Chapter 1

1.0.0. Introduction

Parkinson's disease (PD) is a neurodegenerative disease with a prevalence estimated at 780 individuals per 100 000 within an Australian population (Chan, et al., 2005). The cardinal motor symptoms of PD are tremor (resting and/or action tremor), rigidity, slowing of movement (bradykinesia) and, with disease progression, postural instability (Gelb, Oliver & Gilman, 1999). Although these motor characteristics play a crucial role in the diagnosis of PD, non-motor deficits are also associated with the disease. Of interest, both cognitive deficits and mood disturbances may be a consequence of PD. For example, individuals with PD are at a greater risk of developing dementia, particularly at the end-stage of their disease (Buter, et al., 2008) and they also have a higher incidence of depressed mood than their typically developing peers (Nicoletti, et al., 2010). Indeed, Papapetropoulos, Gonzalez, Lieberman, Villar and Mash (2005) found that 51% of autopsy confirmed patients with PD were diagnosed with dementia prior to death and 43% had been diagnosed with depression.

1.2.0. Cognitive deficits in PD(ND)

Not all individuals with PD develop dementia; however having PD increases an individual's risk for developing cognitive impairments. Between 19-30% of newly diagnosed individuals with PD who are not demented (PDND) will have a mild cognitive impairment, which indicates that their cognitive skills are inferior to

typically aging peers in at least one domain of cognitive functioning (Aarsland, Brønnick, Larsen, Tysnes & Alves, 2009; Elgh, et al., 2009). As will be discussed, the cognitive domains typically impaired in individuals with PDND primarily include deficits in executive functioning, memory, speed of information processing and visuospatial abilities.

1.2.1. Frontal lobe dysfunction in PDND

On neuropsychological tests, individuals with PDND demonstrate significant executive deficits in skills such as organisation and planning (e.g., poorer performance on the Rey Osterrith Figure task), problem-solving abilities (e.g., Tower of London task), abstract thinking (e.g., metaphorical speech, Raven's progressive matrices) and mental flexibility (e.g., Trail Making Test) than their typically aging peers (McKinlay, Dalrymple-Alford, Grace & Roger, 2009; Volpato, Signorini, Meneghello & Semenza, 2009; Stefanova, Kostic, Ziropadja, Ocic & Markovic, 2001; Farina, et al., 2000). Additionally, working memory deficits (e.g., 2-back test, reading span) and generation and fluency skills (e.g., verbal semantic fluency, non-verbal Ruff Figural Fluency) have also been found to be impaired in individuals with PDND (Barnes & Boubert, 2008; Kensinger, Shearer, Locascio, Growdon & Corkin, 2003; Fama, et al., 1998).

1.2.2. Memory deficits in PDND

Memory deficits have been described in terms of the individual with PDND having either a retrieval deficit, whereby the person has difficulty recalling information that has been stored in memory (Muslimović, Post, Speelman & Schmand, 2005; Stefanova, et al., 2001), or that the individual with PDND has inefficient encoding

abilities, which hinders the amount of information they can learn to then store into memory (Brønnick, Alves, Aarsland, Tysne & Larsen, 2011). The type of memory disturbance in PDND is therefore not associated with a loss of stored information, but rather, a difficulty in either getting information into or retrieving information from memory stores.

1.2.3. Cognitive slowing and visuospatial deficits in PDND

Psychomotor slowing is frequently found in individuals with PDND (e.g., poorer performance on Trail making test- trails A, electronic tapping test; Elgh, et al., 2009), which is not surprising considering that motor deficits are a symptom of PD and that psychomotor tasks typically require fine motor control of the hand. However, poorer than peer performance by PDND patients on neuropsychological tasks that do not rely upon controlled hand movements (e.g., naming of colour patches or word reading reaction times) suggest that the underlying cognitive processes of individuals with PDND may be substantially slowed compared to typically aging peers (McKinlay et al., 2009; Muslimović, et al., 2005). Lastly, there is evidence that visuospatial deficits may also manifest in individuals with PDND. For example, Muslimović and associates (2005) demonstrated that individuals with PDND had poorer visuospatial judgement than healthy peers on non-motor tasks measuring visuospatial ability (e.g., a Judgement of line orientation task).

A range of cognitive skills have been found to be impaired within PDND populations, however, at the individual level, a person with PDND is unlikely to demonstrate all of these deficits. That is, for those with a new diagnosis of PD, the majority of individuals with a mild cognitive deficit will have a deficit in one

cognitive domain only (Aarsland, et al., 2010). In addition to cognitive dysfunction, individuals with PD are at a higher risk of mood disturbances.

1.3.0. Mood disturbances in PDND

Incidence of depression has been studied in individuals with PDND. Research indicates that clinically defined major depression occurs in approximately 22-26% of individuals with PDND, with sub-clinical threshold depression occurring in an additional 29% of patients (Nation, Katzen, Papapetropoulos, Scanlon & Levin, 2009; Ehrt, Brønnick, Leentjens, Larsen, & Aarsland, 2006). Additionally, individuals with PDND may also show changes in mood with increased apathy. Individuals with PDND endorsed significantly higher apathetic behaviour, indicating that they demonstrated less initiative-taking behaviours in their daily lives than typically developing peers (Zgaljardic, et al., 2006). In line with this finding, individuals with PDND have demonstrated less perseverance than their peers on mentally challenging tasks, giving-up on attempting to solve problems when the complexity of the task increased (Schneider, 2007). These results indicate that depression and apathy may be problematic issues for individuals with PD, in addition to difficulties in motor or cognitive functioning.

1.4.0. BG circuits and motor, cognitive and affective functioning

While conceptually different, the motor, cognitive and affective deficits in PD have all been attributed to the same underlying degenerative process that occurs within the brain in PD. As described by Mandir and Vaughan (2000) degeneration and loss of dopamine (DA) containing neurons in PD primarily occurs within a small brain

region called the substantia nigra. The substantia nigra provides dopaminergic input to the basal ganglia (BG) and loss of dopaminergic input from the substantia nigra in PD disrupts the functional processes of the BG. The BG includes deep subcortical brain structures such as globus pallidus, striatum (including the caudate nuclei and putamen), subthalamic nucleui and some authors also include the substantia nigra (Galvan & Wichmann, 2008; Anderson, Costantino, & Stratford, 2004). The BG are anatomically and functionally related to five independent circuit loops and have a reciprocal relationship to other cortical and subcortical brain regions (Mandir & Vaughan, 2000; Alexander, DeLong & Strick, 1986), which contribute to the motor, cognitive and affective functioning of a person (Mega & Cummings, 1994).

Motor movement is primarily mediated by the 'motor circuit' and 'oculomotor circuit'. The motor circuit involves reciprocal interaction between the BG with the supplementary and primary motor cortices of the frontal lobe (Alexander & Crutcher, 1990). The motor circuit is involved in controlling overt motor movement, including limb and orofacial movement (Alexander, DeLong & Strick, 1986) and the motor deficits of PD are believed to be the result of disruption to this motor circuit (Galvan & Wichmann, 2008). The 'oculomotor circuit' mediates oculomotor movement (e.g., gaze fixation, gaze shifting and saccadic movement) and results from the interaction between the BG to the frontal eye fields, prefrontal and posterior parietal cortex (Lencer, et al., 2004; Cummings, 1993).

Cognitive and affective functioning is primarily mediated by the three remaining circuits. The 'dorsolateral prefrontal circuit', involves the reciprocal interaction between the BG with the dorsolateral prefrontal cortices, and is involved in cognitive

abilities such as executive function, working memory ability and planned motor action sequences (e.g., in response to the environment) (Zgaljardic, et al., 2006; Cummings, 1993). The 'orbital frontal circuit' is associated with monitoring of behavioural responses (such as impulse control and lability) and mood (e.g., irritability) (Mega & Cummings, 1994). The orbital frontal circuit includes interaction between the BG with the lateral orbitofrontal regions (Cummings, 1993). Behaviour such as apathy, attention regulation and intentional/motivated behaviour is mediated by the BG with the anterior cingulated gyrus and limbic structures (including the amygdalae and hippocampi), forming the 'anterior cingulate circuit' (Zgaljardic, et al., 2006; Mega & Cummings, 1994; Cummings, 1993).

Research therefore suggests that the domains of motor movement, cognition and mood are interrelated, raising the possibility that therapeutic interventions of PD targeting one domain may have subsequent generalisation effects upon the other domains. Interventions to treat PD, however, tend to primarily focus upon improving the motor deficits of the disease, with minimal focus upon targeting cognitive impairments.

1.5.0. Therapeutic interventions of PD

Non-pharmacological interventions (e.g., physiotherapy, occupational and speech therapy) primarily focus upon improving the motor skills of people with PD to improve their daily functioning (See Gage & Storey, 2004). Some interventions do include measures of mood and general wellbeing, with a subjective estimation of the individual's cognitive functioning obtained from general quality of life (QoL) measures, however these measures are are often secondary generalisation measures

in which physical-based interventions primarily target improving physical outcomes (e.g., Reuter, et al., 2011; Dereli & Yaliman, 2010). To date, there appears to be a lack of therapeutic interventions specifically targeting cognitive impairment in individuals with PD. Furthermore, there is also a lack of objective psychometric measures used to assess cognitive function, even as a generalisation measure. Continued research exploring the effects that interventions may have upon improving functioning across physical, mood and cognitive domains would be both beneficial for individuals with PD and instructive for researchers- informing the research hypothesis that these independent domains of functioning (i.e., movement, cognition and mood) are interlinked at a neurological level.

1.6.0. Rodent models of PD

An alternate method to explore the protective effects of non-pharmacological interventions for PD, demonstrating not only treatment efficacy at an overt behavioural level (i.e., alleviation of motor symptoms) but also neural protection at a covert level within the brain, is through rodent models of PD. The loss of dopamine within the BG and subsequent dysfunction in the operation of the BG circuits are reproduced in rodent models of PD. To mimic the dopaminergic loss within the BG in humans, rodents are administered neurotoxins that target dopaminergic neurons, such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), into sites of the BG (e.g., often the substantia nigra or striatum) to induce selective cell death of these neurons (Ferro, et al., 2005; Sauer & Oertel, 1994).

Rodent models of PD produce motor deficits (e.g., inducing both reduced stride length and spontaneous locomotor activity; Metz, Tse, Ballermann, Smith & Fouad, 2005), and dysfunctional electrical brain activity (within and between cortical and subcortical regions of the brain; Sharott, et al., 2005) that mimic similar deficits noted in humans with PD. Additionally, rat models of PD that use low doses of neurotoxins injected within the BG produce neural loss but do not induce motor impairment in these animals (e.g., Srinivasan & Schmidt, 2003). These models are referred to as early-stage PD models and are believed to replicate the pre-clinical stages of PD in humans (Ferro, et al., 2005; Da Cunha, et al., 2001). To date, few rodent models of PD explore whether or not cognitive deficits are produced in these models. However, limited evidence has emerged that demonstrate that cognitive deficits are produced in early-stage PD rat models. Principally, working memory deficits have been established in early-stage PD rats (Braga, Kouzmine, Canteras & Da Cunha, 2005; Bellissimo, et al., 2004), and another study has supported the notion that learning and memory is impaired in these rodents (Da Cunha, et al., 2001).

Pharmacological treatments have often been used on 6-OHDA rat models of PD to either demonstrate their efficacy in treating the induced motor deficits in PD rats (Lundblad, et al., 2002), or to trial drug treatments to counteract the side-effects (e.g., dyskinesia) that are induced by chronic administration of PD medication (L-DOPA/benserazide; Paquette, et al., 2009; Gerlach, van den Buuse, Blaha, Bremen & Riederer, 2004). A non-pharmacological intervention that may be effective in the treatment of both cognitive and motor deficits in rat PD subjects is exposure to environmental enrichment (EE).

1.7.0. Environmental enrichment (EE)

In rodent studies of EE, stimuli used for enrichment may include giving subjects accessibility to increased social interaction (e.g., by increasing the number of subjects housed together); stimuli such as toys, tunnels and nesting materials; exercise equipment; or even music (e.g., Goldberg, Haack, & Meshul, 2011; Sutoo & Akiyama, 2004). While one category of stimulation can be administered to rodents (e.g., a running wheel as a form of exercise treatment), complex environmental enrichment (CEE) may also be administered by providing two or more forms of enriching stimuli within the subject's environment (e.g., chew toys plus exercise equipment) (Harburger, Nzerem & Frick, 2007; Faherty, Shepard, Herasimtschuk & Smeyne, 2005).

1.8.0. EE protects against motor, cognitive and mood disturbances in central nervous system challenged rodents

Exposure to CEE has been shown to attenuate locomotor, cognitive and mood disturbances in central nervous system (CNS) challenged rodents. For example, in mice provided 2-3 months of CEE, locomotor hyperactivity induced by the acute administration of the psychostimulant cocaine was attenuated and CEE reduced sensitised locomotor activity to repeated drug administration following a forced abstinence (Solinas, Thiriet, El Rawas, Lardeux & Jaber, 2009). Complex enrichment also protected against short-term memory impairment in rats that were delivered a neonatal left-side hypoxic ischemic injury. Three weeks of CEE post-injury maintained recognition memory on the novel object recognition task for treated rats, while non-enriched rats showed deficient short-term recognition

memory ability (Pereira, Strapasson, Nabinger, Achaval & Netto, 2008). Lastly, depressive symptoms (reduced sucrose consumption) can be ameliorated by exposure to CEE in stressed rats. For example, rats administered CEE for 10-days, following a period (21-days) of chronic stress, prevented depressive symptoms in these animals. For, rats given CEE continued to prefer drinking a sucrose solution but sucrose consumption declined in non-enriched rats stressed (Veena, Srikumar, Raju & Shankaranarayana Rao, 2009).

Due to the existing evidence that EE has been shown to protect against behavioural and neural dysfunctions elicited in rodent models of neurodegenerative conditions (see Laviola, Hannan, Macrí, Solinas & Jaber, 2008) and that CEE has been able to protect against motor, cognitive and mood dysfunction that are similarly predicted in a rodent model of PD, CEE would be an ideal candidate to trial as a treatment for the motor and non-motor deficits of PD rodents.

1.9.0. EE and rat models of PD

There are limited studies that have reported using EE to improve motor functioning in PD rats. In the study by Tarjiri and colleagues, PD rats that were provided exercise (i.e., forced treadmill running for one month post-lesion), had significantly improved motor movement, i.e., forepaw asymmetry, than PD rats that did not have exercise enrichment (Tarjiri, et al., 2010). The rodents in this study had been administered a unilateral injection of 6-OHDA within the BG (striatum) and in addition to motor improvement, exercise also reduced the degree of cell death within the striatum and increased neuroplasticity within the brain, with new cell migration

occurring at the lesion site in the exercise enriched group versus the control rats (Tarjiri, et al., 2010).

Complex environmental enrichment has also been used as a treatment for early-stage PD rats. Jadavji and Metz (2009) found that CEE improved fine motor skills in early-stage PD rats, acting as both a preventative measure for PD and as a form of therapeutic intervention following the acquisition of PD. In the study, rats were placed in CEEs for either 6-weeks prior to surgery, which involved unilateral injection of 6-OHDA into the nigrostriatal bundle, while other subjects received complex enrichment for approximately 3-weeks only after surgery. Environmental stimuli included increased social interaction (by increasing the number of subjects housed together), larger cages (three-tiered cage) with interchangeable toys and equipment to interact with and novel food exposure. Behavioural testing demonstrated that CEE improved fine-motor performance (skilled reaching) whether or not treatment was given before or after surgery (Jadavji & Metz, 2009). These results suggest that CEE may act as either a preventative intervention for PD or a therapeutic intervention for the progression of PD.

These above studies indicate that EE improve motor functioning of PD rats however, to date, the ability of EE to improve cognitive functioning of early-stage PD rats has not been explored. In light of the evidence from rodent models of PD we can identify that motor deficits can be elicited by dopaminergic loss to the BG; however the range of cognitive deficits that are induced by lesions in PD models has not been thoroughly studied. Considering the breadth (e.g., executive functioning, memory, visuospatial ability) and intricacies of cognitive dysfunction (e.g., retrieval versus

encoding memory deficits) in humans with PDND, more exploration of the cognitive functioning of PD in rodent models may uncover similar cognitive disturbances across the cognitive domains that are observed in humans with PD. Likewise, the types of affective changes associated with disruption to BG functioning has not been adequately addressed in PD rodents.

Before exploring a rat model of PD that induces intracerebral dopaminergic cell loss, the ability for cognitive and affective changes to be elicited and treated with CEE can be explored via a rodent model that produces transient disruption to dopaminergic functioning via administration of antagonists which target DA receptors.

1.10.0. DA receptor antagonism and motor, cognitive and affective disturbances

Dopamine is an important catecholamine within the CNS that has been found to mediate locomotor activity, cognitive functioning, mood and coping ability (de la Mora, Gallegos-Cari, Arizmendi-García, Marcellino & Fuxe, 2010; Andrzejewski, Spencer & Kelley, 2006; Coco & Weiss, 2005). Dopamine may produce behavioural changes through activity at the D_1 receptor subtype (which includes the D_1 and D_5 receptors) or on D_2 receptor subtype (D_2, D_3 and D_4 receptors) (Jaber, Robinson, Missale & Caron, 1996). Dysfunction of dopamine systems and receptor activity has not only been found to be associated with PD but also plays a role in other developmental (e.g., attention deficit/ hyperactivity disorder) and mental health disorders (e.g., drug-taking behaviour, binge eating and schizophrenia) (Bello &

Hajnal, 2010; van der Kooij & Glennon, 2007; Pierce & Kumaresan, 2006; Ohara, 2007).

The role of DA in motor and non-motor behaviour is evident in rodents when dopaminergic functioning is transiently disrupted by the administration of DA receptor antagonists. For example, spontaneous locomotor activity is reduced in rats administered a D_1 receptor antagonist (Meyer, Cottrell, Van Hartesveldt & Potter, 1993), or in those co-administered D_1 and D_2 receptor antagonists (Kiyatkin, 2008). Recognition memory is also impeded in mice administered a D_1 receptor antagonist (Nagai, et al., 2007), while rodents administered either D_1 or D_2 receptor antagonists demonstrate anhedonic behaviour by drinking less sucrose when under the influence of the drug (Duong & Weingarten, 1993; Schneider, Gibbs & Smith,1986).

That motor, cognitive and affective changes can be induced in a rodent with temporarily disrupted dopaminergic functioning raises the possibility that these deficits could be elicited in a PD rat model. The effects of DA receptor antagonism to impair cognitive and affective measures, such as recognition memory and sucrose consumption tests, are also important trials to examine the likelihood of reducing these skills in an early-stage PD model. Furthermore, DA antagonist models allow for the examination of possible treatments, such as CEE, to reverse antagonist-mediated cognitive and affective deficits which may then be applied to an early-stage PD model in rats.

1.11.0. Hypotheses

The aim of this paper was to test the following hypotheses: 1) Administration of DA antagonists would induce motor, memory and anhedonic dysfunction in drug-treated rats, 2) A brief period of CEE provided to rats prior to DA challenge will reverse the locomotor, cognitive and affective dysfunction as induced by DA receptor antagonism, 3) An early-stage rat model will produce, in aged rats, no gross locomotor deficits but cognitive (memory) and affective disturbances due to dopaminergic lesions of the BG, and 4) If the early-stage PD model shows reduced memory or hedonic processing, CEE treatment will reverse the behavioural and affective deficits.

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Chapter 1

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Chapter 2

The effect of complex environmental enrichment on locomotor, memory and affective function following dopamine receptor antagonism in rats.

2.1.0. Introduction

The role of dopamine (DA) receptor activity on cognitive and behavioural function has been intensely studied. There are two dopamine receptor families which consist of the D_1-like receptor family (D_1 and D_5 DA receptor subtypes) and the D_2-like receptor family ($D_{2\text{-}4}$ receptor subtypes) (Jaber, Robbinson, Missale & Caron, 1996). Through manipulation of DA availability at these DA receptors sites, researchers have discovered the diverse and important role DA plays in modulating behaviour. As will be discussed, DA is involved in functions such as locomotor movement, learning and memory ability, and engagement in rewarding activities.

Dopamine and DA receptors, particularly D_1 receptors, have been clearly established to play an important role in motor movement (Dreher & Jackson, 1989; Beninger, 1983). Blocking DA receptors by the administration of selective D_1 receptor antagonists can decrease spontaneous locomotor activity or moderate hyperactivity induced by psychomotor drugs. For example, the administration of SCH23390, which has high selectivity as a D_1 receptor antagonist, reduced spontaneous locomotor activity in drug naïve rats (Meyer, Cottrell, Van Hartesveldt & Potter, 1993) and attenuated hyperactivity that was elicited by the administration of psychostimulant drugs (Hall, Powers & Gulley, 2009; Daniela, Brennan, Gittings, Hely & Schenk, 2004; Schindler & Carmona, 2002). Likewise, SCH23390 was

found to significantly reduce dyskinetic symptoms in semi-Parkinsonian rats that had been elicited by chronic administration of L-DOPA (a precursor of DA) (Taylor, Bishop & Walker, 2005). These studies highlight the well-described view that locomotor movement is essentially moderated by the presence and availability of DA within the central nervous system (CNS) and that the administration of SCH23390 can modulate this behaviour.

In relation to DA and memory processing, the durability of an encoded memory can depend upon the presence and availability of DA at the time of the learning event. The modulation of DA receptors prior to a learning event has been shown to either enhance or impede memory. For example, activation of DA receptors by the administration of the D_1 receptor agonist SKF 81297 prior to an encoding phase (Time 1: T1) on a novel object recognition (NOR) task improved long-term recognition memory of rats (at the recall phase Time 2: T2) but, impaired memory performance when rats were tested after a short delay period (Hotte, Naudon & Jay, 2005). Dopamine antagonism, on the other hand, has been shown to induce memory deficits in rodents. Antagonism of the D_1 receptor in mice prior to the learning trial on a NOR task, impaired recognition memory in these subjects (Nagai et al., 2007); a result similarly replicated by Besheer, Jensen and Bevins (1999) with rats using high dose SCH23990. These studies suggest the level of DA availability within the CNS at the time of a learning event may either enhance or impede learning processes and therefore later memory recall performance.

Dopamine also modulates engagement in naturally rewarding behaviours such as sucrose consumption. Rodents prefer drinking sucrose solutions to water and other

sweet liquids such as sacchrin (Hajnal & Norgren, 2001; Yu, Silva, Sclafani, Delater & Bodnar, 2000, respectively). Manipulation of DA within the CNS alters this sucrose drinking behaviour in rodents. Hajnal and Norgen (2001) demonstrated that by directly increasing DA availability to the nucleus accumbens (via a DA transporter blocker) sucrose consumption was significantly increased in rats, while D_1 and D_2 antagonists each infused concurrently into this region with the transporter blocker attenuated this behaviour.

The systemic administration of DA antagonists has also shown to alter sucrose consumptive behaviour. Both D_1 and D_2 receptor antagonists administered peripherally (via either intraperitoneal or subcutaneous injections) have been found to reduce sucrose consumption under both sham and real feeding conditions in rats (Hajnal, De Jonghe & Covasa, 2007) and in real feeding mice (Dym, Pinhas, Robak, Sclafani & Bodnar, 2009). The reduction in sucrose consumption following systemic administration of the DA antagonists is believed to be centrally mediated and not a systemic effect of the drug upon the peripheral nervous system (PNS). For instance, pimozide is a DA receptor antagonist that acts upon D_2 receptors within the CNS and PNS, while the antagonist domperidone targets peripheral DA receptors (Duong & Weingarten, 1993). Duong and Weingarten administered intraperitoneal injections of these drugs into rats and found that domperidone did not reduce real or sham feeding sucrose consumption in these animals yet, pimozide administration did. The authors therefore concluded that it was the effect of pimozide within the CNS that produced behavioural changes in sucrose consumption, rather than the action of DA antagonists upon the PNS.

Dopamine, therefore, plays an important central role across a number of animal behaviours. Importantly, dopamine antagonism within the brain may disrupt locomotor ambulation, memory performance or decrease engagement in naturally rewarding activities (such as sucrose consumption). Of interest to this paper is whether complex environmental enrichment (CEE) may protect against the locomotor, cognitive and behavioural deficits induced by DA antagonism.

Types of environmental enrichment (EE) may include social enrichment (where rodents are group housed), exposure to music, exercise (including treadmill running or voluntary running wheel exercise) and interactive play equipment (such as toys, climbing equipment and tunnels, etc). Complex environmental enrichment typically involves at least two of these forms of enrichment (e.g., Harburger, Nzerem & Frick, 2007; Faverjon et al., 2002). Exposure to enriched environments has been shown to preserve cognitive, behavioural and neural functioning in typically aging rodents and in rodent models of acquired brain injury, developmental disorder and neurodegenerative conditions (e.g., Harburger, et al., 2007; Mora, Segovia & del Arco, 2007; Griesbach, Hovda, & Gomez-Pinilla, 2009; Hoffman, et al., 2008; Dhanushkodi, Bindu, Raju & Kutty, 2007; Pamplona, Pandolfo, Savoldi, Prediger & Takahashi, 2009; Faherty, Shepard, Hermasimtschuk & Smeyne, 2005; and, Arendash, Garcia, Costa, Cracchio, Wefes & Potter, 2004). More specifically, CEE has been shown to have direct influences upon locomotor, memory and sucrose preference behaviours.

In relation to locomotor movement, typically-developed rodents housed in complex enriched environments have been found to habituate more quickly to novel contexts.

Rats given CEE demonstrate less spontaneous locomotor activity and reduced exploratory behaviour over time compared to subjects provided minimal to no enrichment (Varty, Paulus, Braff & Geyer, 2000; Hoffmann, Schütte, Koche & Schwabe, 2009). Alternatively, CEE appears to improve locomotor functioning in subjects with a compromised CNS, including movement disorders in rodent models of neurological conditions. For example, CEE improved spontaneous locomotor activity in genetically bred mice with Rett syndrome and improved skilled beam walking (in relation to both accuracy and speed) in Parkinsonian rats that had had intracranial unilateral lesions induced within either the substantia nigra or striatum (Nag, et al., 2009; Urakawa, et al., 2007, respectively).

Complex environmental enrichment can either enhance memory recall ability in typically aging rodents or improve memory performance in CNS-challenged rodents. In typically developing rats, Leal-Galicia, Castañeda-Bueno, Quiroz-Baez and Arias (2008) found that group-housed rats that were given intermittent access to interactive play arenas throughout youth (over an 18 month period) sustained good memory performance in their late adulthood. The authors found that elderly rats (aged 21 months) who were not provided CEE in youth demonstrated poor recognition memory ability on the NOR task, while the previously enriched subjects demonstrated intact memory by preferring to spend >50% of their time exploring a novel object over that of a familiar object at T2 (Leal-Galicia, et al., 2008). Environmental enrichment can also improve memory performance following CNS injury. For example, rats that were raised in an enriched environment following a postnatal hypoxic brain injury where protected against a memory deficit, as indicated by performance on the NOR task (Pereira, Strapasson, Nabinger, Achaval & Netto,

2008). These results suggest that CEE may act as both a preventative or treating intervention for negative changes that occur within the CNS that also impacts upon memory functioning.

Finally, CEE can affect sucrose consumptive behaviour. Typically-aging rats raised in a complex enriched environment drink less sucrose water than isolated or group housed rats (Brenes & Fornaguera, 2008). Conversely, CEE can protect against depressive symptoms (e.g., decreased sucrose consumption) produced by chronic stress. In a study by Veena, Srikumar, Raju and Shankaranarayana Rao (2009), group housed rats were given an additional six hours/day of CEE exposure for 10 days, following 21 days of chronic stress. This short-term EE 'treatment' prevented depressive behaviours in rats: returning sucrose consumption preference, over that of tap water consumption, in CEE treated subjects.

The evidence that EE can reduce or ameliorate locomotor, cognitive and affective behavioural deficits in CNS challenged rats is growing, however, there is no consensus among studies in EE methodology. In the abovementioned studies the EE protocols differed in relation to the types of enrichment given (e.g., social or exercise enrichment), the complexity and means of enrichment given within each condition (e.g., the methods and stimuli used under the same heading of 'social' or 'exercise' enrichment), the interval of enrichment exposure (e.g., rearing/housing environments versus intermittent exposure), and at what stage EE is given (e.g., pre- or post- CNS insult/challenge). The aim of this paper is to establish whether short-term CEE given prior to CNS challenge protects against the transient impairment in locomotor

activity, novel object recognition memory or sucrose consumption, as induced by DA receptor antagonism.

2.2.0. Methods

2.2.1. Animals

A total of 96 male Sprague Dawley rats were obtained from the Animal Resources Centre, Perth, Western Australia (32 subjects/experiment). All rats were initially single-housed with minimal enrichment (i.e., standard enrichment- woodchip bedding, paper towel and wooden chew block. Box dimensions: box w 28cm x h 27cm x d 16cm; lid 28cm x 11cm x 26 cm in addition to a food trough of 28cm x – 6cm on a gradient up to 0cm x 15cm) in a 12-hr light-dark cycle (lights on 6.20am) with *ad libitum* access to food and water. Following one-week acclimatisation and an additional 7-8 days of handling, rats began experiment 1, 2 or 3. At this time rats weighed on average 338 ± 14 g. Animal protocols were approved by Macquarie University Animal Ethics Committee and were performed in accordance with animal use and care procedures outlined by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC edition 7, 2004).

2.2.2. Apparatus

2.2.2.i. Experiment 1. Locomotor chambers.

Locomotor chambers (custom built by University of Sydney running Macbench software) were standard operant conditioning boxes (25.0cm x 50.0 x 30.0cm) placed inside wooden, fan-ventilated sound-attenuating boxes (58.0 x 67.0 x 60.0cm). The operant conditioning boxes were made of aluminium (roof and two

side walls) and clear Perspex (front and back wall). Flooring was made of metal rods (6mm diameter) spaced 15mm apart. Locomotor activity was recorded as the number of beam-breaks between two infrared detectors built into the walls of the chamber. The number of beam breaks was logged by "Workbench Mac" software on a Macintosh computer (McGregor, 1996). Cameras were also placed above the operant conditioning boxes to allow for visual monitoring of the subjects during the experiment.

2.2.2.ii. Experiment 2. Novel object arena and objects

The novel object arena (50cm x 50 cm x 50cm, black painted plywood box) was situated in a dark room and lit by red light (60watt red light bulb angled across the top of the arena) with an overhanging camera to record live-feed activity within the arena.

Objects used as either the novel or familiar objects for each test day were: test day one- a small glass jam jar with lid and a small Milo drink tin. Both items had been cleaned, with the labels removed, and weighted to minimise the object being tipped over by the rat. On test day 2, the objects were a ceramic oil burner and small conserve glass jar (with rubber ring removed and weighted); test day 3- a ceramic toothbrush holder and small casserole dish with lid glued on; and on test-day 4- a small glass soft drink bottle and a small rectangular loose leaf tea tin (both cleaned, labels removed and weighted). Objects were bought in duplicate and chosen for size (e.g., items of a similar diameter; heights that allowed rats to explore the entire object), and ease of cleaning to eliminate odour.

2.2.2.iii. Experiment 3. Sucrose consumption boxes

Sucrose consumption testing occurred in boxes identical to those used for single housing. These sucrose test boxes contained woodchip flooring and a water bottle filled with 7% sucrose solution (sucrose mixed with tap water).

2.2.2.iv. Environmental enrichment: group home boxes and arena.

Group housing was conducted in larger cages (box: 36cm x 19cm x 61cm; lid: 36cm x 10m x 61cm, with 7cm food trough) than the single housing cage, and included PVC tubes in addition to paper towel and wood blocks provided in the cage with standard housing.

Enrichment arenas were 120 litre opaque plastic storage containers (45cm x 40cm x 70cm) filled with ~2cm depth of wood chip flooring. Large rectangular holes were cut into the lids of the containers and were covered with wire mesh. Stimuli used to enrich the arena included PVC pipes, a running wheel, dangling bells (used in domestic bird cages), woodblocks and strips of paper (coloured A4 paper and paper towel), and an assortment of plastic, rubber or fabric children's and animal toys (e.g., a dog pull rope, miniature tennis balls, etc.). Items were chosen to increase the subjects' interaction with the material, for example, objects that each subject could climb on, hide in, chew, or play with.

2.3.0. Procedures

2.3.1. Environmental Enrichment

Rats in the CEE condition were housed in groups of 2-4 subjects per cage. If at any stage the rats appeared to be excessively distressed, or acted aggressively toward each other, then the most stressed/aggressive rat was returned to single housing and was excluded from the study (n=1, Experiment 2). Subjects housed together in the CEE treatment condition were also placed in a complex enriched arena for an hour each day for 14 days. Enrichment stimuli were regularly added to the arena over the 14 days, and organisation of the items were rearranged each day. Following this, subjects were returned to single housing conditions before testing began the next day.

Subjects in the standard enrichment (SE) condition remained in single housing as described above. On days of enrichment for the CEE group, the SE subjects were handled for approximately one minute each.

2.3.2. Experiment 1: Locomotor activity

Subjects in Experiment 1 (locomotor activity) were given one day to habituate to the locomotor procedures used on test days. This involved subjects being given a sham (vehicle, 1ml/kg, i.p.) injection before being immediately placed within the locomotor chamber (based on procedures by Meyer, Cottrell, Van Hartesveldt & Potter, 1993). Locomotor activity level (i.e., the number of beam breaks made across the infrared beam) was then recorded for a two hour period. Subjects underwent the same procedure the following day and these data were used as pre-treatment

locomotor activity. From this data subjects were blocked into two groups by matching two subjects with similar levels of pre-treatment locomotor activity (at the two-hour time point) and designating one subject to group A and the other to group B. The housing conditions of subjects within the blocked groups were then taken into consideration. Ideally, at least two subjects within each group had to have been housed next to each other in the home room. This was to attempt to increase familiarity and decrease aggression between subjects if they were to end up housed together in the CEE condition. Each blocked group A or B was then randomised as either the complex environmental enrichment (CEE) or standard enrichment (SE) groups. Once randomised, environmental enrichment procedures commenced for the CEE group.

Following 14 days of enrichment treatment, rats underwent their test days where they were injected with either vehicle or SCH23390 and immediately placed within the locomotor chamber. Once the rat was placed in the chamber, the level of locomotor activity was recorded at every 15-minute interval over a two-hour period. On test day one, subjects underwent a baseline (vehicle injected) test day, followed by three ascending drug dose order test days, with a final test day of a followed-up (vehicle) baseline test. A post-drug baseline test was conducted to establish if subjects had habituated to the test procedure with repeated testing, and to ensure that no long-term drug effects impacted upon behaviour. The five drug test days of Experiment 1 were administered 48-hours apart.

2.3.3. Experiment 2: Novel Object Recognition (NOR)

As no pre-treatment tests were given, subjects in Experiment 2 (NOR test) were randomised into CEE or SE groups in consideration of their home housing conditions alone. Rats were blocked into units of 3-4 if they were housed consecutively in the homeroom, and then each unit block was randomised into treatment conditions. Subjects in the CEE group underwent environmental treatment before testing began. Following treatment, subjects were habituated to the novel object arena over two days. On day one of habituation, subjects were taken to a room separate to the behavioural arena and injected with vehicle before returning to their homeroom. Following a 30-minute period from injection, each subject was taken to the NOR test room and placed in the empty arena for 30-minutes. On habituation day two, the same procedures occurred as on habituation day one, with the exception that subjects were placed in the empty arena for 20-minutes. The number of days and length of habituation to the novel object arena was a compromise from other studies that had used habituation days ranging from six-minute arena exposure the day before testing, up to 30-minute exposure to the empty arena over three days before testing (Silvers, Harrod, Mactutus & Booze, 2007; and Sutcliffe, Marshall & Neill, 2007, respectively).

Subjects participated in four test days: day one was a baseline (vehicle) test day; day two and three were two drug test days of ascending dose-order; and the fourth day, a follow-up baseline test.

On test days, subjects were injected with either vehicle or drug before returning to their home cage. Following a 25-minute period, the rat was taken to the NOR room

and habituated to the empty novel object arena for five minutes. The rat was then taken out and two identical objects were placed in diagonal corners of the arena, approximately 8-10 cm away from the walls. The subject was then replaced back into the arena, and was free to explore the two objects for five minutes (T1). The rat was then returned to its home-room for 30-minutes (inter-trial interval) before re-exposure to the NOR arena (T2): this time with one object from T1 (i.e., a familiar object) and a novel object, which were positioned in the same corners of the arena as in T1. Each session was video-taped and recorded with the experimenter out of the room. Time in seconds spent exploring either the novel or familiar object each minute over a three-minute period was recorded. Test days were 48-hours apart and each test day used unique familiar and novel objects. Objects used as novel or familiar objects were counterbalanced across treatment groups, as was the position of the novel object at T2. Following each trial, the arena and objects were cleaned with 10% alcohol solution.

Object exploration was defined as the rat's nose touching or pointing towards an object less than 2 cm away, actively sniffing, licking or chewing an object. Sitting upon an object or using an object to maintain balance when rearing was not sufficient to be considered exploratory behaviour.

2.3.4. Experiment 3: Sucrose consumption

Subjects in Experiment 3 (sucrose consumption) were given one day to habituate to sucrose testing procedures. Based upon procedures by Duong and Weingarten (1993), subjects were taken from their homeroom and sham injected, in a separate room to where behavioural testing took place, before being returned to their

homeroom for two hours. Following this interval, subjects were taken to the test room, placed in a sucrose test box and given one hour access to the sucrose solution. Sucrose bottles were weighed prior to and post testing and the difference in weight was used to calculate the amount of sucrose consumed within the hour. The next day, pre-treatment baseline testing began. The same procedures occurred as on the habituation day, and the amount of sucrose consumed after the hour was recorded. Forty-eight hours later, a second baseline test day was conducted and sucrose consumption was recorded. Rats were blocked into groups where four consecutive subjects had been housed next to each other and were then randomly assigned into treatment groups. Enrichment procedures then commenced.

On test days, rats were taken from their homeroom and injected with either vehicle or drug before returning to their homeroom. Again, two hours later, rats were taken to a separate testing room and placed in the sucrose test box for one hour and the amount of sucrose consumed was recorded. Each drug dose was tested over two days, 48 hours apart. The commencement of the next drug dose occurred five days after the last test day (i.e., test day 2 of drug dose X). Drug doses given were baseline vehicle injection, three drug doses in ascending order, and post-drug baseline (vehicle).

Taking two measures of sucrose consumption at both pre-treatment baseline and post-enrichment testing was loosely based upon procedures described by Grippo, Na, Johnson, Beltz and Johnson (2004) who obtained two sucrose test days for baseline measurement before testing sucrose consumption following a period of CNS challenge (i.e., a 4-week period experiencing chronic mild stressors). This current

study continued measuring two days of testing under each post-enrichment condition to explore whether or not sucrose drinking behaviour was consistent over time under different levels of DA antagonism. Another primary difference between the current design and that by Grippo and associates is that subjects in this study were not deprived of food or water prior to sucrose testing. In the present study, subjects were able to demonstrate their preference for sucrose simply by consuming the solution during testing at a time when they were not hungry or thirsty (for they had *ad lib.* access to food and water prior to testing).

2.3.5. Drugs

For both Experiment 1 and 2, R(+)-SCH23390 hydrochloride (SCH23390; Sigma-Aldrich) was dissolved in distilled water and injected subcutaneously in each rat at a volume of 1.0 ml/kg. Doses were administered as an ascending dose response curve as 0.00 (control vehicle), 0.03, 0.06, 0.10 and with a final re-examination of baseline behaviour 0.00mg/kg (Experiment 1), and doses 0.00, 0.03, 0.10 and 0.00 mg/kg (Experiment 2). Drug dosage and method of administration used in Experiment 1 were based upon similar dose ranges administered by Meyer et al. (1993). Methods used in Experiment 2 were then guided by doses given in Experiment 1.

For Experiment 3, pimozide (Sigma-Aldrich) was dissolved into 5% acetic acid vehicle in 0.9% saline to the highest concentration of 0.5mg/ml with serial dilutions performed using saline as the diluent. Doses of pimozide were administered intraperitoneally in ascending order 0.00 (baseline), 0.05, 0.3, 0.50 and 0.00 (post-drug baseline) mg/kg. Method of drug administration and drug dosage was based upon methods applied by Duong and Weingarten (1993).

In each experiment, post-enrichment performance (with vehicle injection) was compared to testing baseline performance to observe whether subjects habituated to test procedures over repeated testing or whether there were any long-term drug effects.

2.3.6. Data analyses

The effect of environmental enrichment, drug effects and behaviour performance in locomotor, novel object recognition and sucrose consumption were each analysed using repeated measure analysis of variance with Bonferroni adjustments (statistical package PASW (SPSS) version 18). Significant level was set at $p<0.05$. *A priori* or post hoc analyses were made with one-way ANOVA for multiple comparisons, using Bonferroni adjustment, or paired t-test comparisons for single comparisons. Significance levels were adjusted according to the number of comparisons made. Results are presented as mean and standard error of mean (SEM).

2.3.6.i. Experiment 1

After treatment, locomotor activity levels of the CEE group were significantly lower than that of the SE group (See Results- 2.4.1.ii). To control for changes in locomotor activity levels, each group's locomotor activity under drug and post-drug baseline conditions were compared to their post-enrichment baseline performance. Locomotor activity presented therefore represent changes in each groups' level of activity under varying drug dose conditions from their post-enrichment treatment baseline test performance.

Analysis of the locomotor data included: a) pre-enrichment group comparison of locomotor activity; b) post-enrichment group comparison of locomotor activity to examine the effects of CEE upon locomotor activity; c) the specific drug effect on locomotor activity was analysed at the one hour time point (i.e., bin 4) between the two groups. In this analysis both drug (dose 1, 2, 3 and post-drug baseline) and treatment groups served as the independent between-subject variables, and locomotor activity (at the one-hour period) as the dependent variable; d) a detailed analysis of the effect of CEE on locomotor activity over a 2-hour period under each drug dose condition was conducted. In this manner, the eight (15-minute) time bins served as the independent within-subject variable, and the two treatment groups (CEE and SE) as the independent between-subject variable for each analyses; e) finally, the total locomotor activity of subjects at post-drug baseline was compared to their locomotor activity at post-enrichment baseline testing. This was to establish whether long-term behavioural changes occurred with repeated locomotor drug testing. In this instance the independent within subject variable was day (post-enrichment baseline and post-drug baseline), and between subject variable group (CEE and SE).

Two subjects from the CEE group were dropped from all post-enrichment analyses as they spent a large portion of their time attending to moving equipment (trays) under the skinner boxes across test days. Information from post-drug baseline assessment was lost for one subject from the SE condition due to technical difficulties.

2.3.6.ii. Experiment 2

Novel object preference is often obtained by looking at the total length of time a subject spends with each novel and familiar object (e.g., Ballaz, Akil & Watson, 2007); however, this calculation may become biased if rodents become less active due to the effects of D_1 antagonists. To minimise a potential impact of altered baseline locomotor activity following enrichment and decreased locomotor activity under the influence of SCH23390, preference for the novel object over that of the familiar object in this study was calculated by obtaining the percentage of the total time the subject spent with the novel object over the entire exploration period with both the familiar and novel objects at T2. The formula used was: novel object preference = time spent with the novel object / (time spent with novel object + time spent with the familiar object) x 100; as described by O'Brien, Lehmann, Lecluse & Mumby (2006). Additionally, if subjects did not explore either object within a given minute time bin they were given a percentage score of 50%, which indicated that the subject had spent an equal amount of time with both objects for that period (otherwise a score of 0% would have indicated the subject had spent 100% of their exploration time examining the familiar object during T2).

In Experiment 2, data were analysed to obtain: a) the potential impact of CEE altering novelty exploration was analysed by exploring any difference between the groups in NO preference at post-enrichment baseline testing; b) drug effect upon novel object preference between the two groups for the entire 3-minute exploration time at T2; c) novel object preference at each 1-minute time bin was compared between groups at the different drug dose conditions. Analysis was conducted with both group (CEE and SE) and drug dose (baseline, dose 1, dose 2 and post-drug

baseline) as the independent between-subject variables, while time (three one-minute time bins) was the independent within-subject variable. d) A separate analysis was performed to look at potential long-term changes in behaviour due to repeated drug testing. In this case, day (post-enrichment and post-drug baseline) served as the within subject variable and group (CEE and SE) as the between-subject variables.

One subject was removed from the study entirely due to aggressive behaviour in the CEE housing condition. Data were also lost for one SE subject at low-dose SCH23390 and another from the CEE group at post-drug assessment, due to technical difficulties.

2.3.6.iii. Experiment 3

Data analysis included: a) pre-enrichment sucrose consumption comparison between the two treatment groups; b) potential changes in sucrose consumption as a function of CEE was explored by comparing sucrose consumption between groups at post-enrichment baseline; c) analysis of sucrose consumption was then performed using both group (CEE & SE) and drug dose (baseline, doses 1, 2, 3, and post-drug baseline) as the independent between-subject variables, while day (Day 1 and Day 2) was the independent within-subject variable. As there was no significant main effect for day (see Results- 2.4.3.ii), another analysis was performed using the two abovementioned between subject variables, and the average sucrose consumed over the two days under each dose condition became the dependent variable; d) post-drug and post-enrichment consumptive behaviour was conducted to explore drug or habituation effects. No subjects were excluded from analyses.

2.4.0. Results

2.4.1. Enrichment, locomotor activity and DA antagonism

2.4.1.i. Locomotor activity of the treatment groups prior to CEE

Statistical analysis demonstrated that there was no significant difference between the total locomotor activity of the CEE group (mean= 1199.06, SEM= 84.99) or SE group (mean= 1330.25, SEM= 147.07) prior to treatment, t(30)= 0.77, *p*= 0.45.

2.4.1.ii. The effect of complex enrichment upon locomotor behaviour

At post-enrichment baseline testing, the total locomotor activity levels of CEE subjects (mean= 1055.50, SEM= 114.94) over a 2-hour period was significantly lower than the SE group's (mean= 1580.38, SEM= 111.26) locomotor activity, t(30)=3.28, *p*= 0.00.

2.4.1.iii. Comparison of drug dose on locomotor activity of treated subjects at 1-hour.

At the one hour time point, a significant main effect for SCH23390 dose was found, indicating that the DA antagonist attenuated locomotor activity, F(3,26)= 5.01, *p*= 0.01. Using a stringent adjusted *p*-value of 0.01, there was no significant difference in the activity of subjects under either low, medium or high SCH23390 doses (all p-values were n.s.); however, there was a trend for locomotor activity to be lower under high-dose SCH23390 compared to post-drug locomotor levels (*p*= 0.03). Additionally, a significant group effect indicated that SCH23390 did not decrease the locomotor activity of the CEE group to the same degree as it affected the SE group, F(1,28)= 13.49, *p*= 0.00. The difference in locomotor activity between the treatment groups was significant with the administration of low-, medium- or high-dose

SCH23390 (all *p*-values were < 0.00); although there was no significant group difference in locomotor activity at post-drug baseline (p= 0.09). Likewise, a lack of a significant interaction effect, demonstrated the consistent trend in activity by CEE and SE groups over assessments under different SCH23390 dose conditions, F(3,26)= 1.72, *p*= 0.19. See Figure 1.

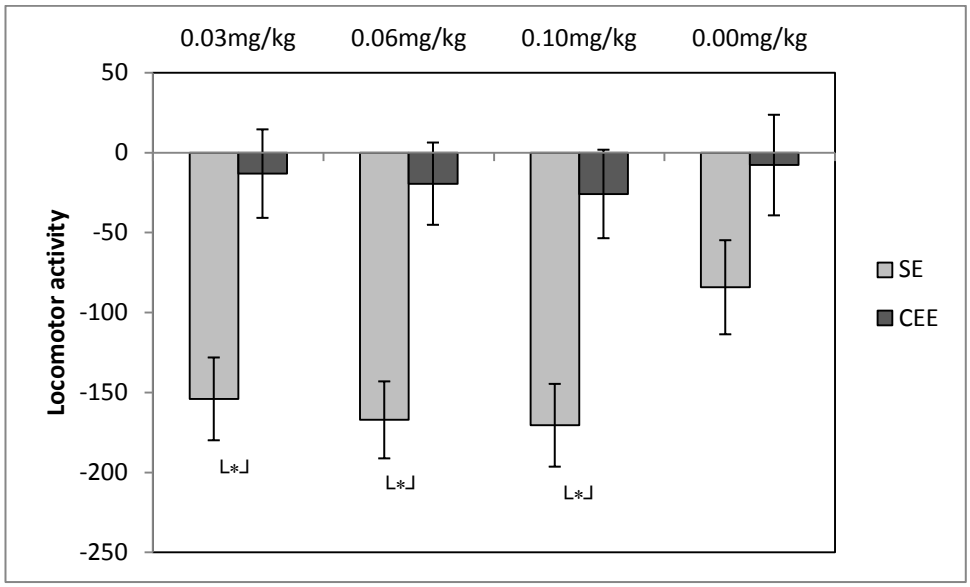


Figure 1. Difference in cumulative locomotor activity from baseline performance at 1 hour into testing under different SCH23390 dose conditions between CEE and SE treatment groups. Significance at adjusted p-value *=p<0.01

2.4.1.iv. The effect of SCH23390 doses and CEE on locomotor activity over a 2-hour period

A significant group effect indicated that the locomotor activity of animals in the CEE group was less affected by the low dose of SCH23390 (0.03mg/kg) in attenuating locomotor activity than subjects in the SE group, F(1,28)=13.96, *p*=0.00. Additionally, during the 2-hour period following low dose SCH23390 administration, the locomotor activity of CEE subjects was significantly closer to their baseline performance than those of SEE subjects at time bin 45-minutes

(F(1,28)= 7.879, *p*=0.009) and 60-minutes (F(1,28)= 13.82, *p*=0.001). See Figure 2.a below. There was a significant time effect (F(7,22)= 5.56, *p*= 0.00), indicating a change in locomotor activity over the two-hour assessment. There was no significant time by group interaction effect, F(7, 22)= 02.07, *p*= 0.09, suggesting that CEE and SE groups each behaved in a consistent manner over time.

At the medium dose of SCH23390 (0.06 mg/kg), a significant group effect indicated that the locomotor activity of subjects in the CEE group were less affected by administration of the DA antagonist than the SE subjects, F(1,28)= 26.51, *p*= 0.00. The locomotor activity of the CEE group was significantly closer to baseline activity than the SE group at time bin 30-minutes (F(1,28)= 13.28, *p*=0.001), 45-minutes (F(1,28)= 11.59, *p*=0.002), and at 60-minutes (F(1,28)= 17.57, *p*=0.000). See Figure 2.b. A significant effect for time occurred (F(1,28)= 26.51, *p*= 0.00); however, there was no significant interaction effect between time and group, F(7,22)= 1.59, *p*= 0.19.

Again, at the highest dose of SCH23390 (0.10 mg/kg), a significant group effect indicated that the locomotor activity of animals in the CEE group were less affected by SCH23390 than SE subjects, F(1,28)= 25.77, *p*=0.00. More specifically over the 2 hour period, the locomotor activity of CEE subjects was closer to baseline locomotor activity than the SE group at time bin 30-minutes (F(1,28)= 14.27, *p*= 0.001), 45-minutes (F(1,28)= 9.78, *p*= 0.004), and at 60-minutes (F(1,28)= 14.58, *p*= 0.001). See Figure 2.c. Again, a significant time effect was found (F(7, 22)= 11.75, *p*= 0.00), although no significant interaction effect between time and group occurred, F(7,22)= 1.36, *p*= 0.27.

Following drug testing, a post-drug assessment with administration of 0.00 mg/kg of SCH23390 indicated no significant difference between the CEE and SE groups in locomotor activity from their baseline performance, $F(1,27)= 0.61$, $p= 0.44$). Likewise, there was no significant difference in activity levels between treatment groups across any time bins over the 2-hour period (all *p*-values were n.s.). See Figure 2.d. Furthermore, there was no significant effect for time ($F(7, 21)= 0.74$, $p= 0.64$) or interaction effect ($F(7, 21)= 0.71$, $p= 0.67$), indicating that the locomotor activity of both groups was similar between each other over time.

2.4.1.v. The impact on locomotor activity with repeated testing

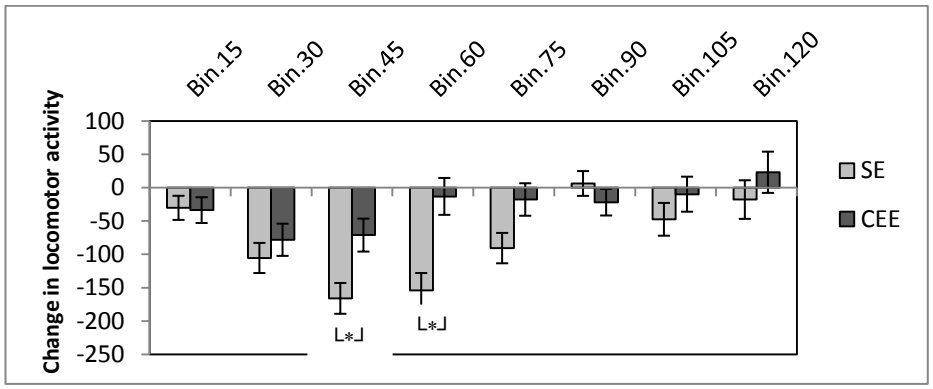
There was no significant assessment effect between post-enrichment and post-drug locomotor activity levels, $F(1,27)= 2.38$, $p= 0.14$, suggesting that subjects did not habituate to repeated locomotor testing over the assessment periods. A significant group effect indicated that CEE subjects were less active than SE rats, $F(1,27)= 17.36$, $p= 0.00$; however, this pattern of behaviour was consistent at post-enrichment and post-drug assessment points between treatment groups, as no significant interaction effect occurred, $F(1,27)= 0.61$, $p= 0.44$. To summarise, CEE subjects had lower post-enrichment locomotor activity than SE subjects, and again at post-drug assessment, but the level of activity of each group did not differ across assessment sessions.

2.4.2. Environmental enrichment, novel object preference and DA antagonism

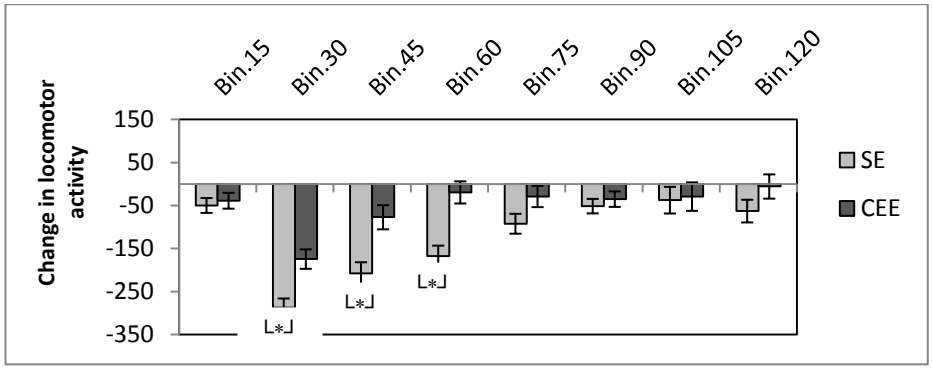
2.4.2.i. The immediate effects of complex enrichment upon novel object preference

Post-enrichment (at baseline test), there was no significant difference in novel object preference between the CEE group (mean= 66.35%, SEM= 5.10) and the SE group

(mean= 62.62%, SEM= 3.90), t(29)= -0.59, *p*= 0.56, indicating that enrichment did not enhance novelty exploration or memory performance.



a)



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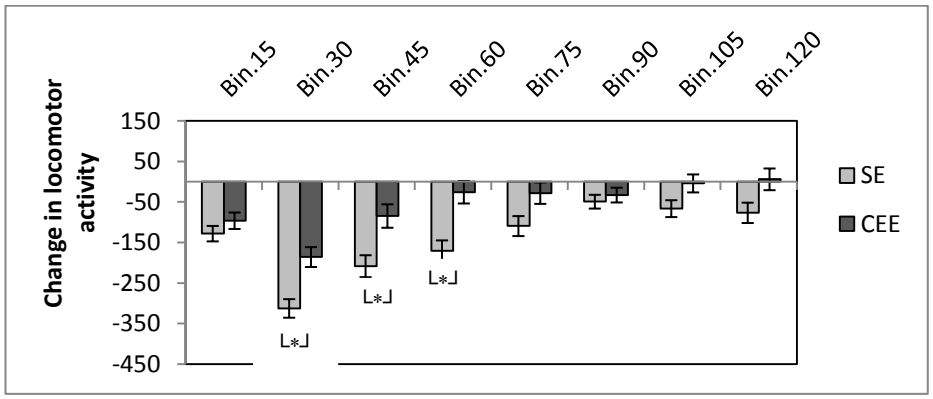

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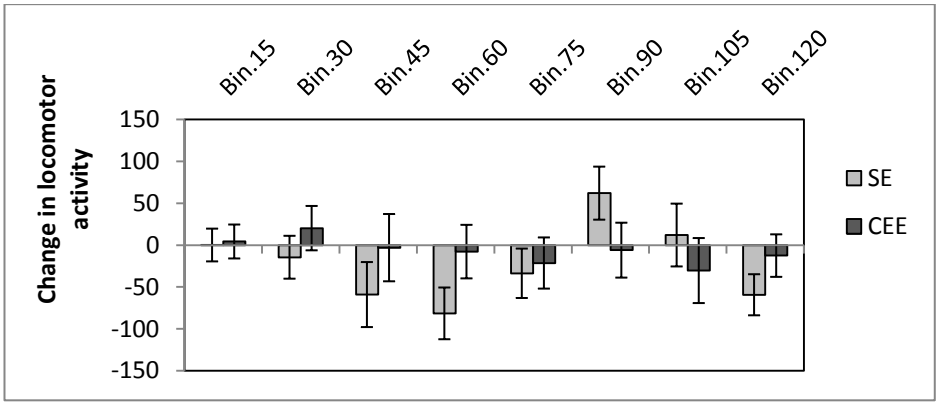

d)

Figure 2. Changes in cumulative locomotor activity within each 15-min time bin from baseline performance under varying doses of SCH23390 between rodents who received complex enriched environment or standard enrichment treatments. 1. a) SCH23390 dose 0.03 mg/kg; b) 0.06 mg/kg; c) 0.10 mg/kg; d) post-drug 0.00 mg/kg. Significance at adjusted p-value *= $p<0.006$.

2.4.2.ii The impact of drug on novel object preference for total exploration time

Over the entire 3-minutes of exploration time at T2 across drug test days, the CEE group (mean= 65.70%, SEM= 2.66) on average spent significantly more time exploring the novel object than the SE group (mean= 57.43%, SEM= 2.57), F(1, 27)= 5.01, *p*= 0.03. There was no significant effect for dose condition (F(3, 25)= 1.59, *p*= 0.22) or interaction effect, F(3, 25)= 0.81, *p*= 0.50, indicating that each group tended to perform in the same manner across assessment test points. See Figure 3.

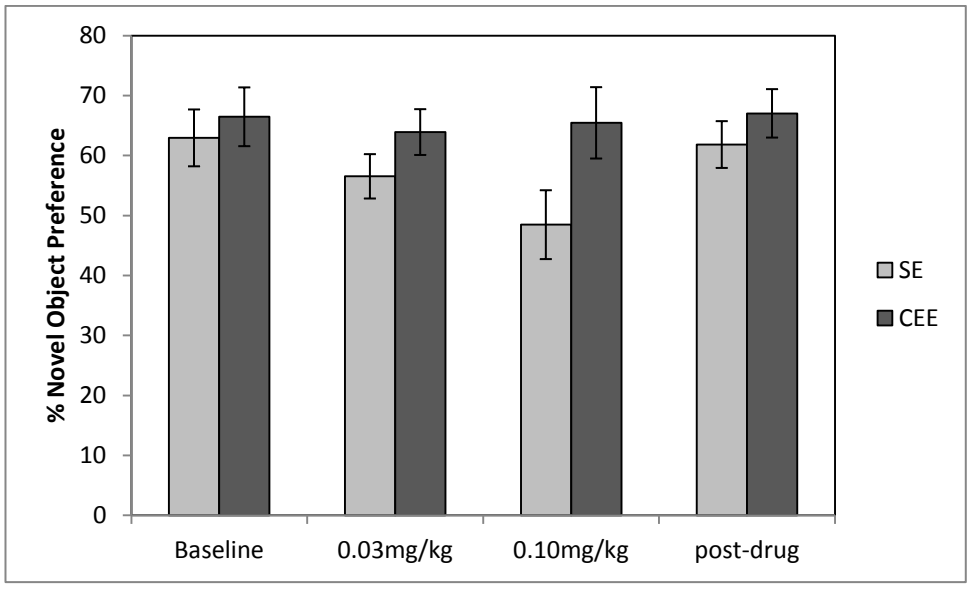

Figure 3. Percent preference for the novel object for the total exploration time (3-min duration) at T2 under varying SCH23390 dose conditions for CEE and SE rodents. Dose conditions: baseline (0.0mg/kg), 0.03mg/kg, 0.1mg/kg and post-drug (0.0mg/kg).

2.4.2.iii. The effect of CEE vs. SE on novel object preference per minute under all test conditions

Overall, there was no significant group effect between treatment groups in novel object preference $F(1,27)= 1.29$, $p= 0.27$. Both CEE and SE conditions demonstrated similar preference levels for the novel object over the familiar object.

2.4.2.iv. Novelty preference changes over the three minute exploration period

A significant time effect was demonstrated ($F(2, 26)= 8.24$, $p= 0.00$). Using an adjusted p-value of $p< 0.02$, novel object preference was significantly greater within the 1st minute of testing compared to the 3rd minute of testing ($p= 0.00$). Novel object preference within the 2nd minute of testing was not significantly different from either the 1st minute ($p= 0.03$) or the 3rd minute of testing ($p= 1.00$). See Table 1.

	Mean (%)	SEM
1st minute	68.22	2.50
2nd minute	57.75	3.28
3rd minute	54.49	3.04

Table 1. Average percent preference of both groups for the novel object over the 3-minute exploration time at T2 on the NOR task. Using an adjusted p-value of p= 0.02, novel object preference was significantly higher within the 1st-minute of testing compared to the 3rd-minute of testing.

2.4.2.v. Novel object preference varies between groups over time and is affected by antagonist dose

A significant dose by time by group interaction effect was found, $F(6,22)= 2.79$, $p= 0.04$. As novel object preference was high for the 1st minute and dropped significantly by the 3rd minute, sig differences between the two treatment groups were compared at the 1st and 2nd minute of testing at T2, even though performance at

the 3rd minute is shown. The adjusted *p*-value for statistical significance was therefore $p < 0.025$. There was no significant difference between the treatment groups in novel object preference over the first 2-minutes of testing at post-enrichment baseline testing, nor at low dose SCH23390 (see Figure 4.a and 4.b). At high dose (0.10 mg/kg) SCH23390, the significant difference between the groups occurred at the 2nd minute time bin, $F(1,29) = 6.23$, $p = 0.02$, whereby the DA antagonist blunted novel object preference of the SE group (see Figure 4.c). At post-drug baseline there was a trend for the CEE group to engage in greater novel object preference within the 1st minute than the SE group, $F(1,28) = 4.91$, $p = 0.04$ (see Figure 4.d).

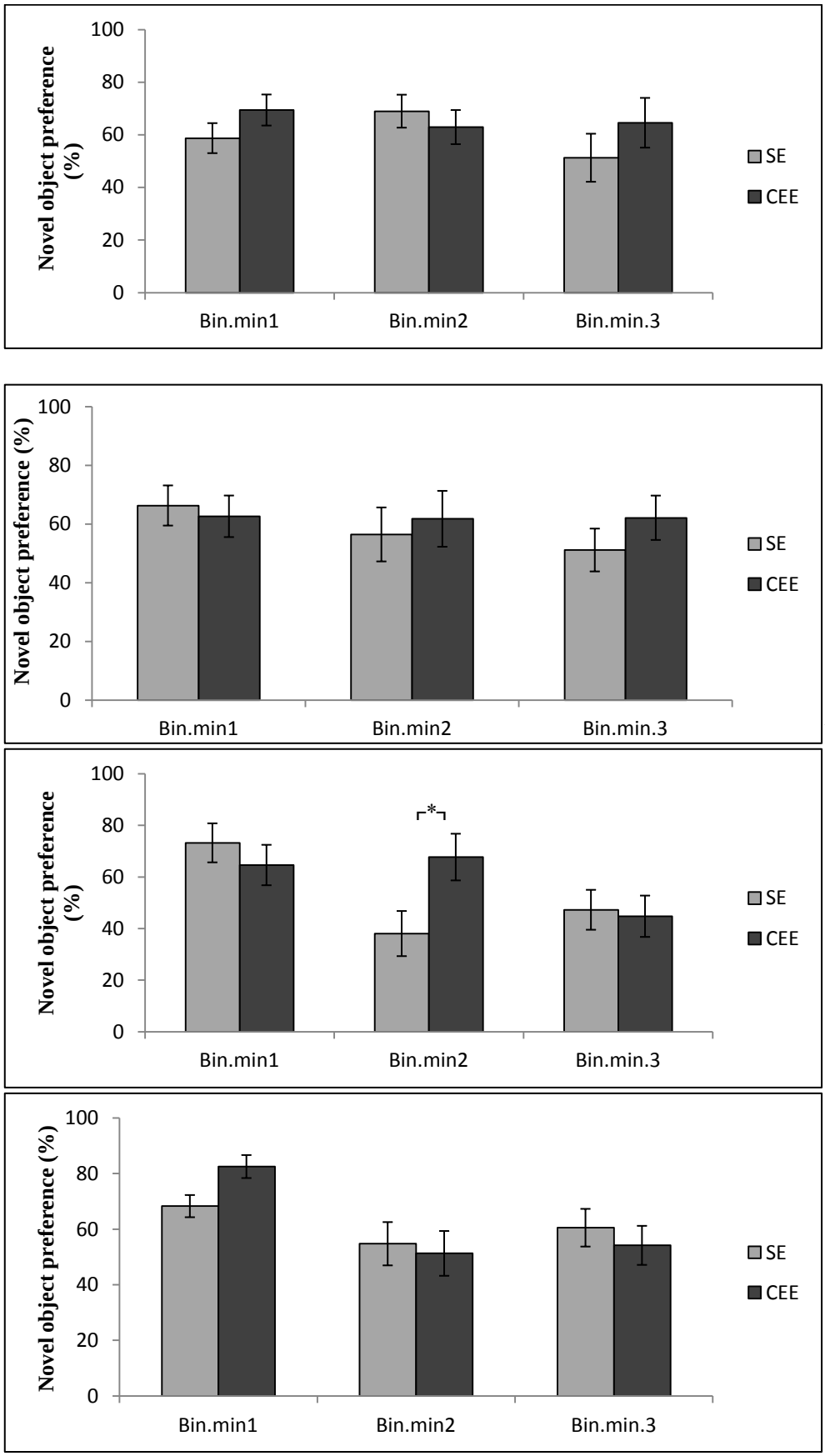

Figure 4. Percent preference for the novel object per minute over the total exploration time under varying SCH23390 doses between CEE and SE rodents. 2.a) baseline 0.00mg/kg; b) 0.03 mg/kg; c) 0.10 mg/kg; d) post-drug 0.00mg/kg. Significance at adjusted p-value *= $p<0.025$.

2.4.2.vi. CEE and SE subjects maintain novelty preference over repeated testing

Overall, there was no significant time effect between the novel object preference exploration time from initial post-enrichment (mean= 64.64%, SEM= 3.29) and post-drug assessment (mean= 63.63%, SEM=2.82), $F(1,28)= 0.05$, $p= 0.83$, indicating that CEE and SE subjects did not habituate to repeated testing in the NOR task. See Figure 3. Likewise there was no significant group effect ($F(1,28)= 0.78$, $p= 0.39$) or group by time interaction effect ($F(1,28)= 0.00$, $p= 0.98$).

Examining the novel object preference within the 1st minute of testing at post-enrichment to post-drug assessments, a significant group effect occurred, $F(1,28)= 5.49$, $p= 0.03$. As no significant interaction effect occurred, $F(1, 28)= 0.01$, $p= 0.94$, this indicates that the CEE group consistently spent greater time exploring the novel object within the 1st minute of testing at both post-enrichment and post-drug assessment points than the SE group. Individual comparison of each treatment group demonstrated that novel preference did not differ between post-drug and post-enrichment baseline for the CEE group ($t(14)= -1.70$, $p= 0.11$) or the SE group ($t(14)= -1.41$, $p= 0.18$), demonstrating no change in novel object preference over time for each group.

2.4.3. Environmental enrichment, sucrose consumption and DA receptor antagonism

2.4.3 i. Pre-enrichment sucrose consumptive behaviour between treatment groups

There was no significant difference in the amount of sucrose consumed by the CEE group (mean= 8.5ml) compared to the SE group (mean= 8.5ml) at pre-treatment baseline assessment ($F(1,30)= 0.00$, $p= 1.00$). There was no significant day effect,

F(1,30)= 0.01, *p*= 0.92), or interaction effect, F(1,30)= 1.32, *p*= 0.26, which indicated that subjects in both treatment groups consumed similar amounts of sucrose over the two test days before enrichment treatment commenced.

2.4.3.ii. The effect of complex enrichment on sucrose consumption

As there was no significant effect for day on sucrose consumption following post-enrichment testing, F(1,30)= 0.05, *p*= 0.82, further analysis used the mean sucrose consumed over the two days of testing at each dose condition.

Immediately following enrichment treatment, there was no significant difference in sucrose consumed by subjects in the CEE group (mean= 13.47, SEM= 1.24) or the SE group (mean= 15.38, SEM= 1.23) at post-enrichment baseline testing, t(30)= 1.10, *p*= 0.28, indicating that CEE did not alter sucrose preference (Figure 5).

2.4.3.iii. Pimozide, CEE and sucrose consumption

A significant main effect for drug dose was found (F(4,27)= 48.93, *p*= 0.00). Using an adjusted *p*-value of $p<0.008$, sucrose consumption significantly increased under low-dose pimozide from baseline consumption (*p*= 0.001) but, sucrose consumption under the influence of medium-dose pimozide did not differ from the sucrose consumed at post-enrichment baseline testing (*p*= 1.00). Sucrose consumption under the influence of the highest dose of pimozide was significantly less than the amount consumed at post-enrichment baseline testing (p= 0.0002, Figure 5). Additionally, sucrose consumption under low-, medium- and high-drug doses were significantly lower than at post-drug assessment (all p-values < 0.000). Overall, there was no difference between treatment groups in the amount of sucrose consumed (F(1,30)=

0.53, *p*= 0.47) or any demonstrated group by drug dose interaction effect (F(4,27)= 0.89, *p*= 0.48). This indicated that CEE did not attenuate the effects of pimozide administration on sucrose consumption because both groups drank similar amounts of sucrose at each test session.

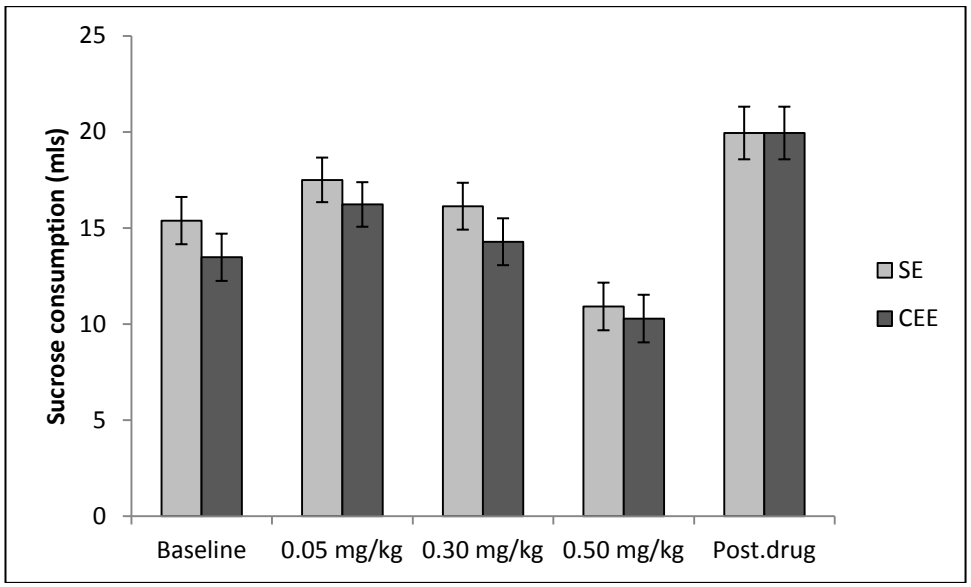

Figure 5. Sucrose consumption by CEE and SE subjects under varying doses of pimozide. Using a stringent adjusted p-value of $p<0.008$, under high-dose pimozide sucrose consumption was significantly lower than at post-enrichment baseline. Consumption under low-, medium- and high-dose pimozide was also significantly lower than at post-drug testing.

2.4.3.iv. Sucrose consumptive behaviour following repeated testing

Subjects consumed more sucrose at the post-drug baseline (mean= 19.94ml, SEM= 0.97) than at the initial post-enrichment baseline assessments (mean= 14.42ml, SEM= 0.87), F(1,30)= 104.99, *p*= 0.00, indicating that all subjects continued to drink sucrose even with repeated exposure to the solution. Likewise, there was no significant group or interaction effect (both p-values were n.s.).

2.5.0. Discussion

This study demonstrated that a brief period of complex environmental enrichment had differential protective effects on behaviour in response to a challenge with dopamine receptor antagonism. In comparison to SE, CEE was found to protect against attenuation of locomotor activity as induced by dopamine D_1 receptor antagonism with SCH23390 administration. Likewise, CEE altered novelty preference in rodents during and following SCH23390 administration. Complex environmental enrichment did not, however, protect against attenuation of sucrose consumption in rats challenged with the dopamine D_2 receptor antagonist pimozide.

2.5.1 Locomotor activity

Rats provided with CEE demonstrated reduced spontaneous locomotor activity following treatment compared to subjects continuing with a SE housing condition. A reduction in locomotor activity in rats following environmental enrichment treatments has been previously reported by other studies (e.g., Hoffmann et al., 2009; Del Arco, et al., 2007). The present study differs from these reports in that the reduction in locomotor activity in rodents occurred after only a brief enrichment period of social housing and 14-days of one-hour access to an enriched arena, rather than a lengthy period (e.g., 2- 3 months) of complex enrichment before behavioural testing occurred.

Interestingly, the impact of CEE to reduce spontaneous locomotor activity was also relatively long lasting. Subjects in the CEE treatment condition still engaged in less locomotor behaviour than SE subjects at sham post-drug baseline testing for at least one week (9 days) following their last day of enrichment. Additionally, there was no

difference in locomotor activity from post-enrichment to post-drug baseline testing between the two treatment groups, indicating that there were no significant changes in locomotor activity as a function of repeated testing, or carry-over effects of repeated drug administration. The effective wash-out of the drug is also best illustrated by Figure 1.d, which shows that subjects continued to perform close to their baseline activity levels at post-drug baseline over the two-hour testing period.

At a neural level, CEE may reduce locomotor activity in rats by eliciting neural changes within the motor circuit of the brain. Complex environmental enrichment significantly decreases D_1 receptor density in the prefrontal cortex (PFC), a brain region associated with mediation of locomotor movement (e.g., Hall, Powers & Gulley, 2009), which may play a role in decreasing the spontaneous locomotor activity of enriched rats compared to non-enriched subjects (Del Arco, et al., 2007).

The current paper further demonstrated that locomotor activity was attenuated by all administrated doses of SCH23390, even from a dosage as low as 0.03 mg/kg. In fact, the low dose of SCH23390 was as effective in reducing locomotor activity levels at the one hour time point as the medium to high doses employed. Meyer and associates (1993) demonstrated that locomotor activity was attenuated from a range of SCH23390 doses of 0.05- 0.20 mg/kg, but locomotor activity was potentiated by a small dose of 0.01mg/kg. In line with Meyer and colleagues (1993), the current findings further refine the dose response curve of SCH23390 administration to decrease locomotor activity.

Of great importance, CEE was found to attenuate the degree to which SCH23390 administration reduced locomotor activity in rodents. Although SCH23390 decreased locomotor activity, subjects who had undergone brief CEE prior to behavioural testing were more resistant to this effect and were significantly more likely to perform closer to their baseline (post-enrichment) activity level than the SE control subjects. This protective effect was generally evident from time bin 30-mintues up to the 60-minute time bin at low-, medium- or high-doses of SCH23390.

The protective effects against DA receptor antagonism by CEE upon dopamine-mediated locomotor functioning are likely to have resulted from the ability of CEE to regulate neural functioning within the motor circuit of the brain. In rodent models, for example, exercise promotes neuroplastic changes within brain regions of the motor circuit, such as the BG (substantia nigra and striatum) and motor cortex of the frontal lobe, by regulating proteins that are involved in synaptic density and neural structure (Ferreira, Real, Rodrigues, Alves & Britto, 2010). Functioning of the motor circuit is also likely supported by an increase in brain derived neurotrophic factor (BDNF) within the striatum (Bezard, et al., 2003) as neurotrophins act to promote the functioning of neurons. Along with decreased D_1 receptor density within the PFC as previously mentioned, CEE also reduces the density of DA transporters (DAT), which mediate dopamine re-uptake within the synaptic cleft, and DAT functioning within the PFC of rodents and further reduces DAT density within the striatum (Bezard et al., 2003; Zhu, Apparsundaram, Bardo, & Dwoskin, 2005; Wooters, et al., 2011, respectively). Furthermore, CEE has been shown to increase extracellular DA within the nucleus accumbens (a ventral portion of the striatum of the BG) of enriched rats (Segovia, Del Arco, De Blas, Garrido & Mora, 2010).

The protective effects of CEE on locomotor activity against D_1 receptor antagonism are therefore likely to be at least three-fold. Functioning of the motor circuit is likely to be improved via neuroplastic changes and processes that support cell functioning (e.g., BDNF) as induced by CEE. Decreased DAT, or their functioning, would act to increase the availability of circulating DA at the synaptic level, effectively reducing the chance of the D_1 receptor antagonist to be active at D_1 receptor sites and induce behavioural changes. This effect may be further enhanced by reduced D_1 receptor number in the PFC, where D_1 receptor agonism is known to enhance locomotor activity (Del Arco, et al., 2007). Lastly, increased DA within the nucleus accumbens would likely enhance locomotor activity as D_1 (and to a modest effect D_2) receptor agonism in this region increases locomotor activity in rats (Meyer, 1993; Dreher & Jackson, 1989).

2.5.2 Novel Object Recognition

Unlike locomotor activity, CEE did not alter novel object preference behaviour between groups immediately following enrichment treatment (at baseline test). Both CEE and SE subjects demonstrated a strong preference for the novel object (i.e., time spent with the novel object was >50% of their total exploration activity at T2), which indicated intact memory for the familiar object from T1. This finding is not surprising given that Varty and associates (2000) found exploratory behaviour (i.e., holepoke of rats noses in holes along the walls and floor of a test chamber) between CEE rats and isolated (non-enriched) subjects to be both high and equivalent in frequency within the first 10 minutes of testing between the two groups. Our current

study further supports the notion that CEE exposure does not alter exploratory behaviour of subjects when measured for a brief period of time.

Complex environmental enrichment did, however, alter novel object exploratory behaviour in subjects under the influence of SCH23390. Our results also highlight the importance of examining behaviour at frequent intervals to uncover when and how novel object preference alters between CEE and SE rats.

When the exploratory behaviour of CEE and SE subjects was totalled over the entire 3-minutes of testing for each test day, results indicated that CEE subjects engaged in significantly higher novel object exploration than SE subjects. However, analysis of the data on a minute by minute basis revealed that CEE altered novel object preference in CEE and SE subjects only under certain dose conditions and at particular times over the exploration period. While novel object preference was similar over time under post-enrichment and low-dose SCH23390, under high-dose antagonism, novel object preference was blunted in SE subjects within the 2^{nd}-minute of testing compared to the continued high novel preference demonstrated by the CEE subjects over the 2-minutes of exploration at T2.

The change in novel object preference between the SE and CEE subjects following D_1 receptor challenge cannot be explained by a recognition memory deficit hypothesis, which the NOR task aims to measure. Within the first minute of testing, both treatment groups demonstrated intact memory for the familiar object at T2 by preferring to spend over 50% of time exploring the novel item over that of the familiar object under all drug conditions. Only once exploratory behaviour is

examined over time does a change in exploration preference between the two treatment groups occur. Novel object preference within the 2nd-minute of testing was significantly reduced only in SE subjects under high-dose SCH23390, with their attention switching primarily to the familiar object. This may suggest that D_1 receptor antagonism affected the motivational drive of SE subjects to interrupt their behaviour in exploring the novel item, which did not occur in CEE subjects.

Areas of the brain involved in motivation and persistent effort-based behaviour include the nucleus accumbens and anterior cingulate cortex where DA antagonism can attenuate effort-based behaviour of rodents (See Salamone, Correa, Farrar & Mingote, 2007; Walton, et al., 2009). For example, with administration of SCH23390 intracranially within the nucleus accumbens of rats, subjects will no longer prefer to obtain food from a high reward (4 pellets) arm on a T-maze if they need to climb a steep barrier to obtain the reward. Rather, rats will take food from a low reward arm (2 pellets) that is more easily accessible (i.e., no barrier; Schweimer & Hauber, 2006). Similarly, this effect was also found in rats given subcutaneous injections of SCH23390 or the D_2 antagonist haloperidol (Bardgett, Depenbrock, Downs, Points & Green, 2009). In the present study, CEE subjects demonstrated intact motivated behaviour to persistently explore the novel object over a 2-minute period under high-dose SCH23390, while novel object preference of the SE subjects subsided within this time. This may suggest that SE subjects were more susceptible to the effects of D_1 receptor antagonism in altering their motivation to persist with exploring the novel object.

As previously mentioned, CEE increases the extracellular presence of DA within the nucleus accumbens and may therefore be one mechanism by which motivation of CEE subjects in the present study was protected against dopaminergic challenge. The neural events that provided protection against motivational changes in CEE subjects in the current study are not fully elucidated and require further research. The impact of CEE to alter motivation in these subjects, however, is not only supported by persistent novelty preference under high-dose DA antagonism in CEE subjects but also in the trend for increased motivated behaviour in CEE rats following drug challenge. For example, CEE subjects tended to engage in higher novel object exploration than SE rats within the 1st minute of testing at post-drug assessment.

The present study suggests that CEE may produce a protective effect on changes to motivated behaviour induced by dopamine receptor antagonism. These findings may also indicate that the NOR test procedures used in this study provide a novel measure to test the resiliency in motivated behaviour both during and following a DA receptor challenge, rather than the NOR task being used to test recognition memory ability.

A memory deficit induced by dopamine D_1 receptor antagonism and any protective effects of CEE, may have been found between treatment groups if the ITI period in this study had been extended. For example, Nagai and colleagues (2007) demonstrated that a brief ITI period (e.g., of 1-hour) did not identify a memory deficit induced by D_1 receptor antagonism, however, a memory deficit was evident at a later time interval (i.e., following a 24-hour delay interval). Therefore, the 30-

minute ITI used in the current study may have been too short to demonstrate memory deficits induced by DA receptor antagonism.

Furthermore, the results demonstrate that although rats were subjected to locomotor attenuation as induced by SCH23390 (as seen in Experiment 1), SCH23390 did not impede novel object preference behaviour in rats- both groups on average were found to spend 68% of their time exploring the novel item over that of the familiar object within the 1st-minute of testing. As novelty preference was most evident within the first minute of testing at T2, a difference in memory ability between CEE and control rats administered a D_1 receptor antagonist in future research would best be identified within the first 1-2 minutes of testing. Additionally, further research intending to explore motivational changes in CEE rats using the NOR task would best examine behaviour on an interval basis to observe alterations in exploratory behaviour between the novel and familiar objects over time by treated subjects, rather than averaging exploratory behaviour over a defined period.

2.5.3 Sucrose consumption

Both treatment groups consumed the same amount of sucrose at post-enrichment baseline testing, indicating that CEE does not alter spontaneous sucrose consumption. From the findings of Brenes and Fornaguera (2008) it was expected that the CEE group would consume less sucrose solution than the standard enriched rodents due to enrichment treatment effects alone. Two considerations may explain the difference in sucrose consumption between the two studies. Firstly, the complex enrichment treatment in the present study may have been too brief to show changes to sucrose consumption. In Brenes and Fornaguera's study, rats were raised in

treatment group conditions (CEE, group-housed or isolated) for approximately 30 days before initial behavioural testing, while the present study employed 14 days of CEE. Secondly, it may be that the standard enrichment treatment provided in the current study (wooden chew blocks, paper towel and handling) was more stimulating than conditions provided to the isolated rats in Brenes and Fornaguera's experiment. Unfortunately details of the isolation conditions were too brief to extrapolate further inferences.

In accordance with Duong and Weingarten (1993) the current study found that pimozide significantly reduced real-feeding sucrose consumption in rats. Moreover, it was demonstrated that there was no difference in sucrose consumption over the two days of testing at each assessment point, indicating consistency in consumptive behaviour under each drug dose condition.

Although only the highest dose of pimozide was found to significantly reduce sucrose consumption in rats compared to their post-enrichment baseline consumption levels in the current study, the lower doses of pimozide (particularly the medium-dose) may have also substantially reduced sucrose consumption. This effect may not have been captured because the post-enrichment performance was used as a baseline measure, which may not be an accurate indicator of consumptive behaviour which tended to increase over repeated testing.

Rada, Avena and Hoebel (2005) demonstrated that rats provided daily access to sucrose increased their consumption of sucrose over time (over 21 days of behavioural testing). Wojnicki, Stine and Corwin (2007) also established that rats

given an intermittent schedule of access to sucrose solution (i.e., sucrose exposure every second day of the work week) were more likely to engage in bingeing behaviour, drinking more than subjects provided limited daily access to sucrose. Together, it would be expected that there would be a clear upward trend in sucrose consumption over the testing period used in the present study. As can be seen in Figure 4, sucrose consumption significantly increased from post-enrichment to low-dose pimozide treatment and to post-drug assessment, indicating an upward trend for sucrose consumption to increase over time. As expected, pimozide pre-treatment produced a dose-dependent decrease in this behaviour that was not different between the SE or CEE exposed groups. Due to the ascending effect of repeated sucrose consumption, a greater effect of pimozide may have been detected if the drug treated groups were compared to control drug-naive subjects under the same sucrose access schedule.

Of interest, CEE did not protect against the attenuation of sucrose consumption as induced by pimozide. Perhaps a lengthier enrichment period may have been necessary to increase any potential protective effect that complex environmental enrichment can provide. Alternatively, CEE may not specifically protect against disruption to sucrose consumption as induced by the D_2 receptor antagonist pimozide. As demonstrated in Experiment 1, and by other authors, CEE protects against the behavioural effects (reduced spontaneous locomotor activity) induced by the D_1 receptor antagonist SCH23390. Pimozide, on the other hand, preferentially targets D_2 receptors (Schneider, Gibbs & Smith, 1986). Complex environmental enrichment might best protect against behaviours moderated by D_1 receptor functioning, rather than D_2 receptors. This hypothesis is difficult to test, however, as

research is limited in exploring the effects of CEE upon D_2 receptor function. Bardo and Hamer (1991) examined the effects of CEE upon both D_1 and D_2 receptors within the BG (including the nucleus accumbens, caudate-putamen and substantia nigra) and medial PFC of rats. The authors found that 30 days of CEE did not alter the number of either D_1 or D_2 receptors in any brain region studied. A lack of identified change in D_2 receptor function due to CEE may result from the possibility that either D_2 function is not altered by CEE, meaning that CEE would therefore not alter behaviours mediated by D_2 receptors, or that research has not yet identified the type or location of change to D_2 receptor functioning by CEE.

Additionally, D_2 receptor antagonism may not play a straight-forward action upon reducing sucrose consumptive behaviour. Research suggests that D_2 receptors play a role in the association of a reward-value paired with the sucrose and in this manner antagonists can affect sucrose consumption. For example, Yu and associates (2000) looked at the ability of SCH23390 and the D_2 receptor antagonist raclopride to reduce sucrose feeding in a conditioned flavour preference task. Rats were exposed to two flavoured solutions, one (flavour A) was paired with sucrose and became the positively reinforced conditioned stimulus CS^+ (CS^+), while the other flavour (B) was paired with saccharin- the negatively reinforced CS (CS^-). In real feeding rats that were not food or water restricted, both DA receptor antagonists reduced consumption of the CS^+ (without altering CS^- consumption), but rats were more sensitive to raclopride D_2 antagonism than SCH23390 at a smaller dose (Yu, et al., 2000). Additionally, Hajnal, De Jonge and Covasa (2007) found that only an obese strain of rat were more sensitive to the effects of the D_2 receptor antagonist raclopride in reducing both sham and real feeding compared to control lean subjects,

while antagonism by SCH23390 decreased sucrose consumption in both obese and control strains equally (Hajnal, De Jonge & Covasa, 2007).

Together, these results suggest that D_2 antagonism may interfere more with the associated positive reinforcing effects associated with consuming sucrose, while D_1 receptor antagonism can best explore the general effects of sucrose feeding behaviour. If there is an effect for CEE to alter D_2 receptor function, this effect may be more relevant to sucrose consumption that is associated with a positive reinforcement effect. Changes to general sucrose consumptive levels between SE and CEE treatment groups, as measured in the current study, may have been identified following challenge with D_1 receptor antagonism.

2.6.0. Conclusion

This study demonstrated that short-term CEE given prior to DA challenge protects against the attenuating effects of the D_1 antagonist SCH23390 on horizontal locomotor behaviour. CEE also appeared to enhance persistence in motivated behaviour of enriched subjects to thoroughly explore a novel item when challenged under high-dose SCH23390. Additionally, there was a trend for higher motivated exploration of novelty by CEE subjects that carried over to post-drug challenge. Lastly, CEE did not attenuate the degree to which the dopamine D_2 receptor antagonist pimozide reduced spontaneous sucrose consumptive behaviour, indicating a possible differential effect of CEE across dopamine receptor subtypes and varied behaviours.

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Chapter 2

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Chapter 3

Motor and non-motor functioning following modest nigral dopamine depletion: a model of early stage PD in aged rats.

3.1.0. Introduction

Approximately 3.5% of an Australian population aged ≥55 years within the city of Sydney has Parkinson's disease (PD), with a prevalence of approximately 780 per 100 000 (Chan, et al., 2005). A diagnosis of PD is established from the presence of cardinal motor deficits (e.g., tremor, rigidity and bradykinesia), however, other cognitive and non-motor disturbances are also evident, even at a time of early diagnosis (Muslimović, Post, Speelman, & Schmand, 2005; Gelb, Oliver & Gilman, 1999). Cognitive deficits frequently observed in non-demented individuals with Parkinson's disease (PDND) include impairments in executive functioning (e.g., problem-solving and mental flexibility), working memory ability, language (e.g., verbal fluency), memory (e.g., encoding information into or retrieving information from memory) and psychomotor and cognitive slowing (Brønnick, Alves, Aarsland, Tysnes, & Larsen, 2011; Elgh, et al., 2009; Muslimović, et al., 2005; Brand et al., 2004; and Katai, Maruyama, Hashimoto & Ikeda, 2003). Other non-motor changes that may be noted in individuals with PDND include reduced persistence and motivation to engage in mentally challenging tasks (Schneider, 2007), and an alteration in mood. Changes in mood may include depression (Costa, Peppe, Carlesimo, Pasqualetti, & Caltagirone, 2006) and/or a higher degree of apathy compared to typically aging peers (Zgaljardic, et al., 2006).

Brain regions that are involved in motor, cognitive and affective functions incorporate those of the basal ganglia (BG). The BG includes such structures as the substantia nigra, globus pallidus, caudate, putamen, and subthalamic nucleus and is functionally related to many cortical and subcortical structures by five anatomically separate circuit loops (Mandir & Vaughan, 2000). Principally, the 'motor circuit' loop involves interaction between the BG with supplementary and primary motor cortices of the frontal lobe and its function is to mediate motor movement (Galvan & Wichman, 2008; Alexander, DeLong & Strick, 1986). The 'oculomotor circuit' involves communication between the BG with the frontal eye fields, prefrontal and posterior parietal cortex and serves to mediate oculomotor movement (e.g., fixation and shifting of eye gaze and saccadic eye movement; Lencer, et al., 2004; Cummings, 1993). The dorsolateral prefrontal lobes reciprocally communicate with the BG to form the 'dorsolateral prefrontal circuit' and play a role in mediating cognition such as executive functioning, working memory ability, and planned motor responses (Zagaljardic et al., 2006; Mandir & Vaughan, 2000; Cummings, 1993). Interaction between the lateral orbitofrontal regions and BG form the 'lateral orbital frontal circuit' and it is believed to be associated with behavioural response monitoring (e.g., impulse control, lability) and mood (e.g., irritability, mania) (Zgaljardic et al., 2006; Mega & Cummings, 1994; Cummings, 1993). Lastly, the 'anterior cingulated circuit' involves communication between the BG, the anterior cingulated gyrus and limbic structures (such as the amygdalae and hippocampi) (Cummings, 1993). This circuit primarily mediates apathy and intentional/motivated behaviour (Mega & Cummings, 1994; Cummings, 1993).

Substantial depletion of dopamine (DA) within the BG in Parkinson's disease (for example, due to loss of dopaminergic neurons in the substantia nigra) disrupts the functioning of the circuit loops of the BG (Galvan & Wichmann, 2008, Mandir & Vaughan, 2000). Consequently disruptions to these circuits have been proposed to explain the motor, cognitive and mood changes observed in PD (Galvan & Wichmann, 2008; Zgaljardic, et al., 2006; Remy, Doder, Lees, Turjanski, & Brooks, 2005).

Rodent models of Parkinson's disease involve the injection of neurotoxins, such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), within the substantia nigra and/or striatal regions of the BG to induce the loss of dopaminergic neurons similar to that are observed in humans with PD (e.g., Ferro, et al., 2005; Cicchetti, et al., 2002). Motor deficit symptoms replicating PD may be induced in rodents if sufficient DA loss occurs. Motor impairments are elicited in rats if approximately ≥55-80% of dopamine are depleted within the striatum or >50% of dopaminergic neurons are lost within the substantia nigra (Fornaguera, & Schwarting, 1999; Lee, Sauer, & Björklund, 1996). Early disease state of PD has also been developed in rodent models. These 'early-stage' PD models use low-dose administration of either 6-OHDA or MPTP and produce only fine motor deficits and induce less pronounced dopaminergic loss within the BG than other (more advanced disease state) models of PD (e.g., Ferro, et al., 2005; Bellissimo, et al., 2004).

One benefit of rodent PD models is that they can be used to trial therapeutic interventions to treat behavioural symptoms resulting from the dopaminergic lesions.

Interventions typically explore how pharmacological treatment can attenuate motor deficits in PD rodents (e.g., Lundblad, et al., 2002). More recently, research has focused upon environmental enrichment (EE) as a non-pharmacological treatment for motor deficits in rodent PD models (Laviola, Hannan, Macrì, Solinas & Jaber, 2008). Enriching stimuli in rat studies may include conditions such as social interaction, physical exercise, materials for rodents to engage with (e.g., toys and tunnels) or exposure to music (e.g., Dhanushkodi, Bindu, Raju & Kutty, 2007; Faverjon, et al., 2002). Jadavji and Metz (2009) explored whether or not EE could protect against fine motor deficits in early-stage PD rodents that did not have gross ambulatory deficits. Rodents allocated to the enrichment treatment were housed in a three-storey cage that had a variety of toys and novel foods that they could interact with and freely explore. Enrichment continued for six-weeks prior to surgery, which involved unilateral infusion of 6-OHDA within the nigrostriatal bundle. Compared to standard housed rats, the EE subjects demonstrated significant improvement in motor tasks post-surgery such as skilled reaching (reaching for food pellets) and ladder walking and they also had significantly reduced apomorphine-induced rotation than control rodents. In addition to protection against behavioural dysfunction, immunohistochemistry analysis of the BG in subjects demonstrated that EE protected against dopaminergic cell loss as induced by 6-OHDA administration (Jadavji & Metz, 2009). Altogether, these results indicated that EE is a valuable therapeutic intervention that can protect against motor dysfunction in PD rodents and minimises neural death of dopaminergic neurons.

While the impact of EE to attenuate motor disturbances in rodent models of PD is currently under study, the possibility of EE to protect against cognitive deficits in

early PD rats has not been investigated. Indeed, few cognitive deficits have been established in early-stage PD rat models and these studies use MPTP to induce these changes. Working memory deficits have been established in early-stage PD rats using a variety of tests (e.g., Y-maze alternate-arms task and spatial working memory water maze tasks; Braga, et al., 2005; Miyoshi, et al., 2002), however, other cognitive deficits, such as short- or long-term memory impairment, have not been firmly established.

For example, the ability of rats to remember familiar objects and hence spontaneously prefer to spend more time exploring a novel object, was examined in a recent early-stage PD rat study by Sy and associates (2010). In this study, rats were each exposed to three (familiar) objects over three consecutive days. On the third day, 5-minutes after familiar object exposure, MPTP and sham rats were each exposed to a novel object in conjunction with two familiar items. MPTP rats spent significantly less time than sham rats exploring the novel object over a 5-minute period, indicating that early-stage PD rats had short-term memory impairment (Sy, et al., 2010).

An alternative interpretation of the results of Sy and associates' (2010) study is that memory was not impaired in MPTP subjects, rather, the performance of early-stage PD rats was impeded by a dysfunction of motivated behaviour, whereby subjects became apathetic and less inquisitive over time- even with the introduction of novelty within the environment. For example, Sy and colleagues (2010) further reported that over the three days of exposure to the familiar objects, exploration of a familiar object by all rats decreased with repeated exposure. Closer examination of

the presented figures indicated that the percent exploration of a familiar object was substantially lower in MPTP rats compared to controls over time (statistical analysis not reported) and, with the introduction of the novel item on day 3 of testing, exploratory behaviour of MPTP rats did not recover while novel exploration of sham animals increased in response to novelty. It is difficult therefore to distinguish whether MPTP subjects demonstrated a significant memory impairment, whereby early-stage PD rats did not recognise a novel item following a 5-minute delay-period from having seen familiar-objects, or whether MPTP subjects lost motivation to engage in exploratory behaviour over time and had an additional apathetic response to novelty. Further research into memory functioning of early-stage PD rats is required to explore these issues.

The present study was an investigation to discover whether an early-stage PD model using 6-OHDA administration to the substantia nigra in aged rats could produce cognitive and/or affective deficits in these subjects, without inducing gross locomotor impairment. Assessment of cognitive skills focused upon those affected by dopamine receptor antagonism in Chapter 2, i) memory ability via the novel object recognition task and ii) mood (anhedonia) and motivation was explored by observing sucrose consumption between 6-OHDA and sham subjects. Additionally, comparison of dopaminergic cell survival between early-stage PD and control rats was examined using immunohistochemical analysis of tyrosine hydroxylase immunoreactivity (THir) within neurons in the substantia nigra.

3.2.0. Methods

3.2.1 Animals

Twenty-two male Sprague Dawley rats (n= 11/treatment group) were obtained from the Animal Resources Centre, Perth, Western Australia. All rats were single-housed with minimal enrichment (i.e., woodchip bedding, paper towel and wooden chew block) in a 12-hr light-dark cycle (lights on 6.20am) with *ad libitum* access to food and water. Home box dimensions: box w 28cm x h 27cm x d 16cm; lid 28cm x 11cm x 26 cm in addition to a food trough of 28cm x –6cm on a gradient up to 0cm x 15cm. Following one-week acclimatisation, rats were handled frequently (~ three times a week) until the end of the study. Rats were approximately 12 months of age at the time of surgery and weighed on average 641 +/- 13g. Animal protocols were approved by Macquarie University Animal Ethics Committee and performed in accordance with animal use and care procedures outlined by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC edition 7, 2004).

3.2.2 Surgery

On surgery day, subjects were anaesthetised with isoflurane. To protect against noradrenaline depletion, 6-OHDA rats were then injected with desipramine (20mg/kg, i.p., Sigma), while sham rats were injected with saline. 6-OHDA (2µg per injection site diluted in 0.1% ascorbic acid) or vehicle (0.1% ascorbic acid) was infused bilaterally into the substantia nigra. Guide cannulae (26 Ga) were placed through two drill holes and lowered to 1mm above the nigral region, using stereotaxic co-ordinates adapted from Fornaguera, Schwarting, Biox and Houston

(1993): anterioposterior (AP) -5.0mm from bregma, mediolateral (ML) +2.0mm and dorsoventral (DV) -8.2mm. At this stage, 33 Ga injection cannulae were guided down to exit 1mm below the larger guide cannulae into the substantia nigra. 6-OHDA or vehicle was then injected (volume 1µL at the rate of 1µL/5-minutes) driven by a microinjection pump and Hamilton microsyringe. The injected substance was allowed to infuse for 5-minutes before injectors and cannulae were carefully removed and scalp wound sutured. Following surgery, animals recovered in a quiet room, were provided heat pads and monitored for two hours before being returned to their home room. Rats were also given pain relief (Flunixin 0.5mg/kg, s.c.) immediately following surgery and two consecutive days post-surgery. The low dose of 6-OHDA reproduced the dose used in an experiment by Srinivasan and Schmidt (2003), who found that this dose did not produce locomotor impairment in early-stage PD rats.

3.2.3 Apparatus

3.2.3.i. Experiment 1. Sucrose consumption boxes

Sucrose consumption testing occurred in clear Perspex chambers (dimensions: w 23.5.cm x h 31 cm x d 35.5cm). Flooring was made of metal rods (6mm diameter) spaced 1.5cm apart. The roof was a hinge lid made of clear Perspex that could be locked in place when closed. L-shaped glass cylinders filled with 7% sucrose solution (sucrose mixed with tap water) were fitted to the side of the box, with the short-end protruding into the chamber. Rats were able to drink the solution by lapping from a hole in the topside component of the protruding cylinder.

3.2.3.ii. Experiment 2. Locomotor chambers

Locomotor chambers (custom built by University of Sydney running Macbench software) were standard operant conditioning boxes (25.0cm x 50.0 x 30.0cm) placed inside wooden, fan-ventilated sound-attenuating boxes (58.0 x 67.0 x 60.0cm). The operant conditioning boxes were made of aluminium (roof and two side walls) and clear Perspex (front and back wall). Flooring was made of metal rods (6mm diameter) spaced 1.5cm apart. Locomotor activity was recorded as the number of beam-breaks between two infrared detectors built into the walls of the chamber. The number of beam breaks was logged by "Workbench Mac" software on a Macintosh computer (McGregor, 1996). Cameras were also placed above the operant conditioning boxes to allow for visual monitoring of the subjects during the experiment.

3.2.3.iii. Experiment 3. Novel object arena and objects

The novel object arena (50cm x 50 cm x 50cm, black painted plywood box) was situated in a dark room and lit by red light (60watt red light bulb) angled across the top of the arena, which allowed an overhanging camera to record live-feed activity within the arena. Objects used as either the novel or familiar objects were either a small glass jam jar with lid or a small Milo drink tin. Both items had been cleaned, labels removed and weighted to minimise the object being tipped over by the rat. Objects were bought in duplicate and chosen for size (e.g., items of a similar diameter; heights that allowed rats to explore the entire object), and ease of cleaning to eliminate odour.

3.2.4 Procedures

3.2.4.i. Experiment 1- Sucrose consumption

Twelve days prior to surgery subjects in Experiment 1 (sucrose consumption) were given one day to habituate to sucrose test procedures. Subjects were taken from their homeroom to a separate test room where they were each placed into a sucrose test box and given one hour access to the sucrose solution. The amount of sucrose consumed at the end of that hour was recorded but not included for analysis. The following two consecutive days constituted pre-surgery baseline sucrose tests. The same procedures occurred as on the habituation day, yet this time the amount of sucrose consumed each day was recorded and used in analyses. If a subject had not consumed any solution over these three days (habituation day and baseline day 1 and 2), this subject was dropped from the experiment. Post-surgery, sucrose consumption was again tested over two consecutive days at two separate time points. Rats were followed-up at two time points following surgery. The first post-surgery assessment session began 10 days following surgery and the follow-up assessment began 21-31 days post-surgery.

Taking two measures of sucrose consumption at baseline (pre-surgery) testing was loosely based upon procedures described by Grippo, Na, Johnson, Beltz and Johnson (2004). In the present study, two test measures were continued to be monitored at post-surgery and follow-up time points allows us to monitor stability in sucrose consumption by subjects at each time point. Additionally, in contrast with Grippo and associates' study, subjects were not deprived of food or water prior to testing. In this manner, subjects were able to demonstrate their preference for sucrose simply by

consuming the solution during testing at a time when they were not hungry or thirsty (i.e., having had *ad lib.* access to food and water prior to testing).

3.2.4.ii. Experiment 2- Locomotor activity

Nine days prior to surgery, baseline locomotor testing (Experiment 2) was conducted. In Experiment 2, subjects were taken from their homeroom to a unique test room and placed within the locomotor chamber. The total locomotor activity level of each subject (i.e., the number of beam breaks made across the infrared beam) was then recorded at 30-minute intervals over a two-hour period and used as a pre-surgery baseline measure. Locomotor activity was again recorded at two time points following surgery: 20-days post-surgery, and a follow-up test was conducted 65-75 days post surgery.

3.2.4.iii. Experiment 3- Novel object recognition

Experiment 3 (novel object recognition testing) occurred 24-33 days post surgery. Subjects were initially habituated to the novel object arena over two consecutive days. On the first habituation day each subject was taken to the NOR test room and placed in the empty arena for 30-minutes. On habituation day two, the same procedures occurred as on habituation day one, with the exception that subjects were placed in the empty arena for 20-minutes.

Novel object recognition testing began the following day. The rat was taken to the NOR room and habituated to the empty novel object arena for five minutes. The rat was then taken out and two identical objects were placed in diagonal corners of the arena, approximately 8-10 cm away from the walls. The subject was then replaced

back into the arena, and was free to explore the two identical objects for five minutes (T1). The rat was then returned to its home-room for 30-minutes (inter-trial interval: ITI) before re-exposure to the NOR arena (T2): this time with one object from T1 (i.e., a familiar object) and a novel object, which were positioned in the same corners of the arena as in T1. Each session was video-taped and recorded with the experimenter out of the room. Time in seconds spent exploring either the novel or familiar object each minute over a three-minute period was recorded. Objects used as novel or familiar objects were counterbalanced across treatment groups, as was the position of the novel object at T2. Following each trial, the arena and objects were cleaned with 10% alcohol solution.

Object exploration was defined as the rat's nose touching or pointing towards an object less than 2 cm away, actively sniffing, licking or chewing an object. Sitting upon an object or using an object to maintain balance when rearing was not sufficient to be considered exploratory behaviour of the object. The number of days and length of habituation to the novel object arena was a compromise from other studies that had used one habituation day (with a six-minute arena exposure) the day before testing began, to 30-minute arena exposure periods over three days before testing (Silvers, Harrod, Mactutus & Booze, 2007; and Sutcliffe, Marshall & Neill, 2007, respectively). As male rats have demonstrated poor novel object recognition memory with an ITI greater than an hour (Sutcliffe, Marshall & Neill, 2007) a shorter ITI of 30-minutes was used in this study as it was predicted that 6-OHDA would induce memory impairment in lesion rats.

Pre-surgery		**Post-surgery**			
Week 1-2		Week 1	Week 2-4	Week 5-8	Week 9- 10
1. Sucrose testing 2. Locomotor testing	Surgery		1. Sucrose testing 2. Locomotor testing 3. Novel object recognition 4. Sucrose testing (follow-up)		Locomotor (follow-up)

Figure 6. Timeline of the test order of experiments performed by 6-OHDA and sham rats pre- and post-surgery.

3.2.4.iv. Tyrosine hydroxylase (TH) immunohistochemistry

Tyrosine hydroxylase immunoreactivity (THir) was conducted in 6 rats/group. Within 7-14 days post behaviour testing, rats were deeply anaesthetised with an overdose of Lethobarb (pentobarbitone sodium, 325 mg/mL, 1-1.5 mL, i.p). Subjects were injected intracardially with 1 mL of 1:10 dilution of heparin in 5% sodium nitrate, before transcardinal perfusion with 300mls phosphate buffered saline (PBS). Subjects then received transcardinal perfusion of 370mls 4% paraformaldehyde (PFA). Brains were removed and stored in fresh PFA in a 4ºC fridge overnight. Brains were then blocked with the midbrain stored in 30% sucrose solution for 4 days (receiving fresh 30% sucrose solution on day 2). Brain sections were then placed in freezing solution and stored in a minus 20 ºC freezer until sectioning for THir.

In preparation for immunostaining for TH, blocked brains were rinsed in phosphate buffer + 0.1% Tween20 (PBT). Brains were mounted to a vibrotome stage and lesion brains were marked using a scalpel. The substantia nigra was cut into 50µm thick sections and placed sequentially into four pots with PBT. Brain slices from one lesion and one sham rat were then combined into the same pot and stored in a -20ºC freezer in freezing solution until required.

For activation, a pot was agitated for 10 minute in PBT, agitated in 5x SSC/Tween20 for 10 minutes and then placed in fresh 5xSCC/Tween20 before being put into a 58ºC oven overnight. Pots were then washed in cold Tris-Phosphate Buffer Saline (TPBS) and agitated 3x 30-minutes at room temperature in this solution. A master primary antibody mix of 3µl mouse & TH antibody (Sigma) + 10% normal horse serum (NHS) + TPBSm (TPBS and 0.05% methylate) was then added 1ml/pot and agitated for 1-hour before being placed in a fridge of 4ºC for 48-hours. Pots were then washed and agitated in cold TPBS 3x 30-minutes, before a secondary antibody was added. A master mix of the secondary antibody (1:500/ml donkey anti-mouse Cy3 (Jackson Immunoresearch) + 5% NHS + TPBSm) was added to each pot, agitated for 1 hour, before being refrigerated for 12 hours. Each pot was washed in cold TPBS for 3x 20-minutes, before brain slices for each rat was mounted onto glass non-gelatinised slides. Vectorshield was added to each slide to cover the brain slices before coverslips were applied.

Images of brain slices were captured with an Axiocam MRMA camera on a Zeiss Z1 microscope. Images were viewed on an attached PC computer using Axiovision software.

3.2.5 Statistical analyses

Baseline and post-surgery behavioural data were analysed using repeated measures ANOVA, Bonferroni adjusted, and Student's *t*-test (statistical package PASW (SPSS) version 18). Statistical significance was set at $p<0.05$ for all analyses. In the sucrose consumption test one 6-OHDA subject was dropped from the study as they did not consume the sucrose solution at the time of baseline testing and one sham

and another 6-OHDA subject were each lost from analysis due to leakage of the solution from the sucrose cylinder. On locomotor testing, at both baseline and post-surgery testing, data from a sham rodent was lost at each assessment due to technical difficulties. No animals from the NOR task were lost from analyses.

TH-immunohistochemical brain analysis was performed using a one-tailed Student's *t*-test. Densitometry of THir neurons in the substantia nigra (pars compacta) was conducted by taking the average pixel density (grey levels) from 37567μm^2 square probes of the region of interest (ROI). Specifically, to control for illumination variations between ROI images and background noise, for each rat a square probe was taken from the ROI in each hemisphere on two brain slices (four data points per brain). Additionally, a reading was taken from the background in each hemisphere per brain slice. The THir density for each ROI was then calculated as a percentage of background and the average value was used for analysis. Any ROI probe that deviated more than 100% of the average for that brain was removed and the remaining 3 probes were averaged. This criterion affected two 6-OHDA rats where only 3 probes were averaged for each brain. One subject was lost from analysis from the sham group due to damage to the brain slices during THir preparation.

3.3.0. Results

3.3.1. Experiment 1

At pre-surgery baseline testing, no significant group effect ($F(1,17)= 0.01$, $p= 0.94$) or interaction effect ($F(1,17)= 0.01$, $p= 0.91$) occurred between 6-OHDA and sham treatment groups, demonstrating that both groups consumed equivalent quantities of

sucrose over the two consecutive days of testing. A significant day effect occurred ($F(1,17)= 20.72$, $p= 0.00$) to indicate that both groups drank significantly more sucrose on day 2 compared to day 1 of pre-surgery baseline testing ($p=0.00$, Figure 7).

Following surgery, there was no significant group effect ($F(1,17)= 0.95$, $p= 0.34$), time by group interaction effect ($F(1,17)= 0.63$, $p= 0.44$), day by group interaction effect ($F(1,17)= 0.50$, $p= 0.49$) or 3-way (time by day by group) interaction effect demonstrated ($F(1,17)= 2.45$, $p= 0.14$). As can be seen in Figure 7, both groups tended to drink similar amounts of sucrose on consecutive days and at each post-surgery assessment (post-surgery and at follow-up). No other main effect for day, time or interaction effect was noted (all p-values >0.05).

As there was no significant day effect on post-surgery performance, a second analysis was performed using the mean sucrose consumed over the two days at each post-surgery sucrose assessment was conducted. As with the above results, no significant group effect ($F(1,17)= 0.95$, $p= 0.34$), time effect ($F(1,17)= 0.59$, $p= 0.45$) or time by group interaction effect was noted ($F(1,17)= 0.63$, $p= 0.44$), indicating that sucrose consumptive behaviour was similar between treatment groups over time (post-surgery).

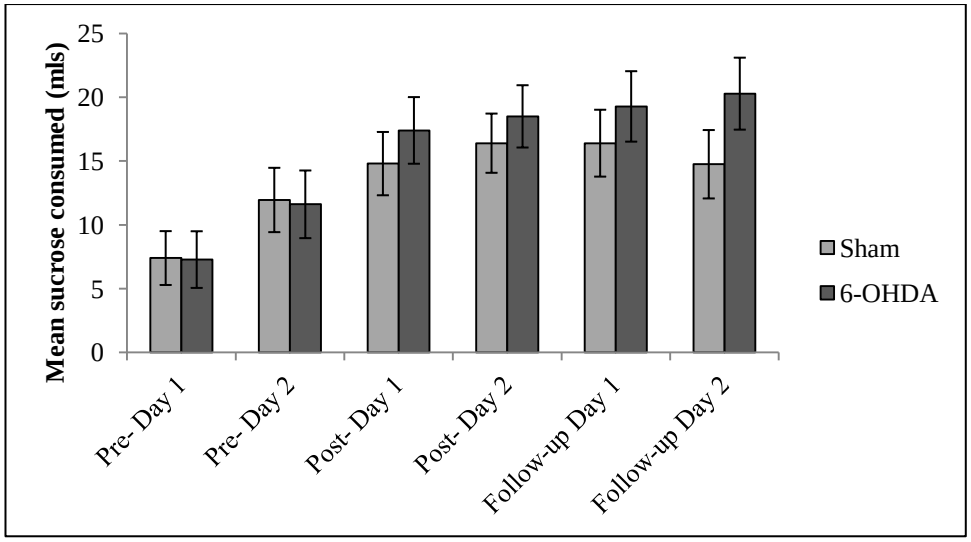

Figure 7. Two consecutive days of sucrose consumption of 6-OHDA and sham subjects at three time points: pre-surgery baseline, post-surgery and follow-up testing. At pre-surgery assessment, both groups drank significantly more sucrose on Day 2 compared to Day 1 of testing ($p<0.05$).

3.3.2. Experiment 2

Pre-surgery baseline testing demonstrated no significant difference in locomotor activity between 6-OHDA and sham rats, $t(19)= 1.17$, $p= 0.26$, which indicated that both groups demonstrated equivalent spontaneous locomotor activity prior to surgery (Figure 8).

Post-surgery, there was no significant group effect for locomotor activity level ($F(1,19)= 0.15$, $p= 0.70$) or post-surgery time by group interaction effect ($F(1,19)= 0.05$, $p= 0.82$), indicating that the locomotor activity levels of each treatment group were similar from post-surgery to follow-up testing. Overall, there was a trend for locomotor activity to decrease over time at post-surgery to follow-up assessment ($F(1,19)= 4.25$, $p= 0.05$, Figure 8).

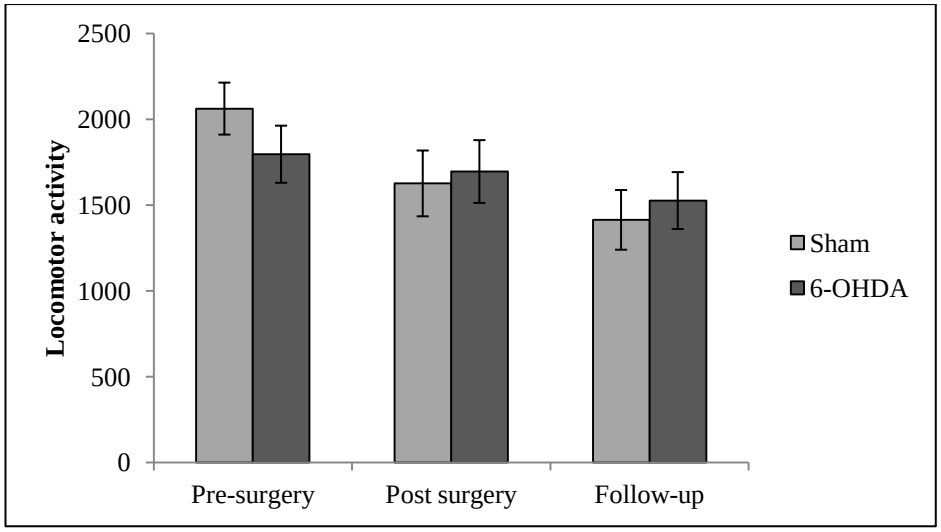

Figure 8. Locomotor activity at baseline, post-surgery and follow-up between 6-OHDA and sham rats.

3.3.3. Experiment 3

There was no main effect for group in novel object preference, F(1,20)= 2.62, *p*= 0.12. Both the 6-OHDA group (mean= 54.66%; SEM= 4.77) and sham group (mean= 65.57%, SEM 4.77) preferred spending over 50% of their time exploring the novel object over the familiar object. Novel object preference was most evident within the first two-minutes of exploration time (See Figure 9) A significant effect for time was found, F(2,19)= 10.62, *p*= 0.00, whereby novel object preference within the first- (mean=74.01%, SEM= 3.38) and second- minute (mean= 67.90%, SEM= 6.09) time bins were not significantly different from each other (*p*= 1.00), but both were greater than novel preference within the third-minute time bin (mean= 38.43%, SEM= 7.30) (both *p*-values <0.05). No significant interaction effect occurred between the exploration time and treatment groups (F(2,19)= 0.23, *p*= 0.80), which indicated that both groups explored the novel object in a similar fashion over time. Additionally, there was no significant difference in novel object preference at the 1st minute of testing at T2 between the two groups (t(20)= 1.78, *p*= 0.09).

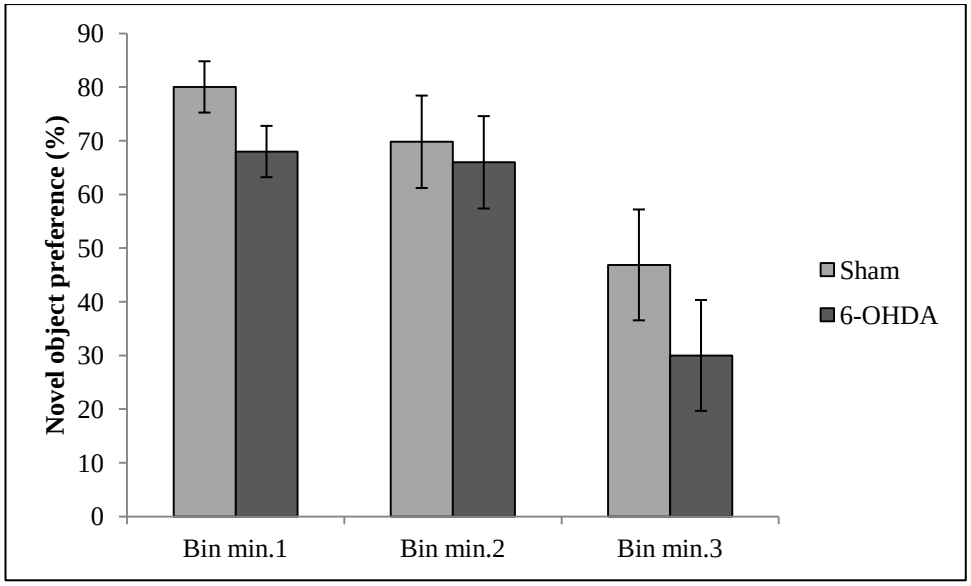

Figure 9. Percent preference for a novel object by 6-OHDA and sham rats over the total exploration period with exposure to both novel and familiar objects at T2.

3.3.4. Immunohistochemistry

Quantitative densitometry indicated that there was a significant decrease in the density of TH-immunoreactive fibres within the substantia nigra pars compacta between 6-OHDA and sham subjects (p= 0.03, Figure 10).

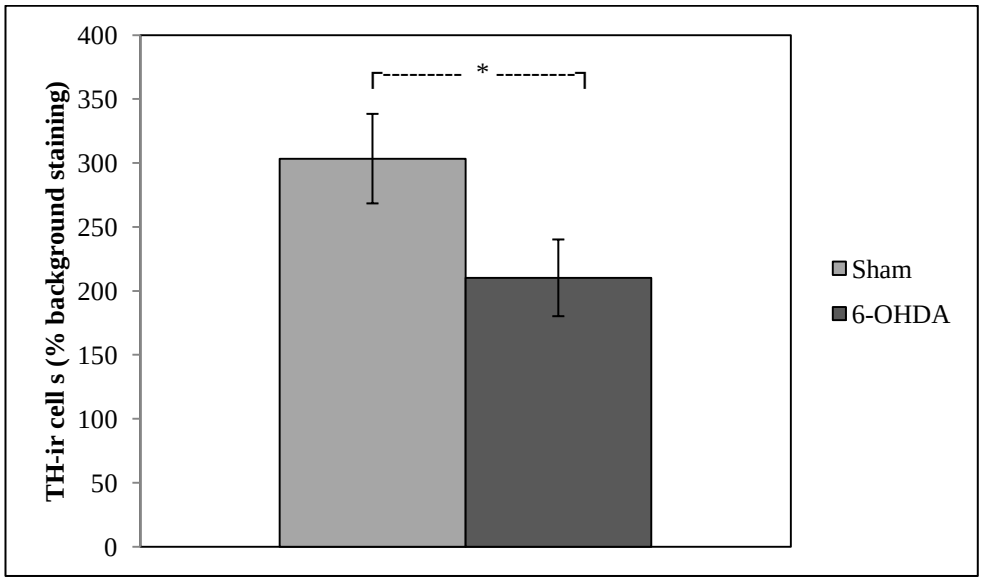

Figure 10. Density of TH cell bodies within the substantia nigra (pars compacta in 6-OHDA and sham rats. *= significance at p<0.05.

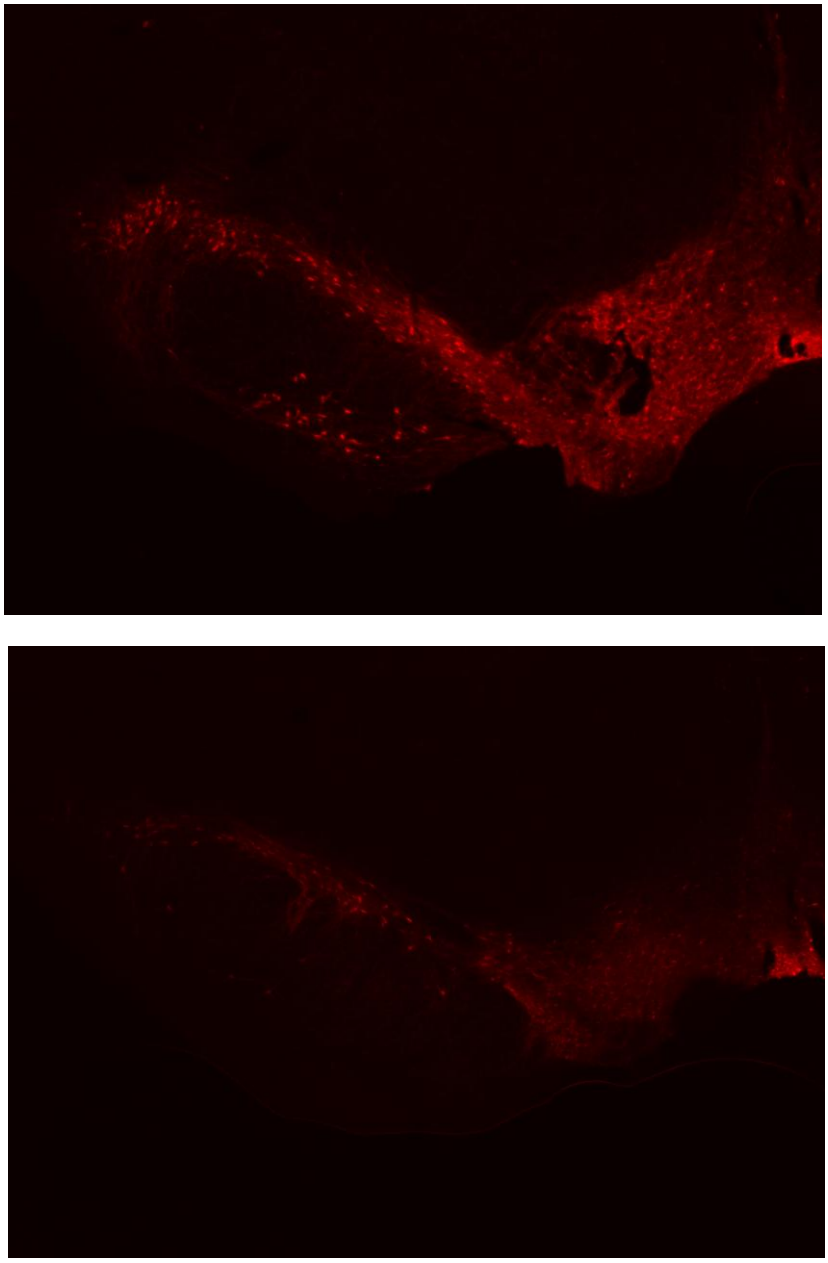

Figure 11.Tyrosine hydroxylase (TH)-immunoreactive cells within the substantia nigra of sham (top) or 6-OHDA (bottom) rats.

3.4.0. Discussion

The purpose of this study was to determine if an early-stage PD rodent model using 6-OHDA could be produced in aged rats. The model was anticipated to induce cognitive and/or affective disturbances in these subjects without eliciting gross locomotor deficits.

3.4.1. Gross motor functioning in an early-stage PD rat model

A successful model of early-stage PD in rodents is one in which dopaminergic lesions to the BG does not create gross motor disturbances in subjects. Results demonstrated that the dose of 6-OHDA administered to aged subjects was sufficient to produce modest dopaminergic cell loss within the substantia nigra (approximately 30% depletion) and this neural loss was not sufficient enough to induce differences in locomotor activity between lesion and sham subjects. These results support similar behavioural findings by Srinivasan and Schmidt (2003) using the equivalent dose of 6-OHDA in younger rats and also supports the findings of Lee and associates (1996), previously mentioned, who found that motor impairment was not observed in 6-OHDA lesioned rats with <50% depletion of dopaminergic neurons within the substantia nigra. This lack of gross motor impairment between treatment groups supports the use of 6-OHDA as a valid neurotoxin to study early-stage PD in aged rats.

3.4.2. Cognitive functioning in the early-stage PD rat model

Examination of cognitive dysfunction in this early-stage PD model focused upon long-term recognition memory retention in aged 6-OHDA and sham rats using the NOR task. Both lesion and sham subjects demonstrated intact memory for the familiar object over a 30-minute period, as, both groups preferred exploring the novel object at T2 and had high (>50%) novel object preference within the first 1- to 2-minutes of testing. As recognition memory of early-stage PD rats was intact on the NOR task following a 30-minute interval in the present study, it is unlikely that the reduced novelty exploration following a 5-minute ITI in early-stage PD rats was due

to a memory impairment in Sy et al.'s study (2010). With intact memory in the present study and equivocal loss of DA neurons within the substantia nigra between the two studies, it is likely that the poorer performance on memory testing by early-stage PD rats in Sy et al.'s study compared to controls may have been confounded by motivational differences between the two groups.

For instance, as was demonstrated in the current study, novel exploration was most intense over the first 1-2 minutes of exposure to objects at T2 and decreased significantly within the 3rd minute of testing, a result similarly found in the D_1 receptor antagonised rats in Chapter 2. As the novel object exploration of lesion subjects was totalled over a 5-minute period rats in Sy et al.'s (2010) study, the difference in exploratory behaviour between the treatment groups may have been a result of reduced motivation of early-stage PD subjects to maintain engagement in exploratory behaviour over the 5-minute test period compared to sham rats. As the exploratory behaviour of rats at intervals over the 5-minute period was not reported, support for a memory deficit between early-stage PD and sham rats in Sy et al.'s study cannot be fully supported.

The current study used the same ITI and interval analysis of novel object preference at T2 in the current study as with Chapter 2, to allow a differentiation between a possible motivational deficit (as proposed in Chapter 2) from a memory deficit (as proposed by Sy, et al., 2010) in 6-OHDA rats. As stated above, both aged 6-OHDA and sham rats demonstrated intact memory on the NOR task. While not statistically significant, there is a general trend for 6-OHDA to blunt the extent of novel object exploration in early-stage PD rats compared to sham rats, particularly within the first

minute of testing. Examining novel object preference over intervals may help to disclose motivational differences in early-stage PD rats in future studies, when using an ITI that does not impede the subjects' memory capacity.

To test for a memory deficit in early-stage PD rats using the NOR task, as argued in Chapter 2, the ITI would likely need to be extended given that D1 receptor antagonism impairs long-term memory of rodents on the NOR task following a 24-hour period but not following a shorter 1-hour ITI (Nagai, et al., 2007). As DA appears to play an important role in long-term memory ability, a lengthy ITI would likely be required to demonstrate impairment in memory functioning in early-stage PD rodents.

Consequently, it was demonstrated that early-stage PD rats are motivated to perform the NOR task, so the NOR test would be a valid tool for measuring memory ability in this model. Future testing for potential memory impairment in the 6-OHDA rats should be conducted with a lengthy ITI. Current results indicate that a memory deficit should be more clearly noted within the first 1- to 2-minutes of exploration at T2, as both treatment groups in this study displayed preference for the novel object at this time.

3.4.3. Early-stage PD rats and mood

Early-stage PD rats in this study did not demonstrate anhedonia due to dopaminergic lesion. Both 6-OHDA and sham rats consistently consumed the same amount of sucrose as each other, both before and any time following surgery. Dalla and associates (2008) found that male rats increase their sucrose consumption when

given intermittent exposure to sucrose solution. The lack of difference found between the present treatment groups may be due to a natural rise in sucrose consumption on an intermittent schedule, which motivates both groups to consume large amounts of sucrose.

Although not significant, it can be observed that sham subjects tended to consume less sucrose than 6-OHDA subjects post-surgery. It would be interesting to explore more fully the pattern of sucrose consumptive behaviour between treatment groups over more consecutive days at each assessment point at post-surgery and at follow-up. Measuring a greater number of sucrose consumption test days (e.g., over 5 days) may be a more sensitive measure to identify a difference in motivated behaviour between treatment groups to drink the solution. Lengthier consecutive testing may demonstrate that 6-OHDA subjects continue to binge more than their sham counterparts while sham animals may habituate to drinking sucrose (i.e., demonstrated by either a plateau or drop in consumption over time). 6-OHDA subjects may be more inclined to continue to consuming sucrose over time as consumption of sucrose increases DA within the basal ganglia (Hajnal & Norgren, 2001). Therefore, 6-OHDA rodents may continue to drink more sucrose than sham subjects over consecutive days to further engage surviving dopaminergic processes within the brain.

Altogether, sucrose consumptive behaviour in early-stage PD rodents may not demonstrate anhedonic behaviour. Further testing, involving an increase in the number of consecutive days of testing may demonstrate a difference in the motivation of treatment groups to consume sucrose solutions.

3.4.4. Methodological considerations

The ability of 6-OHDA to produce an early-stage model of PD in aged rats with associated cognitive (i.e., memory) and motivational dysfunction requires further support with future research. In the present study, the methods employed did not detect memory or affective disturbances in the early-stage PD rats with modest DA depletion (30%). While research suggests that changes to the methodology of the NOR and sucrose tests will likely uncover differences in cognitive and motivated behaviours between the treatment groups, other methodological considerations concerning the early-stage PD model itself must be examined.

A lack of clear cognitive and affective disturbances within the early-stage PD rats in the present study may have occurred for several reasons. Firstly, the dose of 6-OHDA used may not have been substantial enough to elicit non-motor behavioural disturbances in the early-stage PD subjects. As previously mentioned, changes in locomotive behaviour are dependent upon the extent of DA loss within the BG (Fornaguera & Schwarting, 1999). The low dose of 6-OHDA administered to rats in this study may not have diminished dopaminergic neurons to a threshold great enough to elicit disruption to the BG to then create cognitive or affective disturbances. Indeed, Hajnal, De Jong & Covasa (2007) found that both real and sham sucrose consumption in typically developing rats was only suppressed once a certain threshold of both D1 and D2 receptor antagonism was met. It is difficult to estimate the required percent loss of dopaminergic neurons within the substantia nigra to produce a cognitive deficit in the 6-OHDA rats. Other studies that report working memory deficits in early-stage PD rats, using MPTP, report that 20-40% of

dopaminergic cell loss was evident within the BG, however, neural loss was reported within the striatum rather than the substantia nigra specifically (e.g., Braga, Kouzmine, Canteras & Da Cunha, 2005; Miyoshi, et al., 2002).

Secondly, the length of time from surgery to post-surgery testing may have been too great to establish clear memory or affective deficits in early-stage PD rats. Although single administration of 6-OHDA has been found to induce both immediate and progressive DA denervation within the BG (recorded up to 28-days post-injection; Blandini, Levandis, Bazzini, Nappi & Armentero, 2007; Cicchetti, et al., 2002), Steiner and Kitai (2001) found that despite a continued loss of DA denervation within the BG, behavioural deficits (i.e., decreased locomotor activity) improved over a period of 21-days. Similar to other behavioural testing, cognitive and affective changes in an early-stage PD model may best be observed after recovery from administration of the 6-OHDA lesion, for example approximately 2-weeks after surgery (e.g., Metz, Tse, Ballermann, Smith & Fouad, 2005). On the other hand, sucrose consumption by sham subjects in the current study tended to reduce over time compared to 6-OHDA rats and this effect was still evident at follow-up (more than 20 days post-surgery).

Thirdly, an additional complication with PD lesion models is that a consistent administrated dose of a DA neurotoxin into the BG does not create uniform depletion in targeted brain regions across subjects. For example, Fornaguera and Schwarting (1999) administered unilaterally 4µg of 6-OHDA into the substantia nigra of rats. Despite administration of this consistent dose, the amount of DA depletion within the neostriatal region varied greatly between subjects so that

behavioural analysis was conducted in accord with group allocation based upon the degree of DA loss within their BG (e.g. >80% DA loss, 80-55% loss, 55-35% loss, <35%). In the present study, only the effect of 6-OHDA lesion on THir of dopamine cell bodies was measured and not the resulting effect on DA loss in terminal regions. Cognitive and affective changes in early-stage PD rats may require post-mortem grouping, into percent loss of dopaminergic neurons within the BG, for clear evidence of non-motor dysfunction to be uncovered in 6-OHDA rodents.

Changes to the methodological procedures of the current memory and sucrose tests should also be considered before assuming that a higher dose of 6-OHDA is required to produce cognitive or affective changes in aged early-stage PD rats. In relation to memory testing, results from the present study indicate that with a modest depletion of DA 1) early-stage PD subjects are motivated to perform the NOR task, 2) memory deficits were not detected in the first 2-minutes of testing at T2 when novel exploration is typically high, and 3) a longer ITI (greater than 30-minutes) may be required to establish whether 6-OHDA lesion impair long-term memory recall in early-stage PD rodents. Furthermore, sucrose consumption testing needs to be construed as a test of apathy and motivation to engage in a pleasurable activity. Both treatment groups equally consumed the sucrose solution following surgery, however following repeated exposure to sucrose over consecutive days (at separate assessment points post-surgery), the sham rats tended to reduce their sucrose intake in comparison to the 6-OHDA treated subjects. These results tentatively suggest that motivation to drink sucrose may change over time in the different groups. Once the behavioural measures have been refined, dose-dependent effects of 6-OHDA

administration would then determine to what extent DA lesions impede cognitive and/or affective functioning in early-stage PD rodents.

3.5.0. Conclusion

Rodent models of early-stage PD have recently demonstrated that these models are able to produce fine motor dysfunction in rats but, to date, research into the cognitive functioning of early-stage PD rats is limited. The current study supports the use of 6-OHDA to produce early-stage PD (loss of 30% DA) in aged rats and suggests methodological considerations to further refine memory and motivational investigations in these subjects. With confirmation of the range of fine motor and non-motor dysfunction produced in early-stage PD models, research can then focus upon treatment for these disturbances. One promising field of non-pharmacological intervention is environmental enrichment, which has been shown to be beneficial to both locomotor and neural dysfunction in rodent models of PD. Once motor and non-motor dysfunctions have been reliably demonstrated in early-stage PD rat models, it would be intriguing to see the potential for EE to act as either a preventative or treating intervention as a future research direction.

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Chapter 3

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Chapter 4

4.0.0 Discussion

In preparation of developing an early-stage PD rat model with induced cognitive and/or affective dysfunction, Chapter 2 explored the behavioural (locomotor), cognitive (memory) and affective (anhedonic) impact of DA antagonism upon rats. Furthermore, the effectiveness of complex environmental enrichment (CEE) to protect against these motor and non-motor deficits induced by either D_1 or D_2 receptor antagonist administration was examined.

Results from Chapter 2, Experiment 1, indicated that brief CEE (of 14 days) protected against motor and non-motor dysfunction in rats as induced by administration of the D_1 receptor antagonist SCH23390. In line with Meyer, Cottrell, Van Hartesveldt (1993), it was found in the locomotor activity task of Experiment 1 that treatment with SCH23390 attenuated spontaneous locomotor activity in rats. The current study further demonstrated that CEE protected against the degree to which SCH23390 impacted upon locomotor behaviour. Under all dose conditions of SCH23390 the locomotor activity of CEE subjects was significantly less affected by dopamine D_1 receptor challenge than control (i.e., standard enriched: SE) rats.

Interestingly, despite D_1 receptor antagonism attenuating locomotor activity of subjects, as demonstrated in Experiment 1, SCH23390 did not impede the natural preference of novel object explorative behaviour in either CEE or SE rats on the novel object recognition (NOR) task. In the NOR task of Experiment 2, neither CEE

nor control rats demonstrated a recognition memory deficit as both groups preferred spending greater than 50% of their time exploring the novel over the familiar object at T2. Rats did, however, differ in relation to the degree of novel object exploration they engaged in overtime at T2. Following high-dose SCH23390 administration during the encoding phase at T1, CEE subjects continued to sustain a significantly high preference for novel object exploration from the 1st to 2nd minute of exploration at T2. This was in contrast to SE rats who switched preference from the novel item during the 1st min of testing to that of the familiar object during the 2nd minute of exploration at T2. As memory recall was not impaired in CEE or SE rats (with an ITI of 30-minutes), it appeared that CEE increased motivation in exploratory behaviour following DA challenge as CEE subjects demonstrated greater persistence in engaging in novel exploratory behaviour, which the control rats did not. Additionally, even at post-drug testing CEE subjects demonstrated more motivated and persistent behaviour in exploring the novel object within the 1st minute of testing at T2 than control rats.

Results from Experiment 1 and 2 suggest that CEE protected against motor deficits and increased motivated behaviour in enriched subjects challenged by the D_1 receptor antagonist SCH23390. As CEE also increased motivation of subjects' following drug challenge, this indicated the generalised benefits of CEE on non-motor behaviour following DA challenge.

CEE did not, however, protect against the attenuation of sucrose consumption as induced by D_2 receptor antagonism (Experiment 3). Both CEE and control rats were motivated to drink equivalent amounts of sucrose solution post-enrichment.

Pimozide, however, reduced sucrose consumption in all subjects and CEE did not protect against this effect on sucrose consumptive behaviour. A single bottle free-choice sucrose consumption task was used in this experiment to test the anhedonic effects of pimozide on sucrose consumption. Results suggest that CEE did not protect against an anhedonic response to D_2 receptor challenge.

The sucrose test has commonly been used as a measure of anhedonia (Li, Zheng, Liang & Peng, 2010; Herrera-Pérez, Martínez-Mota & Fernández-Guasti, 2008; Willner, 2005). As elegantly reviewed and argued by Berridge (1996), the anhedonia hypothesis proposes that a reduction in a subject engaging in a pleasurable experience (e.g., sucrose consumption) occurs because the salience of a reward has been diminished. However, often DA antagonist studies measure changes in motivational drives that reduce sucrose intake in these animals and then wrongly infer that the results indicate changes in affective experience towards sucrose (Berridge, 1996). The single bottle free-choice sucrose task used in Experiment 3 was assumed to measure the degree of pleasure the subject obtained from sucrose by the amount of sucrose consumed by the animal. This assumption was made because the methods employed limited potential motivational difficulties, such as, effort-based and hunger motivated behaviour, for rats had easy access to the sucrose solution that did not require complex or difficult motor skills to obtain it and animals were sated immediately prior to testing (inferred as they were not food or water deprived). Results do suggest that CEE did not protect against anhedonic effects of D2 antagonism on sucrose consumption; however, increasing the number and type of measures used to capture anhedonic responses in subjects in future studies may help to support these assumptions.

Indeed, additional measures obtained during sucrose feeding may also help to differentiate the underlying influences (including both affective responses and motivational drives) upon consumptive behaviour in DA-challenged rats. For example, obtaining the percent preference for drinking sucrose over non-flavoured water in a two-bottle free choice task (e.g., Brenes & Fornaguera, 2008; Baker & Bielajew, 2007) may demonstrate a liking of the reward, even though total fluid intake may be reduced in subjects due to the effects of DA-antagonism. Moreover, obtaining tongue licking behaviour of rats during consumption may also provide measures about pleasurable experience and motivation differences. The amount of licks of the sucrose solution by a rat may be assumed to indicate how much a subject enjoys the reward while the number of licking bouts, i.e., the number of times a rat goes to engage in consumption, is proposed to indicate the degree of motivated behaviour the animal has to engage in the activity (D'Aquila, 2010). With careful use of the sucrose consumption test, measures obtained may provide information about the affective experience of subjects but may also be expanded to test for and differentiate between drives beyond that of the anhedonia hypothesis.

Presently, however, the results obtained in Chapter 2 suggest that CEE differentially protected against motor and motivation disturbance as induced by D_1 receptor antagonism but, did not protect against hedonic behavioural disturbances associated with D_2 receptor antagonism.

Importantly, although DA receptor antagonists administered in Chapter 2 were injected systemically, it is proposed that the behavioural changes induced by the

drugs had their actions within the central nervous system (CNS) of the subject and not the peripheral nervous system (PNS). The effect of pimozide within the CNS to reduce sucrose consumptive behaviour was demonstrated by Duong and Weingarten (1993). The authors found that sucrose consumption was reduced in rats given i.p. injections of pimozide when receptors of the PNS were blocked with domperidone and consequently indicated that the drug effect must be occurring within the CNS. The behavioural effect of SCH23390 to work at receptors within the CNS is also strongly inferred from previous research. For example, while infusion of a D_1 receptor agonist into the nucleus accumbens increases spontaneous locomotor activity of rats, peripheral (i.p.) injection of SCH23390 (administered either prior to or post D_1 agonism) will attenuate the effect of the agonist on locomotor activity (Dreher & Jackson, 1989). As CEE protected against the effects of D_1 receptor antagonism on locomotor and novel object preference, it is likely that CEE has altered and protected DA functioning through centrally mediated mechanisms (discussed in Chapter 2- 2.5.1.).

Given that CEE protects against motor impairment as induced by D_1 receptor antagonism (Chapter 2, Experiment 1) and protects against both fine-motor deficits and increases cell survival within the BG of early-stage PD rats (Jadavji, Kolb & Metz, 2006), it would be beneficial to explore the ability of CEE to treat cognitive or affective disturbances in early-stage PD rodent models. Rodent models of PD induce dopaminergic cell loss within the BG to replicate similar changes that occur in the disease process of humans with PD. Rodent models of PD have been able to reproduce analogous behavioural symptoms (such as motor deficits) that are seen in people with PD (e.g., Metz, Tse, Ballermann, Smith & Fouad, 2005). Early-stage PD

rat models using the dopaminergic neurotoxin MPTP have been able to induce some cognitive dysfunctions (e.g., working memory deficits; Braga, et al., 2005; Bellissimo, et al., 2004) that commonly occur in individuals with PDND.

In the current paper, a rat model of PDND using modest 6-OHDA lesions in the substantia nigra on aged rats was produced that did not induce overt locomotor deficits to early-stage PD subjects (Chapter 3 Experiment 2). Unfortunately, results demonstrated that this level of dopamine depletion in the early-stage PD rats did not induce cognitive (memory) or affective disturbances, using tests previously shown to be modulated by dopamine receptor antagonism (Chapter 2).

In relation to cognitive functioning of early-stage PD rats, results from Chapter 3 Experiment 3 indicated that both the early-stage PD and sham rats had intact recognition memory on the NOR task. This result was not entirely expected considering that Sy and colleagues (2010) found that early-stage PD rats displayed a recognition memory deficit following a 5-minute ITI. The lack of memory impairment observed in early-stage PD rats in the current study raised questions as to whether poor performance on memory testing by Sy et al.'s rats may have been confounded by lower motivation of lesioned rats to persistently explore the novel object over a 5-minute period at T2. By employing a 30-minute ITI and by analysing the data at minute-intervals over a 3-minute period in the current Experiment (as used in Chapter 2) the NOR task was used to differentiate between memory and motivational differences between 6-OHDA and sham rats. As stated, recognition memory was intact in both early-stage PD and sham rats however there was a trend of less persistent novel object exploration by the early-stage PD rats within the 1st-

minute of testing than the sham rats, even though novel object preference was still >50%.

A memory deficit may not have been found in the early-stage PD rats or in the D_1 antagonised rats of Chapter 2 Experiment 1 because of the short delay period used in the experiments (i.e., an ITI of 30-minutes). Previous research demonstrates that D_1 receptors are involved in modulating long-term memory ability, with D_1 receptor antagonism impairing long-term recognition memory in rodents (with a 24-hour ITI) but not short-term memory ability (using a 1-hour ITI) on the NOR task (Nagai, et al., 2007). A memory deficit in early-stage PD rats would therefore be most evident with the NOR task following a lengthy ITI, which future studies may uncover.

Additionally, in the sucrose consumption test (Experiment 1) with early-stage PD rats in Chapter 3 neither group demonstrated anhedonic behaviour. Both sham and 6-OHDA rats consumed the equivalent amount of sucrose over two consecutive days at each assessment point. Given that Wojnicki, Stine and Corwin (2007) found that rats spontaneously binge on sucrose when exposed to the solution on an intermittent schedule, compared to rats with everyday exposure, it is reasonable to assume that the infrequent sucrose exposure given to rats in Experiment 1 lead to similar (binge-type) consumptive behaviour in both early-stage PD and control subjects. That is, with infrequent exposure to sucrose, coupled with only a short (2-day) monitoring period, both treatment groups likely engaged in binge-type feeding and this may have obscured any significant motivational differences between the groups to consume sucrose. Although not significant, there was a trend for sham rats to consume less sucrose than 6-OHDA rats over time (particularly by Day 2 of the

follow-up assessment). Given that both control and D_1 receptor antagonised rats from Chapter 2 Experiment 3 tended to drink more sucrose over time, it is possible that sham rats may have been less motivated than 6-OHDA rats to consume sucrose. This trend may have been identified if further exposure to sucrose was examined in each group over a number of days at each post-surgery assessment point.

The sucrose consumption test in this instance may be an appropriate task to explore motivational differences between early-stage PD and control rats rather than being used as a measure of anhedonia. For example, sucrose is a natural reward that rats will self-administer and increase their workload (i.e., lever press) to obtain sucrose, just as rats do for other drug rewards such as cocaine or alcohol (Grimm, Shaham & Hope, 2002; Czachowski, Legg & Samson, 2003, respectively). Although the sucrose consumption test using is not typically used as a test of motivation, by increasing the number of consecutive days of consumption recorded on the sucrose test this may produce a sensitive measure to capture motivational differences between early-stage PD and sham rats to consume sucrose over time at post-surgery assessments. Although systematic and intra-accumbens DA receptor antagonism typically leads to decreased sucrose seeking behaviour in rats (Grimm, et al., 2011), sucrose consumption following modest DA depletion of Experiment 1 may have tended to increase in an effort to restore DA levels within the nucleus accumbens (Hajnal & Norgren, 2001). Early-stage PD rats may therefore be more motivated to consume higher levels of sucrose than sham rats over time to help engage surviving dopaminergic functions within the BG.

Due to the lack of clear cognitive or affective disturbances in the early-stage PD rats of Chapter 3 a reversal of non-motor deficits with an intervention treatment was not performed. Further research needs to be conducted to uncover cognitive and/or affective (e.g., motivational) changes within 6-OHDA early-stage PD rats before intervention to reverse these deficits can be explored. As discussed in Chapter 2 (2.5.1.), CEE may be one effective therapy for these deficits as CEE has been shown to support BG and DA functioning within the brain of PD rodents. For example, CEE has been demonstrated to increase neuroplastic changes within the motor circuit of the BG, increase brain derived neurotrophic factors to support cell functioning, and alter DA release within the nucleus accumbens and DA transporters within the PFC of enriched rodents (Ferreira, et al., 2010; Bezard, et al., 2003; Segovia, et al., 2010; Zhu, et al., 2005, respectively).

Finding effective interventions that can be applied to treat or prevent the progression of PD in humans is very important given the prevalence of this disease and our aging population. Within our most populous nations, the projected estimate of PD in individuals aged over 50 years is expected to be between 8.7- 9.3 million people by 2030, which is more than twice the estimated prevalence of PD in 2005 (Dorsey et al., 2007). For each individual, the level of disability to perform activities of daily living and poorer quality of life experienced by individuals with PD is strongly associated with the severity of their motor symptoms, their degree of cognitive dysfunction and depressive or anxious mood state (Braam, et al., 2009; Rosenthal, et al., 2010; Klepac, Trkulja, Relja & Babíc, 2008; Muslimović, Post, Speelman, Schmand & de Haan, 2008; Weintraub, Moberg, Duda, Katz & Stern, 2004). Simply, the development of efficacious therapeutic interventions to protect against the motor,

cognitive and affective disturbances experienced by individuals with PD is necessary to improve the lives of these individuals.

A few recently published rehabilitation interventions for people with PD do replicate the principles of CEE used in rodent models, including increasing social interaction combined with increasing physical conditioning. For example, the impact of 13-weeks social dancing upon motor function was assessed in individuals with PD. Results indicated that dancing improved walking and balance skills in PD participants and post-intervention, subjective improvement in mood was reported by participants that had received treatment (Hackney & Earhart, 2010). Also, Reuter and associates (2011) gave individuals with PD group sessions of walking or relaxation/stretching techniques for 3-sessions/week for 6 months. Walking therapies were shown to improve motor functioning of participants and subjective reports of improved cognition (increased concentration and memory) were reported by participants in all intervention groups (Reuter, et al., 2011).

Given that dopaminergic dysfunction involving the BG leads to the motor and non-motor disturbances in PD (Zgaljardic, et al., 2006; Cummings, 1993), it would be beneficial to explore the generalisation effects of treatments (including CEE) for PD patients upon each motor, cognitive or affective domain of functioning- using measures that specifically capture elements of these domains, rather than using subjective reports or basic screening measures. Exploring the impact of an intervention across a range of motor and non-motor domains alters the goal for an intervention to improve one domain (e.g., motor) of functioning. Focused monitoring of treatment effects (on both targeted and generalised domains of function) will

validate the impact and efficacy of the intervention upon the patient's (physical) functioning, in addition to, demonstrating the impact of the intervention upon their general wellbeing. Additionally, development of fine-motor, cognitive and affective dysfunction in early-stage PD rats would also inform this line of investigation.

Rodent models of PD demonstrate that depletion of DA within the BG can lead to motor and cognitive symptoms that are also similarly experienced by people with PD. As discussed, CEE has been shown to improve both behavioural (e.g., motor) and neural changes (e.g., prevent cell death) within the brain of rodents. Rodent models of PD and the success of interventions in these subjects can help to inform treatment options of PD in humans and can also inform theories regarding the underlying disease process of the disease as experienced by individuals.

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Chapter 4

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Appendix – ARA Approval Forms

MACQUARIE UNIVERSITY ~ SYDNEY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2007/039
Full Approval Duration: 2 January 2008 to 1 January 2009

To: **Ms Louise Hunt (PI)**
Department of Psychology
Macquarie University
Phone: 0428 819 046
Fax: (02) 9850 7759
Email: louise_ruby@yahoo.com.au

Associate investigator/s
Dr Jennifer Cornish Phone: (02) 9850 9467

Other participant (s)
Dr Judi Homewood Phone: (02) 9850 6215
Mr Wayne McTegg Phone: (02) 9850 7757

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: COGNITION AND AFFECT IN EARLY PARKINSONIAN RATS: AN INVESTIGATIVE EARLY PARKINSON'S DISEASE MODEL

Type of animal research and description of project:
Neuro-pharmacological research: The aim of the study is to determine whether cognitive and affective behaviours are assayable using an animal model of early Parkinson's disease whereby no locomotor disturbances are incurred. Rats purchased from external source and housed in standard tubs with *ad libitum* access to food and water. Seven days handling, weighing, text tail marking. Day 7: habituated to sucrose water. Day 8-9: baseline sucrose preference test. Day 10: baseline locomotor activity test. *Surgical procedure:* bilateral injection into caudate putamen with saline, 2 microg or 4 microg of 6-OHDA. One week post-surgical recovery. Four subjects euthanased by rapid decapitation; 12 subjects continue to behavioural studies – sucrose preference testing, locomotor testing, novel object recognition test, holeboard testing. Euthanased by rapid decapitation at completion and brain taken for histological analysis. **All surgical procedures and behavioural testing to be conducted in accordance with details provided in approved application.**

Species of animal: *Rattus norvegicus* (Hooded Wistar or Sprague Dawley)

Number: 48 total

Location: Macquarie University, Central Animal House Facility, F9A

Amendments considered by the AEC during last period: N/A

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions: N/A

(This authority has been issued as the above conditions have been addressed to the satisfaction of the AEC)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **2 January 2008 to 1 January 2009**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a FINAL report at the end of this period.**

Date: 3-1-08

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY ~ SYDNEY

ANIMAL RESEARCH AUTHORITY

AEC Reference No.: 2007/039 – 2
Original Approval Duration: 2 January 2008 to 1 January 2009
EXTENSION APPROVED UNTIL 01 JANUARY 2010

To: **Ms Louise Hunt (PI)**
Department of Psychology
Macquarie University
Phone: 0428 819 046
Fax: (02) 9850 7759
Email: louise_ruby@yahoo.com.au

Associate investigator/s
Dr Jennifer Cornish Phone: (02) 9850 9467

Other participant (s)
Dr Judi Homewood Phone: (02) 9850 6215
Mr Wayne McTegg Phone: (02) 9850 7757

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: COGNITION AND AFFECT IN EARLY PARKINSONIAN RATS: AN INVESTIGATIVE EARLY PARKINSON'S DISEASE MODEL

Type of animal research and description of project:
Neuro-pharmacological research: The aim of the study is to determine whether cognitive and affective behaviours are assayable using an animal model of early Parkinson's disease whereby no locomotor disturbances are incurred. Rats purchased from external source and housed in standard tubs with *ad libitum* access to food and water. Seven days handling, weighing, text tail marking. Day 7: habituated to sucrose water. Day 8-9: baseline sucrose preference test. Day 10: baseline locomotor activity test. *Surgical procedure:* bilateral injection into caudate putamen with saline, 2 microg or 4 microg of 6-OHDA. One week post-surgical recovery. Four subjects euthanased by rapid decapitation; 12 subjects continue to behavioural studies – sucrose preference testing, locomotor testing, novel object recognition test, holeboard testing. Euthanased by rapid decapitation at completion and brain taken for histological analysis. **All surgical procedures and behavioural testing to be conducted in accordance with details provided in approved application.**

Species of animal: *Rattus norvegicus* (Hooded Wistar or Sprague Dawley)

Number: 48 total

Location: Macquarie University, Central Animal House Facility, F9A

Amendments considered by the AEC during last period: N/A

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions:
1. Euthanasia of rats by inducing deep anaesthesia with pentobarbitone sodium followed by intracardial perfusion and fixation with saline and formalin 10% as an alternative to rapid decapitation in up to eight rats per treatment group.
2. Anaesthesia using isoflurane gas
3. Twelve month extension in time

(This authority has been issued as the above conditions have been addressed to the satisfaction of the AEC)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **2 January 2009 to 1 January 2010**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a FINAL report at the end of this period.**

Date: 17-12-08

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY

AEC Reference No: 2009/002

Full Approval Duration: 01 APRIL 2009 TO 31 MARCH 2010 (12 months)

To: Ms Louise Hunt
Dept of Psychology
Macquarie University
Phone: 0428 819 046
Email: lousie_ruby@yahoo.com.au

Associate Investigator(s)
Dr J Cornish Phone: (02) 9850 9467
Dr J Homewood Phone: (02) 9850 6215

Animal Technician
Mr W McTegg Phone: (02) 9850 7757

Is authorised by:

MACQUARIE UNIVERSITY to conduct the following research:

Title of the project: COMPLEX ENVIRONMENTAL ENRICHMENT AND PROTECTION OF COGNITIVE SKILLS, REWARD INCENTIVE AND LOCOMOTION FOLLOWING DOPAMINE ANTAGONISM

Type of animal research and description of project: Research (Neurophysiology / Pharmacology) – This study aims to investigate whether improvement in cognitive skill on a novel object memory task, and maintenance of sucrose consumption and locomotor activity, occurs in subjects that engage in complex enriched environments (CCE) in early adulthood, compared to those in standard enriched housing. Rats will participate in baseline performance tests and will be divided in to a complex enriched environment group (n=16) and standard enriched environments (n=16). Rats will be tested on performance in their test condition task, following administration of one-of-four DA antagonist dose levels. Animals will be euthanased by rapid decapitation at end for histological analysis of brain tissue.

Species of animal: *Rattus novegicus* (Sprague Dawley) male 200 gm

Number: 96 rats per year for one year

Location: Central Animal House Facility, Macquarie University, NSW 2109.

As approved by and in accordance with the establishment's Animal Ethics Committee.

MACQUARIE UNIVERSITY AEC

Approval was granted subject to compliance with the following conditions: N/A

(This authority has been issued as the above condition (s) has been addressed to the satisfaction of the AEC)

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This authority remains in force from **01 APRIL 2009 to 31 MARCH 2010**, unless suspended, cancelled or surrendered, **and will only be renewed upon receipt of a FINAL report at the end of this period.**

Dr Darren Burke
Acting Chair of AEC, Macquarie University

Date: 2.4.09

Form C (issued under part IV of the Animal Research Act, 1985)