The Role of the Dopamine D_1 Receptor in the Negative Symptoms of Schizophrenia

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Abstract

Diminished motivation is a core feature of schizophrenia that has been linked to impaired functional outcomes. A mechanism thought to contribute to diminished motivation is impaired anticipatory pleasure. Impaired anticipatory pleasure is associated with disrupted reward prediction and reduced engagement in reward-seeking behaviours. To investigate the role of the dopamine D_1 receptor in anticipatory pleasure, D_1 mutant rats and WT rats performed five experiments. Reward prediction was examined using the anticipatory locomotion experiment and successive negative contrast experiment. It was found that D_1 mutant rats have impaired anticipatory responses to expected reward. However, as the WT rats did not show the expected response to an alteration in reward expectation, it was impossible to assess the role of the D_1 receptor. Together, these findings suggest that the D_1 receptor may be involved in aspects of reward prediction. Reward-seeking behaviour was examined using the social approach experiment, scent marking experiment, and the separation induced vocalization experiment. It was found that the D_1 mutant rats have an impaired ability to engage in social and sexual reward-seeking behaviours, but have relatively normal ability to engage in maternal reward-seeking behaviours. Together, these findings indicate that the D_1 receptor is involved in certain aspects of reward-seeking behaviours. In conclusion, there is compelling evidence that a D_1 receptor dysfunction is a likely contributor to diminished motivation in schizophrenia.

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CHAPTER 1: Introduction

Schizophrenia is a severe psychiatric disorder that affects between 0.4% to 0.7% of the general population (American Psychiatric Association; APA, 2013). This psychiatric disorder is markedly heterogeneous, consisting of *positive* symptoms (e.g. delusions, hallucinations), *negative* symptoms (e.g. avolition, anhedonia), and *cognitive* symptoms (e.g. impairments in attention, and problem solving).

Negative symptoms have long been considered a core feature of schizophrenia (Kraeplin, 1921). Recent years has seen a resurgence of interest in negative symptoms. This was driven by the recognition that negative symptoms contribute to impaired functional outcomes in schizophrenia (Kirkpatrick et al., 2006; Rabinowitz et al., 2012). Negative symptoms are generally thought to consist of two domains: the diminished motivation domain and the diminished expression domain (Messinger et al. 2011; Strauss, Waltz, & Gold, 2014). To explain the link between negative symptoms and impaired functional outcomes, most researchers have focused on the diminished motivation domain. A mechanism thought to contribute to diminished motivation is impaired anticipatory pleasure (Gard et al., 2006; Horan, Kring & Blanchard, 2006, Kring et al., 2011). Impaired anticipatory pleasure can reflect disruptions in the reward prediction and/or reward anticipation systems. These disruptions subsequently lead to reduced engagement in reward-seeking behaviours (Gard et al., 2007; Heerey & Gold, 2007).

Unfortunately, there is a lack of research addressing the neurobiological mechanisms underlying impaired anticipatory pleasure. The current study aims to investigate the role of the dopamine D_1 receptor in anticipatory pleasure, focusing both on reward prediction, and reward-seeking behaviour. Reward prediction was assessed using the anticipatory locomotion task and the successive negative contrast task. Reward-seeking behaviours were assessed using the social approach task, the scent marking task, and the maternal separation induced

ultrasonic vocalization task. These tasks were performed by two experimental groups: homozygous dopamine D_1 mutant rats and wild type control rats. To provide context for this study, the following topics are reviewed: 1) negative symptoms, 2) reward processing, 3) consummatory and anticipatory pleasure, 4) dopamine, 5) dopamine and reward processing, and 6) dopamine, consummatory and anticipatory pleasure.

CHAPTER 2: Background and Literature Review

Negative Symptoms

Negative symptoms have long been considered a core feature of schizophrenia (Kraepelin, 1921). There are five consensus based negative symptoms. These include: *avolition* (diminished motivation and goal-directed behaviour), *anhedonia,* (diminished ability to experience pleasure), *asociality* (reduced engagement in social activity and decreased interest in forming relationships with other), *restricted affect* (reduced emotional expression), and *alogia* (diminished verbal production and spontaneous speech). Negative symptoms have been linked to impaired functional outcomes (Kirkpatrick et al., 2006; Rabinowitz et al., 2012). Impaired functional outcomes include: unemployment, social isolation, difficulty living independently, and poor quality of life. Unfortunately, current pharmacological treatments are virtually ineffective at treating negative symptoms (Fusar-Poli et al., 2015). In order to develop new effective treatments and improve functional outcomes, it is necessary to understand the mechanisms underlying negative symptoms.

In recent years, much research has focused on the conceptualization of negative symptoms. Accumulating evidence suggests that negative symptoms contain two domains: the diminished motivation domain and the diminished expression domain (Messinger et al., 2011; Strauss et al., 2014) (refer to figure 1). The *diminished motivation* domain reflects reduced motivation and pleasure across a range of life domains. It consists of three symptoms: avolition, anhedonia, and asociality. Conversely, the *diminished expression* domain reflects reduced verbal and non-verbal expression, and communicative output. It consists of two symptoms: restricted affect and alogia. Although investigation of both domains is critically important, the diminished motivation domain is considered to be of greater importance. This is because the diminished motivation domain, but not the

diminished expression domain, is strongly predictive of functional outcomes in schizophrenia (Foussias, Mann, Zakzanis, Van Reekum & Remington, 2009; Foussias & Remington, 2010; Kiang, Christensen, Remington & Kapur, 2003).

Unfortunately, the psychological and neurobiological processes contributing to diminished motivation in schizophrenia remain poorly understood. Recent advances in affective neuroscience have allowed the field to make considerable strides in conceptualizing the likely contributors to diminished motivation. The following section reviews the psychological and neurobiological processes that have received the most attention.

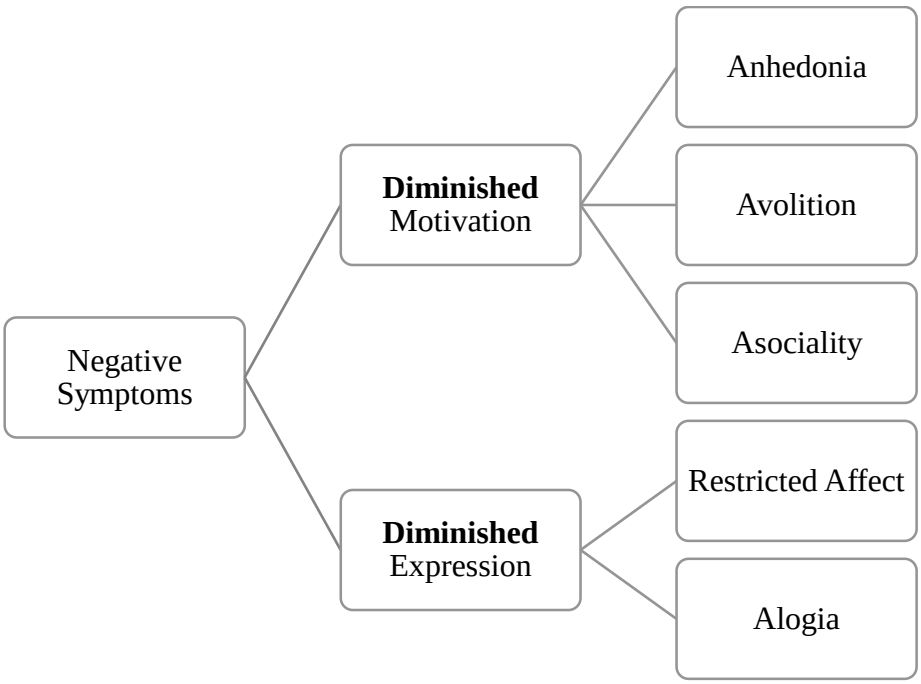


Figure 1. Graphic representation of two domains of negative symptoms

Psychological Processes Contributing to Diminished Motivation

For many years, diminished motivation was thought to reflect the impaired ability to experience pleasure. Thus, patients with schizophrenia do not engage in rewarding activities because they do not find such activities enjoyable. This theory was developed based on the finding that patients with schizophrenia report experiencing lower levels of pleasure than healthy controls on self-report trait measures and interview-based measures (see (Horan et

al., 2006) for review). Recent research has challenged this theory. In short, studies have now found that patients report experiencing similar levels of positive emotion than healthy controls in laboratory-based studies and experience sampling studies (Gard et al., 2006; Kring & Moran, 2008).

Reward Processing

Despite having normal levels of pleasure, it is clear that patients with schizophrenia engage in fewer reward-seeking behaviours aimed at obtaining reward (Gard et al., 2007; Heerey & Gold, 2007). In order to understand why normal levels of pleasure do not translate into reward-seeking behaviours, it is helpful to turn to the reward processing literature. An important concept within this literature is that there are multiple component processes required for translating reward information into reward-seeking behaviour. Two major components are: consummatory pleasure and anticipatory pleasure (Berridge & Robinson, 1998; Gard et al., 2007; Klein, 1987). *Consummatory pleasure* refers to the experience of pleasure in the moment (liking) whereas *anticipatory pleasure* refers to the experience of pleasure from expecting a future enjoyable outcome (wanting). An impairment in these component processes would result in reduced engagement in reward-seeking behaviour (Barch & Dowd, 2010). If an organism does not experience pleasure from reward, they will be less motivated to seek out similar reward in the future (consummatory pleasure). Similarly, if an organism does not anticipate reward when present with a prediction cue, they will be less motivated to seek out the associated reward (anticipatory pleasure). The following section reviews the functioning of the consummatory and anticipatory pleasure components processes in schizophrenia.

Consummatory Pleasure

A large body of evidence suggests that patients with schizophrenia have relatively intact hedonic experiences (see (Kring & Moran, 2008) for review). Laboratory-based studies found that self-reported emotional responses to affect eliciting stimuli were comparable for patients and controls (Aghevli, Blanchard & Horan, 2003; Berenbaum & Oltmanns, 1992). Similarly, experience-sampling studies found that emotional responses to daily life events were comparable for patients and controls (Gard, et al., 2006). Further, startle responses following the presentation of pleasant stimuli were similarly reduced in patients and controls (Kring, Germans Gard & Gard, 2011; Volz, Hamm, Kirsch & Rey, 2003). Moreover, memory was enhanced for positive stimuli in both patients and controls (Hall, Harris, McKirdy, Johnstone & Lawrie, 2007; Horan et al., 2006). Together, these findings suggest that patients with schizophrenia have relatively intact hedonic experiences. Thus, there is compelling evidence that the reduced engagement in reward-seeking behaviour does not reflect impaired hedonic experience in schizophrenia.

Anticipatory Pleasure: Reward Prediction Component

Anticipatory pleasure can be further parsed into two components. The first component is reward prediction. *Reward prediction* refers to the ability of reward predictive cues to trigger anticipatory responses in expectation of that reward. Reward prediction is mediated by the midbrain dopamine (DA) system, particularly the projections to ventral and dorsal striatum (Schultz, Dayan & Montague, 1997). A number of imaging studies have examined the role of the striatum in the reward prediction by looking at the neural response to reward-predicting cues. Studies show reduced ventral striatum responses to reward-predicting cues in patients with schizophrenia compared to controls (Juckel et al., 2006; Waltz et al., 2010). This result was found in medicated and patients treated with first generation antipsychotics, but not in patients treated with second generation antipsychotics. Notably, the severity of

negative symptoms was found to predict the reduction in ventral striatum responses to reward-predicting cues (Juckel et al., 2006).

A number of imaging studies have examined the role of the striatum in reward prediction by looking at prediction error responses. *Prediction error response* refers to the increase in striatal response to unpredicted occurrence of rewards (positive prediction error) and the decrease in striatal response when predicted rewards are omitted (negative prediction error). Some studies show blunted prediction error responses in patients with schizophrenia compared to controls (Murray et al., 2008; Schlagenhauf et al., 2014). However, other studies show intact prediction error responses in patients (Simon et al., 2010; Waltz et al., 2010). Notably, even when prediction error responses were not different from controls, their magnitude was predicted by the severity of negative symptoms (Waltz et al., 2010). Together, these studies suggest that while patients with schizophrenia may have reduced reward prediction the magnitude of this reduction is likely influenced by individual differences in the severity of negative symptoms and medication. Thus, there is compelling evidence that reduced engagement in reward-seeking behaviour in schizophrenia may reflect impaired reward prediction in schizophrenia.

Anticipatory Pleasure: Reward Anticipation Component

The second component of anticipatory pleasure is reward anticipation. *Reward anticipation* refers to the experience of pleasure in anticipation of future reward. A growing number of studies have found that patients with schizophrenia have reduced reward anticipation (Gard et al., 2006; Gard et al., 2007; Mote, Minzenberg, Carter & Kring, 2014), through with some exceptions (Tremeau et al., 2010; Tremeau, Antonius, Nolan, Butler & Javitt, 2014). For example, experience-sampling studies found that patients report experiencing lower levels of pleasure in anticipation of future enjoyable activities, compared to healthy controls (Gard et al., 2006; Gard et al., 2007). Similarly, studies using the

Temporal Experience of Pleasure Scale (TEPS) found that patients report lower levels of trait anticipatory pleasure, compared to healthy controls (Mote et al., 2014). Together, these findings suggest that patients with schizophrenia appear to have reduced reward anticipation. Thus, there is emerging evidence that reduced engagement in reward-seeking behaviour may reflect an impaired reward anticipation in schizophrenia.

Summary

There is compelling evidence that reward processing contributes to diminished motivation in schizophrenia. Reward processing contains two components: consummatory pleasure and anticipatory pleasure. Evidence suggests that schizophrenia involves intact consummatory pleasure but impaired anticipatory pleasure (Horan et al., 2006; Kring et al., 2011). Anticipatory pleasure contains two components: reward prediction and reward anticipation. Evidence suggests that schizophrenia involves impaired reward prediction (Juckel et al., 2006; Waltz et al., 2010) and anticipation (Gard et al., 2006; Mote et al., 2014). Impaired reward prediction and anticipation is manifested as a reduced engagement in reward-seeking behaviours aimed at obtaining reward (Gard et al., 2007; Heerey & Gold, 2007).

Figure 2. Pathways to motivational deficit in schizophrenia

Neurobiological Mechanisms Contributing to Diminished Motivation

Dopamine

Many researchers have attempted to determine the neurobiological mechanisms contributing to diminished motivation in schizophrenia. A mechanism that has received considerable attention is the dopamine (DA) system. The DA system is involved in regulating a multitude of functions within the central nervous systems including: movement, reward, motivation, learning, and cognition (Zhang, Xiong, Zhen & Zhang, 2009).

DA mediates its effect through five different G protein-coupled receptors. These receptors have been divided into two families based on similarity in structure, pharmacology, and coupling. These include the *D_1- like* receptor family and the *D_2- like* receptor family (Zhang et al., 2008). The *D_1-like* receptor family consists of D_1 and D_5 receptor subtypes whereas the *D_2-like* receptor family consist of D_2, D_3, and D_4 receptor subtypes. The current study is focus on the D_1 receptor. The D_1 receptor is the most abundant and widespread of the five DA receptors. High levels of D_1 receptors are found in the caudate putamen, nucleus accumbens and olfactory tubercle (Missale, Nash, Robinson, Jaber & Caron, 1998). Lower levels of D_1 receptors are expressed in the amygdala, globus pallidus, substantia nigra, ventral tegmental area, hippocampus, hypothalamus, thalamus, and prefrontal cortex (Missale et al., 1998). The following section reviews the role of the DA system in motivation and reward processing.

Dopamine and Motivational Processing

For many years, the DA system was thought to mediate motivated behaviour. This theory was developed based on the finding that administration of DA antagonists reduced food-reinforced lever pressing (Blackurn, Phillips & Fibiger, 1987; Salamone et al., 1991; Wise, Spindler & Gerberg, 1978). Despite the popularity of this theory, it has been

challenged on a number of accounts. Considerable evidence demonstrates that DA manipulations affects some, but not all, aspects of motivated behaviour. For example, DA antagonism or depletion affects behavioural activation, exertion of effort, and sustained task engagement (Salamone & Correa, 2012). However, DA antagonism or depletion fail to affect primary food motivation or appetite (Salamone & Correa, 2012). Together, these findings highlight the importance of distinguishing between different aspects of motivated behaviour.

Seeking Phase and Consummatory Phase

Motivated behaviour can be distinguished into two temporal phases. These include: the seeking phase and the consummatory phase (Ikemoto & Panksepp, 1999). During the *seeking phase*, an organism engages in reward-seeking behaviour. Reward-seeking behaviour refers to behaviours aimed at obtaining reward (e.g. exploration, approach, instrumental responding). During the *consummatory phase*, an organism engages in consummatory behaviours. Consummatory behaviour refers to behaviours aimed at the final consumption of reward (e.g. eating, drinking, sexual copulation). These temporal phases of motivated behaviour are differentially affected by DA manipulations.

Accumulating evidence suggest that DA manipulations affect reward-seeking behaviours but do not affect consummatory behaviour. For example, administration of haloperidol (DA antagonist) decreased food-reinforced lever pressing but increased food intake (Salamone et al., 1991). Similarly, administration of primozide (a non-selective DA antagonist) decreased the number of entries into a food niche to obtain food, but had no effect on food intake (Blackburn et al., 1987). Moreover, administrated of primozide and haloperidol decreased preparatory sexual behaviours but had no effect on the initiation of copulation (Pfaus & Phillips, 1991). Together, these findings suggest that DA plays an important role in reward-seeking behaviour but not consummatory behaviour.

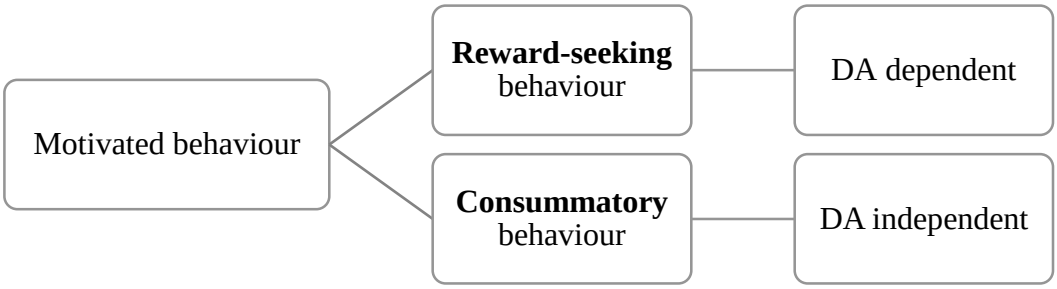


Figure 3. Involvement of DA in components of motivated behaviour.

Whilst it is clear that DA plays an important role in reward-seeking behaviour, the role of receptor subtypes remains poorly understood. Nonetheless, emerging evidence suggests that the D_1 receptor may be involved in reward-seeking behaviours. For example, administration of SCH 23390 and SKF 83566 (D_1 receptor antagonists) decreased food-reinforced lever pressing for food but increased chow consumption (Cousin, Wei & Salamone, 1994; Salamone, Arizzi, Sandoval, Cervone & Aberman, 2002). In addition, administration of SCH 23390 has been found to block reinstatement of food-seeking behaviour (Ball, Combs & Beyer, 2011). Moreover, administration of SCH 23390 has been found to reduce sucrose-seeking behaviour (Grimm et al., 2011). Together, these findings indicate that the D_1 receptor may be involved in reward-seeking behaviour. However, a major limitation of the aforementioned studies is their reliance on pharmacological manipulations of the D_1 receptor. Available D_1 receptor ligands cannot discriminate between D_1 and D_5 receptor subtypes (Undieh, 2010). Thus, from such pharmacological studies, we are unable to confidently conclude that the D_1 receptor mediates reward-seeking behaviour.

Dopamine and Reward Processing

In order to understand the role of DA in reward-seeking behaviour, it is helpful to return to the reward processing literature. As discussed above, reward-seeking behaviour can

be subdivided into: consummatory pleasure and anticipatory pleasure (Berridge & Robinson, 1998; Gard et al., 2007; Klein, 1987). The following section reviews evidence for the role of DA in consummatory and anticipatory pleasure.

Consummatory Pleasure

For many years, the DA system was thought to mediate the ability to experience pleasure (Wise et al. 1978). This theory was developed based on the observation that the DA system becomes activated by the presentation of rewards, such as food, water, sex, and drugs of abuse (e.g. Argona et al., 2006; Becker et al., 2001; Robbins & Everitt, 1998). It received further support from the observation that administration of neuroleptic treatment (DA antagonist) reduced the subjective reports of pleasure in patients (Healy, 1989) and normal subjects (Belmaker & Wald, 1977).

Since its publication, this theory has been challenged. One such challenge has come from taste reactivity studies. Taste reactivity studies assess the hedonic or aversive reaction patterns to tastes and thus are considered to directly measure consummatory pleasure. Palatable solutions, such as sucrose, elicit hedonic reactions (e.g. tongue protrusion, mouth movements) whereas aversive solutions, such as quinine, elicit aversive reactions (e.g. gaping). Studies have repeatedly found that manipulation of the DA system fails to alter hedonic or aversive reactions. For example, suppression of the DA system (via neurochemical lesion, 6-OHDA; or receptor blocking drugs, haloperidol) failed to decrease hedonic reaction to sucrose (Berridge & Robbinson, 1998; Pecina, Berridge & Parker, 1997). Similarly, activation of the DA system (via electrical brain stimulation reward; or receptor activating drugs, amphetamine) failed to increase hedonic reactions to sucrose (Tindell et al. 2005; Wyvell & Berridge, 2000). Together, these findings suggest that DA does not play an important role in consummatory pleasure.

Anticipatory Pleasure

As explained before, anticipatory pleasure can be further subdivided into two separate components: reward prediction and reward anticipation. A growing body of evidence suggests that the DA system mediates reward prediction (Berridge, 2004; Schultz et al., 2007). Studies show that DA neurons in the ventral and dorsal striatum initially fire in response to reward (Schultz, 1993). However, after repeated cue-reward pairings, DA neurons fire in response to reward-predictive cues rather than the reward itself (Schultz et al. 2007). Thus when a reward is not predicted, the DA neurons fire strongly (positive prediction error), while when a predicted reward is omitted, there is a reduction in DA neuron firing (negative prediction error). In line with this, micro dialysis studies show that DA transmission in the medial prefrontal cortex is enhanced when rats are presented with environmental cues preceding palatable food (Bassareo & Di Chiara, 1997). Moreover, voltammetry studies show that increased DA release when rats are presented with a cue that signals food reward (Phillips et al., 1993). Together, these findings suggest that DA plays an important role in reward prediction.

Dopamine and Incentive Salience

Reward prediction contributes to reward-seeking behaviour. It does so though its role in the attribution of incentive salience (Berridge, 2004). *Incentive salience* is a type of motivation attributed to reward-predicting cues. Its attribution transforms the neural representation of the reward-predicting cue from a sensory representation into an attractive and wanted incentive. The attribution of incentive salience requires Pavlovian learning. During Pavlovian learning, a neutral stimulus cue is followed by the presentation of reward. After repeated pairings, the cue (conditioned stimulus; CS) becomes a predictor of the reward (unconditioned stimulus; US). Following Pavlovian learning, the CS is attributed with incentive salience.

When attributed with incentive salience, the CS acquires two distinct wanting properties. These include: the cue-triggered wanting feature and the motivational wanting magnet feature (Berridge & Robinson, 2009). Both wanting features contribute to reward-seeking behaviour. The *cue triggered wanting feature* drives the willingness to exert effort to obtain reward. The *motivational wanting magnet* drives the willingness to approach the reward. The following section reviews evidence for the role of the DA system in these these wanting properties.

Cue Triggered Wanting

An encounter with the CS triggers wanting for the US. The cue triggered wanting feature can be assessed using the Pavlovian-Instrumental Transfer (PIT) experiment. PIT refers to the influence of the CS on instrumental responding towards US. In PIT experiments, animals presented with the CS show increased instrumental responding in effort to obtain the US (Dickson & Balleine, 1994). Considerable evidence demonstrates that DA manipulations affects the cue triggered wanting feature. For example, administration of DA antagonists has been found to decrease cue-triggered instrumental responding (Dickinson, Smith & Mirenowicz, 2000). Conversely, DA agonists have been found to increase cue-triggered instrumental responding (Wyvell & Berridge, 2000; Wyvell & Berridge, 2001). Thus far, one study has examined the role of DA D_1 receptor in the cue triggered wanting feature. They found that administration of SCH 23390 (D_1 receptor antagonist) decreased cue-triggered instrumental responding (Lex & Hauber, 2008). Although this points to the potential role of the D_1 receptor in this component, further research is required.

Motivational Wanting Magnet Feature

When a CS is attributed with incentive salience, it triggers not only wanting for the US, but also wanting for the CS itself. The motivational magnet feature can be assessed using the Pavlovian auto shaping experiment. In this experiment, animals have been found to seek,

approach, and intensely sniff, nibble and bite the CS that predicts sucrose US (Dickson & Balleine, 2000). Emerging evidence demonstrates that DA manipulations affect the motivational magnet feature. For example, administration of DA antagonists has been found to decrease instrumental approach behaviour (Dalley et al., 2002; Everitt, Cardinal, Hall, Parkinson & Robbins, 2000). Thus far, no study has examined the role of DA D_1 receptor in the cue triggered wanting feature.

Summary

In summary, there is compelling evidence that the DA system contributes to diminished motivation in schizophrenia. A mechanism thought to contribute to diminished motivation is impaired anticipatory pleasure (Gard et al., 2006; Horan et al., 2006). Impaired anticipatory pleasure reflects disruptions in reward prediction. Evidence suggests that DA plays an important role in reward prediction (Berridge, 2004; Schultz, 2007). This indicates that DA dysfunction may contribute to disruption in reward prediction in schizophrenia. These disruptions are manifested as a reduced engagement in reward-seeking behaviours (Gard et al., 2007; Heerey & Gold, 2007). Evidence suggests that DA plays an important role in reward-seeking behaviour (Blackburn et al., 1987; Salamone et al., 1991). Taken together, these findings indicate that DA dysfunction may contribute to diminished motivation in schizophrenia. Yet the role of individual receptors, especially the DA D_1 receptor is still largely unexplored.

Animal Models of Diminished Motivation

Whilst the role of DA dysfunction in the motivational impairments in schizophrenia is becoming increasingly recognized, the role of the D_1 receptor subtypes remains unclear. This is because there are currently no pharmacological agents that work exclusively on the D_1 receptor (Zhang et al., 2008). For example, SCH 23390 is arguably the most selective D_1

receptor antagonist available. However, SCH 23390 has been found to have a high affinity for the D_5 receptor and serotonin 5-HT_2 receptors (Bischoff, Heinrich, Sonntag & Krauss, 1986).

In order to study the role of the D_1 receptor in the regulation of anticipatory pleasure, the current study used D_1 mutant rats. These D_1 mutant rats have a point mutation at the D_1 receptor (Smits et al., 2006). This point mutation was generated using ENU (N-ethyl-N-nitrosourea) mutagenesis. Briefly, ENU acts by transferring its ethyl group onto nucleobases. These ethylated nucleobases cause mistaken identity during DNA replication. Mistaken identity can result in a single base pair change. In D_1 mutant rats, the single base pair change is a thymine to adenine change at position 1215 of the D_1 receptor gene. This change results in a single amino-acid change. In D_1 mutant rats, the single amino-acid change is an isoleucine to serine change at position 116 of the D_1 receptor protein. Previous studies in our laboratory have shown that this leads to a 50% reduction in D_1 binding and a strongly reduced behavioural response to D_1 agonists and antagonists. Thus, the D_1 mutant rats provide a usual animal model for investigating the role of the D_1 receptor subtype in the regulation of anticipatory pleasure.

Validating Animal Models of Diminished Motivation

Animal models are validated using behavioural assays. These behavioural assays are used to characterize constructs relevant to the diminished motivation in schizophrenia. Unfortunately, modelling diminished motivation in animals has proven difficult for two main reasons. First, motivational deficits are characterized by decreases in behavioural responding. There are number processes that may contribute to changes in behavioural responding, not all of these related to motivation. For example, an animal might respond less in a task because they are satiated, fatigued, or have motor impairments. Conversely, an animal might respond more to a task because they are more hyperactive. Thus, it is difficult to interpret increases or

decreases in behavioural responses as specific changes in motivation. A second reason why modelling motivational deficits has proven difficult is because motivated behaviour is not the result of a unitary process. Rather, it is the result of multiple component processes as discussed at length above, including: hedonics, reward anticipation, reward prediction, reward valuation and effort valuation (Barch & Dowd, 2010). Consider the simple act of a rat pressing a lever for food reward. The rat must 'like' the reward (i.e. hedonics). They must be able to predict that pressing the lever will result in the presentation of reward (i.e. reward anticipation and prediction). Lastly, they must be able to compute the cost associated with pressing the lever relative to the benefit of the reward (i.e. reward and effort valuation). Thus, it is difficult to determine the specific process contributing to behavioural responding. Fortunately, a wealth of behavioural assays that dissects motivational processes have been developed over the years. The follow section reviews some of these behavioural assays as they relate to diminished motivation in schizophrenia.

Anticipatory Locomotion Experiment

The anticipatory locomotion experiment can be used to assess reward prediction. More specifically, it can be used to assess the anticipatory response in expectation of reward. This experiment involves measuring the locomotor activity prior to the conditioned presentation of reward. Studies have consistently shown that after repeated presentation of reward, animals demonstrate increase locomotor activity. For example, male rats show increased exploration behaviour prior to the introduction of a sexually receptive female rat (Barr, Fiorino & Phillips, 1999; Pfaus & Phillips, 1991). Similarly, rats show increased rearing behaviour prior to the introduction of palatable food (Barbano & Cador, 2005). Increased locomotor activity provides a measure of reward prediction (Barbano & Cador, 2005; Barr et al., 1999; Pfaus & Phillips, 1991). A significant reduction in anticipatory locomotion is considered to reflect an impairment in reward prediction.

Successive Contrasts Experiment

The successive contrasts experiment can be used to assess reward prediction. More specifically, it can be used to assess the response to alteration in expected reward. This experiment involves measuring consummatory behaviour following an unexpected shift in the value of reward. Animals shifted from a reward of high value (e.g. 32% sucrose solution) to a reward of low value (e.g. 4% sucrose solution) consume less solution than animals that received only the reward of low value (Barr & Philips, 2002). This phenomenon is referred to as successive negative contrast (SNC). In line with this, animals shifted from a reward of low value (e.g. 4% sucrose solution) to a reward of high value (e.g. 32% sucrose solution) consume more solution than animals that received only the reward of high value (Flaherty, 1999). This phenomenon is referred to as successive positive contrast (SPC). The successive contrast effects emerge after the *unexpected* alteration in reward and the *unexpected* downshift or upshift in reward value. A significant reduction in the magnitude of the SNC or SPC effect is considered to reflect a deficit in reward prediction.

Social Approach Experiment

The social approach experiment can be used to assess the ability to engage in reward-seeking behaviour aimed at obtaining social reward. This experiment involves measuring *the amount of time spent* in a chamber containing a conspecific versus a chamber containing an object (sociability phase), as well as the time spent in a chamber containing an unfamiliar conspecific versus a familiar conspecific (social novelty preference phase). Studies demonstrate that animals spend more time in the chamber containing a conspecific versus an object (Nadler et al., 2004). Likewise, animals spend more time in the chamber containing an unfamiliar conspecific versus a familiar conspecific. The tendency to spend time in the chamber containing a conspecific (versus object) and the chamber containing an unfamiliar conspecific (versus familiar) provides a measure of consummatory behaviour. Thus, a

significant reduction in the amount of time spent in these chambers is considered to reflect a deficit in consummatory behaviour.

In addition, this experiment involves measuring *the number of entries* into a chamber containing a conspecific versus a chamber containing an object (sociability phase), as well as the number of entries into a chamber containing an unfamiliar conspecific versus a familiar conspecific (social novelty preference phase). Studies demonstrate that animals engage in a greater number of entries into the chamber containing a conspecific versus an object (Nadler et al., 2004). Similarly, animals engage in a greater number of entries into the chamber containing an unfamiliar conspecific versus a familiar conspecific. The tendency to enter the chamber containing a conspecific (versus object) and the chamber containing an unfamiliar conspecific (versus familiar) provides a measure of reward-seeking behaviour. Thus, a significant reduction in the number of entries into these chambers is considered to reflect a deficit in reward-seeking behaviour.

Scent Marking Experiment

The scent marking experiment can be used to assess the ability to engage in reward-seeking behaviour aimed at obtaining sexual reward. Rodent communicate through olfactory signals. An important source of olfactory signals is scent marking, the deposition of urinary pheromone traces in strategic environmental location (Arakawa, Blanchard, Arakawa, Dunlap & Blachard, 2008). Scent markings contain a large amount of information including the following: species, sex, age, individual identity, dominance status, and reproductive status. Contact with chemosensory cues in scent markings induce changes in physiology and behaviour. Such changes include: accelerated puberty, blocking pregnancy, and inhibiting aggression (Hurst, 1990).

Scent markings have two main functions. First, it functions as a *negative advertisement* to exclude other adult male from territory and prevent potential competition

for females. Accordingly, dominant male mice (Desjardins, Maruniak, & Bronson, 1973) and dominant male rats (Taylor, Griffin, & Rupich, 1988) deposit more scent marks than subordinate males. Second, scent marking functions as a *positive advertisement* to attract mates. Accordingly, male mice (Arakawa, Arakawa, Blanchard & Blanchard, 2007) and male rats (Manzo, Garcia, Hernandez, Carrilo & Pacheco, 2002) deposit more scent marks to adult females than towards juvenile females or juvenile males. Together, these functions serve to maximize the probability of mating. Thus, the deposition of scent markings can be considered a reward-seeking behaviour aimed at obtaining sexual reward. A significant reduction in the number of scent marking is considered to reflect a deficit in sexual reward-seeking behaviour.

Separation Induced Vocalization Experiment

The separation induced vocalization (SIV) experiment can be used to assess the ability to engage in reward-seeking behaviour aimed at obtaining maternal care. Rodent communicate through acoustic signals. An important source of acoustic signals is ultrasonic vocalizations (USVs) (Arakawa, et al., 2008). Rats emit three distinctly different vocalizations, depending on their age, environmental conditions, and affective states.

Adult rats emit 22 kHz vocalizations in aversive situations including: exposure to predators, exposure to inescapable pain such as food shocks, or during social defeat (Portfors, 2007). These negative state-associated vocalizations function as alarm calls, warning the colony about the presence of predators or other dangerous. Adult rats also emit 50 kHz vocalizations in appetitive situations, including: during sexual behaviour, during male agonistic behaviour, or during juvenile play (Portfors, 2007). These positive state-associated vocalizations function to increase affiliate and social cooperating behaviour.

Rat pups emit 40 kHz vocalizations when separated from their mothers (Brudzynski, Kehoe, & Callahan, 1999). There has been considerable debate about the function of

separation induced vocalizations (SIVs). According to one theory, pups emit SIVs in order to communicate with their mother. When pups are isolated from their mother, their body temperature rapidly cools. This cooling produces imminent danger to the pup if they are not retrieved quickly. Therefore, SIVs function as a communication signal to gain attention from the mother. According to another theory, pups emit SIVs as an acoustic byproduct of laryngeal braking (Blumberg & Alberts, 1990). Laryngeal braking is caused by an abdominal compression reaction that increases blood flow return to the heart. An abdominal compression reaction occurs in response to a cold stimulus. Therefore, pup SIVs are produced as a consequence of movement related to external stimuli.

Regardless of whether pup emit SIVs to communicate with their mother or whether they are merely an accidental by-product, SIVs initiate maternal searching and retrieval behaviour. Thus, SIVs can be considered a reward-seeking behaviour aimed at obtaining maternal care. A significant reduction in the number of SIVs emitted is considered to reflect a deficit in maternal reward-seeking behaviour.

Current Study

Aims and Hypothesis

The current study aims to investigate the role of the DA D_1 receptor in anticipatory pleasure. Anticipatory pleasure was assessed using the following five experiments: anticipatory locomotion, successive negative contrasts (SNC), social approach, scent marking, and separation induced vocalizations (SIVs) The specific aims and hypothesis for each of these are discussed in turn.

The aim of the anticipatory locomotion experiment was to examine the role of the DA D_1 receptor in reward prediction. If the D_1 receptor is involved in reward prediction, then D_1 mutant rats should show reduced anticipatory locomotor activity compared to WT rats.

Reduced anticipatory locomotor activity would indicate an impaired anticipatory response in expectation of reward.

The aim of the SNC experiment was to examine the role of the DA D_1 receptor in reward prediction. If the D_1 receptor is involved in reward prediction, then D_1 mutant rats should show a reduced SNC effect compared to WT rats. Reduced SNC effect would indicate an impaired response to alterations in expected reward.

The aim of the social approach experiment was to examine the role of the DA D_1 receptor in the ability to engage in reward-seeking behaviour aimed at obtaining social reward. If the D_1 receptor is involved in reward-seeking behaviour, then D_1 mutant rats should make fewer entries into the social zone (sociability phase) and the unfamiliar zone (social novelty phase), compared to WT rats. Fewer entries into the social zone and the unfamiliar zone would indicate an impaired ability to engage in social reward-seeking behaviour. Second, if the D_1 receptor is not involved in consummatory behaviour, then D_1 mutant rats and WT rats should spend a similar amount of time in the social zone (sociability phase) and in the unfamiliar phase (social novelty phase), compared to WT rats. Similar time in the social zone and the unfamiliar zone would indicate a normal ability to engage in social consummatory behaviours.

The aim of the scent marking experiment was to examine the role of the DA D_1 receptor in the ability to engage in reward-seeking behaviour aimed at obtaining sexual reward. If the D_1 receptor is involved in reward-seeking behaviour, then D_1 mutant rats should deposit fewer scent markings compared to WT rats. Fewer scent markings would indicate an impaired ability to engage in sexual reward-seeking behaviour. Second, if the D_1 receptor is involved in reward-seeking behaviour, then D_1 mutant rats should deposit a similar number of scent-markings around social odour (estrus female urine) and non-social odour (lemon essence). Similar scent-marking around social and non-social odour would

indicate an impaired ability of sexual reward to elicit an increase in reward-seeking behaviour.

The aim of the SIVs experiment was to examine the role of the DA D_1 receptor in the ability to engage in reward-seeking behaviour aimed at obtaining maternal care. If the D_1 receptor is involved in reward-seeking behaviour, then D_1 mutant rats should emit fewer SIVs, compared to WT rats. Fewer SIVs would indicate an impaired ability to engage in maternal reward-seeking behaviour.

CHAPTER 3: Anticipatory Locomotion Experiment

Methods

Animal subjects

Test subjects were adult male D_1 mutant Wistar rats (n = 7) and adult male wild-type (WT) Wistar rats (n = 7). All rats were bred in the vivarium of Victoria University of Wellington. They were housed in groups of three to five in standard polycarbonate cages. Water was available *ad libitum* at all times except during the experiment. The housing room had a controlled temperature of temperature of 21 °C, humidity of 55%, and was maintained on a 12-h light/dark cycle with lights on at 0700 h.

All rats were placed on food deprivation one week prior to the experiment. During food deprivation, food intake was limited to 15 g per day. This reduced body weight of the rats to approximately 85% of their free feeding weight.

Apparatus

The anticipatory locomotion experiment was conducted in seven locomotor activity chambers (Med Associates Inc., USA; model ENV-515) made from Plexiglas (dimensions: 42 x 42 x 30 cm). These chambers were equipped with two banks of eight photoelectric infrared cells on each of the internal walls of the chamber. Photocells were 2.5 cm above the chamber floor and were spaced 2.5 cm apart. Each chamber was interfaced with a computer that recorded the following variables: *total activity* (total horizontal beam breaks for each photocell), *ambulation* (cross-over between the inferior beams), and *rearing* (breaks of beams placed high).

During testing, a red light was illuminated and white noise was continually present to mask extraneous disturbances. After testing, the chambers were cleaned and wiped down with Virkon S disinfectant.

Procedure

The experiment contained two phase; the habituation phase and the anticipation phase. During the habituation phase, rats were placed in the activity chambers for 60 minutes daily, for 5 days. Activity data was collected at 5 minute intervals during the first 30 minutes. Rats were fed in their home cages at varying and unpredictable intervals (2-6 hours) after testing. During the anticipation phase, rats were again placed in the activity chambers for 60 minutes daily, for 5 days. Activity data was collected at 5 minute intervals during the first 30 minutes. Rats were fed in their chambers 30 minutes after their introduction into the chamber.

Statistical Analysis

Data were analyzed using a mixed-factor analysis of variance (ANOVA). The between subject factor was genotype (WT, HOM) and the within subject factor was day (day 1, day 2, day 3, day 4, day 5) or time (5 min, 10 min, 15 min, 20 min, 25 min, 30 min). Data were analyzed using SPSS software. A p value of $< .050$ was considered as statistically significant. Data was expressed as mean ± S.E.M.

Results

Habituation Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype on distance travelled and rearing during the habituation phase. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated for rearing, $\chi 2$ (9) = 27.35, $p <$

.050. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, $\varepsilon = 0.62$.

There was no main effect of genotype for distance travelled, F (1, 12) = 0.00, p = .976; or rearing, F (1, 12) = 1.11, p = .313. Distance travelled was similar for WT rats (M = 986.72; SD = 107.29) and HOM rats (M = 991.42; SD = 107.29). Rearing was similar for WT rats (M = 24.26; SD = 6.18) and HOM rats (M = 33.46; SD = 6.18). There was significant main effect of day for distance travelled, F (4, 48) = 27.02, $p < .050$; and rearing, F (2.46, 29.53) = 16.31, $p < .050$. Distance travelled and rearing decreased over the habituation phase. There was a significant interaction between days and genotype for distance travelled, F (4, 48) = 6.37, $p < .050$. This indicates that the decrease in distance travelled during the habituation phase was dependent on genotype. However, there was no interaction between days and genotype for rearing, F (2.46, 29.53) = 0.77, p = .499. Rearing decreased similarly in HOM and WT rats.

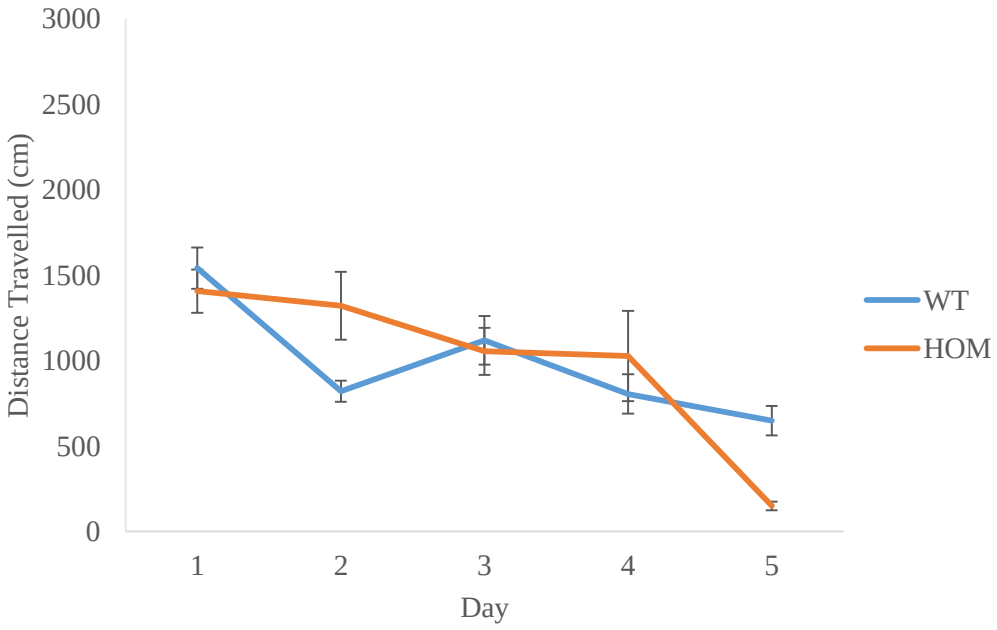


Figure 4. Distance travelled during the habituation phase. Vertical lines represent ±1 standard error of the mean.

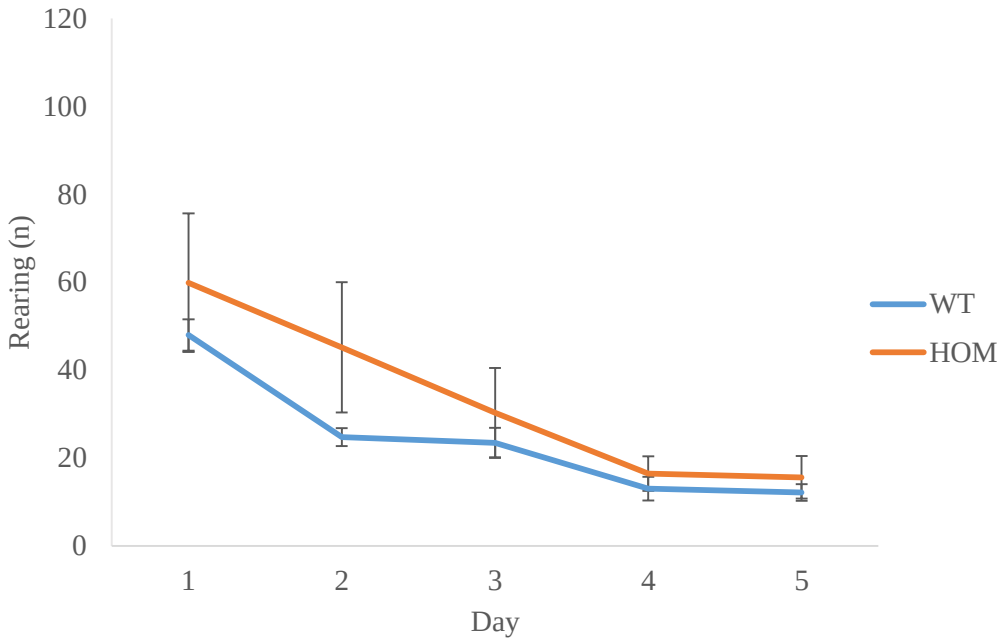


Figure 5. Rearing during the habituation phase. Vertical lines represent ±1 standard error of the mean.

Anticipation Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype on distance travelled and rearing during the anticipatory phase. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated for rearing, $\chi 2$ (9) = 38.01, p < .050. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, ε = 0.46.

There was no main effect of genotype for distance travelled, F (1, 12) = 2.75, p = .123. Distance travelled was similar for WT rats (M = 1634.99; SD = 175.35) and HOM rats (M = 1224.10; SD = 175.35). There was a significant main effect of genotype for rearing, F (1, 12) = 9.59, p < .050. Rearing was greater for WT rats (M = 49.03; SD = 6.58) than for HOM rats (M = 19.40; SD = 6.58). There was a significant main effect of days for distance travelled, F (4, 48) = 14.23, p < .050; and rearing, F (1.85, 22.16) = 5.40, p < .050. Distance travelled and rearing increased over the anticipation phase. There was no interaction between

days and genotype for distance travelled, F (4, 48) = 0.41, p = .802. Distance travelled increased similarly in HOM and WT rats. However, there was a significant interaction between days and genotype for rearing, F (1.85, 22.16) = 4.01, p = .035. This indicates that rearing during the anticipation phase was dependent on genotype.

Simple effect analyses were used to further examine the interaction between genotype and rearing. These analyses indicated that for WT rats, there was a significant main effect of day, F (4, 24) = 4.90, p < .050. Rearing increased across the anticipation phase. However, for HOM rats, there was no main effect of day, F (4, 24) = 2.65, p = .058. This suggests that WT rats engaged much more in anticipatory rearing than HOM rats.

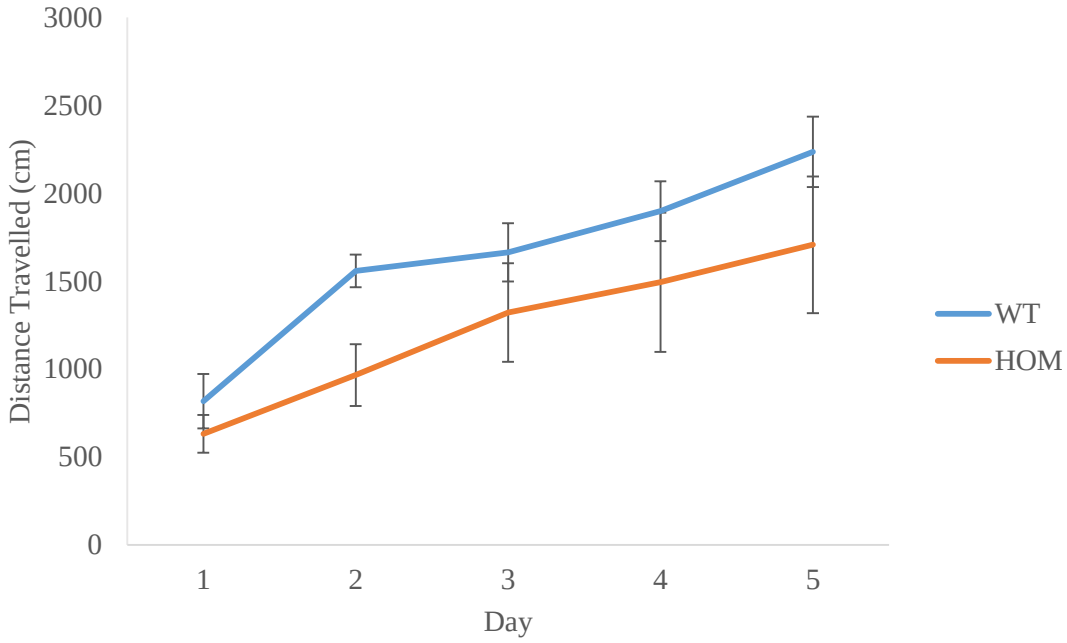


Figure 6. Distance travelled during the anticipation phase. Vertical lines represent ±1 standard error of the mean.

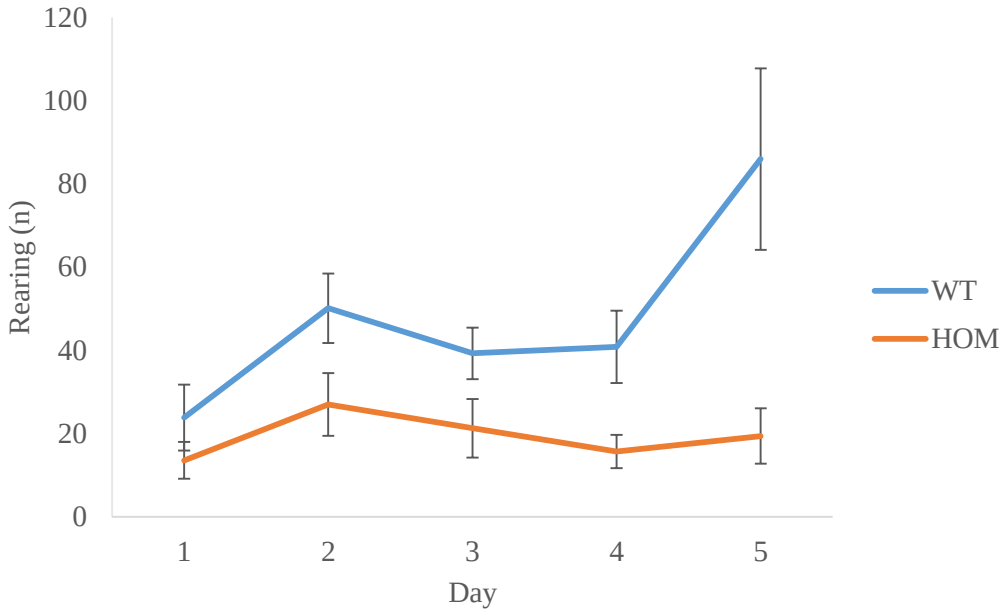


Figure 7. Rearing during the anticipation phase. Vertical lines represent ±1 standard error of the mean.

Anticipation Phase: First Session

A mixed-factor ANOVA was also conducted to determine the effect of genotype on distance travelled and rearing during the first session of the anticipation phase, using blocks of 5 minutes as the within subject factor. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated for distance travelled, $\chi 2$ (14) = 47.85, $p < .050$; and rearing, $\chi 2$ (14) = 49.72, $p < .050$. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon for distance travelled, $\varepsilon = 0.54$; and rearing, $\varepsilon = 0.50$.

There was no main effect of genotype for distance travelled, F (1, 12) = .96, p = .347; or rearing, F (1, 12) = 1.29, p = .279. Distance travelled was similar for WT rats (M = 817.37; SD = 411.75) and HOM rats (M = 631.88; SD = 285.51). Rearing was similar for WT rats (M = 23.88; SD = 20.96) and HOM rats (M = 13.57; SD = 11.65). There was a significant main effect of time for distance travelled, F (2.67, 32.08) = 26.45, $p < .050$; and rearing, F (2.52, 30.17) = 5.42, $p < .050$. Distance travelled and rearing decreased over the first session of the anticipation phase, indicative of the normal habituation to the locomotor boxes. There was no

interaction between time and genotype for distance travelled, F (2.67, 32.08) = 1.48, p = .241; or rearing, F (2.52, 30.17) = 1.60, p = .216. This indicates that the distance travelled and rearing during the first session was not dependent on genotype.

Anticipation Phase: Last Session

A mixed-factor ANOVA was conducted to determine the effect of genotype on distance travelled and rearing during the last session of the anticipation phase, again using blocks of 5 minutes as the within subject factor. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated for rearing, $\chi 2$ (14) = 55.48, $p < .050$. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, ε = 0.38.

There was no main effect of genotype for distance travelled, F (1, 12) = 1.46, p = .250. Distance travelled was similar for WT rats (M =2236.26; SD = 529.86) and HOM rats (M = 1707.00; SD = 1030.94). There was a significant main effect of genotype for rearing, F (1, 12) = 8.52, $p < .050$. Rearing was greater for WT rats (M = 86.00; SD = 57.69) than HOM rats (M = 19.43; SD = 17.63). There was a significant main effect of time for distance travelled, F (5, 60) = 7.37, $p < .050$. The distance travelled decreased significantly over the last session of the anticipation phase. There was no interaction between time and genotype for distance travelled, F (5, 60) = .53, p = 750; and rearing, F (1.88, 22.53) = 1.01, p = .376. This indicates that distance travelled and rearing during the last session was not dependent on genotype.

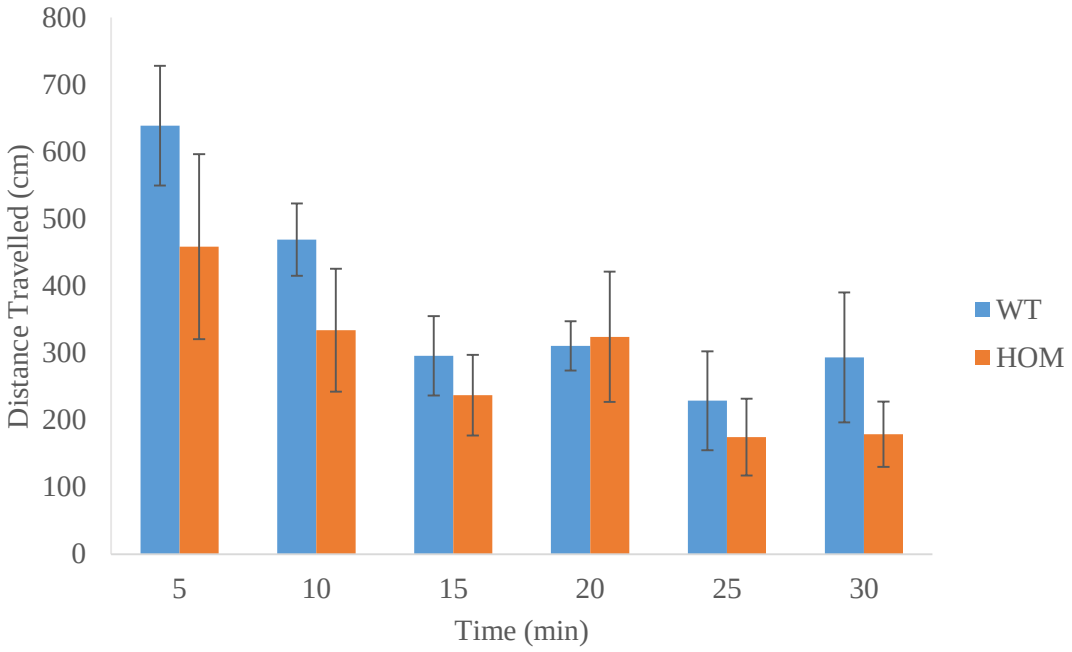


Figure 8. Distance travelled during the last session of the anticipation phase. Vertical lines represent ±1 standard error of the mean.

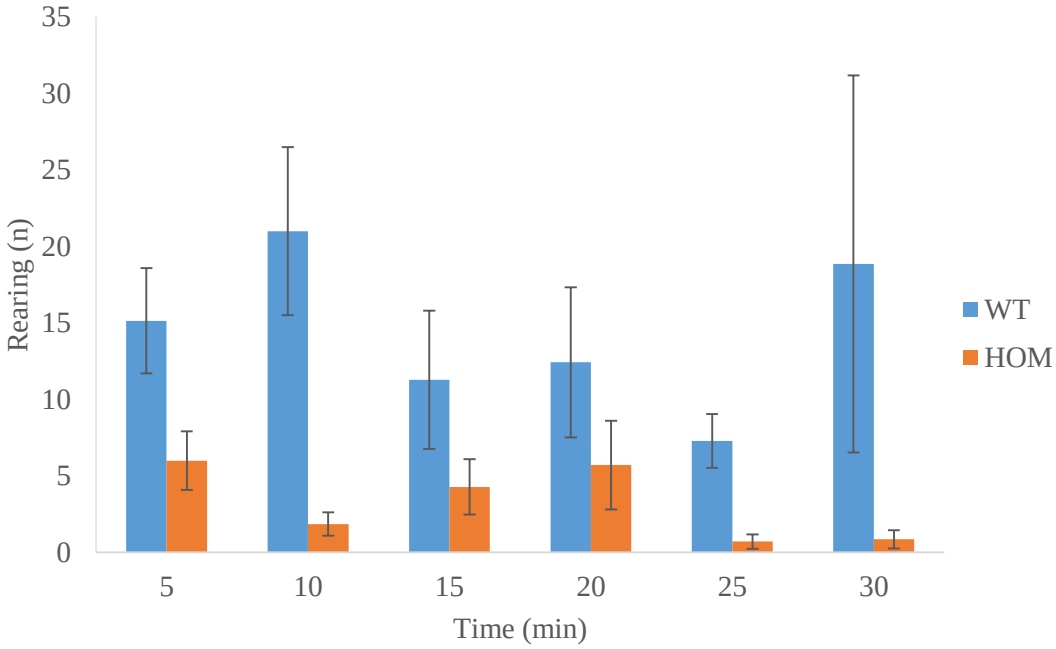


Figure 9. Rearing during last session of the anticipation phase. Vertical lines represent ±1 standard error of the mean.

Discussion

The aim of this experiment was to determine the role of the DA D_1 receptor in anticipatory locomotor activity. Anticipatory locomotor activity was considered a measure of reward prediction. Important to note, no difference between D_1 mutant rats and WT rats were found in distance travelled during the habituation phase. This suggests that locomotor activity in D_1 mutant rats is normal. This is an important finding as the DA system is highly implicated in movement and motor activity (Beninger, 1983). The fact that the D_1 mutants did not show a reduction in motor activity could be due to either compensatory mechanisms or due to the fact that the mutation only reduced D_1 binding by about 50%. In any case, this finding shows that reductions in locomotor activity is not a confounding factor in this and other experiments presented here.

The hypothesis that D_1 mutant rats would show reduced anticipatory locomotor activity was supported. It was found that D_1 mutant rats engaged is significantly less anticipatory rearing behaviour prior to conditioned food presentation, compared to WT rats. Unfortunately, there is currently no research on the role of the DA D_1 receptors in food anticipatory locomotor activity. However, there is considerable research on the general role of the DA system in anticipatory locomotor activity. This research has produced inconsistent findings. Some studies show that administration of DA antagonists reduced anticipatory activity (Barbono & Cador, 2006; Blackburn et al., 1987; Salamone, 1988). In contrast, other studies show that administration of DA antagonists had no effect on anticipatory activity (Jones & Robbins, 1992; Mistlberger & Mumby, 1992). A factor that may account for these inconsistent findings is food palatability. Studies in which animals were presented with palatable food showed that DA antagonists reduced food anticipatory locomotor activity (Blackburn et al., 1987; Barbano & Cador, 2006; Salamone, 1988). In contrast, studies in which animals were presented with normal chow showed that DA antagonists had no effect

on food anticipatory activity (Bardona & Cador, 2006; Jones & Robbins, 1992; Mistlberger & Mumby, 1992). Together, these studies suggest that food palatability may be an important factor in the dopaminergic modulation of anticipatory activity. Interestingly, in the present experiment we used normal chow. Given, that only the WT rats developed anticipatory rearing behaviour, thus suggests that perhaps the D_1 receptors are involved anticipation whereas D_2 receptors are involved in food palatability. In line with this, Buck and colleagues (2014) found that SCH 23390 (D_1 receptor antagonist) dose dependently blocked anticipatory 50 kHz vocalizations.

The finding that the D_1 mutant rats engaged in less anticipatory rearing behaviour is consistent with the theory that the DA D_1 receptor is involved in reward prediction. A large body of evidence suggests that the DA system becomes activated in anticipation of expected reward. Electrophysiological studies show that DA neurons in the ventral and dorsal striatum initially fire in response to reward (Schultz, 1998). After repeated cue-reward pairings, DA neurons fire in response to reward-predictive cues rather than the reward itself (Schultz, 2007). In line with this, voltammetry studies show that increased DA release when rats are presented with a cue that signals food reward (Phillips et al., 1993). Moreover, micro dialysis studies show that DA transmission in the medial prefrontal cortex is enhanced when rats are presented with environmental cues preceding palatable food (Bassareo & Di Chiara, 1997). Given that the prefrontal cortex contains many more D_1 than D_2 receptors, this makes a role for the D_1 receptors in reward prediction more likely. Together, these findings suggest that the DA system plays in important role in the reward prediction.

More generally, the finding that D_1 mutant rats engaged in less anticipatory rearing behaviour is consistent with the theory that the DA D_1 receptor is involved in incentive salience. Incentive salience is a type of motivation attributed to reward-predicting cues (Berridge, 2004). When attributed with incentive salience, reward-predicting cues are

transformed into wanted incentives that elicit motivated behaviour. In the current study, rats were placed in an activity chamber followed by the presentation of food. After repeated pairings, the activity chamber may take on the incentive properties of this food, thus eliciting reward-seeking behaviour (likely reflected in anticipatory activity).

Contrary to the hypothesis, no difference between D_1 mutant rats and WT rats was found for distance travelled prior to conditioned food presentation. A possible explanation for this finding is that rearing may be exclusively regulated by reward anticipation whereas distance travelled may be only partially regulated by reward anticipation. There are at least two other factors that may contribute to the development of anticipatory activity. The first factor is homeostatic state of the rats (food-restricted vs. food-satiated). Studies show that food-restricted rats, but not food satiated rats, developed anticipatory activity prior to food presentation (Barbano & Cador, 2005; Barbano & Cador; 2006). The second factor is the circadian clock of the rat. The circadian clock is entrained when animals are exposed to restricted feeding schedules that allow them to feed at a fixed time each day (as is the case in the current experiment). Studies show that mice lacking circadian clocks (via $Per2^{Brdm1}$ mutation or $Bmal^{-/-}$ mutation) fail to develop food anticipatory locomotion (Fuler, Lu & Saper, 2008).

Important to note is that anticipation can be both positive and negative. *Positive anticipation* refers to the anticipation of positive stimuli (e.g. food, sex) whereas *negative anticipation* refers to the anticipation of negative stimuli (e.g. food shock, tail pinch). There is considerable evidence indicating that D_1 receptors are necessary for coding positive stimuli whereas D_2 receptors are necessary for coding negative symptoms (Young, Moran, Joseph, 2005). In this experiment, only positive stimuli were used (i.e. food reward). Whilst the current findings support the theory that D_1 receptors code positive stimuli, it would be useful to examine negative stimuli.

In conclusion, this experiment revealed that the DA D_1 receptor are involved in anticipatory rearing behaviour prior to conditioned food presentation. This finding supports the theory that the DA D_1 receptor plays an important role in reward prediction, particularly in anticipatory responses to expected reward.

CHAPTER 4: Successive Negative Contrasts

Methods

Animal subjects

Test subjects were adult male D_1 mutant Wistar rats (n = 18) and adult male wild-type (WT) Wistar rats (n = 20). All rats were bred in the vivarium of Victoria University of Wellington. They were housed individually in standard polycarbonate cages. Water was available *ad libitum* at all times except during the experiment. The housing room had a controlled temperature of temperature of 21 °C, humidity of 55%, and was maintained on a 12-h light/dark cycle with lights on at 0700 h.

All rats were placed on food deprivation one week to the experiment. During food deprivation, food intake was limited to 15 g per day. This reduced body weight of the rats to approximately 85% of their free feeding weight.

Apparatus

The successive negative contrast experiment was conducted in the home cages. The sucrose solutions were administered in white plastic drink bottles with non-leak stoppers in the drinking tubes. These non-leak stoppers were designed to ensure that fluid intake calculations were as accurate as possible.

The 32% sucrose solution was prepared by mixing 32g of commercial sugar for every 68g of distilled water. The 4% sucrose solution was prepared by mixing 4g of commercial sugar for every 96g of distilled water.

Procedure

Rats were randomly assigned to one of two different groups: the 32-4 group and 4-4 group. Rats assigned to the 32-4 group received 32% sucrose solution during the pre-shift

phase and received 4% sucrose solution during the post-shift phase. Rats assigned to the 4-4 group received 4% sucrose solution during the pre-shift phase and continued to receive 4% sucrose solution during the post-shift phase. Two days prior to the experiment, both groups were given access to their respective sucrose solution for one hour, in their home cages.

The experiment consisted of two phases: the pre-shift phase and the post-shift phase. During the pre-shift phase, both groups were given access to their respective sucrose solution for 5 minutes per day, for 10 days. During the post-shift phase, both groups were given access to 4% sucrose solution for five minutes per day, for 8 days.

Statistical Analysis

Data were analyzed using a mixed-factor ANOVA. The between subject factor was genotype (WT, HOM) and group (4-4, 32-4). The within subject factor was day. Data were analyzed using SPSS software. A p value of $< .050$ was considered as statistically significant. Data was expressed as mean ± S.E.M.

Results

Pre-Shift Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype and group on sucrose intake during the pre-shift phase. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated, $\chi 2$ (44) = 140.45, $p < .050$. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, $\varepsilon = 0.65$.

There was a significant main effect of day, F (5.82, 197.82) = 18.97, $p < .050$. Sucrose intake increased over the pre-shift phase. There was no main effect of genotype, F (1, 34) = 0.00, $p = .970$. Sucrose intake was similar for WT rats ($M = 4.44$; $SD = 0.44$) and HOM rats ($M = 4.46$; $SD = 0.47$). There was a significant main effect of group, F (1, 34) = 13.98, $p < .050$. Sucrose intake was greater in the 32-4 group ($M = 5.65$; $SD = 0.46$) than in

the 4-4 group (M = 3.24; SD = 0.46). There was no interaction between day and genotype, F (5.82, 197.82) = 1.34, p = .242; or between day and group, F (5.82, 197.82) = 1.66, p = .135. More important, however, there was a significant interaction between day, genotype, and group, F (5.82, 197.82) = 2.19, p < .050. This indicates that the sucrose intake was dependent on day, genotype and group. Therefore, the data were split dependent on the pre-shift sucrose concentration and mixed-factor ANOVAs were used to further examine this interaction.

4-4 group

Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated, $\chi 2$ (44) = 108.77, p < .050. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, ε = 0.45. In the 4-4 group, there was a significant main effect of genotype, F (1, 17) = 4.46, p < .050. Sucrose intake was greater for HOM rats (M = 4.17; SD =.64) than WT rats (M = 2.32; SD = 0.60). There was a significant main effect of day, F (4.07, 69.24) = 5.01, p < .050. Sucrose intake increased over the pre-shift phase. There was a significant interaction between day and genotype, F (4.07, 69.24) = 2.70, p < .050. This indicates that the increase in sucrose intake was dependent on day and genotype.

Simple effect analyses were used to further examine the interaction between day and genotype. These analyses indicated that there was a significant main effect of genotype on day 5, F (1, 17) = 5.78, p < .050; on day 6, F (1, 17) = 6.00, p < .050; on day 8, F (1, 17) = 8.17, P < .050; on day 9, F (1, 17) = 4.57, p < .050; and on day 10, F (1, 17) = 4.89, p < .050. Sucrose intake was greater for WT rats than HOM rats on day 5, day 6, day 8, day 9, and day 10.

32-4 group

Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated, $\chi 2$ (44) = 88.68, p < .050. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, ε = 0.75. In the 32-4 group, there was no main effect of

genotype, F (1, 17) = 3.64, p = .074. Sucrose intake was similar for HOM rats (M = 4.76; SD = 0.69) and WT rats (M = 6.56; SD = 0.65). There was a significant main effect of day, F (6.73, 114.43) = 16.30, p < .050. Sucrose intake increased over the pre-shift phase. There was no interaction between day and genotype, F (6.73, 114.43) = .65, p < .705. This indicates that the increase in sucrose intake was not dependent on day and genotype.

Post-Shift Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype and group on sucrose intake during the post-shift phase. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated, $\chi 2$ (27) = 62.12, p < .050. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon, ε = 0.79.

There was a significant main effect of day, F (5.56, 188.88) = 6.64, p < .050. Sucrose intake increased over the post-shift phase. There was no main effect of genotype, F (1, 34) = 1.98, p = .169. Sucrose intake was similar for WT rats (M = 5.03; SD = 0.53) and HOM rats (M = 6.12; SD = 0.56). There was no main effect of group, F (1, 34) = 2.42, p = .129. Sucrose intake was similar in the 32-4 group (M = 6.18; SD = 0.59) and the 4-4 group (M = 4.97; SD = 0.59). There was no interaction between day and genotype, F (5.56, 188.88) = .61, p = .708; between day and group, F (5.56, 188.88) = 1.24, p = .290; or between day, genotype and group, F (5.56, 188.88) = 1.17, p = .324. This indicates that sucrose intake was not dependent on genotype, or group.

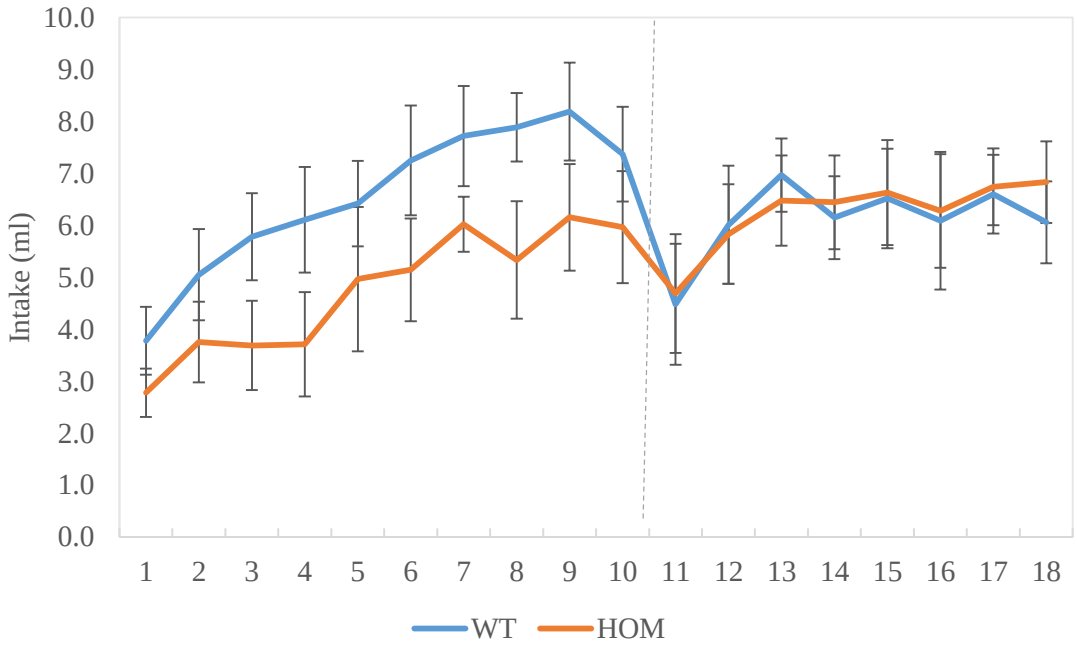


Figure 10. Sucrose intake in the 4-4% group. Vertical lines represent ±1 standard error of the mean

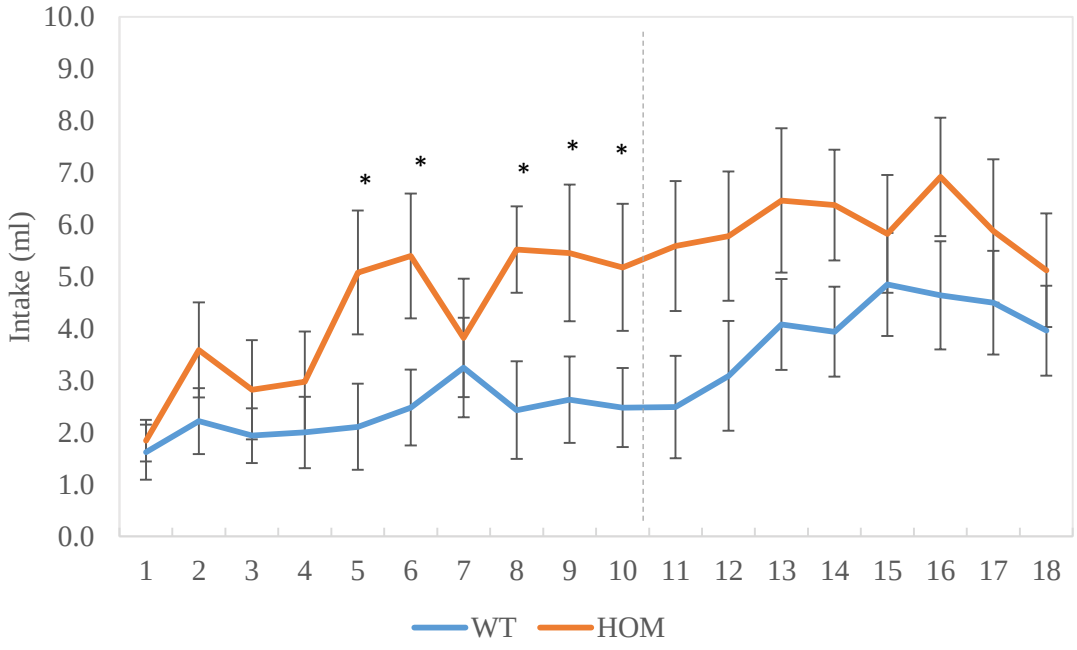


Figure 11. Sucrose intake in the 32-4% group. Vertical lines represent ±1 standard error of the mean. * $p < .050$.

Pre-shift Day 10 and Post-Shift Day 1

A mixed-factor ANOVA was conducted to determine the effect of genotype and group on sucrose intake on pre-shift day 10 and post-shift day 1. There was a significant main effect of day, F (1, 34) = 11.18, $p < .050$. Sucrose intake decreased from pre-shift day 10 and post-shift day 1. There was no main effect of genotype, F (1, 34) = 1.73, $p = .198$. Sucrose intake was similar for WT rats ($M = 4.21$; $SD = 0.60$) and HOM rats ($M = 5.36$; $SD = 0.64$). There was no interaction between day and genotype, F (1, 34) = 3.23, $p = .081$; or between day, genotype, and group, F (1, 34) = 1.17, $p = .287$. However, there was a significant interaction between day and group, F (1, 34) = 16.78, $p < .050$. This indicates that sucrose intake was dependent on day and group. Therefore, the data were split dependent on the pre-shift sucrose concentration and mixed-factor ANOVAs were used to further examine this interaction.

4-4 group

There was no main effect of day, F (1, 17) = .38, $p = .547$. Sucrose intake was similar on pre-shift day 10 and post-shift day 1. There was a significant main effect of genotype, F (1, 17) = 5.42, $p < .050$. Sucrose intake was greater for HOM rats ($M = 5.38$; $SD = 0.90$) than WT rats ($M = 2.49$; $SD = 0.86$). There was no interaction between day and genotype, F (1, 17) = .34, $p = .566$. This indicates that sucrose intake was not dependent on day or genotype.

32-4 group

There was a significant main effect of day, F (1, 17) = 22.12, $p < .050$. Sucrose intake decreased from pre-shift day 10 to post-shift day 1. There was no main effect of genotype, F (1, 17) = 0.24, $p = .634$. Sucrose intake was similar for WT rats ($M = 5.93$; $SD = .848$) and HOM rats ($M = 5.33$; $SD = 0.89$). There was no significant interaction between day and genotype, F (1, 17) = 3.31, $p = .086$. This indicates that in the decrease in sucrose intake was not significantly dependent on genotype, although there was a clear trend.

Discussion

The aim of the present study was to determine the role of the DA D_1 receptor in the SNC effect. The SNC effect is considered a measure of reward prediction. It was hypothesized that D_1 mutant rats would show reduced consummatory SNC effect, compared to WT rats. Contrary to this hypothesis, it was found that both D_1 mutant rats and WT rats failed to show a consummatory SNC effect. Although both D_1 mutant rats and WT rats reduced their consumption following the downshift from 32 to 4% sucrose solution, their consumption was not less than D_1 mutant rats and WT rats that received only the 4% sucrose solution.

Unfortunately, there is currently no previous research on the role of the DA D_1 receptors in the SNC effect. Thus, it is impossible to determine whether these findings are consistent with previous research. However, there is considerable research on the general role of the DA system in the SNC effect. This research has provided consistent evidence demonstrating that DA manipulation affect the magnitude of the SNC effect. For example, administration of amphetamine (indirect DA agonist) has been found to *attenuate* the instrumental SNC effect (Phelps, Mitchell, Nutt, Marston & Robinson, 2015). The number of nose-poke responses was only slightly decreased after the down-shift from a four pellet to a one pellet reward. Conversely, administration of alpha-flupentixol (a non-selective DA antagonists) has been found to *potentiate* instrumental SNC effect (Phelps et al., 2015). The number of nose-poke responses was greatly decreased after the down-shift from a four pellet to a one pellet reward. In line with this, rats on amphetamine withdrawal showed a potentiated consummatory SNC effect (Barr & Phillips, 2002). The number of licking responses was greatly decreased after the down-shift from a 32 to 4% sucrose solution. Together, these findings suggest that the DA system is involved in regulating the magnitude of the SNC effect. Unfortunately, they do not reveal the specific role of the DA D_1 receptor in this function.

The finding that WT rats failed to show a SNC effect does not allow us to draw any conclusions regarding the role of DA D_1 receptor in the SNC effect (Genn, Ahn & Phillips, 2004; Tobler, Fiorillo & Schultz, 2005). At present, it is unclear why the WT rats did not show a SNC effect. Most experiments examining the SNC effect use lever presses or number of licks (using a lick-o-meter). It is possible that measuring total consumption was not sensitive enough to detect subtle differences in overall drinking patterns. Previous research in our laboratory showed that D_1 mutant rats failed to lever press for reward (i.e. sucrose pellets). Thus, using operant chambers with levers was not an option in the present experiment.

Several researchers suggest that the SNC effect is mediated by a number of different mechanisms (Phelps et al., 2015). Two such mechanisms include: the motivation to respond to obtain a reward (e.g. response latency) and the motivation to collect a reward (collection latency). These two mechanisms are mediated by different neural systems. Several studies demonstrate that manipulation of the DA system impairs response latency but does not impair collection latency. For example, administration of amphetamine (indirect DA agonist) has been found to *attenuate* the instrumental SNC effect on response latency but have no effect on collection latency (Phelps et al., 2015). Likewise, administration of alpha-fluxenthixol (DA antagonist) has been found to *potentiate* the SNC effect on response latency but has no effect on collection latency (Phelps et al., 2015). Taken together, these findings suggest that the DA system is involved in the motivation to respond to obtain reward but is not involved in the motivation to collect reward. The current experiment only measured the motivation to collect reward.

As mentioned above, D_1 mutant rats failed to lever press for sucrose pellets. However, when presented with sucrose pellets in the home cage, they quickly consume them (Hanna Squire Buchanan, unpublished PhD thesis). This finding supports the notion that DA (and

indeed the D_1 receptor) is involved in the motivation to respond but not the motivation to collect reward.

Although not directly relevant to the SNC effect, it was found that sucrose intake during the pre-shift phase was similar for D_1 mutant rats in the 4-4 group and those in the 32-4 group. However, sucrose intake during the pre-shift phase was greater for WT rats in the 32-4 group than those in the 4-4 group. A potential explanation for this finding is that D_1 mutant rats have a deficit in their ability to represent the value of (moderate) reward. If they are unable to represent reward value (i.e. high value- 32% sucrose solution; low value- 4% sucrose solution), then they will likely consume a similar amount of each.

In conclusion, this experiment failed to detect a clear SNC effect in WT rats, thus preventing any conclusions regarding the role of the DA D_1 receptors in the response to unexpected alterations in reward. Future research, using more sensitive measures is needed to assess the SNC effect in WT rats and to investigate whether the D_1 receptors is involved in this aspect of reward prediction.

CHAPTER 5: Social Approach Experiment

Methods

Subjects

Target subjects used as 'strangers' were juvenile male Wistar rats. Test subjects were adult male D_1 mutant Wistar rats (n = 11) and adult male wild-type (WT) Wistar rats (n = 7). All rats were bred in the vivarium of Victoria University of Wellington. They were housed in groups of three to five in standard polycarbonate cages. Food and water was available *ad libitum* at all times except when under experimental restrictions. The housing room had a controlled temperature of temperature of 21 °C, humidity of 55%, and was maintained on a 12-h light/dark cycle with lights on at 0700 h.

Apparatus

The social approach was conducted in a T-maze from black acrylic plastic (arm dimensions: 50 cm long, 20 cm wide, 25 cm high walls). This box contained removable partitions separating the box into three chambers. During testing, the T shaped box was centered on a table to minimize environmental conditions that could produce side preference (e.g. gradients in light, temperature, and sound).

Cylindrical wire mesh cages were used to contain stranger rats (diameter: 10.5 cm). A weighted cup was placed on top of the wire cage to prevent the stranger rat from escaping or the test rat from climbing on top. Before the start of each test session, the T shaped box was cleaned with a 70% ethanol-30% water solution, and dried with paper towels.

Procedure

The social approach task consisted of three phases; habituation, sociability, and social novelty. During the habituation phase, the test rat was placed into the T-maze and allowed to

freely explore for 10 minutes. After 10 minutes, the test rat was removed from the T-maze and placed back in a polycarbonate cage.

The sociability phase was conducted immediately after the habituation phase. During this phase, two cylindrical wire mesh cages were placed into the T-maze, one in each side chamber. A male rat (stranger) was placed under one of these cages while the other cage remained empty. The test rat was placed back into the T-maze and allowed to explore for 5 minutes. After 5 minutes, the test rat was removed from the T-maze and placed back in the polycarbonate cage.

The social novelty phase was conducted immediately following the sociability phase. During this phase, a second male rat (unfamiliar stranger) was placed under the previously empty cage while the first male rat (familiar stranger) remained in the other cage. The test rat was placed back into the T-maze and allowed to explore for 5 minutes. After 5 minutes, all rats were removed from the T-maze and returned to their home cages. The behaviour of the test rat was measured using Ethovison XT. The time spent in each zone and the number of zone entries were analyzed.

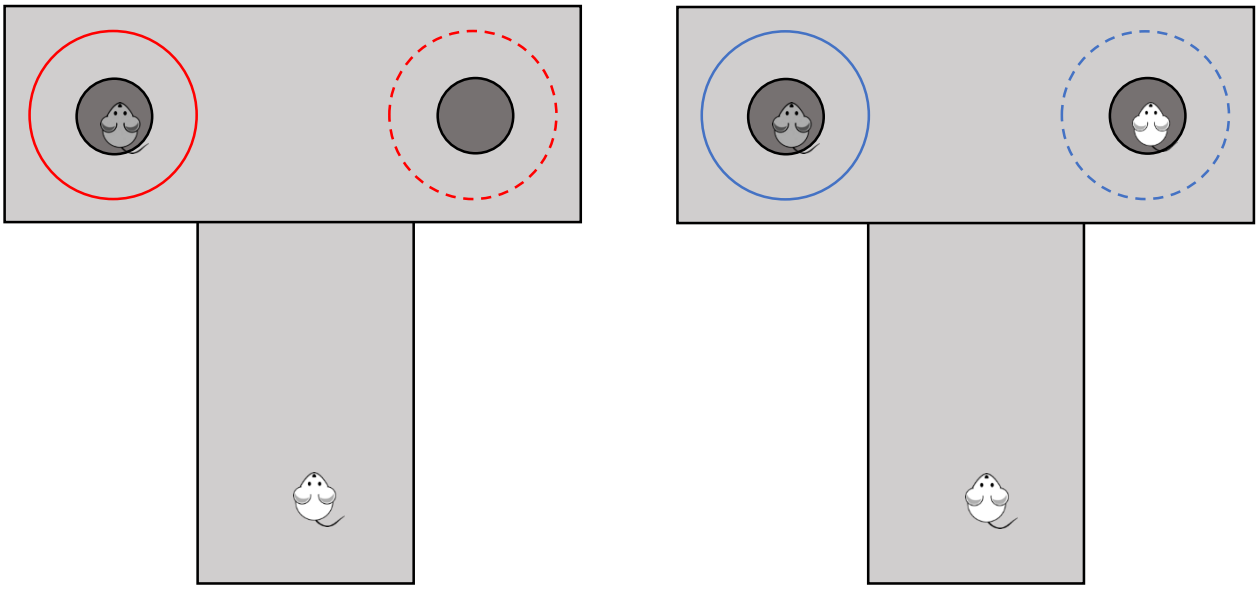


Figure 12. Social approach experiment

Statistical Analysis

Data were analyzed using a one-way ANOVA (distance travelled), and a mixed-factor ANOVA (number of zone entries). The between subject factor was genotype (WT, HOM) and the within subject factor was zone type (social, non-social; unfamiliar, familiar). Data were analyzed using SPSS software. A p value of $< .050$ was considered as statistically significant. Data was expressed as mean ± S.E.M.

Results

Total Distance Travelled

A one-way ANOVA was conducted to determine the effect of genotype on the total distance travelled. There was no main effect of genotype for the habituation phase, F (1, 16) = 1.88, p = .189; the sociability phase, F (1, 16) = .27, p = .611; or the social novelty

preference phase, F (1, 16) = 2.57, p = .125. The total distance travelled during all three phases was similar for WT and HOM rats.

Number of Zone Entries: Sociability Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype and zone type on the number of zone entries. There was a significant main effect of genotype, F (1, 16) = 6.51, p < .050. The number of zone entries was greater for WT rats (M = 28.43; SD = 42.56) compared to HOM rats (M = 9.14; SD = 10.92). There was a significant main effect of zone type, F (1, 16) = 9.34, p < .050. The number of zone entries were greater in the social zone (M = 28.11; SD = 36.77) than in the non-social zone (M = 5.17; SD = 9.41). There was a significant interaction between genotype and zone type, F (1, 16) = 4.23, p < .050. Simple effect analyses were used to further examine the interaction between genotype and zone type.

A one-way ANOVA revealed that there was a significant main effect of genotype for the number of entries into the social zone, F (1, 16) = 5.65, p < .050. The number of entries into the social zone were greater for WT rats (M = 51.00; SD = 12.32) than HOM rats (M = 13.55; SD = 9.83). There was no main effect of genotype for the number of entries into the non-social zone, F (1, 16) = 0.06, p = .812. The number of entries into the non-social zone were similar for WT rats (M = 5.86; SD = 3.66) and HOM rats (M = 4.73; SD = 2.92).

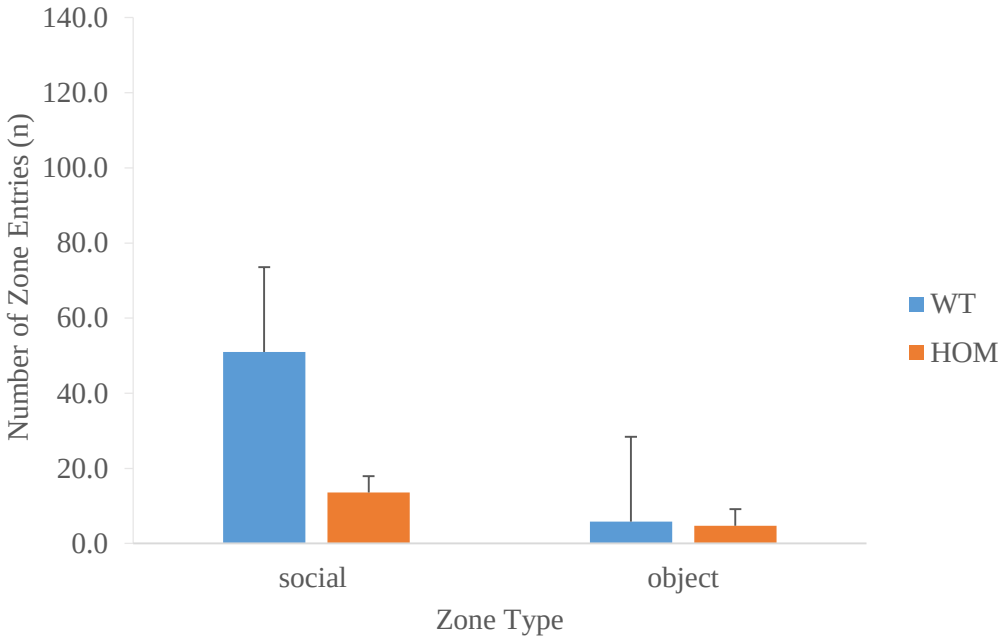


Figure 13. Number of entries into the social and non-social zone during the sociability phase. Vertical lines represent ±1 standard error of the mean.

Number of Zone Entries: Social Novelty Preference Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype and zone type on the number of zone entries. There was a significant main effect of genotype, F (1, 16) = 6.89, $p < .050$. The number of zone entries was greater for WT rats (M = 46.71; SD = 77.15) than HOM rats (M = 10.46; SD = 14.18). There was a significant main effect of zone type, F (1, 16) = 5.92, $p < .050$. The number of zone entries was greater in the unfamiliar zone (M = 40.50; SD = 69.61) than in the familiar zone (M = 8.61; SD = 8.80). There was a significant interaction between genotype and zone type, F (1, 16) = 4.50, $p < .050$. This indicates that the number of unfamiliar and familiar zone entries was dependent upon genotype. Simple effect analyses were used to further examine the interaction between genotype and group.

A one-way ANOVA revealed that there was significant main effect of genotype for the number of entries into the unfamiliar zone, F (1, 16) = 5.61, $p < .050$. The number of

entries into the unfamiliar zone was greater for WT rats (*M* = 83.71; *SD* = 23.33) than HOM rats (*M* = 13.00; *SD* = 18.61). However, there was no main effect of genotype for the number of entries into the familiar zone, *F* (1, 16) = 0.17, *p* = .684. The number of entries into the familiar zone was similar for WT rats (*M* = 9.71; *SD* = 3.41) and HOM rats (*M* = 7.91; *SD* = 2.72).

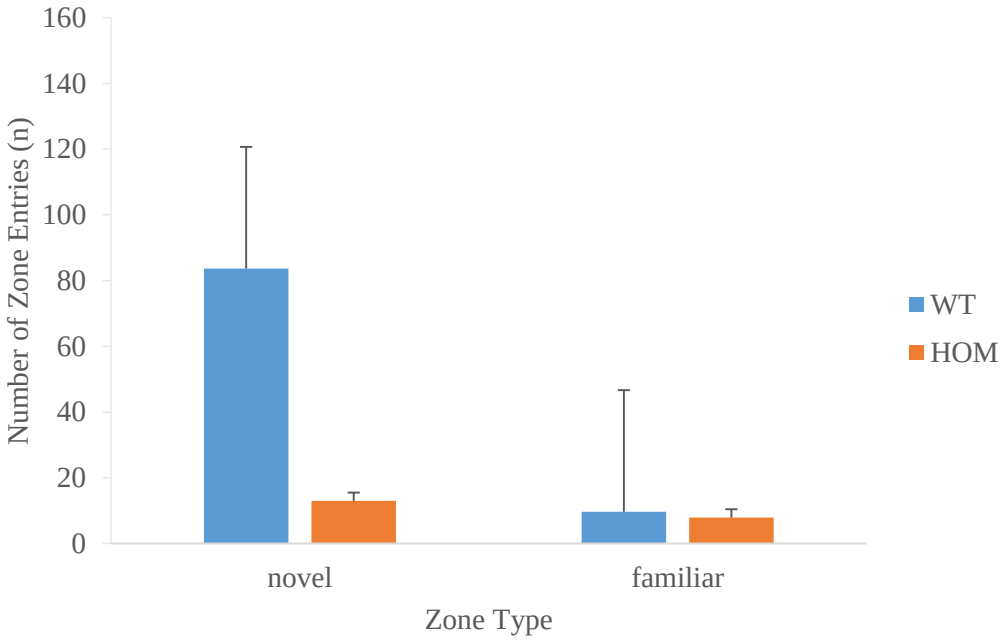


Figure 14. Number of entries into the familiar and non-familiar zone during the social novelty preference phase. Vertical lines represent ±1 standard error of the mean.

Time Spent in Zone: Sociability Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype and zone type on the duration of zone entries. There was no main effect of genotype, *F* (1, 16) = 0.05, *p* = .835. The duration of entries was similar for WT rats (*M* = 110.31; *SD* = 109.23) and HOM rats (*M* = 113.97; *SD* = 123.04). There was a significant main effect of zone type, *F* (1,16) = 4.90, *p* < .050. The duration of entries was greater in the social zone (*M* = 166.84; *SD* = 107.34) than in the non-social zone (*M* = 58.26; *SD* = 100.36). There was no interaction

between genotype and zone type, F (1, 16) = .00, p = .963. This indicates that the duration of entries in the social and non-social zone was not dependent on genotype.

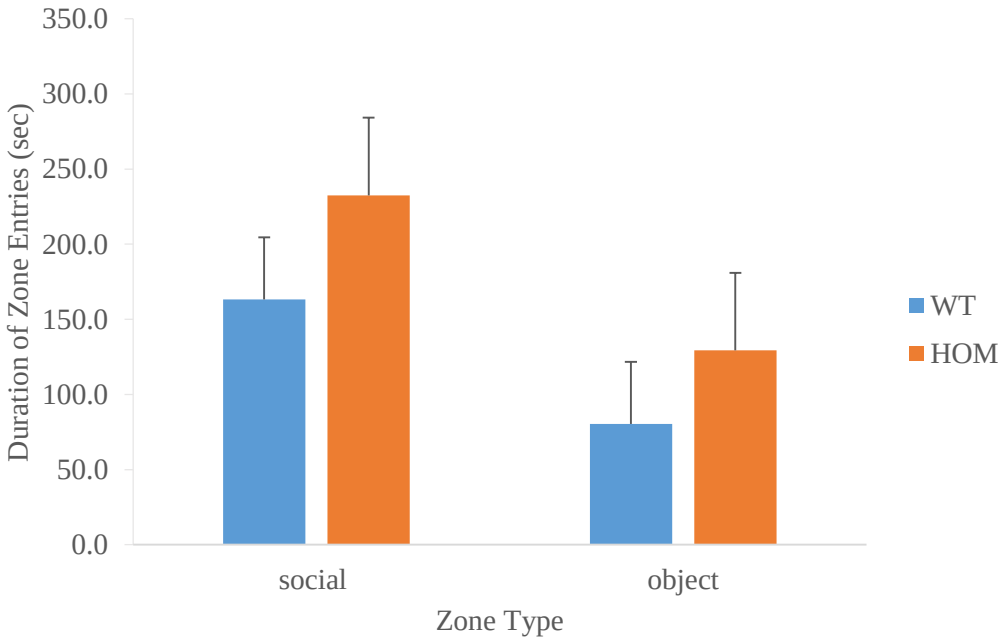


Figure 15. Duration of zone entries in the social and non-social zone during the sociability phase. Vertical lines represent ±1 standard error of the mean.

Time Spent in Zone: Social Novelty Preference Phase

A mixed-factor ANOVA was conducted to determine the effect of genotype and zone type on the duration of zone entries. There was no main effect of genotype, F (1, 16) = 0.97, p = .339. The duration of entries was similar for WT rats (M = 108.00; SD = 94.49) and HOM rats (M = 122.83; SD = 116.78). There was no main effect of zone type, F (1, 16) = 0.10 p = .758. The duration of entries was similar for the unfamiliar zone (M = 125.18; SD = 101.41) and familiar zone (M = 108.95; SD = 116.85). There was no interaction between genotype and zone type, F (1, 16) = 0.00, p = .990. This indicates that the duration of entries in the unfamiliar and familiar zone was not dependent on genotype.

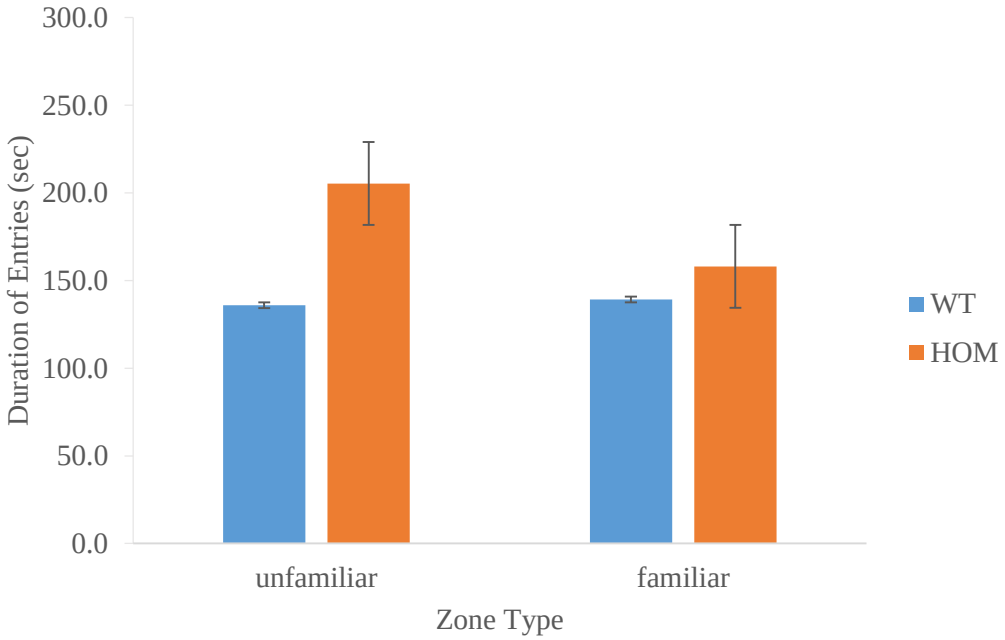


Figure 16. Duration of entries in the unfamiliar and familiar zone during the social novelty preference phase. Vertical lines represent ±1 standard error of the mean.

Discussion

The aim of the social approach experiment was to determine the role of DA D_1 receptors in social reward-seeking behaviour and social consummatory behaviour. The number of entries into the social zone (sociability phase) and the unfamiliar zone (social novelty phase) was considered a measure of social reward-seeking behaviour. The amount of time spent in the social zone and unfamiliar zone was considered a measure of social consummatory behaviour.

Important to note, no difference between D_1 mutant rats and WT rats were found in distance travelled during all three phased of the experiment (habituation, sociability, social novelty preference). This suggests that locomotor activity in D_1 mutant is normal, in line with the results from the experiments in chapter 3, and further emphasize that reduced locomotor activity in D_1 mutant rats is not a confounding factor.

The first hypothesis that the D_1 mutant rats would make fewer entries into the social zone and the unfamiliar zone was supported. It was found that the D_1 mutant rats made significantly fewer entries into the social zone (sociability phase) compared to WT rats. Similarly, it was found that D_1 mutant rats made significantly fewer entries into the unfamiliar zone (social novelty phase) compared to WT rats.

Unfortunately, there is currently no research on the role of the DA D_1 receptor in social reward-seeking behaviour. However, there is considerable research on the role of the DA D_1 receptor in general social behaviour. This research has produced a complex array of findings. For example, administration of D_1 receptor antagonists (SCH 23390) has been found to reverse amphetamine induced social isolation in monkeys (Ellenbroek, Willemen & Cools, 1989). Similarly, intra-accumbens infusions of D_1 receptor antagonist (SCH 23390) has been found to increase time spent in social interaction in female (but not male) mice (Campi, Greenberg, Kapoor, Ziegler & Trainor, 2014). In contrast, intra-accumbens infusion of D_1 receptor agonist (SCH 38393) has been found to decrease time spent in social interaction in female (but not male) mice (Campi et al., 2014). Adding to the complexity, intra-accumbens infusion of D_1 receptor agonist has been found to prevent the formation of new pair bonds in prairie voles (Aragona et al., 2006). However, once a pair bond is formed, D_1 receptor agonist facilitated the maintenance of these bonds (Aragona et al., 2006).

Together, these findings suggest that the DA D_1 receptor may play a role in social behaviour. However, the exact nature of this role remains unclear. The inconsistent findings may be partly due to differences in species and methodologies used. A major limitation of the aforementioned studies is their reliance on pharmacological manipulations of the D_1 receptor. There are currently no pharmacological agents that work exclusively on the D_1 receptor (Zhang et al., 2008).

In addition to research on social behaviour, there is considerable research on the role of the DA D_1 receptor in general reward-seeking behaviour. This research has provided consistent evidence demonstrating that D_1 receptor manipulations affect reward-seeking behaviour. For example, administration of D_1 receptor antagonists (SCH 23390 and SKF 83566) decreased food reinforced lever pressing (Cousin et al., 1994; Salamone & Correa, 2002). Similarly, administration of D_1 receptor antagonist (SCH 23390) blocked reinstatement of food-seeking (Ball et al., 2011) and reduced sucrose seeking behaviour (Grimm et al., 2011). Together, these findings indicate that the D_1 receptor may be involved in reward-seeking behaviour. Again, a major limitation of the aforementioned studies is their reliance on pharmacological manipulations of the D_1 receptor. More generally, these findings support the theory that the DA D_1 receptor is involved in the ability of social reward to elicit reward-seeking behaviour.

The second hypothesis that the D_1 mutant rats and WT rats would spend a similar amount of time in the social zone and the unfamiliar zone was also supported. No difference between D_1 mutant rats and WT rats was found in time spent in the social zone (sociability phase). In addition, no difference between D_1 mutant rats and WT rats was found in time spent in the unfamiliar zone (social novelty phase).

Unfortunately, there is currently no research on the role of the DA D_1 receptor in social consummatory behaviour. However, there is substantial research on the role of the DA system in general consummatory behaviour. This research has consistently found that manipulation of the DA system does not affect general consummatory behaviour. For example, administration of DA antagonists had no effect on food intake or sexual copulation in rats (Blakburn et al., 1987; Pfaus & Phillips, 1991; Salamone et al., 1991). Together, these findings support the theory that the DA D_1 receptor is not involved in the ability of social reward to elicit consummatory behaviour.

Previous studies using the social approach paradigm consider the duration of zone entries to be one of the most useful parameters. In these studies, sociability is defined as the tendency to spend *more time* in the social zone compared to the non-social zone. Preference for social novelty is defined as the tendency to spend *more time* in the unfamiliar zone compared to the familiar zone. The number of zone entries was merely considered to be a measure of general exploratory activity.

To our knowledge, the current study is the first to indicate that duration of zone entries and number of zone entries assess different aspects of social approach behaviour. In this study, time spent in the social and unfamiliar zone was considered to be a measure of *social consummatory behaviour* (behaviour aimed at the final consumption of social reward). Conversely, number of entries into social and non-familiar zone was considered a measure of *social reward-seeking behaviour* (behaviour aimed at obtaining social reward).

A limitation of the current study is that it does not differentiate between active social behaviour and passive social behaviour. *Active social behaviour* refer to behaviours that involve directly interacting with other animals (e.g. sniffing, grooming, chasing). Conversely, *passive social behaviour* refer to behaviours that do not involve directly interacting with other animals (e.g. sitting or lying with bodies in contact). Recent research in our laboratory has found that D_1 mutant rats show reduced active social behaviour but normal passive social behaviour in a social interaction task (Homberg et al., 2016). To confirm the true social nature of the time spent with conspecific, future studies could score the number of sniffs directed towards the conspecific (versus object) and the unfamiliar conspecific (versus familiar). Nonetheless, the number of entries into the social and unfamiliar zone could be seen as more related to active social behaviour, whereas the time spent in the social and unfamiliar zone could be seen as more related to passive social behaviour. This would make the findings of the current experiment in line with those of the social interaction task.

In conclusion, this experiment revealed that the DA D_1 receptor is involved in the number of entries into the social and unfamiliar zone. This finding supports the theory that the DA D_1 receptor plays an important role in the ability of social reward to elicit reward-seeking behaviour. This experiment also revealed that the DA D_1 receptor is not involved in the time spent in the social and unfamiliar zone. This finding supports the theory that the DA D_1 receptor does not play an important role in the ability of social reward to elicit consummatory behaviour. Nevertheless, future research in needed to ascertain the true social nature of the time spent in zone.

CHAPTER 6: Scent Marking

Methods

Subjects

Subjects were adult male WT Wistar rats (*n = 7*) and adult male D_1 mutant Wistar rats (*n* = 11). Rats were bred in the vivarium of Victoria University of Wellington. The rats were housed in groups of three to five in standard polycarbonate cages. Food and water was available *ad libitum* at all times. The housing room had a controlled temperature of temperature of 21 °C, humidity of 55%, and was maintained on a 12-h light/dark cycle with lights on at 0700 h. The laboratory animal care principles of the Victoria University of Wellington Animal Breeding Facility were followed.

Apparatus

The scent marking experiment were conducted in a circular open field box made of black acrylic plastic (diameter: 80 cm). During testing, the open field was centered on a table to minimize environmental conditions that could produce side preference (e.g. gradients in light, temperature, and sound).

Two circular sheets of qualitative filter paper (diameter: 32 cm) were placed on the floor of the open field. These sheets of filter paper effectively absorbed drops of female urine and lemon essence. Before the start of each test session, the open field was cleaned with a 70% ethanol-30% water solution, and dried with paper towels.

The sheets of filter paper were treated with Ninhydrin spray (Beijing Bulant Police Equipment co., ltd, Beijing, China) to allow for visualization of scent marks as purple spots.

Previous Female Experience

To provide a standardized prior history of social experience, adult male rats were exposed to adult female rats of the same strain. Each adult male rat was placed in a clean polycarbonate cage together with an adult female rat for 5 minutes. After 5 minutes, the male rats were returned to their home cages. Socialization occurred 6-9 day before testing.

Reproductive Cycle

Subjects were exposed to urine from an adult female rat in the estrus stage of their reproductive cycle. To determine the stage of their reproductive cycle, female rats underwent a vaginal smear. During this vaginal smear, the tip of a plastic pipette was filled with 0.2 ml of distilled water and inserted approximately 3-5 mm into the rat vagina. The distilled water was released from the pipette and then immediately drawn back into it. This vaginal smear was placed on a glass microscope slide and observed under a microscope. The stage of the cycle was determined based on the proportion of three types of cells: epithelial cells, cornified cells, and leukocytes (see figure 1). A proestrus smear consists predominately of epithelial cells. An estrus smear consists predominately of cornified cells. A metestrus smear consists of equal proportion of leukocytes, cornified, and epithelial cells. A diestrus smear consists predominately of leukocytes.

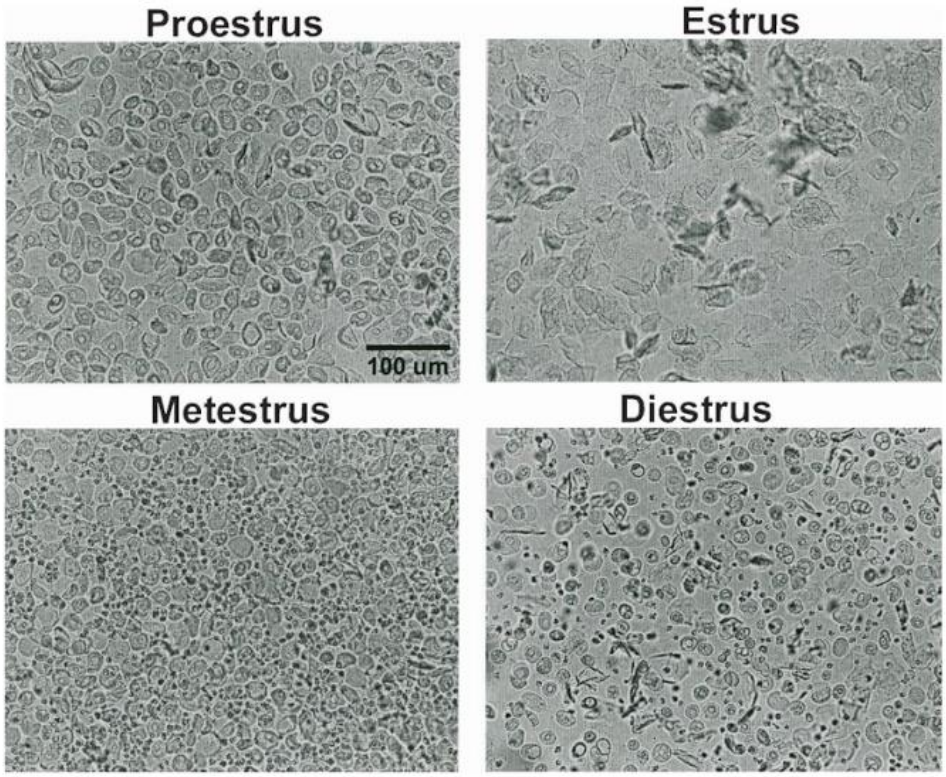


Figure 17. Vaginal Smear (Hubscher, Brooks & Johnson, 2005)

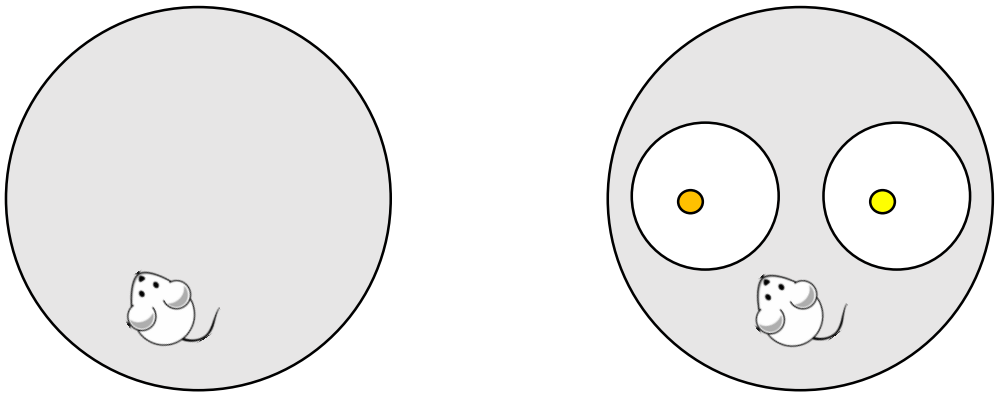
Female urine collection

Urine was collected from adult female rat in the estrus stage of their reproductive cycle. The act of handling the female was sufficient to cause urination. Urine was collected using a 1.0ml Eppendorf tube. The time of urine collection was recorded to ensure urine no older than one hour was used for the experiment.

Procedure

The experiment was conducted in two phases: habituation, and odour exposure. During the habituation phase, adult male rats were placed in the open field for 15 minutes. After 15 minutes, the rat was placed back in a clean polycarbonate cage. Any feces deposited by the rat were removed from the open field. The odour exposure phase was conducted immediately after the habituation phase. During this phase, the open field was lined with two sheets of qualitative filter paper, one at each end. On one sheet of filter paper, thirty

microliters of estrus female urine (social odour) was pipetted. On the other sheet of filter paper, thirty microliters of lemon essence (non-social odour) was pipetted. The same rat was returned to the open field for 5 minutes. After 5minutes, the rat was removed from the open field, and transported back to their home cage.



a) Habituation phase b) Odour exposure phase

Figure 18. Scent marking experiment.

Scent marking behaviour

At the end of experimental session, the marked sheets of filter paper were treated with Ninhydrin spray and left to dry for about 12 hours to allow for visualization of scent marks as purple spots. Scent marks were counted by placing a transparent grid (40 cm^2) divided into squares (1cm^2) sheet on top of the sheet of filter paper. The total number of grids containing scent marks were counted. Pools of urine larger than four square grids were not included in the count.

Statistical Analysis

Data were analyzed using a mixed-factor ANOVA. The between subject factor was genotype (WT, HOM) and the within subject factor was odour type (social, non-social). Data

was analyzed using SPSS software. A *p* value of < .050 was considered as statistically significant. Data was expressed as mean ± S.E.M.

Results

Number of scent marks in the presence of social and non-social odours

A mixed-factor ANOVA was conducted to determine the effect of genotype and odour type on number scent marks. There was a significant main effect of genotype, F (1, 18) = 10.07, $p < .050$. The number of scent marks was greater for WT rats (M = 30.11; SD = 3.72) than HOM rats (M = 14.18; SD = 3.37). There was no main effect of odour type, F (1, 18) = 0.19, p = .669. The number of scent marks was similar for the social odour (M = 21.27; SD = 3.44) and non-social odour (M = 23.02; SD = 2.97). There was no interaction between genotype and odour type, F (1, 18) = 0.38, p = .546. This indicates that the number of scent marks around social and non-social odours was not dependent on genotype.

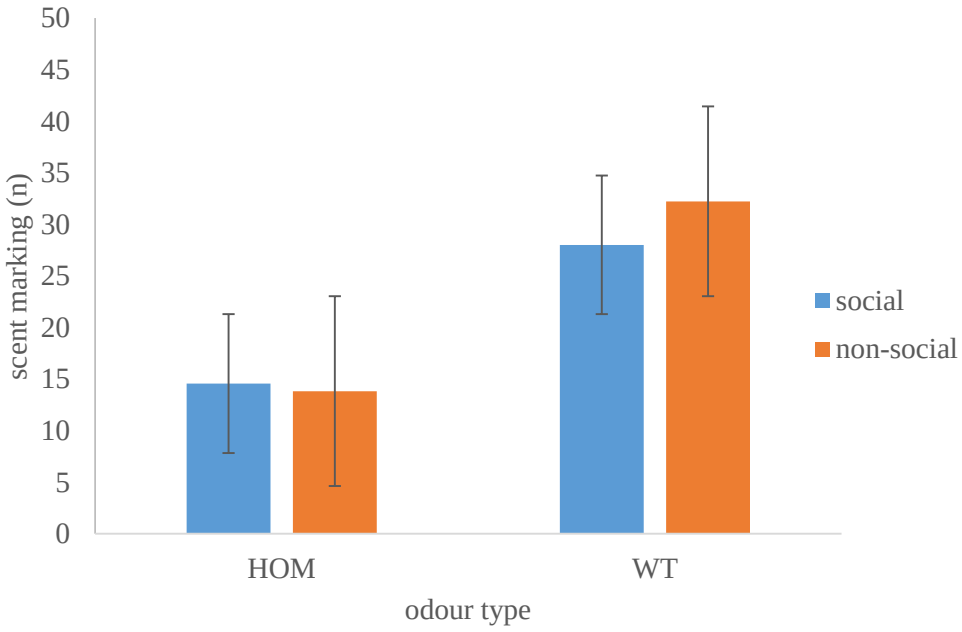


Figure 19. Number of scent markings deposited around social and non-social odours

Discussion

Rodent communicate through olfactory signals. An important source of olfactory signals is scent marking (Arakawa et al., 2008). Scent markings have two main functions, namely, *negative advertisement* (to exclude other adult male from territory and prevent potential competition for females) and *positive advertisement* (to attract mates) (Arakawa et al., 2008). Together, these functions serve to maximize the probability of mating. Thus, the deposition of scent markings can be considered a reward-seeking behaviour aimed at obtaining sexual copulation.

The aim of the current experiment was to examine the role of the DA D_1 receptor in the ability to engage in sexual reward-seeking behaviour. First, it was hypothesized that D_1 mutant rats would deposit fewer scent markings compared to WT rats. Second, it was hypothesized that D_1 mutant rats would deposit a similar number of scent markings around the social odour (estrus female urine) and the non-social odour (lemon essence).

The first hypothesis that D_1 mutant rats would deposit fewer scent markings was supported. It was found that D_1 mutant rats deposited significantly fewer scent markings than WT rats. Unfortunately, there is currently no research on the role of DA D_1 receptor in the deposition of scent markings. Thus, we are unable to determine whether the current findings are consistent with previous research.

The finding that D_1 mutant rats deposit fewer scent markings supports the theory that the DA D_1 receptor is involved in the ability to engage in reward-seeking behaviour. Nevertheless, perhaps other characteristics of D_1 mutant rats reduced the deposition of scent markings. For example, D_1 mutant rats may have a higher level of anxiety. If they have a higher level of anxiety, they are likely to reduce scent-marking behaviour. However, recent research in our laboratory suggest that anxiety and general exploratory activity levels are normal in D_1 mutant rats (Homberg et al., 2016). Moreover, in all previous experiments, there

were no difference between D_1 mutant rats and WT rats in distance travelled, or rearing. Thus, the reduced deposition of scent markings by D_1 mutant rats is unlikely to be due to anxiety related inhibition.

The second hypothesis that D_1 mutant rats would deposit similar scent markings around the estrus female urine (social odour) and lemon essence (non-social odour) was not supported. It was found that both D_1 mutant rats and WT rats deposited a similar number of scent markings around social odour and the non-social odours. This finding does not allow us to draw any conclusions regarding the role of the DA D_1 receptor in the ability of social odour to elicit increased sexual reward-seeking behaviour. In the previous experiment (social approach), the WT rats, but not the D_1 mutant rats, were able to differentiate between a social stimulus and a non-social stimulus. At present, it is unclear why this was not the case in the current experiment. The cues in the current experiment (odours only) were much less conspicuous than in the social approach experiment, which may have contributed to the lack of differentiation.

A limitation that may have confounded the results this experiment is that the female urine used may not have been collected during the estrus phase of the reproductive cycle. The reproductive cycle of rats contains four phases, namely, proestrus, estrus, metestrus, and diestus. The phase of this cycle was determined according to the proportion of three different types of cells in a vaginal smear. It was difficult to distinguish the proestus and estrus phase given the similarity in their appearance. A number of studies demonstrate that adult males are more attracted to urine from sexually receptive females in proestrus than diestrus (Davies & Bellamy, 1971). This may explain why D_1 mutant rats and WT rats deposited a similar number of scent markings around the social and non-social odour. They may have an equal preference for female diestrus urine and lemon essence.

In conclusion, this experiment revealed that the DA D_1 receptor is involved in the number of scent marking deposited. This finding supports the theory that the DA D_1 receptor plays an important role in the ability to engage in sexual reward-seeking behaviour. Unfortunately, WT rats deposited a similar number of scent marking around the social and non-social odour. This prevents any conclusions about the role of the DA D_1 receptor in the ability of social odour to elicit increased sexual reward-seeking behaviour.

CHAPTER 7: Maternal Separation Induced Vocalizations

Methods

Subjects

Subjects were infant wild-type (WT) Wistar rats (n = 9) and infant D_1 mutant Wistar rats (n = 12). Infant rats were bred in the vivarium of Victoria University of Wellington. Each litter was housed with the mother in standard polycarbonate cages. Food and water was available *ad libitum* at all times. The housing room had a controlled temperature of temperature of 21 °C, humidity of 55%, and was maintained on a 12-h light/dark cycle with lights on at 0700 h. The laboratory animal care principles of the Victoria University of Wellington Animal Breeding Facility were followed.

Apparatus

USVs were recorded by a condenser microphone (audible range, 20 Hz to 16 k Hz) connected to a preamplifier, an ultrasound detector (25 ± 4 kHz), filter and amplifier (Ultravox 4-channel system; Noldus Information Technology) and data acquisition software (Ultravox 2.0; Noldus Technology).

A cylindrical wire mesh cage filled with fresh bedding was used to contain the pups (diameter: 10.5 cm). A clamp stand was used to hold the microphone over the midpoint of this wire mesh cage.

Procedure

On postnatal days 4, 7, 10 and 14, pups were individually separated from their mothers and were transported to the test room. Once in the test room, pups were placed in a cylindrical wire mesh cages filled with bedding. The USVs emitted during maternal separation were recorded for 5 min.

Statistical Analysis

Data were analyzed using a mixed-factor ANOVA. The between subject factor was genotype (WT, HOM) and the within subject factors was PND (4, 7, 10, 14). Data were analyzed using SPSS software. A p value of < .050 was considered as statistically significant. Data was expressed as mean ± S.E.M.

Results

Total number of SIVs over PNDs

A mixed-factor ANOVA was conducted to determine the effect of genotype on the total number of SIVs over postnatal days. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated for total number, $\chi 2$ (5) = 20.40, p = .001. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon for total number, ε = 0.84.

There was a significant main effect of PND for total number, F (2.53, 93.60) = 27.24, p < .050. The total number of SIVs changed over PNDs. There was no main effect of genotype for total number, F (1, 37) = 0.91, p = .347. The total number of SIVs was similar for WT pups (M = 326. 58; SD = 24.31) and HOM pups (M = 300.18; SD = 13.20). There was no interaction between PNDs and genotype for total number, F (2.53, 93.60) = .38, p = .731. This indicates that the change in the total number of SIVs over PNDs was not dependent on genotype.

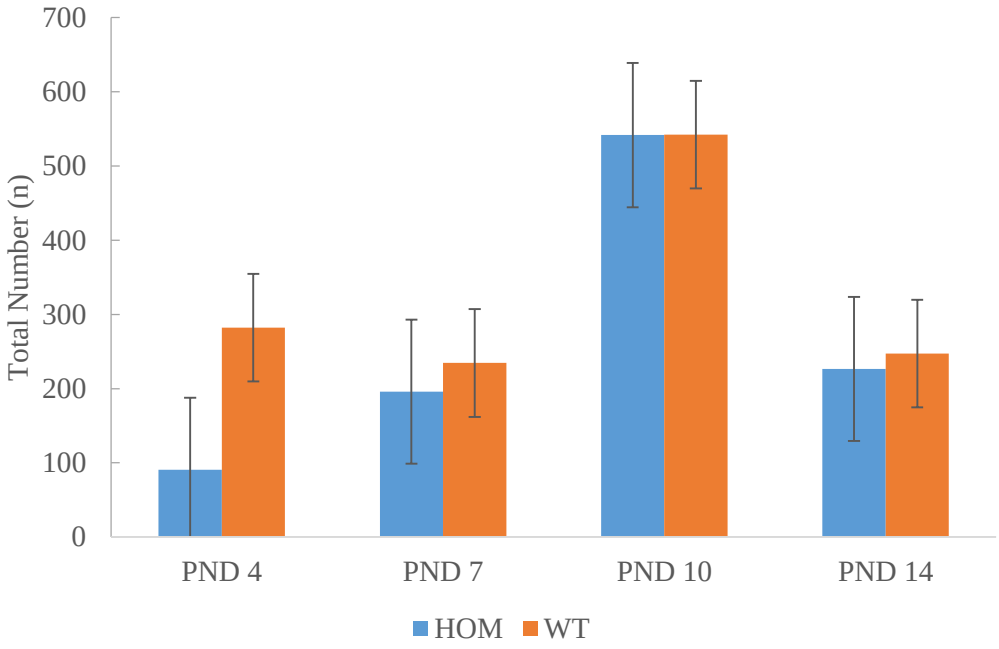


Figure 20. Total number of SIVs on PND4, 7, 10 and 14

Mean duration of SIVs over PNDs

A mixed-factor ANOVA was conducted to determine the effect of genotype on the mean duration of SIVs over postnatal days. There was a significant main effect of PND for mean duration, F (3, 111) = 6.38, p < .050. The mean duration of SIVs changed over PNDs. The was a significant main effect of genotype for mean duration, F (1, 37) = 21.06, p < .050. The mean duration of SIVs was greater for WT pups (M = 88.39; SD = 2.53) than for HOM pups (M = 75.16; SD = 1.39). There was no interaction between PNDs and genotype for mean duration, F (3, 111) = 1.03, p = .38. This indicates that the change in mean duration of SIVs over PNDs was not dependent on genotype.

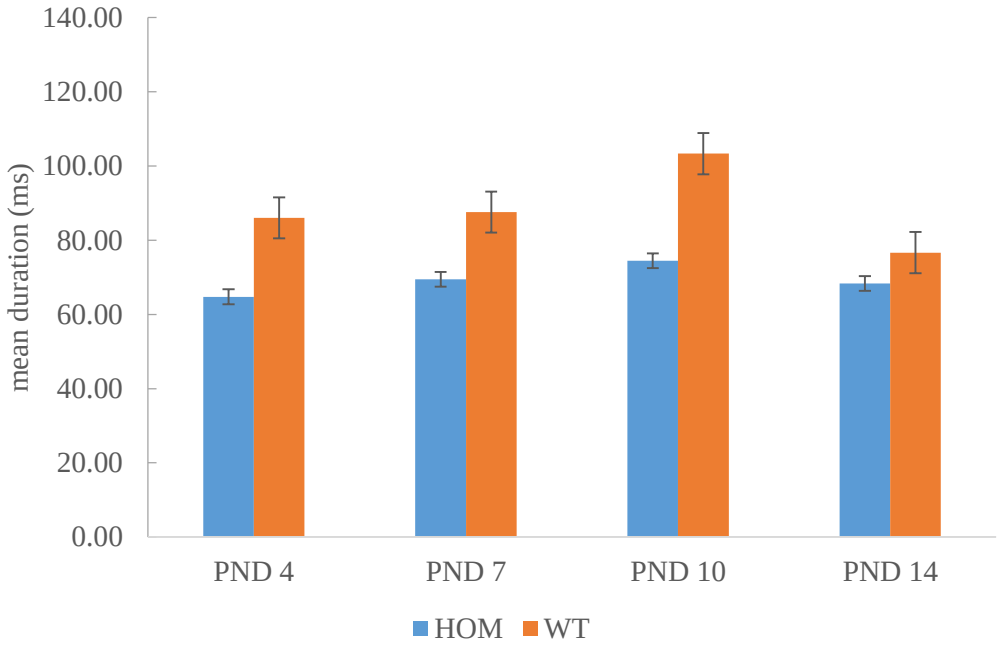


Figure 21. Mean duration of SIVs on PND 4, 7, 10 and 14

Sum duration of SIVs over PNDs

A mixed-factor ANOVA was conducted to determine the effect of genotype on the sum duration of SIVs over postnatal days. Mauchly's Test for Sphericity indicated that the assumption of sphericity had been violated for sum duration, $\chi 2$ (5) = 24.60, p = .000. Therefore, the degrees of freedom were corrected using Huynh-Feldt estimates of epsilon for sum duration, ε = 0.80.

There was a significant main effect of PND for sum duration, F (2.39, 88.38) = 38.23, p < .050. The sum duration of SIVs changed over PNDs. There was a significant main effect of genotype, F (1, 37) = 4.80, p < .050. The sum duration of SIVs was greater for WT pups (M = 30.01; SD = 2.23) than for HOM pups (M = 24.43; SD = 1.22). There was no interaction between PNDs and genotype for sum duration, F (2.39, 88.38) = .42, p = .692. This indicates that the change in sum duration of SIVs over PNDs was not dependent on genotype.

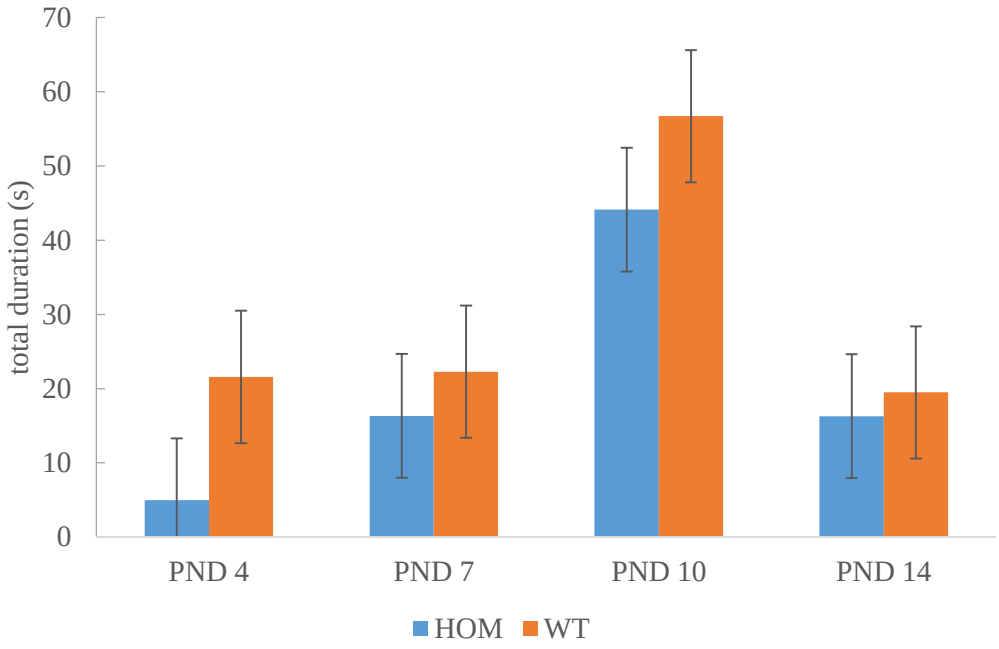


Figure 22. Sum duration of SIVs on PND 4, 7, 10, and 14

Discussion

Infant rats isolated from their mother emit 40 kHz vocalizations (Brudzynski et al. 1999), to elicit maternal searching and maternal retrieval. Thus, the emission of SIVs can be considered a reward-seeking behaviour aimed at obtaining maternal care.

The aim of the SIV experiment was to determine the role of the DA D_1 receptor in the ability to engage in reward-seeking behaviour aimed at obtaining maternal care. The hypothesis that D_1 mutant pups would emit fewer SIVs compared to WT pups was not supported. No difference between D_1 mutant pups and WT pups was found for the number of SIVs emitted across PNDs 4, 7, 10, and 14.

These findings are inconsistent with previous research demonstrating that the DA D_1 receptor is involved in the number of SIVs emitted. For example, systematic injection of D_1 receptor agonist (SKF 81297) was found to reduce SIVs (Dastur, McGregor & Brown, 1999). However, systematic injection of D_1 receptor antagonist (SCH 23390) was found to have no effect on SIVs (Dastur et al., 1999; Muller, Moore, Myers & Shair, 2009). There are four

main factors contributing to the inconsistency between the current study and previous research. First, the current study recorded SIVs on PND 4, 7, 10, and 14, whereas previous research recorded SIVs on PND 11 or 12. A large body of evidence suggests that SIVs depend strongly on age (Brudzynski et al., 1999). Thus, the difference may reflect different developmental stage of the animal. Second, in the current study, rat pups underwent maternal separation on four PND days (i.e. PND 4, 7, 10, 14) whereas in previous research, rats underwent maternal separation on a single PND (i.e. PND 11 or 12). It is possible that the reunion with the mother influenced subsequent vocalizations. Third, previous studies relied on the pharmacological manipulation of the D_1 receptor. As mentioned earlier, there are currently no pharmacological agents that work exclusively on the D_1 receptor (Zhang et al. 2008). And fourth, the D_1 mutant rat model only involves a 50% reduction in D_1 receptors binding, and it is conceivable that this would leave enough functionally active D1 receptor to allow for a normal total number of SIVs.

The finding that D_1 mutant pups emitted a normal number of SIVs across PNDs 4, 7, 10, and 14, fails to support the theory that DA D_1 receptors are involved in the ability to engage in reward-seeking behaviour aimed at obtaining maternal care. A possible explanation is that SIVs may not measure reward-seeking behaviour. Rather, it may reflect the anxiety level of the pups. Anxiolytic compounds (decrease anxiety) have been found to decrease the number of SIVs whereas anxiogenic compounds (increase anxiety) have been found to increase the number of SIVs (Wohr & Schwarting, 2008). Additionally, pups from a high-anxiety strain have been emit more SIVs than pups from a low-anxiety strain (Naito et al., 2001). Recent research in our laboratory suggest that D_1 mutant rats have normal levels of anxiety and general exploratory activity (Homberg et al., 2016). Moreover, in all previous experiments, D_1 mutant rats show normal horizontal exploratory behaviour bouts, distance travelled, and

rearing behaviour. Together, these findings suggest that the number of SIVs may reflect the normal anxiety level of D_1 mutant pups.

As mentioned, SIVs function to elicit maternal searching and maternal retrieval behaviour (Brudzynski et al., 1999). Aside from the number of SIVs emitted, there are other factors that may influence the effectiveness of SIVs to elicit maternal behaviours. One such factor is the duration of SIVs. The duration of SIVs can range from 10ms to 200ms (Elsner, Suter & Adler, 1990). It is possible that SIVs with a longer duration are more effective at eliciting maternal behaviour than SIVs with a shorter duration. If this theory is correct, then D_1 mutant pups should emit SIVs with a shorter duration than WT pups. This would indicate an impaired ability to engage in *effective* reward-seeking behaviour aimed at obtaining maternal care. In support of this theory, it was found that D_1 mutant pups emitted SIVs with a significantly shorter mean duration and sum duration. This suggests that D_1 mutant rats may engage in less effective reward-seeking behaviour aimed at obtaining maternal care. Further research is needed to confirm that duration of SIVs influence their ability to elicit maternal searching and maternal retrieval behaviour.

This experiment had a number of limitations. First, the emission of SIVs may have been influenced by the maternal care received prior to separation. Studies show that pups of mothers with high maternal responsiveness emit fewer SIVs than pups of mothers with comparatively lower maternal responsiveness (Wohr & Schwarting, 2008). In addition, studies show that high licked pups emit fewer SIVs than low licked pups (Wohr & Schwarting, 2008). Moreover, high licked pups emit SIVs with a lower peak amplitude, shorter duration, and more frequency modulation, than low licked pups. Based on these findings, it seems reasonable to suggest that any differences between D_1 mutant pups and WT pups might reflect differences in maternal care.

Second, the emission of SIVs may have been influenced by environmental and social factors. The emission of infant vocalizations is stimulated by factors such as: decreased ambient temperature (Blumberg, Efimova & Alberts, 1992), rough handling (Oswalt & Meier, 1975), recovery from hypothermia (Hofer & Shair, 1992), and unfamiliar odours (Oswalt & Meier, 1975). Conversely, the emission of infant vocalization is suppressed by factors such as: hypoxia and milk deprivation (Blumberg & Alberts, 1991), familiar nest associated odours (Hofer & Shair, 1987), and unfamiliar adult male odour (Takahashi, 1992).

In conclusion, this experiment revealed that the DA D_1 receptor is not involved in the number of SIVs emitted. However, this experiment revealed that the DA D_1 receptor is involved in the mean duration and sum duration of SIVs emitted. Taken together, these findings fail to support the theory that the DA D_1 receptor plays an important role in the ability to engage in maternal reward-seeking behavior. Nevertheless, they suggest that the DA D_1 receptor plays an important role in the effectiveness of maternal reward-seeking behaviour.

CHAPTER 8: General Discussion

Negative symptoms contribute to impaired functional outcomes in schizophrenia (Kirkpatrick et al., 2006; Rabinowitz et al., 2012). Negative symptoms contain two domains, namely, the diminished motivation domain and the diminished expression domain (Messinger et al., 2011; Strauss et al., 2014). To explain the link between negative symptoms and impaired functional outcomes, most researchers have focused on the diminished motivation domain. A mechanism thought to contribute to diminished motivation is impaired anticipatory pleasure (Gard et al., 2006; Horan et al., 2006). Impaired anticipatory pleasure reflects a disruption in reward prediction and reward anticipation systems. These disruptions are manifested as reduced engagement in reward-seeking behaviour (Gard et al., 2007).

The current study aimed to investigate the role of the DA D_1 receptor in anticipatory pleasure, with a particular focus on reward prediction and reward-seeking behaviour. We chose to examine the reward prediction system for two main reasons. First, the reward prediction system is necessary for the generation of reward-seeking behaviour. Second, the reward prediction system is dependent on the dopaminergic system, which is thought to be disruption in schizophrenia. The following section reviews how our results align with previous research in the schizophrenia research, as well as their implication for future work in this field.

Reward Prediction and Reward-Seeking Behaviour: Summary

In Chapter 3, we examined the role of the DA D_1 receptor in reward prediction by looking at anticipatory response in expectation of reward. It was found that D_1 mutant rats demonstrated reduced anticipatory rearing behaviour prior to conditioned food presentation. Reduced anticipatory rearing behaviour indicates impaired anticipatory response in expectation of reward. Considerable research suggests that patient with schizophrenia have

reduced anticipatory responses to reward-predicting cues (Juckel et al., 2006; Waltz et al., 2010). Together, these findings suggest that a DA D_1 receptor dysfunction may contribute to disruptions in the anticipatory response to reward-predicting cues in schizophrenia.

In Chapter 4, we further examined the role of the DA D_1 receptor in reward prediction by looking at the response to unexpected alterations in reward. It was found that both D_1 mutant rats and WT rats failed to show a SNC effect following the unexpected downshift from 32% to 4% sucrose solution. As WT rats failed to show a SNC effect, no conclusions can be drawn regarding the role of the DA D_1 receptor in the response to unexpected alterations in reward. Substantial evidence suggests that patients with schizophrenia have reduced reward prediction error responses (Murray et al. 2008; Schlagenhauf et al. 2014), though with some exceptions (Simon et al. 2010; Waltz et al. 2010). Further research is needed to determine whether a DA D_1 receptor dysfunction contributes to disruptions in the response to unexpected alterations in reward in schizophrenia.

In Chapter 5, we examined the role of the DA D_1 receptor in the ability engage in reward-seeking behaviour aimed at obtaining social reward. It was found that D_1 mutant rats engaged in fewer entries into the zone containing a conspecific (sociability phase) and the zone containing an unfamiliar conspecific (social novelty phase). Fewer zone entries indicate an impaired ability of social reward to elicit reward-seeking behaviour. Evidence suggest that patients with schizophrenia engage in fewer reward-seeking behaviours (Gard et al., 2007; Heerey & Gold, 2007). Together, these findings suggest that a DA D_1 dysfunction may contribute to reduced engagement in *social* reward-seeking behaviour in schizophrenia.

In Chapter 6, we further examined the role of the DA D_1 receptor in the ability to engage in reward-seeking behaviour aimed at obtaining sexual reward. It was found that D_1 mutant rats deposited a reduced number of scent markings. Reduced scent marking indicates an impaired ability to engage in sexual reward seeking behaviour. In addition, it was found

that both D_1 mutant rats and WT rats deposited a similar number of scent markings around social odour (estrous female urine) and non-social odour (lemon essence). As WT rats failed to show a preference for social odour, no conclusions can be drawn regarding the role of the DA D_1 receptor in the ability of social odour to elicit increased sexual reward-seeking behaviour. Evidence suggest that patients with schizophrenia engage in fewer reward-seeking behaviours (Gard et al., 2007; Heerey & Gold, 2007). Together, these findings suggest that a DA D_1 dysfunction may contribute to reduced engagement in *sexual* reward-seeking behaviour in schizophrenia. However, further research is needed to determine whether a DA D_1 dysfunction contributes to the reduced ability of social reward to elicit increased reward-seeking behaviour in schizophrenia.

In Chapter 7, we examined the role of DA D_1 receptor in the ability to engage in reward-seeking behaviour aimed at obtaining maternal care. It was found that D_1 mutant pups and WT pups emitted a similar number of SIVs. A similar number of SIVs indicates a normal ability to engage in maternal reward-seeking behaviour. In addition, it was found that D_1 mutant rats emitted SIV with a shorter mean duration and sum duration. A shorter duration of SIVs may indicate an impaired ability to engage in effective maternal reward-seeking behaviour. Evidence suggest that patients with schizophrenia engage in fewer reward-seeking behaviours (Gard et al., 2007; Heerey & Gold, 2007). Together, these findings suggest that a DA D_1 dysfunction does not contribute to reduced engagement in *maternal* reward-seeking behaviour in schizophrenia. However, future research is needed to determine whether a DA D_1 dysfunction contributes to the effectiveness of *maternal* reward-seeking behaviour in schizophrenia.

Reward Prediction and Reward-seeking Behaviour: Cortical Contributions

In the experiments described above, we examined the hypothesis that reduction in reward-seeking behaviour result from disruptions in the DA D_1 receptor mediated reward

prediction system. Disruptions in reward prediction system would reduce the ability of reward to drive reward-seeking behaviours. However, this disruption may be exacerbated by impairments in high-level cognitive processes required for translating reward information into motivated behaviour. Three such processes include: value computation, effort computation, and the generation of action plans. *Value computation* refers to the ability to generate, maintain, and update value representations. Value representations are thought to be mediated by the orbitofrontal cortex (OFC) (Wallis, 2007). *Effort computation* refers to the ability to compute the effort necessary to obtain an outcome relative to the value of that outcome. Effort computations are thought to be mediated by the anterior cingulate cortex (ACC) (Salamone, Correa, Farrar & Mingote, 2007). *Generation of action plans* refers to the ability to generate and execute the action plans necessary to obtain valued outcomes. The generation of action plans is thought to be mediated by the dorsolateral prefrontal cortex (DLPFC) (Miller & Cohen, 2001). Interactions between these processes allows an organism to select the action plan most likely to obtain the desired outcome. This, in turn, is translated into motivated behaviour aimed at obtaining that outcome. There is some evidence that disruptions in these processes contribute to diminished motivation in schizophrenia. The following section briefly reviews this evidence.

Value Computation in Schizophrenia. Accumulating evidence suggests that schizophrenia involves impaired value representation. Two experimental paradigms used to examine value representation include: probabilistic reversal learning and the Iowa Gambling Task. Studies using the reversal learning paradigm have provided evidence of impaired performance in schizophrenia (Oades, 1997; Waltz & Gold, 2007). Similarly, studies using the Iowa Gambling paradigm have provided evidence of impaired performance in schizophrenia (Shurman, Horan & Nuechterlein, 2005; Yip, Sacco, George & Potenza, 2009), though with some exceptions (Evans, Bowman & Turnbull, 2005; Turnbull, Evans, Kemish,

Park & Bowman, 2006). Together, these findings provide compelling evidence for impaired value representation in schizophrenia.

Effort Computation in Schizophrenia. Several studies have provided evidence for impaired effort computation in schizophrenia. Two experimental paradigms used to examine physical effort allocation include: the finger tapping task and the grip strength task. Studies using the finger tapping task have consistently found that patients with schizophrenia show reduced effort allocation (Gold et al., 2013; Barch, Treadway & Schoen, 2014). Studies using grip strength have been produced more inconsistent findings. One study found that patients show reduced effort allocation (Hartmann et al., 2015) while another study found no reduction (Docx et al., 2015). Taken together, these findings provide compelling evidence of reduced physical effort allocation in schizophrenia.

Goal-directed behaviour in schizophrenia. A large body of evidence suggests that schizophrenia involves impairments in working memory, context representation, goal maintenance, and planning (Barch & Dowd, 2010; Barch & Ceaser, 2012).

Limitations

Undoubtedly, the D_1 mutant rat model has greatly improved our understanding of the function of the DA D_1 receptor subtype. However, it is important to note that the D_1 mutant rat model has three drawbacks. First, the D_1 mutant rat model is likely to be subject to developmental adaptation (i.e. physiological and neural changes over time). Second, the D_1 mutant rat model involves a brain wide deficit. Interpreting the behavioural changes that result from a brain-wide deficit is complicated by competing effects of DA receptors in different brain regions on neural circuits regulating reward. Studies have found that DA receptors play different roles in mediating reward processes in different brain regions (Self, 2010). For example, DA receptors in the ventral striatal regions mediate primary rewarding effects. In contrast, DA receptors in the neocortical or amygdala regions modulate reward

evaluation, choice, or the formation of conditioned environmental associations that acquire their own rewarding properties. Third, the D_1 mutant rat model only involves a 50% reduction in D_1 receptor binding. This means that the role of the D_1 receptor may have been underestimated. In some cases, it is necessary to have an 80 to 100% reduction to find an effect. Despite these limitations, the D_1 mutant rat model remains a great tool for understanding the function of D_1 receptor subtype.

Conclusion

In conclusion, the current study indicates that DA D_1 receptor is involved in anticipatory pleasure. We found evidence that the D_1 receptor is involved in reward prediction, particularly the anticipatory responses in expectation of reward. We also found evidence that the D_1 receptor is involved in the ability to engage in social and sexual reward-seeking behaviour. Together, there is compelling evidence that a DA D_1 receptor dysfunction is a likely contributor to diminished motivation in schizophrenia. Thus, the DA D_1 receptor might be a useful target for developing effective treatments for motivational impairments in schizophrenia.

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