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Five-choice serial reaction time task performance following catecholamine depletion of rat medial prefrontal cortex: implications for attention deficit in schizophrenia

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Five-Choice Serial Reaction Time Task Performance Following Catecholamine Depletion of

Rat Medial Prefrontal Cortex:

Implications for Attention Deficit in Schizophrenia

By

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MASTER'S THESIS

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Rabia V. Magnusson

July 23, 2010

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A Thesis
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The Faculty of
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Abstract

The symptoms of schizophrenia are highly variable and include a variety of cognitive deficits, including attentional deficit. These cognitive deficits may involve dopamine (DA) underactivity in the prefrontal cortex (PFC) (Weinberger, Egan, Bertolino, Callicott, Mattay, Lipska, et al., 2001). The purpose of this thesis was to test the hypothesis that reduced DA in the PFC alters attention by examining the effects of reduced DA in the medial PFC (mPFC) of rats on a sustained attention task. Rats in the DA-lesioned group were administered 6-hydroxydopamine (6-OHDA) in the mPFC. Following 6-OHDA administration, rats in the DA-lesioned and sham-lesioned group were trained to nose poke into a lit aperture for food reward in the 5-choice serial reaction time task (5-CSRTT), a rodent analogue of the Continuous Performance Task used to assess sustained attention in schizophrenics (Robbins, 2002). The 6-OHDA administration resulted in depletions of both DA (33% of controls) and norepinephrine (NE) (50% of controls). Rats' performance was assessed by comparing the frequency of correct, incorrect, omitted, and premature responses in testing conditions where the duration of stimulus in the 5-CSRTT was randomly varied from 2.5 to 0.25 seconds. No significant differences between lesioned and control rats were observed in any behavioral testing parameters. However, there was a trend toward increased numbers of omissions and reduced correct responses seen in lesioned rats especially at the shortest stimulus durations. The trend supports the hypothesis that reduced DA in the PFC contributes to attentional dysfunction in schizophrenia. Future studies should examine the behavioral effects of 6-OHDA lesions using larger sample sizes. Also, future studies should examine rats' 5-CSRTT performance at stimulus durations less than 0.25 seconds to further explore the trend toward poorer performance seen in 6-OHDA lesioned rats.

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Five-Choice Serial Reaction Time Task Performance Following Catecholamine Depletion of

Rat Medial Prefrontal Cortex:

Implications for Attention Deficit in Schizophrenia

Schizophrenia is a disorder characterized by disorganized thoughts, hallucinations and

delusions, emotional disturbances, and cognitive deficits (Lewis, Hashimoto, & Volk, 2005). It is

believed to be a neurodevelopmental disorder (Rapoport, Addington, Frangou, & Psych, 2005),

with the onset of symptoms generally occurring during late adolescence through early adulthood

(Raedler, Knable, & Weinberger, 1998). Post-mortem analyses of the brains of schizophrenics

have revealed structural abnormalities such as decreased grey matter volume in the prefrontal

cortex (PFC) (Hashimoto, Volk, Eggan, Mirnics, Pierri, Sun, et al., 2003; Shenton, Dickey,

Frumin, & McCarley, 2001). The PFC is not fully developed until early adulthood (Krasnova,

Betts, Dada, Jefferson, Ladenheim, Becker, et al., 2007), which is also when schizophrenia is

typically diagnosed. This has led to the hypothesis that abnormal maturation and function of the

PFC may underlie emergence of the symptoms of schizophrenia (Finlay, 2001).

Schizophrenia is diagnosed in approximately 1% of the world's population (Lewis, Cruz,

Eggan, & Erickson, 2004). Sensory disturbances and psychotic states are common features of the

illness. Due to the action of antipsychotic drugs, the most extensively studied neurotransmitter

system in relation to schizophrenia is the monoamine neurotransmitter dopamine (DA) (Guillin,

Abi-Dargham, & Laruelle, 2007). Antipsychotic drugs ameliorate psychotic symptoms by

blocking the action of DA at post-synaptic D2 receptors (Meisenzahl, Scheuerecker, Schmitt, &

Moller, 2007), which suggests that psychotic symptoms occur because of overstimulation of D2

receptors. Likewise, drugs that increase DA release, such as amphetamine, can induce psychosis and exacerbate the psychotic symptoms of schizophrenia (Abi-Dargham & Moore, 2003).

The relationship between excessive activity of DA and psychosis has given credence to the hypothesis that perturbations in DA neurotransmission may underlie many of the symptoms of schizophrenia (Tzschentke, 2001). However, in addition to symptoms of psychosis, schizophrenics experience a range of cognitive deficits, including working memory and sustained attention (Riehemann, Volz, Stutzer, Smesny, Gaser, & Sauer, 2001). These deficits are generally not remedied by DA receptor antagonists (Radek, Kohlhaas, Rueter, & Mohler, 2010). Schizophrenics tend to display decreased cerebral blood flow in the PFC, which is correlated with reduced DA metabolites in cerebral spinal fluid (Weinberger, Berman, & Illowsky, 1988), a brain region critically involved in working memory and attention (Christakou, Robbins, & Everitt, 2004; Goldman-Rakic, Muly, & Williams, 2000). This has led to the hypothesis that reductions in DA neurotransmission in the PFC may underlie the attentional deficits of schizophrenia (Weinberger et al., 2001).

To test this hypothesis, the present study explored the effects of disrupting DA innervation of rat PFC on a sustained attention task modeled after the Continuous Performance Task (CPT) (Rosvold, Mirsky, Sarason, Bransome, & Beck, 1956) that is used to assess sustained attention in schizophrenics. Before presenting the thesis research itself, the following sections will: 1) outline DA function in the PFC in relation to schizophrenia; 2) describe the nature of cognitive impairment in schizophrenia; and 3) describe the task used to study neural correlates of sustained attention in rodents.

Prefrontal Cortex Dopamine and Attention

An optimal level of D1 receptor stimulation in healthy individuals is required for normal working memory and attention (Goldman-Rakic, Muly, & Williams, 2000). In primates, both over- and understimulation of D1 receptors in the PFC hinders performance in a working memory task (Cai & Arnsten, 1997). Too little or too much DA activity, therefore, may negatively affect working memory and attentional performance.

Lesions to the mPFC using quinolinic acid result in behavioral deficits in a 5-choice serial reaction time task (Chudasama, Passetti, Rhodes, Lopian, Desai, & Robbins, 2003). However, multiple types of neurons are destroyed by quinolinic acid lesions. This non-specific lesion identifies a role of the mPFC but does not identify the effects of disruption to a particular kind of neurotransmission within the mPFC.

Evidence for DA involvement in attention comes from pharmacological manipulations demonstrating behavioral effects following direct mPFC D1 agonism and antagonism. This manifested as both improved and hindered attentional performance depending on baseline performance (Granon, Passetti, Thomas, Dalley, Everitt, & Robbins, 2000). Therefore, the present study aimed to further explore the role of reduced DA on attention in rats using a controlled DA manipulation.

Prefrontal Cortex Dopamine and Schizophrenia

Schizophrenics have reduced density of fibers immunoreactive for tyrosine hydroxlyase, the rate-limiting enzyme involved in DA synthesis, in prefrontal cortical layers 3 and 6, (Akil, Pierri, Whitehead, Edgar, Mohila, Sampson, & Lewis, 1999). Also, schizophrenics display reduced dendritic spine density in post-mortem analyses of the PFC, which may be related to decreases in DA innervation (Glantz & Lewis, 2000). In adult rats, DA depletion (>50%) of the

ventral tegmental area (VTA) induced by the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) results in decreased spine density of layer 5 pyramidal neurons of the mPFC (Wang & Deutch, 2008). This suggests that abnormal DA neurotransmission may influence synaptic structure, resulting in fewer synapses and impaired PFC function.

As noted earlier, schizophrenia may involve excess DA in subcortical structures, yet too little DA in cortical regions (Knable & Weinberger, 1997; Guillin et al., 2007). Specifically, the model involves hypoactivity of the mesocortical DA system, beginning in the VTA and terminating in the PFC, paired with hyperactivity of the mesolimbic pathways, which originate in the VTA and terminate in the nucleus accumbens, hippocampus, and amygdala. Mesolimbic DA overactivity is believed to contribute to psychotic experiences (Goto & Grace, 2007), while mesocortical hypoactivity may contribute to cognitive deficit, including sustained attention (Meisenzahl et al., 2007).

Attentional Deficit in Schizophrenia

The CPT is used to study attentional deficits in schizophrenic patients (Rund, 1998). The CPT involves rapidly presenting visual stimuli to subjects in a non-predictable arrangement of continuously changing patterns, typically letters or numbers (Riccio, Reynolds, Lowe, & Moore, 2002). The classic paradigm involves designating the letter "X" as the target, presented within a series of letters flashed for 1-2 seconds (Rosvold et al., 1956). When the target is detected, subjects respond by pressing a lever. Increasing the complexity of the target adds an additional level of difficulty (e.g., requiring that the letter "A" be paired with "X"; Riccio et al.). The unpredictability of the stimulus presentation requires subjects to remain vigilant to changing task requirements (Hazlett, Dawson, Schell, & Neuchterlein, 2001).

Shortening the stimulus duration, adjusting the frequency of target presentation, and manipulating perceptual aspects of the target (such as blurring or partially occluding) are additional methods of increasing attentional challenge on the CPT (Riccio et al., 2002). Cognitive deficits in schizophrenics become more apparent on tasks requiring elevated levels of attention (Jazbec, Pantelis, Robbins, Weickert, Weinberger, & Goldberg, 2007), and schizophrenics typically require a longer duration of stimulus presentation to perform at control levels (Cattapan-Ludewig, Hilti, Ludewig, Vollenweider, & Feldon, 2005). Similar deficits are seen in patients with frontal lobe damage, suggesting that poor performance on the CPT is related to abnormal PFC function (Riccio et al.).

The 5-Choice Serial Reaction Time Task

The 5-choice serial reaction time task (5-CSRTT) is a rodent version of the CPT used to assess attention in schizophrenic subjects (Robbins, 2002; see Figure 1). The task assesses an animal's ability to remain vigilant to an array of visual stimuli over a 30-minute test session (Carli, Robbins, Evenden, & Everitt, 1983). In this task, rats maintain visual attention to an array of five apertures located on one wall of a test chamber. One of these five apertures is lit on a pseudorandom schedule for a short period of time; rats learn to poke their nose into the lighted aperture to receive a food pellet from a dispenser on the opposite wall of the test chamber. This task becomes especially challenging when the length of time that the aperture is lit is reduced. By placing additional cognitive demands (e.g., shortening the stimulus duration, introducing a noise distracter), the 5-CSRTT provides a method for systematically challenging sustained attention in animals analogous to the CPT in humans.

The 5-CSRTT engages DA pathways terminating in the rat mPFC (Dalley, Cardinal, & Robbins, 2004; Muir, Everitt, & Robbins, 1996). Quinolinic acid lesions of the rat mPFC reduce overall PFC neurotransmission and impair performance on the 5-CSRTT (Passetti, Levita, & Robbins, 2003). Quinolinic acid lesions glutamatergic and GABAergic neurons, but does not reduce tyrosine-hydroxylase-labeled axons in lesion sites (Schwarcz, Whetsell, & Mangano, 1983). The attention deficit caused by quinolinic acid is remedied by administration of a D2 receptor antagonist (Passetti, Levita, & Robbins, 2003). This suggests that the mPFC lesion hinders performance because the DA system is disrupted. However, it is unclear where the DA antagonist is acting in the brain to ameliorate deficits.

Antagonism of D1 receptors in the mPFC improves baseline performance in the 5-CSRTT in unlesioned rats performing below the group median (Granon et al., 2000). Performance-enhancing effects of dopaminergic pharmacological manipulations in the 5-CSRTT suggest that this task assesses cognitive abilities that are dependent on the functional capacity of the mesocortical pathway. Consequently, the 5-CSRTT will be used in the present study as a behavioral paradigm for investigating the effects of neurochemical disruption to the DA system on attentional processes in rodents.

Present Study

The present study explored the cognitive effects of insult to dopaminergic neurotransmission in the PFC in the rat. Specifically, the effects of 6-OHDA administered into the mPFC of rats during the early adulthood period (postnatal day 55) on performance in the 5-CSRTT were assessed at adulthood.

Method

Subjects

Male Sprague-Dawley rats (N = 12; Harlan Sprague-Dawley, Inc., Indianapolis, IN.) were housed in pairs from arrival at postnatal day (PN) 37 until surgery. Rats were randomly assigned to either a sham (n = 5) or lesion (n = 7) condition on the day of surgery. One of the rats in the lesion condition did not recover from surgery; as a result, the study included n = 6 rats in the lesion condition. Until the start of behavioral testing, rats were given free access to tap water and standard rodent chow (Mazuri Rodent Chow, Minneapolis, MN) and were maintained on a 12:12 hour light/dark cycle with lights on at 7:00 AM. During behavioral testing, *ad libitum* access to tap water was continued, but rats were food deprived to 80-85% of free-feeding body weight relative to age-matched controls (~310 grams at start of testing). At all times, rats were treated according to the NIH Guidelines for the Care and Use of Laboratory Animals and Western Washington University's local animal use committee.

Surgery

At PN 55 (body weight range 210-270 grams), each rat was given a systemic injection of equithesin (3.0 ml/kg) 20 minutes prior to placement in the stereotax (David Kopf Instruments, Models 900, 970, and 907, Turjunga, CA). Equithesin anesthesia was supplemented as needed throughout the course of the surgery. The surgical procedure involved bilateral microinjections of the 6-OHDA (MP Biomedicals, lot #5664J; 1.0 μg base/2 μl vehicle, dissolved in ascorbic acid; 2 μl/hemisphere at a flow rate of 0.2 μl/min) or vehicle into the mPFC (+3.2 anteroposterior, ±0.8 mediolateral, and -3.2 dorsoventral from bregma; Paxinos & Watson, 1986). To reduce 6-OHDA destruction of norepinephrine (NE) terminals in the mPFC, the NE

transporter antagonist desipramine hydrochloride (DMI; Sigma D3900-5G; 25 mg base/kg, dissolved in saline immediately prior to use) was administered intraperitoneally 1 hour prior to the 6-OHDA injections. Control subjects received an injection of saline, also delivered intraperitoneally.

Immediately following surgery, animals were placed on a heating pad and monitored until full recovery, and then individually housed for the duration of the study.

Behavioral Testing

Apparatus. Operant conditioning chambers (Model MED-NP9L-B1, Med. Associates Inc., St. Albans, VT; 12.0" L x 9.5" W x 8.25" H) were used to assess sustained visual attention in the 5-CSRTT (see Figure 1). Nose poking into one of nine apertures (2.5 cm^2) located on one wall of the chamber resulted in delivery of a 45 mg food pellet from a dispenser located on the opposite wall. A house light located directly above the food receptacle was illuminated when rats responded performed premature, incorrect, or omitted responses. A stainless steel grid floor was situated above cob bedding, which was replaced prior to the start of each daily session. There were two testing chambers; care was taken to ensure that rats were assigned to the same chamber each day. The operant chambers were controlled by a Windows-based computer and interface (MEDPC-IV; Med Associates Inc., St. Albans, VT) located outside of the testing room.

Protocol. Training in the 5-CSRTT began 2 weeks post-surgery. Food deprivation began the day before the first training session; body weights were assessed prior to each operant session to maintain animals at 80-85% of free-feeding weight. Rats reached this body weight after an average of 3-4 days of limited food intake.

Habituation Phase

The goal of habituation sessions was to acclimate each rat to the testing chamber and to lead them to associate the light in the food receptacle with delivery of food reward. Animals spent 2 days habituating to the testing box. Each habituation session lasted for 30 minutes. During each habituation session, a light in the food receptacle was turned on for 30 seconds, and shut off for the remaining 30 seconds of each 60-second period. If the rat failed to retrieve the pellet within the allotted time period, the house light above the food receptacle was illuminated for 5 seconds. The period of time during which a rat could obtain the food pellet once the light in the receptacle was turned off is referred to as the *limited hold period*. The limited hold period provided an opportunity for a rat to respond correctly even when the light stimulus had been turned off.

Response-Shaping Phase

A 2-day response-shaping phase immediately followed the habituation phase. These sessions were identical to the habituation sessions except that: 1) the shaping sessions lasted for 30 minutes or the completion of 100 trials; and 2) each rat had to poke its nose into the food receptacle to trigger the delivery of a food pellet. During the response-shaping sessions, rats were conditioned to perform a specific nose poke response to the light presented in the food receptacle.

Aperture-Response Phase

Immediately after the response-shaping phase, the aperture-response phase was initiated. During this phase, rats learned to nose poke into one of five apertures located on the wall opposite the food receptacle, rather than into the food magazine. Each session during this phase

lasted for 30 minutes or 100 complete trials. For each trial, a light was illuminated pseudorandomly in one of the five apertures; each rat had to learn to nose poke into the lit aperture. Rats learned to pay attention to the wall of apertures in order to correctly detect the location of the lit aperture, and then to subsequently collect their food reward from the receptacle. Once the food pellet was retrieved, a 5 second inter-trial interval commenced, followed by initiation of the next trial.

When a rat had learned to respond correctly for at least 50 of 100 possible trials over a 30-minute session, the task difficulty was gradually increased by reducing the amount of time that the stimulus aperture was lit on the next testing session. At the beginning of the aperture-response phase, rats had 30 seconds to locate the illuminated aperture. As the rats acquired the task during the aperture-response phase, the length of time that the aperture was illuminated was reduced to a 2.5 second duration.

Probe Trial Phase

After a stable performance was achieved (less than 20% omissions; more than 80% correct responses) at a 2.5 second stimulus duration over 2 consecutive testing sessions, rats began the probe trial phase. During the probe trial phase, the length of stimulus presentation during a given session was decreased from 2.5 seconds to one of four different durations (1.5, 1.0, 0.5, and 0.25 seconds), presented over the course of the next testing session on a pseudorandom schedule. The 5-second limited hold period became especially important during the probe trial phase, as the light in an aperture was only visible for a short amount of time. This made the completion of a nose poke in an aperture while the stimulus was presented difficult, if not impossible. Probe trials were not administered on consecutive testing days, meaning that

each probe trial was separated by at least one day of testing at baseline parameters (2.5-second stimulus duration).

Each rat was presented with each of the four different stimulus durations on three different testing sessions, and each probe trial session continued for 30 minutes or the completion of 100 trials.

Analysis. A *correct response* was defined as a nose poke into an illuminated aperture or during the limited hold period that followed.

Additionally, several different types of error are assessed in the 5-CSRTT (see Figure 1; Dalley, Cardinal, & Robbins, 2004). An *incorrect response* is a nose poke response into any of the four apertures where the stimulus was not presented. A *premature response* is a nose poke made during the inter-trial period (5 seconds) prior to the stimulus presentation. Lastly, an *omission* occurs when no response is made during the period where the aperture is lit or the 5-second limited hold period that immediately followed.

The frequency of correct responses, omitted responses, number incorrect, and number of premature responses made was used to assess performance in lesioned and control rats.

Neurochemical Analyses

Animals were decapitated, their brains removed, and the mPFC rapidly dissected out. The tissue was immediately flash-frozen on dry ice and stored at -80ºC. High pressure liquid chromatography (HPLC) was used to assess the concentrations of dopamine (DA) and norepinephrine (NE) in samples taken from the mPFC of each hemisphere.

High Pressure Liquid Chromatography. Tissue from a single hemisphere was placed in microcentrifuge tubes containing 250 µl of 0.2 M hydrochloric acid, sonicated until

homogenous, and then spun at 14,000 rpm for 25 minutes. The liquid portion of the sample was removed, placed in new microcentrifuge spin-X tubes, and spun at 10,000 rpm for an additional 10 minutes. Tissue samples were then frozen at -80ºC until processed using HPLC.

The mobile phase circulated through the HPLC system at a flow rate of 0.4 mL/minute, and consisted of 75 mM sodium dihydrogen phosphate, 0.025 mM ETDA, 10% acetonitrile, 100 µl /L triethylamine, and 1.7 mM 1-octanesulfonic acid (sodium salt). The mobile phase was vacuum filtered, and adjusted to a pH of 3.0 using 12 N phosphoric acid. A standard solution and tissue samples was injected into an HPLC instrument using an autosampler (ESA Model 540, Chelmsford, MA) and delivery system (ESA Model 580, Chelmsford, MA). In some cases, the tissue samples were manually injected into the HPLC. The standard solution was composed of 10 µl each of aqueous DA, NE, DOPAC, HVA, and 5-HIAA in 10 mL 0.2 M HCl. Tissue samples (20 µl) were injected following analysis of the standard solution. The analytical column (15 cm x 3 mm x 3 µm; Supelco, Model Supelcosil™, Bellefonte, PA) was connected to a coulometric detection system (ESA Coulochem II, Chelmsford, MA). Chromatograms were analyzed on a PC using VARIAN Star Chromatography Workstation (Version 5).

Data Analysis

Each dependent measure (correct responses; omissions; incorrect responses; premature responses) was assessed with repeated-measures ANOVAs across five stimulus durations (2.5, 1.5, 1.0, 0.5, and 0.25 seconds). Furthermore, a Pearson correlational coefficient was calculated to determine if correct or omitted responses were related to degree of catecholamine depletion in the mPFC.

An independent-samples t-test was conducted in order to determine the extent of DA depletion in the mPFC of 6-OHDA treated rats and sham controls. HPLC chromatographs were quantified by establishing the peak height and base distance of retention time spikes for DA and NE. The relative concentrations of neurotransmitters were calculated based on retention time chromatographs calibrated from the standard solution.

Results

There were significant reductions in both DA and NE tissue content in 6-OHDA treated animals relative to controls (for DA, ($t(9) = -7.61$, $p < .001$; for NE $t(9) = 5.83$, $p < .001$; see Table 1). Administration of 6-OHDA in the mPFC reduced DA in the lesioned rats ranging from between 16% and 62% of controls (average DA depletion to 33% of controls, *S.E.M.* = 7.77). NE depletion was slightly less robust, ranging from 26% to 65% of controls (average NE depletion to 50% of controls, *S.E.M.* = 6.29). A two-tailed Pearson correlational coefficient did not reveal a significant association between NE and DA depletion in lesioned rats ($r = .48$, $p = .34$).

Manipulating task difficulty in the 5-CSRTT by shortening the length of stimulus presentation resulted in similar behavioral changes in both lesioned and control animals. As the stimulus duration was decreased, both groups of rats were less able to respond correctly and there was a corresponding increase in the frequency of omitted, incorrect and premature responses (see Tables 2-5).

Repeated-measures ANOVAs revealed significant overall effects of shortening the stimulus duration in both 6-OHDA treated and control rats. As the stimulus duration decreased, percent correct responses decreased in both groups [$F(4, 36) = 64.30$, $p < .001$; see Figure 2; for raw data, see Table 1], and percent omissions increased [$F(4, 36) = 60.11$, $p < .001$; see Figure

3; for raw data, see Table 2]. Furthermore, shortened stimulus durations resulted in a significant increase in the number of incorrect responses [$F (4, 36) = 61.37, p < .001$; see Figure 4], and the number of premature responses [$F (4, 36) = 31.86, p < .001$; see Figure 5] in both groups. The 6-OHDA treated rats tended toward increased omissions at four out of five stimulus durations, and tended toward less correct at three out of five stimulus durations. However, repeated-measures ANOVAs did not reveal a significant interaction of lesion x stimulus duration on percent correct responses [$F (4, 36) = 0.40, p = 0.81$], or percent omitted responses [$F (4, 36) = 1.13, p = 0.36$]. The number of incorrect responses did not differ significantly between groups [$F (4, 36) = 1.30, p = 0.29$], nor did premature responses [$F (4, 36) = 0.17, p = 0.95$]. Furthermore, correlations between the degree of DA and NE depletion and number correct and omitted were not significant at stimulus durations of 0.25, 0.5, 1.0, or 1.5 seconds (data not shown).

Discussion

The catecholamine neurotoxin 6-OHDA administered into the mPFC of rats significantly depleted DA, and to a lesser extent NE. Dopamine lesions to the mPFC resulted in a trend toward increased omissions and reduced correct responses, particularly at more challenging stimulus durations. However, these depletions did not significantly impair rats' performance in the 5-CSRTT.

The lack of effect of 6-OHDA in the present study is surprising given the role of DA and cognition in the PFC (Goldman-Rakic, Muly, & Williams, 2000). A trend toward poorer performance in 6-OHDA treated animals suggests that the nonsignificant results are attributable to small group sizes or lack of sensitivity in the 5-CSRTT to detect attentional dysfunction in

lesioned rats. Alternately, when rats sustain combined NE and DA loss in the mPFC,

extracellular DA concentrations in the mPFC were not different from controls (Venator, Lewis,

& Finlay, 1999). It has been suggested that in the absence of NE reuptake proteins to clear

extracellular DA, concentrations of DA in the synapse are maintained at normal levels.

Quinolinic acid lesions of the mPFC, but not the parietal or cingulate cortex, impair 5-

CSRTT performance, suggesting that sustained attention assessed by this task is dependent on

the mPFC (Muir, Everitt, & Robbins, 1996). By contrast, in the present study behavioral deficits

were not observed despite significant catecholamine depletion in the mPFC. The goal of 6-

OHDA induced DA depletion of the mPFC in the present study was to mimic the type of DA

disruption seen in post-mortem analyses of schizophrenics (Akil et al., 1999). However, it is

possible that 6-OHDA neurotoxic effects are not enough to functionally impair rats in the 5-

CSRTT compared to those with mPFC quinolinic acid lesions, which affects predominantly

glutamatergic and GABAergic neurotransmission in lesioned brain regions (Scharcz, Whetsell,

& Mangano, 1983).

Inherent variability in rats' ability in the 5-CSRTT may have diminished the likelihood of

detecting group differences in the present study, particularly in light of small group sizes. Granon

et al. (2000) found that administration of the D1 agonist SCH 38393 into the mPFC improved

accuracy in rats categorized as exhibiting lower baseline accuracy (<75% correct at a stimulus

duration of 0.5 seconds). Alternately, antagonism of D1 receptors using SCH 23390 impaired

accuracy in rats exhibiting higher baseline accuracy (>75% correct at stimulus durations between

0.5 and 0.25 seconds). The different responses to dopaminergic pharmacological manipulation

between high and low performing rats suggests that DA activity in the mPFC may be vary due to

differences in endogenous DA neurotransmission in low- and high-performing rats. Likewise, behavioral deficits in the 5-CSRTT due to DA depletions may not be detected when rats innately performing at different levels are treated equally. Future studies might train rats in the 5-CSRTT prior to surgical manipulation in order to separate low- and high-performing rats before assessing the behavioral effects of 6-OHDA lesions. This would require larger sample sizes than those used in the present study.

The trend toward increased omissions in the lesioned group emerged in four out of five testing sessions, and was observed in trials with the shortest stimulus durations (0.5 and 0.25 seconds). Taking this into account, the trend toward performance deficit under challenging task circumstances should be explored in future studies utilizing 6-OHDA lesions at even shorter durations (ie., 0.10 and 0.05 seconds). It is possible that the baseline and limited stimulus durations used in the present study (2.5, 1.5, 1.0, 0.5, and 0.25 seconds) did not provide the challenge necessary to detect attentional dysfunction in DA depleted rats.

Another methodological consideration is that the 5-CSRTT testing session lasts for 100 trials or 30 minutes, whichever is longer. As the rats learn to perform at a criterion of >80% correct and <20% omitted responses at a 2.5 second stimulus duration, in nearly all animals the length of the testing session was "timed out," meaning that the animals reached 100 trials before 30 minutes. In many cases, the length was cut almost in half. Future studies could test performance in the 5-CSRTT if rats were required to maintain vigilant for the full 30 minutes under challenging testing conditions with shortened stimulus durations.

In the current study, the NE transporter antagonist DMI was administered to prevent 6-OHDA from damaging NE-containing neurons. Unexpectedly, 6-OHDA-treated animals

sustained ~50% NE depletion in addition to ~67% DA depletion in the mPFC. In previous work, administration of 6-OHDA using a similar protocol decreased NE tissue concentrations by 15-35% (e.g., Venator, Lewis, & Finlay, 1999). Given the larger NE depleting in the present study, it is possible that less NE in the mPFC resulted in masking the DA depletion resulting from 6-OHDA. The reasons for greater NE loss in the present study are unclear, but one possibility is that the batch of DMI used was ineffective for unknown reasons. Despite this unresolved issue, catecholamine depletion did not produce deficits on 5-CSRTT performance.

The aim of this thesis was to study the effects of DA depletions in the mPFC of rats on sustained attention performance. Trends toward poorer performance in lesioned animals should be explored in future studies with larger sample sizes. These trends support a possible role of DA and NE in the mPFC in modulating sustained attention while having no effect on impulsivity, consistent with other reports of DA agonists affecting response selection and not premature responding (Passetti, Dalley, & Robbins, 2003).

The trend in results suggests that DA in the PFC may play a role in attentional dysfunction in schizophrenia. Schizophrenia may involve imbalance of DA neurotransmission of cortical and subcortical sites, posing a challenge in developing pharmaceutical treatments. This challenge is further compounded by the heterogeneity of symptoms in schizophrenia, as both psychotic symptoms and cognitive deficits have considerable detriments in occupational and social settings. Unfortunately, current pharmacological therapy is primarily used to treat psychotic symptoms, and does not directly address the cognitive deficits (Wassef, Dott, Harris, Brown, O'Boyle, Meyer, et al., 1999). Complex interactions both within DA systems and between DA and other neurotransmitter systems in the PFC remain to be elucidated. Further

research is needed in order to elucidate the possible role of DA in cognitive deficits, and to

develop pharmacotherapy to DA pathways under conditions of both possible DA hyper- and

hypoactivity.

Table 1. Means and standard deviations of mPFC tissue dopamine (DA), norepinephrine (NE),

assayed by high-pressure liquid chromatography.

| | Relative Concentrations (expressed as ng/mg tissue wet weight) | |
	DA	NE
6-OHDA (n=6)	0.04 (0.02)**	0.25 (0.08)**
Control (n=5)	0.13 (0.01)	0.48 (0.05)

** *p* < .001

Table 2. Means and standard deviations of correct responses of 6-OHDA treated and sham control rats.

	Stimulus Duration (seconds)				
	2.5	1.5	1.0	0.5	0.25
6-OHDA (n=6)	83.3 ± 5.5	69.2 ± 7.4	64.3 ± 6.7	36.9 ± 10.9	18.1 ± 5.1
Control (n=5)	83.8 ± 3.9	75.6 ± 8.5	64.7 ± 8.2	41.7 ± 5.5	23.9 ± 6.6
Total	83.6 ± 4.6	72.1 ± 8.2	64.5 ± 12.9	39.1 ± 8.8	20.7 ± 6.3

Table 3. Means and standard deviations of omitted responses of 6-OHDA treated and sham

control rats.

	Stimulus Duration (seconds)				
	2.5	1.5	1.0	0.5	0.25
6-OHDA	9.6 ± 4.4	18.7 ± 8.3	23.0 ± 15.7	37.8 ± 15.5	50.3 ± 13.7
Control	10.0 ± 4.1	12.1 ± 7.7	20.3 ± 11.2	29.0 ± 7.3	40.0 ± 12.5
Total	9.8 ± 4.1	15.7 ± 8.4	21.8 ± 13.2	33.8 ± 12.7	45.6 ± 13.6

Table 4. Means and standard deviations of incorrect responses of 6-OHDA treated and control rats.

	Stimulus Duration (seconds)				
	2.5	1.5	1.0	0.5	0.25
6-OHDA (n=6)	4.0 ± 3.2	7.3 ± 4.2	8.2 ± 6.1	16.4 ± 7.8	20.1 ± 8.2
Control (n=5)	3.5 ± 2.3	7.7 ± 5.0	10.4 ± 5.8	19.8 ± 6.4	25.3 ± 8.3
Total	3.8 ± 2.7	7.5 ± 4.3	9.2 ± 5.7	18.0 ± 7.0	22.5 ± 8.3

Table 5. Means and standard deviations of premature responses of 6-OHDA treated and control rats.

| | Stimulus Duration (seconds) | | | | |
	2.5	1.5	1.0	0.5	0.25
6-OHDA (n=6)	3.1 ± 2.4	4.7 ± 1.0	4.4 ± 2.2	8.9 ± 2.7	11.6 ± 3.8
Control (n=5)	2.7 ± 2.6	4.3 ± 4.2	4.6 ± 4.1	9.5 ± 4.0	10.9 ± 5.8
Total	2.9 ± 2.4	4.5 ± 2.7	4.5 ± 3.0	9.2 ± 3.2	11.2 ± 4.8

Figure Captions

Figure 1. Possible responses in the 5-choice serial reaction time task over the 30-minute testing session. (Dalley, Cardinal, & Robbins, 2004).
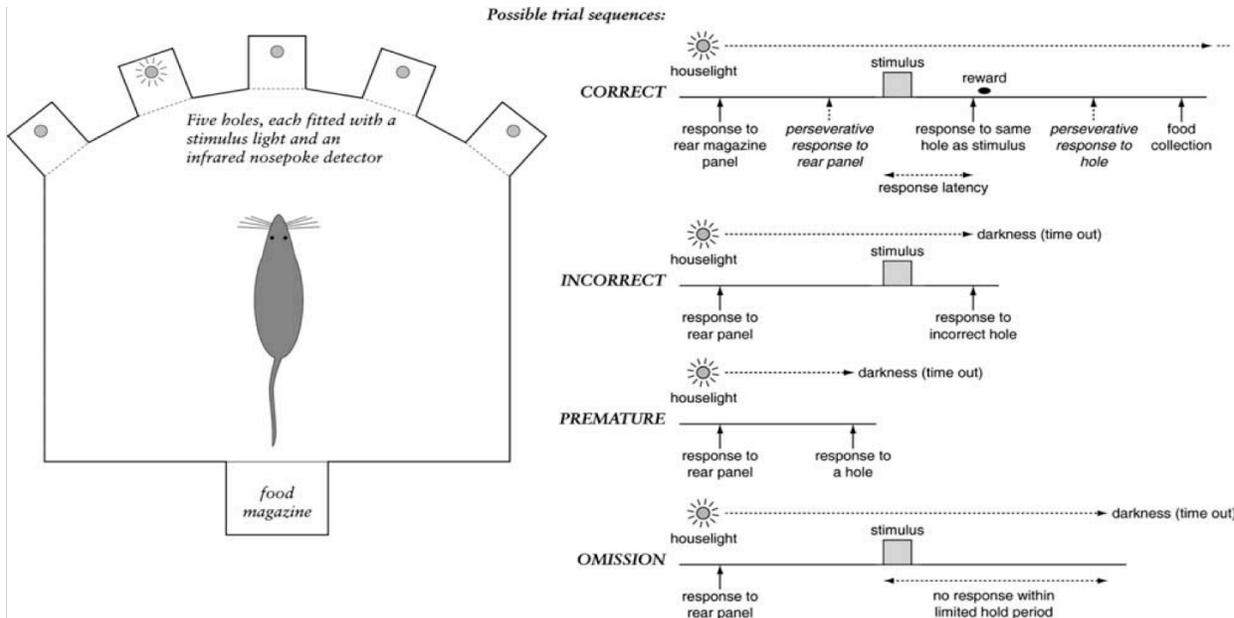
Figure 2. Percent correct* responses in control and 6-OHDA treated animals (error bars = S.E.M.). *% Correct = # Correct / Correct + Incorrect*
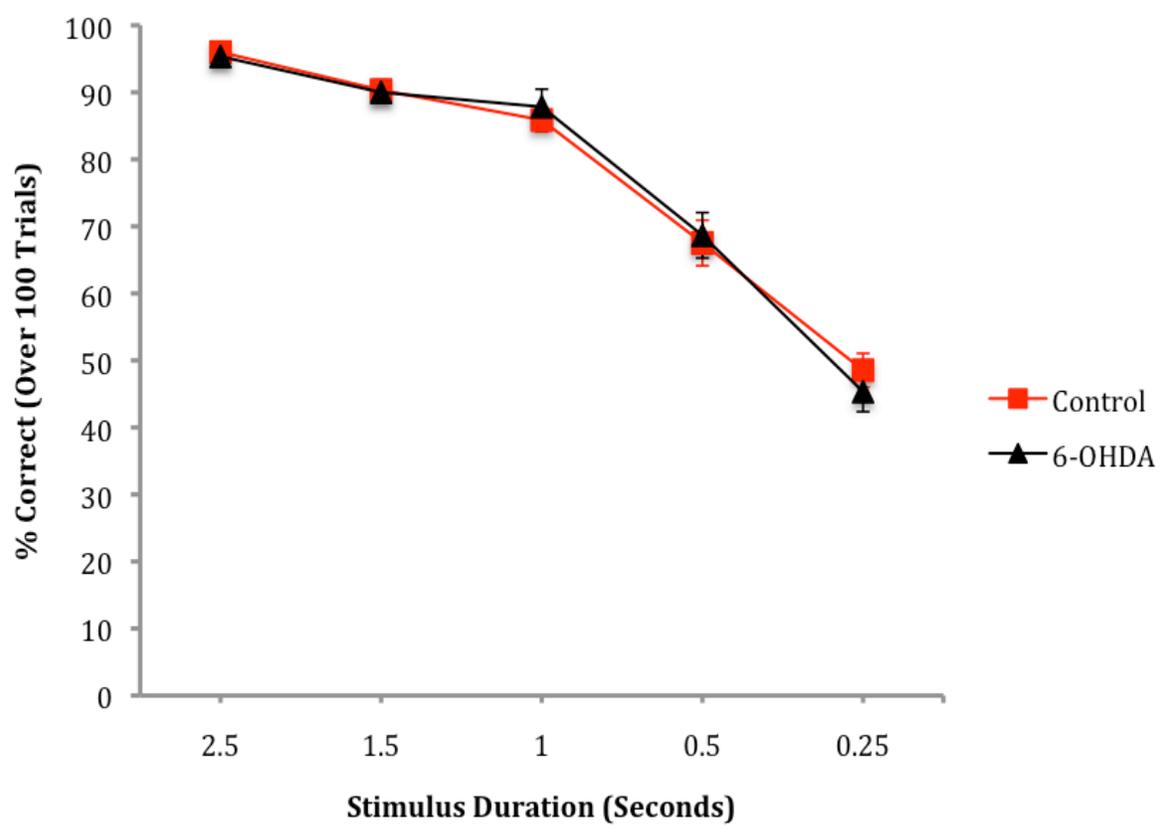
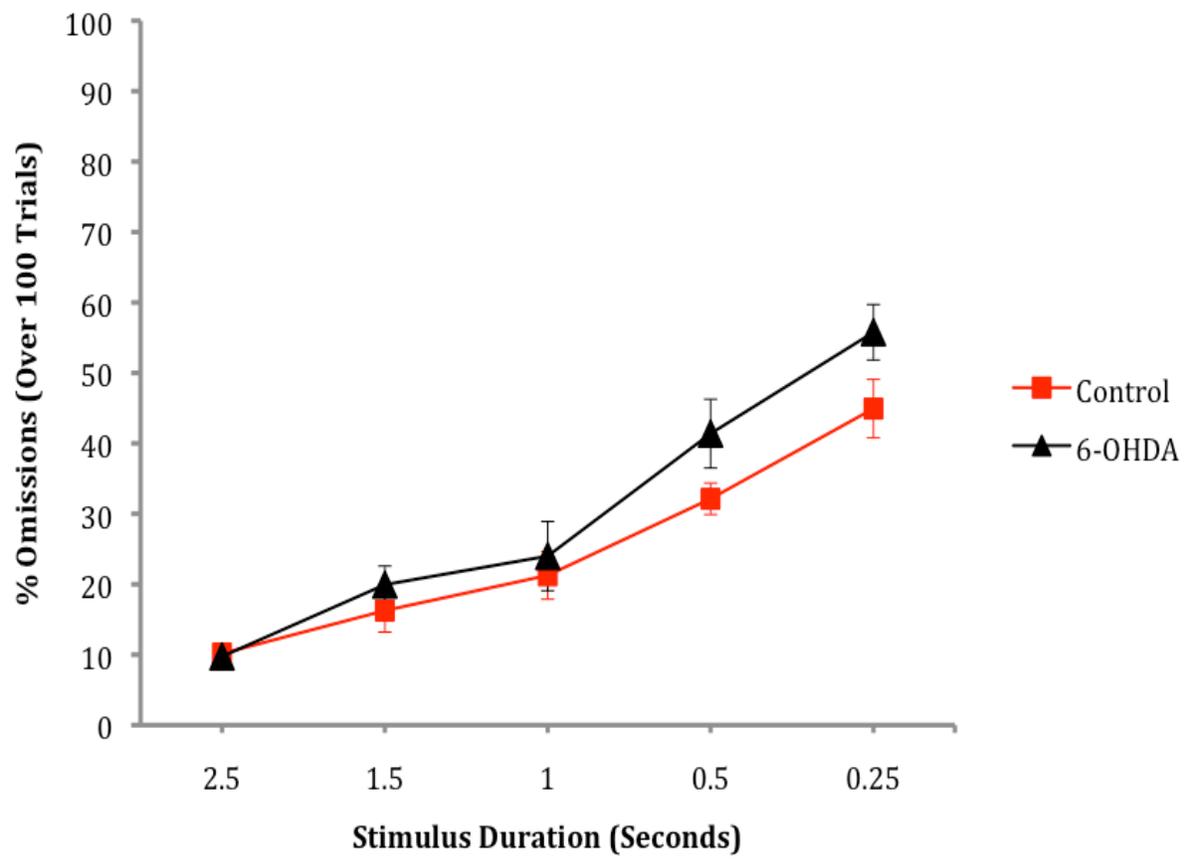*Figure 3*. Percent omissions* in control and 6-OHDA treated animals (error bars = S.E.M.). *% Omitted = # Omissions / Omissions + Correct + Incorrect*
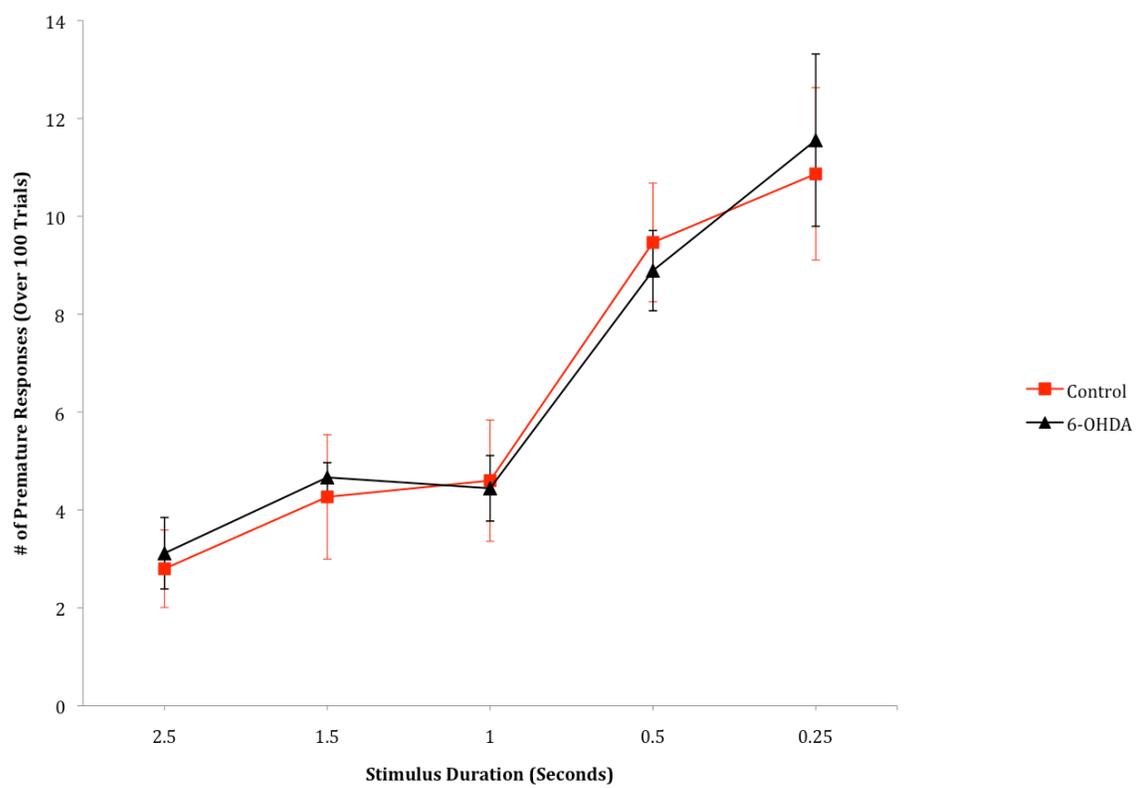
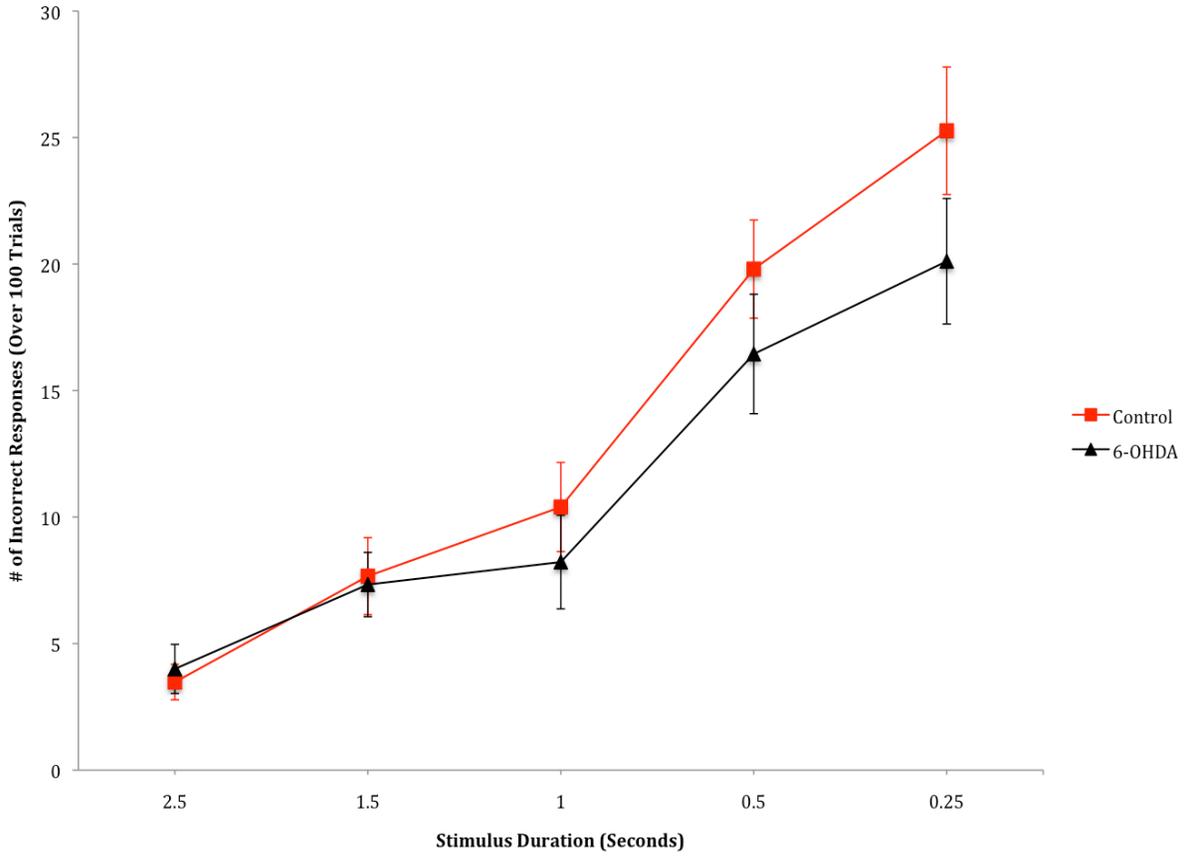*Figure 4*. Number of premature responses in control and 6-OHDA treated animals (error bars = S.E.M.).

Figure 5. Number of incorrect responses in control and 6-OHDA treated animals (error bars = S.E.M.).

Five holes, each fitted with a stimulus light and an infrared nosepoke detector

food magazine

Possible trial sequences:

CORRECT

houselight
stimulus
reward

response to rear magazine panel
perseverative response to rear panel
response to same hole as stimulus
perseverative response to hole
food collection

response latency

INCORRECT

houselight
stimulus
darkness (time out)

response to rear panel
response to incorrect hole

PREMATURE

houselight
darkness (time out)

response to rear panel
response to a hole

OMISSION

houselight
stimulus
darkness (time out)

response to rear panel
no response within limited hold period

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