Running head: DD1R AND COGNITION

The role of the dopamine D1 receptor in cognition

by

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Abstract

The dopamine D1 receptor (DD1R) has been linked to cognitive functioning in various human and animal studies using diverse methods from pharmacological manipulations to brain imaging. Moreover, suboptimal or supraoptimal functioning of the DD1R has been linked to cognitive dysfunction. However, the previous research on this topic has mainly relied on correlational evidence, or the use of drugs that are not selective to the DD1R. Therefore, the current study investigated whether cognitive dysfunction is due to suboptimal functioning of the DD1R. The DD1R mutant rat (Smits et al., 2006) provides an opportunity to examine the role of the DD1R in cognitive functioning. The performance of the DD1R mutant rats was compared to that of littermate control rats (wildtypes). Across five experiments we found tentative evidence to suggest that the DD1R is necessary for normal cognitive ability. First, the DD1R mutant rats were unable to improve their performance when an egocentric strategy was required in the starmaze, using both positive and negative reinforcement. Second, compared to wildtype rats, the DD1R mutants were impaired in learning an allocentric strategy in the starmaze with positive reinforcement when they had been previously trained in an egocentric task. Third, the mutants were unable to improve when an egocentric strategy was required in the Y-maze. Finally, the DD1R mutant rats took longer than the wildtypes to reverse their learning when a baited arm was switched after two weeks of training with a different arm as the baited arm in the T-maze. Despite some of the limitations of the experiments, these initial findings suggest an impairment in cognition. Ideas for future research and applications are discussed.

Key words: Dopamine, D1 receptor, cognition

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1 The role of the dopamine D1 receptor in cognition

Historically the study and treatment of many psychiatric disorders has had a focus on disturbances in emotional processing. A variety of psychiatric disorders were characterised mainly by their impact on the emotional well-being of the individual. As an example, anxiety was mainly characterised by excessive worry and depression by disturbances in mood. Although emotional dysfunction is an important manifestation of a variety of psychiatric disorders, in many of these disorders cognitive deficits are also present and can even be attributing to the maintenance of emotional symptoms (Green, 2006). In the past the cognitive deficits in psychiatric disorders have received less attention in the literature than the emotional deficits (Millan et al., 2012).

However, cognition and emotion are not separate processes. Instead they interact. Cognition can affect mood and mood in turn can change cognitive processing (Pessoa, 2008). As an example, the neurotransmitter dopamine has been implicated in both emotional (Brown & Greshon, 1993) and cognitive (Hauber & Sommer, 2009, Millan, 2006) processes in depression. Depressive mood can be maintained by a cognitive bias to negative thoughts and memories (Disner, Beevers, Haigh & Beck, 2011). Poor cognitive performance due to reduced perception of reward, lack of motivation and inability to maintain sustained effort is often evident in depression (Millan et al., 2012). A disruption of the limbic dopaminergic signalling has been hypothesised as the cause for poor cognitive performance (Hauber & Sommer, 2009, Millan, 2006). More specifically, the dopamine D1 receptor (DD1R) has been implicated in cognitive impairment in both human (e.g. Abi-Dargham et al., 2011, Okubo et al., 1997, Takahashi et al., 2008, Takahashi, Yamada & Suhara, 2012) and animal studies (e.g. El-Ghundi et al., 1999, Roberts, Seymour, Schmidt, Williams & Castner, 2010, Wall et al., 2011). The present study adds converging evidence to the importance of the DD1R in cognition by using a novel DD1R mutant rat model (Smits et al., 2006). Also, the present study overcomes some of the limitations of the previous literature on the topic.

1.1 Cognition

Cognition can be defined as a group of interrelated mental activities (Millan et al., 2012). Millan et al. (2012, p. 141) have listed these mental activities to include: “pre-attentional sensory gating; attention; learning and memory; problem solving, planning, reasoning and judgement; understanding, knowing and representing; creativity, intuition and insight; ‘spontaneous’ thought; introspection; as well as mental time travel, self-awareness and meta-cognition (thinking and knowledge about cognition)”. Taken the amount and diversity of these mental activities the importance of cognition in daily functioning becomes apparent.

The neuroanatomy of cognition is complex due to the diversity of mental functions involved. Multiple distinct neuronal networks interact to form a single aspect of cognition. As an

example, the frontal lobe, basal ganglia and thalamus form a network that connects attention, working memory and executive function (Alexander, DeLong & Strick, 1986). Also, the prefrontal cortex (PFC) has long been associated with higher order cognition, such as problem solving, planning and goal-directed behaviour (Miller, 2000). Cognitive functioning is not only dependent on various brain areas and the complex neuronal circuits between these areas but also involves multiple different neurotransmitters, G-protein coupled receptors and proteins (Millan et al., 2012). The latter group becomes a topic of interest when looking for drug treatments for cognitive deficits. As an example of the various different neurotransmitters that have been identified to play a role in cognitive ability, a review on drugs that enhance cognitive ability in schizophrenia listed 16 novel pharmacological approaches to treat cognitive impairment (Raedler, 2008). The different neurotransmitter systems that can be targeted to enhance cognition include acetylcholine, dopamine, glutamate and serotonin. Also, the cannabinoid CBI receptors, α_2 adrenergic receptors, GABA_A receptors and cytokines have been identified as novel drug targets (Raedler, 2008).

In sum, cognition is a complex concept in terms of theory, neuroanatomy and neurobiology. Therefore, an initial attempt to investigate the underlying causes of cognitive impairment has to focus on specific areas of cognition. Learning and memory are two integral parts of cognition.

1.1.1 Learning.

An organism incapable of learning will not survive long. There are many types of learning, but two distinct types of learning vital for survival are spatial learning and reversal learning because they are needed to navigate in the environment as well as adapt to changing environments (Clark, Cools & Robbins, 2004, O'Keefe & Nadel, 1978). Spatial learning is needed for navigating in novel and familiar environments. One way of characterising spatial learning is dividing it into two distinct processes (O'Keefe & Nadel, 1978). Allocentric learning refers to relying on external cues to find a target; whereas egocentric learning refers to relying on one's own body coordinates (e.g. always turning left-right-left) to reach a target (O'Keefe & Nadel, 1978). Allocentric learning has traditionally been described as involving the use of two different strategies, direction and place learning (Hamilton et al., 2008, 2009, Skinner et al., 2003). Direction learning involves always using a particular direction when moving towards a target (e.g. towards south), whereas place learning involves finding a particular point in the environment using external cues (Hamilton et al., 2008). Therefore, environmental cues are necessary for allocentric learning because these cues help form a cognitive map of the environment which can be stored in memory for later reference. In contrast, environmental cues can be detrimental for egocentric learning because in a complex environment it can become too disrupting to rely on environmental cues to find a constantly

changing target. Instead, egocentric learning relies on the sensorimotor representation of the whole body and its relation to space. The place recognition in egocentric learning is view-dependent. Also, a mental representation of the distance moved, the time spent in the environment and the specific route that has been travelled is needed for accurate egocentric learning (Weniger et al., 2010).

The two main brain structures involved in spatial learning are the hippocampus and the basal ganglia. In the hippocampus, the areas CA1 and CA3 contain place cells (O'Keefe & Dostrovsky, 1971, O'Keefe, 1979) suggesting that these areas are important for allocentric learning. Also, inactivation of the hippocampus using a lidocaine injection hinders allocentric learning in the cross-maze (Packard & McGaugh, 1996). The basal ganglia, and more specifically the caudate nucleus, are necessary for egocentric learning. Support for this argument comes from the finding that a lesion (anatomical or pharmacological) in the caudate nucleus results in an impairment of egocentric learning (Cook & Kesner 1988, Packard & McGaugh, 1996, Potegal, 1972). Also, the medial prefrontal cortex is necessary for learning an egocentric strategy. Rats with lesions in the medial prefrontal cortex had intact allocentric learning, but their egocentric learning was impaired. The opposite pattern of results was found for rats with lesions in the hippocampus (de Bruin, Moita, de Brabander & Joosten, 2001).

More recently Retailleau, Etienne, Guthrie and Boraud (2011) have proposed that the hippocampus and basal ganglia interact during spatial learning. More specifically, based on evidence derived from rodent studies, Retailleau et al. (2011) have proposed three loops that account for different aspects of spatial learning (see Figure 1). The first loop is responsible for allocentric navigation by localisation, and includes projections from the ventral striatum (VS) to the ventral pallidum (VP), which in turn projects to the dorsal hippocampus (DHPC), from where projections reach to the entorhinal cortex (EC) and from there back to the VS. The second loop is responsible for allocentric navigation by external cues, and includes projections from the dorsomedial striatum (DMS) to the entopeduncular nucleus (EN) and from there to the anterior thalamus, which in turn projects to the prelimbic cortex (PLC) and from there back to the DMS. The third loop is responsible for egocentric navigation, and has projections from the dorsolateral striatum (DLS) to the substantia nigra pars reticulata (SNr), which projects to the anterior thalamus and from there the projections reach the sensorimotor cortex (SMC) which in turn projects back to the DLS. All of these three loops are linked by mutual connections (Retailleau et al., 2011).

Figure 1. Three neural loops that are involved in spatial learning. Figure retrieved from Retaillieu et al., 2011, p. 6.

One of the neurotransmitters involved in spatial learning is dopamine. Dopamine from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) project to the DHPC, VS, DMS and DLS, thus affecting each of the three loops (Retaillieu et al., 2011). Support for the vital role of dopamine in spatial learning comes from the finding that depleting dopamine in the dorsal striatal region of rats resulted in an impairment of egocentric learning (Braun, Graham, Schaefer, Vorhees & Williams, 2012). Allocentric learning was also affected in the sense that it took the rats longer to learn the allocentric task compared to controls. However, the rats were able to improve over time with the allocentric task (Braun et al., 2012).

The term reversal learning refers to the ability to adapt to new rules by changing the pattern of responding according to what is beneficial with the new rule (Rolls, 1999). Thus reversal learning is essential in changing environments and impairments of reversal learning can be related to risk-taking and impulsive behaviour (Clark, Cools & Robbins, 2004). Reversal learning is especially relevant in social and emotional behaviours (Rolls, 1999). The ventral prefrontal cortex and the ventral striatum have been linked to reversal learning ability in a variety of rodent, primate and human studies (for a review see Clark, Cools & Robbins, 2004). For instance, reversal learning impairment is apparent in both humans and animals with orbitofrontal lesions (Butter, 1969, Daum, Schugens, Channon, Polkey, & Gray, 1991). Also, Cools, Clark, Owen & Robbins (2002) found significant signal changes in the ventrolateral prefrontal cortex and the ventral striatum in a

functional magnetic resonance imaging study following a reversal task. In terms of neurotransmitters, both dopamine and serotonin are implicated in reversal learning according to pharmacological studies (Barnes et al., 1990, Cools, Barker, Sahakian, & Robbins, 2001, Smith, Neill, & Costall, 1999, Taghzouti, Louilot, Herman, Le Moal & Simon, 1985). For instance, both rats with a lesion in the dopaminergic neurons of the nucleus accumbens (Taghzouti et al., 1985) and serotonin-depleted rats (Nomura, 1992) show reversal learning deficits.

In conclusion, both spatial learning and reversal learning are important aspects of cognition. These two types of learning can be traced to specific brain areas and brain pathways that are distinct from each other. The ventral prefrontal cortex and the ventral striatum are important for reversal learning; whereas the hippocampi, the medial prefrontal cortex and the basal ganglia are important for spatial learning. However, both types of learning rely on normal functioning of the dopaminergic pathways.

1.1.2 Memory.

Memory is an integral part of cognition. First, memory is needed for maintaining information for just a few seconds (short-term memory, STM). Second, memory is needed for maintaining information about facts (semantic) and events (episodic) for longer periods, which can be anything between an hour to several decades (long-term memory, LTM, Baddeley & Warrington, 1970). Third, memory is also important in multitasking when attention is divided between two tasks (working memory, WM, Baddeley, 1992). There are several lines of evidence to suggest that STM, LTM and WM are distinct memory systems (Izquierdo et al., 1998, 2002, Izquierdo, Medina, Vianna, Izquierdo & Barros, 1999, McGaugh, 2000). For instance, some pharmacological manipulations in the hippocampus, entorhinal or parietal cortex affect STM whereas LTM stays intact (Izquierdo et al., 1998, 1999, 2002). Other manipulations affect the two memory systems differently, whereas others affect them in the same manner (Izquierdo et al., 1998, 1999). These findings suggest that STM and LTM are separate processes with some overlapping mechanisms at the receptor level. Similar findings are made when WM is compared with STM and LTM (Izquierdo et al., 1999).

Spatial memory is needed to learn to navigate in the environment. Whereas spatial learning involves the acquisition of new skills to navigate in an environment, spatial memory involves the encoding, storage and retrieval of information about routes, configurations and spatial locations (Burgess, 2008). Bird and Burgess (2008) have identified the brain areas important for spatial memory. Not surprisingly, these areas include the hippocampus, prefrontal cortex and striatum, which are also important in spatial learning (Bird & Burgess, 2008, Retailleau et al., 2011). An additional structure that is important in memory consolidation is the amygdala (McGaugh, 2000).

For instance, long-term retention of information has been found to correlate with the degree of activity of the amygdala during encoding (Cahill, Babinsky, Markowitsch & McGaugh, 1995). There is also some evidence to suggest that the neurotransmitter dopamine is important for memory (Bethus, Tse & Morris, 2010, Espejo, 2003). However, GABA, glutamate, serotonin, and acetylcholine are also involved in memory formation (Hasselmo & Bower, 1993, Tellez, Gomez-Viquez & Meneses, 2012).

In sum, there are three important memory processes (LTM, STM and WM) that are necessary for learning to occur. These processes are involved at different stages of learning. During the initial acquisition of a new skill STM is vital for encoding the information that is necessary for navigation. WM is needed when previous responses within a trial are kept in memory to avoid errors. Finally, LTM is vital for the retention of the learned information over time.

1.1.3 Cognitive deficits in psychiatric disorders.

Cognitive deficits are common in a variety of psychiatric disorders. Often if cognitive deficits are not the cause of the disorder then they are at least a factor in escalating or maintaining symptoms (Millan et al, 2012). Spatial learning deficits are present in various psychiatric disorders such as schizophrenia, Alzheimer's disease and Parkinson's disease (Laczó et al., 2009, Postle, Locascio, Corkin & Crowdon, 1997, Weniger & Irle, 2008). For instance, Laczó et al. (2009) found that subjects with hippocampal mild cognitive impairment had a severe spatial learning deficit similar to that of subjects with Alzheimer's disease. On the other hand, subjects with non-hippocampal mild cognitive impairment showed no such deficit. It has been previously suggested that hippocampal mild cognitive impairment is a prodromal stage of Alzheimer's disease (Dubois & Albert, 2004). Therefore, Laczó et al. (2009) suggested that spatial learning deficits may be used to detect early onset of Alzheimer's disease. Deficits in reversal learning are related to addiction. An inability to reverse a behaviour pattern despite negative consequences is a trademark of addiction (Crews & Boettiger, 2009). In fact, a reversal learning impairment has been found in animals that chronically self-administer cocaine (Schoenbaum and Shaham, 2008) and alcohol (Obernier, Bouldin & Crews, 2002). Also, people who are addicted to cocaine have impaired reversal learning (Schoenbaum and Shaham, 2008). It has been hypothesised that the reversal learning impairment in addiction might be a contributing cause in the disease. Thus, it is possible that addiction could be treated with interventions that target reversal learning (Sofuogly, 2010).

Cognitive impairment, especially memory impairment is also present in depression (Austin, Mitchell & Goodwin, 2001). Moreover, the impairment is not necessarily a result of depression, because it is also present in people who have recovered from depression (Austin et al., 2001). This suggests that cognitive impairment might be one of the contributing factors in the

development of depression. Similarly, cognitive deficits are present in Parkinson's disease (PD, Chaudhuri & Schapira, 2009). In later stages of PD, up to 80 per cent of the patients suffer from dementia (Aarsland, Andersen, Larsen & Lolk, 2003). However, cognitive impairment is also evident in the early stages of PD. Patients with PD have impaired visual perception, spatial and motor perception, executive functioning, as well as visual and verbal memory (Uc, Rizzo, Anderson, Olan, Rodnitzky & Dawson, 2005). Cognitive impairments, along with other non-motor impairments, are a major factor in determining the quality of life of the PD patients (Chaudhuri & Schapira, 2009). Therefore the treatment of these impairments is extremely important.

The cognitive deficits that are observed in a variety of psychiatric disorders have no known single underlying cause. In fact, modern theories on psychological dysfunction often hypothesise that a combination of genetic vulnerability and environmental factors account for the development of many disorders (Burmeister, McInnis & Zöllner, 2008). As an example, family and twin studies have revealed a strong genetic component in the development of schizophrenia, with approximately 50 per cent likelihood of developing the disorder if a monozygotic twin has schizophrenia, when in the general population the disorder has only 0.7 per cent prevalence (Sullivan, Kendler & Neale, 2003). However, although there have been various association studies examining the genetic component of schizophrenia, none of the identified genes are reliably associated with the disorder (Burmeister et al., 2008). Because most of the candidate genes have common allelic variants that only increase the risk of developing the disorder slightly, they cannot be the only cause for schizophrenia (Kellendonk, Simpson & Kandel, 2009). A 'common disease-common allele' model has been proposed, which states that a combination of different common allelic variations leads to a predisposition for schizophrenia. Together with environmental factors, these allelic variations can result in the development of the disorder (Kellendonk et al., 2009).

The most common disease related mutations in the human genome are single nucleotide polymorphisms (SNPs) and copy number variations (CNVs). The latter are duplications and/or deletions of large segments of DNA which often involve several genes (Kellendonk et al., 2009). The underlying cause of cognitive impairment is likely to have a similarly complex cause. Multiple SNPs and CNVs might be related to a genetic predisposition to cognitive deficits, but environmental factors might contribute to the severity of the deficit as well. Despite efforts effective treatments for cognitive deficits are still lacking (Millan et al., 2012, Raedler, 2008). If the cognitive deficits in various psychiatric disorders could be treated, this could possibly help patients with their daily functioning and might alleviate other symptoms as well.

1.2 Dopamine

Dopamine is a catecholamine neurotransmitter that is linked to various different psychological processes. Phillips, Vacca and Ahn (2008, p. 236) listed these processes to include “movement; hedonic reactions to positive reward; provisions of an error detection signal during the acquisition of new learning; response to novel stimuli; provision of reinforcement signals essential for acquisition of new action patterns; and motivation”. Dopamine is also necessary in different types of memory processes (Phillips et al., 2008, Bethus, Tse & Morris, 2010). There are nine major dopaminergic cell groups (for a review see Björklund & Dunnett, 2007). Dopaminergic neurons can be found in the caudate-putamen, nucleus accumbens, lateral septum, amygdaloid complex, hippocampus, prefrontal cortex and anterior pituitary (Nieuwenhuys, 1985, Roth, Wolf & Deutch, 1987)

The four distinct dopamine pathways include the mesolimbic pathway, the nigrostriatal pathway, the mesocortical pathway and the tuberoinfundibular pathway (Lindvall & Björklund, 1978). Dopaminergic neurons projecting from the ventral tegmental area to the nucleus accumbens, septum, olfactory tubercle, amygdala, and piriform cortex form the mesolimbic pathway. Like the mesolimbic pathway, the mesocortical pathway also consists of dopaminergic neurons projecting from the ventral tegmental area. However, in the mesocortical pathway, these projections innervate the medial prefrontal, cingulate and entorhinal cortices. The nigrostriatal pathway consists of dopaminergic neurons projecting from the substantia nigra to the striatum. The tuberoinfundibular pathway includes dopaminergic neurons that project from the arcuate nucleus of the hypothalamus to the intermediate lobe of the pituitary and the hypophyseal portal vessels of the median eminence. While the existence of these four pathways has been known for some time, the function of each of the pathways is less clear (Holmes, Lachowicz & Sibley, 2004). Therefore, much of the research on the function of dopaminergic signalling has concentrated on examining the role of the different dopamine receptors (Holmes et al., 2004).

1.2.1 Dopamine receptors.

All dopamine receptors are G protein-coupled receptors. Early investigations found two types of dopamine receptors, the D1 and the D2 (Garau, Govoni, Stefanini, Trabucchi & Spano, 1978, Keabian & Calne, 1979, Stoof & Keabian, 1981, Onali, Olanas & Gessa, 1984). More recently, dopamine receptors have been divided into two major categories, the D1-like receptors and the D2-like receptors (Seeman, 1980, Seeman & Van Tol, 1994, Sibley, Monsma & Shen, 1993). So far, five dopamine receptors have been identified. Two of the receptors (D1 and D5) belong to the D1-like receptor family, and three of the receptors (D2, D3 and D4) belong to the D2-like receptor family (Seeman, 1980, Seeman & Van Tol, 1994, Sibley, et al., 1993). The signal

transduction of the D1-like receptors is different from the D2-like receptors (Kebabian & Calne, 1979). The D1-like receptors have an excitatory influence on adenylyl cyclase (AC), whereas the D2-like receptors inhibit AC (Undiehl, 2010).

All five dopamine receptors have a distinct distribution in the central nervous system (Missale, Nash, Robinson, Jaber & Caron, 1998). From these receptors, the dopamine D1 receptor (DD1R) is the most abundant, and is rarely expressed together with the dopamine D2 receptor (Missale et al., 1998). The distinct anatomical location and the different signal transduction of the dopamine receptors suggest that each receptor has a specific function of dopamine (Holmes et al., 2004). If these specific functions could be determined, the different dopamine receptor subtypes could be targeted in the treatment of neuropsychiatric diseases (Holmes et al., 2004).

1.2.2 Dopamine and cognition.

The prefrontal cortex (PFC) is especially implicated in cognitive processes such as reasoning, planning and spatial ability (Chao & Knight, 1995, Fuster, 1989, Miller & Cohen, 2001). The PFC has many dopamine neurons (Goldman-Rakic, 1992) and more importantly is very sensitive to dopaminergic signalling (Robbins, 2000). Dopaminergic signalling plays an important role in many aspects of cognition (Bäckman, Nyberg, Lindenberger, Li & Farde, 2006). The evidence for the importance of dopamine functioning in cognition comes from human, animal, electrophysiological, genetic, and pharmacological, as well as neurocomputational studies (for a review see Bäckman et al., 2006). As an example, people who suffer from diseases that affect the dopamine system (e.g. Parkinson's disease, PD) also have deficits in cognitive ability (Brown & Marsden, 1990). Also, PET studies on people with PD have found correlations with dopamine binding and executive functioning as well as episodic memory (Bruck, Aalto, Nurmi, Bergman & Rinne, 2005, Holthoff et al., 1994). In animal studies, cognitive deficits can be elicited by lesioning the dopaminergic system (Boussaud & Kermadi, 1997, Roberts et al., 1994, Simon, Taghzouti & Le Moal, 1986). Also, when the dopamine system is targeted in gene techniques to develop mutant and knockout animals, these animals often have cognitive impairments. For instance, D2 receptor mutant mice are impaired on spatial working memory tasks as well as perceptual discrimination tasks (Glickstein, Hof & Schmauss, 2002, Glickstein, Desteno, Hof, & Schmauss, 2005).

Findings from both human (Luciana & Collins, 1997, Luciana, Collins & Depue, 1998, Luciana, Depue, Arbisi & Leon, 1992) and animal studies (Sawaguchi & Goldman-Rakic, 1991) suggest that pharmacological manipulations of dopamine signalling can have detrimental as well as positive effects on cognitive functioning, depending on whether these manipulations increase or decrease dopamine signalling. However, the pharmacological findings of the effects of dopamine on cognition have also sometimes been mixed. For instance, Kimberg, D'Esposito and Farah (1997)

divided their subjects into two groups according to their working memory (WM) capacity. A D2 receptor agonist (bromocriptine) showed positive effects on the subjects with low WM capacity. However, for subjects with high WM capacity the D2 agonist resulted in detrimental effects on a WM task. In contrast, the administration of a non-selective D1 and D2 agonist (pergolide) resulted in improved WM performance on a delayed task for subjects with high WM capacity but did not improve the performance of subjects with low WM capacity (Kimberg & D'Esposito, 2003).

Contrasting findings on the role of dopamine in cognition can similarly be found among genetic studies. As an example, the gene that codes for catechol *O*-methyltransferase (COMT) has been a gene of interest in this field because COMT inactivates extracellular dopamine (Weinshilboum, Otterness & Szumlanski, 1999). Val108/158Met is a common polymorphism in the COMT gene (Männistö & Kaakkola, 1999) and Val carriers have less dopamine in the prefrontal cortex compared to Met carriers because Val carriers have a higher activity of COMT (Egan et al., 2001). This polymorphism of the COMT gene has been extensively investigated due to the hypothesis that prefrontal dopamine functioning is important for higher order cognitive ability. To date, the evidence of the influence of the COMT polymorphism on cognitive performance has been mixed, with some studies finding that the Met allele is connected with better cognitive performance (e.g. Bishop, Fossella, Croucher & Duncan, 2008), whereas others have found the opposite pattern of results, with a Val allele advantage on cognitive performance (Yeh, Chang, Hu, Yeh & Lin, 2009). A recent meta-analysis has suggested that the COMT val¹⁵⁸met single nucleotide polymorphism has little or no effect on cognitive performance (Barnett, Scoriels & Munafò, 2008).

Although it is not always easy to tease apart the factors that lead to the contrasting findings in the literature on the role of dopamine functioning in cognition, one way to try to explain these findings is with the help of some of the theories of dopamine functioning. The inverted U-shaped theory states that the relationship between dopamine and cognitive performance follows an inverted U-shape (for a review see Seamans & Yang, 2004). According to this theory, too much or too little dopamine will lead to impaired performance. Therefore, it is important to take into account the basal levels of dopamine in pharmacological and genetic studies (Seamans & Yang, 2004, Cools & D'Esposito, 2011). The differences in basal dopamine levels might account for some of the abovementioned contrasting results in pharmacological and genetic studies.

Another theory of dopamine functioning takes into account the phasic and tonic levels of dopamine signalling (Grace, 1991). Dopamine neurons show both basal level tonic patterns from prefrontal cortical afferents as well as phasic bursts from dopamine neurons (Grace, 1991). It is hypothesised that the D2-like receptors are sensitive to the low levels of tonic dopamine release because the D2-like dopamine receptors have a higher affinity to dopamine than the D1-like dopamine receptors (Missale et al, 1998). The dopamine D1 receptor (DD1R) is hypothesised to

respond to phasic bursts of dopamine (Goto & Grace, 2005, Grace, Floresco, Goto & Lodge, 2007) because it has the lowest affinity to dopamine, with up to 10-fold lower affinity than the dopamine D5 receptor (DD5R, Sunahara et al., 1991, Tiberi et al., 1991). Wall et al. (2011) reviewed the evidence from genetic mouse models in light of this phasic/tonic theory. The authors found support for the hypothesis that phasic dopamine release is important for the functioning of the DD1R. Wall et al. (2011) also noted that the DD1R might also partially respond to tonic dopamine signalling because the DD1R knockout mice are impaired in more aspects than the mice with reduced phasic dopamine release. However, it is important to interpret these results with some caution, because it is unclear what types of compensatory mechanisms are occurring in the other dopamine receptors in these knockout mice.

The phasic/tonic theory on dopamine functioning can also help shed light to the contrasting results in the abovementioned studies. For instance, Goto and Grace (2005) found differential roles of the DD1R and the dopamine D2 receptor (DD2R) in the PFC and the hippocampus during phasic and tonic dopamine signalling. Phasic dopamine release via DD1R activation enhanced hippocampus inputs. The authors suggested that this finding in turn could influence spatial learning by affecting the connection between the hippocampus and nucleus accumbens. Contrasting results were found for the DD2R. Both an increase or a decrease in tonic dopamine release influenced the PFC, without having any influence on hippocampus inputs, through DD2Rs. Behaviourally, this DD2R activity is important for set shifting strategies (Goto & Grace, 2005). Therefore, phasic and tonic dopamine signalling might lead to contrasting results depending on which brain areas are most important for the specific task that is used due to the diverging roles of the different dopamine receptors.

As mentioned previously, dopamine is not only involved in cognition, but also various other psychological processes. It is important to note that dopamine is heavily involved in goal-directed behaviours (Sesack & Grace, 2010). The phasic/tonic theory of dopamine functioning has also been investigated with regard to reward. Phasic dopamine release is evident when receiving a reward. In contrast, when an expected reward is lacking, there is tonic dopamine activity (Sesack & Grace, 2010). Also, the same brain areas (basal ganglia, hippocampus, amygdala, prefrontal cortex) are implicated in both goal-directed behaviours and cognition (Retailleau et al., 2011, Sesack & Grace, 2010). Moreover, there are two distinct striatal pathways that are involved in reward and aversive tasks (for a review see Hikida, Kimura, Wada, Funabiki & Nakanishi, 2010). The direct pathway which is hypothesised to be involved in reward-related behaviour includes striatonigral GABA-containing neurons and selectively express dopamine D1 receptors. The indirect pathway, which is hypothesised to be involved in the processing of aversive stimuli, includes striatopallidal GABA-containing neurons and express dopamine D2 receptors (Albin, Young & Penney, 1989,

Alexander & Crutcher, 1990, Gerfen et al., 1990, Surmeier, Ding, Day, Wang & Shen, 2007).

Therefore, motivation to work for reward might be a confounding variable in any studies examining the role of dopamine in cognition.

1.3 The Dopamine D1 Receptor (DD1R) and Cognition

Various species ranging from fruit fly (Gotzes, Balfanz & Baumann, 1994) to rodents (Grilli, Nisoli, Memo, Missale & Spano, 1988, Nisoli, Grilli, Memo, Missale & Spano, 1988) and humans (De Keyser, De Waele, Convents, Ebinger & Vauquelin, 1988, De Keyser, Dierckx, Vanderheyden, Ebinger & Vauquelin, 1988, Dearry et al, 1990) have DD1Rs. The DD1Rs are expressed at higher levels and are also the most widespread dopamine receptor in the brain (Dearry et al., 1990, Weiner et al., 1991). In the central nervous system, the DD1Rs are found in the basal ganglia, olfactory bulb, cerebral cortex, hippocampus and amygdala (Ariano & Sibley, 1994, Bergson et al., 1995, Levey et al., 1993). Levey et al. (1993) found the DD1Rs to be especially expressed in the caudate, which is a part of the striatum, as well as the internal segment of the globus pallidus and the substantia nigra pars reticulata (SNr). Bergson et al. (1995) also found the DD1Rs to be abundant in the neostriatum and the SNr. Bergson et al. (1995) examined the distribution of the DD1Rs compared to the dopamine D5 receptors (DD5Rs). The authors found a differential distribution of these two D1-like receptors in the neostriatum, caudate nucleus, SNr, basal forebrain, cerebral cortex and the hippocampus (Bergson et al., 1995).

The prefrontal cortex (PFC) has more abundant DD1R compared to dopamine D2 receptors (DD2R) in rodents (Dubois, Savasta, Curet & Scatton, 1986), in primates (Lidow, Goldman-Rakic, Gallager & Rakic, 1991) and in humans (Hall et al., 1994). The PFC plays an important role in higher order cognitive functioning (Chao & Knight, 1995, Fuster, 1989, Miller & Cohen, 2001) as well as working memory (Brozoski, Brown, Rosvold & Goldman, 1979). Because the DD1Rs are more abundant in this brain area, it has been suggested that the DD1R has a more central role in cognitive functioning than the other DA receptors (El-Ghundi et al., 1999). On a more molecular level, both long-term potentiation (LTP) and long-term depression (LTD) require D1-like receptors (Centonze et al., 2003, Centonze, Picconi, Gubellini, Bernardi & Calabresi, 2001, Kerr & Wickens, 2001, Shen, Flajolet, Greengard & Surmeier, 2008). LTP leads to greater post-synaptic responses in subsequent excitations of specific synaptic pathways, whereas LTD decreases the subsequent responding. The facilitation and dampening of connections that LTP and LTD offer has been long hypothesised to be an important neuronal mechanism for learning and memory (Ito, 1989, Bliss & Collingridge, 1993). The role of the DD1R in cognition has been investigated in various imaging, pharmacological, genetic and animal studies.

1.3.1 Imaging studies with human subjects.

A relationship between the DD1R and cognition has been established in various imaging studies with human subjects. However, the DD1R functioning seems to correlate more strongly with specific aspects of cognition. Also, some brain areas have received more attention in the literature than others. Based on the anatomical distribution of the DD1R, the prefrontal cortex (PFC) and the striatum have been examined extensively. Using positron emission topography (PET), Takahashi et al. (2008) found an inverted U-shape relationship in healthy subjects between the PFC DD1R binding and performance on the Wisconsin Card Sorting Test (WCST). The WCST is a measure of set shifting and working memory. However, DD1R binding in the hippocampus was not correlated with performance on memory or executive function tasks. Instead, hippocampal DRD2 binding was related to long-term memory (LTM) performance.

The relationship between DD1R functioning in the PFC and cognition has also been investigated in clinical populations. Using PET, Okubo et al. (1997) found a decrease in DD1R in the prefrontal cortex of schizophrenic patients. In contrast, Abi-Dargham et al. (2012) found an increase in the prefrontal DD1R in drug naïve schizophrenic patients. Both studies used PET, but differences in methodology, such as the choice of the radioligand, and the clinical history of the patients might have contributed to these contrasting findings. For instance, the age of the patients at the onset of the disease could potentially be a factor affecting the expression of the DD1R. If the subjects had had schizophrenia for a longer period of time, or had an earlier onset of the disorder in the Abi-Dargham et al. (2012) study compared to the Okubo et al. (1997) study, this might explain why they had increased levels of DD1R, because the CNS would have compensated to the lack of dopamine binding by up-regulating the amount of DD1Rs. However, Abi-Dargham et al. (2012) did not provide information on the clinical history of their subjects, so it is difficult to ascertain what might have contributed to the mixed findings. Nevertheless, DD1R expression is altered in patients with schizophrenia.

In the striatum, Karlsson et al. (2009) found that cognitive processing reduced the radioligand binding in the DD1R in younger subjects, whereas older subjects had no alteration in the binding during a cognitive task. Because a reduction in radioligand binding indicates that there is more endogenous DA competing with the radioligand (Laruelle, 2000), this finding provides support for the notion that striatal DD1Rs are active during cognitive processing in younger subjects (Karlsson et al., 2009). Karlsson et al. (2011) also found strong relationships between specific areas of the striatum and specific cognitive functions. The DD1R binding in the sensorimotor striatum was correlated more strongly with speed, whereas the DD1R binding in the associative striatum was correlated more strongly with general knowledge. Also, Karlsson et al. (2011) found an association between DD1R binding in the hippocampus and executive functioning, speed and general

knowledge. Taken together, these findings suggest a differential role of the DD1R in cognition depending on the cognitive task and the brain area of interest (Karlsson et al., 2011). Also, Takahashi et al. (2010) found that striatal DD1R availability was correlated with decision-making ability in a gambling test in healthy subjects. Lower striatal DD1R activity was correlated with overestimation of low probabilities on the one hand, and underestimating high probabilities on the other hand (Takahashi et al., 2010). In other words, low striatal DD1R activity was related to risky behaviour.

Although it seems that the DD1R is implicated in cognition based on a number of human studies, there are also some limitations to these studies. As mentioned previously, the results of the studies can sometimes be mixed. Some of the mixed results can be accounted for by taken into consideration the use of different methodologies. Also, the radioligands that are commonly used are not entirely selective to DD1R (Takahashi et al., 2012). For instance, the radioligands [^{11}C]SCH23390 and [^{11}C]NNC112 bind to serotonin 2A receptors (5-HT_{2A}) with approximately 10 to 25 per cent affinity (Ekelund et al., 2007, Slifstein et al., 2007). They also bind to the DD5R (Undieh, 2010). Most importantly, all of the studies that can ethically be done with human subjects are based on statistical correlations. However, correlations only detect a relationship between phenomena. They cannot be used to conclude anything about causation. Therefore, other methods are needed to determine the cause and examine whether defective DD1R functioning causes cognitive impairments.

1.3.2 Pharmacological studies.

Various pharmacological studies have examined the role of the dopamine D1 receptor (DD1R) in cognition with the use of DD1R agonists and antagonists. Adding to the converging evidence from the human imaging studies on the role of DD1R in cognition, different pharmacological manipulations of the DD1R have different effects on cognition. The results depend on whether an agonist or an antagonist is used, and which brain area is targeted. However, mixed results can also be found depending on the chosen drug. Using rats, Seamans, Floresco and Phillips (1998) found that injecting the DD1R antagonist SCH 23390 in the medial prefrontal cortex (mPFC) increased the number of errors in a foraging task when the rats had previously learned the task and the drug was administered prior to the test. Yet these infusions had no effect when they were administered prior to the training phase, indicating that DD1R is important in the retrieval of previously encoded information (Seamans et al., 1998).

DD1Rs in the mPFC are also important for working memory (WM) dependent choice behaviour (Sawaguchi & Goldman-Rakic, 1994). Using DD1R antagonists prior to the task showed dose-dependent effects, so that higher doses lead to more impairment in a WM dependent choice

behaviour. Also, these effects were evident until approximately 40 minutes after the injections (Sawaguchi & Goldman-Rakic, 1994). In addition, using a DD1R agonist in the mPFC prior to retrieval can improve performance on a WM task (Floresco & Philips, 2001). More specifically, when WM was impaired by increasing the delay between the initial learning and the test, administration of the DD1R agonist lessened this impairment (Floresco & Philips, 2001). Also, when spatial WM deficits were induced by ketamine injections, administering A77636 (full DD1R agonist) or SKF38393 (partial DD1R agonist) reduced the impairment (Roberts et al., 2010).

An inverted U-shape relationship between DD1R activation and performance has been found in pharmacological studies (Williams & Goldman-Rakic, 1995, Zahrt, Taylor, Mathew & Arnsten, 1997). Zahrt et al. (1997) found that a DD1R agonist lead to impairments in a spatial WM task. Zahrt et al. (1997) found no effect of a DD1R antagonist on performance at low doses. Yet, at high doses, performance was impaired also with the antagonist. These results suggest that DD1R functioning needs to be in an optimal level, and too little or too much activation of the receptors leads to cognitive impairment. The inverted U-shape relationship between DD1R activity and cognitive performance can also be found on a more molecular level (Williams & Goldman-Rakic, 1995, Vijayraghavan, Wang, Birnbaum, Williams & Arnsten, 2007). Williams and Goldman-Rakic (1995) proposed the inverted U-hypothesis of dopamine signalling in the PFC after finding that moderate administration of the DD1R antagonist (SCH39166) resulted in increased firing of the pyramidal cells that was selective to a preferred response, whereas increasing the administration of the antagonist actually abolished this effect. Using rats and monkeys, Vijayraghavan et al. (2007) also found the inverted U-shape relationship in the PFC neurons after stimulating the DD1Rs. When DD1Rs were slightly stimulated by an agonist, responses to irrelevant stimuli in the working memory task were suppressed; leading to better performance. In contrast, when DD1Rs were stimulated with higher levels of agonists, there was a reduction in the delay-related firing of the PFC neurons to all stimuli, resulting in decreased performance.

The major limitation of all of the abovementioned pharmacological studies is that there are currently no drugs that selectively target the DD1R (Holmes et al., 2004, Undieh, 2010, Zhang, Xiong, Zhen & Zhang, 2009). According to Undieh (2010) current knowledge of the function of the DD1R also includes other D1-like receptors because completely selective DD1R drugs have yet to be invented. According to Zhang et al. (2009) the main reason behind the struggle to develop DD1R selective drugs is that the exact three-dimensional orientations of amino acid residues at the dopamine receptor binding sites are still unknown. Therefore, the pharmacological studies examining the role of the DD1R in cognition are actually examining the role of the D1-like receptors, which include both the D1 and the D5 receptors, and possibly other D1-like receptors that have yet to be identified (Undieh, 2010). In a review on DD1R knockout mice Holmes et al. (2004)

stated that the currently used ligands have at most 10-fold selectivity for DD1R compared to DD5R. This lack of selectivity limits the use of these ligands as research tools (Holmes et al., 2004). In sum, although these pharmacological studies add converging evidence of the importance of the DD1R in cognition, the involvement of other D1-like receptors cannot completely be ruled out in these experiments.

1.3.3 Genetic studies.

Some of the polymorphisms of the DD1R have been linked to psychiatric disorders and cognitive impairment. Potkin et al. (2003) examined the DD1R genotypes in treatment-resistant schizophrenic patients before and after clozapine treatment. The authors found that the 2,2 genotype was associated with metabolic decreases in most brain areas, whereas the 1,2 genotype showed no such effects. Also, the patients with the 2,2 genotype significantly improved with clozapine treatment, whereas the patients with the 1,2 genotype actually worsened with treatment (Potkin et al., 2003). Lane et al. (2008) found that the DD1R polymorphism A-48G is associated with the number of preservative errors in the Wisconsin Card sorting task (WCST). Also, a haplotype of the DD1R gene has been linked to alcohol dependence (Batel et al., 2008). Alcohol dependent subjects were more likely to have the T allele of the rs686 polymorphism in the DD1R gene. The severity of the dependency as well as the severity of withdrawal seizures was also linked to the T allele (Batel et al., 2008).

Finally, another haplotype of the DD1R (consisting of the rs265981-C, rs4532-A and rs686-T alleles, also known as C-A-T) has been linked to autism spectrum disorder (ASD) especially in males. The C-A-T haplotype is associated with typical ASD symptoms, such as severe social interaction deficits, impairments in non-verbal communication, and greater amount of stereotypic behaviours (Hettinger, Liu, Schwartz, Michaelis & Holden, 2008). In sum, there is some evidence from genetic studies suggesting a role of the DD1R in cognition. However, it is currently unclear what the functional consequences of the different haplotypes are. It is not known whether and how the different haplotypes lead to a dysfunction of the DD1Rs. Also, as in the case of the human imaging studies, the aforementioned genetic studies also rely on correlational evidence. Therefore, it is not possible to conclude whether polymorphisms in the DD1R gene are a cause of cognitive impairment without the use of animal models.

1.3.4 Rodent studies.

Because the human studies cannot ascertain causation and the pharmacological studies lack DD1R selective drugs, animal studies are necessary to further investigate the role of the DD1R on cognition. There are several techniques that can only be used with animals, including

pharmacological manipulations, lesion studies and gene targeting. One of these techniques includes lesioning a brain area (either anatomical lesioning or pharmacological ablation by using a toxin) and seeing how this affects behaviour. This method can be used to study the role of, for instance dopamine, in a specific brain region. However, the lesion method is not selective to specific receptors. Therefore, when studying the effect of the DD1R specifically, a second technique, gene targeting, is necessary. Mice have been the most popular animals for these genetic models because in order to genetically manipulate a specific gene of interest, embryonic stem cells are needed, and only mice have pluripotent (self-renewing) embryonic stem cells. These stem cells are used in the technique of homologous recombination to knockout a specific gene of interest (Capecchi, 1989).

Although homologous recombination has not been used in rats until recently (Ton, Li, Wu, Yan & Ying, 2010) other techniques, such as N-ethyl-N-nitrosourea-driven target selected mutagenesis (ENU-mutagenesis), can be employed (Zan et al., 2003). Because mice have pluripotent stem cells, they have been superior to rats in gene targeting studies. The techniques that could be used in rats (e.g. ENU-mutagenesis) were not able to target a specific gene of interest. Instead, the techniques used in rats often led to multiple knockouts or mutations and the location of these knockouts and mutations could not be predetermined. The logic behind both the homologous recombination and ENU-mutagenesis techniques is that depleting a gene that codes for a specific receptor will lead to the absence of that receptor. Therefore, any disruption in subsequent behaviour can be accounted for by the absence of that receptor. However, there are some caveats to consider. One of them is possible compensatory mechanisms that the developing brain adopts to overcome the absence of specific receptors (Undieh, 2010). Multiple neurotransmitters interact to form even simple behaviours. Thus, the lack of one receptor can affect many neurotransmitter systems. There are two genetic models that focus on the role of the DD1R; the DD1R knockout mice, and the DD1R mutant rats.

1.3.3.1 DD1R knockout mice.

The general characteristics of the DD1R knockout mice include a 20-25 per cent reduction in body weight compared to wildtype controls (Smith et al., 1998). Although moist food is necessary for the DD1R mice during weaning, they do not require special feeding after weaning to survive (Smith et al., 1998). The DD1R knockout mice have some abnormal brain development. Xu et al. (1994) found that the DD1R knockout mice have a 22 per cent reduction in striatal volume. Studies on the behaviour of the DD1R knockout mice have revealed that these mice also show some abnormal behaviour. DD1R knockout mice have abnormal motor functions, with increased locomotor activity, decreased orofacial movements and grooming, as well as impaired motor coordination (for a review see Holmes et al., 2004). However, some of the results on the motor

function of the DD1R knockout mice are mixed, and it depends on the task used whether these mice show an increase or a decrease in activity (for a review see Wall et al., 2011). The motor reflexes of the DD1R knockout mice appear normal (Drago et al., 1994, El-Ghundi et al., 1999).

The DD1R knockout mice have normal sucrose preference (Wall et al., 2011). El-Ghundi, O'Dowd, Erelik and George (2003) found no difference between DD1R knockout mice and wildtype controls in terms of sucrose preference when given free-choice, they also found that DRD1 knockout mice were unable to learn to press a lever for food, and concluded that these mice have an impaired motivation for working for a food reward. However, this conclusion is confounded by the fact that it is possible that the mice were unable to learn the association between the lever press and the food reward, due to the lack of DD1Rs which might be important in learning and memory.

In terms of spatial learning, the DD1R knockout mice seem to have a deficit in learning an egocentric strategy. The DD1R knockout mice performed poorly on an egocentric T-maze task where the mice have to rely on their own body coordinates to reach a reward (Wall et al., 2011). Similarly, DD1R knockout mice were slower to learn to use the egocentric strategy in a U-maze, were impaired in reversing between two strategies when needed, and were slower to change from an egocentric to an allocentric strategy compared to control mice (Wall et al., 2011). Also, in a Morris Water Maze (MWM) which requires the mice to swim in a circular maze to reach a platform, DD1R knockout mice show severe impairments, with longer escape latencies at acquisition than wildtype or DD1R heterozygous mice (El-Ghundi et al., 1999). Also, DD1R knockout mice were unable to learn the new location of a hidden platform when it was changed from previous trials. Also, when a probe trial was presented some days after the initial learning, the DD1R knockout mice did not distinguish between the previous location of the platform and the other areas in the maze. In contrast, the wildtype mice spent more time in the location where the platform had been previously (El-Ghundi et al., 1999).

These impairments are unlikely due to being stressed because of the water. Ortiz et al. (2010) used a Barnes maze, which is a dry circular maze with holes on the sides. One of the holes is the escape hole. Ortiz et al. (2010) found that the DD1R knockout mice have increased escape latencies and an inability to learn the new location of the escape hole, as well as an impaired long-term memory of the location of the escape hole (measured by a probe trial on day 14) compared to wildtype mice. In sum, the results on the spatial ability of the DD1R knockout mice suggest that the DD1R is important for egocentric learning, reversal learning and long-term memory. Allocentric learning might also be impaired, at least after the mice have learned an egocentric strategy previously.

Working memory has not been extensively examined in the DD1R knockout mice. Quite surprisingly, unlike in the human and pharmacological studies, DD1R knockout mice have similar performance as wildtype mice on a Y-maze task that measures spontaneous alternation, which is used as a measure of working memory (El-Ghundi et al., 1999). However, spontaneous alternation is a very simple task that could be argued to measure short-term memory more than working memory, because it requires memory for the previously visited arms and does not require the simultaneous maintenance of a lot of information in memory. Revisiting arms is considered a working memory error in this task. Due to the low number of arms in the Y-maze, this task might not be sensitive enough to detect subtle differences in working memory. There is some evidence to suggest that prefrontal functioning of the DD1R knockout mice is impaired (El-Ghundi et al., 1999, 2003). Also, DD1R knockout mice have abnormal LTP in the prefrontal cortex (Huang, Simpson, Kellendonk & Kandel, 2004). Further studies are required before conclusions can be made about the working memory capacity of the DD1R knockout mice.

Although the DD1R knockout mice provide a valuable tool in examining the role of the DD1R in cognition, there are also some limitations to consider. Due to the fact that these mice have a complete knockout of the DD1R, it is unclear what types of compensatory mechanisms are at work. For instance, Wang, Stanwood, Grandy and Deutch (2009) found that both DD1R and DD2R knockout mice have abnormal dendrites in the pyramidal cells in the prefrontal cortex. The authors hypothesised that these changes are likely due to abnormal functioning of the dopamine receptors in development, and argued that behavioural data from these mice must be interpreted with caution because there are many changes that can have occurred due to the lack of the dopamine receptors in development.

Furthermore, Kellendonk et al. (2006) found that transient overexpression of DD2Rs in the striatum activates the DD1Rs in the prefrontal cortex. This finding highlights the intertwined functioning of the different dopamine receptors and is a good reminder to keep caution when interpreting results from knockout studies. Another limitation is that compared to mice, rats are more sociable and complex in their behaviour, which makes them more suitable to use as a model for neurocognitive disorders (Baker, 2011). Rats are also used in behavioural tasks more often than mice. For instance, a search on Pubmed.com showed 147 results for set shifting studies on rats, whereas only 60 were done with mice (accessed 4.1.2013).

In conclusion, although there is a considerable amount of evidence from human, pharmacological and rodent studies suggesting that the DD1R has a role in cognition, it is still unclear whether the DD1R is necessary for normal cognition and if so, what specific aspects of cognition the receptor contributes to.

1.3.3.2 DD1R mutant rats.

The DD1R mutant rats are Wistar rats with a missense mutation in the DD1R (Smits et al., 2006). This mutation was established using the technique of ENU-mutagenesis. The DD1R rats have a single amino acid change in the second transmembrane domain. The thymine to adenine change occurred at position 1215 of the DD1R gene, which encodes a G-protein coupled receptor. The single amino acid change results in an isoleucine changing to a serine at position 116 in the DD1R protein (Smits et al., 2006). When examining the sequence alignment of the DD1R, the isoleucine is present in all species that have been investigated thus far (Ellenbroek, personal communication). Because all species that have been investigated have the isoleucine present in the same position, it is likely that a change in this amino acid can critically affect the functioning of the DD1R. In fact, some preliminary findings suggest that the DD1R is affected in these rats. Homberg et al. (unpublished data, cited in Müller et al., 2010) found a 50 per cent decrease in binding to the DD1R using autoradiography with [³H]SCH23390. Also, Homberg et al. (submitted, personal communication) found a significantly reduced cAMP response to SKF81297 as well as no behavioural response to a dopamine D1-like receptor antagonist SCH23390.

Preliminary cognitive tests on the DD1R mutant rats have revealed specific types of cognitive impairment. In the spatial version of the Morris water maze (MWM), the homozygous DD1R mutant rats showed a small but significant deficit in learning to find the hidden platform. When the task was changed so that the rats had to rely on egocentric learning the mutant rats performed extremely poorly and learning was almost non-existent (Youn & Ellenbroek, unpublished data, personal communication). These findings suggest that spatial cognition is impaired in the DD1R mutant rats. Moreover, the finding that egocentric learning is especially compromised in these rats supports the hypothesis of the role of the medial prefrontal cortex and the caudate nucleus in egocentric learning since the DD1Rs are especially abundant in these brain areas (Dubois et al., 1986, Levey et al. 1993).

1.4 The present study

Various human, pharmacological and rodent studies suggest that the DD1R is related to some aspects of cognition. Whether DD1R dysfunction is the cause of spatial and reversal learning deficits still remains unclear. The aim of the present research is to determine whether DD1Rs are important for spatial and reversal learning. DD1R mutant rats are hypothesised to show deficits in spatial learning compared to wildtype rats. Spatial learning will be measured with three different paradigms: the star-maze, the Y-maze and the T-maze. Reversal learning will be measured with the T-maze. The role of motivation will also be explored by using both positive and negative reinforcement. This research may have important implications for the potential future use of the D1

mutant rats as an animal model of cognitive deficits in psychiatric disorders, and the development of novel pharmacotherapies.

2 Pilot 1: Egocentric learning with negative reinforcement

As mentioned above in the chapter on learning, egocentric learning requires relying on one's own body coordinates (e.g. always turning left-right-left) to reach a target (O'Keefe & Nadel, 1978). The first pilot was conducted in order to determine the optimal experimental conditions for egocentric learning in the starmaze with negative reinforcement. The rats had to escape from a negative stimulus (water or ice). Previous research (Rondi-Reig et al., 2006) has found that mice were able to learn to use an egocentric strategy after 10 days of training. Therefore, it was hypothesised that the rats would improve over time by adopting an egocentric strategy to reach the target arm.

2.1 Materials and methods

2.1.1 Subjects.

Nine male Sprague-Dawley rats were used for the first pilots. The rats were between 3-5 months old and were housed in a temperature controlled room (18 degrees Celsius). The rats were housed three rats per cage in the experiment room. The light/dark cycle was reversed with lights on from 7pm to 7am. All rats were given food and water ad libitum. The experiments were conducted during the dark phase of the light/dark cycle. Animal care was according to the guidelines of the Victoria University of Wellington Animal Ethics Committee.

2.1.2 Apparatus.

The starmaze consists of a 5 arm maze with a central hub (see Figure 2) (Rondi-Reig et al. 2006). Each of the arms has a round opening on the right hand side, which can be covered with a sliding door that has small holes at the bottom. In addition, each arm has two doors, one at the end of the arm and one close to where the arm is connected to the central hub. A box was placed at the end of each of the arms, in front of the round opening. Clean bedding was added in each of the boxes. In four of the arms the access to the box at the end of the arm was prevented with the use of a sliding door. Only one arm was open for a rat to enter. Initially water, and later on ice was placed on the bottom of the maze so that it covered the floor of the entire maze.

Figure 2. The starmaze.

2.1.3 Handling and habituation

Rats were handled for three consecutive days. On first day of handling, rats were handled for 5 minutes in pairs. The two following days the rats were handled for 5 minutes each individually. After the three days of handling, the rats were habituated to the apparatus for three days prior to commencing the pilot. On the first day of habituation, the rats explored the apparatus three rats at a time for 10 minutes with access to one randomly chosen arm. On the second day of habituation, the rats explored the apparatus individually for 5 minutes. Again, the rats had access to one randomly chosen arm. Finally, on the third day of habituation water was added at the bottom of the maze and rats could explore the maze for 5 minutes each. For the first two days of the pilot the aversive stimulus was water. On day 3, the aversive stimulus was changed to ice.

2.1.4 Procedure.

Rats were taken from the corner of the experimental room where they were housed and put on a table in the middle of the room in their home cage. In order to teach rats to use an egocentric strategy, the start arm and the target arm changed for each trial. The body movements that a rat had to make to reach from the start to the target stayed the same. As an example, if a rat was placed in Arm 1, the rat would have to move left-right-left to reach the target (box with bedding) in Arm 3 (see Figure 3). On the next trial the same rat might start from Arm 2 and again would need to move left-right-left to reach the target, which this time would be in Arm 4. Each rat had two trials daily for 18 successive days. The time between trials was approximately 5-10 minutes depending on how long the previous rat took to complete the trial. To minimise olfactory cues, the bedding in the boxes was changed after a rat had entered the box, and the ice was moved around after each trial. The side where the rat turned (left-right-left or right-left-right) was counterbalanced across rats. The

order in which the rats were run was counterbalanced across days. The trials were recorded using the Ethovision tracking programme and a recording camera.

Figure 3. Egocentric strategy.

2.2 Results and discussion

We were interested in whether the pilot rats would be able to learn to use an egocentric strategy. We looked at the performance of the rats using several measures (total latency, primary latency, total number of arm visits, primary number of arm visits and total distance moved). Total latency was defined as the total time the rat explored the arena before entering the box. The rat was considered as having entered the box once the animal had placed the front paws inside the box. Total arm visits were defined as the total amount of arms the rat visited before entering the box. Primary latency was defined as the time the rat explored the arena before entering the target arm. The rat was considered as having entered the target arm once it went past the midpoint of the target arm. Primary arm visits was defined as the amount of arms the rat visited before it entered the target arm. We especially wanted to see whether the rats would have significantly lower scores on the last day compared to the first day of the pilot. During the trials when water was used as an aversive stimulus the rats did not enter the box when they first found it on 55% of the trials. Instead the rats continued exploring the maze for several minutes. Therefore we concluded that water was not aversive enough and we started using ice instead of water on Day 3. Thus the following data are from Day 3 to Day 18.

A one-way repeated measures analysis of variance (ANOVA) was conducted to examine whether the rats improved over time. However, the Levene's test was significant for all measures (total distance, total latency, total arm visits, primary arm visits and primary latency). This result indicates that the data violates the assumption of homogeneity. Therefore, Welch's test was used to examine the data. Welch's test revealed a significant effect of days on the total distance the rats moved in the arena, $F(15, 102.42)=2.14, p<0.05$. Planned comparisons revealed that the rats

moved a significantly shorter distance on Day 18 ($M=377.10$, $SE=64.72$) compared to Day 3 ($M=718.53$, $SE=128.24$), $t(17)=3.06$, $p<0.01$, $r=0.60$ (see Figure 4). This finding indicates that over time the rats learned to move a shorter distance before entering the target box.

Welch's test also revealed a significant effect of days on total latency, $F(15, 102.35)=2.12$, $p<0.05$. Planned comparisons revealed that the rats spent less time in the arena on Day 18 ($M=49.86$, $SE=10.81$) compared to Day 3 ($M=111.68$, $SE=24.77$), $t(17)=2.49$, $p<0.05$, $r=0.52$ (see Figure 5). This finding indicates that the rats learned to enter the target box faster over time. Welch's test also revealed a significant effect of days on primary latency, which measures the amount of time the rats took to find the box, $F(15,101.83)=3.96$, $p<0.01$. Planned comparisons revealed that the rats spent significantly less time finding the box on Day18 ($M=115.33$, $SE=24.09$) compared to Day3 ($M=39.00$, $SE=9.97$), $t(17)=3.30$, $p<0.01$, $r=0.62$ (see Figure 6). This finding indicates that the rats learned to reach the target faster over time.

Figure 4. Total distance moved in arena over 16 days of training. Error bars represent one standard error of the mean.

Figure 5. Total latency in arena over 16 days of training. Error bars represent one standard error of the mean.

Figure 6. Primary latency in arena over 16 days of training. Error bars represent one standard error of the mean.

Another measure that can be used to examine improvement over time is the number of arms that the rats visited in each trial. Arm visits were calculated starting with the start arm, and including the target arm. Therefore, if a rat used a perfect egocentric strategy they would visit two arms in total (start arm and target arm). Welch's test revealed a marginally significant effect of days on total arm visits, $F(15, 102.44)=1.71, p=0.06$. Planned comparisons revealed that the rats visited significantly less arms on Day 18 ($M=3.61, SE=0.37$) compared to Day 3 ($M=5.11, SE=0.64$), $t(17)=2.93, p<0.01, r=0.58$ (see Figure 7). This finding is similar to the finding from the total latency in that it shows that rats learned to enter the box faster over time.

Finally, a Welch's test revealed a significant effect of days on primary arm visits, $F(15, 102.40)=2.15$, $p<0.05$. Planned comparisons revealed that rats visited significantly less arms before finding the box on Day 18 ($M=3.39$, $SE=.31$) compared to Day 3 ($M=4.83$, $SE=.60$), $t(17)=3.63$, $p<.01$, $r=0.66$ (see Figure 8). This finding suggests that the rats visited fewer arms over time before finding the target box. This indicates that the rats learned the direction where the target box was. However, it does not show perfect egocentric learning because none of the rats were able to reach the target with just 2 arm visits.

Figure 7. Total number of arm visits over 16 days of training. Error bars represent one standard error of the mean.

Figure 8. Primary number of arm visits over 16 days of training. Error bars represent one standard error of the mean.

The results showed that the rats improved over time in all of the measures that were examined. The most important measures in terms of egocentric learning are the primary latency and the primary arm visits, because the other measures also take into account the exploratory behaviour of the rats. The rats improved quickly in primary latency and their performance plateaued after the first few trials. In terms of primary arm visits, the rats reached their best performance at approximately Day 9 and did not improve greatly after that.

Most importantly none of the rats acquired perfect egocentric learning (always turning left-right-left, or right-left-right, see Figure 3). Even on Day 18 rats still visited on average 3 to 4 arms. To be labelled as using an egocentric strategy the rats would have needed to visit just 2 arms on at least 3 out of the last 5 trials. Therefore the hypothesis was only partially supported. The rats did improve over time, but did not learn to use a perfect egocentric strategy. This finding is inconsistent with previous research (Rondi-Reig et al., 2006). After 10 days of training 80% the wildtype mice in the Rondi-Reig et al. (2006) study had acquired egocentric learning.

Some of the differences between the two studies might account for the different findings. First, we used rats, whereas the previous study (Rondi-Reig et al., 2006) was done with mice. Mice and rats often differ in behavioural tasks (Baker, 2011) and the starmaze was specifically designed for mice (Rondi-Reig et al., 2006). We could have altered the size of the starmaze to account for the bigger size of the rats. However, this would have made the maze too large to fit into the experimental room. The size of the maze might be a problem because the rats might not find the extra work of visiting three arms aversive enough to be motivated to learn the fastest route.

Second, the mice in the previous study (Rondi-Reig et al., 2006) did four trials per day whereas the rats in our study only did two trials per day. However, we tried with three trials a day as well, and there was no significant improvement compared to two trials (data not shown). This led us to conclude that the number of trials would not significantly alter our results. Third, we were unable to block all the external cues due to the nature of our tracking device. The tracking camera was located at the top of the ceiling and in order to track the rats it needed a light source from one side of the room. Therefore we could not cover the sides of the room with black blankets as was done in the previous study (Rondi-Reig et al., 2006). The presence of external cues can be detrimental to egocentric learning. In fact, even with humans, when external cues are increased people tend to rely on these more and switch to an allocentric strategy even when it is not the more efficient strategy to use (Byrne & Henriques, 2013).

Finally, the previous study was done with high levels of water which forced the animals to swim in the maze. The current study used low levels of ice which could have led to an issue of motivation. Although the rats were more likely to go into the box on the first visit to the target arm when there was ice in the maze compared to when there was water in the maze, there were still

approximately 22% of trials where the rats continued exploring the arena. Pilot 2a was conducted to explore whether the task is too hard for the rats, or whether the rats were not motivated enough to reach the target.

3 Pilot 2a: Egocentric learning with positive reinforcement

Pilot 2a was conducted to determine the optimal experimental conditions for egocentric learning with a positive reinforcement, where the rats would receive a food reward (coco pops) when they reached the target. Pilot 2a was also conducted to determine whether the inability to learn the egocentric task in Pilot 1 was due to lack of motivation, or whether the task is simply too difficult for the rats. Based on previous research (Rondi-Reig et al., 2006) it was hypothesised that rats would improve over time by adopting an egocentric strategy to perform the task.

3.1 Materials and methods

3.1.1 Subjects.

Nine male Sprague-Dawley rats were used for Pilot 2a. The rats were between 3-5 months old and were housed in a temperature controlled room (18 degrees Celsius). The rats were housed three rats per cage in the experiment room. The light/dark cycle was reversed with lights on from 7pm to 7am. All rats were given water ad libitum. In an initial version of the pilot it was found that rats would not be interested in the food reward unless they were food restricted (rats would not eat the coco pops in the box). Therefore food intake was restricted to 10 grams per rat per day for a week prior to the start of the experiment. Once rats reached 85 per cent of their ideal body weight they were fed 11-12 grams of food per rat per day. The experiment was conducted during the dark phase of the light/dark cycle. Animal care was according to the guidelines of the Victoria University of Wellington Animal Ethics Committee.

3.1.2 Apparatus.

The apparatus was the same as in Pilot 1 with two differences. First, there was no ice added to the maze and the floor of the maze was dry. Second, there was no bedding in the target box, instead there were 3 coco pops placed at the end of the target box as well as the other boxes to reduce olfactory cues.

3.1.3 Handling and habituation

The handling and habituation was the same as in Pilot 1 with three exceptions. Firstly, the rats were given coco pops prior to the commencement of the experiment in order to get the animals used to the new food. Second, the rats were food restricted, which meant that they were weighted

each day to monitor their weight loss. Third, water was not added on the bottom of the maze during habituation. Instead, coco pops were placed around the maze and in the boxes for the rats to consume freely.

3.1.4 Procedure.

The procedure was the same as in Pilot 1 with four exceptions. First, the weight of the rats was checked and the rats were fed 11 grams of food pellets per rat each day after the two trials. Second, the rats were given one coco pop each prior to the start of the trials each day to remind them what they were working for. Third, the pilot lasted 17 days. Fourth, after each trial the maze was cleaned with 75 per cent ethanol to reduce olfactory cues.

3.2 Results and discussion

We were interested in whether the pilot rats would be able to learn to use an egocentric strategy with a positive reinforcement. We looked at the primary latency and the primary number of arm visits because these were the measures that we determined to best show the learning of the rats. The Levene's test was significant for both measures, indicating that the data were not homogeneous. Therefore Welch's test was used to examine the data. Welch's test revealed a significant effect of days on primary latency, $F(16, 107.12)=10.04, p<0.01$. Planned comparisons showed that the rats were significantly faster to find the target on Day 17 ($M=17.28, SE=8.70$) compared to Day 1 ($M=43.94, SE=5.36$), $t(17)=2.54, p<0.05, r=0.52$ (see figure 9). Welch's test also revealed a marginally significant effect of days on primary number of arm visits, $F(16, 108.17)=1.72, p=.053$. Planned comparisons indicated that the rats visited significantly less arms on Day 17 ($M=3.56, SE=0.22$) compared to Day 1 ($M=4.72, SE=0.49$) before finding the target, $t(17)=2.15, p<0.05, r=0.46$ (see figure 10).

Figure 9. Primary latency over 17 days of training. Error bars represent one standard error of the mean.

Figure 10. Primary number of arm visits over 17 days of training. Error bars represent one standard error of the mean.

The rats improved significantly over time in both of the examined measures. However, the rats were still unable to acquire a perfect egocentric strategy (e.g. always turning left-right-left) because even on Day 17 the rats still visited 3 to 4 arms. There was no problem with motivation in this pilot because the amount of primary and total arm visits was the same (data not shown). This finding suggests that the rats are not able to learn to use a perfect egocentric strategy even with extensive training. The animals learn to perform faster and with fewer arm visits, but they do not learn the optimal route to the target. This finding is inconsistent with our hypothesis and previous

research (Rondi-Reig et al., 2006). It is likely that the differences between our study and the previous work done with the star maze (Rondi-Reig et al., 2006) which were mentioned in the discussion of Pilot 1 account for the fact that the rats were unable to learn the task whereas the mice learned it.

4 Pilot 2b: Allocentric learning with positive reinforcement

Allocentric learning is defined by relying on external cues to find a target (O'Keefe & Nadel, 1978). The target always stays the same from trial to trial, whereas the start position changes. Pilot 2a was conducted to determine the optimal experimental conditions for allocentric learning, when food reward is used as a positive reinforcement. Based on previous research (Rondi-Reig et al., 2006) it was hypothesised that the rats would improve over time by starting to use an allocentric strategy to complete the task.

4.1 Materials and methods

4.1.1 Animals.

The animals were the same as in Pilot 2a.

4.1.2 Apparatus.

The apparatus was the same as in Pilot 2a.

4.1.3 Handling and habituation

The rats were the same as in Pilot 2a, so they were not separately handled and habituated prior to Pilot 2b.

4.1.4 Procedure.

The procedure was the same as in Pilot 2a with four exceptions. First, the rats were given a break for a week during which the weight of the rats was checked and the rats were fed, but they did not enter the maze. During this week the rats were kept at 85 per cent of their initial body weight. Second, instead of rats always having to turn left-right-left (egocentric learning), rats had to learn to always go into the same target arm (allocentric learning). The start arm would change each trial but the target arm would always be the same (see Figure 11). The target arm position was counterbalanced across rats. Third, there were cues around the maze that the rats could use to find their target arm. There was a plastic green net between arms 2 and 3. There was a plastic red devil's fork between arms 1 and 2. And there was a plastic yellow sunflower between arms 1 and 5 (see Figure 11). Also, the lamp where the light came into the room was located between arms 5 and 4. Fourth, the pilot lasted for 16 days.

Figure 11. Allocentric learning with two trials for the same rat. The target is always Arm1 and the start arm changes across trials.

4.2 Results and discussion

We were interested in whether the rats would improve over time and start using an allocentric strategy to find the target. Levene's test was significant for both the primary latency and the primary number of arms visited. Welch's test revealed that there was no significant effect of days on primary latency, $F(15, 102.32)=0.93$, n.s. However, this result is likely due to the fact that the same rats that were used in Pilot 2a were also used in this pilot. Thus, the rats were already moving fast in the maze on Day1 (see Figure 12). Welch's test did however reveal a significant effect of days on primary number of arm visits, $F(15, 102.68)=2.09$, $p<0.05$. Planned comparisons revealed that the rats visited significantly less arms on Day 16 ($M=2.72$, $SE=0.29$) compared to Day 1 ($M=4.11$, $SE=0.36$) before finding the target, $t(17)=3.41$, $p<0.01$, $r=0.64$ (see Figure 13). Moreover, 5 out of the 9 rats acquired allocentric learning by immediately entering the target arm on three out of five successive trials on the last three days.

Figure 12. Primary latency over 16 days of training. Error bars represent one standard error of the mean.

Figure 13. Primary number of arm visits over 16 days of training. Error bars represent one standard error of the mean.

We found evidence to suggest that rats can learn the allocentric strategy over time.

Although there was no improvement in the primary latency, the rats significantly improved in primary number of arm visits and 5 rats acquired allocentric learning. This is consistent with our hypothesis. However, there is a possibility that the rats did not improve as well as they would have if they had not previously been trained with the egocentric task. In the chapter on learning we discussed that the hippocampus is important for allocentric learning, whereas egocentric learning relies more on activation of the basal ganglia and the medial prefrontal cortex (O'Keefe & Dostrovsky, 1971, O'Keefe, 1979, Potegal, 1972, Cook & Kesner 1988, de Bruin et al., 2001). The

involvement of the prefrontal cortex in egocentric learning suggests that this type of learning is harder than allocentric learning, which relies on more lower-level processes.

Also, it has been suggested by Yin and Knowlton (2006) that allocentric learning is similar to medial temporal lobe (MTL) dependent declarative learning in humans, whereas egocentric learning is similar to striatum dependent non-declarative learning. Declarative learning is explicit and flexible, just like allocentric learning. Non-declarative learning is similar to a habit; it is formed over extensive training, just like egocentric learning (Yin & Knowlton, 2006). Therefore, it could be hypothesised that once the animals have learned the harder task (egocentric), they would have difficulty to switch to another task (allocentric). Pilot 2c was conducted to examine whether allocentric learning is compromised by previous egocentric learning.

5 Pilot 2c: Allocentric learning with positive reinforcement using naïve rats

Pilot 2c was conducted simultaneously with Pilot 2b to determine whether rats would be hindered by having completed two weeks of egocentric learning prior to the allocentric task. It was hypothesised that naïve rats would be faster at learning the task compared to rats which had received egocentric training prior to the commencement of the task.

5.1 Method

5.1.1 Subjects.

Same as in Pilot 2a with one exception. In Pilot 2c consisted of 8 male Sprague-Dawley rats naïve to the starmaze.

5.1.2 Apparatus.

The apparatus was the same as in Pilot 2a.

5.1.3 Handling and habituation

The handling and habituation were the same as in Pilot 2a.

5.1.4 Procedure.

The procedure was the same as in Pilot 2b with two exceptions. First, the rats were naïve to the maze and had not done the egocentric learning prior to the start of the experiment. Second, Pilot 2c only lasted for 8 days.

5.2 Results and discussion

We were interested in seeing whether the naïve rats would improve over time faster than the rats that had already been learning an egocentric task prior to the start of the allocentric training.

An 8 (Days: 1-8) x 2 (Study: naïve rats vs. experienced rats) mixed analysis of variance (ANOVA) was used to examine the results for primary latency (see Figure 14). Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(27)=228.82$, $p<0.01$ therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.29$. The significant main effect of days, $F(2.03, 60.91)=6.62$, $p<0.01$, $\eta p^2=0.18$ and the significant main effect of study, $F(1,30)=34.95$, $p<0.01$, $\eta p^2=0.54$ were qualified by an interaction between days and study, $F(2.03, 60.91)=5.17$, $p<0.01$, $\eta p^2=0.15$.

On Day 1 the experienced rats ($M=10.89$, $SE=3.58$) were faster than the naïve rats ($M=72.29$, $SE=21.94$). Levene's test revealed that equal variances could not be assumed, so a corrected independent samples t-test revealed that the difference between the groups on Day 1 was significant, $t(13.69)=2.76$, $p<0.05$, $r=0.60$. Planned comparisons also revealed that on Day 8 there was no significant difference between the experienced rats ($M=6.78$, $SE=1.77$) and the naïve rats ($M=10.36$, $SE=2.49$), $t(30)=1.20$, *n.s.* This finding indicates that the rats get faster the more time they spend in the maze. Since the experienced rats had already received two trials daily for two weeks in the maze prior to the start of the allocentric experiment, they were already performing faster at the start of the experiment than the naïve rats. However, there was no difference between the two groups on Day 8 which suggests that both groups improve in a similar manner over time.

An 8 (Days: 1-8) x 2 (Study: naïve rats vs. experienced rats) mixed ANOVA was used to examine the results for primary arm visits (see Figure 15). Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(27)=68.28$, $p<0.01$ therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.56$. There was a significant main effect of days, $F(3.91, 117.31)=4.59$, $p<0.01$, $\eta p^2=0.13$. The effect of study was not significant, $F(1,30)=.001$, *n.s.* nor was the interaction term, $F(3.91, 117.31)=1.99$, *n.s.* Planned comparisons revealed that there were no significant differences between the two groups on Day 1, $t(17.70)=1.24$, *n.s.* or Day 8, $t(30)=1.48$, *n.s.* The findings suggest that the rats improved over time and the improvement was similar regardless of whether the rats were naïve or experienced.

Figure 14. Primary latency over 8 days of training for naïve and egocentrically trained rats. Error bars represent one standard error of the mean.

Figure 15. Primary number of arm visits over 8 days of training for naïve and egocentric trained rats. Error bars represent one standard error of the mean.

We found that the experienced rats did not significantly differ from the naïve rats in terms of their performance when looking at the primary arm visits. This finding does not support our hypothesis. However, these results are likely due to the fact that the experienced rats did not acquire egocentric learning in the first experiment. Therefore, they did not need to unlearn an egocentric response. Also, the primary arm visits is a less sensitive measure than the primary latency. Because there are only 5 arms to visit in total, there is less variability and it is harder to see improvement with this measure. In terms of primary latency the performance of the experienced rats was superior

to the performance of the naïve rats. This suggests that previous exposure to the maze will affect how fast the rats move in the maze. However, in terms of our experiments this finding is not problematic because we are interested in seeing whether the rats improve over time and we can use the primary arm visits as an alternative measure. Since we do not have to rely just on the primary latency data, the results of Pilot 2c suggest that we can use the same animals for the egocentric and the allocentric experiments if we do not have enough DD1R mutant rats.

6 General discussion for Pilots

We were interested in finding the optimal conditions for rats to learn to use the egocentric and allocentric strategies using positive and negative reinforcement in the starmaze. We found across four pilots that rats are able to learn a faster route, and visit fewer arms prior to reaching the target arm across several days of training. We found the use of ice in the maze to be more effective than the use of water in the negative reinforcement task. We also found that the rats needed to be food restricted in order for them to be motivated enough to learn the task in the positive reinforcement task. As expected, the allocentric task was easier to learn, with 5 out of 9 rats learning the task. In contrast, the egocentric task was too hard for the rats, and all of the animals continued to visit at least 3 arms on their last day of training. We also found evidence to suggest that we can use the same rats for the egocentric and allocentric experiments in case we do not have enough DD1R mutant rats to use for each experiment.

Although the pilot rats were unable to learn a perfect egocentric strategy, the animals improved over time in primary latency and primary arm visits. Also, this improvement was statistically significant, indicating that the rats were able to learn to at least go in the right direction, even if they did not learn the correct pattern of movements. Therefore we decided to run the experiments with the DD1R mutant and wildtype rats. The wildtype rats were expected to improve over time similarly to the pilot rats despite differences in the rat strain (the pilot rats were Spargue-Dawley rats and the wildtype rats were Wistar rats). However, the DD1R mutant rats might not show such an improvement. Therefore, if the DD1R is implicated in these tasks we should be able to detect a difference between the two groups of rats.

7 Experiment 1a: Egocentric learning with negative reinforcement

As has been discussed in the introduction, there is considerable evidence to suggest that the DD1R plays a role in egocentric learning. First, the DD1R is abundant in the brain areas that are involved in egocentric learning (Dubois et al., 1986, Levey et al. 1993). Second, the DD1R knockout mice show an impairment in egocentric tasks (El-Ghundi et al., 1999, Ortiz et al., 2010, Wall et al., 2011). Finally, preliminary data from the DD1R mutant rats suggests that these rats are

impaired in learning an egocentric strategy in the Morris Water Maze (Youn & Ellenbroek, unpublished data, personal communication). Based on previous research (Dubois et al., 1986, El-Ghundi et al., 1999, Levey et al. 1993, Ortiz et al., 2010, Wall et al., 2011) we hypothesised that the DD1R mutant rats would be impaired in learning the egocentric strategy in the starmaze when compared with the wildtype rats. More specifically, it was hypothesised that the DD1R mutant rats would not improve over time in terms of primary arm visits or primary latency.

7.1 Method

7.1.1 Subjects.

Dopamine D1 receptor mutant rats were developed by ENU-mutagenesis (Smits et al., 2006). DD1R mutants were derived from mating rats homozygous to the D1 mutation (+/+). Wildtypes were derived from mating littermates that lacked the mutation (-/-). A total of 28 male and female Wistar rats were used. Out of the 28 rats 14 were D1 mutants (10 males/4 females) and 14 were wildtypes (10 males/ 4 females). The rats were between 3-5 months old and were housed in a temperature controlled room (18 degrees Celsius). The rats were housed in pairs. The light/dark cycle was reversed with lights on from 7pm to 7am. The rats were housed in the experiment room. Genotype of rats was determined by Transnetyx (Cordova, TN, USA). All rats were given food and water ad libitum. The experiments were conducted during the dark phase of the light/dark cycle. Animal care was according to the guidelines of the Victoria University of Wellington Animal Ethics Committee.

7.1.2 Apparatus.

Same as in Pilot 1.

7.1.3 Handling and habituation.

Same as in Pilot 1 with one exception. Ice was added in the maze on day 3 of habituation.

7.1.4 Procedure.

Same as in Pilot 1 with one exception. Ice was used from the start as the aversive stimulus.

7.2 Results and discussion

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task in the starmaze compared to the wildtype rats in terms of primary latency (see Figure 16). First, the light grey line shows that the wildtype rats were getting faster in completing the task over the 16 day period. Second, the dark grey line suggests that there was no such improvement with the DD1R mutant rats. A 16 (Days: 1-16) x 2 (Genotype:

DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary latency. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(119)=658.83$, $p<0.01$ therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.30$. There was a significant main effect of genotype, $F(1, 54)=4.13$, $p<0.05$, $\eta p^2=0.07$. The effect of days was not significant, $F(4.49, 242.55)=.60$, *n.s.* nor was the interaction term, $F(4.49, 242.55)=1.15$, *n.s.*

Planned comparisons revealed that there was no significant difference between the two groups on Day 1, $t(54)=0.08$, *n.s.* On Day 16 the DD1R mutant rats ($M=43.61$, $SE=14.36$) took longer to reach the target than the wildtype rats ($M=14.68$, $SE=3.26$). This result was marginally significant, $t(29.78)=1.97$, $p=0.06$. Planned comparisons also revealed that the wildtype rats were significantly faster on Day 16 ($M=14.68$, $SE=3.26$) compared to Day1 ($M=28.82$, $SE=4.55$), $t(27)=2.62$, $p<0.05$, $r=0.45$. In contrast, the DD1R mutant rats did not significantly improve over time, $t(27)=1.03$, *n.s.* These findings give tentative support for the hypothesis that the DD1R mutant rats are impaired in learning the task.

Figure 16. Primary latency over 16 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task in the starmaze compared to the wildtype rats in terms of primary arm visits (see Figure 17). First, the light grey line shows that the wildtype rats were improving slightly over time. Second, the dark grey line suggests that there was no such

improvement with the DD1R mutant rats. A 16 (Days: 1-16) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary arm visits. There were no significant main effects and the interaction term was also not significant, all F s < 1.6, *n.s.* Planned comparisons revealed that there were no significant differences between the two groups on Day 1, $t(54)=0.66$, *n.s.* On Day 16 the DD1R mutant rats ($M=3.61$, $SE=0.30$) visited more arms than the wildtype rats ($M=3.18$, $SE=0.22$). This finding was marginally significant, $t(54)=1.16$, $p=0.06$. Planned comparisons also revealed that the wildtype rats visited significantly less arms on Day 16 ($M=3.18$, $SE=0.22$) compared to Day 1 ($M=4.18$, $SE=0.35$), $t(27)=2.32$, $p<0.05$, $r=0.41$. In contrast, the DD1R mutant rats did not significantly improve over time, $t(27)=0.83$, *n.s.* Although there was no great improvement over time for either of the groups, only the wildtype rats improved significantly from Day 1. This suggests that the DD1R mutant rats might be impaired to learn an egocentric task. However, none of the rats acquired a perfect egocentric strategy.

Figure 17. Primary arm visits over 16 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

We were interested in finding whether the DD1R mutant rats would be impaired in learning an egocentric task in the starmaze compared to the wildtypes. We found that unlike the wildtype rats, the DD1R mutants did not improve significantly in either of the measures that we examined. This finding is consistent with our hypothesis. The finding also replicates previous research using a different maze and a different negative reinforcement (Youn & Ellenbroek, unpublished data).

However, there are some limitations to these findings. The same problem that was encountered in the pilots is also evident for this experiment. The task is too hard even for the

wildtypes and none of the rats acquired a perfect egocentric strategy. The primary arm visits measure is not a very sensitive measure because there are a limited number of arms in the maze. And there is a problem of a floor effect, because even the wildtypes did not learn the task. Therefore, one possibility for the small but significant differences between the groups is that the wildtypes did not perform as well as they could have if the task had not been too hard. Another possibility is that there is only a subtle difference between the groups because the mutants still might have some functioning of the DD1R. As explained previously, the DD1R follows an inverted U-shape with optimal functioning requiring specific amount of stimulation of the receptor (Williams & Goldman-Rakic, 1995, Zahrt, Taylor, Mathew & Arnsten, 1997). The DD1R mutant rats might have suboptimal DD1R activity, but they might still have some functioning of the receptor. Therefore, the DD1R mutant rats might only show a slight cognitive impairment.

In sum, we found tentative evidence to suggest that the DD1R mutant rats are not able to learn an egocentric task even after extensive training. Due to the limitation of the floor effect, we wanted to explore the same task using the same rats with a slightly altered maze.

8 Experiment 1b: Egocentric learning with negative reinforcement in the Y-maze

Because there was no great improvement in Experiment 1a regarding primary arm visits, we decided to make the task easier for the rats and we ran the same task in a Y-maze. We were interested in seeing whether reducing the choice of arms to just two would improve the performance of the rats, and enable them to learn to learn a perfect egocentric strategy. We were also interested in whether the DD1R mutants would still be impaired in the task despite the fact that the task was made easier. Based on previous research (Levey et al. 1993, Dubois et al., 1986, Wall et al., 2011, El-Ghundi et al., 1999, Ortiz et al., 2010, Youn & Ellenbroek, unpublished data) we hypothesised that the DD1R mutant rats would not improve on the egocentric task, whereas the wildtypes would get better over time.

8.1 Method

8.1.1 Subjects.

Same as in Experiment 1a.

8.1.2 Apparatus.

Same as in Pilot 1 with one exception. Two of the arms of the apparatus were closed with black doors so that the apparatus now formed a Y-shape (see Figure 18). As in Experiment 1a the start arm changed across trials. Therefore, the blocked arms changed also. As an example, if the rat would start in arm 1 and would always need to go left-right-left, then arms 2 and 5 were blocked. In

this case, the target arm would be arm 3 (see Figure 19). Now on the second trial the same rat might start from arm 2 and arms 3 and 1 would be blocked. In this case the target arm would be arm 4 (see Figure 19).

Figure 18. The Y-maze.

Figure 19. Two examples of an egocentric strategy in the Y-maze.

8.1.3 Handling and habituation.

The rats were the same as in Experiment 1a, so they were not separately handled and habituated prior to the start of Experiment 1b.

8.1.4 Procedure.

The procedure was the same as in Experiment 1a with two exceptions. First, two of the arms of the maze were blocked prior to the start of a trial and the ice was only in the parts of the maze where the rats had access. Second, the experiment lasted 15 days.

8.2 Results and discussion

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task in the Y-maze compared to the wildtype rats in terms of primary latency (see Figure 20). First, as in Experiment 1a, the light grey line indicates that the wildtype rats were getting faster in completing the task over the 16 day period. Second, similarly to Experiment 1a, the dark grey line suggests that there was no such improvement with the DD1R mutant rats. A 15 (Days: 1-15) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary latency. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(104)=659.58$, $p<0.01$ therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.35$. There was a significant main effect of genotype, $F(1, 54)=17.22$, $p<0.05$, $\eta p^2=0.24$. The main effect of days was also significant, $F(4.92, 265.79)=3.09$, $p<0.05$, $\eta p^2=0.05$. The interaction term was not significant, $F(4.92, 265.79)=1.56$, *n.s.*

In the planned comparisons, the Levene's test revealed that equal variances could not be assumed, so a corrected independent samples t-test revealed that there was no significant difference between the two groups on Day 1, $t(32.61)=1.50$, *n.s.* On Day 15 the DD1R mutant rats ($M=25.93$, $SE=4.04$) took longer to reach the target than the wildtype rats ($M=6.61$, $SE=1.17$). This result was significant with the correction for unequal variances, $t(31.50)=4.60$, $p<0.01$, $r=0.63$. Planned comparisons also revealed that the wildtype rats were significantly faster on Day 15 ($M=6.45$, $SE=$) compared to Day 1 ($M=12.50$, $SE=2.31$), $t(27)=2.74$, $p<0.05$, $r=0.47$. In contrast, the DD1R mutant rats did not significantly improve over time, $t(27)=0.34$, *n.s.* The planned comparisons give tentative support for the hypothesis that the DD1R mutant rats are impaired in learning the task. The findings are also replicating what was found in Experiment 1a.

Figure 20. Primary latency over 15 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task in the Y-maze compared to the wildtype rats in terms of primary arm visits (see Figure 21). First, the light grey line indicates that the wildtype rats improved by visiting fewer arms over the 16 day period. Second, the dark grey line suggests that there was no such improvement with the DD1R mutant rats. A 15 (Days: 1-15) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was also used to examine the results for primary arm visits. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(104)=160.17$, $p<0.01$ therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\varepsilon=0.70$. There was a marginally significant main effect of genotype, $F(1, 54)=3.98$, $p=0.051$, $\eta p^2=0.07$. The main effect of days was not significant, $F(9.77, 527.68)=1.02$, $p<0.05$, *n.s.* nor was the interaction, $F(9.77, 527.68)=1.11$, *n.s.* Planned comparisons revealed that on Day 1 there was no significant difference between the two groups, $t(54)=1.62$, *n.s.* On Day 15 the DD1R mutant rats ($M=3.00$, $SE=0.22$) visited significantly more arms before finding the target than the wildtype rats ($M=2.36$, $SE=0.14$), $t(54)=2.49$, $p<0.05$, $r=0.32$. This replicates the finding from Experiment 1a.

Planned comparisons also revealed that the wildtype rats did not significantly improve from Day 1 to Day 15, $t(27)=1.06$, *n.s.* This finding was inconsistent with what was found in Experiment 1a. DD1R mutant rats also did not improve over time, $t(27)=0.00$, *n.s.* which is consistent with the results of Experiment 1a. Although the Y-maze was an easier task for the rats, many of them were still unable to learn the egocentric strategy. Approximately 65 per cent of the

wildtype rats learned to use the egocentric strategy, and only approximately 36 per cent of the DD1R mutant rats learned the task (see Figure 22). Although there is a difference between the DD1R mutants and the wildtypes when the rats are characterised as using either an egocentric or other strategy, the difference is not statistically significant, $\chi^2(1)=2.29$, *n.s.*

Figure 21. Primary arm visits over 15 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

Figure 22. Proportion of rats that used the egocentric strategy compared to another strategy for DD1R mutants and wildtypes.

We were interested in finding out whether the DD1R mutant rat would be impaired in learning the egocentric task in a Y-maze when compared with wildtype rats. The results from the primary latency data suggest that the DD1R mutants are impaired, because they were unable to improve over time. In contrast, the wildtype rats became significantly faster to reach the target over

time. This result replicates the findings from Experiment 1a. Also, there was a significant difference between the groups in primary arm visits on the last day of training. The wildtypes performed significantly better than the DD1R mutants. This finding also replicates the result from Experiment 1a. However, there was no improvement in the primary arm visits for wildtypes or DD1R mutants. The lack of improvement for the wildtypes might be due to the fact that this group started with visiting only 2.5 arms on average. With no errors they would have had a mean of 2 arms. Therefore, there appears to be a ceiling effect for the wildtype rats in Experiment 1b; they were not able to improve greatly from their first day performance because they were already performing quite well. In terms of egocentric learning, there was a trend indicating that the majority of the wildtypes learned the task, whereas the opposite was true to the DD1R mutants. However, this trend was not statistically significant. It is possible that our sample size was too small to detect a difference between the groups, especially if the impairment in the DD1R mutants is subtle.

In conclusion, we found some evidence to suggest that the DD1R mutant rats are unable to improve over time in the egocentric task. Although there are some limitations to the findings, we did replicate the results from Experiment 1a and a previous experiment by Youn and Ellenbroek (unpublished data, personal communication). Also, the finding is consistent with the vast amount of literature on the role of the DD1R in cognition (e.g. Abi-Dargham et al., 2011, El-Ghundi et al., 1999, Okubo et al., 1997, Roberts et al., 2010, Takahashi et al., 2008, Takahashi et al., 2012, Wall et al., 2011). Experiment 1c was conducted to explore whether a similar impairment would be evident using an allocentric task.

9 Experiment 1c: Allocentric learning with negative reinforcement

As discussed previously, it is easier to learn an allocentric task compared to an egocentric task (Yin & Knowlton, 2006). Also, previous research (Youn & Ellenbroek, unpublished data, personal communication) suggests that although the DD1R mutant rats take longer than the wildtype rats to perform an allocentric task in the Morris Water Maze, they are able to improve over time. Therefore, it is possible that the DD1R mutant rats will not be impaired in the allocentric task. However, the DD1Rs are abundant in the hippocampus (Levey et al., 1993, Ariano & Sibley, 1994, Bergson et al., 1995), and this brain area is involved in allocentric learning (O'Keefe & Dostrovsky, 1971, O'Keefe, 1979). Also, there is evidence from the DD1R mice literature that suggests that these mice are impaired in learning an allocentric task after having previously learned an egocentric task (Wall et al., 2011). Also, reversal learning seems to be impaired in the DD1R mice (Wall et al., 2011). Because the same rats were used for all the parts of Experiment 1, it was hypothesised that the DD1R mutant rats are impaired in learning this task. More specifically, it was hypothesised that

the DD1R mutant would not improve over time in primary latency or primary arm visits, whereas the wildtypes would improve over time.

9.1 Method

9.1.1 Subjects.

The animals were the same as in Experiments 1a and b.

9.1.2 Apparatus.

The apparatus was the same as in Pilot 2a.

9.1.3 Handling and habituation.

The rats were the same as in Experiment 1a and b, so they were not separately handled and habituated prior to the start of Experiment 1c.

9.1.4 Procedure.

The procedure was the same as in Pilot 2b with two exceptions. First, Experiment 1c lasted for 14 days. Second, ice was used in the maze as an aversive stimulus.

9.2 Results and discussion

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning allocentric task in the star maze compared to the wildtype rats in terms of primary latency. (see Figure 23). First, there was no improvement over time. Both the wildtypes and the mutants did not improve from Day1 to Day14. Second, the mutants seemed to be moving overall slower than the wildtypes. A 14 (Days: 1-14) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary latency. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(90)=279.24$, $p<0.01$ therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.53$. There was a significant main effect of genotype, $F(1, 54)=5.16$, $p<0.05$, $\eta p^2=0.09$. The main effect of days was not significant, $F(6.90, 272.48)=1.48$, *n.s.* The interaction term was also not significant, $F(6.90, 272.48)=1.19$, *n.s.* Furthermore none of the planned comparisons were significant. Regardless of the genotype, all the rats were unable to improve with the allocentric task.

Figure 23. Primary latency over 14 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning allocentric task in the starmaze compared to the wildtype rats in terms of primary arm visits. (see Figure 24). First, there appeared to be no improvement over time. Both the wildtypes and the mutants visited similar amount of arms on Day 1 and Day 14. Second, there seemed to be no difference between the mutants and the wildtypes. A 14 (Days: 1-14) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary arm visits. Neither of the main effects nor the interaction was significant, all $F < 1.1$, *n.s.* Also, none of the planned comparisons were significant. Regardless of the genotype, all rats were unable to learn the task by visiting fewer arms. In fact, none of the rats acquired the allocentric strategy which was defined as visiting only 2 arms on 3 of the 5 final trials. Taken together, the results of Experiment 1c suggest that all the rats were unable to learn the allocentric strategy.

Figure 24. Primary arm visits over 14 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

We were interested in whether the DD1R mutant rats would be impaired in the allocentric task using a negative reinforcement in the starmaze. The results suggest that all the rats regardless of genotype were unable to learn the task. There was no improvement in terms of primary latency or primary arm visits for either of the groups. This is inconsistent with our hypothesis, as well as our findings from Pilot 2. In Pilot 2 the same rats first received egocentric training, then a one week break, followed by allocentric training. The pilot rats were able to improve in the allocentric task regardless of the previous egocentric training.

So why did the rats in Experiment 1c fail to improve? There are three differences between Experiment 1 and Pilot 2. First, the rats in Experiment 1c had first received 31 days of egocentric training in two different versions of the maze (starmaze and Y-maze). This is a much longer training period than the 17 days that the pilot rats received. Second, some of the rats in Experiment 1c had actually acquired egocentric learning in Experiment 1b. Therefore, it is possible that the rats were unable to switch to allocentric strategy after having learned the egocentric strategy (Yin & Knowlton, 2006). Third, Experiment 1c used negative reinforcement, whereas Pilot 2 used positive reinforcement. It is possible that rats are more motivated to learn the task with positive reinforcement.

The results of Experiment 1c also suggest that we should be careful when interpreting the latency data. Even though neither of the groups improved over time, the DD1R mutants took longer to reach the target across days. Also, although the primary latency data suggests that the DD1R mutants are performing worse than the wildtypes, there was no difference in the primary arm visits.

It is possible that the DD1R compromises the ability of the animals to move in the maze. Dopamine is heavily implicated in movement (Phillips et al., 2008). In fact, Müller, Olivier & Homberg (2010) suggested that caution should be taken when interpreting latency data of these animals because they found differences between the male DD1R mutant rats and the male wildtype rats in various tests that measure locomotor activity.

In sum, we found that none of the rats were able to learn the allocentric strategy after extensive egocentric training. Although the DD1R mutants took longer to reach the target overall, the general lack of improvement for both groups in terms of primary latency and primary arm visits prohibits us from making any conclusions about whether the DD1R is important for this task. Future research should investigate the role of the DD1R in allocentric learning by using rats that are naïve to the behavioural tests.

10 Experiment 2a: Egocentric learning with positive reinforcement

Although we have found some evidence to suggest that the DD1R mutant rats are impaired in learning an egocentric strategy in the starmaze, the previous experiments were done using negative reinforcement. We had some concerns from the pilot that the negative reinforcement (ice) was not aversive enough. The rats were not always motivated to enter the box once they found the target arm, and instead kept exploring the maze. Therefore, we conducted the pilot also with a positive reinforcement (food reward). The same concerns we had for the Pilot 1 apply for Experiment 1a and 1b. The total arm visits and primary arm visits were not the same, because some of the rats continued exploring the maze even after they found the box (data not shown). Therefore, we conducted the same experiment with a positive reinforcement to explore whether a lack of motivation was behind the inability of the rats to learn. Based on previous research (Levey et al. 1993, Dubois et al., 1986, Wall et al., 2011, El-Ghundi et al., 1999, Ortiz et al., 2010) we hypothesised that the DD1R mutant rats would be impaired in learning to use an egocentric strategy when compared to the wildtype rats. More specifically, we predicted that the DD1R mutant rats would not improve over time in terms of primary latency or primary arm visits, whereas the wildtype rats would improve.

10.1 Method

10.1.1 Subjects.

A total of 18 male Wistar rats were used for Experiment 2a. Out of the 18 rats 9 were DD1R mutants and 9 were wildtypes. The rats were between 3-5 months old and were housed in a temperature controlled room (18 degrees Celsius). The genotype of the rats was determined in the same manner as in Experiment 1a. Also, the rats were housed in the same manner as in Experiment

1a. All rats were given water ad libitum. The rats were food restricted for a week prior to the start of the experiment until they reached 85 per cent of their normal body weight. The rats were given 10 grams of food per rat per day. After 85 per cent of the body weight had been reached the rats were fed 12 grams per rat per day. The rats were also fed coco pops daily. The experiments were conducted during the dark phase of the light/dark cycle. Animal care was according to the guidelines of the Victoria University of Wellington Animal Ethics Committee.

10.1.2 Apparatus.

The apparatus was the same as in Pilot 2a.

10.1.3 Handling and habituation.

The handling and habituation were the same as in Pilot 1.

10.1.4 Procedure.

The procedure was the same as in Pilot 2a with one exception. The experiment lasted 16 days.

10.2 Results and discussion

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task using a positive reinforcement compared to the wildtype rats in terms of primary latency, (see Figure 25). First, the light grey line indicates that the wildtypes started with a longer latency but improved greatly over time. Second, the dark grey line suggests that the mutants were faster at the start of the experiment and also seemed to improve slightly over time. A 16 (Days: 1-16) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary latency. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(90)=968.56$, $p<0.01$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.20$. The effect of genotype was not significant, $F(1, 34)=2.43$, *n.s.* The main effect of days was significant, $F(3.03, 102.96)=6.69$, $p<0.01$, $\eta p^2=0.16$. The interaction term was not significant, $F(3.03, 102.96)=2.23$, *n.s.*

Planned comparisons revealed no significant differences between the two groups on Day 1, $t(34)=1.39$, *n.s.* On Day 16 the DD1R mutant rats ($M=8.94$, $SE=1.88$) took longer to reach the target than the wildtype rats ($M=4.89$, $SE=0.59$). This result was marginally significant with the correction for unequal variances, $t(20.31)=2.06$, $p=0.05$, $r=0.42$. Planned comparisons also revealed that the wildtype rats were significantly faster on Day 16 ($M=4.89$, $SE=0.59$) compared to

Day 1 ($M=77.56$, $SE=24.32$), $t(17)=2.99$, $p<0.01$, $r=0.59$. In contrast, the DD1R mutant rats did not significantly improve over time, $t(17)=1.77$, *n.s.*

Figure 25. Primary latency over 16 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task using a positive reinforcement compared to the wildtype rats in terms of primary arm visits, (see Figure 26). First, the light grey line shows that the wildtypes started with more arm visits and seemed to improve slightly over time. Second, the dark grey line shows that the mutants visited fewer arms at the start of the experiment and did not seem to improve much over time. A 16 (Days: 1-16) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary arm visits (see figure X). Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(119)=155.94$, $p<0.05$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.63$. Neither of the main effects nor the interaction was significant, all $F_s<1.60$, *n.s.*

None of the planned comparisons were significant either. The means for wildtypes and DD1R mutants suggest a similar pattern of results as in Experiment 1a but this finding did not reach significance. Although the wildtype rats were able to improve over time by performing the task faster, they did not significantly improve in terms of arm visits. Also, the mutant rats did not improve over time in either of the measures. Furthermore, none of the rats acquired the egocentric strategy.

Figure 26. Primary arm visits over 16 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

We were interested in whether the wildtype rats would perform better than the DD1R mutant rats in the egocentric version of the star maze using a positive reinforcement. We replicated some of our previous results with primary latency data. We found that the DD1R mutant rats did not improve significantly over time, whereas the wildtypes did. However, unlike in our previous experiments, we only found a marginally significant difference between the wildtypes and the DD1R mutants on the last day of training. The results might only be marginally significant because we had a smaller sample in Experiment 2 compared to Experiment 1. The smaller sample size might not give enough power to detect differences. The problem with the small sample size is also a possible reason for the lack of significant findings in terms of primary arm visits. Although the pattern of result was the same as in Experiment 1a, none of the comparisons were significant.

In sum, the wildtype rats were getting faster but did not visit fewer arms, whereas the DD1R mutants did not improve in either measure. One possible explanation for the lack of improvement in primary arm visits is a phenomenon called superstitious behaviour (Timberlake & Lucas, 1985). If a rat previously turned left and visited 3 arms before reaching the target and then did the same pattern on subsequent trials they would be considered as performing superstitious behaviour because they are repeating the exact pattern of movement that they were rewarded for on the previous trial. At least 50 per cent of both wildtypes and mutants engaged in this superstitious behaviour (data not shown). Therefore, an idea for future research would be to somehow make the incorrect responses more costly to the rat. For instance, an automatic door could be used that closes if the rat did the wrong pattern of movement before the rat reaches their reward.

Further caveats to this study come from the involvement of the dopamine system in reward related behaviours (Phillips et al., 2008). Previous research with both DD1R knockout mice (El-Ghundi et al., 2003) and DD1R mutant rats (Squire, Harper & Ellenbroek, unpublished data, personal communication) has shown that an impairment of the DD1R leads to an inability to learn to press a lever for food. Therefore, it is possible that the DD1R rats are unmotivated to work for a food reward. However, our results suggest otherwise. If the DD1R mutant rats were impaired in this task due to a lack of motivation to work for food, we should have seen a difference between the wildtype rats and the DD1R mutant rats in primary and total arm visits. More specifically, if the DD1R mutants were not motivated to work for food, they should not have entered the box to consume the coco pops when they first encountered the box. Instead, they should have continued exploring the maze. This would have resulted in a higher amount of total arm visits for the DD1R mutants compared to the wildtypes, because the total arm visits takes into account exploratory behaviour. However, the total arm visit data and the primary arm visit data was identical for both groups of rats (data not shown). Therefore, the DD1R mutant rats seem as motivated to work for food as the wildtype rats.

In sum, we found some evidence to suggest a subtle impairment in the DD1R mutant rats to learn an egocentric strategy in the starmaze using a positive reinforcement when compared with the wildtype rats. However, this result should be replicated with a bigger sample size to see whether the differences observed between the groups are in fact statistically significant.

11 Experiment 2b: Allocentric learning with positive reinforcement

As mentioned in Experiment 1c, previous literature has shown that the DD1R mutant mice are impaired in learning an allocentric task after having previously learned an egocentric task (Wall et al., 2011). Also, reversal learning seems to be impaired in the DD1R mice (Wall et al., 2011). We used the same rats in Experiment 2b as we did in Experiment 2a. Therefore, it was hypothesised that the DD1R mutant rats will be impaired in learning this task. More specifically, it was hypothesised that the DD1R mutant rats would not improve over time in primary latency or primary arm visits, whereas the wildtypes would improve over time.

11.1 Method

11.1.1 Subjects.

The subjects were the same as in Experiment 2a.

11.1.2 Apparatus.

The apparatus was the same as in Pilot 2a.

11.1.3 Handling and habituation.

The rats were the same as in Experiment 2a, so they were not separately handled and habituated prior to Experiment 2b.

11.1.4 Procedure.

The procedure was the same as in Pilot 2b with one exception. Experiment 2b lasted for 14 days.

11.2 Results and discussion

There are two main findings that help us answer the question of whether the DD1R mutant rats were impaired in learning the egocentric task using a positive reinforcement compared to the wildtype rats using primary latency as a measure, (see Figure 27). First, the light grey line shows that the wildtypes seemed to improve consistently over time. Second, the dark grey line shows that the mutants also seemed to improve over time but to a lesser extent than the wildtypes. A 14 (Days: 1-14) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary latency. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(90)=239.90$, $p<0.01$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.45$. The effect of genotype was not significant, $F(1, 34)=3.11$, *n.s.* The main effect of days was significant, $F(5.91, 200.80)=3.09$, $p<0.01$, $\eta p^2=0.08$. The interaction term was not significant, $F(5.91, 200.80)=0.51$, *n.s.*

Planned comparisons revealed no significant difference between the two groups on Day 1, $t(34)=0.86$, *n.s.* On Day 14 the DD1R mutant rats ($M=5.56$, $SE=0.89$) took longer to reach the target than the wildtype rats ($M=3.06$, $SE=0.46$). This result was significant with the correction for unequal variances, $t(25.50)=2.49$, $p<0.05$, $r=0.44$. Planned comparisons also revealed that the wildtype rats were significantly faster on Day 14 ($M=3.06$, $SE=0.46$) compared to Day 1 ($M=7.39$, $SE=1.43$), $t(17)=2.84$, $p<0.05$, $r=0.57$. In contrast, the DD1R mutant rats did not significantly improve over time, $t(17)=1.80$, *n.s.* This finding suggests that the DD1R mutants are impaired in improving in the allocentric task in terms of primary latency after having received egocentric training compared to the wildtype rats.

Figure 27. Primary latency over 14 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

The results for primary arm visits is very similar to the primary latency data (see Figure 28). First, the light grey line shows that the wildtypes seemed to improve consistently over time. Second, the dark grey line shows that the mutants also seemed to improve over time but to a lesser extent than the wildtypes. A 14 (Days: 1-14) x 2 (Genotype: DD1R mutant vs. wildtype rats) mixed ANOVA was used to examine the results for primary arm visits. Mauchly's test for sphericity indicated that the assumption of sphericity had been violated, $\chi^2(90)=117.01$, $p<0.05$, therefore the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, $\epsilon=0.56$. The effect of genotype was not significant, $F(1, 34)=0.31$, *n.s.* The main effect of days was significant, $F(7.24, 245.99)=3.02$, $p<0.01$, $\eta p^2=0.08$. The interaction term was not significant, $F(7.24, 245.99)=0.78$, *n.s.* Planned comparisons revealed no significant difference between the two groups on Day 1, $t(34)=0.27$, *n.s.* or Day 14, $t(34)=1.12$, *n.s.* Planned comparisons also revealed that the wildtype rats visited significantly less primary arms on Day 14 ($M=2.83$, $SE=0.26$) compared to Day 1 ($M=3.89$, $SE=0.41$), $t(17)=2.16$, $p<0.05$, $r=0.46$. In contrast, the DD1R mutant rats did not significantly improve over time, $t(17)=1.25$, *n.s.* Although there were more wildtype rats who learned the allocentric strategy (see Figure 29), this finding was not statistically significant with the Yates' correction for small sample sizes, $\chi^2(1)=0.94$, *n.s.*

Figure 28. Primary arm visits over 14 days of training for DD1R mutant and wildtype rats. Error bars represent one standard error of the mean.

Figure 29. Proportion of allocentric strategy used compared to any other strategy for DD1R mutant and wildtype rats.

We were interested in the potential impairment of the DD1R mutant rats in the allocentric version of the star maze using a positive reinforcement when compared to wildtype rats. Our results suggest that there is a potential impairment. The primary latency data replicated what we had found in the previous experiments with egocentric training. The wildtypes improved over time, whereas the DD1R mutants did not. The primary arm visit data had a similar pattern, the wildtypes improved over time, whereas the DD1R mutants did not. However, there was no difference between the groups on the last day of training. It is possible that our small sample size again prohibited us from finding a statistically significant result for the last day of training, because the pattern of results for

the means of the two groups was the same as for the primary latency data; the wildtypes visited fewer arms than the DD1R mutants. Also, there were more wildtype rats that acquired allocentric learning, although the difference was not significant. In sum, we found a similar pattern of results for the allocentric task with positive reinforcement that we had found previously for the egocentric task using both positive and negative reinforcement. This suggests that the DD1R mutant rats could have a subtle impairment in learning the allocentric task after they have received egocentric training. Moreover, this impairment is not evident in the wildtype rats.

12 Experiment 3: Allocentric, egocentric, and reversal learning in the T-maze

The current knowledge of allocentric and egocentric learning suggests that these two types of learning exist in parallel (Retailleau et al., 2011). In fact, these two types of learning depend on separate brain areas; the hippocampus is important for allocentric learning whereas the basal ganglia are important for egocentric learning (Yin & Knowlton, 2006). Therefore, it is reasonable to expect these two types of learning to compete at different stages of learning. Previous research using the cross-maze has found that the use of an allocentric strategy is more common at the initial stages of learning, whereas an egocentric strategy is adopted after extensive training (Packard, 1999, Packard & McGaugh, 1996). More specifically, the authors used a training procedure where one arm (east or west) was baited with a food reward. After four trials a day for a week, a probe trial was conducted. The probe trial involved changing the start arm, so that if the start had been the south arm during training, on the probe trial the start arm would be the north arm. This procedure enabled the authors to characterise the rats as allocentric or egocentric learners. If the baited arm was the west arm, and if the rats on the probe trial turned to the west arm, they would be characterised as allocentric learners because they were going into the same place as previously. However, if the rats turned left, they would be characterised as egocentric learners because they were relying on their own body coordinates to reach the target (Packard, 1999, Packard & McGaugh, 1996).

Reversal learning can also be examined using the T-maze. After two weeks of training, a reversal protocol can be employed whereby the baited arm is changed. As mentioned previously, the ventral prefrontal cortex and the ventral striatum are important for reversal learning (for a review see Clark, Cools & Robbins, 2004). The DD1Rs are prevalent in these brain areas (Ariano & Sibley, 1994, Bergson et al., 1995, Dubois et al., 1986, Hall et al., 1994, Levey et al., 1993, Lidow et al., 1991).

Based on previous research (Packard, 1999, Packard & McGaugh, 1996) it was hypothesised that at the initial probe trial more rats would be categorised as allocentric learning. After two weeks of training it was hypothesised that there would be a shift to egocentric learning.

Previous research on the learning ability of the DD1R knockout mice suggests that the receptor is important for both egocentric learning in the T-maze as well as reversal learning (Wall et al., 2011). Therefore, it was hypothesised that the DD1R mutant rats would be more likely to use the allocentric strategy on both of the probe trials than the wildtype rats. It was also hypothesised that the DD1R mutant rats would be impaired in reversing a previously learned response.

12.1 Method

12.1.1 Subjects.

The subjects were the same as in Experiment 2.

12.1.2 Apparatus.

The apparatus was a simple T-shaped maze (see Figure 30). The height of the walls on the sides of the maze was 20 cm. The stem of the maze was a metallic colour, whereas the top of the maze was black. The stem of the maze was the start arm. A small plastic cup was placed at the end of the two arms at the top of the maze. The baited arm had the small cup filled with coco pops. In order to avoid olfactory cues, the unbaited arm also had the cup filled with coco pops but the cup was placed upside down, so that the rats could not access the food. Small holes were made on the cup so that the rats were still able to smell the coco pops. There were two environmental cues around the maze. There was a plastic sunflower 25 centimetres from north-west side of the maze (Cue1 in Figure 30). There was a green net 25 centimetres from the north-east of the maze (Cue2 in Figure 30).

Figure 30. The T-maze during the training phase.

12.1.3 Handling and habituation.

The rats were the same as in Experiment 2, so they were not separately handled prior to Experiment 3. The rats were habituated to the new maze for two days. On Day 1 rats were placed in the maze in pairs and allowed to explore the maze for 5 minutes. On Day 2 rats were placed in the maze individually for 5 minutes.

12.1.4 Procedure.

The procedure was adopted from (Packard & McGaugh, 1996). Briefly, the rats were placed at the start arm (stem of the maze) and whether they turned to the baited or the unbaited arm was recorded. If the rat turned to the baited arm, the rat was allowed 5 seconds to feed on the coco pops. If the rat turned to the unbaited arm a correcting procedure was followed whereby the rat was gently guided from the start arm to the baited arm. The correction procedure was only done on Day 1. After each trial the rats were removed from the maze for 30 seconds during which the maze was cleaned with 75% ethanol liquid to erase olfactory cues. All rats received 4 successive trials per day. A trial was recorded as correct if the rat first turned to the baited arm. Whether the baited arm was on the left or the right of the maze was counterbalanced across rats. This training protocol continued for 7 days.

On Day 8, the rats received a probe trial. The maze was turned 180 degrees, so that the start arm was now on the north and the target arms in the south. The cues were kept on the same sides, so that Cue 1 was placed west from the maze and Cue 2 east from the maze (see Figure 31). During the probe trials neither of the arms was baited. A plastic cup was placed at the end of both arms but no coco pops were placed in the cups. On the probe trial rats were categorised as either allocentric (always going towards the environmental cue) or egocentric (always turning left or right) learners. After the probe trial the rats received 7 training days identical to the first training days. On Day 16 another probe trial was performed in the same manner as on Day 8. Finally, for the last 5 days of the experiment the rats had to unlearn their previous response. If the baited arm had been on the east, now the baited arm was on the west (and vice versa).

Figure 31. The T-maze during the probe trial.

12.2 Results and discussion

We were interested in whether the DD1R mutant rats would be impaired in egocentric and reversal learning in the T-maze. We first examine the egocentric learning ability of the DD1R rats compared to the wildtype rats. There are three important findings to help us answer the question of whether the DD1R mutant rats are impaired in egocentric learning. First, there was no difference between the two groups in their ability to learn the location of the baited arm (see Figure 32). This finding is important because it suggests that the DD1R mutant rats were motivated to work for the food reward. Second, on the first probe trial there was no differentiation between allocentric and egocentric learning; there were approximately the same amount of both learners (see Figure 33). Also, there was no difference between the wildtype and the DD1R mutant rats in the proportion of egocentric and allocentric learners. Finally, on the last probe trial there were more egocentric learners compared to allocentric learners, but again there was no difference between the wildtypes and the DD1R mutants (see Figure 34). This finding suggests that the DD1R mutant rats are not impaired in egocentric learning in the T-maze.

Figure 32. The proportion of correct responses for DD1R mutant and wildtype rats over 15 days of training. Note that day 8 is missing since it was a probe trial day.

Figure 33. The proportion of DD1R mutant and wildtype rats that were characterised as allocentric and egocentric learners on the first probe trial on Day 8.

Figure 34. The proportion of DD1R mutant and wildtype rats that were characterised as allocentric and egocentric learners on the second probe trial on Day 16.

We were also interested in the ability of the DD1R to reverse a previously learned response. In order to examine this we looked at the five days of reversal training that the rats received after the two week training period. Figure 35 shows two important findings that help us answer the question of whether the DD1R mutant rats are impaired in reversal learning. First, both the light and the dark grey bars get higher gradually over time, indicating that all the rats learn the new rule. Second, the light grey bar is higher than the dark grey bar, indicating that the wildtype rats acquired the task faster than the DD1R mutants. A 5 (Days:1-5) x 2 (Genotype: DD1R mutant, wildtype) mixed ANOVA using the proportion of correct responses of each rat per day revealed a significant main effect of day $F(4, 64)=27.12$, $p<0.01$, $\eta p^2=0.63$. The main effect of genotype was also significant, $F(1,16)=5.30$, $p<0.05$, $\eta p^2=0.25$. The interaction was not significant, $F(4,64)=1.72$, $p=0.16$. Also, planned comparisons revealed that the wildtypes ($M=0.92$) were performing significantly better than the DD1R mutants ($M=0.72$) on Day 3, $t(16)=2.21$, $p<0.05$. There was also a marginally significant difference between the wildtypes ($M=0.89$) and the DD1R mutants ($M=0.69$) on Day 4, $t(16)=2.11$, $p=0.05$. This suggests that although the DD1R mutant rats are able to learn the new rule, it took them significantly longer to learn it compared to the wildtypes.

Figure 35. The proportion of correct responses for DD1R mutant and wildtype rats over 5 days of reversal training.

We were interested in whether the DD1R mutant rats are impaired in egocentric learning in the T-maze. Also, we wanted to know whether these rats would perform worse than wildtype rats in a reversal task after two weeks of training to turn a certain direction. We found that approximately 55 per cent of the rats were using an egocentric strategy already on the first probe trial. We also found that the DD1R mutant rats were able to use the egocentric strategy as often as the wildtype rats on both probe trials. Furthermore we found that the DD1R mutant rats had a slight but significant impairment in the reversal task using the T-maze.

The first finding was inconsistent with previous research. The most common finding with the T-maze is that on the first probe trial the animals mainly rely on an allocentric strategy, and on the second probe trial they switch to an egocentric strategy (Packard & McGaugh, 1996, Packard, 1999). One possible explanation for this discrepancy in findings is the strain of rat that is used in different studies. Espina-Marchant, Pinto-Hamuy, Bustamante, Morales and Herrera-Marschitz (2009) found that different strains of rats had a different distribution of allocentric and egocentric strategies on the first probe trial (which they performed on Day 11). More specifically, the majority of the A x C rats which are phenotypically similar to wild rats used an egocentric strategy on the first probe trial, whereas from the Long-Evans rats mainly used an allocentric strategy. In the Packard (1999) study the rats were also Long-Evans rats. In the Packard & McGaugh (1996) study the rats were Sprague-Dawley rats. The present study used Wistar rats. It would be interesting to compare the performance of Sprague-Dawley and Wistar rats to determine whether the strain of the rat affects the results. Another way to explore this explanation is to do the first probe trial at an earlier stage, perhaps after four days of training and see whether the rats are using an allocentric

strategy at this stage. Another explanation for the discrepant findings could be that the allocentric cues were not salient enough for the rats. We used the same cues as in Experiment 2a, and it is possible that since the cues were not novel to the rats, the animals did not pay as much attention to them. The experiment could be conducted again using different environmental cues to rule out this explanation.

The second finding was also inconsistent with our hypothesis. We expected the DD1R mutant rats not to use the egocentric strategy as often as the wildtypes. One possible explanation for this finding comes from the nature of the task in the T-maze. The T-maze is one of the simplest mazes in behavioural research, and it is very easy for rats to learn the task. Although the DD1Rs are abundant in both the hippocampus and the basal ganglia (Levey et al., 1993, Ariano & Sibley, 1994, Bergson et al., 1995), it is possible that the DD1R mutant rats have still some functioning of the DD1R. This might be enough to learn a simple task such as the T-maze. Moreover, the DD1R are especially important in the prefrontal cortex (Dubois et al., 1986, Lidow et al., 1991, Hall et al., 1994). Due to the simplicity of the task, it is unlikely that the prefrontal cortex was involved in learning the task.

The third finding was consistent with our hypothesis. Similar to the DD1R knockout mice (Wall et al., 2011), the DD1R mutant rats showed an impairment in unlearning a previously learned response. Although this impairment was only subtle, and was no longer evident after the fifth day of reversal training, this finding suggests that the DD1R is involved in reversal learning. In sum, we found evidence to suggest that although the DD1R is not vital for learning to use an egocentric strategy in the T-maze, it is needed when previously learned behaviour is required to be reversed.

13 General discussion

The primary research question was whether the DD1R is necessary for normal cognitive functioning. The answer is a tentative yes. Although we found no differences between the DD1R mutant rats and the wildtype rats when the task was easy (initial learning phases and two probe trials of Experiment 3), when the task was more complicated the DD1R mutant rats seemed to be either unable to learn (Experiment 1a and b, Experiment 2 a and b) or took longer than the wildtypes to learn (reversal phase of Experiment 3). These findings support the hypothesis that the DD1R is necessary for higher-order cognitive functions. These findings are also consistent with previous research indicating an important role of the DD1R in higher-order cognitive functions that are regulated by the prefrontal cortex (for a review see Floresco & Magyar, 2006).

Based on the inverted U-shape hypothesis of the role of the DD1R functioning in cognition (Takahashi et al., 2008, Williams & Goldman-Rakic, 1995, Zahrt, Taylor, Mathew & Arnsten, 1997), it is likely that the DD1R mutant rats have a suboptimal level of DD1R functioning. This

leads to an impairment in spatial as well as reversal learning tasks. However, the results from the present study cannot address the question of whether these rats are only slightly impaired in these tasks, or whether there is a major impairment. The star-maze tasks were too difficult for even the pilot and wildtype rats to acquire. Therefore, the baseline (the performance of the wildtype rats) that we compared the performance of the DD1R mutant rats to was not optimal. Future studies should aim to use a behavioural task that is challenging enough to engage the prefrontal cortex, but not too challenging so that the wildtype rats are able to learn the task. One such task could be the Y-maze protocol that we used in Experiment 1b. This experiment could be conducted again using naïve DD1R mutant and wildtype rats. Our preliminary results suggested that the wildtype rats were able to learn this task, whereas the DD1R mutant did not improve over time.

Also, because the inverted U-shape relationship between the DD1R activity and cognitive performance is especially evident in working memory tasks (Floresco & Magyar, 2006); future studies could explore the role of the DD1R in a working memory task. The radial arm maze can be used to distinguish the difference between working memory errors and reference memory errors (Olton & Papas, 1979, Wirsching, Beninger, Jhamandas, Boegman & El-Defrawy, 1984). Revisiting an already visited arm during a trial is considered a working memory error in this task, whereas visiting an arm that has never been baited on any trial is considered a reference memory error (Olton & Papas, 1979). Therefore, with the radial arm maze future studies could investigate the role of the DD1R in both working memory as well as reference memory. Also, it would be expected based on the inverted U-shape hypothesis that the DD1R rats homozygous to the mutation (+/+) would have the worst performance because they would have least functioning of the DD1R, whereas the rats heterozygous to the mutation (+/-) would show an intermediate phenotype, and the wildtypes that lack the mutation (-/-) would provide the baseline performance. Therefore, using the three different groups (wildtypes, heterozygous and homozygous DD1R rats) could shed more light to the role of the DD1R in working memory functioning.

If the suboptimal functioning of the DD1R leads to cognitive impairment, what might be the mechanism behind this relationship? As mentioned previously, the DD1Rs have been hypothesised to specifically respond to phasic dopamine release (Wall et al., 2011). This phasic dopamine release has been extensively studied as an important process for reward-related learning (for a review see Regrave, Gurney & Reynolds, 2007). More specifically, the short-duration and short-latency dopamine bursts (Schultz, 1998) have been hypothesised to act as a mechanism for reward prediction errors. These prediction errors are needed to adjust the response so that the animal can receive maximal reward (Bayer and Glimcher, 2005, Nakahara, Itoh, Kawagoe, Takikawa & Hikosaka 2004, Satoh, Nakai, Sato & Kimura, 2003, Schultz, 1998, Schultz, 2006).

There are four lines of evidence to suggest that the phasic dopamine release is important in reward prediction errors. First, any novel sensory stimuli can elicit phasic dopamine responses. However, these responses quickly habituate if the presence of the stimulus does not lead to behaviourally rewarding consequences (Ljungberg, Apicella & Schultz, 1992). Second, if this stimulus that no longer elicits a phasic response is later on paired with a reward, the phasic response returns (Ljungberg et al., 1992). Third, the phasic response elicited by the reward diminishes once the connection has been established between the stimuli and the reward (Pan, Schmidt, Wickens & Hyland, 2005, Schultz, 1998). Finally, if the stimulus is no longer followed by a reward, the spontaneous activity of dopamine neurons is suppressed briefly, as opposed to eliciting a phasic response (Schultz et al., 1997).

The basal ganglia are important brain structures in reinforcement learning (Regrave et al., 2007). Given the abundance of the DD1Rs in the basal ganglia (Ariano & Sibley, 1994, Bergson et al., 1995, Levey et al., 1993), and the hypothesis that the DD1Rs specifically respond to phasic dopamine release (Wall et al., 2011), it is possible that the DD1R mutant rats have an impairment in reward-related learning due to an impairment in phasic dopamine response, which in turn leads to an impairment in reward prediction errors. This would manifest as an inability to learn the correct route in both the negative and the positive reinforcement tasks in the present study, since the target box was paired with a reward in both tasks. In the positive version there was a food reward, and in the negative version the escape from ice was rewarding. Future studies could investigate whether the DD1R mutant rats are impaired in learning tasks that require avoidance of aversive stimuli. For instance, the aforementioned radial arm maze could be used with aversive stimuli in some of the arms. This protocol would shed light to whether the DD1R is important in just reinforcement learning, or whether avoidance learning is also affected.

We found that the DD1R mutant rats were not impaired in their goal-directed behaviour (motivation to work for a food reward). This finding is inconsistent with previous research. Both El-Gundhi et al. (2003) using DD1R knockout mice, and Squire et al. (unpublished data, personal communication) using DD1R mutant rats, found that these animals are unable to learn to press a lever for a food reward. El-Gundhi et al. (2003) concluded that the mutant mice are unmotivated to work for food after ruling out potential impairments in movement ability. However, there is a potential alternative explanation to these results. As mentioned above, it is possible that the suboptimal functioning of the DD1R leads to an inability to learn the connection between the behaviour and the reward (Regrave et al., 2007). But how would this explain our findings? It is possible that the key to understanding this apparent discrepancy is in the type of task that is examined.

The tasks used in the present experiments were all relatively simplistic in terms of the actions that were required in order to acquire the food reward. The rats only needed to be able to walk, enter a box which had a wide opening and eat the food. In the El-Gundhi et al. (2003) experiments and the experiment by Squire et al. (unpublished data, personal communication) the rats needed to learn to press a lever to receive the food reward. The rats naturally walk and explore their surroundings, whereas pressing a lever is a more artificial task which requires more effort. If the inverted U-shape relationship between DD1R activity and performance is applied to these two tasks, it is likely that entering a box to eat a food reward from the ground requires less DD1R activity than learning to press a lever. If the DD1R mutant rats still have some functioning of DD1R this might be enough to learn to collect food from a box. However, even though the DD1R mutant rats learned to enter the box to receive the food reward, they were still impaired in learning the fastest strategy to get to the correct arm (Experiments 2a and b). Therefore it is possible that the DD1R mutant rats are able to learn a simple task, such as entering a box, due to some DD1R activity, but are unable to learn more complex tasks such as pressing a lever or using an egocentric strategy.

We also had an interesting finding when we compared the results from the negative and positive reinforcement tasks. The wildtypes were always overall faster than the DD1R mutants in the negative reinforcement tasks. However, in the positive reinforcement tasks there was no such difference. Also, this difference was only evident in the primary latency data. Although this finding does not relate to cognition, it does shed light to the function of the DD1R. It appears that the DD1R is implicated in movement under aversive conditions. Interestingly, the DD1Rs are expressed in the amygdala (Ariano & Sibley, 1994, Bergson et al., 1995, Levey et al., 1993). The amygdala is a brain structure that is integrally involved in fear response (for a review see Fanselow & Gale, 2006).

However, it is unclear why the lack of DD1Rs in the amygdala would lead to an enhanced fear response. In fact, previous research has suggested that reduced DD1R activity leads to an inhibition of a fear response (Lamont & Kokkinidis, 1998). Also, previous research on the DD1R mutant rats found no difference in their startle response compared to wildtype rats (Müller, unpublished data, from Muller et al., 2010). Another possibility is that the DD1R mutant rats show more behavioural inhibition than the wildtype rats. However, a previous study found no difference between the wildtype rats and the DD1R mutant rats in an elevated plus-maze (Ellenbroek, unpublished data, personal communication). The elevated plus-maze is a common behavioural test that measures behavioural inhibition in rodents (Hogg, 1996).

A third possibility is that the DD1R mutant rats somehow experienced the aversive stimulus (ice) as more aversive than the wildtype rats. In fact, dopamine has an important role as an

endogenous mechanism to increase tolerance to pain (Wood, 2006). More interestingly, this analgesic effect seems to rely on phasic dopamine bursts (Wood, 2006, Wood et al., 2007). It is possible that the DD1Rs are necessary for this analgesic effect, since the DD1Rs are hypothesised to specifically respond to phasic dopamine signalling (Wall, et al., 2011). Therefore, perhaps due to a lack of normal dopamine signalling the DD1R mutant rats perceived the ice as more aversive than the wildtype rats. Freezing is a common fear-response in rats (Fanselow, 1986). If the DD1R mutant rats perceived the ice as more aversive, it is possible that they stayed still for longer periods of time, and therefore had longer primary latencies to reach the target. The data supports this idea. Although the DD1R rats had much longer primary latencies, their primary arm visits were not as distinctively different from that of the wildtypes. This would suggest that the DD1R mutant rats took longer because they stayed still or moved slower in the ice. If the DD1R mutant rats had moved the same pace as the wildtypes they should also have had much higher primary arm visits since it took longer for them to find the target.

Although it is still unclear why the DD1R mutant rats have such a clear impairment in their movement in an aversive task compared to wildtype rats this finding does suggest that latency data from the DD1R mutant rats should be interpreted with caution when using aversive tasks. This finding is consistent with the preliminary locomotor activity findings of Müller & Homberg (unpublished data, from Müller et al., 2010) in the Morris water maze, where the DD1R mutant male rats were less active than the wildtype male rats. Also, the locomotor activity of these mutant rats is decreased compared to wildtypes in the open field, whereas there is no difference in their locomotor activity in the home cage (Olivier & Balemans, unpublished data, from Müller et al., 2010). Therefore, longer latency data of the DD1R mutant rats in comparison to the wildtype rats should not be automatically interpreted as an indication of a cognitive deficit before alternative explanations can be ruled out.

Although the DD1R mutant rats provide a novel model to test the role of the DD1R in cognition, like any other model, the use of these rats also has some limitations. The first limitation is that it is still unclear how the DD1R is affected in these rats. It is still not known whether these rats have some functioning of the DD1Rs. A second limitation is that the DD1R is a part of a larger dopaminergic system and this system itself interacts with other neurotransmitters and proteins (Undiehl, 2010). There is a great possibility that the mutation of these rats has also affected the other systems due to compensatory mechanisms. However, this animal model also has some considerable strength. As mentioned previously, most behavioural tasks were developed for rats (Baker, 2011). Therefore, rats are superior to mice when measuring some complex cognitive functions (Baker, 2011). Also, testing the cognition of the DD1R mutant rats can add converging evidence to the current knowledge on this topic. Furthermore, because these rats only have a mutation in the gene,

rather than a complete knockout of the gene, they potentially have less compensation in their central nervous system. Also, a mutant is closer to the human condition because complete gene deletions are rare in humans and it is more likely that a single nucleotide polymorphism or copy-number variation is affecting the DD1R functioning in humans (Kellendonk et al., 2009).

The potential learning and memory deficits of the DD1R mutant rats are important to determine because they can shed light to the role of the DD1R in human cognitive impairments. As mentioned in the introduction, spatial and reversal learning deficits are evident in a variety of psychiatric disorders ranging from schizophrenia to depression and Alzheimer's disease (Austin et al., 2001, Laczó et al., 2009, Postle et al., 1997, Weniger & Irle, 2008). Also, reversal learning impairments are often evident in addiction (Crews & Boettiger, 2009). If the tentative findings from the present experiments can be replicated using different behavioural tests, then these DD1R mutant rats could be used in the development of novel treatments for cognitive deficits.

In sum, we found tentative evidence to suggest that the DD1Rs are necessary for egocentric learning (Experiment 1a and b, Experiment 2a), learning to switch from egocentric to allocentric learning (Experiment 2b), as well as reversal learning (Experiment 3). Further research should explore the role of the DD1R in cognition using different behavioural tasks. Also, the exact functioning of the DD1R in the mutant rats should be determined. In conclusion, the DD1R mutant rats have the potential to be used as animal models of cognitive deficits in various psychiatric disorders.

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