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An evaluation of neonatal dopamine-depletion in the rat as a model of
Attention-Deficit Hyperactivity Disorder

By

Karin Marie Korth

A thesis submitted in conformity with the requirements for the degree of
Master’s of Arts
Graduate Department of Psychology
University of Toronto

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An evaluation of neonatal dopamine-depletion in the rat as a model of Attention-Deficit Hyperactivity Disorder
Master's of Arts, 1998
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Abstract

The neonatally dopamine-depleted rat exhibits transient spontaneous hyperactivity, which is attenuated by the administration of stimulants. As such, this rat has been proposed as an animal model of Attention Deficit Hyperactivity Disorder. Rats were administered 6-hydroxydopamine (i.c.v.) on post-natal day 3, and tested for hyperactivity, and attention-deficits, using the 5-choice serial reaction time task. The dopamine-depleted group showed juvenile hyperactivity, and impaired choice-accuracy and response time on the 5-choice test. However, 5-choice task performance impairments occurred with a more general behavioural depression and learning deficit. In addition, treatment with amphetamine did not ameliorate these deficits. These results suggest that neonatal dopamine depletion does produce the symptoms of ADHD, however a more substrate-specific lesion producing a subtler DA-dysfunction would provide a more accurate animal model of the disorder.
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Attention Deficit Hyperactivity Disorder

Attention Deficit Hyperactivity Disorder (ADHD) is a developmental disorder characterized by the broadly defined symptoms attention deficit, hyperactivity and impulsivity (DSM-IV, 1994). The disorder persists into adolescence in 50 – 80%, and into adulthood in 30 – 50% of all cases clinically diagnosed in childhood (Barkley, 1997). However, the chief manifestations of the syndrome appear to shift with development, such that ADHD has been subdivided into “predominantly hyperactive-impulsive”, “predominantly inattentive”, and “combined type” (DSM-IV, 1994). The hyperactive-impulsive type appears to emerge first, as it is chiefly found among preschool-aged children, with combined-type and predominantly inattentive-type being diagnosed in older, school-aged children (Barkley, 1997). Whether this is evidence of distinct and independently occurring subtypes of ADHD, a developmental time-course of the disorder, or simply the effect of changing environmental demands on the child is not clear. Moreover, “current research on ADHD is nearly atheoretical … [and] mainly exploratory and descriptive” (Barkley, 1997, p.66). In the absence of an identified core deficit, it is difficult to postulate an underlying organic cause to this disorder. However, the results of this exploratory and descriptive research do converge, giving rise to the “catecholamine hypothesis” of ADHD. The catecholamine hypothesis is currently protean, and does not specify beyond catecholaminergic involvement in the disorder.
ADHD and Catecholamines

Clinical Research

The catecholamine hypothesis is largely based on the observation that ADHD (subtypes notwithstanding) is most commonly and efficaciously treated with the stimulants methylphenidate and d-amphetamine (Barkley, 1977), implying that catecholaminergic, and potentially dopaminergic dysfunction is involved. However the precise nature of this dysfunction is unclear, as these stimulants have a paradoxical calming effect on children with ADHD. Further, both are non-specific drugs, affecting dopaminergic, adrenergic and noradrenergic transmission by releasing and/or inhibiting the reuptake of these neurotransmitters. Examination of catecholamine metabolites in ADHD patients has done little to elucidate the involvement of these neurotransmitters in the disorder. Several examples of findings in this area are provided to illustrate. With regard to dopamine (DA), Shaywitz, Cohen and Bowers (1977) found lowered cerebro-spinal fluid (CSF) homovanillic acid (HVA, the major metabolite of dopamine) levels in ADHD patients. However, Shetty and Chase (1976), found no difference in baseline CSF HVA, but a significant decrease in post-treatment (d-amphetamine) HVA in children with ADHD as compared to controls. Interestingly, this decrease was significantly correlated \( r = -0.94 \) with clinical measures; as post-treatment HVA levels decreased, clinical measures improved.

Regarding the involvement of other catecholamines, both d-amphetamine and methylphenidate have been reported to increase urinary epinephrine levels (Elia et al., 1990). Adding norepinephrine (NE) to the equation, Shekim, Javaid, Dekirmenjian, Chapel & Davis (1982) measured urinary metabolites in children with ADHD who had been divided clinically into “responders” (to amphetamine) and “non-responders”. Not only did these two
groups display different baseline urinary metabolite profiles (only responders’ HVA levels were lower than those of control children), and response to amphetamine (excretion of MHPG, the main noradrenergic metabolite, was only decreased in responders), but the responding group was further subdivided. Specifically, some “responders” exhibited normal levels of MHPG and low levels of HVA, while others displayed the opposite profile. The authors speculate that amphetamine’s therapeutic efficacy in the former is through its effects on presynaptic norepinephrine and dopamine autoreceptors, and in the latter through inhibition of NE and potentiation of DA (affecting NE-DA interaction).

More recently, Castellanos et al. (1994) examined boys diagnosed with ADHD for correlations between CSF HVA and scores on multiple behavioural scales. Significant correlations were found, but they were not consistent across different scales measuring the same behaviour. HVA was found to be positively correlated with activity levels on only two of the four tests measuring hyperactivity, and only one test of aggression. Further, HVA was positively correlated with scores on one inattention scale, but not with either omission error (attention) or commission error (impulsivity) scores on the Continuous Performance Test. MHPG levels were positively correlated only with one measure of aggression, and a “total behaviour problems” score.

It is thus apparent that measures of catecholaminergic metabolites in ADHD are at best inconclusive, providing neither a clear picture of stimulants’ mechanism of action, nor one of the catecholamines’ role in the symptoms of the disorder. One possible explanation for this diversity among ADHD patients, as with any behaviourally diagnosed disorder, is that distinct sub-populations exist whose independent biological anomalies manifest in similar or overlapping behavioural symptoms - in short, that ADHD is actually several as yet
undifferentiated disorders. The division of the disorder into subtypes may be a move in this direction. As the above-mentioned clinical division of ADHD into subtypes is a recent development (Barkley, 1997), these disparate metabolite-study findings may in fact reflect different populations of ADHD patients.

An experiment measuring cerebral blood flow in ADHD children lends indirect support to a specific dopamine theory of ADHD. Lou and colleagues (1989) found hypoperfusion of striatal regions, which constitute a portion of multiple dopaminergic systems. Treatment with methylphenidate increased flow to the striatum, suggesting that methylphenidate increases (dopaminergic) striatal activity in ADHD children (Lou, Henrikson, Bruhn, Borner & Nielson, 1989). This could represent a normalisation of striatal activity by methylphenidate in ADHD.

Finally, indirect behavioural support for the catecholamine (specifically dopamine) hypothesis of attention, and of ADHD, comes from studies of prepulse inhibition (PPI). PPI is the attenuation of the startle reflex following presentation of a sub-threshold stimulus. Children with ADHD in absence of co-morbid disorders such as Tourette's syndrome have shown prepulse-inhibition (PPI) of the startle reflex greater than that seen in control populations (Castellanos Fine, Kaysen, Marsh, Rapoport & Hallet, 1996). The finding that PPI is disrupted in rats by increased dopamine function is a robust one (Davis, Mansbach, Swerdlow, Campeau, Braff & Geyer, 1990); the dopaminergic region most important in modulating prepulse inhibition is the nucleus accumbens (Hoffman & Donovan, 1994). This disruption can be blocked by pretreatment with dopamine antagonists (Hoffman & Donovan, 1994). Haloperidol (a dopamine receptor antagonist) alone potentiates PPI
(Depoortere, Perrault & Sanger, 1997). Thus enhanced PPI in ADHD patients is consistent with a suggested deficit in dopamine function.

Attempts to relate enhanced PPI in ADHD patients to the symptoms of the disorder involve speculation on the nature of the deficit in Attention-Deficit Hyperactivity Disorder. Braff and Geyer (1990) posit prepulse inhibition as indicative of "sensorimotor gating". The inability to "gate" sensory input (disrupted PPI) is said to lead to "sensory flooding" in schizophrenic patients (Swerdlow, Braff, Taaid & Geyer, 1994). When related to ADHD, however, PPI is interpreted differently: it has been proposed that elevated PPI reflects heightened sensitivity to the prepulse (sub-threshold stimulus), and is consistent with hypersensitivity to irrelevant (sub-threshold) environmental stimuli. Thus, this position states that "attention-deficit" is not the result of impaired attending, but rather, impaired filtering (Castellanos et al., 1996). If this is the case, enhanced prepulse inhibition may be a manifestation of the attention "deficit" itself.

Animal Models

Although metabolite studies in this area are thus far inconclusive, and the blood-flow and PPI findings address the dysfunction underlying ADHD only indirectly, further evidentiary support for the dopamine hypothesis of ADHD exists in animal research. One animal model of ADHD involves inducing a dopaminergic deficit early in development. 6-hydroxydopamine, a catecholaminergic neurotoxin, when administered in early life, results in a dramatic depletion of dopamine; pretreatment with a noradrenergic blocker will spare the NE system. Although the significance of this effect is not known, this treatment in neonatal animals also results in serotonergic hyperinnervation (Schallert & Wilcox, 1985). It
is evident that development plays a role here, as the same procedure used in adult animals results in severe behavioural suppression (adipsia, aphagia, apraxia), whereas young animals will nurse normally and appear healthy (Potter & Bruno, 1991). Many experiments have shown that neonatal rat pups given (6-OHDA) lesions exhibit a hyperactivity syndrome not seen in animals given the same treatment in adulthood (Erinoff, MacPhail, Heller & Seiden, 1978). Erinoff et al. (1978) suggest that the maturation of neuronal systems that use dopamine during development is necessary for suppression of locomotor activity. Miller, Heffner, Kotake and Seiden (1981) expanded on this finding to produce a ‘dose-response-curve’ relating magnitude and duration of hyperactivity to the extent of the dopaminergic depletion: clearly, as the level of dopamine decreases, activity level increases.

This spontaneous hyperactivity effect is necessary but not sufficient to qualify neonatal depletion of dopamine as an animal model of ADHD, or even of the hyperkinesis symptomatic of the disorder. One other requirement is responsiveness to treatment. Drugs that alleviate symptoms of ADHD in children should attenuate hyperactivity in these animals. This has been demonstrated for both amphetamine (Shaywitz, Klopper, Yager & Gordon, 1976) and methylphenidate (Shaywitz, Klopper & Gordon, 1978). Contradictory results have also been reported; for example, Pappas, Gallivan, Dugas, Saari and Ings (1980) have also investigated the effects of stimulants on hyperactivity in neonatally DA-depleted rats, and found both amphetamine and methylphenidate to increase activity levels. However, it should be noted that the doses used (1.0 and 2.0 mg/kg, respectively) were relatively high. Thieme, Djikstra and Stoof (1980) conducted a similar experiment, and found amphetamine (0.75 mg/kg) to have no significant effect on 6-OHDA-treated animals. The reason behind the conflicting results of these neonatal lesion experiments is unclear. Surgical procedure
and drug administration methods appear to be similar across studies. One possible explanation is inter-experiment differences in the measurement of activity, but sufficient detail is not provided in these reports to accurately assess this. Notwithstanding, an overview of the research seems to support the validity of the neonatally DA-depleted rat as a model of at least the hyperactivity seen in ADHD.

It should be noted that 6-OHDA-induced hyperactivity appears to require dopaminergic (as opposed to merely catecholaminergic) depletion. Luthman, Fredriksson, Lewander, Gosta and Archer (1989) examined the effects of low and high doses of d-amphetamine and methylphenidate on rats given 6-OHDA as neonates with two different pretreatments: desipramine (a norepinephrine uptake blocker – to produce preferential dopamine depletion) and amfolenic acid (a dopamine uptake inhibitor – to produce preferential norepinephrine depletion). The desipramine-6-OHDA treatment produced spontaneous hyperactivity, the amfolenic acid-6-OHDA treatment did not, suggesting that the hyperactivity is a dopaminergically, and not noradrenergically mediated effect. Low doses of both amphetamine and methylphenidate decreased forward locomotion in the dopamine-depleted group, and actually increased it in the norepinephrine-depleted and control groups.

A second animal model of ADHD in use is the Spontaneously Hypertensive Rat (SHR). Originally intended (and widely used) as an inbred model of essential hypertension, SHR rats display spontaneous hyperactivity that is attenuated by stimulants (Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995). Although the reason for this locomotor activity pattern remains unknown, the SHR has been proposed as a model of ADHD because the behavioural pattern that resembles that seen in ADHD is of endogenous origin; it
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presents in the absence of surgical, drug or environmental manipulation. Interestingly, regarding the DA hypothesis of ADHD, the SHR has altered dopaminergic functioning in terminal areas of the mesocortical, nigrostriatal and mesolimbic DA systems (Russell et al., 1995).

Despite the support the early DA-depletion model provides for the dopamine-deficit hypothesis of ADHD, the disorder involves more than hyperactivity; in fact, as mentioned above, attention-deficit symptoms can present independently of hyperactivity. Therefore, in order to test the validity of the model, the performance of neonatally 6-OHDA-treated rats on a test of attention needs to be examined. It is reasonable to predict that an attentional deficit will arise, as evidence implicating dopamine in attention is strong.

Attention and Dopamine

A role for dopamine in attention is suggested by its apparent involvement in several disease states which of which attention deficit is a component. These include Parkinson’s disease, schizophrenia and ADHD (Clark, Geffen & Geffen, 1987b). Specific to ADHD, the amelioration of attention-deficit by stimulants has been shown to be dopamine-mediated. Levy and Hobbes (1988) reported that pretreatment with haloperidol (a D2 antagonist) to block the performance-enhancing effects of methylphenidate on a vigilance task in ADHD-patients. Consistent with these results, in normal human subjects, droperidol (a dopamine receptor blocker) decreased the target discrimination (hit) rate in a sustained attention task (Clark et al., 1987b). The administration of methylphenidate following droperidol blocked this effect (Clark et al., 1987b).
Manipulations of the dopaminergic system are known to affect the performance of adult animals in attentional tasks. The most dramatic illustration of an attention-deficit is that of hemineglect. Unilateral injection of haloperidol into the striatum causes neglect of the side contralateral to the injection (Hoyman, 1980), as does a unilateral lesion of the striatum (a highly dopaminergic structure) (Marshall, Berrios & Sawyer, 1980). Apomorphine (a DA-agonist) reverses this neglect (Marshall & Gotthelf, 1979). Dopamine itself, injected unilaterally into the striatum, produces an increase in “contralaterally directed behaviour” (Joyce, Davis, & Van Hartesveldt, 1981).

Subtler (and perhaps more relevant to ADHD) deficits have also been produced in rats through DA-ergic manipulation, however the implications of these data are less clear. In a two-choice, attention task involving operant responding, amphetamine and cocaine had effects dependent on both dose and the precise nature of the task (Grilly, Gowans, McCann & Grogan, 1989). Sustained attention (vigilance) was improved by low doses of amphetamine and cocaine, and choice latency was reduced on the vigilance task. Selective attention (involving distracting stimuli) was unaffected by low doses of these drugs, however all doses of both drugs reduced choice latency in this task. Further, high-dose amphetamine disrupted selective attention-accuracy. Thus, there does not appear to be a single action of either dopamine or amphetamine on attention in this case. In accord with this do Brockel and Fowler (1995) report a finding. In an attention task in which animals were trained to respond to only one of three spatial discriminative stimuli, chronic systemic haloperidol treatment led to a decrease in the number of trials completed, and increases in both the percentage of omission errors, and reaction time. Amphetamine reversed these impairments. A high dose of amphetamine alone, however, increased the percentage of
omission errors, leaving reaction time unaffected. It is unclear whether this task measures sustained or selective attention; although the authors refer to it as a sustained attention task, the inclusion of stimuli that are not to be responded to suggests a selective attention component to this task.

One widely used attention task for rats is analogous to a test used to assess attention in humans. The 5-choice serial reaction time test requires a rat to attend to and spatially discriminate visual stimuli occurring at one of five locations and respond correspondingly (described in detail under ‘methods’). Under baseline conditions, this is a test of sustained attention; however distracting stimuli can be added, allowing the test to measure selective attention. This test has been used extensively to investigate the neurochemistry and anatomical structures involved in attention. Cholinergic (Jakala et al., 1992), noradrenergic (Cole & Robbins, 1987), serotonergic (Carli & Samanin, 1992, Jakala et al., 1992, Harrison, Everitt & Robbins, 1997) and dopaminergic (Cole & Robbins, 1989) manipulations have been shown to affect different aspects of performance on this task. Cole and Robbins (1989) performed 6-OHDA lesions of the nucleus accumbens septi of adult rats; they found a transient impairment in speed and impulsivity of baseline responding, but no impairment in discrimination accuracy. Jakala et al. (1992) administered para-chlorophenylalanine (PCPA), an inhibitor of serotonin synthesis with depletion effects lasting approximately 8 days to adult rats; this resulted in a tendency (approaching significance) for the rats’ baseline discriminative accuracy to be impaired, and overall responding to be decreased. Once the task was manipulated to increase the burden on attentional processing, a deficit in discriminative accuracy induced by PCPA was revealed. Jakala et al. (1992) note that frontal cortical dopamine was reduced (42%) by PCPA treatment, and posit that the decrease in
overall responding is 'in line' with a reduction in dopamine, as dopaminergic activation has been shown to cause general behavioural activation in this task (Cole & Robbins, 1989). Finally, Harrison et al. (1997) performed 5,7-DHT lesions (i.c.v.) on adult rats, which resulted in unaltered discriminative accuracy, but increased impulsivity (the number of premature responses). Administration of a DA receptor antagonist (SCH 23390) reversed the impulsivity. This suggests a DA-5-HT interaction, as the authors posit that "increased impulsivity may be mediated by an altered 5-HT-dopamine interaction, with the [5,7-DHT] lesion removing an inhibitory influence over dopamine neurotransmission" (Harrison et al., 1997). Therefore, multiple transmitter systems appear to be involved in performing attentional tasks, as measured by the 5-choice serial reaction time test. However, results derived from the use of this paradigm do lend further support to the notion of a dopaminergic contribution to attention.

Only one study to date has attempted to apply the 5-choice serial reaction time task to an animal model of ADHD. This study did not employ any exogenously induced impairments (i.e. lesions or drug administration), instead dividing rats' baseline performance into 'normal' and 'poor' (Puumala et al., 1996). It was observed that the 'poor' responders (those with low accuracy scores) also had longer stimulus-response latencies than the 'normal' group. This is consistent with the findings of Cole and Robbins (1989), as it suggests a deficit in the timing of responses. Subsequent administration of a low dose (100μg/kg) of methylphenidate was found to slightly improve the performance of poorly performing rats (both impulsivity and accuracy), without altering the performance of normal animals. A high dose (1000μg/kg) was found to increase impulsivity in both groups. More recently, an attempt has been made to establish neurochemical correlates of performance in
non-manipulated rats. Puumala and Sirvio (1998) determined that lowered frontal
dopaminergic transmission, and heightened frontal serotonergic transmission correlate with
impaired 5-choice task performance accuracy, and increased impulsivity.

The Present Study: Objectives and Hypotheses

Most investigations of attention have used animals manipulated as adults. These are
of limited use to the study of ADHD, given the disorder's developmental nature. There are
practical limitations to the examination of attention in animals during development, as
attention tasks generally require operant responding, necessitating training, and the rodent
"juvenile" period is not very long. However, in light of the above-mentioned recent (DSM-
IV, 1994) division of ADHD into sub-disorders based on predominant symptoms, and the
observation that the attention-deficit pattern may outlast the hyperactivity, it would be of
interest to examine attentional performance in the neonatally DA-depleted rat, after the
hyperkinesis has waned. Despite this, very few attempts have been made to extend the
neonatal DA-depletion model beyond hyperactivity.

The present study investigated the effects of neonatal dopamine depletion on several
behavioural markers of ADHD, including, but not limited to, hyperactivity. Thus, neonatally
dopamine-depleted rats were tested using the prepulse inhibition paradigm and the 5-choice
serial reaction time task (as a test of sustained attention).

Given the apparent relationship between dopaminergic transmission and prepulse-
inhibition, it was expected that the dopamine-depleted rats would display elevated pre-pulse
inhibition (greater inhibition of the startle reflex), as the ADHD boys examined by
Castellanos and colleagues (1996) did.
Attentional impairments are most important for the evaluation of the DA-depleted rat as a model of ADHD. For the model to be useful, three criteria must be met: construct, face, and predictive validity. Construct validity is provided by the convergent evidence implicating dopaminergic function in various forms of attention (including studies using the 5-choice task – Cole & Robbins, 1989), and dopaminergic dysfunction in Attention Deficit Hyperactivity Disorder. Thus far, the only face validity established for this model has been the observed hyperactivity during the juvenile period. However, regarding the 5-choice task, it is reasonable to assume that depletion of dopamine would result in performance impairments. The precise nature of these impairments, however, is less easily predicted. The strictest measure of attention in the 5-choice task is ‘accuracy’, consisting of correct responses, incorrect responses (errors of commission), and failures to respond (errors of omission). It was assumed that accuracy would be impaired; as such, the correct response rate would be decreased, and incorrect response and omission rates would be increased in the DA-depleted animals. Regarding measures of behavioural inhibition, it was assumed that the 6-OHDA lesion would produce impulsivity (elevated premature responding), concordant with the known hyperactivity effect. The neonatally dopamine-depleted rat, then, should represent the original conception of ADHD, now classified as “combined type”. Finally, these impairments should be ameliorated by the dopamine agonists used therapeutically in humans (predictive validity); thus a range of doses of amphetamine were tested for their therapeutic efficacy (ability to reverse the attention deficits).
EXPERIMENT 1:

This experiment was conducted to replicate the well-documented locomotor activity-effect of neonatal 6-OHDA lesions, and examine the effects on startle reflex and prepulse-inhibition.

Methods

Subjects

Sixteen Long-Evans hooded rats were used in this study. Timed pregnant dams were ordered (Charles River, Que.), and the pups were born on the premises, and housed as litters with dams until post-natal day (PND) 25. Nursing cages were clear Plexiglas with a wire mesh top. Post-weaning, all animals were individually housed in hanging Plexiglas cages. The housing room was maintained on a 12 h light/dark schedule (lights on at 07:00 h) and at a temperature of 22± 2°C. Food and water were available ad libitum unless otherwise specified. Treatment was assigned by litter, so that pups with depletions did not compete with control pups to nurse. All treatments and tests took place during the light phase.

Surgery

On PND3, the experimental pups were administered 6-OHDA, the control group were given vehicle. All pups were removed from the home cage, given 10mg/kg nisoxetine (to prevent noradrenergic depletion) dissolved in 0.9% saline (s.c.), and returned to the dams for 30 min. Following this, they were refrigerated until cryo-anaesthesia was achieved, and placed in a modified stereotaxic device. A sagittal incision was made, an injector (30GA stainless steel tubing) was lowered 3 mm into the brain, 1.5 mm lateral to bregma. 6-OHDA (150μg in 5μl vehicle) or vehicle (1% ascorbic acid) alone was infused into the lateral
ventricle using a gravity-feed. The injector was held in place for 1 min to allow diffusion from the tip, then raised. This procedure was then immediately repeated on the other side. The incision wound was closed with superglue. The pups were placed in a warmed recovery chamber until consciousness was regained, then returned to the dam.

Locomotor Activity

Apparatus

Activity tests were conducted in four Plexiglas activity chambers (Med Associates Inc., St. Albans, VT) measuring 40 cm long, 40 cm wide, and 28 cm high. Horizontal movement was detected by two arrays of 16 infrared beams. Movement was recorded in five-minute time bins by a 386-SX IBM-compatible computer. The activity score generated was forward locomotion outside of a two-by-two beam square which represented the area occupied by the rat’s body.

Procedure

Each trial was 60 min. One habituation session (60 min.) preceded testing. All sessions were conducted in a quiet room under low light, to create low-anxiety conditions. After baseline activity scores were collected on PND 21, response to a 0.5 mg/kg amphetamine-challenge was measured. Animals were assigned to either amphetamine or vehicle (0.9% saline) in counter balanced order, 72 hours apart. Drugs were given i.p., 10 minutes before the beginning of the test. d-Amphetamine sulphate was obtained from RBI.
Pre-Pulse Inhibition

Apparatus.

Testing was conducted in four SR-Lab Startle Response Systems (San Diego Instruments, San Diego, CA). Each system consisted of a 37.5 x 40.0 x 57.5 cm isolation cabinet housing the startle chamber. The startle chamber consisted of an 8.2 cm Plexiglas cylinder mounted on a 12.5 x 25.5 cm Plexiglas platform with a piezoelectric accelerometer unit attached to the bottom of the platform. The accelerometer detects and transduces motion within the startle chamber and these signals are digitized, rectified, and recorded by an IBM-PC compatible computer interfaced with the startle apparatus. The computer-interface assembly also controls delivery of acoustic stimuli through a speaker mounted above the floor of the isolation cabinet. Chambers were balanced across each of the experimental conditions. Sound levels were measured and calibrated with a sound meter placed within each chamber. Response sensitivities were calibrated using the SR-LAB Startle Calibration System.

Procedure.

Testing began on PND 60. A test session began by placing a subject in the Plexiglas cylinder where the subject was exposed to the background noise (65 dB white noise) for 10 min. After the 10 min acclimation period, each subject was presented with three trials of 120 dB and the responses from these trials were discarded. Following these three trials, each subject was presented with nine iterations of eight different types of trials: no pulse (0 dB), a startle pulse (110 dB, 40 msec broadband burst), and three prepulse intensities (70, 75, 80 dB, 20 msec broadband burst) presented alone or 100 msec preceding a startle pulse. The presentation of trial type was randomized within each of the nine iterations. The
average inter-trial interval was 15 seconds (range 10-20), and the inter-trial interval was randomized across all 72 trials. The startle response was measured every 1 msec for a 250 msec period from the onset of the startle stimulus. The average startle amplitude across the 250 msec measurement period was used as the dependent measure.

**Neurochemical Analysis**

At the end of the experiment, rats were killed by decapitation and brains removed. The hippocampus, striatum, nucleus accumbens and parietal cortex were rapidly dissected, frozen over dry ice, and stored at \(-80^\circ\) C. Monoamines were extracted from brain areas in 0.1 N perchloric acid containing sodium bisulphite as an antioxidant, and were analysed using high performance liquid chromatography (HPLC), with electrochemical detection. The HPLC consisted of a Waters 600 Multisolvent Pump maintaining a flow rate of 0.5 ml/min, a Hichrom 250 X 4.6 mm column with ODS2 5\(\mu\) packing material, an ESA Coulochem 5100A Detector with 5011 Analytical cell and 5020 Guard cell (redox mode: detector 1 = +0.10 V, detector 2 = -0.39 V, Guard +0.40 V), a Thermo Separation Products AS3000 Refrigerated Autosampler, and a Spectra Physics SP 4290 Integrator. The mobile phase was comprised of an aqueous acetate buffer mixture of 0.082 M glacial acetic acid, 0.094 M sodium acetate, 0.124 mM EDTA, 6% methanol, and 0.8 mM sodium octane sulphonate. 100 \(\mu\)l samples of diluted brain area extracts (3:1 to 50:1) were sequentially injected. Peak heights were used to determine the standard and sample monoamines in ng/mg of brain tissue.
Results

Neurochemical Assay

6-OHDA treatment resulted in a mean depletion of striatal dopamine of 93%. DOPAC levels were decreased by a mean of 92%, HVA by 90%. Norepinephrine was depleted by only 10%. Striatal serotonin was elevated by a mean of 90% (Table 1). One 6-OHDA-treated animal showed only a 21% depletion of striatal dopamine, was considered an incomplete lesion, and removed from the sample. All subsequent analyses reflect this.

Locomotor Activity

Activity was examined in twelve 5-minute time bins. These data were analyzed using a 2 × 2 × 12-factor ANOVA. 6-OHDA-treated rats were significantly more active than their sham-lesioned counterparts; amphetamine significantly attenuated the activity levels of the 6-OHDA-treated group, while eliciting locomotor activity in sham-treated animals (F_{1,13} = 28.25, p < 0.001, Figures 1a and 1b).

Startle Response and Pre-Pulse Inhibition

Ten levels of stimulus-intensity were used in the test of startle amplitude. A 2 × 10 ANOVA was used to analyze these data. There was no group difference in response to stimuli at 80 and 85 dB (sub-threshold stimuli, used as the pre-pulse), however 6-OHDA-treated animals showed increased startle amplitude to stimuli ranging from 90 to 120 dB (F = 11.34, p < 0.01, Figure 2). When pre-pulse inhibition was examined, however, a main effect of group (F = 12.34, p < 0.01) and of prepulse stimulus (F= 32.43, p < 0.001) in the absence of a significant group × stimulus level interaction indicates that percent inhibition
was unaffected by lesion, and that the heightened response at each prepulse level was simply a result of greater baseline startle (Figure 3a and 3b).

Discussion

Locomotor Activity

As expected based on previous work (e.g. Erinoff, McPhail, Heller & Seiden, 1978), the dopamine-depleted animals were hyperactive as compared to the control group. A low dose of amphetamine attenuated this hyperactivity. This response to therapeutic intervention is a less robust finding (see Pappas, Gallivan, Dugas, Saari and Ings, 1980, in contrast to Shaywitz, Klopper, Yager and Gordon, 1976), and was the first requirement of this proposed animal model of ADHD. In this study, this requirement has been satisfied.

Startle and Prepulse Inhibition

It is unclear why startle reflex would be heightened in dopamine-depleted animals. Castellanos et al. (1996) did not find group differences in baseline startle amplitude. It is possible that increased startle amplitude in 6-OHDA-treated animals is reflective of a general heightened reactivity to stimuli. That percent-inhibition of the startle-reflex was unaffected by 6-OHDA lesion lends no further credence to the DA-depletion model of ADHD, but is not necessarily damaging, as the connection between PPI and ADHD is at present merely theoretical. It should also be borne in mind that the group difference between ADHD- and normal children has thus far been seen only once, and may be discovered to be typical of only a subset of ADHD-patients. These data do imply, however that the
dopamine-depleted rat does not have the impairment in filtering proposed as the underlying deficit in ADHD.

EXPERIMENT 2

Forty-two Long-Evans hooded rat pups were acquired for this study. Eighteen completed all experiments. Housing conditions and surgical procedures were the same as those used in Experiment 1. Food and water was available ad libitum unless otherwise specified.

Methods

Locomotor Activity

Apparatus

Ten hanging cages equipped with photocell beams were used for this activity study. The cages measure 34 cm × 33 cm × 28 cm, with two photocell beams 3 cm above the cage floor, 11 cm from the front and rear walls. The front and rear walls of the cage were wire mesh, the sides are solid metal, and the floors are solid plastic. The apparatus was controlled, and the data collected by a 286 IBM-compatible computer. Locomotion was recorded as the number of crossovers, defined as the serial interruption of both front and rear beams.

Procedure

Subjects' locomotor activity was measured using 1 hour tests on three separate occasions. All rats were tested on PND 21, based on Experiment 1, as a behavioural index of dopamine depletion. No drug challenge was investigated at this time. Prior to beginning the
5-choice training (PND 45), this activity test was repeated. Upon completion of the 5-choice serial reaction time experiment, a final activity test was conducted to ascertain whether the neonatally DA-depleted animals would now respond typically to amphetamine (with increased activity levels). All rats were re-habituated to the apparatus for 1 hour. Following this, they were given two doses of amphetamine (1.0 and 1.5 mg/kg) plus vehicle alone (0.9% saline), i.p., in counterbalanced order. Drugs were administered 10 min before the onset of the activity test, and tests were conducted 2 days apart. All testing was conducted in a quiet room, under low light.

**Sucrose Consumption**

Ten- percent sucrose solution was used as the reinforcement in the 5-choice task. To habituate subjects to sucrose solution, and to ascertain that there was no difference in the reward properties of the solution between DA-depleted and control animals, baseline consumption was measured in the animals' home cages prior to beginning 5-choice training. The animals were approximately 120 days old. The automated water delivery system was disconnected, and replaced by water bottles for 3 days, to introduce the rats to the bottles. Water intake over 24 hour-periods was measured. Following this, the water was replaced with 10% sucrose (dissolved in tap water), and consumption over 24 hour-periods was measured for 5 days. In the final phase of this experiment, the automated water was reconnected, and sucrose bottles were made available for two hours per day. Sucrose intake was measured daily for 5 days.
5-Choice Serial Reaction Time Test

Apparatus

The attentional task was trained and tested in 5-choice operant boxes by Med Associates (St. Albans, VT). Each box consisted of a chamber 33 cm in length, 31 cm in width and 29 cm in height. Set into the curved rear wall were five 2.5 cm square recesses, 2 cm deep, 2.5 cm off the floor and 2.5 cm apart. Each recess had a 3-watt bulb centered on its rear wall and a photobeam crossing it horizontally, 1 cm in. The front wall of the chamber had a 5 cm square recess, 2.5 cm off the floor, and 3 cm deep. Centered within this magazine was a dipper that can be raised to deliver 0.06 ml of 10% sucrose solution, and then retracted. The magazine was also crossed by a horizontal photobeam, and the rear wall also had a 3-watt bulb. A 3-watt houselight was situated at the top of the front wall. Each chamber was housed in a sound-attenuation box with a fan. The apparatus was controlled, and data collected by an IBM-compatible computer.

Procedure

The 5-choice serial reaction time task requires the rat to discriminate spatially a brief visual stimulus, make a correct response, and then collect a reward. Throughout the session, the houselight was off. The animal was required to attend to the five holes in the rear wall. When any one of them was illuminated, the correct response was a nose-poke into that hole (breaking the photobeam), within a preset time limit (limited hold). If this response was made, the dipper was raised for a limited time, allowing the rat access to the reinforcer. A response in any other hole (an incorrect response) or a failure to respond during the limited hold resulted in a ‘time-out’ (a period before the next trial, with the houselight on), as did responses made at either the holes or the magazine during the inter-trial-interval (ITI). Any
response made during the time-out restarted the time-out period. Responses made at the holes and those made at the magazine during the ITI were scored as ‘premature’ (impulsive), and responses made at the holes immediately after a correct or incorrect response were scored as ‘perseverative’ (perseverative correct and perseverative incorrect respectively). Responses made during the timeout period were simply recorded as timeout responses. Failure to respond within the stimulus duration or limited hold was scored as an omission. Latency to collect the reward after a correct response was recorded as a measure of motivational factors (Puumala, Ruotsalainen, Jakala, Koivisto, Riekkinen & Sirvio, 1996). Latency to respond after stimulus onset (correctly or incorrectly) was also recorded.

Subjects were food-restricted for the duration of this experiment, such that they were given 20 g of lab chow 30 min after the last rats had had been run each day (at approximately 6:00 p.m.) Operant training for this task consisted of three phases. The first 3 days of training were used for ‘magazine-training’ and habituation to the chamber. The chamber was illuminated by the houselight, the magazine light was on, and the water dipper was raised on a random time (RT) 30 s schedule, to a maximum of 60 available rewards. At the end of this phase, all subjects were consistently collecting all 60 reinforcers, and phase two was initiated, introducing the animals to the holes. One of the five holes was illuminated (in random order) until the rat made a correct nose-poke response, at which time the magazine light was turned on and the dipper was raised for 5 s. Immediately after the reward was collected, the next trial began. A session consisted of 30 min or a maximum of 60 trials. After 5 consecutive days of this training schedule, a group difference was noted, such that all sham-lesioned rats completed 60 trials, while the 6-OHDA-treated animals’ mean number of trials completed was 18 ± 9. In an attempt to improve performance in the lesioned
rats, the houselight was turned off, so that the boxes were dark excepting the stimulus lights. A second 5 consecutive days of training followed, to habituate all subjects to the difference. This change improved the experimental group's performance to a mean of 53 ± 6, considered sufficient to progress to the third phase.

The third phase approximated test conditions. All holes were illuminated in random order, the water dipper was raised for each correct response, and a time-out (a period with the houselight on) resulted from each incorrect response. The stimulus duration and limited hold periods were initially set at 60 and 1 s respectively; the time-out period was set at 5 s. Once subjects' performance level was stable at approximately 80% correct responses and no more than 15% omissions, stimulus duration was shortened in successive approximations of final testing parameters (Carli & Samanin, 1992, Cole & Robbins, 1987). The final parameters used for all sham-lesioned animals were a 1 s stimulus duration and a 3 s limited hold. 6-OHDA-treated animals were tested using a 1 s limited hold, and the stimulus duration at which their performance level was optimal (closest to that of the control group). This was 3 s for 5 of the animals, 20 s for two subjects, and 10 s for the eighth (mean stimulus duration = 8 s).

**Drug Challenge**

Four doses of amphetamine (0.25, 0.5, 1.0, and 1.5 mg/kg), plus vehicle (0.9% saline) were administered, i.p., in counter-balanced order. All drugs were given 10 min before testing began. Test days were 72 hours apart, and the rats performed the 5-choice task on the interim-days, to ensure that performance had returned to baseline levels.

Upon completion of this study the rats were decapitated, brains extracted, and neurochemical analysis performed as described for Experiment 1.
Results

Neurochemical Assay

6-OHDA treatment produced a mean 96% depletion in striatal dopamine. Mean DOPAC reduction was 91%, and HVA was reduced by a mean of 94%. Norepinephrine was depleted by only 12%, and striatal serotonin was elevated by 45% (Table 2).

Locomotor Activity:

PND 21

All activity data were collected in twelve 5-minute time-bins, and subsequently analyzed using a $2 \times 12$ ANOVA. 6-OHDA-treated rats were significantly ($F_{11,176} = 30.49$, $p < 0.001$) more active than control-rats, displaying the same activity pattern over 1 hour as those of Experiment 1 (Figure 4a). As subsequent behavioural measures were taken to test an animal model based on juvenile hyperactivity, these locomotion scores were used as a screen, such that the 12 most active rats from the experimental group were selected as the subject pool for following experiments. Ten sham-treated animals were randomly selected to provide a control group.

PND 45

Spontaneous hyperactivity had disappeared by this point ($F_{11,176} = 1.54$, n.s., Figure 4b), as there was no longer a group difference in locomotion.

Sucrose Consumption

Consumption of sucrose solution over two hours did not differ between groups across 5 days of testing ($t_{16} = 1.2$, n.s., Figure 5). Further, the mean amounts consumed for
both groups were always greater than 6 ml, which was the maximum amount available
during the 5-choice test session (100 trials × 0.06 ml).

5-Choice Serial Reaction Time Test

Three of the 6-OHDA-treated subjects failed to learn the 5-choice task at 60s
stimulus duration. As they repeatedly completed zero trials, no performance measures were
available, and thus drug effects were impossible to assess. For this reason they were
removed from the sample. In addition one experimental animal died. Final group sizes for
this experiment reflect this (6-OHDA group: n = 8, control group: n = 10). All 5-choice test
data were analyzed using 2 × 5 ANOVAs; where applicable, post hoc analyses conducted
were Tukey’s HSD tests, adjusted for unequal n.

Measures of Behavioural Activation and Inhibition

The 6-OHDA-lesion significantly reduced the number of trials completed (F_{1,16} =
8.12, p < 0.05). Amphetamine had no main effect, however a trend was noted, such that the
two highest doses (1.0 and 1.5mg/kg) decreased the number of trials completed by sham-
treated rats without affecting the 6-OHDA group (F_{4,64} = 2.084, n.s., Figure 6).

Neonatal administration of 6-OHDA significantly decreased the number of
premature responses (F_{1,16} = 8.58, p < 0.01). Amphetamine had no statistically significant
effect on premature responding in either group (Figure 7). Neonatal treatment and
amphetamine affected neither timeout responses nor perseverative incorrect responses (data
not shown). However, perseverative correct response rate was higher in the 6-OHDA-treated
group (approaching significance, F_{1,16} = 4.38, p < 0.06). Amphetamine had a main effect
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(F\textsubscript{4,64} = 4.25, p < 0.01), decreasing perseverative-correct response rates in both groups (Figure 8).

**Measures of Attention**

Attention is measured in this paradigm through performance accuracy: correct and incorrect responses rates, and failures to respond. Percentage scores (number of responses divided by number of trials completed) were used here, to account for the differences in number of completed trials. In general, the 6-OHDA group showed a deficit, amphetamine impaired performance, and this impairment was greater in the sham-treated animals.

Percent correct responses was significantly lower in the 6-OHDA-treated group (\(F_{1,16} = 5.96, p < 0.05\)). Amphetamine decreased percent correct across both groups of animals (\(F_{4,64} = 8.3, p < 0.001\)), however this disruption was more drastic in the sham-treated group (\(F_{4,64} = 3.22, p < 0.05\), Figure 9). Percent incorrect responses was significantly higher in the 6-OHDA-group (\(F_{1,16} = 19.19, p < 0.001\)). Amphetamine worsened performance across both groups (\(F_{4,64} = 3.7, p < 0.01\)). Approaching significance, this disruption was greater in sham-treated animals (\(F_{4,64} = 2.31, p = 0.07\), Figure 10). Percent omissions was unaffected by 6-OHDA lesion, but was increased by amphetamine (\(F_{4,64} = 3.28, p < 0.05\)). This increase was significantly greater in the sham treated group, at 1.5mg/kg (\(F_{4,64} = 3.72, p < 0.01\), Figure 11).

**Response Latencies**

Latencies were to make correct and incorrect responses and to collecting reward were recorded. Neonatal dopamine deletion increased the latency to respond correctly (\(F_{1,15} = 8.1, p < 0.05\), Figure 12), and to collect reward (\(F_{1,16} = 6.44, p < 0.05\)). Amphetamine had a main effect on latency to collect reward; post-hoc tests revealed this to be an increase the
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sham-group’s latency at the highest dose (F_{4,64} = 6.52, p < 0.001, Figure 13). Latency to respond incorrectly was unaffected by either treatment (6-OHDA versus sham) or amphetamine (data not shown).

**Locomotor Activity**

These data were analyzed using a 2 x 12 ANOVA. Following the completion of 5-choice testing, amphetamine increased activity levels in both sham- and 6-OHDA-treated rats (F_{2,32} = 8.01, p < 0.01). The 6-OHDA-lesion attenuated this effect (approaching significance; F_{1,16} = 3.78, p < 0.07). There was no treatment x drug interaction (Figure 14).

**Discussion**

**Locomotor Activity**

The results of activity tests conducted on PND 21 in these animals are consistent with both the activity data collected in Experiment 1 of this study and previous reports of hyperactivity following neonatal dopamine depletion (e.g. Erinoff, MacPhail, Heller & Seiden, 1978). The animals are not only more active at the beginning of the test session, but show decreased habituation over the course of the session. This hyperactivity naturally wanes, however, and by PND 46, locomotion had decreased, and a normal pattern of habituation across the 1 hour test session had emerged. This is in accord with reports of hyperactivity decreasing with age in ADHD children (Barkley, 1997). For the purposes of this study, this decrease in locomotion also suggests that during the subsequent 5-choice test, performance was not adversely affected by hyperkinesis.
Sucrose Consumption

Sucrose consumption did not differ between groups. To conclude from this that neonatally DA-depleted animals' reward systems are intact would be premature; however, the lack of lesion effect does illustrate that during the 5-choice experiment, the reward properties of sucrose to each group did not affect performance. That each animal consistently consumed more than the maximum amount available during the 5-choice test session (100 x 0.06 ml = 6 ml) implies any performance impairments (for example, a lower number of trials completed) were not due to satiety.

5-Choice Serial Reaction Time Test

The first observation on the DA-depleted rats' performance requiring address is that of a pronounced learning impairment. This was not unexpected, as Heffner and Seiden (1983) found that 6-OHDA treated animals required more than twice as long as sham animals to acquire lever-pressing reinforced on a FR1 schedule. Heffner and Seiden (1983), however, tested this between PND 30 and 45, and thus heightened activity levels at this stage may have confounded performance. In the present study, 6-OHDA-treated rats required longer to reach performance criteria on each training schedule, beginning with phase two (the first exposure to the holes). The greatest improvement in the performance of DA-depleted rats was seen after switching background conditions of the apparatus from light to dark. Two possible explanations for this are: a) the global DA-depletion caused by i.c.v. 6-OHDA affected the functioning of these rats' retinas (Feldman, Meyer & Quenzer, 1997, Diamond, 1998)), so that they could not discern the stimulus lights as easily as sham rats against a light background, and b) testing in the dark evoked some of the behavioural
arousal seen naturally during rats’ “night”, resulting in increased exploratory behaviour. In addition to the three above-mentioned subjects subsequently removed from the experimental group, the 6-OHDA-treated animals required more training sessions at the 60 s stimulus duration to reach performance criteria. While subjects were already familiar with the holes and basic behaviour sequence required of them, this was their first exposure to the full complexity of the task (i.e. the fact that incorrect, premature and perseverative responses and omissions now resulted in punishment, and thus were to be avoided). The sham-operated group had reached, and in fact exceeded the response criterion (80% correct responses) by the second training day (mean = 95 ± 1 %). On the fifth training day, the experimental group had achieved a mean of 84 ± 2% correct. This difference cannot be explained by a motoric deficit, as although the latency to correct response was longer for the 6-OHDA group (8.63 ± 1.88 s versus 2.76 ± 0.22 s), these latencies were far short of the 61 s allowed for response. It is difficult to tease apart attention deficit and learning impairment in this case; it is possible that these animals did not learn as quickly because of a failure to attend. However, in light of the fact that the DA-depleted group did eventually learn the task, but required a greater number of training sessions, it is more plausible that these rats display a learning impairment independent of attentional dysfunction.

It should also be noted that the 6-OHDA group was never successfully shaped to complete this task using a 1 s stimulus duration, as was the sham group. The final parameters used during drug challenge sessions were titrated, chosen so that each animal was performing at an optimal (closest to that of the sham group) level. When attempts were made to reduce the stimulus duration of the experimental group further, rather than
completing the same number of trials but with less accuracy, subjects simply did not perform the task: the number of trials completed dropped, often to as few as 5.

**Effects of Dopamine Depletion**

On 5-choice tests, the 6-OHDA-treated animals showed impairment on multiple measures. They completed fewer trials and had longer response latencies. This cannot be explained as a motoric dysfunction for two reasons. First, in adulthood these rats showed normal locomotor activity levels. Second, although the 6-OHDA group's mean latency to collect reward was longer than that of the sham group, it was considerably shorter than their mean latency to make a correct response. These rats were obviously capable of faster motor response than they displayed when responding at the holes. It is reasonable to conclude, then, that this behavioural slowing is a deficit in either response selection or initiation.

The dopamine-depleted group also committed fewer premature responses. This is not a performance deficit per se, as a premature response is an error. It was an unexpected finding however, (as hyperactivity generally coincides with impulsivity in ADHD – Barkley, 1997), and is potentially misleading. On the surface, lower premature responding rates would imply more accurate performance. However, in light of the deficits shown by these rats across other response measures of this task, it is more likely that decreased premature responding is reflective of a general response suppression or deficit. It must be acknowledged that the “premature responses” score from the 5-choice task is not a very sensitive measurement of impulsivity, as the first premature response made by a subject results in a timeout period; any responses made immediately thereafter are scored as “timeout responses”. Timeout responses however, were unaffected by dopamine depletion. Contradictory to this, the 6-OHDA lesion produced an increase (approaching significance)
in perseverative correct response rates. It is difficult to reconcile this to other measures of behavioural activation, especially in the absence of any effect of DA depletion on perseverative incorrect responses (indicating general perseveration is not the cause). This may be (speculatively) interpreted as a specific form of impaired behavioural inhibition – once the animal has made a correct response, which will immediately result in feedback (the sound of the dipper, magazine light), the deficit lies in ceasing that response.

Neonatal dopamine depletion reduced accuracy, as deduced from two response measures: percent correct (decreased) and incorrect (increased). Percent omissions was not affected by 6-OHDA treatment. The total number of trials completed in this task can be divided into correct, incorrect, and omissions. Thus, as percent correct decreases, percent incorrect, or percent omissions, or both must increase. The observation that in dopamine-depleted animals the reduction in percent correct was made up as an increase in percent incorrect, but not omissions is interesting. This presents a strong case for a real reduction in accuracy, as if the 6-OHDA lesion resulted in a general response-suppression, it would be expected that the animals would simply fail to respond (an increase in omissions). Since these rats showed an increase in percent incorrect responses (i.e. were responding), a true reduction of accuracy is revealed.

It appears then, that neonatal depletion of dopamine did produce an attention deficit. However, this deficit was accompanied by a more general response-impairment, evidenced by the lower number of trials completed and increased response latencies.

Effects of Amphetamine

An overview of amphetamine’s effects on 5-choice task performance presents several unexpected effects. The high doses (1.0 and 1.5mg/kg) of amphetamine decreased
the number of trials completed in sham-treated animals, without affecting the 6-OHDA group. Latency to correct response was unaffected by the stimulant, however latency to collect reward was increased in both groups by the highest dose (1.5 mg/kg). Premature and perseverative correct response rates did not change with amphetamine administration. Accuracy indices were more vulnerable to the effects of amphetamine. Percent correct was decreased at 1.0 and 1.5 mg/kg doses in both groups, though more dramatically in the sham-treated animals. Percent incorrect was increased only in 6-OHDA-treated animals, at the highest dose. Percent omissions was unaffected in the dopamine-depleted group, but increased in the control group at the two highest doses. Although the effects of amphetamine on this task appear bi-directional, and disorganized, one clear conclusion emerges. Notably, no aspects of performance of the 6-OHDA-lesioned animals were improved by amphetamine administration, and in fact several were disrupted. This poses a problem for the neonatally dopamine-depleted rat as an animal model of ADHD, as response to therapeutics is a necessary condition to validate any animal model of a clinical disorder.

**Adult Locomotor Activity**

As juveniles, The dopamine-depleted rats showed spontaneous hyperactivity when compared to control rats. This hyperactivity decreased in response to amphetamine. Following the completion of the 5-choice test, the 6-OHDA-treated rats displayed the opposite pattern of locomotor activity: baseline activity was not heightened in comparison to sham-treated animals, and locomotion was modestly increased in response to amphetamine. Although spontaneous hyperactivity had by this point ceased in these animals, and the 6-OHDA lesion attenuated amphetamine-induced activity, it is difficult to reconcile with the fact that stimulants reduce activity in children with ADHD. Compensatory mechanisms such
as receptor supersensitivity are a possible explanation. It should also be noted that these animals had been exposed to four different doses of amphetamine previous to this test, and thus sensitization cannot be ruled out.

GENERAL DISCUSSION

As an evaluation of an existing proposed animal model of ADHD, this study began by replicating a well-documented effect: neonatal dopamine-depletion produced spontaneous hyperactivity, which was attenuated by the administration of a stimulant (d-amphetamine). However, the results were not as straightforward when these animals were tested for other behavioural effects relevant to ADHD. To assess the overall validity of the dopamine-depleted rat as an animal model of ADHD, two requirements must be met. The animal must present "phenomenological similarities" to the disorder (face validity), and there must be "correspondence between drug actions in the model and in the clinic" (predictive validity) (Willner, 1991).

The lack of group difference in PPI contrasts sharply to the group differences in 5-choice performance. This, however, need not be problematic, as the theoretical relevance PPI has to ADHD lies in selective attention, and the 5-choice test employed in this study examined sustained attention. Nonetheless, it must be concluded that the results of this study do not support the theory of impaired filtering as the core deficit in ADHD.

The 5-choice serial reaction time test revealed an impairment in sustained attention, and it is thus tempting to conclude that the required "phenomenological similarities" (Willner, 1991) have been satisfied. However, if one examines the whole behavioural profile of these rats, a caveat is necessary. The attention deficit appeared to exist with a general behavioural
depression. In interpreting these effects the division of ADHD into subtypes described above becomes important. This behavioural depression, in and of itself, may actually represent true ADHD of the “predominantly inattentive” type, as children diagnosed with this subtype have been described as “lethargic, hypoactive, and passive”, which is interpreted as indicative of a “deficit in speed of information processing” (Barkley, 1997). This interpretation, however, implies that the 6-OHDA-treated rat is an animal model of the “predominantly hyperactive-impulsive” subtype when young, and then shifts completely to represent the “predominantly inattentive” subtype during adulthood. The obvious problems with this explanation are that a) it implies that one animal model represents two independent types of patient (at different times), and b) it leaves no room for any representation of the third subset of ADHD patients (who exhibit hyperactivity and attention-deficit simultaneously), now labeled the “combined type”. Therefore, the dopamine-depleted rat appears to manifest symptoms of ADHD, without accurately representing the syndrome itself.

A more plausible interpretation of these seemingly disparate behavioural manifestations of a single neurochemical manipulation lies in the nature of the manipulation itself. Evidence of altered dopaminergic functioning in ADHD is plentiful, but indirect. Therefore, what specifically is altered, and how, is not yet known. Intracerebroventricular (i.c.v.) administration of 6-OHDA to a neonatal rat produces global devastation of the dopamine system. While all subtypes and symptoms of ADHD appear to be related to, or at least concordant with DA-ergic dysfunction, it is unlikely that the widespread depletion caused by i.c.v. 6-OHDA reflects the dysfunction underlying the disorder.
Examining animal research involving neuroanatomically discrete lesions, it has been found that 6-OHDA-lesion of the mesolimbic DA system (infusion into the nucleus accumbens) produces an increase in response latency, without affecting choice-accuracy, in the 5-choice task (Cole & Robbins, 1989). Consistent with this finding, amphetamine administration in intact rats impairs both choice accuracy and response latency in an attention-switching task, however dopamine-depletion of the nucleus accumbens blocks amphetamine’s effect on response-latency only (Robbins et al., 1986).

Response-latency may be a key measure in applying 5-choice performance to ADHD. It has been suggested that “disorganized response processing appears to underlie the pathology of attention deficit disorder... there appears to be a breakdown in the selective linking of sensory to motor events” (Clark, Geffen & Geffen 1987a). ADHD, then may not simply involve an inability to attend to stimuli, but a more complex impairment involving the interplay between attending and responding to stimuli. This “response processing” is suggested to involve prefrontal structures, which have been shown to become active when one must “mentally manipulate...information and make a response” (Pliszka et al., 1996). The involvement of the mesocortical DA system in attention-deficit is suggested by studies of attentional impairments in patients with phenylketonuria; these patients have depleted dopamine levels in the prefrontal cortex (Diamond, 1998). The idea that attention is related specifically to cortical dopamine is supported by the finding that accuracy measures (but not behavioural activation measures) on the 5-choice serial reaction time test correlated positively with frontal cortical dopamine levels (Puumala & Sirvio, 1998).

If, then, the deficit in ADHD involves an interaction between attention and response, it is possible that the dopaminergic dysfunction underlying ADHD involves interaction between
mesolimbic (nucleus accumbens) and mesocortical (prefrontal cortex) DA systems. This is nicely illustrated by studies using non-transmitter-selective lesions in neonatal rats.

Electrolytic lesions of the medial ventral tegmental area (VTA) decreased DA content within the prefrontal cortex by 42 – 57%, and produced transient juvenile spontaneous hyperactivity (comparable to that seen after i.c.v. 6-OHDA lesions). A lesion of the substantia nigra, reducing striatal DA by 68%, had no locomotor outcome (Heffner, Heller, Miller, Kotake & Seiden, 1983). The authors conclude that mesocortical DA-depletion is necessary to produce hyperactivity. Kalsbeek, de Bruin, Matthijssen and Uylings (1989) expanded on this experiment, comparing thermal lesions of the medial- and complete-VTA. The medial VTA-lesion led to a “moderate” DA depletion in the prefrontal cortex, and produced hyperactivity. Destruction of the entire VTA produced an “almost complete” depletion of prefrontal cortical dopamine, and a partial depletion of nucleus accumbens-DA. Animals with this lesion were significantly hypoactive. Thus, it appears that in addition to a depleted mesocortical system, a relatively intact mesolimbic system is required for hyperactivity to occur. Kalsbeek et al. (1989) suggest that the nucleus accumbens is a “key structure in the initiation of open field activity”, and the prefrontal cortex plays a “modulatory role”. This interaction suggests that distinct symptom-profiles that constitute the sub-types of ADHD are caused by variation among specific and subtle dopaminergic dysfunctions.

The 6-OHDA-treated rat, has, as previously noted, widespread dopaminergic depletion. While not well understood, compensatory mechanism are a known phenomenon in these animals (two examples are the striatal serotonergic hyperinnervation, and preservation of normal ingestive behaviour seen only when 6-OHDA is administered neonatally). This non-
specific depletion has produced symptoms which, when examined individually, provide face validity to the model. In combination, however, these symptoms do not represent any one subtype of ADHD child. Furthermore, amphetamine was "therapeutically efficacious" only when applied to hyperactivity, and did not improve attentional performance, a fact that calls the predictive validity of the animal model seriously into question. Here again, it is possible that the magnitude of the i.c.v. 6-OHDA lesion is to blame, for two reasons. First, the brains of these rats do not remain static after 6-OHDA lesions; this is evidenced by the amphetamine-induced increase in activity seen in adulthood. It is reasonable to speculate that some compensation for the depletion occurs over the course of development. As it is unlikely that the presumed DA-dysfunction in ADHD children is widespread reduction ranging from 80 – 95%, it is equally unlikely that the compensation occurring in these rats is equivalent to the compensation occurring in the brains of ADHD-children. This may explain the stimulant-induced hyperactivity the 6-OHDA-treated rats exhibited in adulthood, an effect not generally reported in clinical literature. Second, since mesocortical and mesolimbic dopaminergic structures interact (Lindvall & Bjorkland, 1983, Kolb, 1991), and both appear to contribute to the symptomology of ADHD, stimulant treatment may normalize the behaviour of ADHD children through multiple mechanisms (Shekim, Javaid, Dekermenjian, Chapel & Davis, 1982), resulting in the restoration of balance in the system (Shenker, 1992). Through the ablation of all brain dopamine, however, 6-OHDA has produced a system within which there is nothing to balance.

In conclusion, the results of this study tentatively support the neonatally dopamine-depleted rat as an animal model of ADHD, as several symptoms of the disorder were observed. The i.c.v. 6-OHDA-treated rat’s utility, however, is limited by the widespread
nature of the lesion that is produced. As an animal model of a human disorder, the 6-OHDA-rat cannot elucidate specific mechanisms or neuroanatomical structures involved in either the deficits or therapeutics of ADHD, and thus the posited interactions between distinct dopaminergic systems are still entirely speculative. A potentially more informative technique would involve the depletion of discrete dopaminergic systems and/or structures, in an attempt to more accurately represent subtypes of the disorder, and elucidate the variation in dysfunction that gives rise to these subtypes.
Figure Captions

Figure 1: Juvenile locomotor activity under two conditions: saline, (all time intervals except 1 and 2: p < 0.01), and 0.5mg/kg amphetamine (drug:group interaction p < 0.001).

Figure 2: Baseline startle response: stimulus intensities 90 – 120, p < 0.01.

Figure 3: Response to prepulse-pulse in absolute startle values (group effect p < 0.01), and percent inhibition (n.s.)

Figure 4: Locomotor activity at PND 21 (p < 0.001 across all time intervals). and PND 46 (n.s.).

Figure 5: Mean sucrose consumption (across 5 days) over a two-hour period (n.s.)

Figure 6: Number of trials completed. Between group difference p < 0.05), within-group difference (sham at 1.5mg/kg) p < 0.05, (Tukey’s HSD).

Figure 7: Premature responding. (group effect p < 0.01).

Figure 8: Perseverative responding. Approaching significance (p < 0.06) across all doses of amphetamine.

Figure 9: Percent correct responses. (* = between groups, p < 0.05, + = within sham group drug effect, p < 0.05, Tukey’s HSD).

Figure 10: Percent incorrect responses. (* = between group effect, p < 0.001, + = within lesion group drug effect, p < 0.05).

Figure 11: Percent omissions. (+ = within sham group drug effect, p < 0.01, Tukey’s HSD).

Figure 12: Latency to respond correctly. (p < 0.05, across all doses of amphetamine).

Figure 13: Latency to collect reward. (intergroup effect: p < 0.05, across all doses), Intragroup (sham) effect: + = p < 0.001, at 1.5 mg/kg amphetamine.

Figure 14: Post 5-choice task activity test. Intergroup effect: p < 0.07, (approaching significance), Drug effect: * = p < 0.01.
References


support for the dopamine depletion model of minimal brain dysfunction.

*Psychopharmacology, 70*, 41-46.


Table 1: Striatal monoamine levels (ng/mg tissue), Experiment 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DOPA</th>
<th>NE</th>
<th>DOPAC</th>
<th>DA</th>
<th>5-HIAA</th>
<th>HVA</th>
<th>5-HT</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>0.0167</td>
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Table 2: Striatal monoamine levels (ng/mg tissue), Experiment 2

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<th>DOPAC</th>
<th>DA</th>
<th>5-HIAA</th>
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<tr>
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<td>0.0217</td>
<td>0.0244</td>
<td>0.0785</td>
<td>0.0836</td>
<td>0.0098</td>
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</table>
Figure 1a: Juvenile activity, saline

Ambulatory counts

5-minute intervals

- Lesion
- Sham
Figure 1b: Juvenile activity, amphetamine

- Lesion
- Sham

5-minute intervals

Ambulatory counts
Figure 2: Baseline startle response

- Sham
- Lesion

Average startle response

Stimulus intensity (dB)
Figure 3a: Prepulse inhibition, absolute values

Startle amplitude

Sham
Lesion

Stimulus intensity (dB)
Figure 3b: Percent inhibition of startle amplitude

Prepulse intensity (dB)

Percent inhibition

Sham
Lesion

70
75
80
Figure 4a: Activity, PND 21

Crossovers

5-minute intervals
Figure 4b: Activity, PND 46

Crossovers

5 minute intervals

Lesion
Sham
Figure 5: Mean 2 hour sucrose consumption
Figure 6: Number of trials completed

Amphetamine (mg/kg)

Lesion
Sham
Figure 7: Number of premature responses

![Graph showing the number of premature responses at different amphetamine (mg/kg) dosages for lesion and sham groups.](image-url)
Figure 8: Number of perseverative correct responses

![Graph showing the number of perseverative correct responses against different doses of amphetamine (mg/kg). The graph compares lesion and sham conditions.](image)
Figure 9: Percent correct responses

Percent correct responses over different doses of amphetamine. The graph shows the effect of amphetamine (mg/kg) on percent correct responses for lesion and sham groups. The asterisks indicate significant differences between the groups.

- Lesion
- Sham

Amphetamine (mg/kg)

Percent
Figure 10: Percent incorrect responses
Figure 11: Percent omissions

[Graph showing percent omissions against amphetamine (mg/kg) dosage, with lines indicating Lesion and Sham conditions.]
Figure 12: Correct response latency

Lesion
Sham

Amphetamine (mg/kg)

Seconds
Figure 13: Latency to collect reward

Seconds

Amphetamine (mg/kg)

Lesion
Sham

*
Figure 14: Activity levels: 1 hour totals

Neonatal Treatment

Crossovers

Lesion

Sham

0
1
1.5