Palatability and animal models of schizophrenia

Emma Sian Lydall

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

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PUBLICATIONS AND CONFERENCE PRESENTATIONS

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CHAPTER ONE

1.1. The manifestation of schizophrenia

Schizophrenia is one of the most serious mental disorders of humankind. It affects about 1% of the population worldwide and has devastating consequences, including suicide in 10% of sufferers (e.g. Lewis & Lieberman, 2000). Schizophrenia also has serious social impact with sufferers accounting for more than one third of the homeless population in western society (Folsom & Jeste, 2002).

The clinical features of schizophrenia are highly disabling and fall into the three symptom clusters outlined below.

- **Positive symptoms** (additional to normal human experience)

- Hallucinations (usually auditory), delusions (often of a paranoid nature), irrational thoughts and communication (often perceived to be under someone else's control), abnormal behaviour, including aggressive behaviour and stereotyped movement.

- **Negative symptoms** (lacking relative to normal human experience)

- Blunting of emotions, social withdrawal, avolition (reduced motivation), anhedonia (reduced capacity to experience pleasure).

- **Cognitive symptoms**

- Deficits of attention and memory.

The name 'schizophrenia' means 'divided mind' and it was first used by Eugen Bleuler in 1911 following his observation that patients seemed to alternate between normal and abnormal mental states. By this, he referred to psychotic episodes that

commonly characterise schizophrenia. It is during these episodes that the positive symptoms are at their most severe. These episodes are often separated by periods in which individuals are not obviously psychotic, but tend to experience more of the negative symptoms. The negative symptoms of schizophrenia are the most difficult to treat and are often considered to predict the long-term course of positive symptoms (e.g. Pogue-Geile & Harrow, 1985). Patients who experience predominantly negative symptoms are often the most severely ill and tend to have the poorest prognosis. For example, in a longitudinal study, Ho, Nopoulos, Flaum, Arndt, and Andreasen (2004) showed that negative symptom severity is moderately and significantly correlated with later unemployment, financial dependence on social service agencies, impairment in the ability to perform household duties, inability to maintain relationships and to participate in recreational activities. Pogue-Geile and Harrow (1985) also found that blunted affect and poverty of speech predicted poor outcome in terms of symptom severity, social functioning, independence of others and rehospitalisation. Similarly, Wieselgren, Lindstrom and Lindstrom (1996) found that negative symptoms measured using the Comprehensive Psychopathological Rating Scale (CPRS) predicted poor social functioning 5 years after hospitalisation during a patient's first psychotic episode. The implications of such findings are even more severe when it is considered that feelings of hopelessness and poor functioning are significant risk factors for suicide in schizophrenia (Cohen, Test, & Brown, 1990; Kaplan & Harrow, 1999).

Schizophrenia manifests itself as three main subtypes, based on features present during psychotic episodes:

- **Paranoid Schizophrenia**

- Delusions predominate.

- **Disorganised Schizophrenia**

- Disorganised behaviour and speech, lack of/inappropriate emotional expression, early age of onset and steadily worsening course without substantial remission periods.

- **Catatonic Schizophrenia**

- Unusual movement, gesturing and catatonia.
- Meaningless repetition of phrases.

1.2 The aetiology of schizophrenia

The causes of schizophrenia are far from fully established, although the work of Gottesman (1991) has clearly illustrated a genetic link. Gottesman ranked relatives of individuals with schizophrenia in terms of what percentage of genes they shared with the sufferer. The chances that a person will have the disorder declines as the proportion of genes shared with the sufferer decreases, so that first-degree relatives, such as offspring, who share 50% of the sufferer's genes, have a higher incidence of schizophrenia than second-degree relatives, who share 25% of the sufferer's DNA. Despite this genetic component, studies of monozygotic twins showing a 45% concordance rate despite sharing 100% of genes with a sufferer, demonstrate that genetic factors are not the only cause (e.g. Kallmann, 1946).

Schizophrenia is often associated with anatomical abnormalities in the brain, including enlarged ventricles reflecting loss of surrounding brain tissue, thinning of the cortex of the medial temporal lobe, reduction in the size of the anterior hippocampus and disturbance in blood flow to the temporal lobe (Ananth, et al., 2002; Shenton, Dickey, Frumin, & McCarley, 2001; Vita & De Peri, 2007; Vita, De Peri, Silenzi, & Dieci, 2006). These abnormalities are seen most commonly in

patients who have predominantly negative symptoms (Ho, et al., 2003; Maj, Starace, & Kemali, 1987; Pearlson, Garbacz, Breakey, Ahn, & Depaulo, 1984; Williams, Reveley, Kolakowska, Ardern, & Mandelbrote, 1985). To highlight some examples, Mathalon, Sullivan, Lim, and Pfefferbaum (2001) showed that prefrontal grey matter decline and expansion of the frontal sulci was associated with greater negative symptom severity. Similarly, Ho et al. (2003) found that decreased frontal lobe volume white matter volume and enlargement of the frontal sulci and lateral ventricles was associated with higher levels of negative symptoms. Furthermore, patients with poor outcome showed the largest lateral ventricular enlargement. This may suggest that the increased brain structure abnormalities in individuals with greater negative symptom severity could account for the increased illness severity and poorer prognosis in these patients.

Interestingly, Suddath, Christison, Torrey, Casanova and Weinberger, (1990) discovered that when one person in a pair of monozygotic twins is affected by schizophrenia, only the affected individual displays evidence of the above-mentioned structural abnormalities using MRI scans. Since genes alone cannot account for these structural abnormalities, it has been suggested that genetic factors are necessary for schizophrenia to develop, but environmental challenges, such as maternal viral infection (e.g. Brown, 2006; Boksa, 2008), or childhood abuse (e.g. Janssen et al., 2004) must also occur for the disorder to develop. However, the exact combination of aberrant genes and environmental insults most likely to produce the disease state remains unclear (Lewis & Lieberman, 2000).

In addition to the structural abnormalities evident in schizophrenia, abnormalities in synaptic transmission have also been implicated. In particular,

changes in dopaminergic and glutamatergic transmission have formed the basis of the two main theories relating to schizophrenia.

1.3 The dopamine hypothesis of schizophrenia

There are four major dopaminergic pathways in the brain: the mesolimbic pathway, the mesocortical pathway, the nigrostriatal pathway and the tuberoinfundibular pathway. The mesolimbic pathway transmits dopamine from the ventral tegmental are to the nucleus accumbens. It is important in memory and motivating behaviours (e.g. Pezze & Feldon, 2004). The mesocortical pathway runs from the ventral tegmental area to the frontal cortex and surrounding structures. There is some evidence that malfunctions of this pathway may contribute to hallucinations and disordered thinking (Silbersweig, Stern, Frith, Cahill et al. 1995). The nigrostriatal pathway transmits dopamine from the substantia nigra to the striatum and is involved in motor control (Ungerstedt & Arbuthnott, 1970). The tuberoinfundibular pathway runs from the hypothalamus to the pituitary gland and influences the secretion of certain hormones, including prolactin (This may be implicated in the weight gain induced by antipsychotic medication (Gudelsky, Koenig, Simonovic, Koyama, et al. 1987).

Interestingly, it has been found that dopaminergic pathways can interact producing bi-directional effects on dopamine in different brain areas. Projections from the prefrontal cortex regulate extracellular dopamine via their connections with the ventral tegmental area. However, by labelling anterograde and retrograde projections between these areas, Carr and Seasack (2000) observed that afferents from the prefrontal cortex project to mesoprefrontal dopaminergic neurones, but not mesoaccumbal dopaminergic neurones. On the other hand, prefrontal afferents project to mesoaccumbel GABA-ergic neurones, but not mesoprefrontal GABA-ergic

neurones. In this way, prefrontal cortex activity can result in an increase in prefrontal dopamine release alongside a simultaneous decrease in dopamine release from the nucleus accumbens. The effect has been observed practically by Jackson, Frost and Moghaddam (2001) who found that accumbal dopamine release was reduced when the prefrontal cortex was electrically stimulated at levels produced during performance of cognitive tasks.

The dopamine hypothesis proposed by Carlsson (1978) suggests that excessive dopamine synthesis and release underlies the symptoms of schizophrenia. Two main findings underlie this theory. Firstly, it has been found that amphetamine, a drug that enhances neurotransmission via the release of dopamine, can produce symptoms that can be difficult to distinguish from a psychotic episode when taken by humans (e.g. Hollister, 1986). Secondly, drugs that are effective in treating the positive symptoms of schizophrenia often block D_2 receptors (e.g. chlorpromazine) or prevent neuronal dopamine storage (e.g. reserpine). In fact, Seeman, Lee, Chauwong and Wong (1976) examined a large number of antipsychotics and found a strong correlation between clinical potency and ability to bind to D_2 receptors.

Imaging studies have provided further evidence for increased dopamine release in schizophrenia. For example, Laurelle (2001) measured binding of a dopamine antagonist, raclopride, to striatal D_2 receptors. When injected, amphetamine caused release of dopamine, which in turn displaced raclopride, reducing the signal intensity. In participants with schizophrenia, this displacement was greater, indicating that amphetamine induced greater dopamine release in sufferers than controls. This effect was absent in sufferers during remission periods, further implicating dopamine in positive symptomatology.

Despite these findings, the fact that newer drugs, such as clozapine, have little effect on D_2 receptors indicates that increased dopamine release is not fully responsible for the symptoms of schizophrenia. This has led to a revision of the original model, suggesting that dopamine up-regulation in the mesolimbic system, which runs from the ventral tegmental area to many components of the limbic system, gives rise to the positive symptoms of schizophrenia. It further suggests that negative symptoms are produced by dopamine down-regulation in the mesocortical system, which runs from the ventral tegmental area to the prefrontal cortex, an area involved in motivation, attention and social behaviour (Weinberger, 1987).

Weiner (2003) also cites evidence that bi-directional effects on dopamine release are responsible for different facets of schizophrenia. Latent inhibition is disrupted in rats by admisnistration of amphetamine, an agent which causes dopamine release (Angrist, Sathananthan, Wilk & Gershon, 1974). However, latent inhibition is potentiated by antipsychotic drugs which reduce dopamine release (Weiner & Feldon, 1987). Weiner suggests that since latent inhibition is imapired during the acute phases of schizophrenia, and intact during the chronic phases, bi-directional effects on dopamine may be involved.

1.4 The glutamate hypothesis of schizophrenia

The revised version of the dopamine hypothesis may provide a useful explanation of the neurotransmitter abnormalities underlying the symptoms of schizophrenia. However, it is difficult to test this theory directly via administration of dopamine receptor antagonists in humans or animals because such drugs cannot produce the bi-directional effects on dopaminergic transmission that the model suggests are required to produce both positive and negative symptoms. Developing treatment strategies

based on this theory would pose an even greater problem since it is difficult to envisage how dopamine-altering pharmacological agents could produce opposing effects on dopamine activity in different brain areas.

Glutamate is another neurotransmitter implicated in schizophrenia (e.g. Moghaddam, 2003), as it has been suggested that N-methyl-D-aspartate (NMDA) (glutamate) receptor hypofunction may underlie the symptoms of the disorder. This idea has arisen from findings that NMDA receptor antagonists such as phencyclidine (PCP) and s-(+)-ketamine produce a psychotic state that is more reminiscent of schizophrenia than dopaminergic psychostimulants, such as amphetamine, since both negative and cognitive symptoms are induced (e.g. Javitt & Zukin, 1991; Lahti, Weiler, Michaelidis, Parwani, & Tamminga, 2001). Treatment with NMDA antagonists can also exacerbate psychosis in schizophrenic patients (Lahti, Koffel, Laporte, & Tamminga, 1995). In addition, post-mortem studies have found reduced densities of glutamate receptor mRNAs in the frontal cortex of schizophrenics who did not receive neuroleptic treatment for over six months prior to their death (Sokolov, 1998).

NMDA receptor antagonists, such as PCP, bind both to NMDA receptors and dopaminergic neurone terminals in the nucleus accumbens. When PCP binds to NMDA receptors it inhibits the release of dopamine. However, at the dopaminergic neurone terminals of the nucleus accumbens, PCP acts in a similar way to amphetamine by increasing dopamine release and reducing reuptake (Javitt, 2010). The ability of NMDA receptor antagonists to produce contrasting effects on dopamine release suggests that the glutamate hypothesis is superior to the hypothesis that relies on dopamine alone. It is possible that NMDA receptor hypofunction

produces the effects outlined in the revised dopamine hypothesis. However, the glutamate hypothesis also attempts to identify the cause of dopamine dysregulation.

The glutamate hypothesis has broadened the number of neurotransmitter systems considered in drug hunting efforts. For example, NMDA receptor antagonists such as PCP also bring about a reduction in parvalbumin-containing neurones in the prefrontal cortex (Cochran, Fujimara, Morris & Pratt, 2002). This is also evident in the post-mortem brains of schizophrenia patients (e.g. Beasley & Reynolds, 1997; Reynolds, 2000). Parvalbumin is known to be a calcium binding protein found in selective subpopulations of GABA-ergic neurones (Reynolds et al, 2000). Support for the role of GABA in schizophrenia has also come from evidence that mRNA encoding the enzyme responsible for synthesising GABA from glutamate, glutamate decarboxylase (GAD), is reduced in the prefrontal cortex in schizophrenia (Volk, Austin, Pierri & Sampson, 2000). Additionally, post-mortem investigations have also revealed a deficit in a subset of GABA-ergic neuronal terminals determined by GAT-1, a GABA uptake transporter (Woo, Whitehead, Melchitzty & Lewis, 1998).

1.5 The treatment of schizophrenia

Antipsychotic drugs are usually considered to fall into two categories. The drugs originally developed tend to have their effects via blockade of dopamine receptors and are known as *typical* antipsychotics. This drug group includes the phenothiazines (e.g. chlorpromazine), butyrophenones (haloperidol), and the thioxanthenes. The newer drugs are commonly referred to as *atypical* antipsychotics. Although there is some debate as to what defines an atypical drug (e.g. Remington, 2003), most atypical compounds have different pharmacological action compared with typical drugs. Additionally, typical antipsychotics often produce disabling motor problems due to

D2 receptor blockade in the basal ganglia (e.g. Parkinsonism and tardive dyskinesia - involuntary tongue and trunk movements). However, by avoiding potent D_2 blockade, some of the atypical treatments produce fewer motor side effects (Leucht, Pitschel-Walz, Abraham, & Kissling, 1999).

Crow (1980) first drew the conclusion that the positive symptoms of schizophrenia are often ameliorated by available antipsychotics, whereas the negative symptoms are usually treatment resistant. This followed evidence from Johnstone, Frith, Gold, & Stevens (1979) who found that *cis*-flupenthixol had no greater efficacy against negative symptoms than its inactive isomer. More recently evidence has arisen suggesting that atypical antipsychotics are superior to typical antipsychotics in the treatment of negative symptoms (see King, 1998, for a review). For example, Chouinard et al. (1993) and Marder and Meibach (1994) found that risperidone (an atypical drug) produced a greater decrease in negative symptoms than haloperidol, when assessed using the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) negative subscale and the Brief Psychiatric Rating subscales. Similarly, the atypical drugs olanzapine and amisulpiride have also been shown to be more effective thaN haloperidol in reducing negative symptoms (Beasley, et al., 1996; Freeman, 1997). However, the assessment of the efficacy of pharmacological treatments against negative symptoms is difficult because it is difficult to determine whether improvements are the result of treatment of the pathophysiology specifically related to negative symptoms or whether the improvement is secondary to the treatment of positive symptoms. For example, a reduction in social avoidance could be secondary to a decrease in paranoia. Miller, Perry, Cadoret and Andreasen (1994) sought to disentangle these negative symptom treatment effects by measuring correlations between improvements in negative symptoms and improvements in positive and

disorganised symptoms. They found that negative symptoms (assessed using SANS) improved with clozapine treatment, but this improvement was not correlated with improvement in positive symptoms, depression or drug-induced extra-pyramidal side-effects. Although there was a correlation between negative symptoms and disorganised symptoms, Miller et al. suggested that these symptoms change in parallel but are independent of each other. Using this evidence, they proposed that clozapine's therapeutic effect on core negative symptoms is at least partially mediated via a direct effect on the pathology associated with negative symptoms, and therefore was independent of its effects on positive symptoms.

Despite the above evidence, there has been some debate over the advantages of atypical drugs, with a large-scale study suggesting that atypical drugs do not provide greater relief of symptoms, even though they reduce the risk of extra-pyramidal side-effects (Geddes, et al., 2000). Geddes suggested that the reason for the apparent superiority of atypical drugs may be because prescribed doses of typical drugs are often too high and therefore cause disabling side-effects. However, using another large-scale meta-analysis Davis, Chen and Glick (2003) found that the efficacy of atypical drugs over typical drugs was not biased by the dose used. Davis et al argued that the claim made by Geddes et al. (2000) asserting that differences in the efficacy and tolerability of typical and atypical drugs is not apparent after controlling for the higher than recommended doses of typical drugs is unfounded because tolerability problems were measured in terms of adherence to medication, which would be lower with less effective drugs.

Whether or not atypical drugs are more effective than typical drugs in the treatment of schizophrenia, the need for better medications is undisputed. Lack of patient adherence to prescribed medications indicates that no currently available drug

is sufficiently effective. Adherence to antipsychotic treatment is in fact a major problem, causing poorer functional outcomes in patients and great economic costs due to increased relapse rates and hospitalisations (e.g. Ascher-Svanum, et al., 2006; Fenton, Blyler, & Heinssen, 1997). In a study by Ascher-Svanum et al. (2008) only 35% of patients treated with haloperidol were found to be adherent to their medication, compared to 61% adherence with atypical antipsychotics. This suggests that adherence is better with atypicals than typicals, but nevertheless, adherence to all available oral antipsychotics is far from optimal.

A number of studies have shown that one of the most crucial factors determining adherence is the effectiveness of the treatment (e.g. Freudenreich, Cather, Evins, Henderson, & Goff, 2004; Rettenbacher, et al., 2004). Kikkert et al. (2006) found that contrary to the assumption widely held by healthcare professionals, patients consider efficacy of treatment to be more important than severity of side-effects in terms of their decision to continue taking their medication. In light of this, since most antipsychotics are effective against positive symptoms but are largely ineffective against negative symptoms, it is plausible that problems with patient compliance may be due primarily to the lack of treatment efficacy against negative symptoms. In support of this view, it has been found that objective quality of life measures such as participation in activities and maintenance of personal relationships are predicted most strongly by the presence and severity of negative, rather than positive, symptoms (Narvaez, Twamley, McKibbin, Heaton, & Patterson, 2008). Quality of life is now increasingly acknowledged to be as important as treatment outcomes in schizophrenia patients (e.g. Ruggeri, et al., 2005). The fact that currently available treatments are only effective in targeting positive symptoms is therefore a major problem. Indeed, even in studies that have found atypical antipsychotics to be

superior to typical drugs, the efficacy of atypical treatments is still far from adequate. For example, although the study by Miller et al. (1994) (cited above) showed that clozapine was more effective than haloperidol in the treatment of negative symptoms, the improvement in these symptoms only reached 31%. This further supports the fact that treatments for negative symptoms are urgently needed in order for patient adherence and personal suffering to be improved.

1.6 The importance of animal models in treatment development

Schizophrenia has proved to be a challenging disorder to model in animals since its origin is unknown and its diagnosis relies on behavioural abnormalities that may only be expressed in humans. It is also difficult to discern the fidelity of animal models since no obvious biological marker exists (Lipska & Weinberger, 2000). Nevertheless, animal models of schizophrenia have been used to investigate the pathology of the disease and to the aid the development of new treatments (e.g. Lewis & Lieberman, 2000; Lipska & Weinberger, 2000; Morris, Cochran, & Pratt, 2005). Most of the available models reflect the pathology and behavioural manifestations of the positive symptoms of schizophrenia. This is reflected by the fact that the majority of treatments developed for schizophrenia have been most effective against the positive symptoms. However, in order to screen new treatments to assess their potential efficacy against negative symptoms in a pre-clinical setting, it is of great benefit to have animal models that reliably reflect those aspects of the disease. This is particularly difficult in animals since negative symptoms are often described as emotional in nature.

One main objective of this body of work is to attempt to identify an analogue of the negative symptom, anhedonia, in an animal model of schizophrenia. An

important feature of a model is that it should not be impractical to use in large scale studies such as those generally used in pharmaceutical research. If a reliable indicator of anhedonia could be generated in animals, this could provide an invaluable screen for the development of therapeutic compounds.

1.7 Anhedonia in schizophrenia

Anhedonia is commonly understood as a diminished capacity to experience pleasure. Classic descriptions of schizophrenia, provided by Kraeplin (1919) and Bleuler (1911) emphasised the presence of anhedonia. At a later date major theorists such as Rado (1956) and Meehl (1962) proposed that anhedonia may be one of the four cardinal symptoms of schizophrenia. Meehl further suggested that anhedonia is an indicator of genetic vulnerability necessary for the onset of schizophrenia and also predicts the severity of the social impairments that will be experienced by a sufferer. However, more recently, Pelizza and Ferrari (2009) found that out of a sample of 80 schizophrenia sufferers, anhedonia scores were only significantly higher than controls in 45% of patients. Positive correlations of anhedonia with negative and disorganised symptoms were found, indicating that Meehl's hypothesis of anhedonia being a necessary factor in the aetiology of schizophrenia is limited, at most, to subgroups characterised by high levels of negative and disorganised symptoms. However, since this subgroup is the most problematic to treat and has the poorest prognosis in terms of functional outcome, the study of anhedonia is of great importance.

Anhedonia is often classified into different forms. Chapman, Chapman and Raulin (1976) drew a distinction between two types of anhedonia, physical and social, and devised scales of assessment for each. The physical assessment scale included a variety of pleasures such as viewing a sunset, eating and drinking, whereas the social

anhedonia scale contained pleasures associated with spending time with others and having friendships. Chapman et al. confirmed that people with schizophrenia generally have significantly higher levels of physical and social anhedonia than controls. Andreasen (1982) subsequently included these two forms of anhedonia in the anhedonia-asociality subscale of the Scale for the Assessment of Negative Symptoms (SANS), the most frequently used measure of anhedonia in modern clinical research.

Another distinction that has been made between different forms of anhedonia relies on the distinction between pleasure that occurs at the time of a particular experience (consummatory pleasure) and pleasure that occurs in advance of an experience (anticipatory pleasure) (Gard, Gard, Kring, & John, 2006). Gard et al. (2006) reported that people with schizophrenia experience lower levels of anticipatory pleasure than controls, but display intact consummatory pleasure.

A major factor that hampers the diagnosis and development of treatments for anhedonia stems from the fact that diagnostic scales of assessment most commonly do not differentiate between the different forms of the symptom outlined above. Additionally, diagnostic tools tend to obscure the boundaries between purely hedonic factors and other capacities that are also often impaired in schizophrenia, such as motivational and cognitive factors (Horan, Kring, & Blanchard, 2006). For example, the SANS questionnaire contains items that enquire about the frequency of engagement in physically or socially enjoyable activities. However, the ratings provided by such questions may reflect cognitive deficits, motivational issues, financial constraints or paranoia, for example, rather than an inability to experience pleasure per se.

The occurrence of cognitive impairment in schizophrenia has been well-documented. In particular, memory impairment and deficits in executive function have been frequently highlighted. Impairments in both immediate and delayed recall have been found during acute psychotic episodes and during remission (e.g. Mohamed, Paulsen, O'Leary, Arndt, et al., 1999; Hoff, Riorden, O'Donnell, Morris, et al., 1992). Verbal and spatial memory deficits also appear to be essential components of schizophrenic pathology (e.g. Saykin, Shtasel, Gur, Kester, et al., 1994; Joyce, Hutton, Mutsata, Webb, et al., 2002).

People with schizophrenia have difficulties with problem-solving and planning (Hutton, Puri, Duncan, Robbins, et al., 1998). Aspects of executive functioning such as sequencing, organisation and flexibility show high levels of impairment in patients with first episode schizophrenia compared with controls (Mohamed et al, 1999).

There is much evidence that although anhedonia is correlated to other symptoms (Blanchard, Mueser, & Bellack, 1998; Pelizza & Ferrari, 2009), it is in fact a distinct aspect of schizophrenia (Horan et al, 2006). For example, anhedonia and depression often show some overlap and can be difficult to differentiate clinically, but numerous factor analysis studies show that anhedonia and depression constitute separate factors in schizophrenia patients (Hafner, Loffler, Maurer, Hambrecht, & van der Heiden, 1999; Kitamura & Suga, 1991; Kulhara & Avasthi, 2003). In addition, anhedonia in schizophrenia also differs from anhedonia present in major depression, since in schizophrenia, anhedonia is stable over time and tends to remain during remission from psychotic symptoms, whereas anhedonia in depression tends to co-occur with depressive episodes (Pelizza & Ferrari, 2009). Anhedonia is also frequently confused with apathy. Although people with anhedonia show a reduction

in positive emotions, the majority show equal or greater levels of negative emotions (e.g. Suslow, Roestel, Ohrmann, & Arolt, 2003), indicating that most do not experience global apathy. The fact that amelioration of psychotic symptoms in schizophrenia is not correlated with improvement in negative symptoms also demonstrates conclusively that anhedonia is not associated with positive symptoms (Miller et al, 1994). For this reason, animal models that reflect the positive symptoms of schizophrenia, or any of the other negative symptoms, are not adequate to serve as a pre-clinical screening tool for drugs with potential to treat anhedonia.

1.8 Treatment of anhedonia

Most of the available treatments for schizophrenia, and particularly typical antipsychotics, are D_2 receptor antagonists. The revised dopamine hypothesis suggests that positive symptoms may result from an increase in dopaminergic activity in the mesolimbic system, whereas negative symptoms may result from a decrease in dopaminergic activity in the mesocortical system. However, neuroimaging has shown that reduced dopaminergic activity in the mesolimbic system is associated with severe negative symptoms. This bi-directional relationship between mesolimbic dopamine activity and schizophrenia symptoms may account for the worsening of negative symptoms caused by drugs that alleviate the positive symptoms (Juckel, et al., 2006). Indeed, it has been reported that just a single dose of haloperidol or respiridone can induce negative symptoms in healthy individuals (Artaloytia, Arango, Lahti, Sanz, Pascual, Cubero, et al., 2006). On the basis of such findings, it has been suggested that drugs targeting neurotransmitter systems other than the dopaminergic system may be more effective in the treatment of anhedonia (Erhart, Marder, & Carpenter, 2006; Wolf, 2006). As mentioned previously, the development of appropriate

treatments for anhedonia will be greatly enhanced by the generation of pre-clinical models of the symptom.

1.9 Choosing a modelling method

Traditionally, animal models of schizophrenia have involved manipulation of the dopaminergic neurotransmitter system (Lipska & Weinberger, 2000). This has meant that most antipsychotic drugs produced to date have their effects via the dopaminergic system. The side effects of these drugs, along with their inability to treat the non-psychotic symptoms of schizophrenia have led to the development of new models in recent years.

Neurodevelopmental Models

Neurodevelopmental models attempt to reflect the hypothesised aetiology of schizophrenia. A number of studies have implicated developmental insults as contributory factors in the disease process. These factors include malnutrition (e.g. Susser & Lin, 1992), obstetrical complications during pregnancy or birth (e.g. Dalman, Allebeck, Cullberg, Grunewald, & Koster, 1999), and maternal influenza (Adams, Kendell, Hare, & Munkjorgensen, 1993). When these factors are imposed in animals, aspects of schizophrenia-like behaviour and pathology result. For example, gestational malnutrition has been shown to negatively affect learning and cognition (Tonkiss & Galler, 1990).Obstetrical complications have been reflected in animals by carrying out caesarean birth or inducing anoxia during birth, resulting in changes in dopamine activity in the mesolimbic system in adulthood (e.g. ElKhodor & Boksa, 1997). Additionally, pre-natal exposure to influenza has been reported to cause disorganised hippocampal pyramidal cells, consistent with what is seen in

schizophrenia, in some of the offspring of mice exposed to the virus (Cotter, et al., 1995).

Other neurodevelopmental models do not seek to reflect the causes of the disease, but to produce disease-like pathology by disrupting neurogenesis. An example of this is the methylazoxymethanol acetate (MAM) model. This model relies upon disruption of neurodevelopment by administration of a DNA methylating agent to pregnant rats on gestational day 17 (GD17). At this stage in development MAM exposure affects development of the frontal and temporal cortices, resulting in neuroanatomical and behavioural features resembling those in schizophrenia. Neuroanatomically, GD17 MAM exposure produces reductions in cortical thickness in the medial pre-frontal cortex and the hippocampus (Flagstad, et al., 2004; Le Pen, et al., 2006). In the human condition, similar reductions in cortical thickness have been found in the pre-frontal cortex and the temporal lobe (Wright, et al., 2000).

Other developmental models of schizophrenia have relied upon neonatal lesioning of the hippocampus. It has been found that neonatal lesions of the ventral hippocampus produce increased mesolimbic dopamine transmission, enhanced sensitivity to NMDA receptor antagonists, and impairments in latent inhibition and social behaviours which, in similarity to the disease state, appear only in adulthood (see Lipska & Weinberger, 2000 for a review).

Genetic Models

Genetic epidemiological studies have suggested that genetic factors are largely responsible for individual variation in susceptibility to schizophrenia. The susceptibility genes for which the most supporting data is available are those encoding

dysbindin (DTNBP1) and neuregulin 1 (NRG1) (Owen, Craddock, O'Donovan, 2005).

Dysbindin binds to both α- and β- dystobrevin, which are components of the dystrophin glycoprotein complex (Benson, Newey, Martin-Rendon, Hawkes, et al., 2001). Dysbindin is reduced in the schizophrenic brain within glutamatergic neurones of the hippocampus (Straub, 2002), and a reduction in glutamate release has been found in cultured neurones with reduced DTNBP1 expression (Numakawa, Yagasaki, Ishimoto, Okada, et al., 2004). This suggests that DTNBP1 may contribute to schizophrenia risk by altering glutamate release.

Neuregulin 1 encodes around fifteen proteins with a wide range of functions in the brain, including, neurotransmission, axon guidance, glial differentiation, myelination and synaptogenesis (Corfas, Roy & Buxbaum, 2004). Impairment in any of these functions could increase an individual's susceptibility to schizophrenia.

A more recent discovery concerns the involvement of the gene ZNF804A in schizophrenia. O'Donovan, Craddock, Norton et al. (2008) carried out a genome-wide association study reported evidence for association between rs1344706 within ZNF804A (encoding zinc-finger protein 804A). Proteins with this zinc-finger domain were originally identified as DNA-binding molecules with a role in transcription. In addition, the mouse homologue of ZNF804A has recently been suggested to be involved in the regulation of early neurodevelopment (Chung, Lee, Deocaris, Min et al., 2010). Although much work is still to be done to understand the role of this gene, its genome-wide significance is attracting much research activity (Donohoe, Morris & Corvin, 2010)

Schizophrenia has also been associated with several chromosomal abnormalities (MacIntyre, Blackwood, Porteous, Pickward, et al., 2003). For example,

adults with 22q11 deletion syndrome have an increased risk for schizophrenia (Williams & Owen, 2004). Candidate genes mapping to this region include catechol-O-methyltransferase (COMT) and proline dehydrogenase (PRODH) (Kirov, Williams, Preece, et al., 2004). Additionally, a chromosomal translocation has been implicated in schizophrenia, disrupting two genes on chromosome 1: DISC1 and DISC2. DISC1 appears to be related to neuronal migration and intracellular transport, compatible with a role in schizophrenia (Ozeki, Tomoda, Kleiderlein, Kamiya, et al., 2003).

Genetic models have been produced on the basis that genetic abnormalities are associated with predisposition to schizophrenia. Such models have been used to investigate the involvement of neurotransmitter receptors in schizophrenia and have been useful for the replication of social anhedonia. For example, Mohn, Gainetdinov, Caron and Koller (1999) created a mouse model of schizophrenia by producing a mutant strain with reduced numbers of NMDA receptors. Unlike control animals, these mice tended to avoid social contact with one another, an abnormality that was reversed by acute clozapine treatment. Genetic models have the added benefit that they can be used to study the interaction between developmental and genetic factors in the aetiology of schizophrenia.

NMDA receptor antagonism

Pharmacological models produced by acute and sub-chronic administration of NMDA antagonists such as PCP, s-(+)-ketamine and (+)MK-801 have become popular in recent years. These models have evolved from ideas surrounding the glutamate hypothesis of schizophrenia (see section 1.4). Morris et al. (2005) suggested that NMDA antagonists, and in particular PCP, are purported to be unrivalled in their ability to mimic both the positive and negative symptoms of schizophrenia and the

underlying pathology of the disorder. In humans, acute treatment with NMDA antagonists (particularly PCP and s-(+)-ketamine) can induce positive symptoms (e.g. Krystal, et al., 1994; Malhotra, et al., 1996) and cognitive deficits (Malhotra et al, 1996). The translational capacity of this treatment into a pre-clinical setting has also been revealed. For example, in rodents, acute doses of NMDA antagonists can induce effects which may relate to schizophrenic symptomatology, such as augmented stereotyped behaviours and hyperlocomotion (e.g. Castellani & Adams, 1981; Verma & Moghaddam, 1996)[1]. Additionally, Dunn and Killcross (2007) observed that conditional discrimination performance is disrupted by acute administration of PCP. In a conditional discrimination task, animals learn to make an appropriate response according to the particular stimulus that is presented to them. For example, in this study, animals were reinforced if they pressed a left lever in response to a click, or a right lever in response to a tone. Impairment in this task can be likened to a cognitive deficit in humans with schizophrenia whereby difficulty is experienced in using environmental cues to inform goal-directed behaviour (e.g. Stratta, Daneluzzo, Bustini, Casacchia, & Rossi, 1998; Stratta, Daneluzzo, Bustini, Prosperini, & Rossi, 2000). Dunn and Killcross proposed that PCP disrupts conditional discrimination performance by increasing extracellular levels of dopamine in the pre-frontal cortex. This is supported by their finding that drugs that are antagonists of D_1 receptors (abundant in the pre-frontal cortex) are able to attenuate this deficit, whereas antagonists of D_2 receptors (almost absent from the pre-frontal cortex) are not.

Sub-chronic administration of PCP in rodents is thought to be even more relevant than acute treatment as a model of schizophrenia as it appears to have greater capacity to produce the non-psychotic symptoms of schizophrenia (Morris, et al.,

[1] Hyperlocomotion is thought to reflect dysfunction within the striatal dopaminergic system, related to the positive symptoms of schizophrenia.

2005). For example, in humans, repeated exposure to PCP produces long-term cognitive deficits (e.g. Cosgrove & Newell, 1991) and negative symptoms (Javitt & Zukin, 1991). This thought to be because, in contrast to acute PCP treatment, sub-chronic treatment produces a reduction in glucose utilisation and blood flow in the prefrontal cortex (hypofrontality). This hypofrontality is exhibited in schizophrenia and it correlates with deficits in cognitive function (e.g. Buchsbaum, et al., 1990).

In addition to these pathological changes, rats that receive withdrawal from sub-chronic PCP treatment also show changes in social behaviour (Sams-Dodd, 1998) that are closely related to the abnormalities observed in schizophrenia (but see also Jenkins, Harte, McKibben, Elliott, & Reynolds, 2008). In addition, rats treated sub-chronically with PCP also exhibit cognitive deficits in terms of conditional discrimination performance (e.g. Dunn & Killcross, 2006). Dunn and Killcross (2006) also attribute this deficit to disturbance in pre-frontal dopaminergic activity since sub-chronic PCP treatment reduces levels of prefrontal dopamine. In their investigation cognitive function was improved following clozapine treatment, which is known to restore pre-frontal dopamine, but not by haloperidol, which has an opposite effect on dopamine activity. Dunn and Killcross suggested that the sub-chronic PCP model therefore has significant predictive validity since the efficacy of clozapine but not haloperidol in the amelioration of cognitive deficits in rats may reflect the superiority of certain atypical antipsychotics in the treatment of cognitive symptoms in humans.

Attempts to model schizophrenic anhedonia using the NMDA antagonist PCP have already been made. It has been claimed that an analogue of anhedonia is present in rats twenty hours after a large (15mg/kg) dose of PCP (Baird, Turgeon, Wallman, & Hulick, 2008; Turgeon & Hoge, 2003). This was inferred from a decrease in the amount of sucrose consumed voluntarily by PCP-treated animals. Decreased

voluntary consumption of a sucrose solution is a commonly used indicator of anhedonia in stress-induced models of human affective disorders (e.g. Papp & Moryl, 1994; Papp, Willner, & Muscat, 1991; Rygula, et al., 2005; Willner, Lappas, Cheeta, & Muscat, 1994; Zurita, Martijena, Cuadra, Brandao, & Molina, 2000; Zurita & Molina, 1999). However, the assumption that general consumption reflects a hedonic response is problematic since the amount an animal consumes may reflect a number of ingestive and motor factors that do not necessarily relate to the value or liking of a reward. Unfortunately, there are also problems with the face validity of a schizophrenia model produced by withdrawal from an acute high dose of PCP such as that produced by Turgeon and Hoge (2003) since the pathology produced by administration of large doses of PCP is inconsistent with that seen in schizophrenia (Olney, Labruyere, & Price, 1989).

Despite the limitations of the above studies, it is plausible that NMDA antagonists do in fact have the potential to produce anhedonia in animals. Indeed, the similarity between the pathology brought about by sub-chronic PCP treatment and that seen in schizophrenia, along with the numerous reports of the effectiveness of NMDA antagonists in producing negative and cognitive symptoms in both humans and animals (e.g. Javitt & Zukin, 1991; Lahti et al. 2001; Dunn & Killcross, 2006) suggest that models produced via NMDA antagonist administration hold great promise as a model of anhedonia. In addition, NMDA antagonist treatment is relatively practical to implement in large scale pharmacological screening studies, and could therefore be more useful in drug development than other, more labour-intensive models. For these reasons, pharmacological treatment with NMDA antagonists was selected as a primary modelling method throughout this thesis in order to explore the presence of anhedonia.

1.10 NMDA antagonist mechanism of action

NMDA receptors act at selective ion channels and are therefore known as ionotropic glutamate receptors. They are tetramers comprised of at least one NR1 subunit in combination with different numbers of NR2 and NR3 subunits. Each subunit can exist as one of a number of isoforms, thereby allowing the formation of many different NMDA receptors with distinct pharmacological properties (Lynch & Guttmann, 2001). Unusually for the ionotropic glutamate receptors, glutamate is not the only agonist of the NMDA receptor. Glycine is a co-agonist and both transmitters must bind in order for the receptor to function (Johnson & Ascher, 1987). At resting membrane potentials NMDA receptors are inactive due to a voltage-dependent block of the channel pore by magnesium ions, preventing ion flow through it. Sustained activation of AMPA receptors depolarises the post-synaptic cell, releasing the channel inhibition and thus allowing NMDA receptor activation and cation influx, thereby enabling a variety of signalling cascades (Lynch and Guttmann, 2001).

The NMDA receptor antagonist PCP is a non-competitive antagonist and is frequently referred to as an 'open channel blocker'. Open channel blockers enter the NMDA receptor-associated ion channel only when it is already open, and become trapped inside the channel when the agonist unbinds. Javitt and Zukin (1991) suggested that, despite PCP's other action on potassium and sodium channels, it is the blockade of NMDA receptor-associated calcium channels that underlie its psychotomimetic properties. S-(+)-ketamine is a weaker blocker, and (+)MK-801 a more potent blocker, of these same channels (Ellison, 1994).

1.11 Measurement of hedonic responses in animals

Traditionally, assessment of the hedonic value of rewards has relied upon measurements of general consumption, preference, or willingness to make instrumental responses. The assumption behind such tests is that the palatability of a reinforcer is inextricably linked to the amount an animal consumes. Berridge (1996) has suggested that consumption actually reflects how much an animal wants a reinforcer, whereas palatability reflects how much an animal likes it. Via a review of taste reactivity studies he has suggested that 'wanting' and 'liking' are two separate components of reward value.

Taste reactivity was developed by Grill and Norgen (1978b) to provide a more informative measure of hedonic responses in rats than general consumption. This method involves infusing a small quantity of a taste solution directly into a rat's oral cavity and video-recording the response for frame by frame analysis. Grill and Norgen examined sucrose, NaCl, HCL and quinine, and found that the responses to these taste stimuli were consistent within and between rats. Responses to quinine, a substance known to be bitter and unpalatable, involved gaping and increased body movement, whereas responses to sucrose, known to be sweet and palatable, were characterised by patterns of mouth movements and tongue protrusions. Since reactions to palatable (e.g. sweet) and unpalatable (e.g. bitter) solutions are well characterised, the taste reactivity test allows the palatability of novel tastants to be assessed by examining whether their taste reactivity profiles are a better match to those produced by sucrose or quinine.

There are also examples of taste reactivity changing because of learning. For example, when a palatable tastant is paired with lithium chloride administration in rats, consumption of that solution is subsequently reduced. Taste reactivity testing

shows that the tastant also becomes aversive under such circumstances (Berridge, Grill, & Norgren, 1981; Grill & Norgren, 1978a; Parker, Hutchison, & Riley, 1982). However, Parker (2003) cites evidence that although a number of stimuli can cause a reduction in the consumption of a solution it is paired with, stimuli that do not produce nausea do not produce an aversion to the taste of the solution. This is true in the case of foot-shock, for example (Pelchat, Grill, Rozin, & Jacobs, 1983). Parker (2003) suggests that the reduction in consumption brought about by nausea is related to a conditioned disgust response in which an animal learns to dislike the tastant, whereas reduced consumption brought about by pairing with other aversive stimuli is related to a conditioned fear response in which the palatability of the tastant is unchanged. Findings indicating that reduced consumption is not always accompanied by palatability changes support the idea that taste reactivity responses reflect palatability (or liking) rather than characteristic responses to the sensory properties of a tastant. That is, a learned association between an aversive stimulus and a taste should not change the sensory properties of the tastant, but it can change the hedonic reaction to it. Such observations are consistent with the ideas of Berridge (1996), who suggested that palatability is not reflexive, but instead reflects both the sensory properties of a tastant and its prior associations.

There are also instances where conditioned associations produce an increase in palatability using taste reactivity testing. For example, Myers and Sclafani (2001b) showed that pairing flavoured saccharin with intragastric glucose infusion resulted in an increase in the palatability, indicated by an increased number of positive responses.

Taste reactivity studies, examining brain and pharmacological manipulations have also indicated that wanting (linked to the motivation to consume) and liking

(linked to palatability) do not always co-vary and can frequently be manipulated separately (Berridge, 1996). For example, Berridge, Venier and Robinson (1989) brought about ablation of the dopaminergic neurones of the substantia nigra by treating rats with 6-hydroxydopamine. The subsequent depletion of dopamine caused rats to become aphagic. However, their hedonic reactions to sweet and bitter solutions remained intact, indicating that wanting had changed independently of liking. Berridge (1996) cites another dramatic example of this dissociation in rats who received electrical stimulation of the lateral hypothalamus, a treatment which potentiates feeding. Taste reactivity tests showed that hedonic reactions were not potentiated and unexpectedly, aversive reactions were enhanced (1991).
Data from such taste reactivity studies led Berridge (1996) to conclude that consumption (linked to wanting) is controlled mainly by mesotelencephalic dopamine neurotransmitter systems, whereas palatability (linked to liking) appears to be linked to opioid and GABA neurotransmitter systems. The idea that dopamine is not closely linked to liking, is not inconsistent with the fact that typical antipsychotics are not effective in the treatment of anhedonia.

Taste reactivity studies conclusively demonstrate that consumption and palatability do not always co-vary. In relation to the study of anhedonia, palatability (linked to liking) would have greater relevance than consumption (linked to wanting). Therefore, for the investigation of anhedonia in animal models, measures of general consumption are not sufficient. However, despite its advantages over general consumption measures, the taste reactivity method has limitations that prevent it from being effective for examining the presence of anhedonia. The most crucial of these is that taste reactivity tests can only provide categorical information as to whether something is palatable or aversive to an animal, but it cannot give information on how

palatable a substance is. Since anhedonia is characterised by a reduction in the capacity to experience pleasure, but may not make normally pleasurable experiences aversive, it is unlikely that taste reactivity will enable the detection of anhedonia.

Another way in which the value of a reward can be measured, with more direct relevance to anhedonia, is to examine its palatability via microstructural analysis of licking during ingestion. Rats rarely show continuous consumption of a liquid. Instead they perform repeated clusters of licks separated by pauses. The number of licks in each cluster (cluster size) has a positive, monotonic relationship with the concentration of a palatable solution such as sucrose (Davis & Smith, 1992; Hsiao & Fan, 1993; Spector, Klumpp, & Kaplan, 1998). Cluster size has a negative monotonic relationship with the concentration of an unpalatable solution such as quinine (Spector & St John, 1998). These findings have led to the idea that cluster size may be a useful measure of reinforcer palatability and hence, reward value. Importantly, cluster size is not simply a reflection of the amount consumed. For example, although cluster size increases with increased sucrose concentration, the amount consumed varies in an inverted U-shaped function (e.g. Ernits & Corbit, 1973). This relationship means that it is possible to select many pairs of different concentrations in which each solution, when offered to a rat, gives rise to different cluster size measurements, whilst overall consumption may be the same. In addition, studies of conditioned taste aversion have also shown that palatability and consumption can dissociate. For example, Dwyer, Boakes, and Hayward (2008) showed that consumption of a palatable saccharin solution is reduced by pairing with amphetamine administration, lithium administration or forced wheel running. However, only lithium administration and wheel running produced a reduction in cluster size indicative of reduced palatability. This is consistent with Parker's

suggestion that palatability is only reduced by stimuli that induce nausea (Parker 2003). In contrast, Myers and Sclafani (2001a) demonstrated that flavour preference learning can increase the palatability of the CS+ flavour using lick cluster size measures in addition to their concurrent analysis of taste reactivity. Moreover, a flavour conditioning study by Dwyer (2008) showed that palatability responses can be modified in situations even when consumption is not. In this study, conditioned stimulus (CS) flavours were paired with 2% and 16% maltodextrin as the unconditioned stimuli (US). CS1 was paired with 2% maltodextrin and CS2 was paired with 16% maltodextrin. Both flavours were then tested in combination with both maltodextrin concentrations and it was found that animals consumed more CS1 than CS2 when paired with 2% maltodextrin, but not when paired with 16% maltodextrin. On the other hand cluster size was always greater with CS1 than with CS2. In summary, there is a double dissociation between cluster size measures of palatability and measures of consumption whereby some treatments affect cluster size but not consumption, while others affect consumption but not cluster size.

In addition to the parallels between palatability measurements made using microstructural analysis and those made using taste reactivity testing, the lick analysis method also benefits from its sensitivity to drug treatments. For example, Higgs and Cooper (2005) investigated the effects of the benzodiazepine receptor agonist, midazolam maleate, on the licking microstructure of water-deprived rats during ingestion of sodium chloride solutions. Midazolam increased the amount of solution consumed by increasing the size of lick clusters rather than by increasing the number of lick clusters. Therefore the analysis of licking suggests that the benzodiazepine receptor agonist increased intake by increasing the perceived palatability of the solution. Other drugs that increase consumption may do so in different ways. For

example, morphine causes an increase in the number of lick clusters produced but does not increase cluster size (Higgs & Cooper, 1998). Microstructural analysis of licking is therefore a very useful method for discerning the mode of action of different drugs.

The sensitivity of microstructural analysis to small changes in hedonic value, in addition to the fact that lick recordings can be automated make this method more accurate and less cumbersome than the taste reactivity method for examining hedonic responses. In the absence of changes in the test solution, any change in palatability indicates an effect on animal itself. Therefore, the lick analysis method provides a very useful tool for exploring the presence of anhedonia in pre-clinical animal models, and so is the primary measurement used for the experiments presented in this thesis.

1.12 Using the lick analysis method

The lick analysis method relies on the automated recording of each lick made by each rat. From this data the number and size of lick clusters and the average inter-lick interval (ILI) within clusters can be extracted. Throughout this thesis, licks separated by an interval of less than 0.5s are considered to fall within the same cluster. If this interval is exceeded, further licks mark the beginning of a new cluster. This cluster definition was utilised by Davis and Smith (1992). A 1s criterion has been used by other researchers (e.g. Spector, et al., 1998). However, this makes little difference as most pauses greater than 0.5s are also greater than 1s (e.g. Davis & Smith, 1992; Spector et al. 1998).

The ability to calculate the average interval between each lick is a major advantage of this method. ILI normally shows very little variability, except when

motoric changes are induced: for example, by changes in posture (Weijnen, 1998), or disturbances in motor pattern generation in the hind brain (Aja, Sahandy, Ladenheim, Schwartz, & Moran, 2001). Since an increase in ILI is likely to impact upon cluster size measurements, it is very useful to be able to monitor this parameter when using drugs that may produce motor abnormality. For this reason, ILI was monitored for all experiments in this thesis. However, since it generally indicates motor problems, and does not reflect hedonic value, ILI will only be reported when drug-induced changes, indicative of motor abnormality, arise.

1.13 Summary

To conclude, the introduction to this thesis has discussed the importance of anhedonia as a negative symptom of schizophrenia and has highlighted the limitations of currently available treatments in ameliorating this symptom. The lack of animal models able to reliably reflect the negative symptoms of schizophrenia has hindered progress in the development of adequate treatment. The assessment of anhedonia in an animal model of schizophrenia will require a sensitive measure of hedonic reactions. I have suggested that microstructural analysis of licking during ingestion provides such a method by giving an index of palatability that is sensitive to small changes in hedonic value. The advantages of pharmacological models relying on NMDA receptor antagonist treatment in rodents has also been discussed since this method has translational validity and is efficient to produce, making it useful for large-scale screening of therapeutic compounds. Therefore, the main focus of this thesis will be to examine NMDA receptor antagonist models of schizophrenia using microstructural analysis of licking. The initial work presented will describe my attempts to use lick analysis in conjunction with operant schedules of reinforcement.

Subsequent chapters will focus on the effects of NMDA receptor antagonists on lick analysis measures during voluntary consumption in the absence of operant responding.

CHAPTER TWO

Effects of operant responding on drinking behaviour

2.1 Introduction

Assays incorporating various schedules of instrumental responding have been used to compare the *in vivo* effects of NMDA antagonists to examine their potential to model the motivational and cognitive deficits present in schizophrenia. For example, Gilmour et al. (2009) compared a selection of NMDA antagonists, including PCP, s-(+)-ketamine and (+)MK-801 using a series of variable interval (VI) schedules of reinforcement. In an operant VI schedule, animals are rewarded after a variable period of time if they press an 'active' lever following this interval. It was found that s-(+)-ketamine produced a dose-dependent decrease in instrumental output, whereas PCP and (+)MK-801 produced bi-directional effects in which lower doses increased responding and higher doses eventually decreased responding. It is possible that the performance disparity produced by these drugs may reflect differences in motivation to obtain reinforcers. It is also possible that decreased responding may be secondary to a drug-induced reduction in the perceived hedonic value of the reinforcer, which may be likened to anhedonia.

There are, however, several reasons why decreased instrumental responding cannot be unambiguously interpreted in terms of reduced hedonic value. Firstly, the physical effort involved in lever pressing may mean that this measure will be affected by any motoric changes induced by drugs. Secondly, it has been shown that with continued operant training, performance can become habitual rather than goal-directed. This means that a cue such as a lever may elicit a response regardless of the outcome value, once a stimulus-response habit has been formed (Dickinson &

Balleine, 2002). A third reason why changes in instrumental responding cannot be confidently used for the purposes of studying anhedonia stems from the apparent dissociation between 'wanting' and 'liking' discussed earlier (Berridge et al, 1996). Lever pressing is an appetitive response that more closely reflects motivation to obtain a reinforcer than the reinforcer's hedonic value per se, and therefore this measure alone is not adequate to infer changes in an animal's capacity to experience pleasure. For these reasons, it was considered that a useful initial step in attempting to assess hedonic value would be to combine operant responding with the lick analysis technique described in the introduction by training rats to press a lever to gain access to a sucrose solution.

Preliminary training sessions were carried out to determine whether food-restricted rats would reliably press a lever to gain access to a sucrose solution. Fixed ratio (FR) schedules were used for this purpose. In a fixed ratio schedule a reinforcer is presented to an animal after they have made a specified number of lever presses. During the preliminary training sessions the fixed ratio response requirement was gradually increased from one lever press (FR1) to 50 lever presses (FR50). Interestingly, it was observed that the size of lick clusters during ingestion of the sucrose reinforcer increased as the response requirement was raised. This increase in cluster size may have been due to the extra experience of drinking in the experimental apparatus. However, it could also be reflective of an effect whereby the more effort rats expended to obtain the reinforcer, the greater its perceived palatability. Although the latter would be an important and interesting effect, it would also imply that the measurement of hedonic value using operant schedules has a major confound. Therefore, Experiment 1 sought to determine whether increased reinforcer experience

or increased effort was responsible for the increased cluster size observed with increasing response requirement.

2.2 Experiment 1

Method

Subjects

Experiment 1 was conducted in the behavioural neuroscience laboratory at the School of Psychology, Cardiff University. 12 male hooded Lister rats supplied by OLAC, Bicester, UK were used. They were housed in pairs on a 12 hour / 12 hour light / dark cycle and experimental procedures were performed 4 hours into the light portion of the cycle. The animals were maintained on a food restricted diet with ad libitum water access for the duration of the experiment. Their initial free-feeding weights ranged 285-330 grams. Daily food rations were placed in the home cages 30 minutes after completion of the experimental session. Food rations were monitored and adjusted to maintain the animals at no less than 85% of their free-feeding weights.

Apparatus

Testing was carried out in a room containing 4 operant chambers measuring 30×24×21cm. These chambers were housed in light and sound attenuation boxes. The chamber floor consisted of 19 steel rods measuring 4.8mm in diameter. Two of the chamber walls were clear perspex and two were aluminium. A lever was positioned on the right hand side of one of the aluminium walls and a hole was positioned 5cm above the grid floor on the left hand side to allow rats to access solutions during experimental sessions. Solutions were given in 50ml bottles attached to stainless steel

drinking spouts. These were fastened to motorised holders that positioned the metal spout level with the wall of the chamber, and retracted it when required. A contact-sensitive lickometer registered the time of each lick to the nearest 0.01s. This was recorded by a computer using MED-PC software (Med Associates Inc., St Albans, VT, USA). Licks separated by an interval of less than 0.5s were considered to fall within the same cluster. The number and size of lick clusters, the average inter-lick interval (ILI) within clusters, and the number of licks produced during each reinforcer presentation were extracted from the data recorded.

The amount of solution consumed by each animal was measured by weighing the drinking bottles before and after each session. The reinforcer was a 10% weight/weight sucrose solution.

Procedure

Rats received daily (Monday-Friday) 30 minute sessions throughout training and testing. In the first eleven sessions rats were trained to press a lever to gain 60 seconds of sucrose access on random ratio (RR) schedules. During RR schedules reinforcement is presented after an average number of responses. During training, the average number of responses required for each reinforcer was increased from 1 to 50. Once animals were responding reliably on the RR50 schedule they received alternating sessions with RR10 and RR50 response ratios for four days. This was counterbalanced so that half of the animals received RR10 schedules on days 1 and 3, and half received RR50 schedules. This was reversed on days 2 and 4. Because animals were able to earn more rewards on the RR10 than the RR50 schedule, data analysis was performed on the first five rewards of each day only.

Results

Statistical significance was set at $p<.05$ throughout this thesis. Figure 1A shows the mean number of licks per cluster in both the high response requirement (RR50) and low response requirement (RR10) conditions. Figure 1B shows the mean number of licks produced per 60 second reinforcer. The lick cluster size measure of palatability was significantly higher in the high response requirement condition than in the low response requirement condition ($t(11)=4.60$, $p<.001$). By contrast, there were more licks/reinforcer when the response requirement was low than when the response requirement was high ($t(11)=2.73$, $p=.020$).

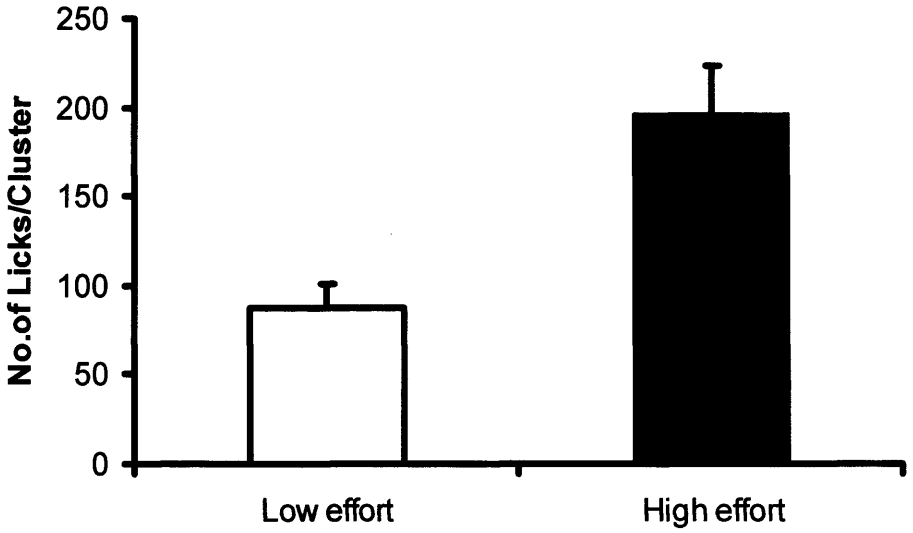
Summary of Experiment 1

Experiment 1 demonstrated that rats can be trained to press a lever to obtain moderately extended access to a sucrose solution. Additionally, it has shown that rats required to press a lever 50 times (high effort) to receive a sucrose reinforcer perceive this reward to be more palatable than when they press a lever only 10 times (low effort). This effect suggests there is a confound in the combined use of operant schedules and lick analysis to investigate anhedonia in pre-clinical models of schizophrenia.

2.3 Experiment 2

Although the findings of Experiment 1 complicate the use of operant responding in the measurement of hedonic value, the behavioural effect of reinforcer palatability increasing with effort is both unexpected and particularly interesting and thus warrants exploration. In fact, the effect bears striking resemblance to the human

1A.



1B.

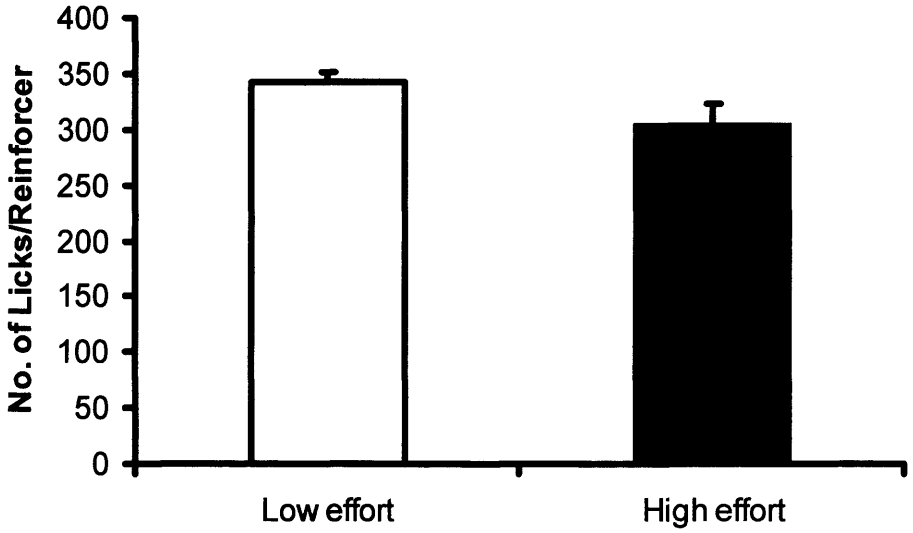


Figure 1: Shows the mean number of licks/cluster (Panel A) and licks/reinforcer (Panel B) with standard error of the mean (SEM), in low effort (RR10) and high effort (RR50) conditions.

phenomenon whereby greater value is placed on the things worked hardest for. For this reason, I will now explore theoretical accounts relating to this effect.

According to Festinger (1957), this tendency can be attributed to cognitive dissonance: a state of psychological discomfort that occurs when there is a discrepancy between a person's attitude and their actions. In such circumstances, humans try to reduce this dissonance by modifying their attitudes to complement their behaviour.

Festinger and Carlsmith (1959) paid people either $1 or $20 to take part in an essentially boring task and then tell a potential participant that the task was interesting. Intriguingly, when completing a questionnaire immediately following the task, participants paid $1 judged the task as more interesting than those paid $20. Festinger and Carlsmith argued that since $1 was not sufficient payment to justify the participants' actions, they instead altered their perception of the task to reduce the dissonance that was produced by behaving in a way that conflicted with their attitude.

More recently, evidence has been found that conscious reasoning is not required for cognitive dissonance-like effects. Lieberman, Ochsner, Gilbert, & Schacter (2001) showed amnesics a number of art prints and asked them to rate each picture. They were later shown sets of these prints. Each set comprised of two pairs of prints, previously rated equal by the participants. The participants were then asked to choose which pair they would prefer to put up in their house. This requisite forced participants to perform a counter-attitudinal behaviour as they were asked to choose between things that they had previously described as equal. When asked to re-rate the initial two groups of art prints, participants rated the chosen prints higher and the rejected prints lower than they had in their initial rating. Interestingly, amnesics

showed as much behaviour-induced change in their ratings as controls, despite having no explicit memory of the prints they had previously been asked to choose between, or the choices they had made. Festinger (1957) proposed that dissonance is produced when there is a discrepancy between a person's attitudes and their behaviour. This produces psychological discomfort which may be resolved by that person changing their attitudes. According to this theory, a person must attribute the psychological discomfort experienced to a particular behaviour. This suggestion seems to rely on the person remembering their previously held attitudes or their counter-attitudinal behaviour. However, since the amnesic participants in this study have no memory of their initial picture ratings or the choices they subsequently made, this suggests that behaviour-induced attitude change may be more automatic than cognitive dissonance theory posits. Interestingly, similar effects have been observed using an adaptation of this paradigm in monkeys and young children, again raising the possibility that complex cognitive reasoning may not be required (Egan, Santos, & Bloom, 2007). However, West, Beckman, Vonk and Jett (in press) suggest that this effect does not exist in non-primates. In experimental trials, a variety of primate and non-primate species were given choices between two equally preferred food items and then presented with the rejected item and another equally preferred item. In control trials, animals were presented with one accessible and one inaccessible food item. Following this they were presented with the previously inaccessible item and a novel item. It was found that primates preferred the novel item in experimental but not control trials. West et al. suggested that the primates resolved cognitive dissonance by reducing the value of items they rejected. However this did not occur in non-primate species.

Another interesting effect that has been explained in terms of cognitive dissonance reduction is 'Effort Justification', in which greater value is given to rewards that are harder to obtain. In a classic study by Aronson and Mills (1959), young women underwent 'severe' or 'mild' initiation to join a discussion group. Participants in the severe initiation condition were asked to read aloud a sexually explicit passage, whereas those in the mild initiation group were given much less embarrassing material to read. When asked to rate the discussion group, the severe initiation group's ratings were significantly higher than those of the mild initiation group. That is, manipulating the effort required to join the group changed the participants' perceived value of the outcome of their behaviour. Aronson and Mills suggested that the group whose initiation process was more difficult increased their valuation of the discussion group to resolve the dissonant relationship produced by undergoing severe embarrassment in order to join a discussion group of little value. There is some ambiguity as to whether the original proponents of cognitive dissonance theory believed that reasoned thought is necessary for behaviour-induced attitude change. However, the language used implies that this may be the case.

One way to investigate whether complex cognitive processing is required for effort justification is to determine whether this effect occurs in non-human animals. A number of studies indicate that some avian species show preference for stimuli associated with high effort tasks. For example, Kacelnik and Marsh (2002) found that starlings trained to fly either a short or long distance to reach different coloured response keys, which could be pecked to obtain a food reward, tended to prefer the coloured key that followed the highest effort (longest flight) when given a choice between both response keys. Similarly, Clement, Feltus, Kaiser, and Zentall (2000) presented pigeons with two different simple simultaneous discriminations, one that

followed 20 pecks to an initial stimulus, and one that followed a single peck. Following this, pigeons showed a preference for the discrimination stimuli that followed the highest effort (20 pecks). However, these studies assess preference for stimuli that precede food reinforcement and do not measure the value of the reinforcer itself. There is therefore a fundamental distinction between these animal-based studies and those demonstrating effort justification in humans.

The suggestion that such an effort justification effect can occur in rats was raised by Lawrence and Festinger (1962), who manipulated the effort required for a reward by altering the incline of a ramp leading to a goal box containing food. The behavioural measure of reward value used in this experiment was resistance to extinction. Lawrence and Festinger asserted that since the high effort response took longer to extinguish, the rats must have learned more about these trials than the low effort trials. However, they suggested that since the reinforcer was the same in both trials, learning could have only been improved if animals perceived the reinforcer that followed high effort as more valuable. However, their behavioural measure of reward value, resistance to extinction, has subsequently been shown to be an inadequate measure of response strength, as it is thought to indicate 'what' is learned during a task rather than 'how much' is learned (Mackintosh, 1974). This point is illustrated by the fact that, in an operant context, extinction of a response is faster when an animal previously received large reinforcers than when they had received small reinforcers. Mackintosh argues that this effect can be explained by the idea that discrimination between training (where the reinforcer is present) and extinction (where it is absent) is easier when the reinforcer is large than when it is small. Mackintosh further illustrates the point that resistance to extinction is an indicator of what is learned rather than how much by referring to the fact that partial reinforcement leads to

slower extinction of a response than continuous reinforcement. Similarly, this can be explained by the fact that it is easier to discriminate continuous reinforcement (where the reinforcer is present on every trial) from extinction (when it is never present) than it is to discriminate partial reinforcement (where the reinforcer is present on some trials but not others) from extinction. The effect reported by Lawrence and Festinger therefore provides insufficient evidence that effort increases the value of cues associated with rewards by causing an animal to learn more about them. In addition, Mackintosh's suggestion can directly explain the effect observed by Lawrence and Festinger. Since a low effort response holds little salience, if the reinforcer is removed from this training situation the difference between training and test situations is large and more obvious than if a reinforcer is removed from a training condition that requires high effort, which holds greater salience. That is, the reinforcer would form a greater part of the whole training situation when the effort requirement is low and so its removal would create a greater generalisation decrement.

Several studies have failed to find effort-induced changes in reward value in rats. Armus (2001) and Jellison (2003) manipulated the effort required to press a lever to obtain sucrose pellets. Rats were rewarded with either grape or bacon flavoured sucrose pellets according to the degree of effort required. The value of these flavoured pellets was then assessed in a Y maze (Armus, 2001) or a T maze (Jellison, 2003) in which the flavoured pellets associated with different effort requirements served as rewards for choosing particular maze arms. Animals showed no preference for arms containing reinforcers associated with high effort. The failure to find a significant effect of effort on reward value may have been due to the vast differences between the operant training context and the maze context used for testing, which may have prevented any effort-induced changes in flavour preference from

transferring to the test context. Indeed, as noted by Zentall and Singer (2007), by the time the rats had learned about the new test context, any effort-induced flavour preference obtained during operant training may have reached extinction. In addition, Jellison (2003) noted that rats showed poor discrimination between grape and bacon flavoured pellets in preliminary experiments. This fact, coupled with the knowledge that rats tend to alternate spontaneously when visiting maze arms, suggests that Y and T mazes may not provide sufficiently sensitive tests for detecting effort-induced changes in the value of rewards.

However, the combination of operant responding and lick analysis employed in Experiment 1 provides an appropriate method to assess whether rats are subject to the effort justification effect. If this is the case then rewards that follow large numbers of lever presses (equivalent to the severe initiation in Aronson and Mill's 1959 study) should be perceived to be of greater value than rewards that follow few lever presses (equivalent to the mild initiation). Experiment 1 showed that this is indeed the case. However, the effort involved in the lever press response requirement has two obvious components: work and waiting time, because it takes more work and more time to press a lever 50 times than 10. Therefore Experiment 2 investigated the possibility that differences in the interval between reinforcers in low and high ratio schedules may account for the changes in reward palatability seen in Experiment 1, using a procedure in which rats either worked (lever pressed), or just waited to be rewarded.

Method

Subjects and Apparatus

16 male Hooded Lister rats supplied by OLAC Bicester, UK were used. Husbandry procedures and experimental apparatus were the same as those detailed for Experiment 1.

Procedure

The rats received daily (Monday-Friday) 30 minute sessions throughout training and testing. Rats were pre-trained to press a lever to gain 60 seconds of sucrose access on fixed ratio (FR) schedules. During FR schedules reinforcement is presented after a specific number of responses. During training, the number of responses required for each reinforcer was increased from 1 to 50. Once animals were responding reliably on the FR50 schedule they were run in four conditions: a low effort master condition, a low effort yoked condition, a high effort master condition, and a high effort yoked condition. When run in the low and high effort master conditions, rats were required to make 10 or 50 lever presses respectively to receive a 60 second presentation of sucrose. Rats in the corresponding yoked conditions received reinforcement at the same time as the master animals but did not have access to the lever (See Table 1). By making some rats work to obtain the reinforcer, whilst corresponding animals simply waited the same length of time, the effects of time and work on reinforcer value could be isolated.

Each rat was tested twice in all four conditions and the order in which rats were assigned to each condition was counter-balanced. The animals were re-trained on FR10 and FR50 schedules after every two test days to ensure that they continued

Table 1:

Design of experiment 2.

Condition	Description
Low effort master	Fixed response requirement of 10 lever presses
High effort master	Fixed response requirement of 50 lever presses
Low effort yoked	No lever pressing required. Rats reinforced at the same time as the low effort master
High effort yoked	No lever pressing required. Rats reinforced at the same time as the high effort master

to lever press. The data presented here was averaged over the two tests in each condition.

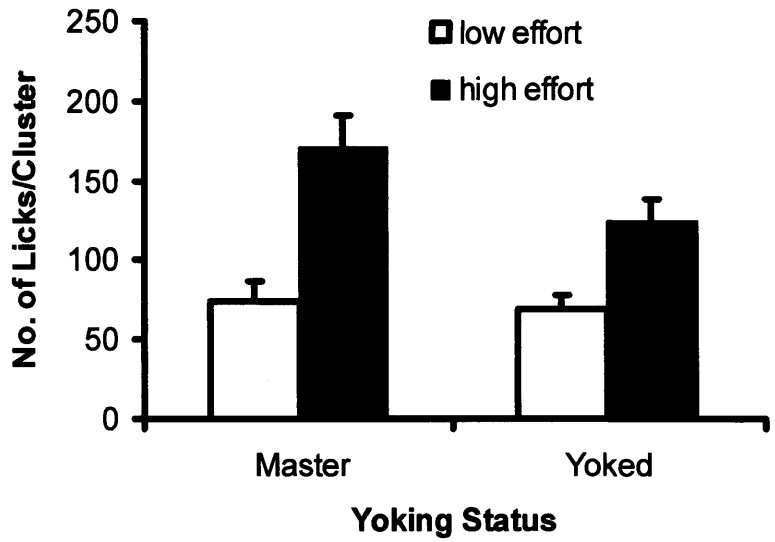
Results

A two way ANOVA was carried out with effort level and master/yoked status as the within-subjects variables. Figure 2A shows the mean number of licks/cluster in the yoked and master conditions, for both the low and the high effort conditions (FR10 and FR50). Overall, when in the high effort (FR50) conditions rats produced significantly more licks/cluster than rats in the low effort (FR10) conditions, $F(1,15)=27.92$, $p<.001$. Palatability also differed significantly between master and yoked conditions, with licks/bout being higher in the master conditions. $F(1,15)=13.33$, $p=.002$. Crucially, an interaction occurred between the two independent variables, $F(1,15)=8.7$, $p=.01$, whereby the palatability difference between the high and low effort conditions was greater in the master condition than in the yoked condition. The number of licks/reinforcer (Figure 2B) was unaffected by effort requirement, $F(1,15)=1.17$, $p=.298$, or yoking status, $F(1,15)=1.88$, $p=.191$, and there was no interaction, $F<1$. The number of reinforcers obtained in a session differed according to effort condition, $F(1,15)=60.5$, $p<.001$, with a mean of 12.6 obtained in the low effort condition and 8.8 in the high effort condition.

Summary of Experiment 2

As with Experiment 1, Experiment 2 showed that the sucrose reinforcer was perceived as more palatable in the high effort condition than in the low effort condition. Experiment 2 also showed that the palatability difference between the high

2A.



2B.

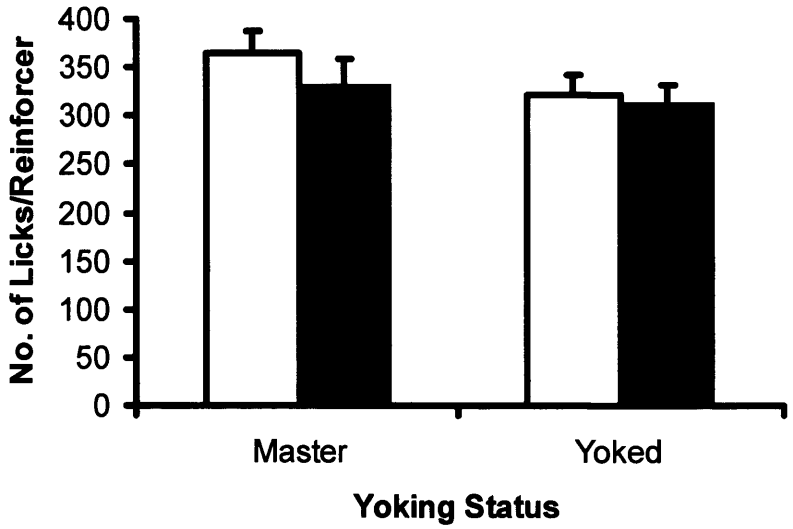


Figure 2: Panel A shows the mean number of licks/cluster with SEM, as a factor of effort level (FR10 v FR50) and yoking status. Panel B shows the mean number of licks/reinforcer with SEM, as a factor of effort level and yoking status.

and low effort conditions was increased when the animals had to work (lever press) for the reinforcer, rather than just wait for it.

2.4 Discussion

Experiments 1 and 2 indicate that rats perceived a sucrose reinforcer as more palatable in the high effort condition than in the low effort condition. The finding that the palatability difference between the high and low effort conditions was further enhanced when the animals had to work (lever press) for the reinforcer suggests that although the greater intervals between reinforcers in the high effort condition increased the palatability of the reinforcer, the effect of operant work was greater than that of time alone. This effect does not appear to be a physiological compensatory mechanism to alleviate thirst or energy expenditure since the amount consumed per reinforcer was not increased by raising the effort requirement. These results suggest that like humans, rats place more value on the things they work hardest for. Such an effort justification effect in humans would likely be attributed to mechanisms such as cognitive dissonance. Although appealing to such a theory to account for the rats' behaviour is possible, one of the guiding principles of comparative psychology is to interpret an animal's behaviour in the simplest possible terms (Morgan, 1894).

In an experiment by Clement et al. (2000), pigeons were required to peck a white light either once or twenty times to receive one of two different simultaneous colour discrimination problems. Pecks on the correct colour of each discrimination (S+) were rewarded with food. Following training, when pigeons were given a choice between the two positive (S+) colours, they showed a preference for the colour that followed the greatest effort during training. Clement et al. (2000) proposed that such a result could be attributed to 'within-trial contrast'. This account suggests that the

value of a positive stimulus is judged relative to the motivational state of the animal prior to its presentation. A twenty-peck response requirement is presumably more aversive than a single peck requirement. Therefore the appearance of the S+ that normally follows twenty pecks may be a relatively greater improvement in conditions (a greater contrast) than the appearance of the S+ that normally follows one peck. In this way a preference may develop for the outcome that follows higher effort. The experiment by Clement et al. is not a direct replication of the human effort justification effect, since the value of the reinforcer itself (the food reward) was not measured. However, the theoretical interpretation offered for their findings is interesting. If the results of this experiment were to be interpreted according to a contrast theory account, the palatability difference that emerged between master and yoked conditions indicates that an increase in the interval between reinforcers is aversive. However, an increase in the work required for reinforcement is even more aversive than an increase in the waiting time. Contrast provides one example of a parsimonious account of the behaviour I report here (and of other examples of effort justification) that does not require the involvement of any effortful cognitive mechanisms.

The findings of Clement et al. (2000) have been extended to demonstrate that other aversive events, besides effort, also enhance preference for discriminative stimuli. These include longer delay to reinforcement (DiGian, Friedrich, & Zentall, 2004), and the absence of reinforcement (Friedrich, Clement, & Zentall, 2005). Increased effort and hunger have both been found to induce changes in preference for discriminative stimuli in starlings (Kacelnik & Marsh, 2002; Marsh, Schuck-Paim, & Kacelnik, 2004). However, up until now, research that has shown significant effects of effort on reward value has assessed preference for stimuli that precede

reinforcement rather than measuring the value of primary reinforcers. In most cases this is necessary because ensuring that the objective values of different primary reinforcers are equal is difficult. However, in examples of real-life cognitive dissonance discussed by social psychologists (e.g. Aronson & Mills, 1959) these intermediate stimuli are not the point of interest. The novel method employing analysis of licking in combination with operant responding has enabled quantification of the effects of effort on the value of the primary reinforcer itself. This has provided the first direct analogue of the human effort justification phenomenon in animals. The discovery that behaviour-induced changes in perceived reward value are not unique to humans calls into question the use of complex cognitive theories to account for this intriguing phenomenon in general. It is a reminder that Lloyd Morgan's (1894, p.53) cannon "In no case may we interpret an action as the outcome of the exercise of a higher psychical faculty, if it can be interpreted as the outcome of one which stands lower in the psychological scale," should be borne in mind when examining human as well as non-human behaviour.

The finding that effort enhances reward value in rats also highlights a problem that may confound operant studies that use an animal's willingness to expend effort as a measure of reward value. If animals expend different levels of effort in a task, this may influence the hedonic value of a reinforcer. For instance, animals that are more inclined to respond for a reinforcer may experience a boost in the palatability of that reinforcer, which may in turn increase responding further. If animals are less inclined to respond, perhaps because of an anhedonic affect that lowers the palatability of reinforcers, these animals will not experience an effort-induced increase in palatability and therefore, any difference between control and drug-treated animals will be increased. This suggests that lever pressing should not be combined with

analysis of licking in my attempts to assess anhedonia in pre-clinical models of schizophrenia. For this reason, the following three chapters have assessed hedonic responses in using the lick analysis method in the absence of operant responding.

CHAPTER THREE

Acute NMDA receptor antagonists and drinking behaviour

3.1 Introduction

It is well documented that antagonism of NMDA receptors via a single administration of agents such as PCP, s-(+)-ketamine and (+)MK-801 can produce behavioural and cognitive deficits in healthy volunteers that closely mimic schizophrenia (Javitt & Zukin, 1991; Krystal, et al., 1994). Such treatments can also exacerbate psychosis in schizophrenic patients (e.g. Lahti, et al., 1995)

PCP was originally marketed as an anaesthetic agent that has its effects without causing respiratory or circulatory depression (Domino, Chodoff, & Corssen, 1965). However, it was soon found that other symptoms of PCP treatment, including paranoia and auditory hallucinations, were difficult to distinguish from those of schizophrenia (Smith, 1980). In rodents, acute doses of PCP can induce effects which may relate to schizophrenic symptomatology, such as augmented stereotyped behaviours and hyperlocomotion (e.g. Castellani & Adams, 1981; Verma & Moghaddam, 1996). PCP acts on a range of receptors and ion channels. However, Javitt and Zukin (1991) considered all of these actions and proposed that it is the NMDA antagonist properties of PCP that are responsible for its psychotomimetic actions, via the blockade of calcium channels (see section 1.10). S-(+)-ketamine also acts on NMDA receptors, although it is a weaker open channel blocker than PCP. Acute s-(+)-ketamine treatment is reported to produce schizophrenia-like symptoms in healthy volunteers, including performance deficits in verbal fluency, word recall and the Wisconsin Card Sorting test (Krystal, et al., 1994)

(+)MK-801 produces a more selective and potent blockade of NMDA receptors than either PCP or s-(+)-ketamine. Owing to its cardiovascular side-effects, (+)MK-801 is not used for human studies, but it has been found to produce similar effects to PCP when administered to pigeons, rats and monkeys (Koek, Woods, & Winger, 1988). However, whereas PCP is purported to provide a 'full' model of schizophrenia, unrivalled in its ability to mimic both the positive and negative symptoms of schizophrenia (Morris, et al., 2005), evidence that (+)MK-801 can produce effects that parallel the non-psychotic symptoms of schizophrenia is far more limited (e.g. Seillier & Giuffrida, 2009). Despite this, (+)MK-801 is often used interchangeably with PCP in the production of pre-clinical animal models of schizophrenia whilst, due to its relative safety, s-(+)-ketamine is the drug of choice for modelling aspects of schizophrenia in human volunteers (e.g. Passie, Karst, Wiese, Emrich, & Schneider, 2005).

Gilmour et al. (2009) argued that it is inaccurate to assume that these NMDA receptor antagonists are equivalent and that inferences made about the behavioural effects of one drug can be applied to drugs with similar pharmacological action. In their study, a selection of NMDA antagonists, including PCP, s-(+)-ketamine and (+)MK-801, were systematically compared using a series of variable interval (VI) schedules of reinforcement. In an operant VI schedule, animals are rewarded after a variable period of time if they press an 'active' lever following this interval. It was revealed that the way in which various NMDA antagonists affect operant responding can differ greatly. For example, in a simple VI schedule, s-(+)-ketamine produced a dose-dependent decrease in instrumental output, whereas PCP and (+)MK-801 produced bi-directional effects in which lower doses increased responding and higher doses eventually decreased responding.

It is possible that the performance disparity produced by these drugs may reflect differences in the hedonic value of reinforcers. For instance, decreased responding may reflect an amotivational state produced by the drug or may instead be secondary to a reduction in the perceived hedonic value of the reinforcer. Since the effects of PCP, s-(+)-ketamine and (+)MK-801 are not as generalisable as often presumed, it is possible that the potential of each of these drugs to produce a model of schizophrenic anhedonia may vary considerably. For this reason, Experiment 3 examined the effects of single doses of these drugs on drinking behaviour, using the lick analysis technique described previously as an index of perceived reinforcer value. This experiment was conducted as an initial screen to examine whether there are any obvious effects of these drugs on hedonic value, which may either account for the effects observed on operant behaviour, or may indicate which of these drugs may hold the most potential for the pre-clinical modelling of anhedonia.

3.2 Experiment 3

Experiments 3a, 3b and 3c examined the effects of single doses of PCP, s-(+)-ketamine and (+)MK-801 on drinking behaviour.

Methods

Subjects

Experiment 3 was conducted in the behavioural neuroscience laboratory at the School of Psychology, Cardiff University. Forty eight male hooded Lister rats supplied by OLAC, Bicester, UK were used. Husbandry details were as reported in chapter 2. The initial free-feeding weights of the animals ranged 291-340 grams. Daily food rations were placed in the home cages 30 minutes after completion of the experimental

session. Food rations were monitored and adjusted to maintain the animals at no less than 85% of their free-feeding weights.

Apparatus

Testing was carried out in a room containing 16 drinking chambers. These were white plastic boxes measuring 32×15×12cm with metal grid floors and wire mesh lids. Fluids were given in 50ml cylinders attached to stainless steel drinking spouts. These spouts were positioned on the left hand side of the chamber at the beginning of each experimental session. A contact sensitive lickometer registered the time of each lick to the nearest 0.01s. This was recorded by a computer using MED-PC software (Med Associates Inc. St. Albans, USA). The amount of fluid consumed by each animal was measured by weighing the drinking bottle before and after each session. The solutions used for this experiment were 4% and 16% (weight / weight) sucrose, made using deionised water.

Drugs

The drugs used in this experiment were the non-competitive NMDA receptor antagonists, phencyclidine hydrochloride (Experiment 3a), S-(+)-ketamine hydrochloride (Experiment 3b), and (+)MK-801 hydrogen maleate (Experiment 3c), supplied by Sigma-Aldrich, UK. These drugs were made into solution using 5% (weight / volume) glucose. PCP was formulated at 1 mg/ml, S-(+)-ketamine was formulated at 5mg/ml and (+)MK-801 was formulated at 0.05 mg/ml. All drugs were injected at 1ml/kg. The sterile glucose solution was also used for vehicle injections. The drug doses reported refer to the base weight of compounds. These doses were chosen as their effects on operant responding is known (Gilmour et al. 2009).

Training

All animals received access to 4% or 16% sucrose in the lickometer cages for 10 minutes each day (Monday-Friday). The sucrose concentration given to each animal remained the same throughout the experiment. Animals received 16 days of pre-training to ensure that a steady level of consumption was reached prior to the first drug test. Animals then received weekly acute drug tests (on Fridays), preceded by four days of pre-training sessions (Monday-Thursday). PCP was given on the first week, MK-801 on the second, and ketamine on the third. Drug testing was carried out between-subject and animals were assigned to drug or vehicle treatment groups on the basis of the pre-training data obtained the day before each drug test. There were four conditions for each drug test: 4% sucrose with drug, 4% sucrose with vehicle, 16% sucrose with drug and 16% sucrose with vehicle.

Data Analysis

The lick cluster criterion used was the same as that used in chapter 2. The data were analysed using a two-way analysis of variance (ANOVA) with drug/vehicle treatment and sucrose concentration as between-subject factors. The data for two animals were removed from the analysis of the (+)MK-801 drug test because an abnormally high volume per lick calculation indicated that there was either a leakage of the drinking bottles or a failure with the lick recording apparatus. The data removed for one of the animals came from the 16% sucrose, vehicle-treated group. The other came from the 4% sucrose, (+)MK-801 treatment group.

Results of Experiment 3

Figure 3 shows the results of Experiment 3a with PCP. Figure 4 shows the results of Experiment 3b with s-(+)-ketamine. Figure 5 shows the results of Experiment 3c with (+)MK-801. Each figure includes the mean amount of sucrose consumed (panel A), the mean number of licks/cluster (panel B) and the mean ILI (panel C), for 4% and 16% sucrose, after acute drug treatment.

Experiment 3a

Overall consumption of sucrose was numerically but not significantly decreased following PCP treatment, $F(1,44)=3.24$, $p=.070$. Consumption of the 16% solution was higher than consumption of the 4% solution, $F(1,44)=17.65$, $p<.001$. There was no interaction between sucrose concentration and drug treatment, $F(1,44)=1.15$, $p=.290$.

PCP treatment produced an overall decrease in licks/cluster, $F(1,44)=14.89$, $p<.001$. Licks/cluster during consumption of 16% sucrose was numerically, but not significantly, higher than licks/cluster for 4% sucrose, $F(1,44)=3.50$, $p=.068$. There was no interaction between drug treatment and sucrose concentration, $F<1$.

ILI was significantly increased by PCP treatment, $F(1,44)=9.38$, $p=.004$. ILI was also lower for 16% sucrose than for 4% sucrose, $F(1,44)=4.13$, $p=.048$. No interaction occurred between drug treatment and sucrose concentration, $F<1$.

Experiment 3b

S-(+)-ketamine significantly decreased overall sucrose consumption, $F(1,44)=4.87$, $p=.033$. Consumption of 16% sucrose was higher than consumption of 4% sucrose,

$F(1,44)=10.45$, $p=.002$. There was no interaction between drug treatment and sucrose concentration, $F<1$.

S-(+)-ketamine treatment produced an overall decrease in licks/cluster, $F(1,44)=5.67$, $p=.022$. Licks/cluster was higher during consumption of 16% sucrose than during consumption of 4% sucrose, $F(1,44)=17.25$, $p<.001$. No interaction occurred between drug treatment and sucrose concentration, $F<1$.

S-(+)-ketamine significantly increased ILI, $F(1,44)=93.84$, $p<.001$. There was no significant effect of concentration on ILI, $F(1,44)=3.73$, $p=.060$, and no interaction between drug treatment and sucrose concentration, $F<1$.

Experiment 3c

(+)MK-801 produced no effect on sucrose consumption, $F<1$. Consumption of 16% sucrose was higher than 4% sucrose, $F(1,42)=6.94$, $p=.012$. There was no interaction between drug treatment and sucrose concentration, $F=1.82$, $p=.184$.

Licks/cluster was unaffected by (+)MK-801 treatment, $F<1$. Unexpectedly, there was no effect of sucrose concentration, $F<1$. There was also no interaction between drug treatment and sucrose concentration, $F<1$.

(+)MK-801 produced a marginal decrease in ILI, $F(1,42)=3.86$, $p=.056$. ILI was also lower for 16% sucrose than for 4% sucrose, $F(1,42)=7.91$, $p=.007$. There was no interaction between drug treatment and sucrose concentration, $F<1$.

Summary of Experiment 3

In Experiment 3a, the cluster size distinction between 4% and 16% sucrose was not as strong as would be expected. This is likely to be due to the between-subject design of the experiment as cluster size is very variable between animals. PCP and s-(+)-

ketamine produced a similar decrease in cluster size at the doses selected. However, neither decrease can be interpreted as an anhedonic effect because of the concurrent increase in inter-lick interval (ILI). ILI normally shows very little variability, except when motoric changes are induced. It is therefore possible that the reduced cluster size brought about by these drugs is an artefact of motor abnormality. In order to assess whether hedonic deficits are present in the absence of motor abnormality, lower drug doses may need to be tested.

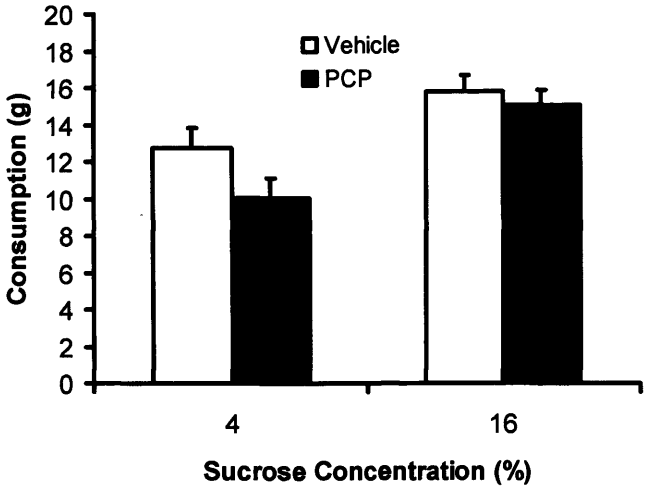
(+)MK-801 produced a different drinking behaviour profile, with no effects on the hedonic measures of consumption and lick cluster size, but a trend towards an increase in the rate of licking. This result is somewhat surprising given that (+)MK-801 and PCP appear to have similar effects on operant responding (Gilmour et al., 2009). This disparity may have arisen if the drug doses used here were not equivalent.

3.3 Experiment 4

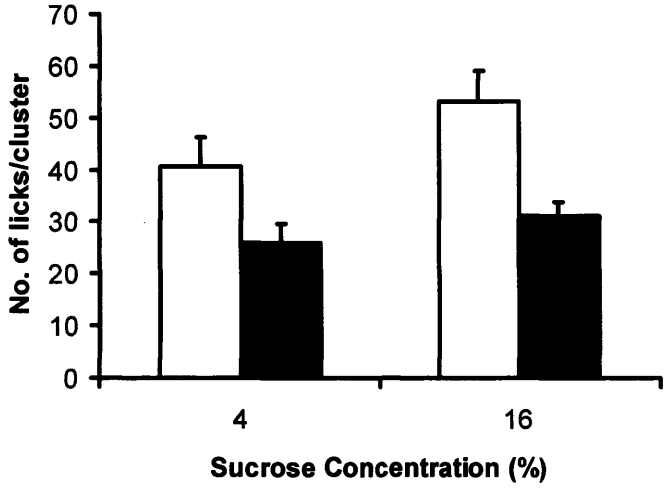
In light of the results of Experiment 3, Experiment 4 investigated the effects of PCP and (+)MK-801 on the hedonic value of a sucrose reward by means of dose-response experiments.

PCP was selected for this experiment as it is the most popular pharmacological agent used for modelling schizophrenia (Morris, et al., 2005). It has also been claimed that an analogue of anhedonia is present in rats twenty hours after a large (15mg/kg) dose of PCP (Turgeon & Hoge, 2003; Turgeon & Hulick, 2007; Baird, et al., 2008). This was inferred from a decrease in the amount of sucrose consumed voluntarily by PCP-treated animals (see section 1.9). Since microstructural analysis of licking provides a detailed method of measuring reward value, sensitive to motor abnormality, Experiment 3 was able to show that a moderate dose of PCP

3A.



3B.



3C.

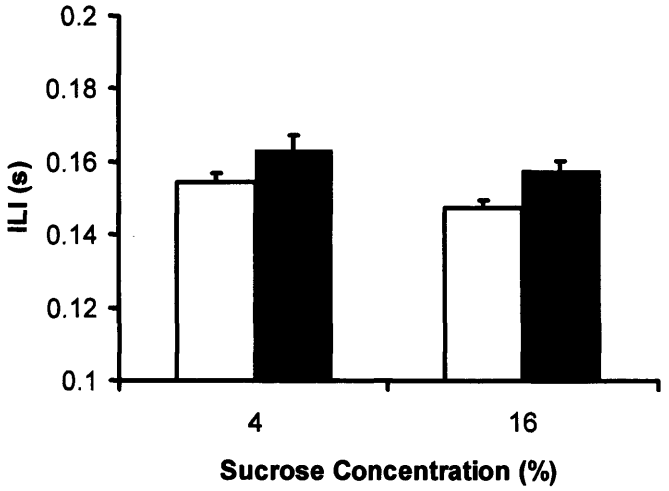
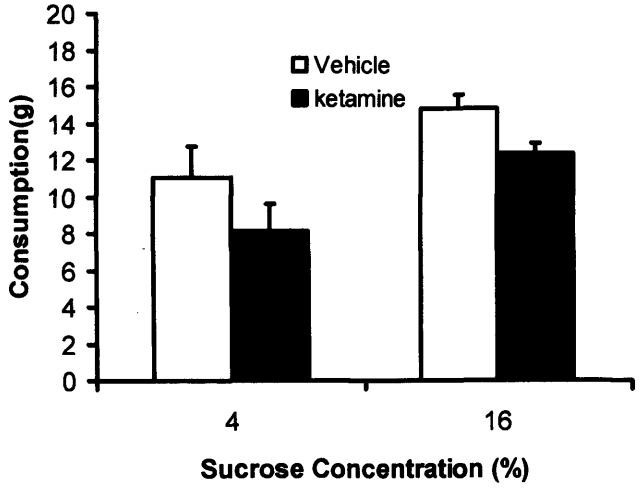
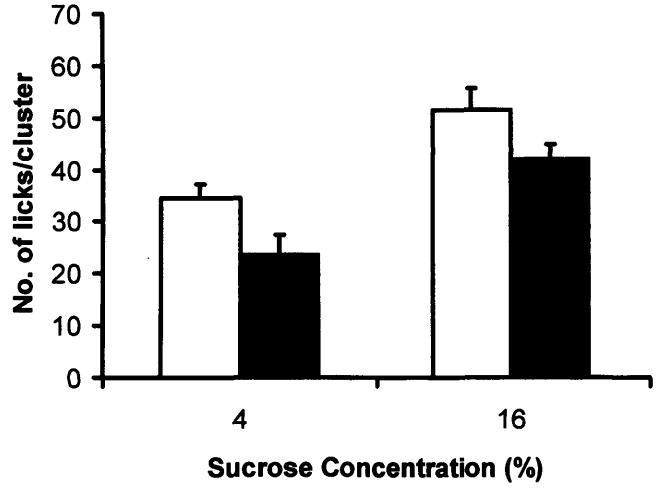


Figure 3: Shows the amount consumed (Panel A), mean number of licks/cluster (Panel B) and mean inter-lick interval (ILI) (Panel C), with SEM, for 10min exposure to 4% and 16% sucrose, following treatment with 1mg/kg PCP or vehicle.

4A.



4B.



4C.

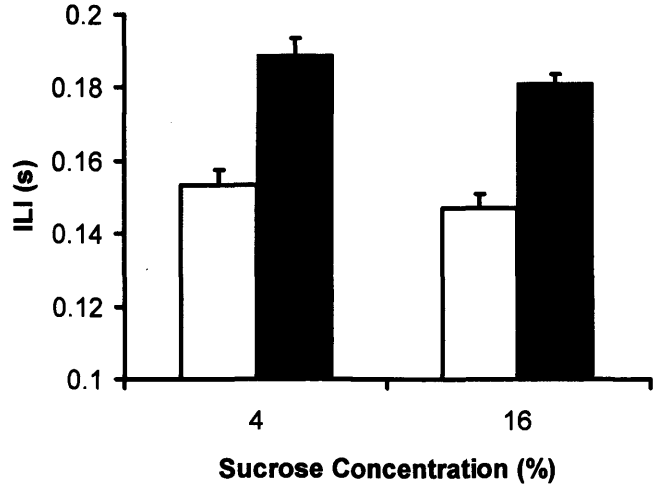
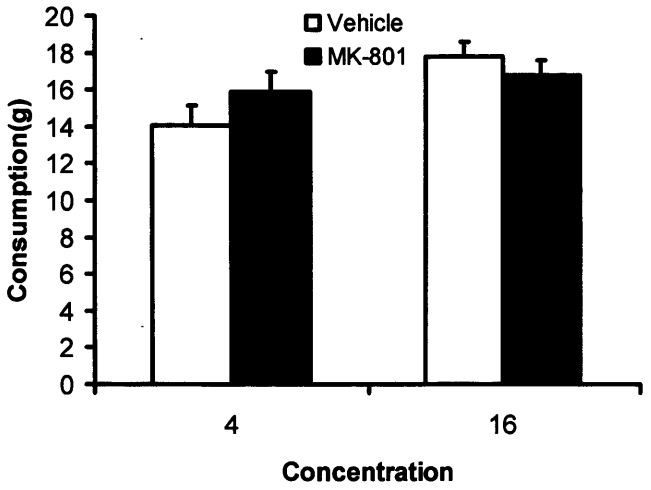
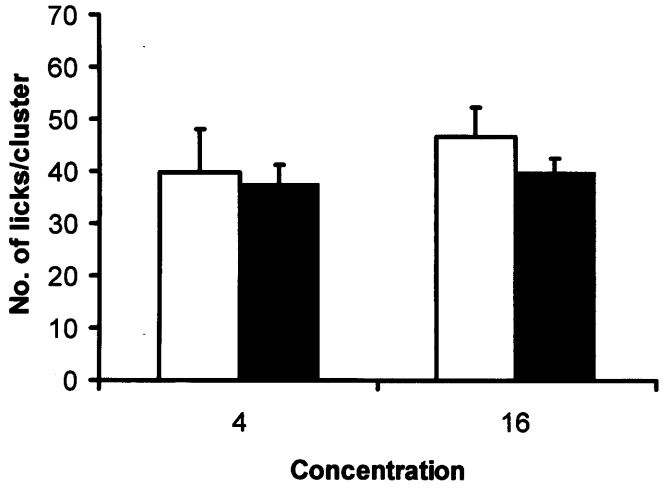


Figure 4: Shows the amount consumed (Panel A), mean number of licks/cluster (Panel B) and mean inter-lick interval (ILI) (Panel C), with SEM, for 10min exposure to 4% and 16% sucrose, following treatment with 5mg/kg ketamine or vehicle.

5A.



5B.



5C.

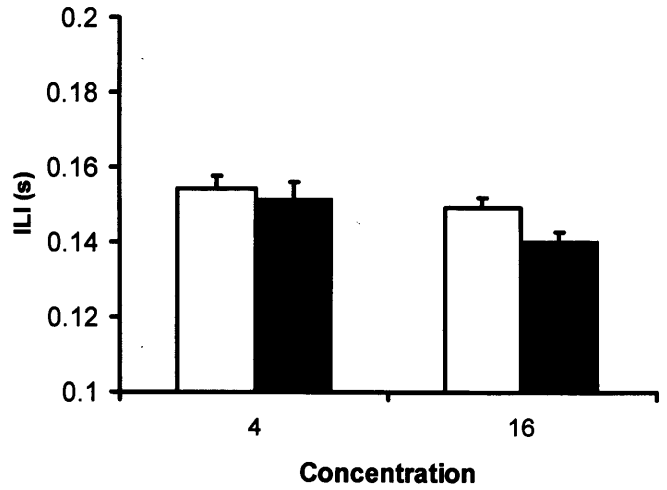


Figure 5: Shows the amount consumed (Panel A), mean number of licks/cluster (Panel B) and mean inter-lick interval (ILI) (Panel C), with SEM for 10min exposure to 4% and 16% sucrose, following treatment with 0.05mg/kg MK-801 or vehicle.

produces motoric changes. By combining this method with a range of PCP doses, Experiment 4 sought to discover whether evidence of reduced reward value was present at doses low enough to avoid motor changes. This would provide a better informed judgement of whether PCP produces deficits in reward function.

Although (+)MK-801 produced no hint of a hedonic deficit in Experiment 3, this drug is most frequently used interchangeably with PCP in pre-clinical studies and so was also selected for examination in this experiment.

Subjects and Apparatus

Thirty-two male hooded Lister rats were used. Supplier, husbandry and experimental apparatus details were the same as those in Experiment 3. The rats' initial free-feeding weights ranged 308-360 grams.

Drugs

PCP hydrochloride (Experiment 4a) and (+)MK-801 hydrogen maleate (Experiment 4b), supplied by Sigma-Aldrich, UK, were used. These drugs were made into solution using 5% (weight / volume) glucose. PCP was formulated at 0.25 mg/ml; 0.5 mg/ml; 1 mg/ml and 2.5 mg/ml. (+)MK-801 was formulated at 0.0125 mg/ml; 0.025 mg/ml; 0.5 mg/ml and 0.1 mg/ml. All drugs were injected at 1mg/kg. The sterile glucose solution was also used for vehicle injections. The drug doses reported refer to the base weight of compounds.

Training

All animals received access to a 10% sucrose solution in the lickometer cages for 10 minutes each day (Monday-Friday). A single intermediate sucrose concentration of

10% was selected for this experiment because no drug × concentration interactions were observed in Experiment 3. Training and test sessions began approximately 2.5 hours into the light part of the light / dark cycle. Animals received 13 days of pre-training to ensure that a steady level of consumption was reached. Following pre-training, animals received daily sessions for 5 days at a time.

Dosing

On the fifth day of each training block, animals received a drug dose. Injections were given sub-cutaneously with a pre-treatment time of 30 minutes. Between each set of 5 days animals received a seven day rest period in which no training or testing was carried out, in order to minimise drug tolerance.

16 of the animals received drug tests with PCP (Experiment 4a) and 16 received (+)MK-801 (Experiment 4b). The animals in each drug condition were divided so that half received drug doses in order of increasing size and half received the drug in decreasing doses. This manipulation controlled for order effects such as the effect of increasing body weight on response to a drug.

Data Analysis

The lick cluster criterion used was the same as in previous experiments and the same parameters were extracted from the data recorded. The data was analysed using repeated-measures analyses of variance (ANOVA) with 'dose' as a single factor. Planned contrasts compared each individual dose to vehicle for all parameters. The data for four animals were removed from the analysis, two because of blocked bottle spouts in the 2.5mg/kg PCP test, and the others because of blocked spouts in the 0.05mg/kg or 0.25mg/kg (+)MK-801 tests. Another two animals were removed from

the analysis of the PCP data, and one was removed from the (+)MK-801 analysis, due to failure to drink at the highest drug doses. No equipment malfunction was identified in these latter cases, suggesting that the rats' failure to drink may have been due to drug effects. However, since no licks were made, it was not possible to obtain cluster size or ILI data for these animals. Neither replacing the missing data with the mean for that condition nor restricting the analysis to exclude the highest dose affected the pattern of results reported here.

Results of Experiment 4

Figure 6 shows the results of Experiment 4a including the mean amount of sucrose consumed (panel A), the mean number of licks per cluster (panel B) and the mean ILI (panel C) at each dose of PCP. PCP produced overall significant effects on consumption, $F(4,44)=19.15$, $p<.001$, cluster size, $F(4,44)=6.68$, $p<.001$, and ILI $F(4,44)=7.0$, $p<.001$. With respect to consumption, for the 0.25 mg/kg dose the volume consumed was above that for vehicle, $F(1,11)=11.45$, $p=.006$, and the 2.5mg/kg dose below, $F(1,11)=37.04$, $p<.001$. Although cluster size was numerically higher than vehicle after 0.25mg/kg, this difference was not significant, $F(1,11)=2.8$, $p=.123$. The 2.5mg/kg dose significantly reduced cluster size, $F(1,11)=7.1$, $p=.022$. ILI was significantly increased by 0.5mg/kg, 1mg/kg, and 2.5mg/kg, with the smallest $F(1,11)=5.3$, $p=.042$.

Figure 7 shows the results of Experiment 4b including the mean amount of sucrose consumed (panel A), the mean number of licks per cluster (panel B) and the mean ILI (panel C) at each dose of (+)MK-801. (+)MK-801 produced overall effects on consumption, $F(4,48)=14.92$, $p<.001$, cluster size, $F(4,48)=10.17$, $p<.001$, and ILI, $F(4,48)=5.29$, $p<.001$. Consumption was increased relative to vehicle by 0.025mg/kg,

$F(1,12)=5.86$, $p=.032$, and 0.05mg/kg, $F(1,12)=7.67$, $p=.017$. Consumption for the 0.1mg/kg dose was below that for vehicle, $F(1,12)=11.02$, $p=.006$. Cluster size was numerically higher than vehicle after 0.0125mg/kg, but this was not significant, $F(1,12)=1.8$ $p=.205$, and was significantly reduced after 0.1mg/kg, $F(1,12)=39.2$ $p<.001$. Interestingly the increase in consumption with the 0.05mg/kg dose was not mirrored by an increase in cluster size which was numerically but not significantly lower than vehicle at this dose, $F(1,12)=4.3$, $p=.061$. This suggests that something other than palatability changes was responsible for this increase in consumption. ILI was significantly increased by the 0.1mg/kg dose, $F(1,12)=11.7$, $p=.005$.

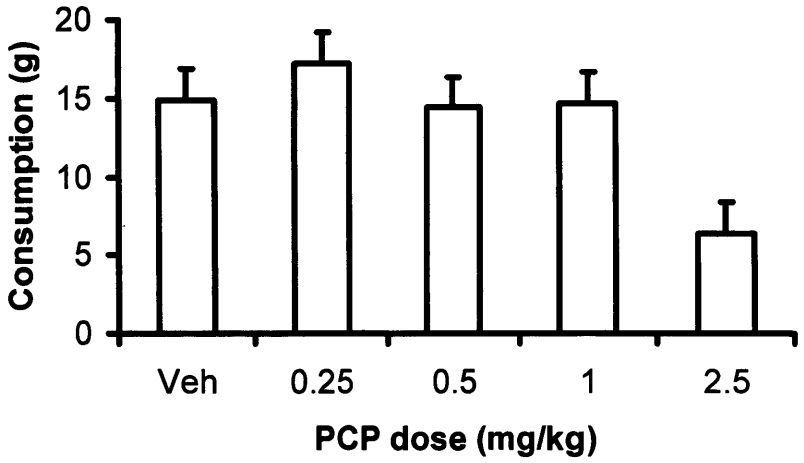
Summary of Experiment 4

In Experiment 4, there was evidence of decreased cluster size following administration of both PCP and (+)MK-801, but only at the highest dose tested, when ILI was increased. As mentioned previously, raised ILI is indicative of confounding motor deficits. Therefore, this experiment provides no evidence of reduced reward value following acute treatment with either PCP or (+)MK-801.

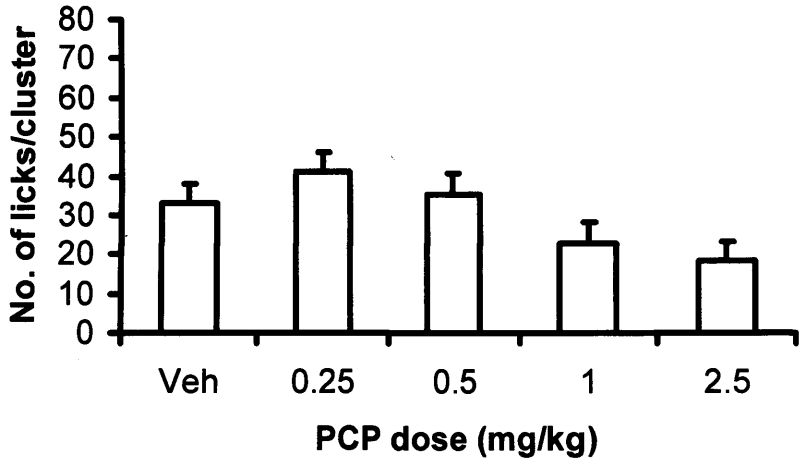
3.4 Experiment 5

Although the results of Experiment 4 suggest that anhedonia is not present following acute PCP and (+)MK-801 treatment, it has been suggested that NMDA receptor antagonist-induced hedonic deficits may be detectable when a reward has a lower hedonic value. Vardigan, Huszar, McNaughton, Hutson and Uslaner (2010) found that when (+)MK-801-treated rats were given a choice between sucrose and water, consumption of a 7% sucrose solution was increased relative to controls, whereas consumption of a 0.8% sucrose solution was decreased. In addition, in the case of

6A.



6B.



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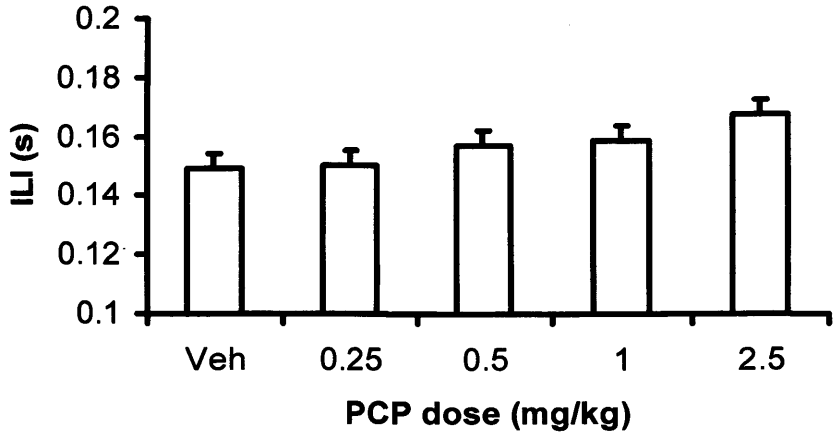
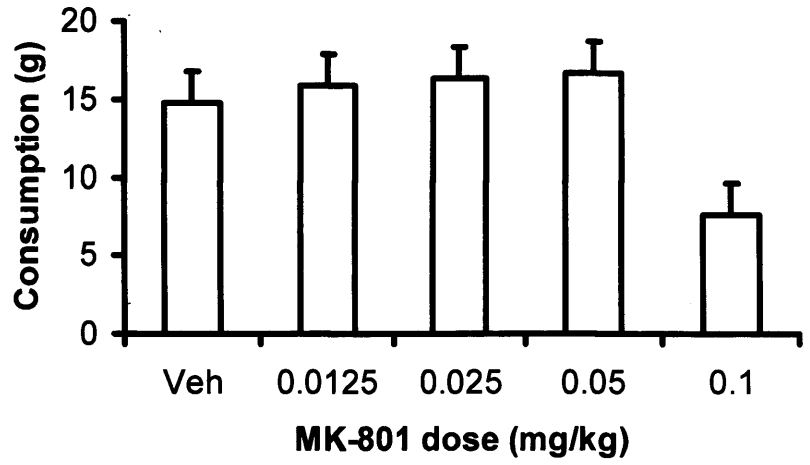
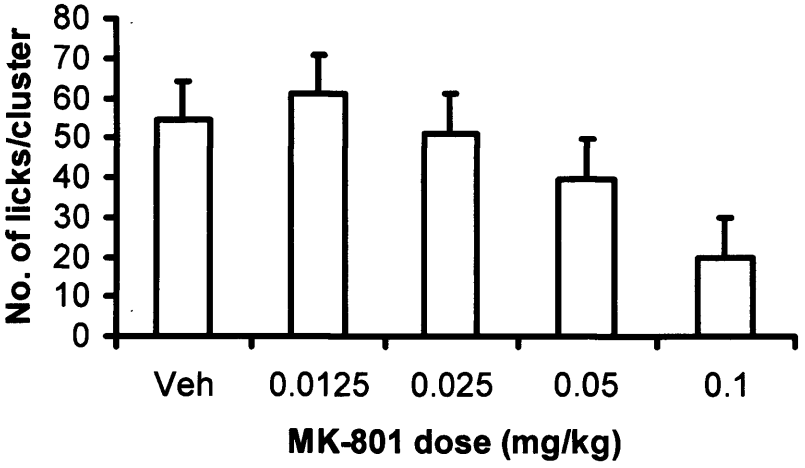


Figure 6: Shows the data obtained from an acute dose-response experiment using PCP. Panels A-C illustrate the mean amount of sucrose consumed, the mean number of licks per bout, and the mean ILI respectively, with SEM, as a function of PCP dose (mg/kg).

7A.



7B.



7C.

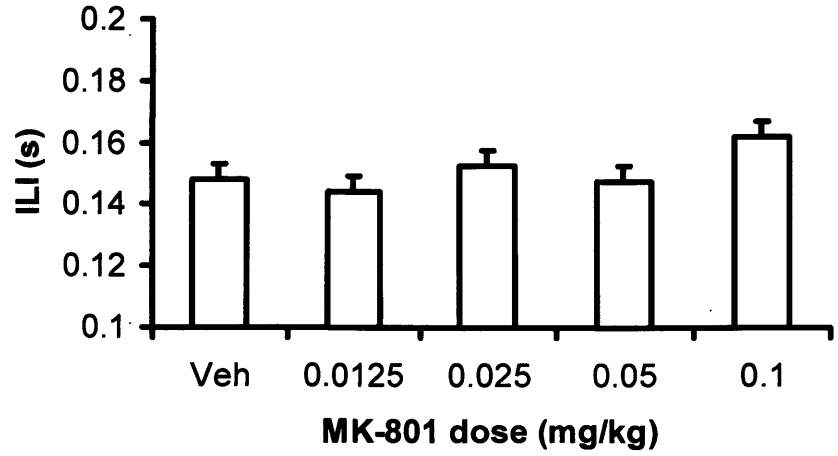


Figure 7: Shows the data obtained from an acute dose-response experiment using PCP. Panels A-C illustrate the mean amount of sucrose consumed, the mean number of licks per bout, and the mean ILI respectively, with SEM, as a function of MK-801 dose (mg/kg).

PCP, a preliminary test suggested that when mildly water-restricted rats are presented with a choice between 1% sucrose and water, PCP-treated animals show a slight reduction in their sucrose/water consumption ratio relative to controls (see appendix 1). Therefore, Experiment 5 used microstructural analysis of licking to investigate whether acute PCP and (+)MK-801 produce hedonic deficits that are evident at concentrations lower than those tested previously in this chapter.

Methods

Subjects and Apparatus

Forty eight male hooded Lister rats supplied by Charles River, Margate, Kent, UK, were used in this experiment. Half of these animals were used for Experiment 5a and half were used for Experiment 5b. Husbandry and experimental apparatus details were the same as those in the previous two experiments. Animals' free-feeding weights ranged 338-388 grams.

The sucrose solutions used for test sessions were 1%, 3% and 10% sucrose, made weight/weight using deionised water. 8% maltodextrin (weight/weight) was used for pre-training.

Drugs

PCP hydrochloride (Experiment 5a) and (+)MK-801 hydrogen maleate (Experiment 5b), supplied by Sigma-Aldrich, UK, were used. These drugs were made into solution using 5% (weight/volume) glucose. PCP was formulated at 1 mg/ml and (+)MK-801 was formulated at 0.05 mg/ml. The sterile glucose solution was also used for vehicle injections. The drug doses reported refer to the base weight of compounds.

Training

Training and test sessions were carried out Monday-Friday, 4 hours into the light period of the light/dark cycle. Training commenced with 5 pre-training sessions in which all rats received 10min daily access to 8% maltodextrin in the lickometer cages to enable them to gain experience of drinking in the apparatus. Following this, rats were given daily 10min sessions (Mon-Thurs) in which they received access to the solution with which they would receive drug testing at the end of the week.

Dosing

On the fifth day of exposure to each test solution (Friday), twelve of the animals received acute, sub-cutaneous PCP injections and twelve received vehicle injections (Experiment 5a) 30min prior to their daily session in the lickometer cages. Experiment 5b was run in parallel and involved the injection of twelve animals with (+)MK-801 and twelve with vehicle. After a 2-day washout, the animals received another test session in which rats previously treated with drug received vehicle, and those previously treated with vehicle received drug treatment. Following this, animals received a 6-day washout period prior to training with the next solution to be tested. The three sucrose concentrations to be tested were given to half of the animals in ascending order and half in descending order.

Data Analysis

The data were analysed using repeated measure analysis of variance (ANOVA), with drug treatment and sucrose concentration as within-subject factors. In Experiment 5a, one rat was re-tested in the 10% sucrose PCP condition and, in Experiment 5b, two

rats were re-tested in the 10% sucrose (+)MK-801 condition as their data was lost due a lick recording problem. This replacement data was included in the analysis, although removing it had no effect on the outcome.

Results of Experiment 5

Experiment 5a

Figure 8 shows the amount of sucrose consumed (panel A), the mean number of licks/cluster (panel B), and the mean ILI (panel C), for 1%, 3% and 10% sucrose, following PCP and vehicle treatment.

The amount of sucrose consumed increased with increasing concentration, $F(2,46)=68.34$, $p<.001$, and was unaffected by PCP treatment, $F<1$. There was no interaction between drug treatment and sucrose concentration, $F<1$.

The number of licks/cluster increased with increasing concentration, $F(2,46)=43.15$, $p<.001$. PCP treatment significantly decreased licks/cluster, $F(1,23)=47.53$, $p<.001$. However, there was no interaction between drug treatment and sucrose concentration, $F(2,46)=2.92$, $p=.064$.

The PCP-induced decrease in lick cluster size cannot be taken to indicate an anhedonic effect since PCP also increased ILI, $F(1,23)=12.22$, $p=.002$. ILI was unaffected by sucrose concentration, $F<1$, and no interaction occurred between sucrose concentration and drug treatment, $F(2,46)=1.79$, $p=.177$.

Experiment 5b

Figure 9 shows the amount of sucrose consumed (panel A), the mean number of licks/cluster (panel B), and the mean ILI (panel C), for 1%, 3% and 10% sucrose, following MK-801 and vehicle treatment.

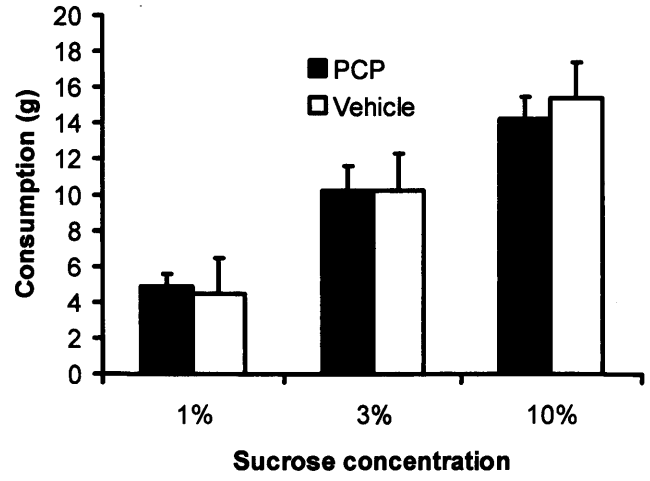
The amount of sucrose consumed increased with increasing concentration, $F(2,46)=34.36$, $p<.001$, and was unaffected by (+)MK-801 treatment, $F<1$. There was no interaction between drug treatment and sucrose concentration, $F<1$. The number of licks/cluster increased with increasing concentration, $F(2,46)=25.99$, $p<.001$. (+)MK-801 treatment had no effect on licks/cluster, $F<1$, and there was no interaction between drug treatment and sucrose concentration, $F<1$.

There was a significant effect of sucrose concentration on ILI, $F(2,46)=10.48$, $p<.001$. ILI was reduced following (+)MK-801 treatment, $F(1,23)=16.21$, $p<.001$. No interaction occurred between sucrose concentration and drug treatment, $F<1$. The significant effect of sucrose concentration on ILI arose because ILI was increased for 3% sucrose. There is no obvious reason why this occurred. However, the fact that there was no interaction between sucrose concentration and drug treatment supports the conclusion that (+)MK-801 produced no effect on cluster size independent of motor effects.

Summary of Experiment 5

Experiment 5 demonstrated that, even at low sucrose concentrations, there is no evidence of a PCP-induced reduction in cluster size occurring independently of motor abnormality indicated by raised ILI. Similarly, the consumption and palatability of low sucrose conentrations were also unaffected by (+)MK-801 treatment. In Experiment 5 the effect of sucrose concentration on cluster size was stronger than in Experiment 3. This is probably because of the within-subject design of the experiment.

8A.



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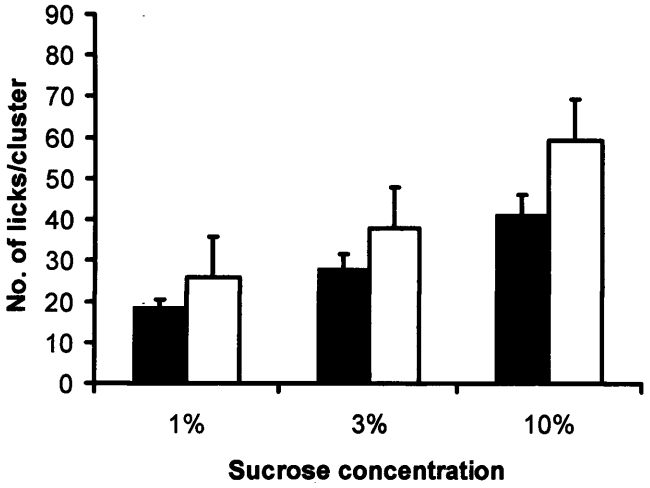

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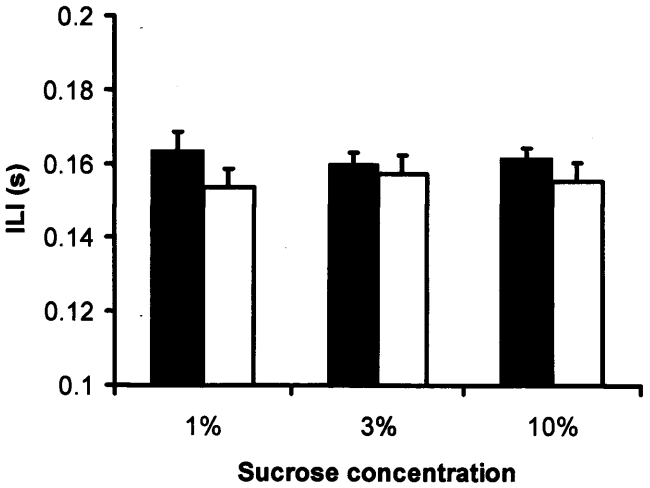
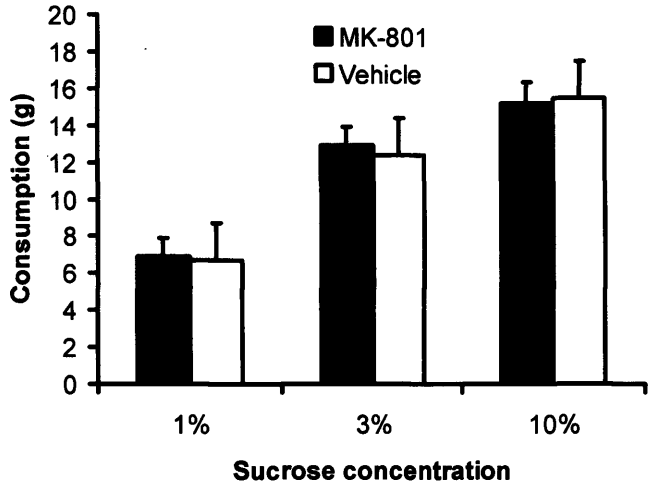
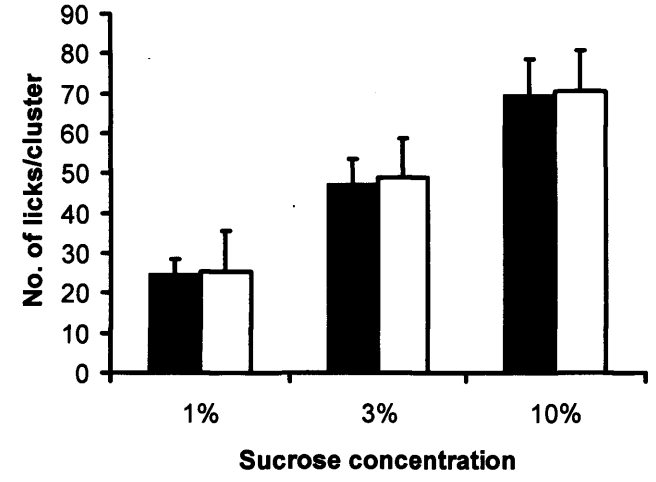


Figure 8: Panels A-C show the mean amount of sucrose consumed, the mean number of licks per bout, and the mean ILI respectively, with SEM, for 10min exposure to 1%, 3% and 10% sucrose, following acute treatment with 1mg/kg PCP and vehicle.

9A.



9B.



9C.

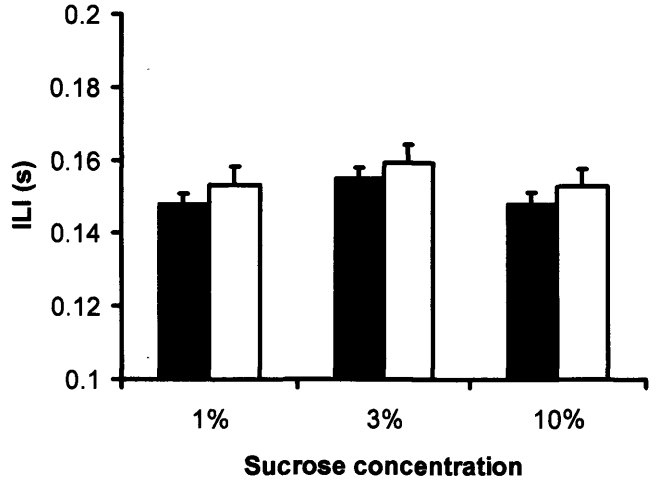


Figure 9: Panels A-C show the mean amount of sucrose consumed, the mean number of licks per bout, and the mean ILI respectively, with SEM, for 10min exposure to 1%, 3% and 10% sucrose, following acute treatment with 0.05mg/kg MK-801 and vehicle.

3.5 Discussion

The main aim of the current set of experiments was to investigate whether evidence of anhedonia could be found in rodents following acute administration of selected NMDA receptor antagonists. Experiment 3 examined the effects of single doses of PCP, s-(+)-ketamine and (+)MK-801 on drinking behaviour and suggested a possibility that PCP and s-(+)-ketamine may reduce reward value, as indexed by reduced lick cluster size. However, this experiment was limited by the fact that the doses selected produced motor deficits. Since a single dose was selected for each drug, the discrepancy between (+)MK-801 and the other two drugs may have been due to a lack of equivalence between the doses selected, especially since PCP and (+)MK-801 have bi-directional effects on operant responding (Gilmour et al. 2009). Therefore, Experiment 4 compared the dose-response effects of PCP and (+)MK-801 on drinking behaviour. It was reasoned that if changes in reward value underlie the effects on operant responding produced by PCP and (+)MK-801, then the patterns of palatability and general consumption generated by the acute drug treatment given in Experiment 4 would mimic the drug effects on lever pressing. This was generally found to be the case. At the highest doses tested, both drugs decreased sucrose consumption, where similar doses also decreased operant responding. There was also evidence of decreased lick cluster size at high doses of both drugs. However, this was only seen at doses which increased the ILI. ILI normally shows very little variability, except when motoric changes are induced: for example, by changes in posture (Weijnen, 1998), or disturbances in motor pattern generation in the hind brain (Aja, et al., 2001). The fact that cluster size decreased only when ILI was increased suggests that the reduced cluster size was an artefact of motor effects induced by the drugs.

Therefore, we have no evidence here for reduced palatability, only motor abnormality.

Evidence from the literature suggests that PCP, in particular, may have potential for the pre-clinical modelling of anhedonia. PCP has been shown to induce a state in humans that closely reflects the positive, negative and cognitive symptoms of schizophrenia (e.g. Allen & Young, 1978; Adler, et al., 1999), and has therefore been favoured as a pharmacological agent for modelling schizophrenia in animals (Morris et al. 2005). Turgeon and Hoge (2003) found that voluntary sucrose consumption was decreased in rats who received a 15mg/kg injection of PCP 20 hours prior to testing. In Experiment 4a, this decrease in sucrose consumption was replicated following acute administration of 2.5mg/kg PCP (our highest dose). It should be noted however, that no significant decreases in consumption were observed at the lower doses used in Experiments 3 and 5. Several authors have suggested that decreased sucrose consumption in animal models of affective disorders indicates the presence of anhedonia (e.g. Papp, et al., 1991; Papp & Moryl, 1994; Rygula, et al., 2005; Willner, et al., 1994; Zurita, et al., 2000; Zurita & Molina, 1999). However, the use of microstructural analysis to examine palatability in Experiments 3-5 has provided a much more informed understanding of the processes underlying acute PCP-induced decreases in sucrose consumption, enabling us to determine whether or not they really are indicative of decreases in reward value. Using this method, I could find no evidence for reduction in reward value following acute treatment with either PCP or (+)MK-801 that was not confounded by the presence of motor abnormality. In fact, low to moderate doses of both drugs produced an increase in sucrose consumption and a trend towards an increase in palatability. Experiment 4 therefore provided no evidence that the acute effects of either PCP or (+)MK-801 include the production of

anhedonia. Further, these results suggest that decreased sucrose consumption alone cannot be taken to indicate anhedonia, thereby highlighting a fundamental flaw with one way in which hedonic deficits are frequently assessed.

It is of course possible that the reduced sucrose consumption seen by Turgeon and Hoge (2003) (see also, Baird et al. 2008) on withdrawal from acute treatment with 15mg/kg PCP was due to the presence of anhedonia, since Baird et al. (2008) found no evidence of motor abnormality following this protocol. In addition, Turgeon and Hullick (2007) found that sub-chronic pre-treatment with the atypical antipsychotic clozapine prevented the PCP-induced reduction in sucrose consumption, further suggesting that the decreased consumption reflected a negative symptom. However, in addition to the problems that arise from using general consumption as a measure of anhedonia, the validity of this dosing regimen as a model of schizophrenia is also questionable, since high doses of PCP have been shown to cause degeneration of pyramidal neurones in the retrosplenial cortex. This is not consistent with human post-mortem studies, which show that it is the interneurones that degenerate in schizophrenia (Olney, et al., 1989). The neurodegeneration caused by large doses of PCP raises the possibility that the ability of sub-chronic clozapine to reverse PCP-induced decreases in sucrose consumption may rely on the neuroprotective properties of clozapine. The effectiveness of clozapine as a neuroprotective agent has been demonstrated in several *in vitro* studies reporting decreased apoptosis and neurodegeneration among hippocampal and other cell populations, in response to challenges such as growth medium deprivation (e.g. Bai, et al., 2002; Qing, Xu, Wei, Gibson, & Li, 2003; Bastianetto, Danik, Mennicken, Williams, & Quirion, 2006).

A potential limitation of Experiment 4 is that only one, relatively high concentration of sucrose was used in testing. It has been suggested that NMDA receptor antagonist-induced hedonic deficits may be detectable when a reward has a lower hedonic value. Vardigan, et al. (2010) found that when (+)MK-801-treated rats were given a choice between sucrose and water, consumption of a 7% sucrose solution was increased relative to controls, whereas consumption of a 0.8% sucrose solution was decreased. Therefore, Experiment 5 used microstructural analysis of licking to investigate whether acute PCP and (+)MK-801 produce hedonic deficits that are evident at concentrations lower than those tested in Experiments 3 and 4. Again, no evidence of hedonic deficit was found with either drug in this experiment.

Vardigan et al. claimed that (+)MK-801 alters hedonic experience by shifting the inverted U-shaped concentration-consumption relationship to the right. Although such a shift may well indicate an attenuation of either a sensory or hedonic response to sucrose, the fact that only three concentrations of sucrose were tested in their experiment does not allow this claim to be validated. A further concern is that consumption of the 7% sucrose solution was raised in their experiment. However, the dose used was 0.3mg/kg, which is far higher than the highest used here in Experiment 4, following which motor abnormality was evident via a significant decrease in consumption and significant increase in ILI.

In summary, the experiments presented here demonstrate that acute NMDA antagonist treatment produces no evidence of reduced reward value as indexed by palatability and consumption. This may reflect a disparity between the neurochemical changes induced by this drug and those thought to be responsible for the non-psychotic symptoms of schizophrenia. Acute administration of non-competitive NMDA receptor antagonists is associated with increased forebrain dopamine

transmission (e.g. Schilstrom, Nomikos, Hertel, Nisell, & Svensson, 1996; Jentsch, Tran, Le, & Roth, 1997). This increase is thought to underlie the positive symptoms of schizophrenia since haloperidol, a typical antipsychotic effective in ameliorating positive symptoms, acts to reduce DA levels. On the other hand, atypical antipsychotics, such as clozapine, consistently elevate DA levels in the pre-frontal cortex (Moghaddam & Bunney, 1990). It is therefore more likely that the negative symptoms of schizophrenia may be reflected in animals following a reduction in frontal DA transmission, an effect that can be brought about by sub-chronic NMDA receptor antagonist administration (Jentsch, Taylor, et al., 1997; Jentsch, Tran, et al., 1997).

CHAPTER FOUR

Antipsychotic treatment and licking microstructure/sucrose preference

4.1 Introduction

There is some evidence to suggest that atypical antipsychotics are superior to typical antipsychotics in the treatment of negative symptoms (see section 1.3). Another major advantage which has led to the increased usage of atypical drugs is that they produce less extrapyramidal side effects (Leucht, et al., 1999). Although all effective antipsychotics are dopamine D_2 receptor antagonists, D_2 antagonism is also responsible for the severe extrapyramidal side-effects associated with typical antipsychotics (Kerwin, 1994). It has been proposed that the benefits of atypical over typical drugs occur because of the relatively low D_2 receptor occupancy produced by atypical drugs. Receptor occupancy is suggested to be mediated via the relative affinities of these drugs (i.e. the rate at which the drugs move on to and off of the D_2 receptors). Atypical drugs such as clozapine show low D_2 receptor affinity, whereas typical drugs such as haloperidol have much higher affinity (e.g. Kapur & Seeman, 2001; Seeman, 2002).

Despite the advantages of atypical antipsychotics, they are associated with greater weight gain than traditional drugs (e.g. Stanton, 1995; Allison, et al., 1999; Kraus, et al., 1999; Fell, Neill, Rao, & Marshall, 2005; Fell, Neill, Williams, Reynolds, & Marshall, 2003). The mechanisms underlying antipsychotic-induced weight gain are unclear, and may occur via a stimulation of food intake, or an endocrine disturbance that increases fat deposition (Baptista, 1999). There have been reports that clozapine increases food intake in rats (e.g. Antelman, Black, & Rowland, 1977). It is possible that this effect may be due to a drug-induced increase in the

perceived hedonic value of foods. Indeed, this may account for the restoration of voluntary sucrose consumption observed by Turgeon and Hulick (2007) when clozapine pre-treated animals received large doses of PCP.

The aim of the work contained in this thesis is to attempt to identify a pre-clinical analogue of anhedonia, which may be used for the screening of therapeutic compounds. However it is important for the study of antipsychotics that the independent effects these drugs on reward value are known, particularly in light of the suggestion that antipsychotics may increase food intake.

Using microstructural analysis of licking, Hartfield, Moore and Clifton (2003) investigated ingestive behaviour following acute clozapine, olanzapine and haloperidol administration in rats. They found that clozapine and olanzapine significantly increased intake of a high-calorie fat solution, whereas haloperidol decreased intake. Cluster size was unaffected by clozapine and olanzapine treatment, but was decreased by haloperidol treatment. Hartfield et al. suggested that these differences reflect the greater weight gain tendency with atypical antipsychotics and that delayed post-ingestive satiety and intact palatability are the mechanisms responsible for hyperphagia after treatment with these drugs. However, it is possible that these effects are limited to fat solutions, whereas throughout the experiments in this thesis sucrose has been used, in line with other published work investigating the presence of anhedonia. Therefore, the current experiment investigated the effects of clozapine and haloperidol on the hedonic value of sucrose.

4.2 Experiment 6

Methods

Subjects and Apparatus

Twenty-four male hooded Lister rats were used. Supplier, husbandry and experimental apparatus details were the same as those used for Experiments 3-5. The rats' initial free-feeding weights ranged 300-345 grams.

Drugs

Clozapine (Experiment 6a) and haloperidol (Experiment 6b), supplied by Sigma-Aldrich, UK, were used. Clozapine was dissolved in lactic acid before adding the appropriate amount of deionised water to make a solution of 0.3mg/ml. The solution was sonicated and neutralised with 1M NaOH. Haloperidol was made into a 0.1mg/ml solution using 5% (weight / volume) glucose. Both drugs were injected at 1ml/kg. These drugs were selected because they were used by Hartfield et al. and were representative of typical and atypical drugs and their differing pharmacology. The doses were chosen because they were the middle of the three doses used by Hartfield et al., thereby allowing for comparison of the drugs' effects on fat and carbohydrate solutions. The sterile glucose solution was also used for vehicle injections. The drug doses reported refer to the salt weight of compounds.

Training

All animals received access to a 4% sucrose solution in the lickometer cages for 20 minutes each day (Monday-Friday). 4% sucrose was selected for this experiment as it was reasoned that anti-psychotic induced weight gain could be secondary to an

increase in the palatability of sweet tastants. For this reason a lower sucrose concentration was chosen than used previously so that the test would be more sensitive to palatability increase. The sucrose solution was presented on the left and right side of the cage on alternate days. Training and test sessions began approximately 2 hours into the light part of the light / dark cycle. Animals received 9 days of pre-training to ensure that a steady level of consumption was reached. Following pre-training, animals received daily sessions for 5 days at a time.

Dosing

On the fifth day of each training block, half of the animals received a drug dose (clozapine on week 1 and haloperidol on week 2), and half received vehicle. After a two day washout, the treatments were reversed so that each animal received drug treatment. Injections were given via the intra-peritoneal route with a pre-treatment time of 30 minutes. Between each set of five days animals received a seven day rest period in which no training or testing was carried out, in order to minimise drug tolerance.

Data Analysis

The lick cluster criterion used was the same as used previously and the same parameters were extracted from the data recorded. The data was analysed using ANOVA with drug treatment as a within-subject factor. The data for one animal was removed from the analysis of the clozapine drug test and the data for two animals were removed from the analysis of the haloperidol drug test because an abnormally low volume per lick calculation indicated that there was a blockage of the drinking

bottles. This blockage occurred following vehicle treatment in Experiment 6a and haloperidol treatment in Experiment 6b.

Results of Experiment 6

Figures 10 and 11 show the results of Experiments 6a and 6b, including the mean amount of sucrose consumed (panel A), the mean number of licks/cluster (panel B) and the mean ILI (panel C), for 4% sucrose, after acute treatment with clozapine and haloperidol respectively.

Experiment 6a

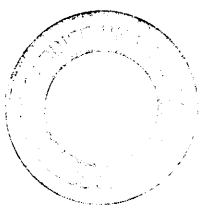
Clozapine treatment had no effect on consumption, $F(1,22)=1.21$, $p=.284$., or licks/cluster, $F<1$. However, ILI was increased by clozapine, $F(1,22)=18.15$, $p<.001$.

Experiment 6b

Haloperidol decreased consumption, $F(1,21)=53.61$, $p<.001$, and also decreased licks/cluster, $F(1,21)=31.15$, $p<.001$. Haloperidol had no effect on ILI, $F<1$.

Summary of Experiment 6

In Experiment 6, clozapine had no effect on the consumption and palatability of sucrose, but produced some motor abnormality, indicated by an increase in ILI. Haloperidol produced no evidence of motor effects but produced a decrease in the value of sucrose, indicated by decreased consumption and palatability.



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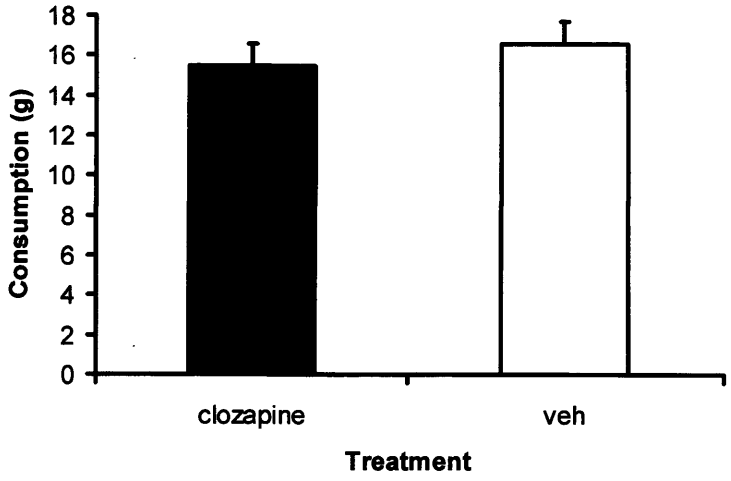

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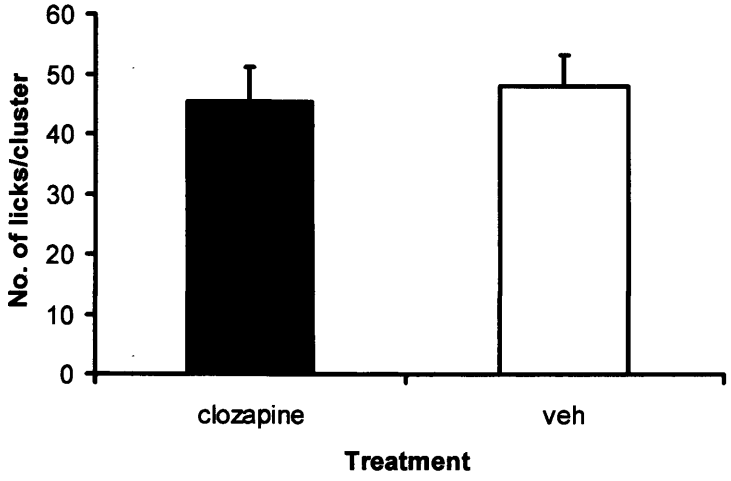


10C.

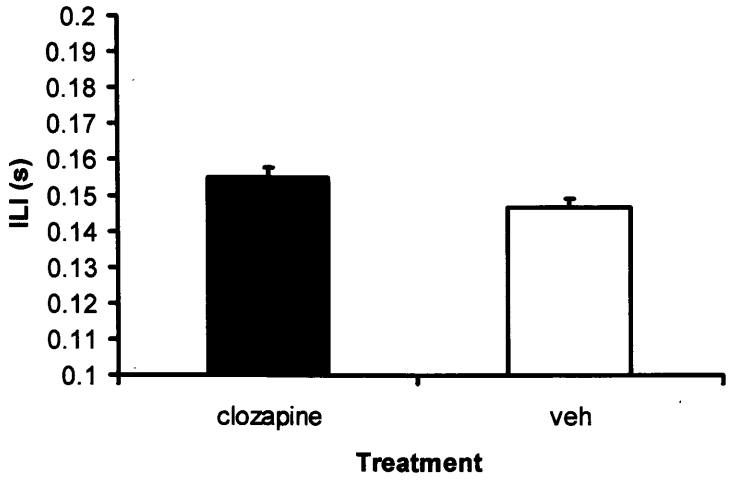
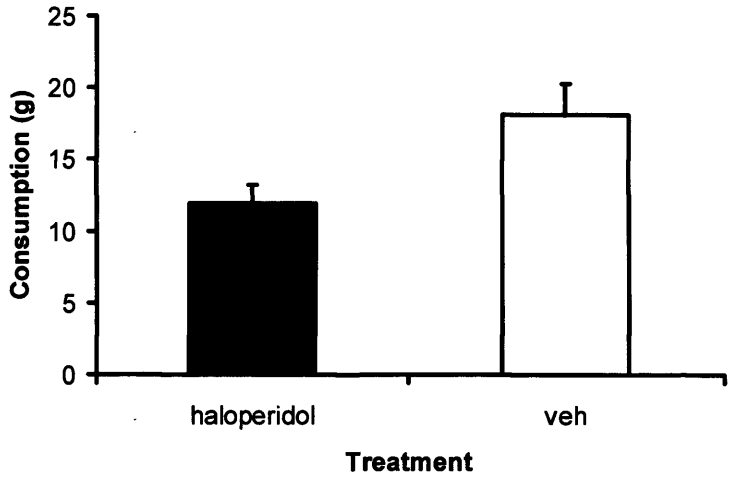


Figure 10: Shows the consumption (Panel A), licks/cluster (Panel B) and ILI (Panel C) for 20min access to 4% sucrose, following acute treatment with clozapine and vehicle, with SEM.

11A.



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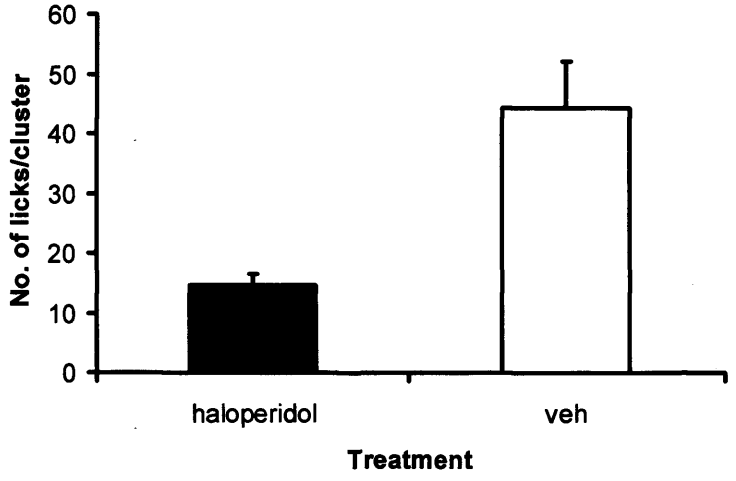

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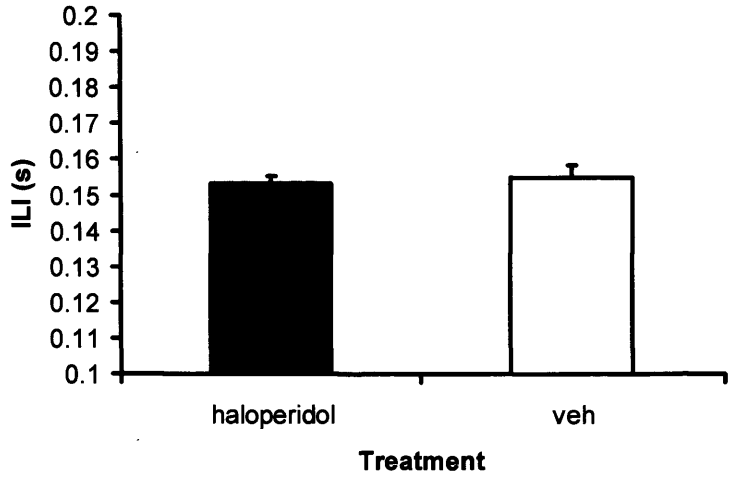

Figure 11: Shows the consumption (Panel A), licks/cluster (Panel B) and ILI (Panel C) for 20min access to 4% sucrose, following acute treatment with haloperidol and vehicle, with SEM.

4.3 Experiment 7

Under certain circumstances, it has been suggested that assessing an animal's preference for sucrose over water may provide a more sensitive test of reward value since it is less sensitive to confounds, such as large motor deficits, than consumption tests using a single solution. For this reason, preference tests have been used to study anhedonia in animal models of chronic mild stress (Willner, 2005). Experiment 7 therefore examined the effects of clozapine and haloperidol on the preference of sucrose over water.

Subjects and Apparatus

This experiment followed Experiment 6 and used the same subjects and apparatus.

Drugs

Clozapine (Experiment 7a) and haloperidol (Experiment 7b) were used in this experiment at the same doses used in Experiment 6a and 6b.

Training and testing

Animals received a 9 day rest period before re-training for this experiment commenced. They then received four pre-training sessions with 4% sucrose, the same as those in Experiments 6a and 6b. Following this, animals were given a choice between 1% sucrose and water following 4.5 hours of water deprivation. In Experiment 7a half of the animals received clozapine treatment and the other half received vehicle injections. After a 2-day washout, the treatment groups were reversed so that all animals received clozapine treatment. There was a 7-day washout

period before pre-training for Experiment 7b began. Pre-training and testing for Experiment 7b followed the same format as Experiment 7a.

Data Analysis

For this experiment the parameter of interest was the amount of water and sucrose consumed. Lick analysis data is not reported as during preference testing animals tend to alternate between available solutions making lick cluster data unreliable.

The data is reported in figure 12. Two methods of analysis were used. The first method was ANOVA with drug treatment and reinforcer type (sucrose/water) as within-subject factors. The second method calculated the mean sucrose preference ratios following drug and vehicle treatment. In this case, the sucrose preference ratio is the amount of sucrose consumed as a proportion of the total amount consumed (both sucrose and water). This ranges between 1 (only sucrose consumed) and 0 (no sucrose consumed, only water). A paired samples t-test was then carried out on these ratios.

Results of Experiment 7

Experiment 7a

In the clozapine test there was an overall effect of reinforcer whereby animals drank more sucrose, $F(1,23)=147.35.18$, $p<.001$. There was no overall effect of clozapine treatment on consumption, and there was no drug treatment × reinforcer interaction, $Fs<1$. There was no significant difference between the sucrose preference ratios for vehicle-treatment and clozapine-treatment conditions; $t < 1$.

Experiment 7b

In the haloperidol sucrose preference test, overall consumption of sucrose was higher than consumption of water, $F(1,23)=26.65$, $p<.001$. There was no overall effect of drug treatment, and no treatment × reinforcer interaction, Fs<1. However, in terms of the sucrose preference ratio, there was a significant reduction in the haloperidol condition compared to the vehicle condition, $t(23)=2.95$, $p=.007$.

Summary of Experiment 7

In Experiment 7, clozapine produced no effect on the consumption of sucrose and water when presented simultaneously. Clozapine also produced no effect on the ratio of sucrose consumption relative to water consumption. Although haloperidol produced no overall effect on the consumption of sucrose and water, there was a reduction in the ratio of sucrose consumption relative to water consumption.

4.4 Discussion

On initial interpretation, the results of Experiment 6a and 7a suggest that clozapine does not increase sucrose intake, palatability, or preference, but causes some motor abnormality, indicated by raised ILI. However, in the experiments carried out previously in this chapter, raised ILI has always been accompanied by decreased lick cluster size. Since cluster size was not decreased following increased ILI for both fat (Hartfield, et al., 2003) and sucrose, it is possible that clozapine does enhance palatability of sucrose, but that this is masked by motor disturbance.

The absence of an increase in sucrose consumption stands in some contrast to the findings of Hartfield et al. (2003). However, if increased consumption is due to a reduction of a post-ingestive satiety signal then the session length used in the current

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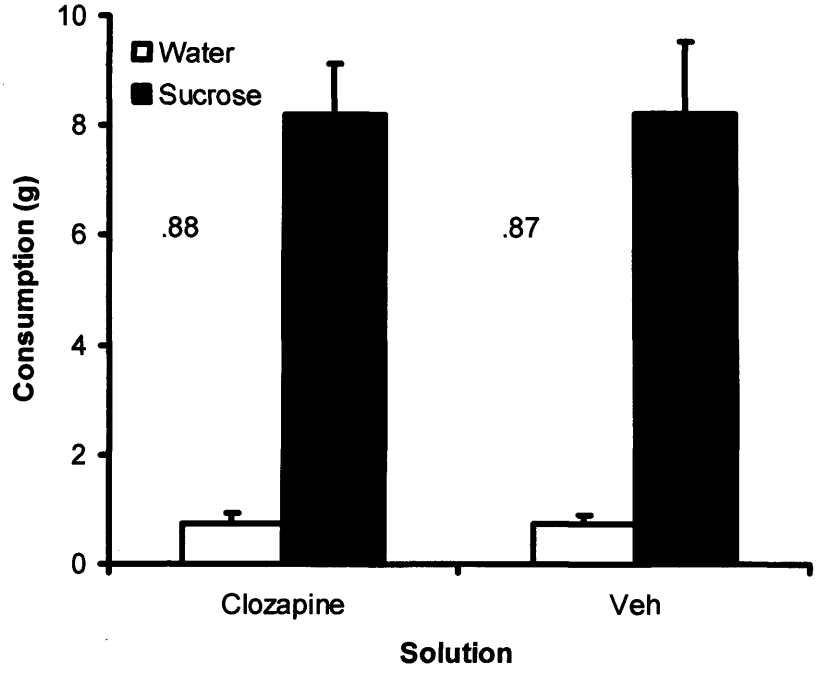


12B.

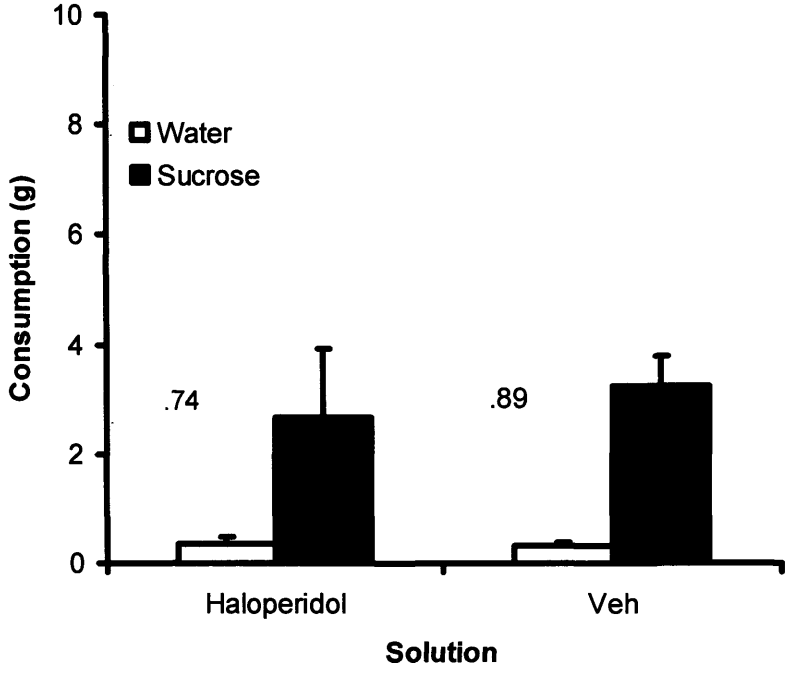


Figure 12: Shows the mean consumption of water and 1% sucrose when presented simultaneously for 20min following acute treatment with 0.3mg/kg clozapine and vehicle (Panel A), or 0.1mg/kg haloperidol and vehicle (Panel B), with SEM. Numbers beside each pair of bars represent the sucrose preference ratios, indicating the amount of sucrose consumed as a proportion of the total amount consumed (both sucrose and water).

investigation may have been too short to reveal this (the session length used here was 20min, but 30min sessions were used by Hartfield et al.). The deprivation state of the animals used in the study of Hartfield et al. may also play a part in the difference observed. In their study animals received ad libitum food, whereas in the current study animals were food restricted to 85% of their free-feeding weight. If clozapine increases food intake by reducing satiety, this rise in consumption would be more difficult to detect in animals that are hungry at the point of testing than it would be in sated animals. Additionally, the test solutions used may also play a part in the difference between the results of current experiment and those of Hartfield et al. Hartfield et al used a 10% fat solution whereas the current experiments used either 4% or 1% sucrose. Fat has more than twice as many calories as sucrose, and the higher caloric density of the fat solution used by Hartfield et al. would be more satiating than the sucrose used here. Therefore, an effect mediated by reducing satiety may be more apparent in the previous study than it would be here.

Experiment 6b showed that haloperidol decreases both sucrose intake and lick cluster size, consistent with the reports of Hartfield et al. (2003). Although the normal preference for sucrose over water was not reversed by haloperidol, a sensitive analysis using sucrose preference ratios rather than overall consumption revealed that haloperidol decreased the ratio of sucrose intake over water compared to vehicle-treated animals. In this experiment, the decreases in consumption, palatability and sucrose preference could not be attributed to motor deficits as ILI was unaffected. Haloperidol is commonly associated with weight gain, although this is to a lesser extent than atypical drugs such as clozapine. In light of the current results it seems more likely that this weight gain is mediated via an altered endocrine response that increases fat deposition, because experimental consumption was reduced rather than

increased. This is consistent with data from Fell et al. (2003) and Fell et al. (2005) who found that haloperidol increased weight gain in adult and juvenile rats without increasing food intake.

Hartfield et al. (2003) suggested that haloperidol decreases intake of fat solutions in rats by reducing their perceived palatability. The results of the current investigation support this conclusion. Sucrose solutions are normally highly palatable to rats. The decreased palatability brought about by haloperidol treatment may reflect the exacerbation of negative symptoms such as anhedonia, often reported with use of typical antipsychotics. The implications of these results for drug discovery will be considered in section 7.4 of the general discussion.

CHAPTER FIVE

Sub-chronic PCP treatment and reward value

Section One: Sub-chronic PCP treatment and consummatory reward value

5.1 Introduction

Experiment 4 provided evidence that although acute treatment with NMDA antagonists can decrease cluster size, this only occurs when ILI is increased. As described in chapter 3, raised ILI is indicative of confounding motor deficits. However, several authors have suggested that sub-chronic PCP treatment is more relevant than acute treatment for modelling the non-psychotic symptoms of schizophrenia (Morris, et al., 2005). This is because, in contrast to acute PCP treatment, sub-chronic treatment produces a reduction in glucose utilisation and blood flow in the prefrontal cortex (hypofrontality). This hypofrontality is exhibited in schizophrenia and it correlates with deficits in cognitive function (e.g. Buchsbaum, et al., 1990). In addition to these pathological changes, rats given sub-chronic PCP treatment also exhibit cognitive deficits (Dunn & Killcross, 2006) and changes in social behaviour (e.g. Sams-Dodd, 1998, but see also Jenkins, et al., 2008). Rats treated sub-chronically with PCP are also sensitised to the effects of amphetamine on locomotor behaviour (Jentsch, Taylor, & Roth, 1998).

In light of this evidence, Experiment 8 sought to investigate the effects of sub-chronic PCP treatment on the value of sucrose rewards.

5.2 Experiment 8

Method

Subjects

Experiment 8 was conducted in the behavioural neuroscience laboratory at the School of Psychology, Cardiff University. Forty eight male Hooded Lister rats were used. Their free-feeding weights ranged 296-338 grams. Supplier and husbandry details were as in chapters 2 and 3

Apparatus

The apparatus used for drinking tests were the same as in chapter 3 Animals were pre-trained to drink in the lickometer cages using 8% maltodextrin prior to PCP treatment. Maltodextrin was used so that responses to sucrose following PCP treatment were not influenced by any memory of the hedonic value of the pre-training solution. Three concentrations of sucrose were used in the test stage: 4%, 8%, and 16%. Either before or after these drinking tests, rats received locomotor activity assessments to test for augmented hyperlocomotion following amphetamine injection in the PCP treated animals, an effect seen consistently following this injection protocol. The apparatus used for this were white plastic boxes with grid floors and lids. The boxes measured 48cm x 31cm x 18cm. Two infra-red beams spanned the boxes. When these beams were interrupted by the movement of an animal, a signal was recorded by a linked computer.

Drugs

PCP supplied by Sigma-Aldrich UK was used in this experiment. This was made into a 5mg/ml solution using 5% (w/w) glucose. D-Amphetamine sulphate from the same supplier was also dissolved in 5% glucose to obtain a 1mg/ml solution. These doses refer to the salt weight of the compounds. Drugs were injected at 1ml/kg.

Dosing and Testing

All animals received access to an 8% maltodextrin solution in the lickometer cages, 10 minutes each day for 10 days, to ensure that a steady level of consumption was reached. Training and test sessions began at approximately 9.30am every morning (2.5 hours into the light phase of the light/dark cycle).

Following pre-training, twenty four of the rats received sub-cutaneous injections of 5mg/kg PCP twice daily (8.00hrs and 19.00hrs) for seven days, whilst twenty four rats received vehicle injections. Following a seven-day washout period, rats were given three concentrations of sucrose in the lickometer cages: 4%, 8% and 16%. Each concentration was given for three consecutive days, and the order of sucrose presentations was balanced so that half of the animals received the sucrose concentrations in ascending order and half received them in descending order.

Locomotor activity testing

In addition to the drinking tests, rats received locomotor activity assessments to test for PCP-induced augmented hyperlocomotion following amphetamine injection. Half of the animals in both PCP and vehicle treated groups received this testing prior to the drinking tests, and the rest received locomotor activity testing following the drinking tests. The rats were placed in cages for a 30 min habituation period in which

movements were recorded by the number of interruptions that occurred to infra red beams spanning the cages. They were then given 1mg/kg amphetamine sub-cutaneously, and their activity was recorded for a further 60 min.

Data Analysis

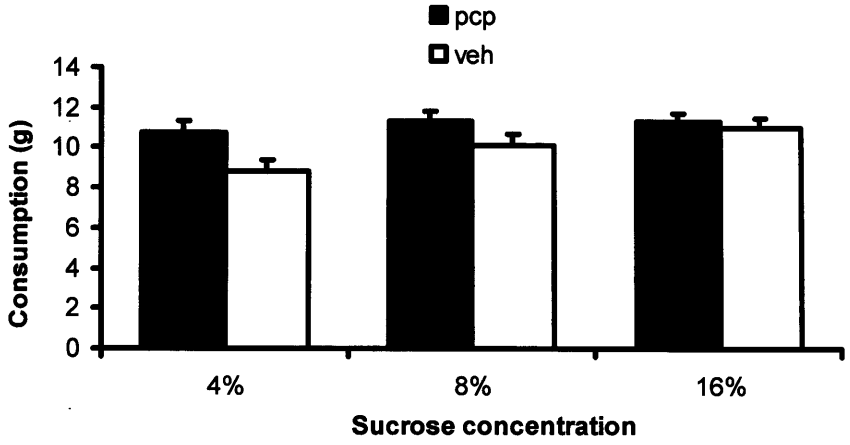
The parameters measured via analysis of licking were the same as those described in chapters 2 and 3. Data from the drinking tests was analysed using mixed ANOVA with sucrose concentration as a within-subject factor, and drug treatment as a between-subject factor. The data used for analysis was averaged over the second and third session with each sucrose concentration. The data for the first session was not used in order to minimise contrast effects that may have occurred as a result of changing the sucrose concentration.

The apparatus used for locomotor activity testing counted the number of movements made and grouped them into ten-minute bins. The data was analysed using mixed ANOVA with 'bin' as a within-subject factor, and drug treatment as a between-subject factor. One animal was removed from the analysis of Experiment 8 due to a failure in lick recording during one of the drinking test sessions.

Results of Experiment 8

Figure 13 shows the results of Experiment 8, including the mean amount of sucrose consumed (panel A), the mean number of licks per cluster (panel B) at each sucrose concentration. Increasing sucrose concentration produced an overall increase in the amount consumed, $F(2,90)=8.08$, $p<.001$. Pairwise comparisons show that rats consumed significantly more 16% sucrose than 4%, $F(1,45)=12.49$, $p<.001$, and more

13A.



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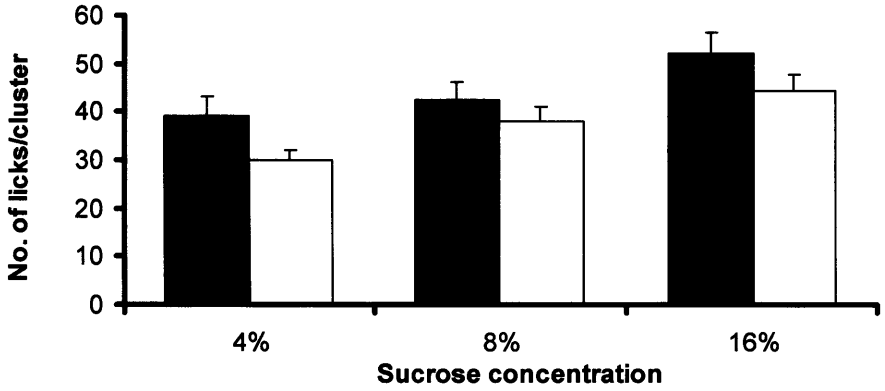


Figure 13: Shows the lickometric data obtained from consumption of three sucrose concentrations follow sub-chronic PCP treatment. Panels A and B illustrate the mean amount of sucrose consumed and the mean number of licks per bout respectively, with SEM.

8% sucrose than 4%, $F(1,45)=9.21$, $p=.004$. Consumption did not differ significantly between 8% and 16% sucrose, $F(1,45)=1.414$, $p=.241$. Although consumption was numerically higher after PCP treatment this difference was not significant, $F(1,45)=3.82$, $p=.057$. There was no interaction between sucrose concentration and drug treatment, $F(2,90)=2.61$, $p=.079$. With respect to the number of licks per cluster, increasing sucrose concentration produced an overall significant increase in cluster size, $F(2,90)=17.24$, $p<.001$. Pairwise comparisons show that cluster size was significantly greater for 16% sucrose than for 4%, $F(1,45)=29.92$, $p<.001$, and 8% $F(1,45)=13.90$, $p<.001$. Cluster size was also greater for 8% sucrose than for 4%, $F(1,45)=5.81$, $p=.02$. PCP treatment had no effect on cluster size, $F(1,45)=3.22$, $p=.079$, and there was no interaction between these independent variables, $F<1$.

Locomotor activity testing

Figure 14 shows the activity counts recorded, grouped into 10 minute bins across the session. Locomotor activity testing confirmed that the PCP treatment sensitised animals to the effects of amphetamine on locomotor activity. There was a significant effect of bin, $F(8,360)=7.62$, $p<.001$, and PCP treatment, $F(1,45)=4.59$, $p=.038$. There was a bin × PCP treatment interaction, $F(8,360)=2.61$, $p=.009$. Simple effects show that prior to amphetamine injection (bins 1-3) the pattern of activity in PCP and vehicle treated animals is similar (Fs <1). Following amphetamine injection, there was a significant difference between levels of activity in PCP and vehicle treated animals at bins 6, 7, and 8, smallest $F(1,45)= 4.77$, $p=.034$. The difference between patterns of activity in bins 4, 5 and 9 did not reach significance, largest $F(1,45)= 1.86$, $p=.179$.

14.

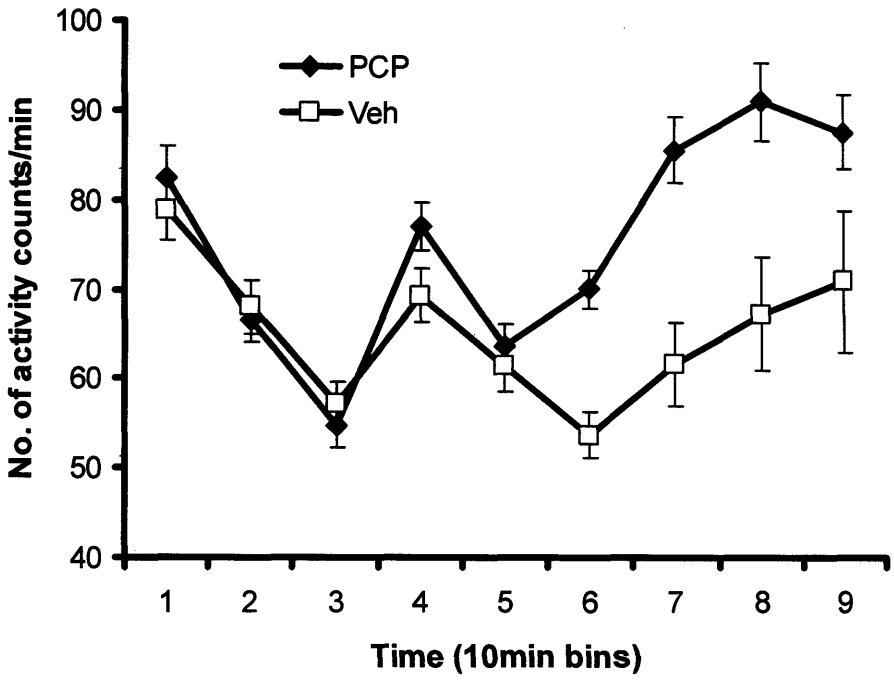


Figure 14: Graph shows the mean number of activity counts (with SEM) made over a 90min session, before and after administration of 1mg/kg amphetamine (given between bins 3 and 4). Counts are grouped into 10min bins.

5.3 Discussion

The main aim of this experiment was to investigate whether evidence of anhedonia could be found in rodents following sub-chronic administration of PCP. It is plausible that the existence of an identifiable analogue of consummatory anhedonia may be observed more readily following long-term PCP treatment than following acute treatment. Jentsch and Roth (1999) reviewed evidence that long-term PCP abuse in humans has been associated with reduced frontal blood flow (Hertzman, Reba, & Kotlyarov, 1990). A corresponding decrease in blood flow is seen in individuals with schizophrenia. Since schizophrenia sufferers frequently show deficits in cognitive tasks relying on frontal lobe function (e.g. spatial working memory deficits; Pantelis, et al., 1997), chronic PCP treatment may indeed be more relevant for modelling the non-psychotic symptoms of schizophrenia. The expectation that long-term PCP exposure may be important for the generation of a schizophrenia-like pattern of changes in the rat brain has been confirmed by Cochran et al. (2003), who found reduced glucose utilisation in the pre-frontal cortex, reticular nucleus of the thalamus and auditory system, in addition to decreased prefrontal parvalbumin expression – changes that resemble those in schizophrenia. However, despite this biological validity, we found no evidence of a reduction in reward value following our sub-chronic dosing regimen. Indeed, sub-chronic PCP treatment produced an overall trend towards an increase in the consumption and palatability of sucrose solutions.

It is improbable that this outcome reflects problems with the dosing regimen used, since the sensitisation effect of PCP on amphetamine-induced hyperlocomotion (previously reported by Jentsch et al. 1998) was evident in this experiment. It is also unlikely that the regimen used here is inappropriate for modelling the non-psychotic symptoms of schizophrenia, since evidence of cognitive deficits have been found

using this protocol. For example, using this protocol, Dunn & Killcross (2006) found impaired performance on tasks that required the use of conditional information to direct responding. This effect may reflect the inability to use task-setting cues to guide goal-directed behaviour in the human condition of schizophrenia. This deficit was reversed by the atypical drug clozapine, but not by the typical antipsychotic haloperidol, thereby mirroring clinical findings.

There is some evidence that withdrawal from chronic PCP treatment using higher doses can produce anhedonic effects using an intracranial self-stimulation paradigm (Olney, et al., 1989; Spielewoy & Markou, 2003). However, since such high doses are known to cause pathology that is not produced in schizophrenia (Olney, et al., 1989), any anhedonic effects may well be a manifestation of neural damage that is inconsistent with the disorder. It has also been reported that some effects of sub-chronic PCP withdrawal may be more apparent following a shorter (24 hour) washout period (Jenkins, et al., 2008) although withdrawal effects remained consistent after the shortest test interval (and there are other reports that the length of withdrawal interval was without effects (Noda, Yamada, Furukawa, & Nabeshima, 1995). In addition, the effects reported by Turgeon and Hoge (2003) were observed 20 hours after acute treatment. However, a seven day washout period was selected for the current study in order to maximise the predictive validity of the model. The persistent nature of effects produced by sub-chronic PCP treatment is one of the reasons that long-term PCP exposure is favoured above acute treatment as a model of schizophrenia (Jentsch & Roth, 1999). A transient anhedonic-like effect would not accurately reflect the disease state.

In light of the fact that cognitive deficits occur following the treatment regimen used in this study, our findings suggest that consummatory anhedonia can be

dissociated from cognitive deficits following sub-chronic PCP treatment. On one hand, it is possible that our inability to find evidence of reduced reward value could indicate that sub-chronic PCP treatment in rodents provides an incomplete model of negative schizophrenic symptomatology. On the other hand, the occurrence of cognitive deficits without the negative symptom of anhedonia may reflect an absence of anhedonia as a primary symptom of the disorder itself. This is an idea considered by several authors who suggest that individuals with schizophrenia experience amotivation (Foussias & Remington, 2010), or a deficit in the ability to experience and express all emotions (emotional blunting) (Romney & Candido, 2001), rather than anhedonia per se.

As the results presented here and in chapter 3 do not appear to reflect PCP-induced anhedonia, it is useful to examine whether other aspects of reward value are affected by acute and sub-chronic PCP treatment. Berridge (1996) proposed that an experience of a reward contains two distinguishable components – 'liking' and 'wanting'. In light of the current measures, palatability may be associated with liking, whereas consumption, linked to appetite and motivation, may be associated with wanting. Berridge also cites evidence that these are controlled by independent neurotransmitter systems and thus can be separately measured and manipulated pharmacologically. This may mean that animals treated acutely with PCP 'want' a reinforcer less, and may explain why lever pressing is reduced following acute PCP treatment (Gilmour, et al., 2009). If an animal 'wants' a reinforcer less, they may be less willing to work for it. This does not necessarily mean that the animal 'likes' the reinforcer less when it is presented. This may account for our inability to find evidence for a decrease in reward value (consummatory anhedonia) via our analysis of palatability in Experiments 3-5. However, this suggestion cannot apply to sub-

chronic PCP treatment as there was a marginal increase in the consumption of animals treated sub-chronically with PCP in the current experiment.

In summary, although sub-chronic PCP treatment is generally thought to be more relevant for modelling the non-psychotic symptoms of schizophrenia, microstructural analysis of licking yielded no evidence of reduced reward value. Therefore, the symptoms induced by both acute and sub-chronic PCP treatment do not seem to include the production of anhedonia. This may reflect either a weakness of PCP-induced animal models of schizophrenia, or it may suggest that consummatory anhedonia is not actually a primary symptom of the disorder.

Section two: Sub-chronic PCP treatment and the anticipation of reward

5.4 Introduction

Research from both social psychology and neuroscience has suggested that the experience of pleasure has two distinct components; *consummatory* and *anticipatory*. Consummatory pleasure refers to the in-the-moment pleasure experienced when a person is engaging in an enjoyable activity, whilst anticipatory pleasure is the pleasure experienced in anticipation of things to come. Gard et al. (2006) developed two different scales to measure individual trait dispositions in anticipatory and consummatory pleasure experiences. They found that the scales measured distinct constructs, with individual differences in anticipatory pleasure being more closely related to motivational processes than consummatory pleasure. They also found that there was less than 10% overlap between individuals showing diminished anticipatory pleasure and those showing a consummatory pleasure deficit. This distinction has also been supported by functional neuroimaging studies where it has been found that the

pre-frontal cortex is activated during consumption of a reward, whereas the nucleus accumbens (but not the pre-frontal cortex) is activated in anticipation of the reward.

A debate has ensued concerning the relevance of these two distinct pleasure types in the psychopathology of schizophrenia. This has arisen because of discrepant findings regarding the manifestation of anhedonia. For example, people with schizophrenia often report that they experience lower levels of pleasure than controls (Miller, 1987), and engage in fewer enjoyable activities (Devries & Delespaul, 1989). However, some laboratory studies have not found that people with schizophrenia experience less pleasant emotion than controls. For example, Berenbaum and Oltmanns (1992) found that individuals with schizophrenia did not differ from controls in their self reports of consummatory pleasure whilst watching comic film clips, even though they were less facially expressive. Furthermore, Kring (1999) took electromyographical recordings from facial muscles and found that people with schizophrenia do not differ from controls in their unobservable muscle responses to pleasant stimuli, even though these movements were less likely to reach the threshold at which they could be detected visually.

To reconcile these findings, it has been suggested that people with schizophrenia can and do experience comparably normal levels of consummatory pleasure, but experience less pleasure than normal in anticipation of future events. Evidence from Gard, Kring, Gard, Horan, and Green (2007) supports this hypothesis. In this study, participants made consummatory pleasure ratings whilst conducting everyday tasks. At the same time, they were required to indicate what they looked forward to doing and how much they thought they would enjoy the activity. Participants with schizophrenia did not differ from controls in their reports of consummatory pleasure, but exhibited deficits in anticipatory pleasure, particularly

concerning goal-directed activities. Furthermore, anticipatory (but not consummatory) pleasure deficits were related to clinical ratings of anhedonia, including scores on the Behavioural Activation Scale (BAS) measuring reward sensitivity (developed by Carver & White, 1994), and anhedonia scores on the Schedule for the Assessment of Negative Symptoms (SANS) scale (developed by Andreasen, 1982). Additional evidence in favour of the idea that people with schizophrenia have deficits in their ability to anticipate the value of future rewards comes from a study of delayed discounting by Heerey, Robinson, McMahon and Gold (2007). In a delayed discounting test, participants are asked to judge the subjective value of a reward according to how long they have to wait for it. Heerey et al. found that people with schizophrenia discounted the value of delayed rewards at a greater rate than controls. In light of these findings, it is possible that the inability to find evidence of consummatory anhedonia in rats following sub-chronic PCP treatment may reflect an absence of this symptom in the disorder itself. Therefore, the experiments detailed in this section investigated the occurrence of anticipatory anhedonia in this model using an anticipatory contrast paradigm.

Negative anticipatory contrast occurs when rats receive brief, sequential access to two solutions, once each day. Repeated pairing of the two solutions allows animals to anticipate what the second solution will be, and this affects their intake of the first solution. If the second solution is preferred over the first, intake of the first solution is suppressed (see Flaherty, 1996 for a full review). In the first demonstration of this effect rats were given 3-min access to 0.15% saccharin, followed by 5-min access to 32% sucrose after a 15-second inter-solution interval. Control animals had access to saccharin only and increased their consumption of this solution across days.

However, in animals receiving 32% sucrose, this rise in saccharin consumption across days was reduced (Flaherty, 1996).

It is now known that the magnitude of the negative anticipatory contrast effect can be influenced by several factors. For example, the greater the hedonic value of the second solution, the greater the contrast effect (e.g. Flaherty & Checke, 1982). Also, when the inter-solution interval exceeds 1-min, the contrast effect is decreased with increasing interval (Flaherty & Checke, 1982). In addition, contrast is enhanced when the duration of access to the second solution is longer than the first solution, (Flaherty, 1996). With these findings in mind, the following experiment was designed to optimise the negative anticipatory contrast effect.

Using this paradigm, it is possible to detect whether PCP produces a deficit in an animal's ability to anticipate the value associated with future rewards. A failure to expect the second solution, or to accurately predict its value, will bring about a reduction in the anticipatory contrast effect, manifested by an increase in consumption of the first solution when presented repeatedly across days.

5.5 Experiment 9

Methods

Subjects

This experiment was carried out as a follow-up to Experiment 8. Twenty four animals were used from the initial cohort. The husbandry details, prior drug treatment of these animals, previous sucrose exposure, and locomotor activity testing were described in section 4.2.

Apparatus

Testing was carried out in a room containing six automated drinking chambers measuring 30×24×21cm. The chamber floor consisted of 19 steel rods measuring 4.8mm in diameter. Two of the chamber walls were clear perspex and two were aluminium. Two holes were positioned 5cm above the grid floor on the left and right hand side of one of the aluminium walls to allow rats to access solutions during experimental sessions. Solutions were given in 50ml bottles attached to stainless steel drinking spouts. These were fastened to motorised holders that positioned the metal spout level with the wall of the chamber, and retracted them when required. A contact-sensitive lickometer registered the time of each lick to the nearest 0.01s. This was recorded by a computer using MED-PC software (Med Associates Inc., St Albans, VT, USA). The solutions used for this experiment were 4% and 32% sucrose, formulated (weight/weight) using deionised water.

Dosing and testing

The anticipatory contrast test sessions discussed here began 20 days after the final sub-chronic PCP injections described in section 4.2. Rats were given daily anticipatory contrast sessions in which they received 3min access to a 4% sucrose solution presented on the left side of the automated chamber. This was followed by the presentation of either a 4% or 32% sucrose solution for 6min, after a 4 second pause. The concentration of this second solution remained consistent throughout the experiment. For half of the animals in the PCP and vehicle treated groups the second solution concentration was 4%, and for the other half of the animal so it was 32%.

Test sessions began at approximately 9.30am every morning and were conducted for 18 consecutive days.

Data Analysis

The parameters recorded and the lick cluster criterion used for this experiment were the same as those used in previous chapters. The anticipatory contrast data obtained from both the first and second solutions presented was analysed via mixed analysis of variance (ANOVA) with 'block' as the within-subject factor, and second solution concentration and drug treatment as between-subject factors. The data from all eighteen days of testing was used to analyse the effects of sub-chronic PCP treatment on acquisition of the anticipatory contrast effect. This was averaged into two-day blocks prior to analysis.

Results

Figures 15 shows the amount of sucrose consumed (panel A) and the mean number of lick/cluster (panel B) for both the first solution presented. Figure 16 shows the same data for the second solution presented. The first solution presented was always 4% sucrose and the second was either 4% or 32% sucrose. In terms of the amount consumed of the first solution, there was an overall increase in consumption across days, $F(8,160)=48.15$, $p<.001$, and consumption was higher when the second solution was 4% sucrose than when it was 32% sucrose, $F(1,20)=19.98$, $p<.001$. There was a significant interaction between 'block' and second solution concentration consistent with the observation that consumption rose more steeply across days when the second solution was 4% sucrose than when it was 32% sucrose, $F(8,160)=8.94$, $p<.001$. Sub-chronic PCP treatment had no effect on consumption, $F<1$, there was no interaction

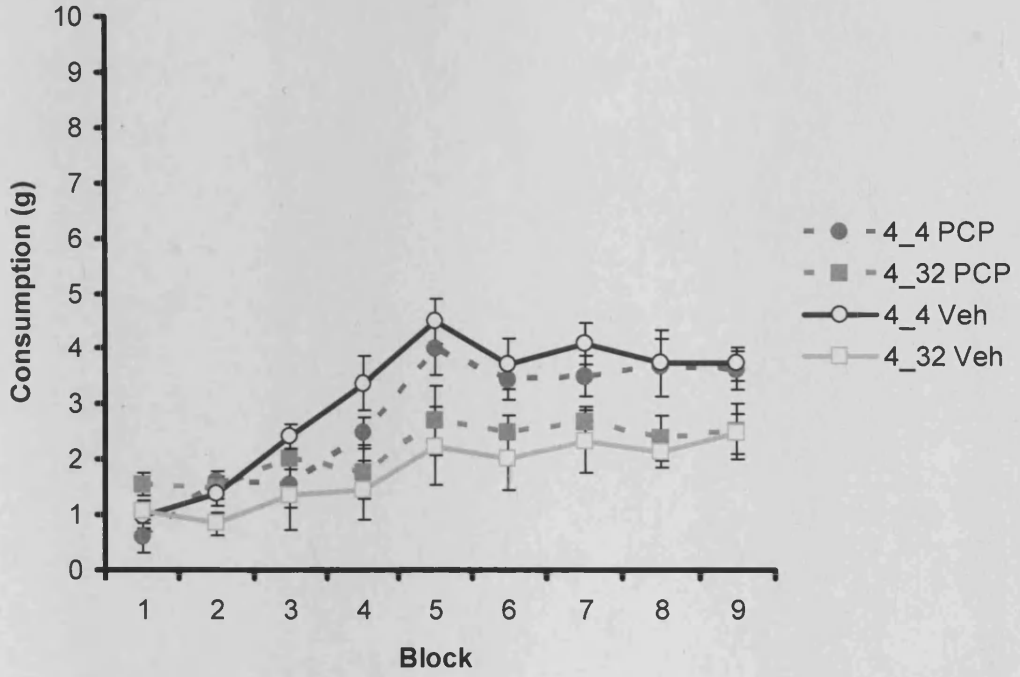
between day and sub-chronic treatment, $F<1$, no interaction between treatment and second solution concentration, $F(1,20)=3.60$, $p=.072$, and no three-way interaction with second solution concentration, $F < 1$.

With respect to licks/cluster, there was an overall increase across days, $F(8,160)=12.47$, $p<.001$. Licks/cluster was also higher when the second solution was 4% sucrose than when it was 32% sucrose, $F(1,20)=8.46$, $p=.009$. There was no interaction between day and second solution concentration, $F(8,160)=1.11$, $p=.358$. There was no effect of sub-chronic PCP treatment on the number of licks/cluster, $F<1$. There was also no interaction between day and sub-chronic treatment, no interaction between drug treatment and second solution concentration, $F<1$, and no three-way interaction with second solution concentration, Fs<1.

The amount consumed of the second solution presented increased across days, $F(8,160)=43.20$, p<.001. Consumption of the 32% solution was significantly higher than the 4% solution, $F(1,20)=9.61$, $p=.006$. An interaction occurred between day and solution concentration whereby consumption of the 32% sucrose rose more steeply over time than consumption of the 4% solution, $F(8,160)=2.31$, $p=.023$. Sub-chronic PCP treatment had no effect on consumption, $F<1$. No interaction occurred between day and sub-chronic treatment, $F(8,160)=1.30$, $p=.245$, or between drug treatment and second solution concentration, $F<1$, and there was no three-way interaction with second solution concentration, $F<1$.

The number of licks/cluster for the second solution increased across days, $F(8,160)=4.51$, $p<.001$. Unexpectedly, there was no significant difference in licks/cluster between the 4% and 32% second solution concentrations, $F(1,20)=3.41$, $p=.080$. However, there was an interaction between day and second solution concentration whereby licks/cluster showed a larger increase across days when the

15A.



15B.

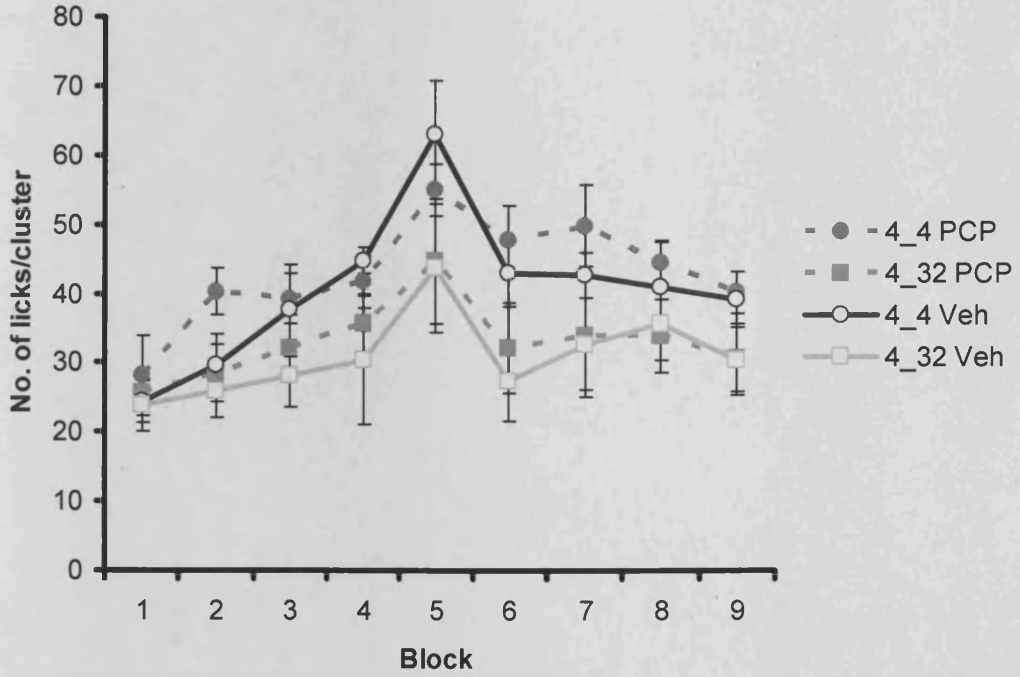
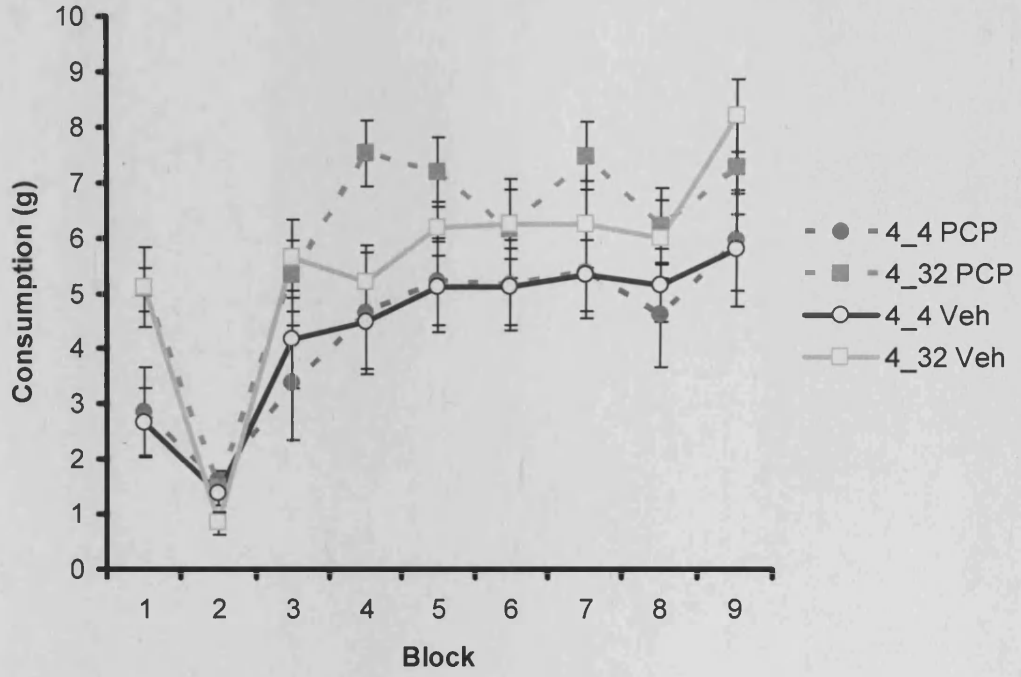


Figure 15: Graphs show the consumption (Panel A) and number of licks/cluster (Panel B), with SEM, for a 4% sucrose solution consistently presented prior to either 4% sucrose or 32% sucrose, for animals treated sub-chronically with PCP or vehicle.

16A.



16B.

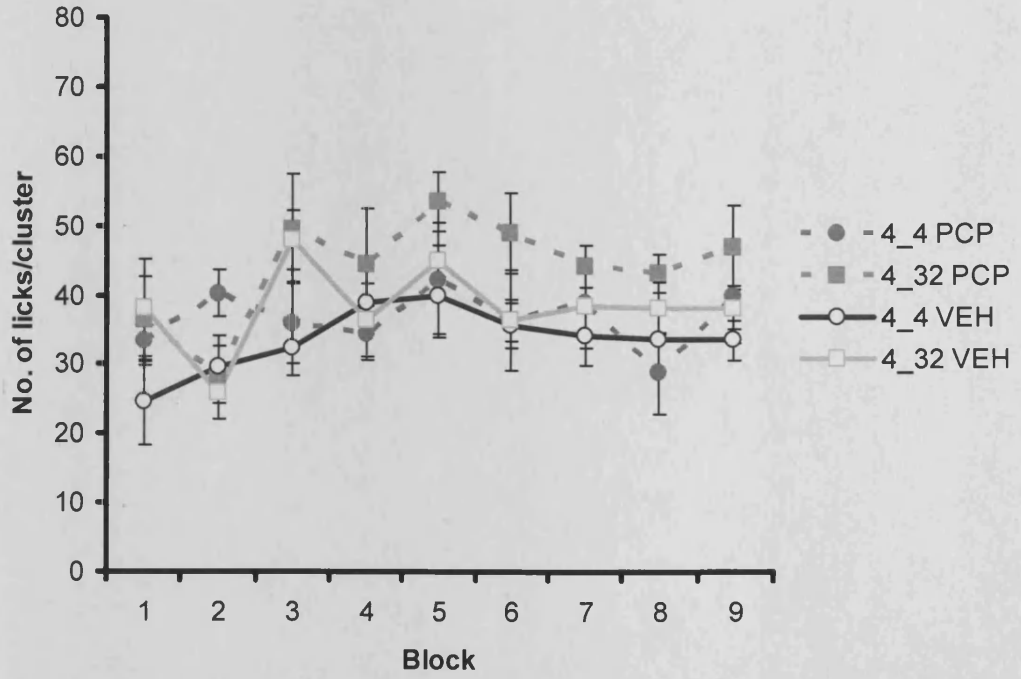


Figure 16: Graphs show the consumption (Panel A) and number of licks/cluster (Panel B), with SEM, for 4% and 32% sucrose solutions presented after 4% sucrose, for animals treated sub-chronically with PCP or vehicle.

solution was 32% sucrose than when it was 4% sucrose, $F(8,160)=2.32$, $p=.022$. Sub-chronic PCP treatment had no effect on the number of licks/cluster, $F(1,20)=1.81$, $p=.194$. There was no interaction between day and sub-chronic treatment, $F<1$, no interaction between drug treatment an second solution concentration, $F<1$, and no three-way interaction with the second solution concentration, $F(8,160)=1.10$, $p=.367$.

Summary of Experment 9

The results of Experiment 9 showed that anticipatory contrast effects could be seen both in terms of overall consumption and palatability. This was evident from the lower consumption and cluster size measurements associated with the first solution presented when it was consistently followed by a more palatable solution. This contrast effect was not affected by prior sub-chronic PCP treatment.

5.6 Experiment 10

Experiment 9 was conducted to follow an earlier experiment and the test sessions began 20 days after sub-chronic PCP treatment, by which time, any effects of PCP withdrawal may have attenuated. The rats used in Experiment 9 had also received prior exposure to sucrose solutions. Since the conditions of Experiment 9 were not optimal, Experiment 10 was necessary to confirm that sub-chronic PCP treatment does not affect anticipatory contrast. The conditions used for Experiment 10 were the same as Experiment 9 except that the animals used were experimentally naïve. For this reason, fewer sessions were required to obtain a stable anticipatory contrast effect.

Methods

Subjects and Apparatus

Forty eight male hooded Lister rats supplied by OLAC, Bicester, UK were used in this experiment. Husbandry details and apparatus used were the same as those in Experiment 9.

Dosing and testing

Prior to testing, 24 rats received sub-cutaneous injections of 5mg/kg PCP twice daily (0800 and 1900hours) for 7 days, whilst 24 rats received 5% glucose vehicle injections. Following a 7-day washout period, all rats were exposed to daily experimental sessions, the same as those in Experiment 9. Test sessions began at approximately 9.30am every morning and were conducted for 8 consecutive days.

After the acquisition phase of the experiment, test sessions were continued for a further 3 days. On the first and third of these days all animals received a sub-cutaneous vehicle injection. On the second day all animals were given a 1mg/kg injection of PCP.

Locomotor activity testing

All rats received locomotor activity assessments to test for PCP-induced augmented hyper-locomotion following amphetamine treatment. For half of the animals, this testing took place before they experienced the anticipatory contrast schedule. The rest of the animals were tested at the end of the experiment. The equipment and procedure for this testing was the same as that recorded for Experiment 8.

Data Analysis

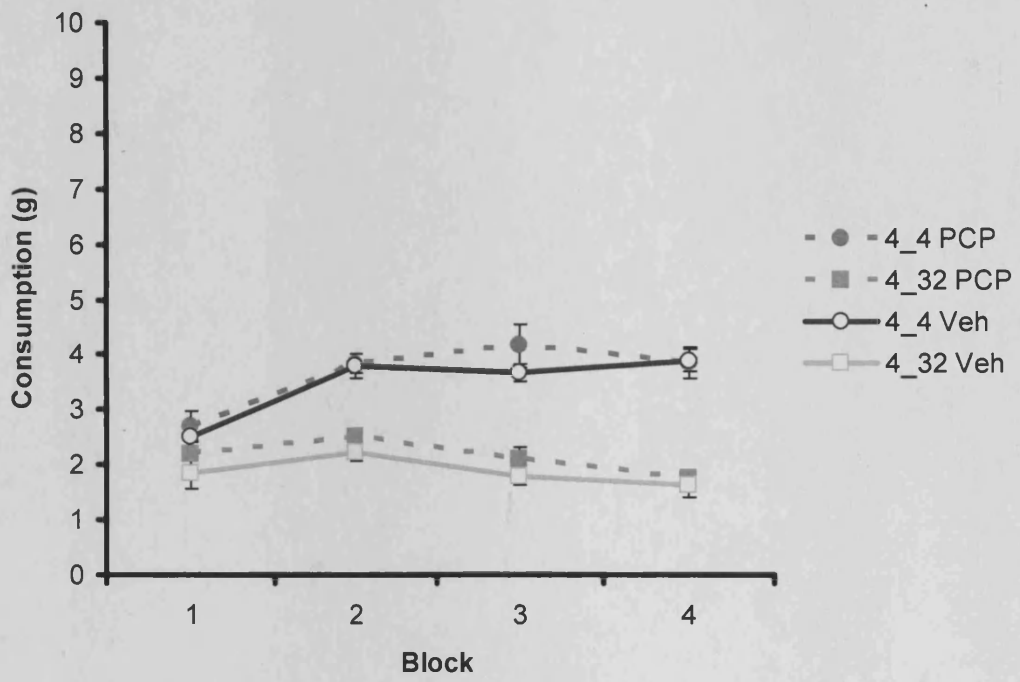
The data from the acquisition phase of the experiment was analysed using the same methods as those reported in Experiment 9. The data from the last three days of testing (the acute PCP test) was analysed using a mixed ANOVA with acute treatment as the within-subject factor and sub-chronic treatment as the between-subject factor. The vehicle data used was averaged across the first and third day (i.e. before and after acute PCP treatment).

During each session, the apparatus used for locomotor activity testing counted the number of movements made by an animal and grouped them into 10min bins. This data was analysed using a mixed ANOVA with 'bin' as a within-subject factor and drug treatment as a between-subject factor.

Results

Figures 17 shows the amount of sucrose consumed (panel A) and the mean number of lick/cluster (panel B) for the first solution presented. Figure 18 shows the same data for the second solution presented. The first solution presented was always 4% sucrose and the second was either 4% or 32% sucrose. In terms of the amount consumed of the first solution, there was an overall increase in consumption across days, $F(3,132)=12.22$, $p<.001$, and consumption was higher when the second solution was 4% sucrose than when it was 32% sucrose, $F(1,44)=103.01$, $p<.001$. A significant interaction between 'day' and second solution concentration indicated that consumption rose more steeply across days when the second solution was 4% sucrose than when it was 32% sucrose, $F(3,132)=13.55$, $p<.001$. Sub-chronic PCP treatment had no effect on consumption, $F(1,44)=2.04$, $p=.161$, there was no interaction between day and sub-chronic

17A.



17B.

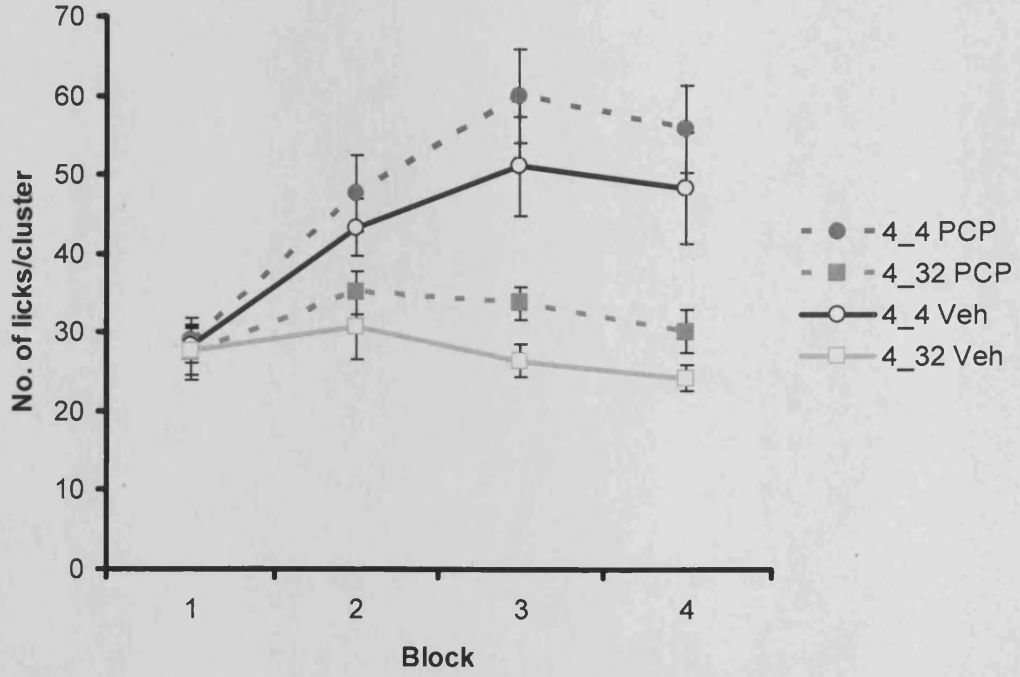
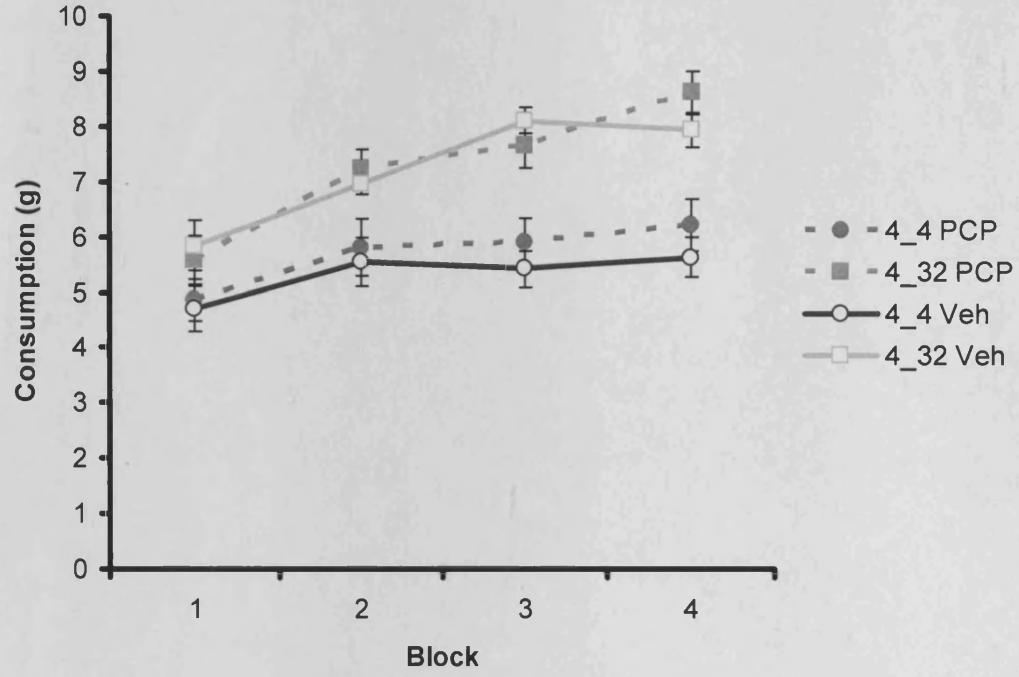


Figure 17: Graphs show the consumption (Panel A) and number of licks/cluster (Panel B), with SEM, for a 4% sucrose solution consistently presented prior to either 4% sucrose or 32% sucrose, for animals treated sub-chronically with PCP or vehicle.

18A.



18B.

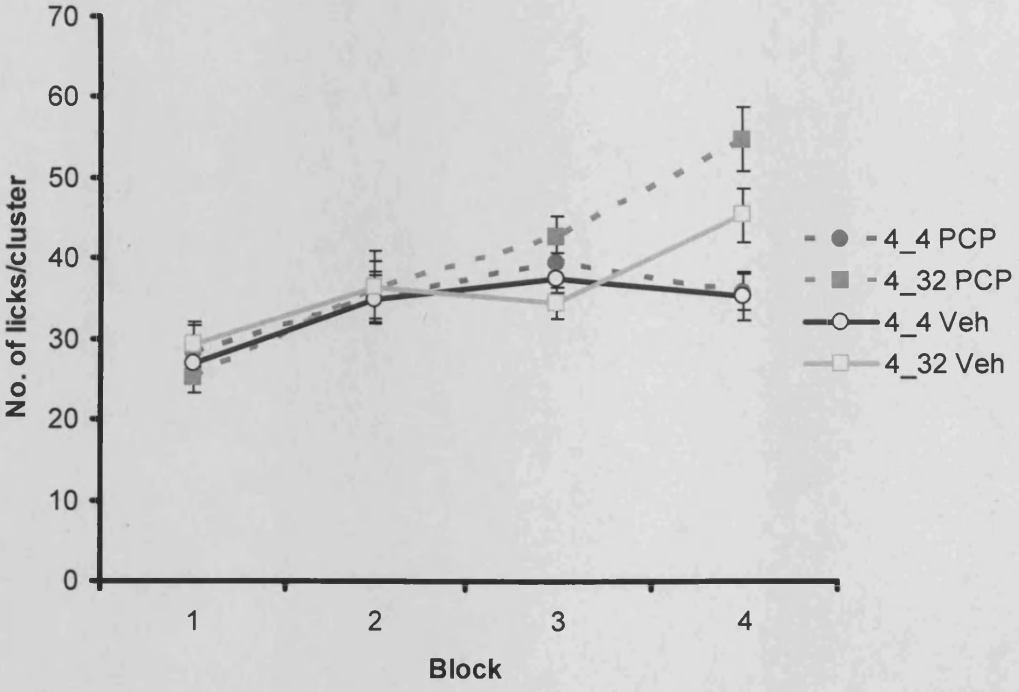


Figure 18: Graphs show the consumption (Panel A) and number of licks/cluster (Panel B), with SEM, for 4% and 32% sucrose solutions presented after 4% sucrose, for animals treated sub-chronically with PCP or vehicle.

treatment, no interaction between drug treatment and second solution concentration, and no three-way interaction with second solution concentration, Fs<1.

With respect to licks/cluster, there was an overall increase across days, $F(3,132)=17.09$, $p<.001$. Licks/cluster was also higher when the second solution was 4% sucrose than when it was 32% sucrose, $F(1,44)=26.89$, $p<.001$. An interaction occurred between day and second solution concentration whereby the number of licks/cluster rose across days only when the second solution was 4% sucrose (i.e. the same concentration as the first solution), $F(3,132)=13.71$, $p<.001$. There was no effect of sub-chronic PCP treatment on the number of licks/cluster, $F(1,44)=2.45$, $p=.125$. There was also no interaction between day and sub-chronic treatment, $F(3,132)=1.24$, $p=.193$, no interaction between drug treatment and second solution concentration, $F<1$, and no three-way interaction with second solution concentration, $F<1$.

The amount consumed of the second solution presented increased across days, $F(3,132)=26.14$, $p<.001$. Consumption of the 32% solution was significantly higher than the 4% solution, $F(1,44)=39.39$, $p<.001$. An interaction occurred between day and solution concentration whereby consumption of the 32% sucrose rose more steeply over time than consumption of the 4% solution, $F(3,32)=4.53$, $p=.005$. Sub-chronic PCP treatment had no effect on consumption, $F<1$. No interaction occurred between day and sub-chronic treatment, or between drug treatment and second solution concentration, and there was no three-way interaction with second solution concentration, Fs<1.

The number of licks/cluster increased across days, $F(3,132)=24.21$, $p<.001$. Unexpectedly, there were no significant differences in licks/cluster between the 4% and 32% second solution concentrations, $F(1,44)=3.40$, $p=.072$. However, there was

an interaction between day and second solution concentration whereby licks/cluster showed a larger increase across days when the solution was 32% sucrose than when it was 4% sucrose, $F(3,132)=7.15$, $p<.001$. Sub-chronic PCP treatment had no effect on the number of licks/cluster, $F(1,44)=1.02$, $p=.319$. There was no interaction between day and sub-chronic treatment, $F(3,132)=1.59$, $p=.197$, or between drug treatment and second solution concentration, $F<1$, and there was no three-way interaction with the second solution concentration, $F(3,132)=1.48$, $p=.222$.

Post-acquisition acute PCP test

Figure 19 shows the consumption (panel A) and number of licks/cluster (panel B), with SEM, following administration of acute PCP and vehicle treatment, for the first solution presented. Figure 20 shows this data for the second solution presented. Data was collapsed over sub-chronic PCP treatment conditions as there were no significant effects or interactions involving the sub-chronic PCP factor (see below). Acute PCP treatment had no effect on the amount consumed of the first solution presented, $F(1,44)=1.08$, $p=.305$. Consumption of the first solution was lower when it preceded 32% sucrose than when it preceded 4% sucrose, $F<1$. There was no acute treatment × second solution concentration interaction, demonstrating that, in terms of consumption, acute PCP treatment did not alter the anticipatory contrast effect acquired, $F<1$. Additionally, there was no effect of prior sub-chronic treatment on consumption of the first solution, and no interaction between sub-chronic treatment and second solution concentration. There was no interaction between acute PCP treatment and sub-chronic treatment and no 3-way interaction with second solution concentration (all Fs < 1). This demonstrates that, in terms of consumption, the

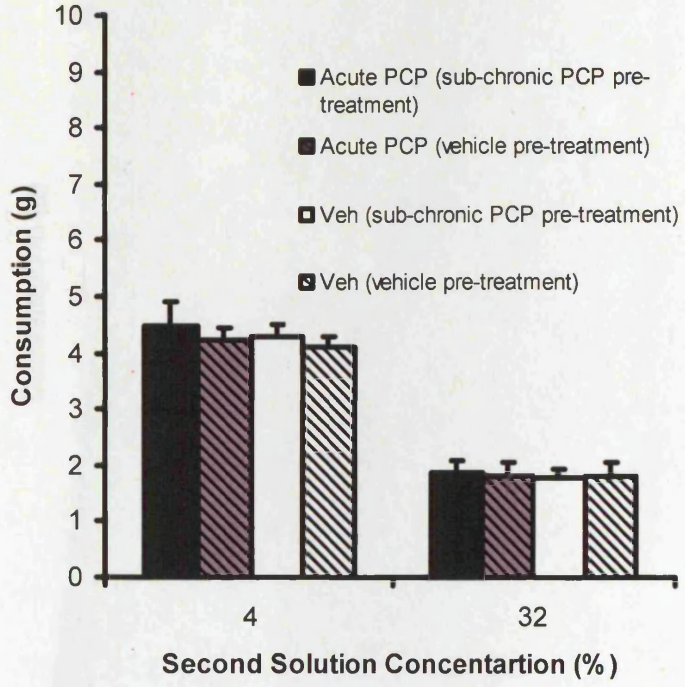
absence of an effect of acute PCP treatment on anticipatory contrast was true for both animals treated sub-chronically with PCP and those treated with vehicle.

Acute PCP treatment produced no overall effect on the number of licks/cluster during consumption of the first solution, $F<1$. The number of licks/cluster during consumption of the first solution was lower when it preceded 32% sucrose than when it preceded 4% sucrose, $F(1,44)=34.13$, $p<.001$. There was no acute treatment × second solution concentration interaction, demonstrating that, in terms of consumption, acute PCP treatment did not alter the anticipatory contrast effect acquired, $F(1,44)=1.01$, $p=.321$. Again, there was no effect of sub-chronic treatment on licks/cluster during consumption of the first solution, $F(1,44)=1.96$, $p=.169$ There was no interaction between sub-chronic treatment and second solution concentration, no interaction between acute PCP treatment and sub-chronic treatment, and no 3-way interaction with second solution concentration, Fs<1. This demonstrates that, as with consumption, the absence of an effect of acute PCP treatment on anticipatory contrast in terms of licks/cluster was true for animals treated sub-chronically with PCP and with vehicle.

With respect to the second solution presented, consumption of 32% sucrose was higher than 4% sucrose, $F(1,44)=31.74$, $p<.001$. Consumption was not affected by acute PCP treatment, $F<1$. There was no acute treatment × solution concentration interaction, $F<1$, and no acute treatment × sub-chronic treatment interaction, $F(1,44)=1.75$, $p=.193$.

The number of licks/cluster was numerically higher for 32% sucrose than 4% sucrose, but this did not reach significance, $F(1,44)=2.72$, $p=.106$. Acute PCP treatment did not affect licks/cluster, $F(1,44)=2.71$, $p=.107$. There was no acute

19A.



19B.

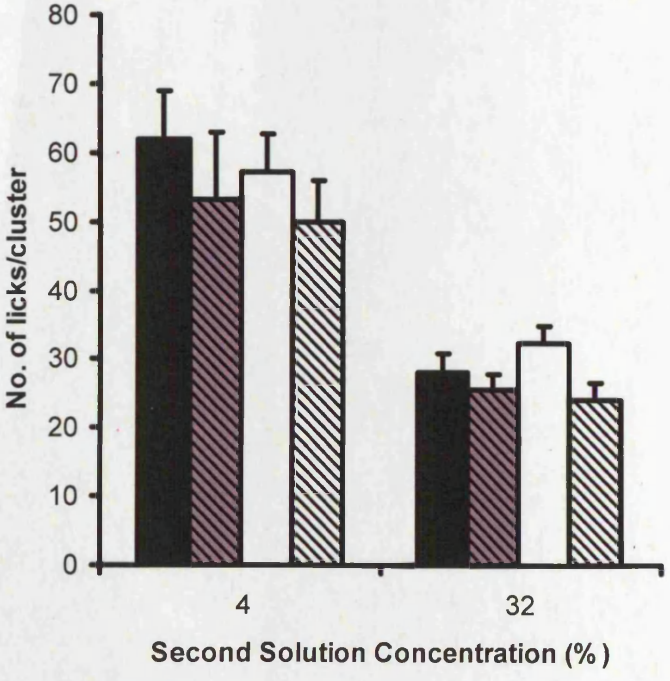
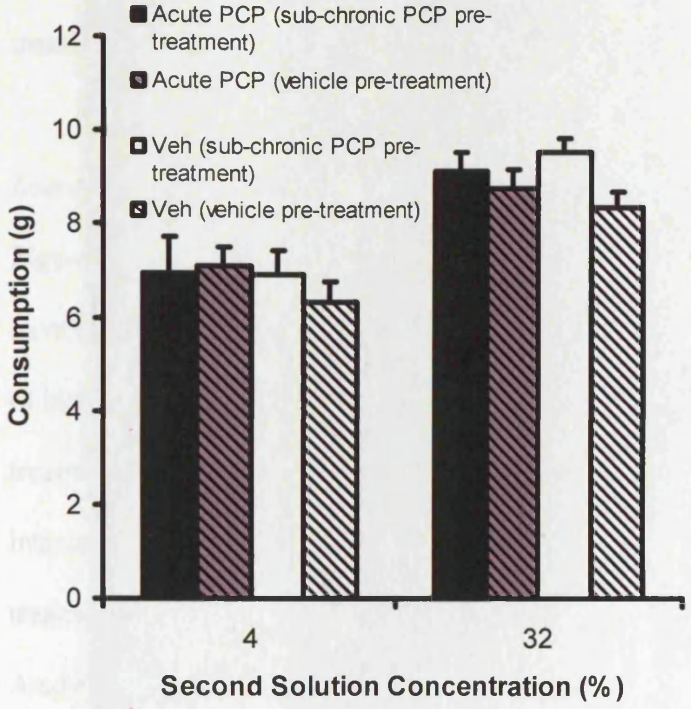


Figure 19: Graphs show the consumption (Panel A) and number of licks/cluster (Panel B), with SEM, following administration of acute PCP and vehicle treatment, for a 4% sucrose solution consistently presented prior to either 4% sucrose or 32% sucrose. Data is shown for sub-chronic PCP pre-treatment and vehicle pre-treatment conditions.

20A.



20B.

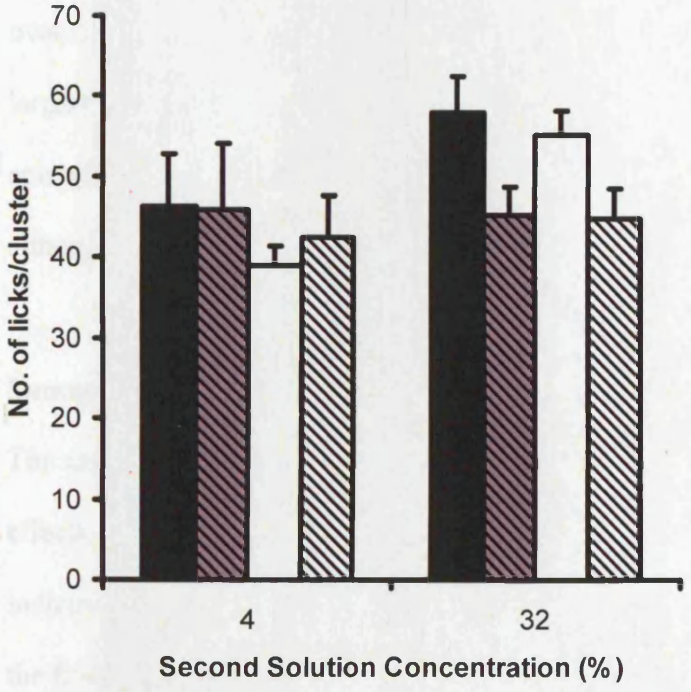


Figure 20: Graphs show the consumption (Panel A) and number of licks/cluster (Panel B), with SEM, following administration of acute PCP and vehicle treatment for 4% and 32% sucrose solutions presented after 4% sucrose. Data is shown for sub-chronic PCP pre-treatment and vehicle pre-treatment conditions.

treatment × solution concentration interaction, no acute treatment × sub-chronic treatment interaction, and no 3-way interaction, all Fs<1.

Locomotor activity testing

Figure 21 shows the activity counts recorded during locomotor activity testing. These have been grouped into 10-min bins across the session. There was a significant effect of bin, $F(8,368)=67.39$, $p<.001$. Although there was no overall effect of PCP treatment, $F(1,46)=2.04$, $p=.160$, there was a significant bin × PCP treatment interaction, $F(8,368)=2.94$, $p=.004$, suggesting that withdrawal from sub-chronic PCP treatment sensitised animals to the effects of amphetamine on locomotor activity. Analysis of simple effects showed that the levels of activity in PCP and vehicle treated animals were similar prior to amphetamine injection (bins 1-3), with PCP-treated animals showing a non-significant decrease in activity relative to controls, largest $F(1,46)=2.22$, $p=.143$. Following amphetamine administration, PCP-treated animals showed numerically higher levels of activity than control animals in all bins, although these differences did not reach significance, largest $F(1,46)=3.83$, $p=.056$.

Summary of Experiment 10

The results of Experiment 10 provide further evidence that anticipatory contrast effects can be seen both in terms of consumption and palatability. Again, this was indicated by the lower consumption and cluster size measurements associated with the first solution presented, when it was consistently followed by a more palatable solution. As in Experiment 9, this contrast effect was not affected by prior sub-chronic PCP treatment. Experiment 10 also showed that, once anticipatory contrast is established, acute PCP treatment has no impact on this effect.

21.

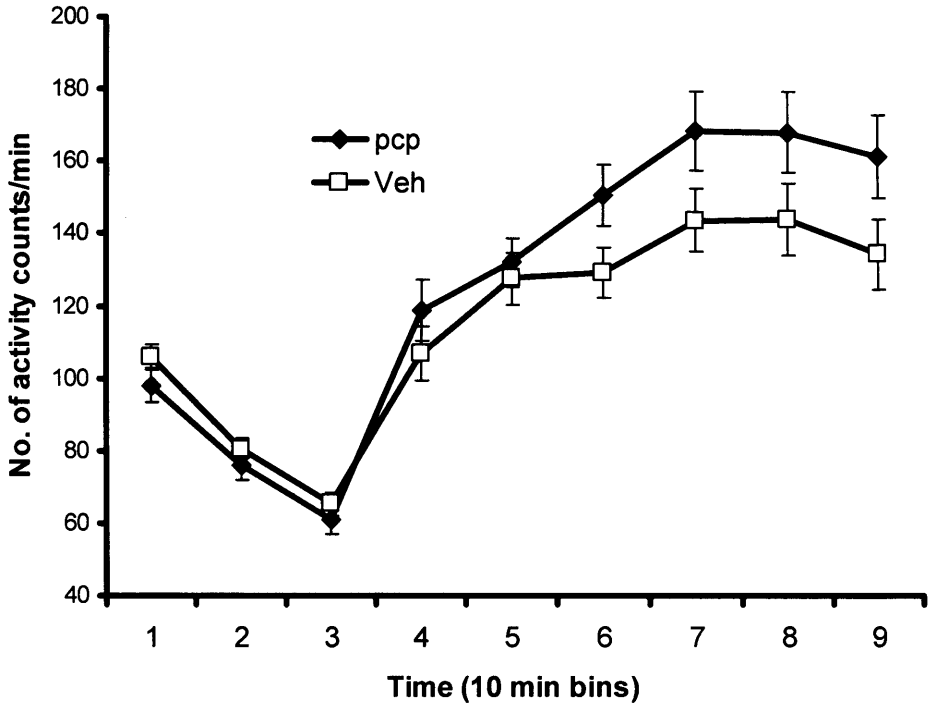


Figure 21: Graph shows the mean number of activity counts (with SEM) made over a 90min session, before and after administration of 1mg/kg amphetamine (given between bins 3 and 4). Counts are grouped into 10min bins. The scale for the No. of activity counts/min extends to 200counts/min because higher activity levels were reached than in the previous locomotor activity test using the same apparatus (Experiment 8).

5.7 Discussion

The primary purpose of the current experiments was to examine the acquisition of a negative anticipatory contrast effect in control and sub-chronic PCP-treated animals. Both Experiment 9 and Experiment 10 showed that the amount consumed of the first solution presented rose less steeply across days when the second solution was 32% than when it was 4%, demonstrating that the perceived hedonic value of the first solution was moderated by the greater value of the second. To my knowledge, there have been no prior reports of detailed lick analysis being used in conjunction with this paradigm. In the current experiments this has revealed a novel finding that the anticipatory contrast effect is also reflected in terms of cluster size, so that the perceived palatability of the first solution is also reduced when it is followed by a solution of greater hedonic value. This suggests that anticipatory contrast produces an effect on perception, such that the hedonic reaction to the first solution is reduced by the expectation of a more palatable alternative.

Interestingly, no overall significant differences were observed between the cluster size of 4% and 32% solutions presented as the second solution in both experiments. Although the experiment had a between-subject design, the difference in concentration between 4% and 32% sucrose is large enough to expect a significant difference in cluster size. The lack of a difference suggests that the differences observed in the consumption and palatability of the first solution may affect how the animals interact with or perceive the second solution. The implications of the anticipatory contrast procedure for responding to the second solution has never been previously considered. However, the current results indicate that this issue warrants further exploration.

Anticipatory contrast effects, both in terms of consumption and palatability, was unaffected by sub-chronic PCP treatment in both Experiments 9 and 10. Following the acquisition phase of Experiment 10, animals were given acute treatment with 1mg/kg PCP. This also produced no changes in the acquired contrast effects. This acute test does not suffer from the motor confound present in my acute tests for consummatory anhedonia (Experiments 3 and 4), in that for anticipatory anhedonia to be evident in this paradigm an animal would need to drink a greater amount of the first solution when treated acutely with PCP. Tests of consummatory anhedonia rely on decreased consumption as a marker of the symptom, but it is difficult to draw conclusions from such an effect as ILI is often increased following acute PCP treatment, raising the possibility that decreased consumption is due to a motor deficit.

The inability to find evidence of anticipatory anhedonia following sub-chronic and acute PCP treatment may highlight a short-coming of PCP treatment as a model of negative symptomology. However, it is also possible that anhedonia may not exist as a primary feature of schizophrenia. (This idea will be addressed in detail in section 7.4 of the general discussion).

Since anticipatory anhedonia does not appear to be a feature of acute and sub-chronic PCP models of schizophrenia, chapter 6 will investigate the presence of motivational deficits.

CHAPTER SIX

Motivation in the sub-chronic PCP and MAM model of schizophrenia

6.1 Introduction

In chapter 4 the suggestion was raised that anhedonia may not be a feature of the sub-chronic and acute PCP models of schizophrenia. If this is the case, this may reflect a failure of PCP models, or it may suggest that anhedonia, when strictly defined as a diminished capacity to experience reward, is not a primary symptom of the disorder. Although diagnostic tools state that anhedonia is a primary symptom of schizophrenia, it is difficult to verify this because it is conceivable that anhedonia may be secondary to other core symptoms such as hallucinations and delusions that are detrimental to the quality of life experienced by sufferers. Avolition (lack of motivation) is a symptom of schizophrenia that appears to be the most closely linked with anhedonia. Indeed, Foussias and Remington (2010) reviewed evidence that measures of anhedonia and avolition are moderately related when assessed using the MATRICS analysis (correlation coefficients between 0.47 and 0.57). The problem of deciphering whether anhedonia is a primary symptom of the disorder is further impacted by the fact that current, frequently used scales of assessment such as SANS, use questions relating to the frequency of engagement in enjoyable activities in order to detect anhedonia (Horan, et al., 2006). Clearly, engagement in recreational activities reflects motivational as well as hedonic factors, and since much of the evidence suggesting that anhedonia is a primary symptom of schizophrenia has utilised such scales, it is possible that motivational deficits are actually the core problem. Evidence from Gard et al. (2007), introduced in section 4.4, is consistent

with this suggestion. In this study the deficits in anticipatory pleasure reported were observed mainly in relation to goal-directed activities (e.g. preparing dinner) rather than non-goal-directed activities (e.g. eating). Schizophrenia sufferers also reported less frequent engagement in such activities. Experiments 9 and 10 showed that PCP does not produce an anticipatory pleasure deficit. However, since Gard et al. found pleasure deficits most commonly occur when rating pleasure associated with goal-directed activities, it is possible that that the apparent pleasure deficits observed by Gard et al. actually reflect a reduction in motivation.

In light of the evidence that anhedonia and avolition may represent different facets of a familiar underlying issue, it is important to assess whether motivational deficits are present in the PCP model, since an abnormal experience of reward may manifest itself in this way.

The current experiments employed a progressive ratio procedure to examine motivation in the sub-chronic PCP model and in an alternative, neurodevelopmental, methylazoxymethanol acetate (MAM) model. In a progressive ratio paradigm the number of lever presses required to obtain reinforcement increases across a session with successive reinforcers. The ratio at which the rat ceases to respond, or the highest ratio obtained within a defined period (the breakpoint) is often used as an index of motivation or reinforcer efficacy. This technique was first used by Hodos (1961) who found that animals achieved a higher breakpoint when the concentration of a palatable liquid reward was increased. Further support for the use of breakpoint measures as an index of motivation has come from findings that breakpoint is influenced by changes in reinforcer magnitude (Skjoldager, Pierre, & Mittleman, 1993), and deprivation state (Ferguson & Paule, 1997). Progressive ratio performance has also been used as a means of assessing the effects of drug treatments on

motivational processes (Zhang, et al., 2005, with typical and atypical antipsychotics). Additionally, it has been used as a tool to discover the brain areas required in motivational processes and the evaluation of reinforcer efficacy (Bezzina, Body, et al., 2008; Bezzina, den Boon, et al., 2008).

Schizophrenia is a disorder in which similar pathology can arise from multiple different etiologies (e.g. environmental and genetic). This is consistent with the fact that a number of methods (e.g. pharmacological and neurodevelopmental manipulation) can be used to produce animal models of schizophrenia. The MAM model relies upon disruption of neurodevelopment by administration of a DNA methylating agent to pregnant rats on gestational day 17 (GD17). At this stage in development MAM exposure affects development of the frontal and temporal cortices, resulting in neuroanatomical and behavioural features resembling those in schizophrenia (see section 1.9). Neuroanatomically, GD17 MAM exposure produces reductions in cortical thickness in the medial pre-frontal cortex and the hippocampus (Flagstad, et al., 2004; Le Pen, et al., 2006). In the human condition, similar reductions in cortical thickness have been found in the pre-frontal cortex and the temporal lobe (Wright, et al., 2000).

The decision to use MAM-exposed animals in addition to the sub-chronic PCP model was based on behavioural evidence reporting that this treatment produces effects analogous to the positive, negative and cognitive symptoms of schizophrenia, which develop during adolescence. For example, it has been suggested that people with schizophrenia experience abnormalities in attending to and filtering relevant sensory information, and that this results in an inability to distinguish self-generated cognitive stimuli from external information, which may underlie aspects of positive symptomatology (see Braff, 1993, for a review). Assessment of latent inhibition is a

technique used to examine attentional processes in animals. Latent inhibition refers to a reduction in the capacity to learn conditioned associations with a stimulus that has been presented previously without any consequence. Flagstad, Glenthoj and Didriksen (2005) found that control rats that received pre-exposure to a conditioning stimulus showed strong latent inhibition, but this was absent in MAM-exposed animals. Gray, Hemsley, & Gray (1992) also showed that latent inhibition is absent in newly-medicated schizophrenia patients, but is restored following chronic antipsychotic treatment. However, Williams et al. (1998) suggest that abolishment of latent inhibition may be an effect of antipsychotic medication that wears off with chronic use.

In addition to the positive symptom profile of MAM-exposed rats, Flagstad et al. (2004) showed that MAM-exposed rats display significantly less social interactions than controls in terms of the amount of time spent in close proximity (less than 20cm) to an unfamiliar rat. They suggested that this effect is analogous to the negative symptom, asociality, although this observation is confounded by the hyperactivity exhibited in MAM-exposed animals. MAM-exposed rats also display deficits in multiple cognitive domains (Featherstone, Rizos, Nobrega, Kapur, & Fletcher, 2007). For example, MAM-exposed animals show deficits in attentional set-shifting, a task in which animals learn an initial rule in order to solve a series of discriminations, but must then shift to using an alternative rule. This shift is analogous to what is required for humans to perform the Wisconsin card-sort test, a task in which people with schizophrenia show impairment (Goldberg, Weinberger, Berman, Pliskin, & Podd, 1987).

It has been suggested that motivational and cognitive deficits in schizophrenia are inextricably linked. Gorissen, Sanz and Schmand (2005) measured mental effort

in schizophrenia sufferers using a word memory test. Participants were also given a selection of cognitive tests. It was found that lower than average scores on the effort test accounted for around one-third of the variance in cognitive test performance. Gorissen et al. proposed that mental effort is a motivational factor, and that their finding highlights the detrimental effect motivational problems can have on cognitive functioning. Since cognitive abnormalities are evident in both the MAM model (Flagstad, et al., 2004) and the sub-chronic PCP model (Dunn & Killcross, 2006) this may further suggest that motivational deficits are likely to be evident in these models.

6.2 Experiment 11

Methods

Subjects

This experiment was carried out at Lilly Research Centre, Surrey. Thirty-two male Wistar rats supplied by Harlan, Bicester, UK, were used. They were housed in groups of four, in individually ventilated cages, on a 12hr/12hr light/dark cycle. Animals were food restricted to no less than 85% of their free-feeding weight, and weighed between 291 and 352 grams at the time of testing. All other husbandry procedures were the same as those reported for experiments carried out at Cardiff School of Psychology. Experimental procedures were carried out Monday-Friday in the light part of the cycle.

Apparatus

Testing was carried out in standard operant chambers (dimensions as reported in section 4.5). These chambers were housed in sound and light attenuation boxes (Med

Associates, US). Two retractable levers were located either side of a recessed magazine where sucrose pellets (Noyes, 45mg, formula P) were delivered from an automatic pellet dispenser. Experimental sessions were controlled and recorded using MedPC IV software.

Drugs

PCP supplied by Sigma-Aldrich UK was used in this experiment. This was made into a 5mg/ml solution using 5% (w/w) glucose. D-Amphetamine sulphate from the same supplier was also dissolved in 5% glucose to obtain a 1mg/ml solution. These concentrations refer to the salt weight of the compounds.

Dosing and testing

Half of the animals were treated sub-chronically with PCP using the same seven-day procedure used in the other sub-chronic experiments reported in this thesis. The other half of the animals received an equal number of 5% glucose vehicle injections. Training began 9 days after the last PCP dose. Rats initially received two 30-min sessions of nose-poke training in which they received pellets on a variable interval (VI 60s) schedule. Following this, rats were assigned a left or right lever and received a fixed ratio 1 (FR1) schedule for two days. Rats then underwent daily training sessions under the progressive ratio schedule using the following exponential progression: 1, 2, 4, 6, 9, 12, 15, 20, 25 32, 40, 50, ⋯.This was developed by Roberts and Richardson (1992), based on the formula $(5\times e^{0.2n})$-5, where n is the position in the ratio sequence. After animals made the required number of presses to be rewarded at a particular ratio, there was a delay of 15 seconds before the lever became operational again. In the first 3 days of training, sessions allowed animals to achieve

the first 10 components of the progression. Following this, animals received sessions in which 20 ratios could be achieved for 6 days.

Locomotor Activity Testing

In addition to the progressive ratio tests, rats received locomotor activity assessments to test for PCP-induced hyperlocomotion in response to amphetamine injection. Half of the animals in both PCP and vehicle treated groups received this testing prior to the progressive ratio tests and half were tested after the completion of these sessions. This testing took place in a completely dark test room. Animals were placed in clear Perspex boxes (40×40×30cm) and their activity was recorded based on infrared fields that spanned 100×100cm. Four boxes were placed on each field and one animal was placed in each box. Overhead infra red cameras (Sanyo VCV-3412P, TrackSys Ltd, UK) tracked the movement of each animal. This was recorded by a Quad compressor unit (Sanyo VDM-801P, TrackSys Ltd, UK) linked to a PC running Ethovision v2.2 Software (Noldus, NL). Animals were initially placed in the boxes for a 30-min habituation period. They were then given 1mg/kg amphetamine sub-cutaneously and their activity was recorded for a further 60-min.

Data Analysis

The data reported is based on the breakpoints achieved. The breakpoint is defined as the last ratio to have been completed by the end of a 45-min session. The analysis of the progressive ratio data was based on an averaged snapshot of two consecutive days. These days were selected as they represented performance towards the end of training on days that fell at the end of a week rather than after a weekend. One rat was removed from the analysis due to failure to acquire the lever-press response.

The measurement of interest for the locomotor activity testing was distance moved (cm). This distance was grouped into 10-min bins and analysed using repeated measures ANOVA with 'bin' as the within-subject factor and 'treatment group' as the between-subject factor.

Results of Experiment 11

Figure 22 shows the progressive ratio breakpoints for sub-chronic PCP-treated and control animals. There was no effect of sub-chronic PCP treatment on the breakpoint achieved, $F<1$. The apparent trend towards increased breakpoint in the PCP-treated animals is not significant and is in the opposite direction to what would be expected if a motivational deficit was present.

Locomotor Activity Testing

Figure 23 shows the distance moved in 10-min bins across the session. There was a significant effect of bin, $F(5,145)=114.82$, $p<.001$. There was no overall effect of PCP treatment, $F<1$. However, there was a significant bin × treatment interaction, $F(5,145)=4.14$, $p=.002$, consistent with a greater increase in locomotor activity in PCP-treated animals relative to controls following amphetamine administration.

Summary of Experiment 11

The analysis of progressive ratio breakpoints in Experiment 11 provided no evidence that sub-chronic PCP treatment produces a motivational deficit, despite the fact that the treatment regimen did produce the expected amphetamine-induced augmented hyperlocomotion that normally occurs following this treatment.

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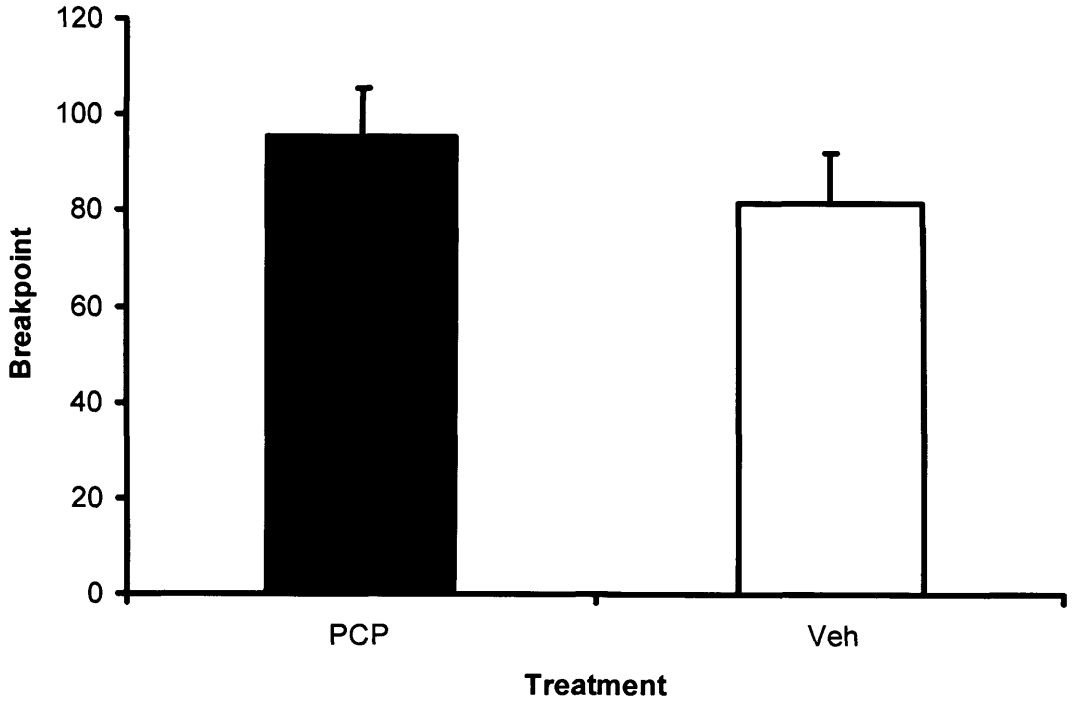

Figure 22: Shows the highest mean response ratios (breakpoints) achieved, with SEM, for animals treated sub-chronically with PCP or vehicle during a progressive ratio schedule.

23.

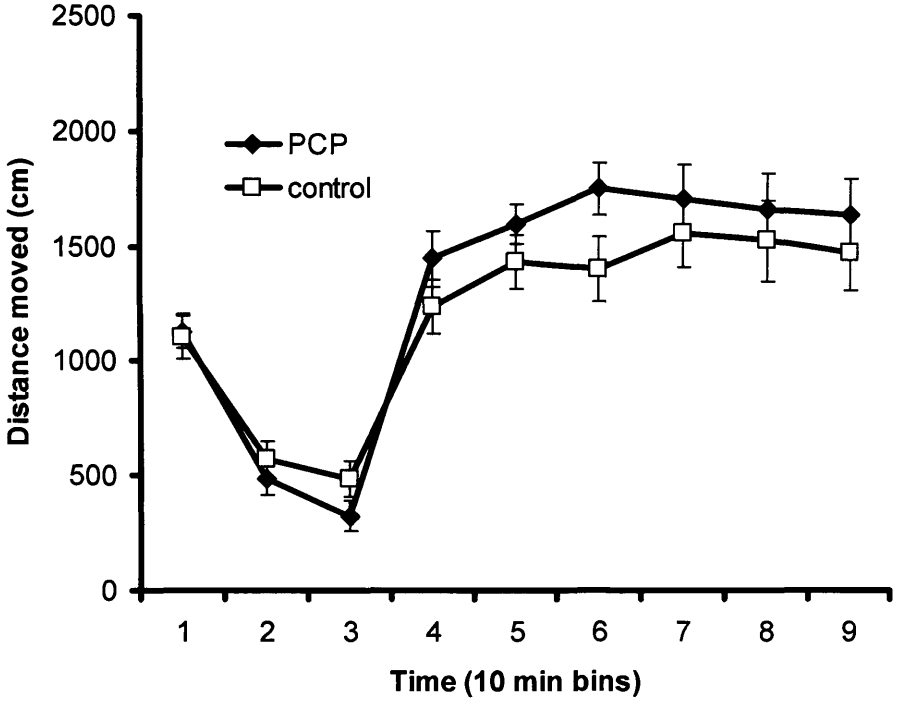

Figure 23: Shows the distance moved by PCP and vehicle-treated rats over a 90min session, before and after administration of 1mg/kg amphetamine (given between bins 3 and 4). Counts are grouped into 10min bins.

6.3 Experiment 12

Thirty-two male Wistar rats were used in this experiment. 16 of these had been exposed pre-natally to methylazoxymethanol acetate (MAM), and 16 were control rats. All rats were bred by in-house at Lilly Research Centre. MAM-exposed rats were generated by injecting pregnant dams intraperitoneally with 28mg/kg MAM on gestational day 17. MAM was supplied by Midwest Research Institute, Kansas City, Missouri. It was formulated using 0.9% saline. Control rats were generated by injecting pregnant dams with saline on gestational day 17.

The offspring were 5 months old and weighed between 327 and 560 grams when testing commenced. They had received no prior operant training. General husbandry procedures were the same as those for Experiment 11.

Apparatus and procedure

This experiment was run in parallel with Experiment 11. The apparatus and procedural details were the same except that 0.1mg/kg (+)MK-801 was used for locomotor activity testing instead of amphetamine. The decision to use this drug was based on the experience of researchers at Lilly Research Centre who had found that (+)MK-801-induced augmented hyperlocomotion provided a reliable positive control for MAM treatment effects. The data were analysed in the same way as Experiment 11.

Results of Experiment 12

Figure 24 shows the mean breakpoints for the progressive ratio experiment. The MAM-exposed rats showed a decrease in breakpoint, consistent with a reduced motivation to work for the reinforcer, $F(1,29)=44.63$, $p<.043$.

Locomotor Activity Testing

Figure 25 shows the distance animals moved grouped into 10-min bins across the session. There was a significant effect of bin, $F(5,145)=22.31$, $p<.001$. MAM-treated rats showed higher levels of activity overall, $F(1,29)=13.79$, $p=.001$, and there was a bin × treatment group interaction indicating that activity levels were augmented in the MAM-exposed rats following (+)MK-801 treatment, $F(5,145)=2.30$, $p=.048$.

Summary of Experiment 12

Experiment 12 showed that breakpoints are lowered in MAM-exposed animals, an effect that may be consistent with decreased motivation. Locomotor activity testing confirmed that the MAM treatment was able to produce augmented hyperlocomotion in response to (+)MK-801, an effect that would be expected after this treatment.

6.4 Discussion

The current set of experiments used analysis of progressive ratio breakpoints as an index of motivational deficits in the sub-chronic PCP and neurodevelopmental MAM model of schizophrenia. Experiment 11 showed that sub-chronic PCP treatment produced no significant effect on progressive ratio breakpoints. In fact, there was a numerical, but not significant, trend towards an increase in breakpoint in the PCP-treated animals. This trend is in the opposite direction to what would be expected if a motivational deficit was present. Therefore, Experiment 11 provided no evidence that a motivational deficit exists in the sub-chronic PCP model of schizophrenia. This cannot be attributed to problems with the dosing regimen since PCP-treated animals

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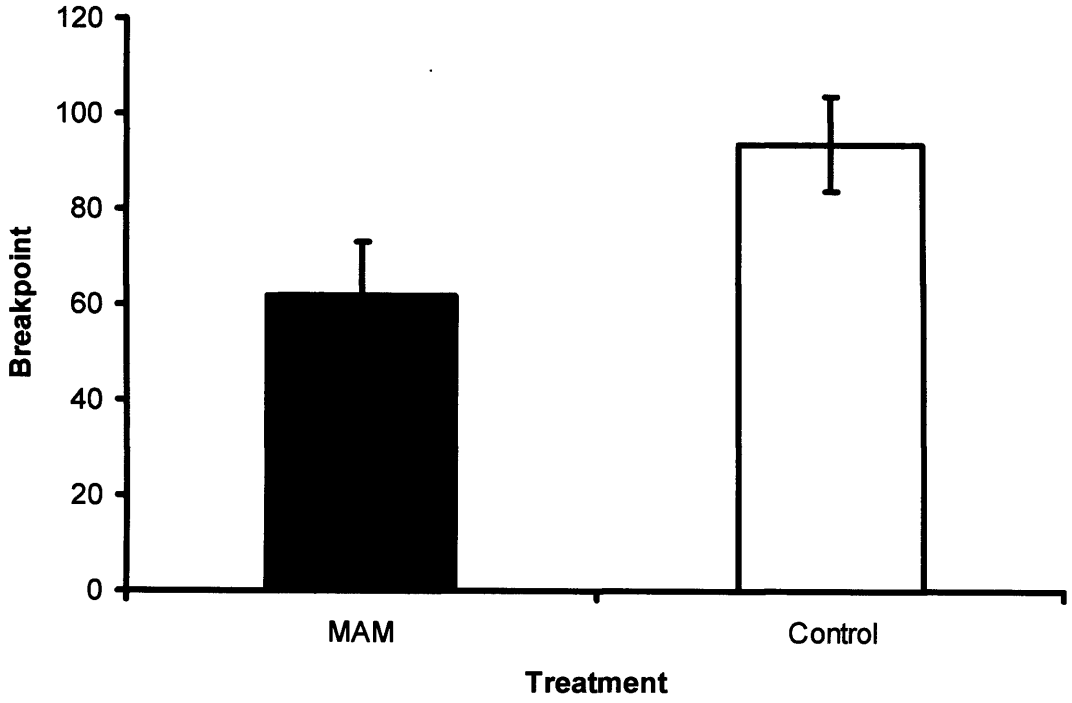

Figure 24: Shows the highest mean response ratios (breakpoints) achieved, with SEM, for animals exposed pre-natally to MAM or vehicle, during a progressive ratio schedule.

25.

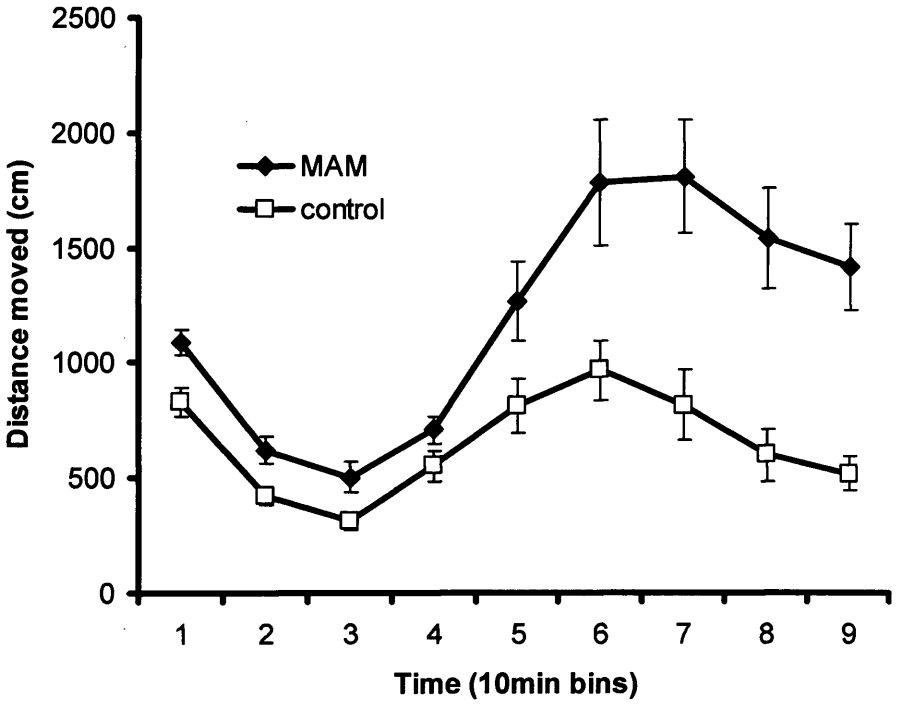

Figure 25: Shows the distance moved by MAM and control rats over a 90min session, before and after administration of 1mg/kg amphetamine (given between bins 3 and 4). Counts are grouped into 10min bins.

showed augmented hyperlocomotion in response to acute amphetamine challenge, an effect that would be expected in these animals.

When considered in conjunction with the data presented in the previous chapters of this thesis, it can be concluded that there is no evidence for hedonic deficits in the sub-chronic PCP model of schizophrenia in terms of consummatory or anticipatory pleasure, or in terms of motivation. The implications of these findings will be discussed in detail in section 7.3 of the general discussion.

Interestingly, Experiment 12 demonstrated that motivation, as indexed by breakpoint, is reduced in MAM-exposed animals. Reduced motor activity cannot account for this effect since locomotor activity testing showed that these animals are hyperactive relative to controls. In addition, there appeared to be no problems with the treatment regimen since MAM-exposed animals showed augmented hyperlocomotion in response to (+)MK-801 treatment, a well documented effect following this treatment. The reduction in breakpoints could, however, represent a satiety confound whereby MAM-treated animals were sated more readily than controls. This cannot be equated to a genuine deficit in the experience of reward since the MAM-exposed animals would effectively experience an effect which would be likened to a milder state of food deprivation relative to controls. The idea that a satiety confound may exist in MAM-exposed rats is consistent with the finding that MAM exposure has been found to cause a 13% decrease in the body weight of rats when they reach adulthood (Greiner, et al., 1992).

A confound of effort may also be present. In chapter 2 it was reported that the palatability of a reinforcer increases with effort expended. That is, an interaction may be observed between the effort expended and reward value, whereby lever pressing makes a reinforcer more palatable. Animals will then be more inclined to press the

lever to gain access to this enhanced reinforcer. This positive feedback may be interfered with by factors that influence initial lever pressing, such as motor problems or a reduction in the initial palatability of the reward. Therefore, if the MAM-treated animals were initially less inclined to press the lever, perhaps because they experienced the reward as less palatable, or because the rats were not as hungry as controls, these animals would not experience the same increase in perceived palatability experienced by controls due to lever pressing. This may enhance the difference in breakpoint between MAM-treated animals and controls, without there being a genuine deficit in motivational processing in the MAM model.

It is indeed possible that the reduced breakpoint observed in MAM-treated animals reflects the negative symptom of avolition seen in human schizophrenia. Lick analysis experiments such as those reported in previous chapters would enable further characterisation of the deficit observed in this experiment and would determine whether MAM-exposed animals experience a reduction in their hedonic capacity. However, since avolition is suggested to be closely linked to schizophrenic anhedonia, the discovery of reduced breakpoints in these animals may indicate that this model has potential to provide an analogue of anhedonia.

CHAPTER SEVEN

7.1 Summary of experimental results

Experiments 1 and 2 combined analysis of licking with schedules of operant responding. It was initially thought that since operant responding is already used as an indicator of motivation in animal assays, its combination with lick analysis would provide a more accurate measure of hedonic value. It was established that rats will produce a lever press response to gain access to a liquid reinforcer. These experiments also demonstrated that when the response required to obtain reinforcement is high, the palatability of the reinforcer is greater than when the response requirement is low. This was discovered by comparing the palatability of a reinforcer when 10 lever presses were required with the palatability of the same reinforcer when 50 lever presses were required. Furthermore, Experiment 2 used a yoking paradigm whereby some rats pressed a lever to obtain the reinforcer, whilst corresponding animals simply waited the same length of time. This showed that although it took longer for a rat to press a lever 50 times than 10 times, this difference in waiting time did not increase the palatability of a reinforcer as much as the requirement to work for the reinforcer.

Experiments 3, 4 and 5 assessed the effects of acute NMDA receptor antagonist treatment on the voluntary consumption and palatability of sucrose solutions. Experiment 3 showed that single intermediate doses of the NMDA receptor antagonists PCP and s-(+)-ketamine produced a decrease in cluster size, but this only occurred in the presence of motor abnormality indicated by raised ILI. (+)MK-801 produced no effect on cluster size or consumption. In order to assess whether a

reduction in consumption or cluster size is present at doses low enough to avoid motor abnormality, PCP was selected for a dose response experiment (Experiment 4a). Since (+)MK-801 is often used interchangeably with PCP in pre-clinical modelling, a dose response experiment was also carried out with this drug (Experiment 4b). There was evidence of reduced consumption and cluster size with both drugs, but again, this only occurred when ILI was increased.

Experiment 5 investigated whether evidence of reduced consumption and cluster size could be found at lower sucrose concentrations than those examined in Experiments 3 and 4. It was found that even at low sucrose concentrations, there was no evidence of a PCP-induced reduction in cluster size occurring independently of motor abnormality. The consumption and palatability of low sucrose concentrations were unaffected by (+)MK-801 treatment. Since no reduction in palatability was observed, Experiments 3, 4 and 5 provided no evidence of anhedonia following acute NMDA receptor antagonist treatment.

Experiments 7 and 8 compared the effects of the typical and atypical antipsychotics clozapine and haloperidol using lick analysis and sucrose preference testing. Clozapine did not affect sucrose intake, palatability, or preference for sucrose over water. Haloperidol, on the other hand, decreased both sucrose intake and lick cluster size. The ratio of sucrose over water intake was also decreased in haloperidol-treated animals relative to controls. Thus current drug treatments for schizophrenia have direct effects on consumption independent of their antipsychotic action.

Experiment 8 examined the effects of sub-chronic PCP treatment on the consumption and palatability of sucrose. As with acute PCP treatment, sub-chronic treatment produced no effect on consumption or cluster size, thus providing no evidence of consummatory anhedonia. Experiments 9 and 10 assessed negative

anticipatory contrast following sub-chronic PCP treatment. Negative anticipatory contrast occurs when rats receive brief, sequential access to two solutions, once each day. Repeated pairing of the two solutions allows animals to anticipate what the second solution will be and this affects their intake of the first solution. If the second solution is preferred over the first, intake of the first solution is suppressed. In both experiments anticipatory contrast effects could be seen, both in terms of overall consumption, and in terms of palatability. This contrast effect was unaffected by prior sub-chronic PCP treatment. Furthermore, Experiment 10 showed that after the anticipatory contrast effect was acquired, it was not affected by acute PCP challenge. Therefore, Experiments 9 and 10 provided no evidence of anticipatory anhedonia. These experiments also provided additional evidence for the absence of consummatory anhedonia since there was no overall effect of sub-chronic PCP treatment.

Experiment 11 used analysis of progressive ratio performance to assess whether motivational deficits are present following sub-chronic PCP treatment. PCP treatment was found to have no affect on the breakpoint (highest response ratio achieved) in this context. However, Experiment 12 investigated the same paradigm in the developmental MAM model of schizophrenia, and found that breakpoint was reduced. This suggests that MAM exposure may provide an analogue of avolition, but this is not the case with PCP treatment.

7.2 Novel effects arising from the use of microstructural analysis of licking

The reliability of the lick analysis method in the measurement of hedonic value has been affirmed by the experiments contained in this thesis. As detailed in the introduction, many authors have reported that during ingestion the number of licks

per cluster (cluster size) has a positive, monotonic relationship with the concentration of a palatable solution such as sucrose (e.g. Davis & Smith, 1992; Davis & Perez, 1993; Hsiao & Fan, 1993; Spector et al. 1998). Similarly, in Experiments 3, 5, 9 and 10, where rats were exposed to more than one concentration of sucrose, cluster size was consistently largest for the highest concentration tested.

The use of lick analysis in the experiments reported has given rise to some very interesting effects demonstrating novel ways in which the palatability of a solution can be changed without altering the concentration itself. For example, Experiments 1 and 2 showed that when rats are required to expend effort, the palatability of a reinforcer is increased. The effort involved in the lever-press response requirement employed in Experiments 1 and 2 has two obvious components: physical work and waiting time, because it takes more work and more time to press a lever 50 times than 10 times. However, Experiment 2 showed that physical work had effects on palatability that were greater than simply imposing a delay prior to reinforcement. This effect could not be accounted for by a physiological compensatory mechanism to alleviate thirst or energy expenditure since the amount consumed during each presentation of the reinforcer was not increased by raising the effort requirement. This discovery has provided the first direct analogue of the human effort justification phenomenon in animals. The fact that the explanation of this effect need not require the use of complex cognitive theories is a useful reminder that scientists should avoid resorting to such theories without direct evidence if simpler alternatives may suffice.

In addition to its implications for the analysis of 'effort' effects per se, the use of lick analysis in combination with operant schedules has also demonstrated an important consideration to be borne in mind when interpreting data from operant

schedules in terms of hedonic and motivational effects. The results of these experiments suggest that an interaction may be observed between the effort expended and reward value, whereby lever pressing makes a reinforcer more palatable. Animals will then be more inclined to press the lever to gain access to the reinforcer. This positive feedback may be interfered with by factors that influence initial lever pressing, such as motor problems or a reduction in the initial palatability of the reward. This means that effects such as reduced breakpoint, which are normally considered to be indicative of reduced motivation, may actually reflect other factors unrelated to motivation. It is possible that this problem can be circumvented by using a reinforcer that is not very palatable in the first instance. However, this confound should not be ignored.

The use of lick analysis in Experiments 9 and 10 has also given new insight into the mechanisms underlying the effect known as anticipatory contrast. Several authors and, in particular, Flaherty (1996) have noted that the consumption of a solution is attenuated when it precedes access to a solution that has greater hedonic value. Experiments 9 and 10 have revealed, for the first time, that the effects of reward anticipation on consumption are also reflected in terms of palatability, such that the palatability of the same solution is lower when that solution precedes a more palatable solution than when it precedes a solution of the same hedonic value. This suggests that, as with simultaneous contrast (Dwyer, Lydall & Hayward, in press), anticipatory contrast appears to reflect a change in the sensory experience of the reward.

The novel findings produced via the employment of lick analysis has demonstrated the potential of this method to provide valuable new insight into the mechanisms underlying long-established behavioural effects. The two novel effects

discussed above emphasise that fact that the palatability of a reinforcer can be changed without changing the concentration of the reinforcer itself. This confirms that measurements of palatability made using lick analysis are not solely determined by the properties of a solution, but by an interaction between the solution, the state of an individual animal, and the context of consumption. This reaffirms the expectation that measurements made using lick analysis should be sensitive to any changes in the hedonic capacity of an animal, such as those that might have been brought about by NMDA receptor antagonist treatment.

7.3 Implications of thesis experiments for the modelling of anhedonia

As previously noted, anhedonia may take two forms. The first of these is consummatory anhedonia, characterised by a deficit in in-the-moment pleasure. The presence of consummatory anhedonia in rodents was assessed following acute treatment with PCP, s-(+)-ketamine and (+)MK-801 (Experiments 3, 4 and 5), and following sub-chronic PCP treatment (Experiments 8, 9 and 10). Although acute NMDA receptor antagonist treatment decreased consumption and lick cluster size at some doses, this should not be interpreted as a hedonic deficit because such decreases were always accompanied by motor abnormality. This highlights a critical flaw in the assumption made by many authors, that reduced consumption alone indicates the presence of anhedonia (e.g. Papp & Moryl, 1994; Papp, et al., 1991; Papp & Moryl, 1994; Rygula, et al., 2005; Willner, et al., 1994; Zurita, et al., 2000; Zurita & Molina, 1999). It also suggests that the interpretation of reduced consumption following large doses of PCP treatment as an analogue of anhedonia (Turgeon & Hoge, 2003) is unsupported.

Although sub-chronic PCP treatment is associated with pathological changes that more closely resemble those that occur in schizophrenia than is acute PCP treatment (e.g. Jentsch & Roth, 1999), sub-chronic administration produced no effect on the hedonic value of sucrose rewards in terms of either consumption or palatability (Experiments 8, 9 and 10). Therefore, no evidence of consummatory anhedonia was found following either acute NMDA receptor antagonist treatment or sub-chronic PCP treatment.

As discussed in chapter 5, there have been suggestions that anhedonia is actually anticipatory in nature. That is, people with schizophrenia experience a reduced capacity to anticipate the pleasure associated with future rewards. However, acquisition of an anticipatory contrast effect was unaffected by sub-chronic PCP treatment. Once the effect was acquired, it was also unaffected by acute PCP challenge. This indicates that anticipatory anhedonia is also not a feature of PCP models of schizophrenia.

It has also been argued that anhedonia is closely linked to motivational deficits in schizophrenia (Foussias & Remington, 2010). However, no evidence of a reduction in motivation was found using progressive ratio analysis in animals treated sub-chronically with PCP. There is therefore no evidence that acute NMDA receptor antagonist or sub-chronic PCP treatment is able to produce any effect that could be considered as an analogue of a hedonic deficit, since there was no consummatory, anticipatory or motivational deficit present in these models.

The decision to use NMDA receptor antagonists in an attempt to identify an analogue of anhedonia was based upon the apparent plausibility of the glutamate hypothesis of schizophrenia, suggesting that N-methyl-D-aspartate (NMDA) (glutamate) receptor hypofunction may underlie the positive, negative and cognitive

symptoms of the disorder (see section 1.4). In humans, NMDA receptor antagonists such as phencyclidine (PCP) and s-(+)-ketamine produce a psychotic state that is more reminiscent of schizophrenia than dopaminergic psychostimulants, such as amphetamine, since negative and cognitive symptoms are also induced (e.g. Javitt & Zukin, 1991; Lahti et al. 2001). The suggestion that the glutamate hypothesis may account for negative symptoms as well as positive symptoms is supported by animal studies showing that acute NMDA receptor antagonist treatment produces other non-psychotic symptoms of schizophrenia, such as increased stereotyped behaviours (Verma and Moghaddam, 1996) and cognitive deficits (Dunn and Killcross, 2006) (see section 1.9). Likewise, sub-chronic PCP treatment is known to produce cognitive deficits (e.g. Cosgrove et al, 1991; Dunn and Killcross, 2006) and also produces pathological changes likened to those in schizophrenia, such as hypofrontality relating to the pre-frontal cortex (see section 1.9). In addition, NMDA antagonists such as PCP are able to produce contrasting effects on dopamine in mesolimbic and mesocortical brain systems. This is thought to be necessary for the production of the whole range of schizophrenic symptoms (Weinberger, 1987). It was therefore expected that NMDA receptor antagonist treatment, and in particular, sub-chronic PCP treatment would provide an 'all-round' model of schizophrenia, as suggested by Morris et al. (2005).

The absence of hedonic deficits following both acute NMDA receptor antagonist treatment and sub-chronic PCP treatment is therefore somewhat suprising, and it seems to suggest one of two possibilities concerning the validity of the glutamate hypothesis. Firstly, it is possible that the glutamate hypothesis provides an accurate account of the mechanisms underlying schizophrenia, but that NMDA antagonists such as PCP are unable to fully reproduce all aspects of brain function

relevant to the hypothesis. Alternatively, it is possible that the glutamate hypothesis is incomplete and is only able to account for the pathology underlying positive and cognitive deficits.

To assert both that the glutamate hypothesis is accurate and that PCP treatment provides a complete model of schizophrenia in animals would raise the controversial idea that anhedonia may not exist as a primary symptom of schizophrenia. This controversial line of thought has received support from Trémeau, Antonius, Ziwich, Butler, Cacioppo and Javitt (2008). Controls and individuals with schizophrenia were presented with series of emotionally evocative pictures and sounds and asked to rate how positive and negative they felt following each stimulus. Prior to each series of stimuli, participants evaluated the degree of pleasure they anticipated from each sub-task. Following the task, in order to rate motivation, participants were asked how willing they would be to repeat the task. Participants with schizophrenia did not differ from controls in terms of anticipatory and in-the-moment pleasure. They also showed greater remembered pleasure than controls. However, they displayed lower correlation between affective and motivational measures. Trémeau et al. have therefore suggested that the reward system deficit seen in schizophrenia lies not in the ability to experience pleasure, but in the ability to translate pleasure into motivational processes.

Foussias and Remington (2010) have reviewed evidence that they believe argues against anhedonia as a core symptom of schizophrenia. They suggest that the apparent anticipatory pleasure deficit in schizophrenia observed by Gard et al. (2006) is closely related to motivation and goal-directed behaviour. They argue that since worse functional outcomes are reported in individuals with prominent negative symptoms, amotivation is likely to be the main contributor in this relationship.

Although they acknowledge that cognitive dysfunction contributes to functional outcome in schizophrenia, there is a strong influence of motivation over cognitive functioning (Gorissen, Sanz & Scmand, 2005). They therefore proposed a model in which all negative symptoms are conceptualised as avolition (lack of motivation). They suggest that other symptoms, such as attentional impairment, inappropriate affect, and poverty of speech content, traditionally believed to be negative symptoms, are more closely related to the cognitive or disorganised symptoms of schizophrenia. They argue that since research has failed to find evidence of diminished emotional experience, despite finding deficits in emotional expression, anhedonia is not a core symptom of schizophrenia, but is related to the engagement of motivational processes which control goal-directed behaviour. They consequently suggest that amotivation is a core negative symptom and that loss of motivation is phenotypically expressed in terms of asociality and alogia, observed clinically as increased social withdrawal. Blunted affect parallels a loss of motivation, but they argue that this is distinguishable from hedonic capacity, which remains intact in schizophrenia, even though this is not complimented by an individual's premorbid capacity for goal-seeking. In this way, avolition is proposed to directly lead to functional decline in schizophrenia. It also plays an indirect role via its influence on cognitive dysfunction.

The progressive ratio paradigm used in Experiment 11 showed no evidence of a motivational deficit following sub-chronic PCP treatment and therefore does not add any support to the suggestion of Foussias and Remington (2010). However, it is possible that all negative symptoms, including motivational and social deficits, may occur secondary to the cognitive deficits observed in schizophrenia. The data presented in this thesis cannot help to clarify this possibility since hedonic measurements were not made in conjunction with cognitively demanding tasks.

However, the suggestion that negative symptoms may occur secondary to cognitive deficits is supported by the striking lack of evidence for negative symptoms in animals following PCP treatment. Indeed, it has been reported that animals treated sub-chronically with PCP show reduced social interaction with unfamiliar rats (Bruins Slot, Kleven, & Newman-Tancredi, 2005; Sams-Dodd, 1998). However, these deficits were observed less than one hour after the last PCP dose. Although Snigdha and Neill (2008) found social interaction deficits in terms of following, sniffing and climbing over unfamiliar rats, 6 weeks after sub-chronic PCP administration in female rats, other studies have not identified social deficits after an extended period of PCP withdrawal (Egerton, et al., 2008; Sams-Dodd, 2004). Indeed, Jenkins et al. (2008) found that sub-chronic PCP treatment actually increased the amount of time rats engaged in social interaction, thus demonstrating that findings in this regard do not provide conclusive evidence for a reduction in social interaction following sub-chronic PCP treatment.

Findings from Heerey, Bell-Warren and Gold (2008) also suggest that reward experience may be intact in schizophrenia and that cognitive problems may be implicated in what appears to be anhedonia. In this study, reward sensitivity was found to be normal when measured using a signal detection task in which participants develop a bias when choosing between two stimuli, one of which is rewarded more often than the other. However, participants were also given a decision-making task in which they received a choice between two gambles, along with information on the reward magnitude, probability of winning, and potential gains and losses. In this task, participants with schizophrenia were less able to weigh up potential outcomes when making their choice. Heerey et al. concluded that people with schizophrenia have reduced ability to utilise working memory in order to draw on their experience of

reward when making decisions. If this is the case, motivational deficits may exist in schizophrenia because the potential rewarding outcomes of activities are not immediately obvious to the individual, rather than because they have reduced capacity to experience pleasure. If this line of thought is accurate, it may explain the absence of hedonic deficits following PCP treatment, even though cognitive deficits are present. However, the lack of a direct correlation between the presence of negative symptoms and positive symptoms following PCP treatment might question the idea that negative symptoms are secondary to other symptoms in schizophrenia.

Using animal models it may not possible to draw a firm conclusion as to whether anhedonia exists as a core feature of schizophrenia. However, further investigation of the MAM model, which showed some evidence of motivational deficits, may go some way towards clarifying this issue. Tests of hedonic capacity such as those carried out with NMDA receptor antagonists in this thesis would identify whether a deficit in the ability to experience pleasure is present. If further testing confirmed that the motivational deficit reported in Experiment 12 is genuine and not a product of altered satiety mechanisms or confounding effects of effort, the presence of a hedonic deficit would support the conclusion that anhedonia is indeed a core symptom of schizophrenia. However, if hedonic deficits were absent but motivational deficits present, this would support the possibility that anhedonia may not exist as a primary symptom in schizophrenia, but that diagnostic scales of assessment actually detect a motivational deficit rather than a hedonic one.

7.4 Implications of thesis findings for drug discovery

The data presented in this thesis have shown that antipsychotic drugs can independently alter hedonic capacity in animals. For this reason, if further

investigations show that anhedonia can be modelled in animals, potential antipsychotic drugs will need to be screened for their effects on hedonic capacity in normal animals prior to testing for their ability to ameliorate anhedonia.

The experiments described in this thesis sought to find an animal analogue of anhedonia. This was deemed important because anhedonia is purported to be a core negative symptom of schizophrenia, and negative symptom severity is associated with poor functional outcome in schizophrenia patients (Ho et al, 2004) (see section 1.1). In addition, current antipsychotic treatments are ineffective in improving negative symptoms, which may account for the great lack of adherence to medication in this disorder (see section 1.8).

The finding that acute NMDA receptor antagonist and sub-chronic PCP treatment does not produce anhedonia in rodents has raised a question as to whether anhedonia does actually exist as a primary symptom of schizophrenia. Further efforts to establish a model of anhedonia should seek to resolve this question. For this reason, tests of hedonic value, such as those described in this thesis with lick analysis, should be carried out with other models of schizophrenia (e.g. the MAM model). If no evidence of hedonic deficits can be found in other models, this may suggest that anhedonia is not a primary symptom as initially suspected, but may be secondary to other symptoms, such as cognitive deficits. If anhedonia is secondary to cognitive deficits, this may be difficult to detect as it would require examination of hedonic capacity during a cognitive task that animal models of schizophrenia experience problems with. The additional confound of effort increasing reward value will present a further obstacle in this endeavour.

As discussed, investigation of hedonic capacity in other models of schizophrenia may elucidate whether anhedonia is in fact a core symptom. The

suggestion that motivational deficits occur in the MAM model raises the possibility that models replicating the developmental aspects of the disorder offer greater potential for the modelling of negative symptoms.

It can, however, be concluded that NMDA receptor antagonist treatment cannot be used to model anhedonia in rodents. It is also now clear that reduced voluntary sucrose consumption, used previously by numerous authors as an indicator of anhedonia, is not an appropriate method by which to assess the presence of the symptom and that analysis of licking provides a superior method.

In short, the examination of hedonic reactions via analysis of licking microstructure has revealed novel behavioural effects whereby the affective value of sucrose is influenced by the context of ingestion. However, despite their plausibility, NMDA receptor antagonist-based models of schizophrenia show no evidence of producing an analogue of anhedonia when assessed using the same methods. Thus attempts to model such symptoms should focus on other models of schizophrenia, such as the neurodevelopmental MAM model.

APPENDIX 1

Sucrose preference test and acute PCP

This experiment used the same subjects, apparatus and procedures as Experiment 6. In brief, following mild water-restriction, animals were given 20min access to both 1% sucrose and water. 30 minutes prior to this experimental session an acute sub-cutaneous dose of PCP (1mg/kg) was administered to half of the animals, whilst the other half received vehicle. After a 2-day washout period, the drug-treatment conditions were reversed.

For this experiment, the parameter of interest was the amount of water and sucrose consumed. The data was analysed in the same way as for Experiment 6. This involved analysing the consumption of both solutions using ANOVA, in addition to calculating sucrose/water preference ratios and comparing the ratios for each treatment group using a paired samples t test.

Results

Table 2 shows the consumption of sucrose and water in the PCP and vehicle treatment conditions. Overall consumption of sucrose was higher than consumption of water following PCP treatment, $F(1,23)=51.28$, $p<.001$. PCP treatment did not affect overall consumption, and there was no drug treatment × reinforcer interaction, Fs<1. There was a significant difference between the sucrose preference ratios for vehicle treatment (M=.8804, SD=.14439) and PCP treatment (M=.7345, SD=.14439) conditions; $t=3.12$, $p=.001$.

Table 2:

	Water Consumption (g)	1% Sucrose Consumption (g)
1mg/kg PCP	Mean= 0.5 SEM= .116	Mean= 3.5 SEM= .590
Vehicle	Mean= 0.3 SEM= .034	Mean= 4.2 SEM= .548

Conclusion

The results of this experiment suggest that although there was no effect of PCP treatment on the overall consumption of water and sucrose. However, when the ratio of sucrose consumption relative to water was examined, it was found that controls drink slightly more sucrose relative to water than controls. This raises the possibility that PCP treatment may reduce an animal's preference for sucrose when presented alongside water following water restriction.

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