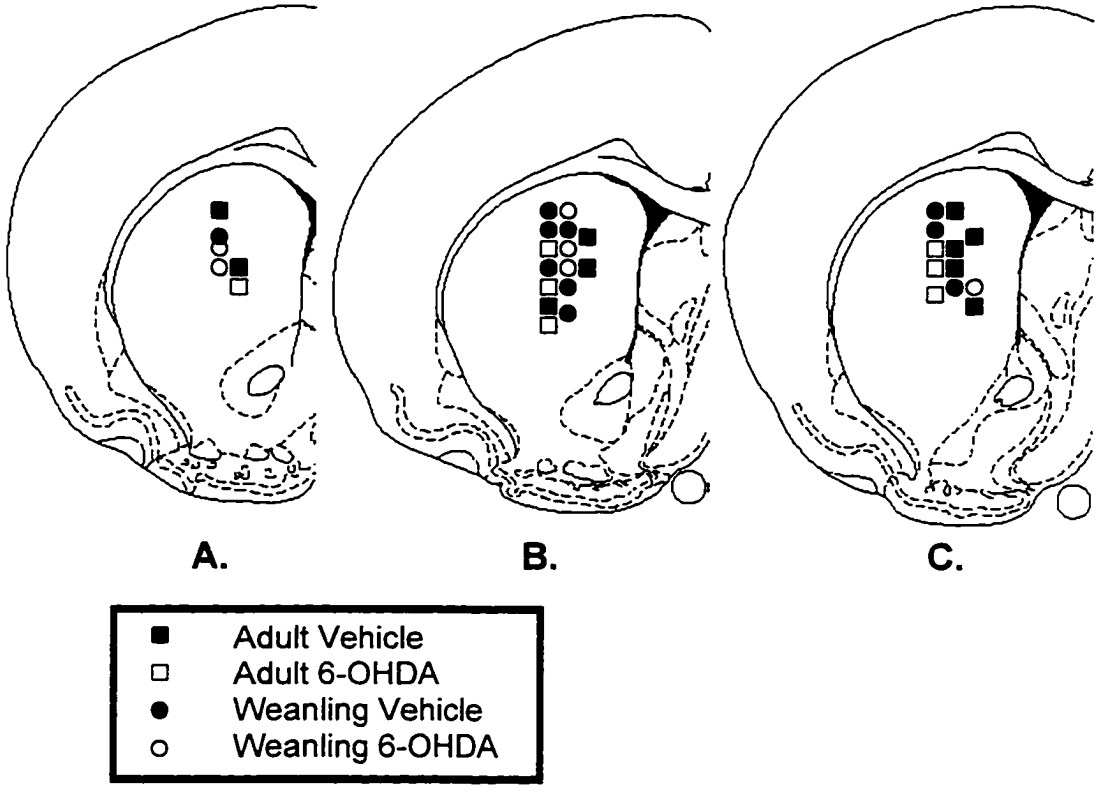

FIGURE 4.3

Placement of the microdialysis probes in Experiment 1. Dorsal sections are taken from (Paxinos & Watson, 1997 CDRom) the following coordinates in mm anterior from Bregma: A. 0.70, B. 0.48, C. 0.20. The legend defines the age and condition of the placement shown.

Figure 4.4 Pre and Post-Operative Akinesia

Figure 4.4 depicts the duration of *spontaneous* akinesia (sec) exhibited by all rats included in Experiment 2. The range of time points on the X-axis represent the last test administered prior to surgery (pre-surg), and the tests administered on each of the eight subsequent days following surgery. Open symbols with dotted lines represent the 6-OHDA treated rats, while closed symbols with solid lines represent the vehicle controls. The adults are represented by boxes, while the weanlings are represented by circles (see legend). Adult VEH = 10; Adult Lx = 7; Weanling VEH = 9; Weanling Lx = 6.

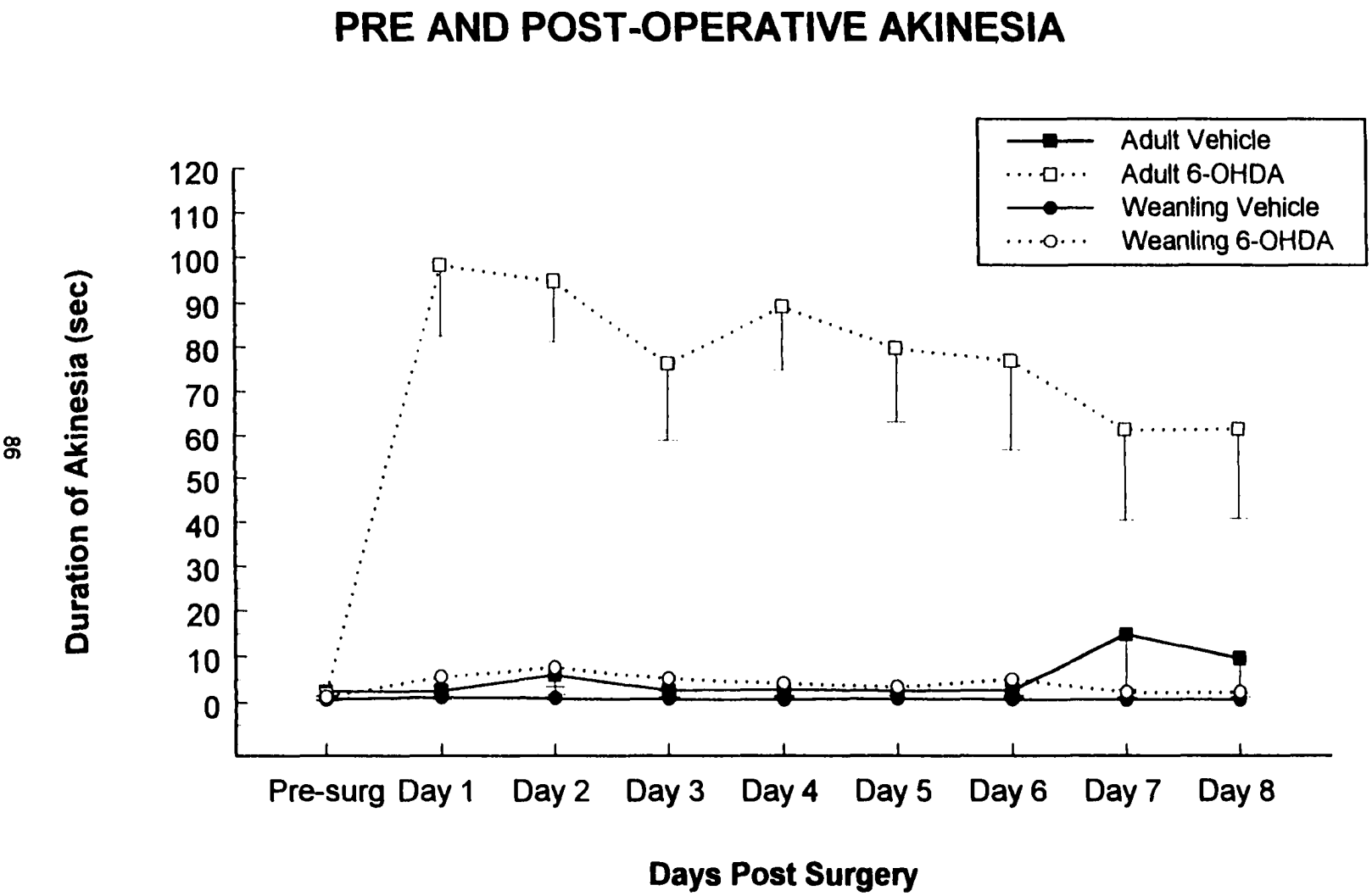
PRE AND POST-OPERATIVE AKINESIA
Adult Vehicle
Adult 6-OHDA
Weanling Vehicle
Weanling 6-OHDA
Duration of Akinesia (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
Pre-surg
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Days Post Surgery

Figure 4.4

Figure 4.5 Pre and Post-Operative Catalepsy

Figure 4.5 depicts the duration of *spontaneous* catalepsy (sec) exhibited by all rats included in Experiment 2. The range of time points on the X-axis represent the last test administered prior to surgery (pre-surg), and the tests administered on each of the eight subsequent days following surgery. Open symbols with dotted lines represent the 6-OHDA treated rats, while closed symbols with solid lines represent the vehicle controls. The adults are represented by boxes, while the weanlings are represented by circles (see legend). Adult VEH = 10; Adult Lx = 7; Weanling VEH = 9; Weanling Lx = 6.

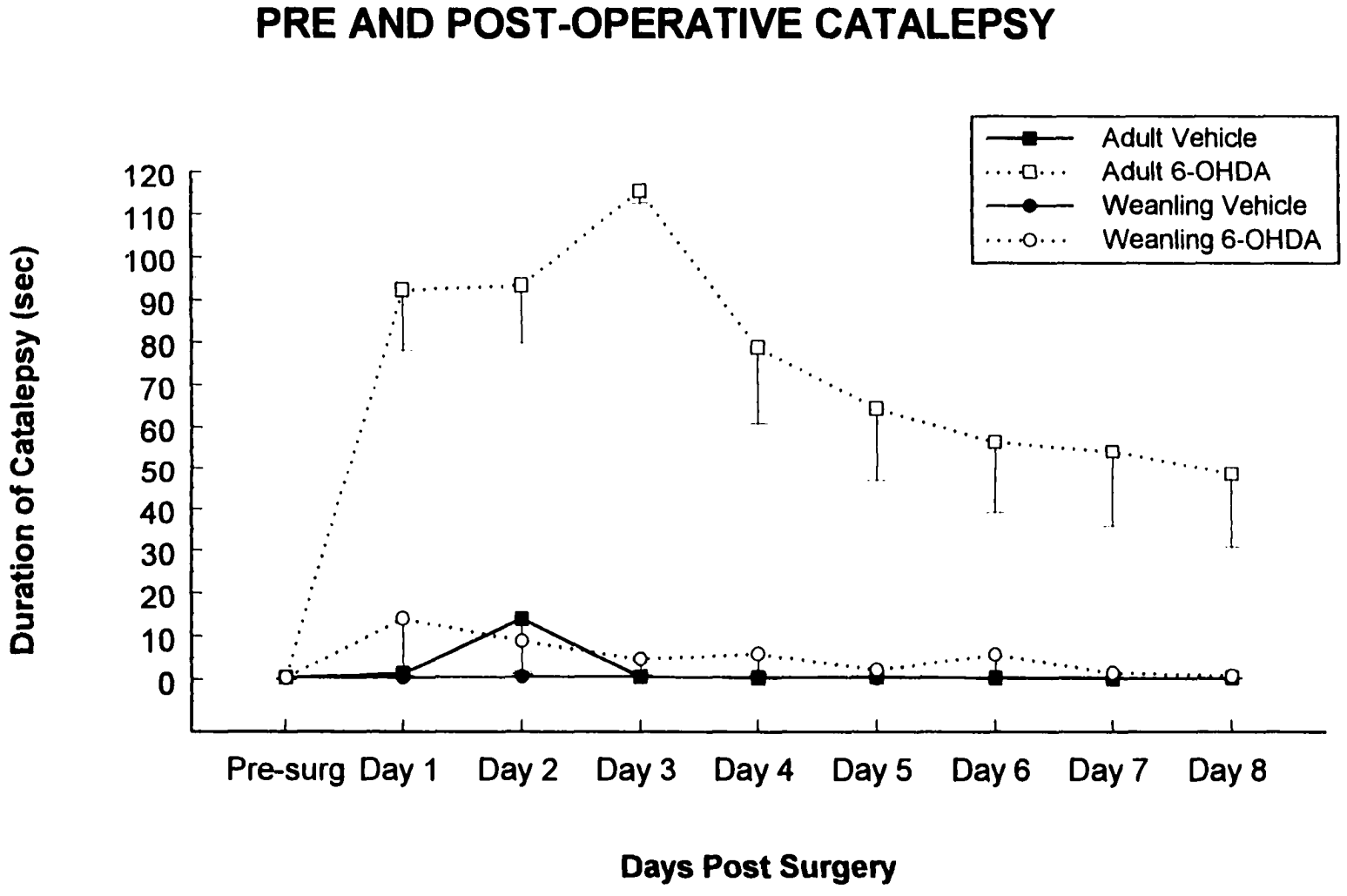
PRE AND POST-OPERATIVE CATALEPSY
Adult Vehicle
Adult 6-OHDA
Weanling Vehicle
Weanling 6-OHDA
Duration of Catalepsy (sec)
120
110
100
90
80
70
60
50
40
30
20
10
0
Pre-surg
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Days Post Surgery

Figure 4.5

Figure 4.6 Pre and Post-Operative Open Field Activity

Figure 4.6 depicts the *spontaneous* open field activity (lines crossed) exhibited by all rats included in Experiment 2. The range of time points on the X-axis represent the last test administered prior to surgery (pre-surg), and the tests administered on each of the eight subsequent days following surgery. Open symbols with dotted lines represent the 6-OHDA treated rats, while closed symbols with solid lines represent the vehicle controls. The adults are represented by boxes, while the weanlings are represented by circles (see legend). Adult VEH = 10; Adult Lx = 7; Weanling VEH = 9; Weanling Lx = 6.

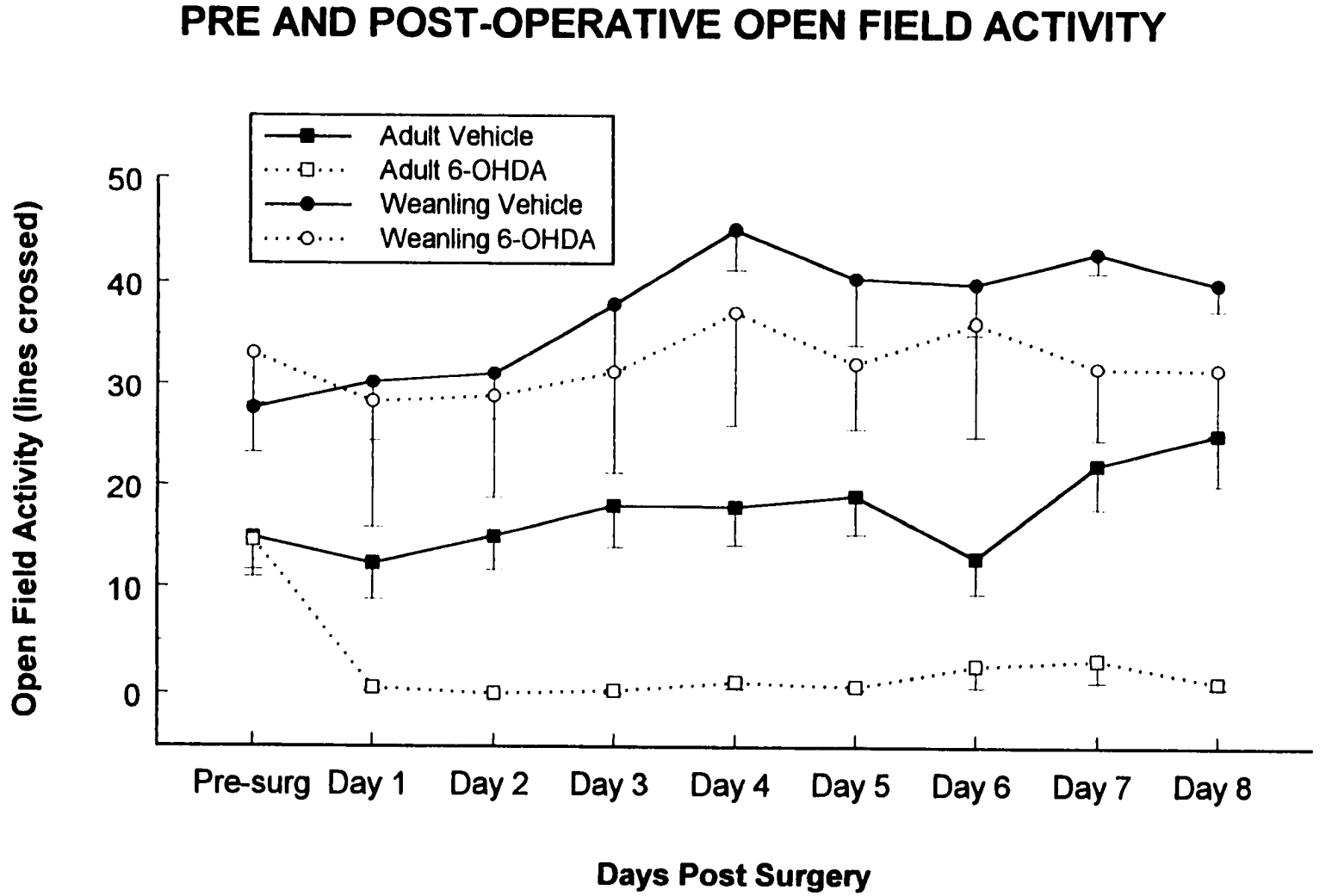
PRE AND POST-OPERATIVE OPEN FIELD ACTIVITY
Adult Vehicle
Adult 6-OHDA
Weanling Vehicle
Weanling 6-OHDA
Open Field Activity (lines crossed)
50
40
30
20
10
0
Pre-surg
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Days Post Surgery

Figure 4.6

Figure 4.7 Pre and Post-Operative Somatosensory Orientation

Plateau Response

Figure 4.7 depicts the *spontaneous* somatosensory response type (1-4) elicited by the force shown in Figure 4.6 for all rats tested in Experiment 2. The range of time points on the X-axis represent the last test administered prior to surgery (pre-surg), and the tests administered on each of the eight subsequent days following surgery. Open symbols with dotted lines represent the 6-OHDA treated rats, while closed symbols with solid lines represent the vehicle controls. The adults are represented by boxes, while the weanlings are represented by circles (see legend). Adult VEH = 10; Adult Lx = 7; Weanling VEH = 9; Weanling Lx = 6.

PRE AND POST-OPERATIVE SOMATOSENSORY ORIENTATION

PLATEAU RESPONSE TYPE

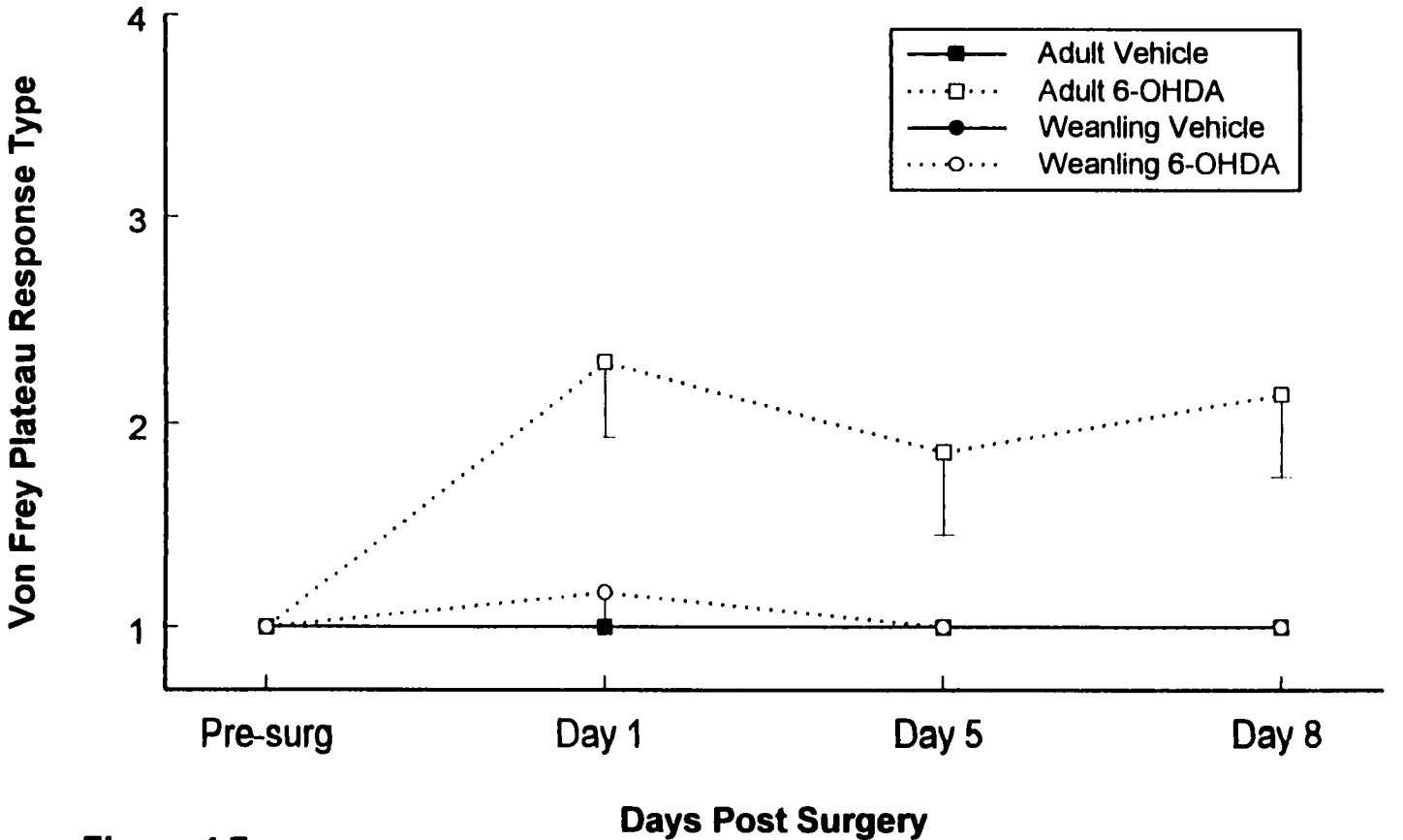

Figure 4.7

Figure 4.8 Pre and Post-Operative Somatosensory Orientation

Force to Plateau Response

Figure 4.8 depicts the *spontaneous* somatosensory sensitivity as demonstrated by the force (grams) of the Von Frey hair required to elicit the plateau response type from all rats tested in Experiment 2. The range of time points on the X-axis represent the last test administered prior to surgery (pre-surg), and the tests administered on each of the eight subsequent days following surgery. Open symbols with dotted lines represent the 6-OHDA treated rats, while closed symbols with solid lines represent the vehicle controls. The adults are represented by boxes, while the weanlings are represented by circles (see legend). Adult VEH = 10; Adult Lx = 7; Weanling VEH = 9; Weanling Lx = 6.

PRE AND POST-OPERATIVE SOMATOSENSORY ORIENTATION

FORCE TO PLATEAU RESPONSE

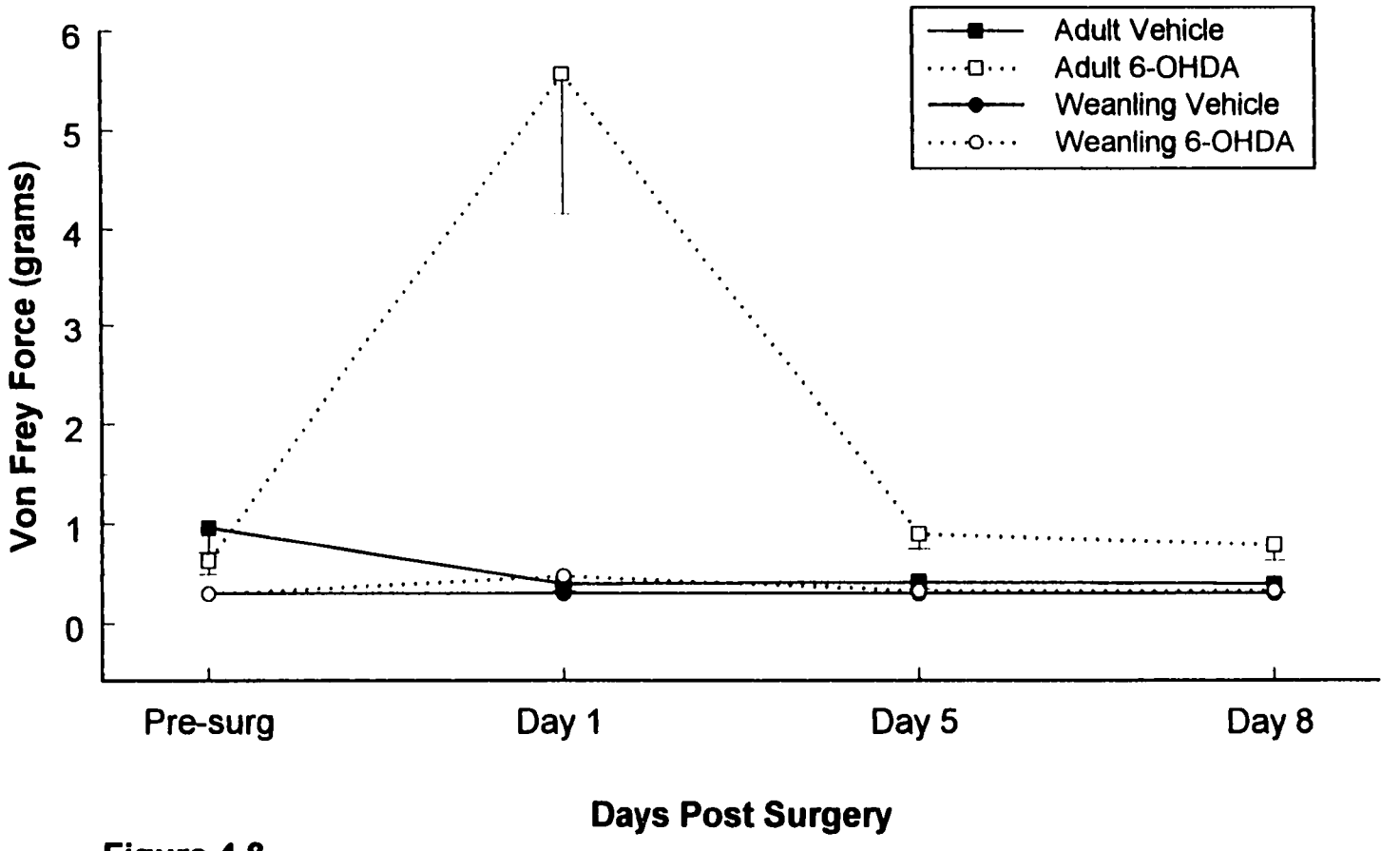

Figure 4.8

Figure 4.9 Pre and Post-Operative Body Weight Percentage

Figure 4.9 depicts the percent of pre-operative body weight exhibited by all rats included in Experiment 2. The range of time points on the X-axis represent the last weight taken prior to surgery (pre-surg), and the weights on each of the eight subsequent days following surgery. Open symbols with dotted lines represent 6-OHDA treated rats while closed symbols with solid lines represent the vehicle controls (weights of vehicles only shown for pre-surg and Day 8 for comparison). The adults are represented by boxes, while the weanlings are represented by circles (see legend). The insert graph shows the mean number of intragastric feedings across 6-OHDA treated rats of either age (adults = boxes, weanlings = circles). Population means were used for this insert, resulting in numbers less than 1, but feedings were typically administered either once or twice per day for any given animal as described in the General Methods. Adult VEH = 10; Adult Lx = 7; Weanling VEH = 9; Weanling Lx = 6.

PRE AND POST-OPERATIVE BODY WEIGHT PERCENTAGE

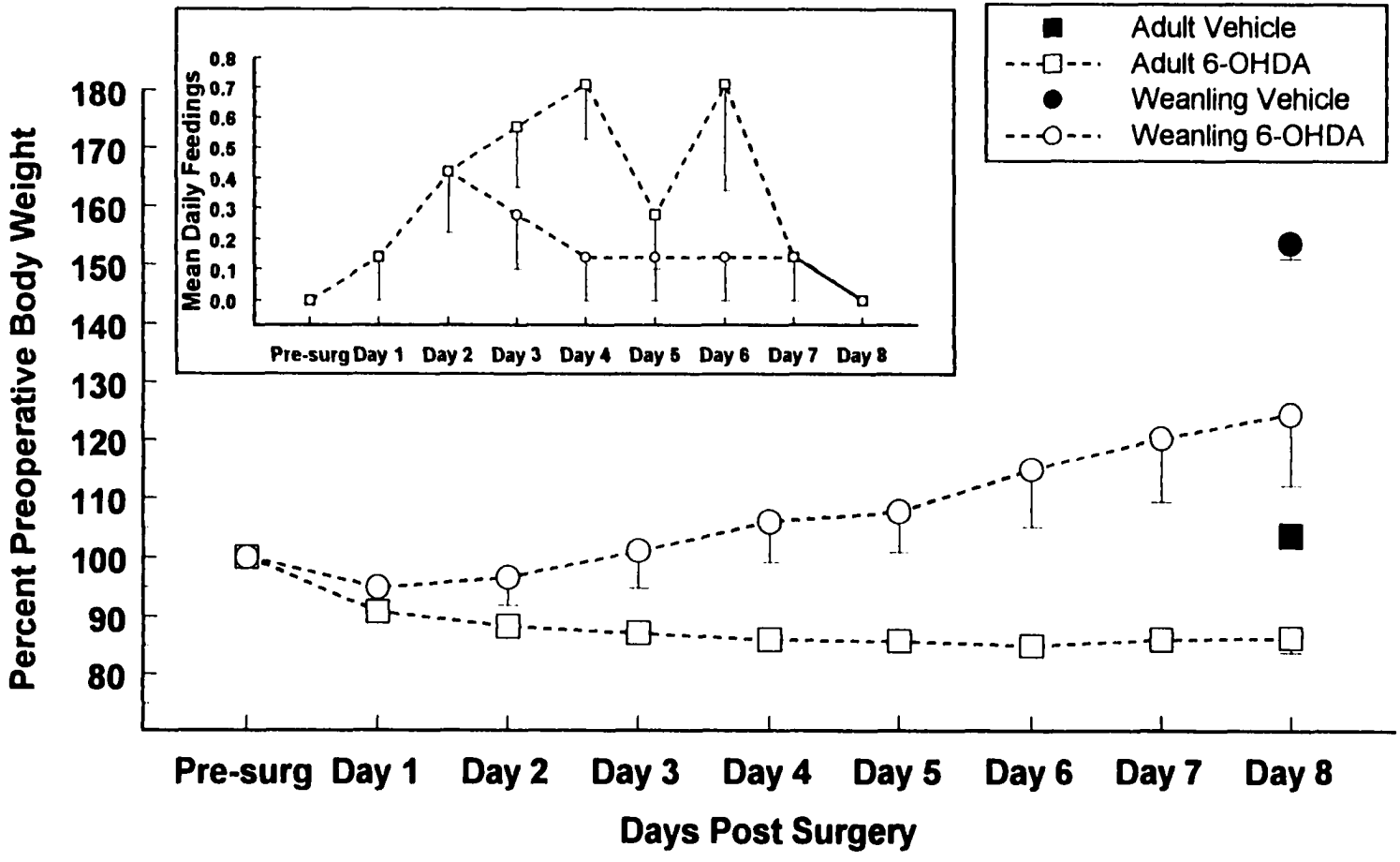

Figure 4.9

Figure 4.10 BASAL DA EFFLUX

Figure 4.10 depicts the *basal DA efflux* obtained from all rats in Experiment 2 as described in the Procedures Section of Experiment 2. The data represents the following n's: Adult VEH = 10, Adult Lx = 7, Weanling VEH = 10, Weanling Lx = 6. The basal DA was obtained from the first dialysis session of each rat, and raw DA values were corrected for the recovery value of the probe used.

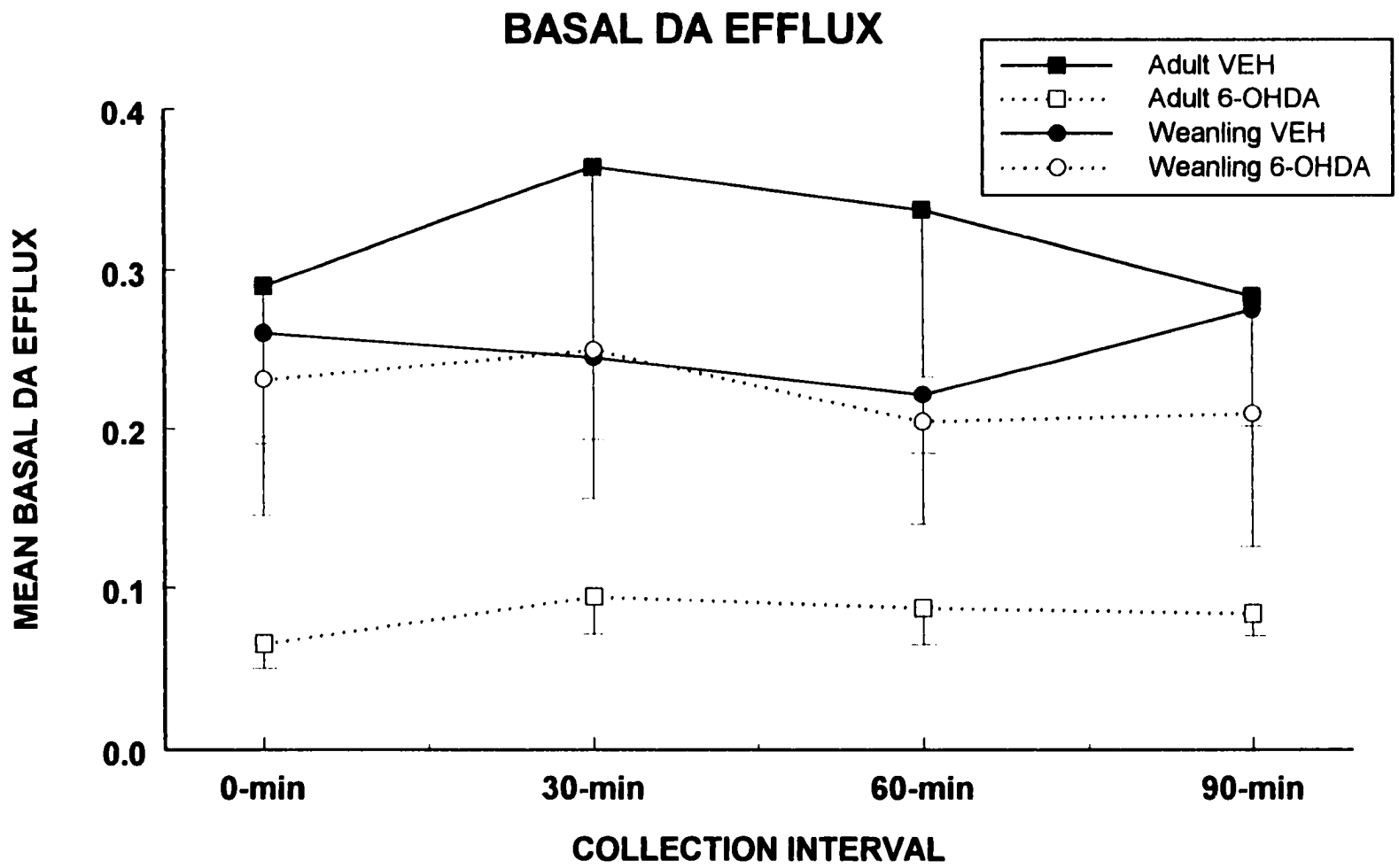
BASAL DA EFFLUX
Adult VEH
Adult 6-OHDA
Weanling VEH
Weanling 6-OHDA
MEAN BASAL DA EFFLUX
0.4
0.3
0.2
0.1
0.0
0-min
30-min
60-min
90-min
COLLECTION INTERVAL

Figure 4.10

Figure 4.11 FRACTIONAL DA EFFLUX

Figure 4.11 depicts the *fractional DA efflux* obtained from all rats in Experiment 2 as described in the Procedures Section of Experiment 2. The data represents the following n's: Adult VEH = 10, Adult Lx = 7, Weanling VEH = 10, Weanling Lx = 6. The fractional DA was obtained from the first dialysis session of each rat, and raw DA values were corrected for the recovery value of the probe used.

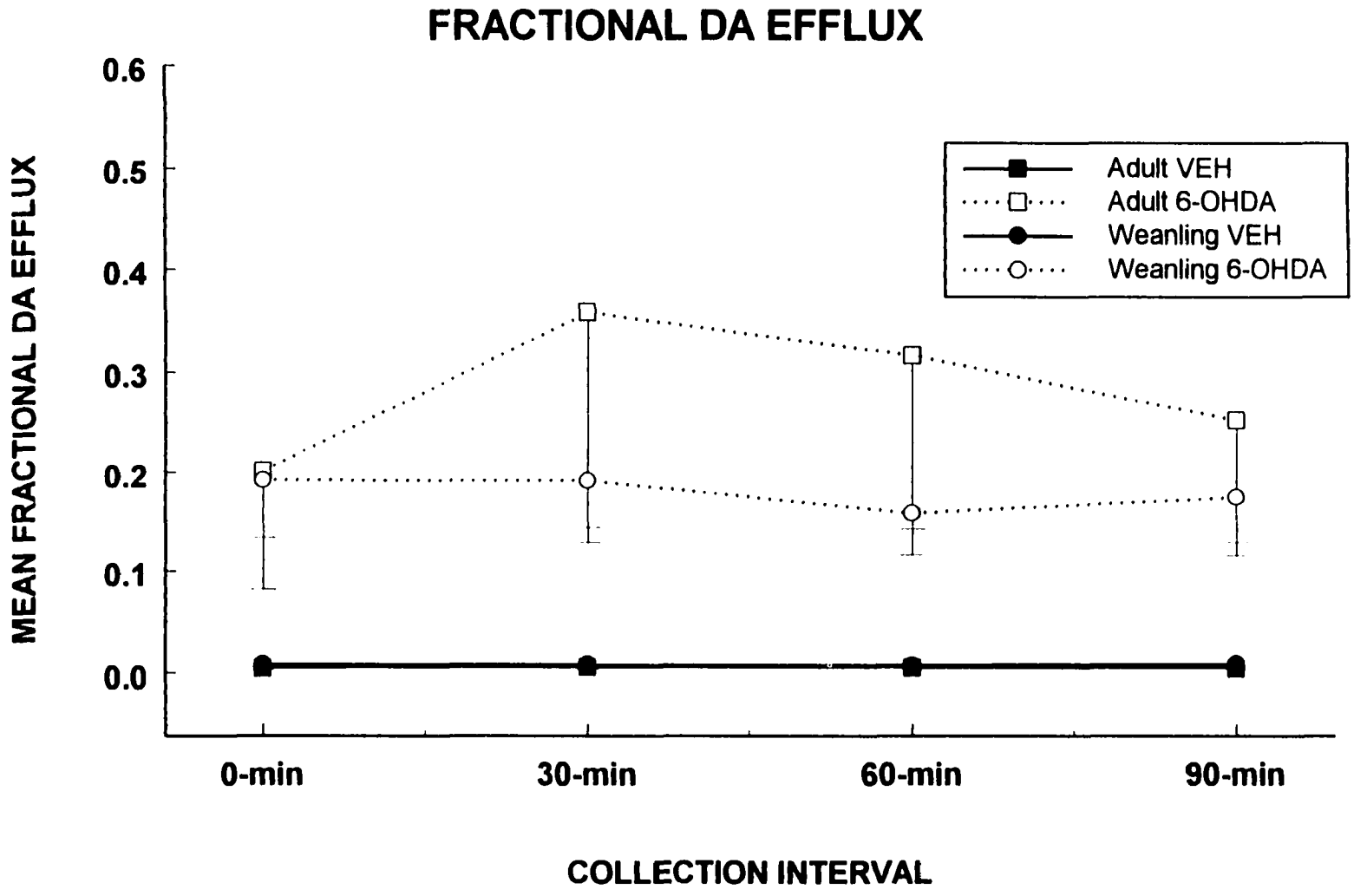
FRACTIONAL DA EFFLUX
MEAN FRACTIONAL DA EFFLUX
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Adult VEH
Adult 6-OHDA
Weanling VEH
Weanling 6-OHDA
0-min
30-min
60-min
90-min
COLLECTION INTERVAL

Figure 4.11

Figure 4.12 BASAL STRIATAL ACh

Figure 4.12 depicts the basal ACh obtained from all rats in Experiment 2. The data represents the following n's: Adult VEH = 10, Adult Lx = 7, Weanling VEH = 10, Weanling Lx = 6. The basal ACh was the median baseline collection (picomoles ACh per 20 µl dialysate), from the first dialysis session of each rat, corrected for the recovery value of the probe used.

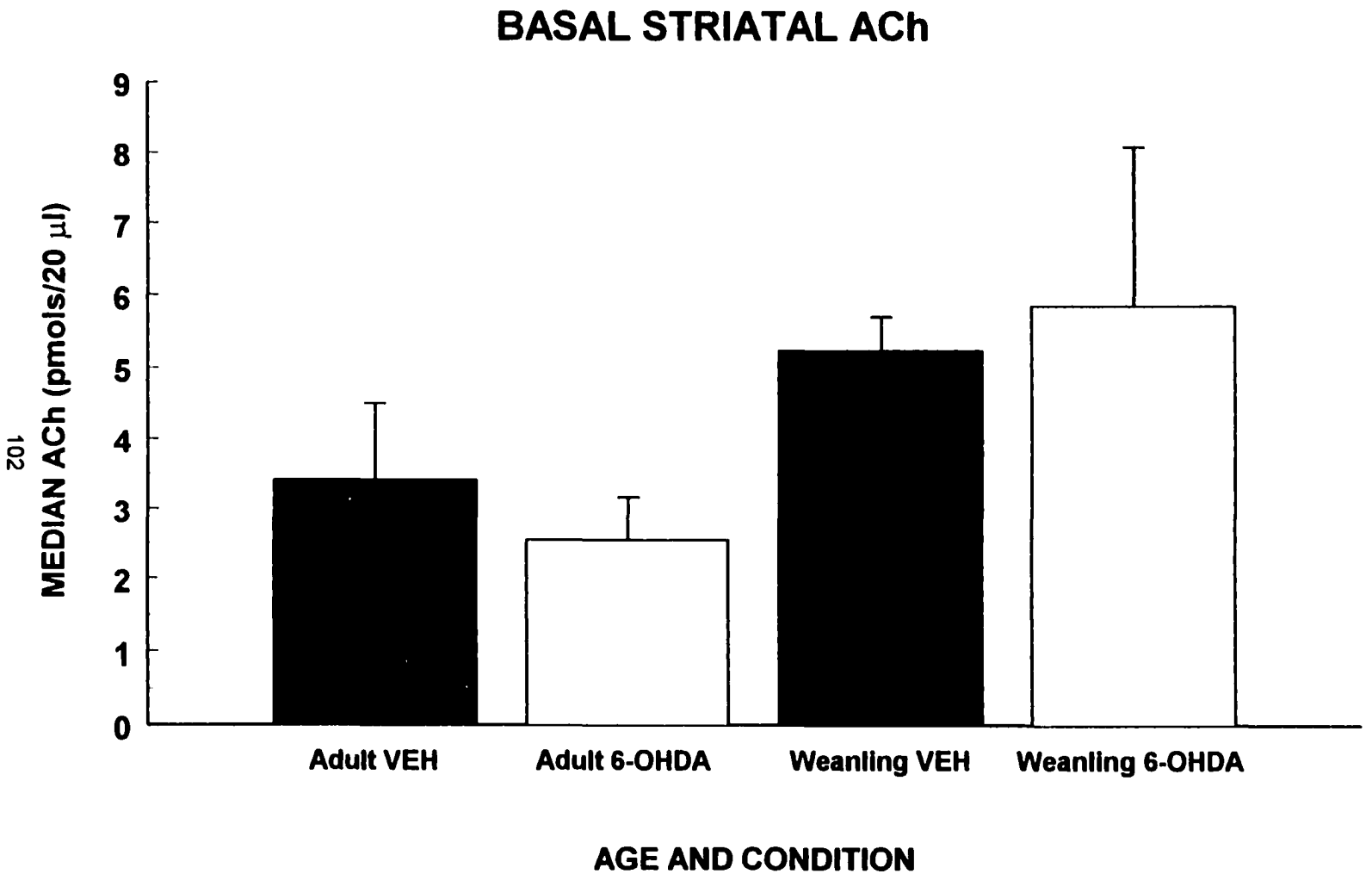
BASAL STRIATAL ACh
MEDIAN ACh (pmols/20 µl)
9
8
7
6
5
4
3
2
1
0
Adult VEH
Adult 6-OHDA
Weanling VEH
Weanling 6-OHDA
AGE AND CONDITION

Figure 4.12

Figure 4.13 Sulpiride Modulation of Striatal ACh

ADULT RATS

Figure 4.13 depicts the effects of 10 or 100 μM sulpiride perfused through the dialysis membrane in only the adult rats. The X-axis represents the last baseline collection (of four). The solid black line depicts the period during which the sulpiride was infused at either 10 or 100 μM sulpiride (10 = dotted lines on graph, 100 = solid lines on graph). At 75 and 90 minutes following probe insertion, two final collections were taken after drug was removed from the perfusion medium. Both 6-OHDA (open symbols) and vehicle (closed symbols) rats are shown. The abscissa depicts the percent change from the median baseline ACh efflux concentration (picomoles per 20 microliter). Mixed numbers of subjects were represented in this graph: VEH 10μM = 5; 6-OHDA 10μM = 5; VEH 100μM = 9, Lx 100μM = 7.

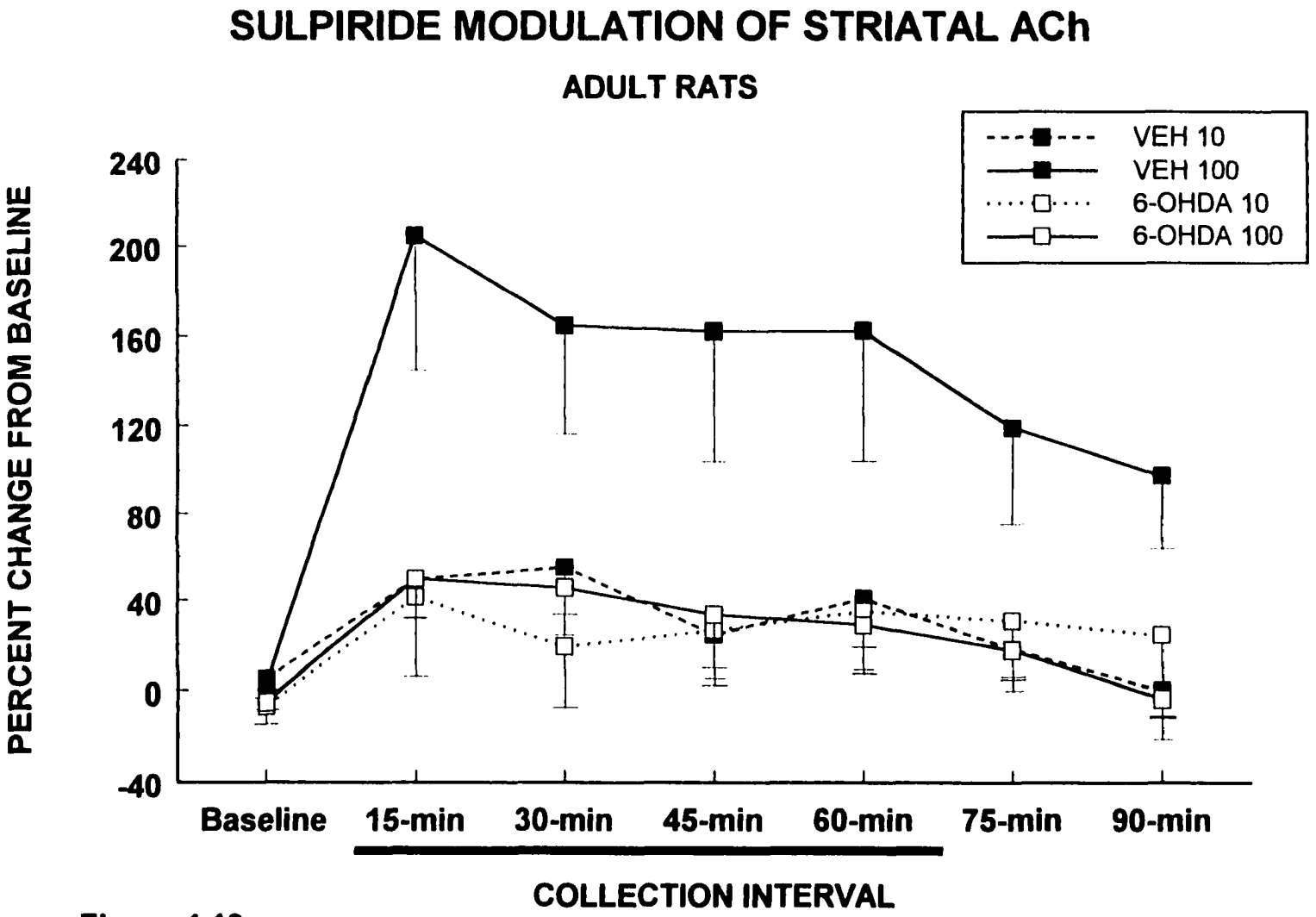
SULPIRIDE MODULATION OF STRIATAL ACh
ADULT RATS
VEH 10
VEH 100
6-OHDA 10
6-OHDA 100
PERCENT CHANGE FROM BASELINE
240
200
160
120
80
40
0
-40
Baseline
15-min
30-min
45-min
60-min
75-min
90-min
COLLECTION INTERVAL

Figure 4.13

Figure 4.14 Sulpiride Modulation of Striatal ACh

WEANLING RATS

Figure 4.14 depicts the effects of 10 (VEH n = 7, Lx n = 4) or 100 (VEH n = 9, Lx n = 6) μM sulpiride perfused through the dialysis membrane in only the weanling rats. The X-axis represents the last baseline collection (of four). The solid black line depicts the period during which the sulpiride was infused at either 10 or 100 μM sulpiride (10 = dotted lines on graph, 100 = solid lines on graph). At 75 and 90 minutes following probe insertion, two final collections were taken after drug was removed from the perfusion medium. Both 6-OHDA (open symbols) and vehicle (closed symbols) rats are shown. The abscissa depicts the percent change from the median baseline ACh efflux concentration (picomoles per 20 microliter).

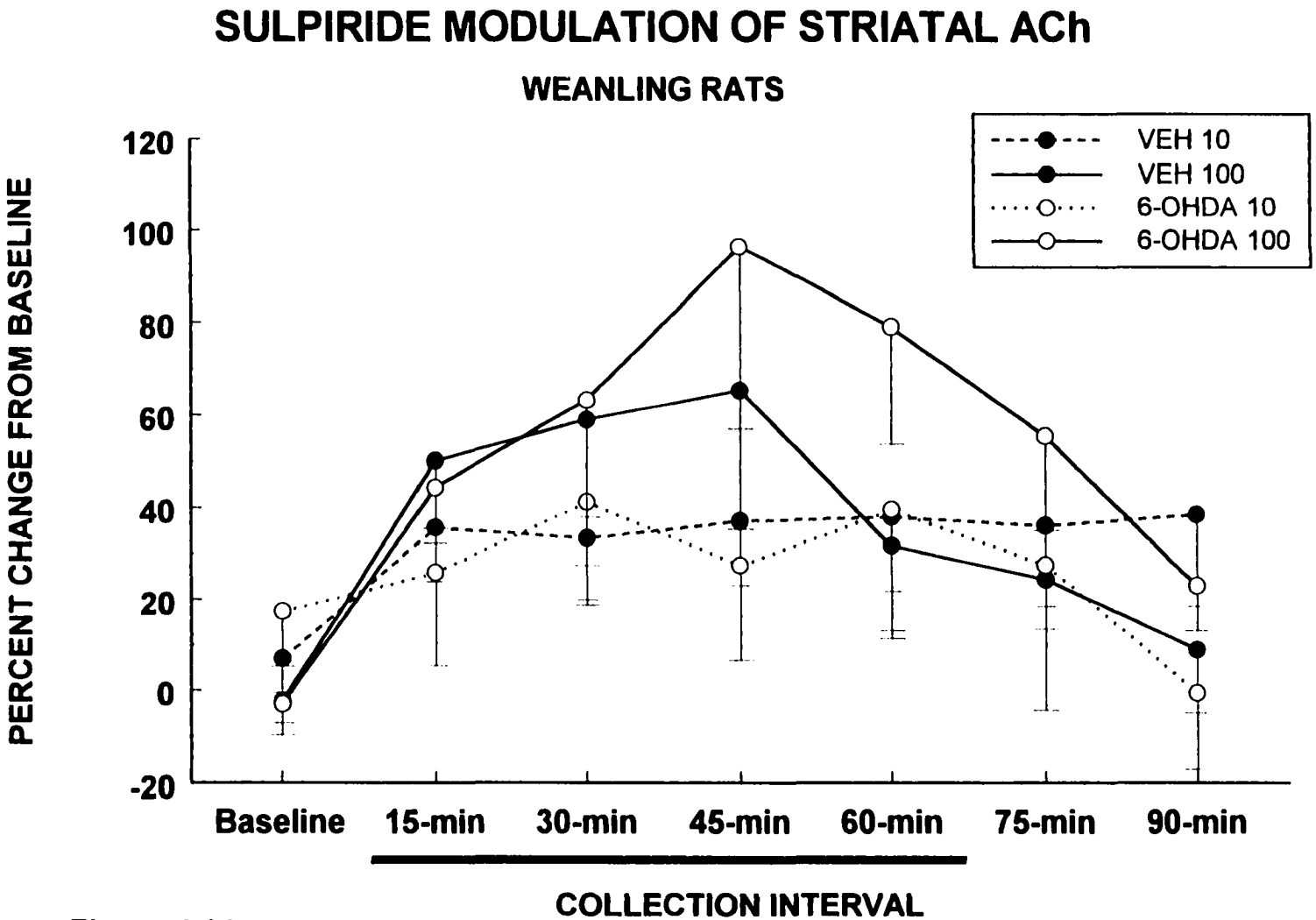
SULPIRIDE MODULATION OF STRIATAL ACh
WEANLING RATS
VEH 10
VEH 100
6-OHDA 10
6-OHDA 100
PERCENT CHANGE FROM BASELINE
120
100
80
60
40
20
0
-20
Baseline
15-min
30-min
45-min
60-min
75-min
90-min
COLLECTION INTERVAL

Figure 4.14

Figure 4.15 Sulpiride Modulation of Striatal ACh

BOTH AGES: 100μM Response

Figure 4.15 depicts the effects of 100μM sulpiride perfused through the dialysis membrane for both adult (VEH n = 9, 6-OHDA n = 7), and weanling (VEH n = 9, 6-OHDA n = 6) rats. The X-axis represents the last baseline (of four). The solid black line depicts the period during which the sulpiride was infused. At 75 and 90 minutes following probe insertion, two final collections were taken after drug was removed from the perfusion medium. Both 6-OHDA (open symbols), and vehicle (closed symbols) rats are shown. The abscissa depicts the percent change from the median baseline ACh efflux concentration (picomoles per 20 microliter).

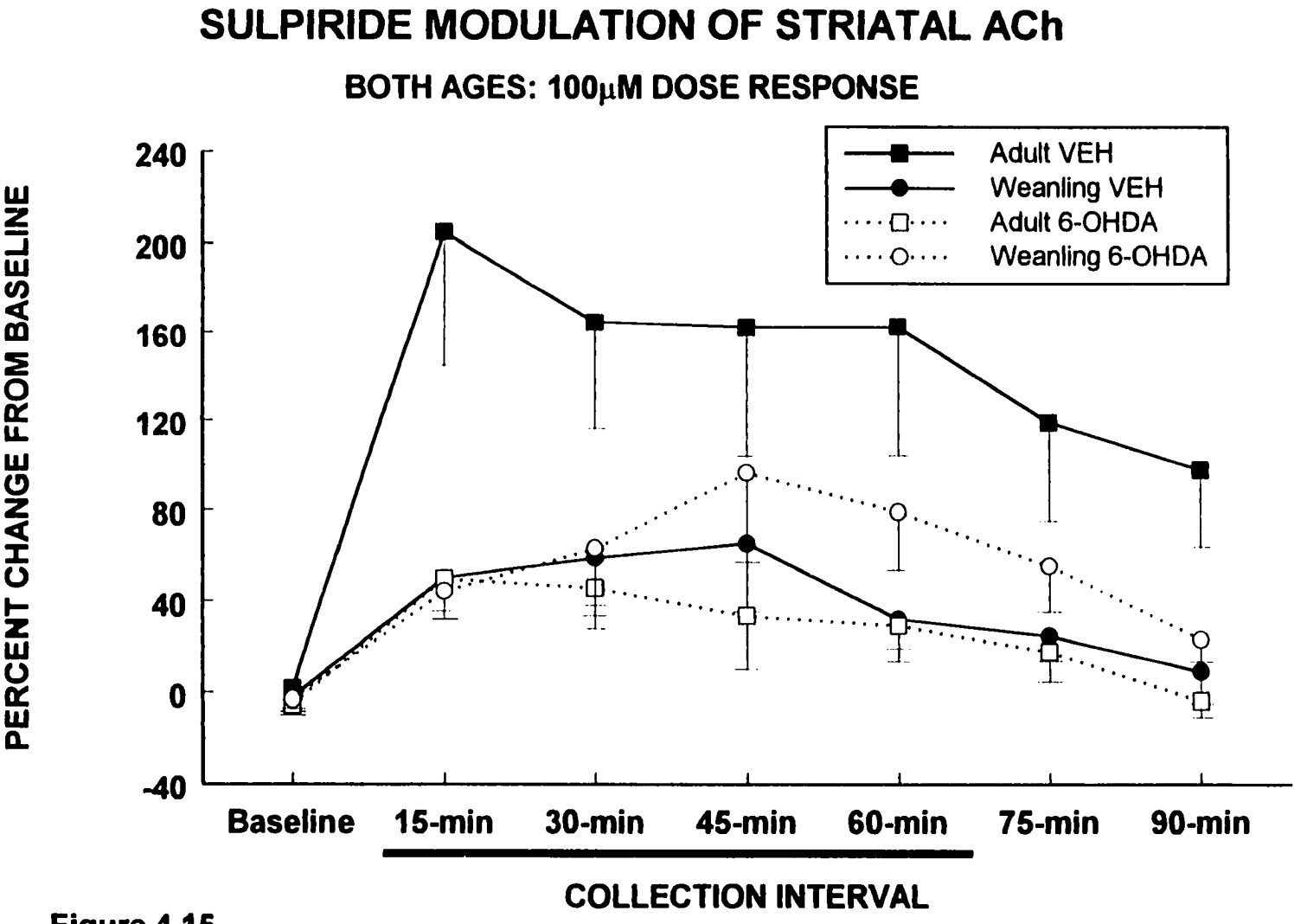
SULPIRIDE MODULATION OF STRIATAL ACh
BOTH AGES: 100μM DOSE RESPONSE
PERCENT CHANGE FROM BASELINE
240
200
160
120
80
40
0
-40
Adult VEH
Weanling VEH
Adult 6-OHDA
Weanling 6-OHDA
Baseline
15-min
30-min
45-min
60-min
75-min
90-min
COLLECTION INTERVAL

Figure 4.15

Figure 4.16 Sulpiride Modulation of Striatal ACh

BOTH AGES: TTX Response

Figure 4.16 depicts the effects of TTX perfused through the dialysis membrane on the basal ACh efflux and on the 100μM sulpiride drug effect. Two representative rats were selected from each AGE/COND cell for this experiment. Each line represents the mean results taken from two representative rats of each cell. The introduction of TTX is represented by the thick broken line. 100μM sulpiride was perfused during the interval marked by the thick solid line as in the other "Sulpiride Modulation" graphs. Two collections were taken before sulpiride, and two after. The abscissa depicts the percent change from the median baseline ACh efflux concentration (picomoles per 20 microliter).

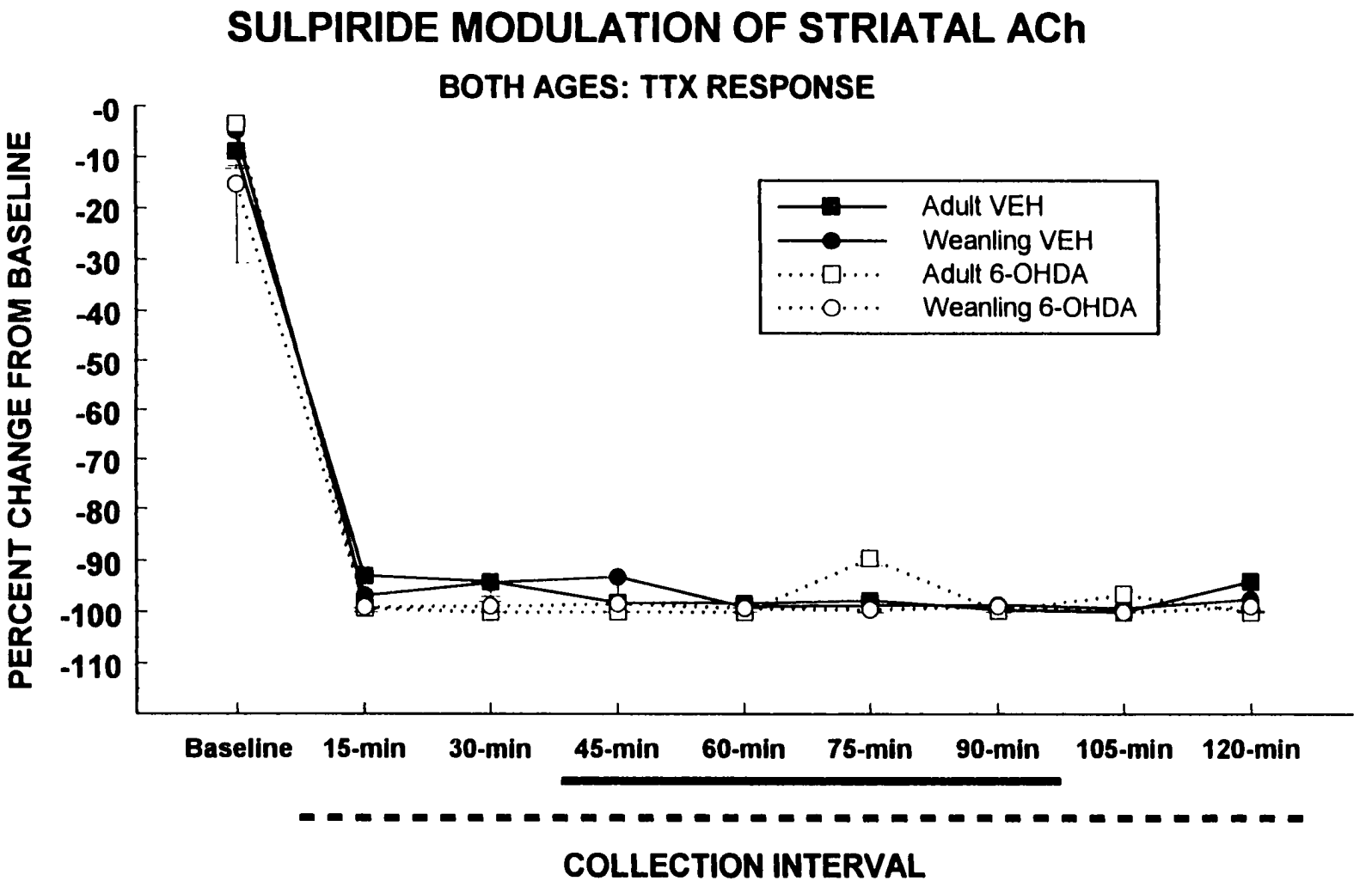
SULPIRIDE MODULATION OF STRIATAL ACh
BOTH AGES: TTX RESPONSE
PERCENT CHANGE FROM BASELINE
-0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
Adult VEH
Weanling VEH
Adult 6-OHDA
Weanling 6-OHDA
Baseline
15-min
30-min
45-min
60-min
75-min
90-min
105-min
120-min
COLLECTION INTERVAL

Figure 4.16

CHAPTER 5

GENERAL DISCUSSION

THE MAIN POINTS

The main overriding question driving this research was: why do rats that are DA depleted as weanlings not seem to suffer the same consequences of sensorimotor deficits as those DA depleted as adults? I've observed how dramatic the differences in sensorimotor effects of DA depletion can be during my time in this laboratory, depending upon the age of the rat at the time of DA depletion. The sensorimotor effects of weanling DA depletion in these experiments were more like *sparing* than the *accelerated recovery* demonstrated by my predecessors. This serves to increase the disparity between rats depleted of DA as weanlings versus those lesioned as adults in these experiments. So is striatal DA involved in supporting sensorimotor behavior by this age? Several studies have demonstrated that DA is clearly involved in sensorimotor behavior by postnatal day 27, even before weaning (day 21) (Van Hartesveldt et al., 1994; Meyer et al., 1993; Stehouwer et al., 1994). Some more subtle behavioral deficits can be revealed in rats unilaterally DA-depleted on postnatal day 21 with more sensitive tests which measure manual dexterity, grasping spatial navigation, tongue use, and limb posture (Abrous et al., 1992; Whishaw et al., 1987). It may very well be that such tests would have revealed deficits in the rats DA-depleted as weanlings in Experiment 2. My own observation that supports this would be that after breaking food pellets into smaller pieces, the weanling DA-depleted rats were more successful in eating the food as evidenced by its more rapid disappearance.

The main points of the two experiments in this dissertation need to be reiterated here. Experiment 1 was designed to test the sensitivity of rats depleted of DA as weanlings to the sensorimotor effects of striatal infusion of the D2-like DA antagonist sulpiride. This experiment was necessary because of the possibility that rats lesioned at this age would be insensitive to local striatal DA antagonism, possibly due to striatal DA activity no longer supporting the sensorimotor behavior they exhibit. This *sensitivity* was demonstrated with both akinesia and catalepsy in both the intact controls and 6-OHDA treated rats. The other interesting main result of Experiment 1 was the *lack of supersensitivity* to the sulpiride infusion in the DA-depleted rats. A reduced DA input into the striatum might be expected to result in a reduction of extracellular DA which would compete with the infused antagonist, resulting in a greater effect of lower doses. This did not happen with the DA-depleted rats of Experiment 1, and one likely explanation for this *lack of supersensitivity* would be that similar amounts of extracellular DA exist in the striata of both DA-depleted and intact vehicle controls at the time of drug infusion.

Experiment 2 demonstrated that *basal DA efflux* was not significantly different between the two conditions (vehicle, 6-OHDA) among the weanling rats. This *basal DA efflux* was significantly decreased in the DA-depleted adult rats compared with their age-matched vehicle treated controls. Therefore it seems that increased extracellular DA is a possible explanation for the lack of supersensitivity to striatal sulpiride infusion in *adult* rats which were DA-depleted as weanlings. DA also seems to be present in concentrations approximating the intact rats in these DA-depleted weanlings much sooner after DA-depletion than after similar lesions of rats *during adulthood.*

Whether this basal extracellular DA present soon after DA-depletion of weanling rats is actively modulating striatal neurons was the point of the second part of Experiment 2. Although none of the important results of this part of the experiment were significant, the data obtained suggests DA modulation of striatal ACh is reduced at this early time following DA depletion of adult rats, but possibly not soon after DA depletion of weanling rats. Although the evidence is not strong enough to reject the null hypothesis, it doesn't seem strong enough to accept the null hypothesis either.

RELATED POINTS

DA modulation of striatal ACh was the focus of the assay of striatal DA transmission following DA depletion in Experiment 2. There was precedence for this choice, namely, that striatal ACh comes exclusively from interneurons, that nigrostriatal dopaminergic terminals have been found in close association with cholinergic neurons (Dimova et al., 1993; Izzo et al., 1988), that anticholinergic drugs can reverse many symptoms of Parkinson's disease (Cedarbaum & Schleifer, 1990), and in-vitro work of Zigmond has already demonstrated a correlation between DA modulation of striatal ACh and sensorimotor recovery (MacKenzie et al., 1989). However this interaction is by no means the sole basis of DA support for sensorimotor behavior. Among the other transmitter systems of the basal ganglia, both glutamate and γ-aminobutyric acid (GABA) also have well established roles in sensorimotor behavior support. Evidence has been found supporting the hypothesis that the motor deficits of Parkinson's disease involve disruptions in glutamatergic striatal inputs, possibly due to overactivity arising from decreased dopaminergic control (Klockgether et al., 1993; Blandini et al., 1996; Greenamyre, 1993; Chase et al., 1996; Calabresi et al., 1996). Glutamate antagonists may therefore be beneficial additions to the traditional therapies given for Parkinsonism (Greenamyre, 1993). Glutamate antagonists can block catalepsy induced by local striatal infusion of the DA antagonist haloperidol in rats (Yoshida et al., 1994). The catalepsy response to acute local striatal sulpiride infusion can be dose-dependently blocked by injection of the $GABA_A$ receptor agonist muscimol into the substantia nigra reticulata, or by injections of the $GABA_A$ receptor antagonist bicuculine into the globus pallidus (Ossowska et al., 1993). Similar catalepsy can also be induced by muscimol injection into the globus pallidus, and this response is also blocked by muscimol injection into the substantia nigra reticulata (see **Figure 1.1**) (Ossowska et al., 1993). These effects all demonstrate the importance of GABA transmission in the basal ganglia for this response.

Experiments which were recently performed by Zigmond and colleagues to address the dopaminergic modulation of striatal [^{3}H]GABA rather than striatal [^{3}H]ACh efflux in vitro, revealed

results very similar to the case of [^{3}H]ACh. Sulpiride induced [^{3}H]GABA release from depolarized striatal slices decreased in association with decreases in tissue DA when slices were taken from rats three days after 6-OHDA. Three weeks after 6-OHDA produced large depletions of tissue DA (>90%) the sulpiride-induced [^{3}H]GABA efflux was normalized to intact levels (Harsing, Jr. et al., 1996). D2 dopamine receptors are typically associated with an inhibition of GABAergic medium spiny striatal neurons, so the D2-like receptor antagonist sulpiride would be expected to induce increased GABA through disinhibition, but only if these neurons are currently being inhibited by endogenous dopamine. So this experiment, like the above-described [^{3}H]ACh efflux experiment, suggests that DA does not only return to the striatal extracellular space after DA depletion, but it also resumes a postsynaptic role on the neurons there.

However the DA modulation of striatal ACh was the assay for striatal DA transmission used in these experiments, so an exploration of the inability to clearly demonstrate condition effects with this assay seems in order. There has been a controversy in the literature regarding the primary DA receptor system through which DA modulates ACh. The D2 receptor is expressed by striatal cholinergic neurons and has been linked to inhibition of ACh release (Stoof et al., 1992; LeMoine et al., 1990; Ikarashi et al., 1997; DeBoer et al., 1996; Stoof et al., 1992), while D1 receptors act to increase ACh release indirectly, likely through corticostriatal inputs (see **Figure 1.1**) (Damsma et al., 1990; Imperato et al., 1994; DeBoer et al., 1992). Part of this controversy involves the effects of *d*-amphetamine, as some studies show that this agent increases ACh efflux in the striatum in vivo (Damsma et al., 1991; Guix et al., 1992). If the effects of *d*-amphetamine primarily result from its induction of DA release from dopaminergic axons, then why might it increase ACh release given that the most direct effect of DA on cholinergic neurons might be expected to be inhibition via D2 receptors? Of course one possibility for this response might well be the effects of amphetamine-induced DA release on striatal D1-like receptors, but why amphetamine-induced DA release would effect one population of striatal DA receptors exclusively remains the crucial question regardless of which receptor response is proposed. Alternatively, the increase in ACh after *d*-amphetamine may depend on the neostigmine used during the experiment (Acquas et al., 1998; De Boer et al., 1996), and have less to do with the

physiological effects of *d*-amphetamine on DA release. The increased basal ACh efflux demonstrated by the present study in the weanling rats of either condition compared with the adult rats might suggest that there is less DA inhibiting cholinergic neurons through D2 receptors. Some studies make bold statements like, "spontaneous release of acetylcholine in striatum is preferentially regulated by inhibitory dopamine D2 receptors," (DeBoer et al., 1996). Other studies suggest that DA depletion will preferentially impair D1 rather than D2 receptor regulation of striatal ACh release (Bertorelli et al., 1992). If D1 receptors are more important in regulating basal striatal ACh efflux which was increased in the weanling rats, then the increased striatal ACh efflux found in these rats (see **Figure 4.12**) may be an indication of increased DA activity in their striata.

Decreased DA in the striatum may also lead to increased ACh efflux via the glutamatergic inputs into this region. Striatal glutamate release is regulated by DA via inhibitory D2-like receptors on glutamatergic terminals (Kornhuber et al., 1986; Yamamoto et al., 1992; Hsu et al., 1995). If DA is decreased in the striatum, glutamate release may therefore increase due to disinhibition, and stimulate cholinergic neurons more. The work of Jane Stewart is related to this same topic but has broader applications. Two papers relating behavioral recovery from partial 6-OHDA lesions of the substantia nigra came out of her laboratory in the past two years. One of them showed that both neurochemical (basal DA restoration), and behavioral (rotation) recovery was blocked by chronic application of D1, but not D2 DA receptor antagonists following a partial unilateral 6-OHDA lesion (Emmi et al., 1997). The other showed that these same components of recovery could be blocked with daily applications of the glutamate antagonists MK-801 and CPP. In fact, the DA efflux measured from intact and 6-OHDA treated rats which were either treated with MK-801 or saline (shown in their Figure 2) looks very similar to the *basal DA* obtained in Experiment 2 and shown in my **Figure 4.10**, such that the adult 6-OHDA treated rats of my experiment may exhibit similar basal striatal DA to those rats which received either chronic MK-801 or CPP. These data imply that either D1 receptor stimulation, or striatal glutamate activity, are very important for recovery of function to proceed following the lesion. The obvious

question then would be whether striatal D1 or glutamate receptor activity is increased in rats DA-depleted as weanlings compared with those DA-depleted as adults.

FUTURE DIRECTIONS

The main concern raised by these experiments is the lack of clear significant effects obtained for the second part of Experiment 2. It seems that more rats need to be tested to more fully represent the ages and conditions of this experiment. It also would be beneficial to explore higher doses of sulpiride in both the adult and weanling rats. One practical problem related to the dose of sulpiride is keeping this drug from precipitating out of solution when it is dissolved in higher concentrations. Sulpiride dissolves well in low pH. When it is put into the aCSF, which is made at a pH between 6.7 and 6.9, it will only stay in solution if it is in moderate concentrations. Therefore, it may be useful to explore alternative D2 antagonists which do not have this solubility problem.

The activated nocturnal phase of the rat circadian cycle presents another opportunity to explore the dynamic capacities of the nigrostriatal system following DA depletion. Greater extracellular DA might be expected during this nocturnal phase. It would be interesting to determine whether circadian rhythm is restored to the DA system soon after depletion of DA in weanling rats.

To more fully examine the correlation between striatal DA transmission and sensorimotor behavior following recovery from DA-depleting lesions, it would also be informative to investigate the effects of intrastriatal sulpiride infusions sooner after DA depletion of weanlings than in the experiments of this dissertation. Rats that are DA-depleted as weanlings recover very rapidly, so implantation of bilateral intrastriatal infusion cannula during the same surgery as when 6-OHDA is administered would allow testing of weanling rats immediately following their recovery of sensorimotor function. It may be that as yet unforeseen developmental factors render DA transmission less critical for sensorimotor function immediately following DA depletion at this early age, despite the present findings that suggest its role in this behavior during adulthood.

Finally, it would be interesting to see whether the DA modulation of striatal ACh differs when the rats tested are matched for their absolute age rather than their relative age, holding the age of testing constant rather than the time post 6-OHDA. It would be interesting if, for example, rats depleted of DA as weanlings but tested as adults become sensitive to 100 μM sulpiride. This would indicate the effects obtained in Experiment 2 were a developmental phenomenon rather than a procedural issue.

APPENDIX

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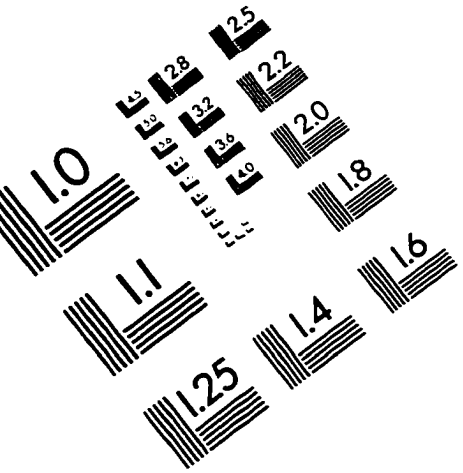

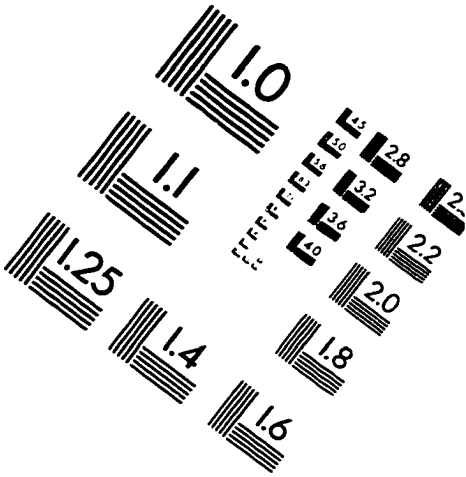

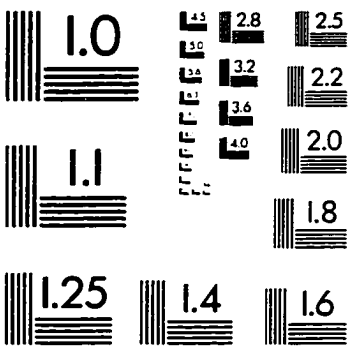

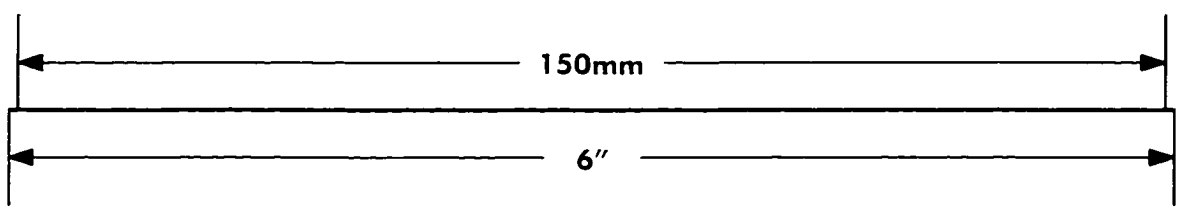

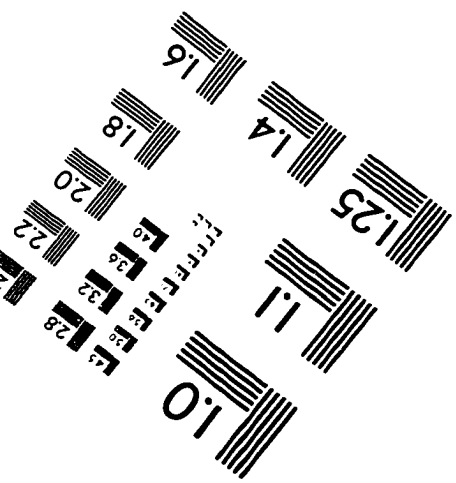

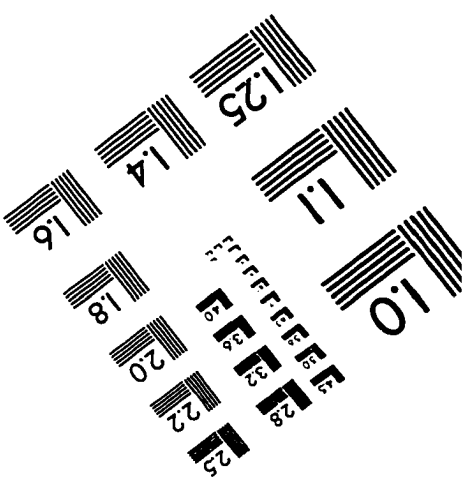

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