# AN INVESTIGATION OF THE NEUROBIOLOGICAL MECHANISMS INVOLVED IN THE CONTROL OF OPERANT BEHAVIOUR BY FOOD REINFORCERS: QUANTITATIVE STUDIES USING THE PROGRESSIVE-RATIO SCHEDULE

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## Abstract

This thesis describes a series of experiments investigating the neural underpinnings of the 'efficacy' or 'value' of food reinforcers in the control of operant behaviour. A number of methods have been devised for measuring reinforcer value. The experiments described in this thesis employed the progressive-ratio schedule, in which the number of responses required to obtain a reinforcer is progressively increased with each successive reinforcer. The performance of rats on this schedule was analysed using a quantitative model of schedule-controlled behaviour, Killeen's (1994) 'Mathematical Principles of Reinforcement' (MPR) model. An advantage of this approach is that MPR provides a theoretical basis for discriminating between the effects of interventions on 'motivational' and 'motor' aspects of operant behaviour. According to MPR, schedule-controlled behaviour may be characterized by an 'activation' parameter, *a*, which measures the reinforcer efficacy or value, a 'response time' parameter, $\delta$, which measures the minimum inter-response time, and a 'coupling' parameter' $\beta$ which expresses the weight in short-term memory assigned to the most recent response.

Chapter 1 reviews the background literature related to the main themes of the project: the neurobiology and behavioural functions of the orexinergic and the dopaminergic systems of the brain, and the use of the progressive-ratio schedule in behavioural neuroscience. Special emphasis is given to MPR and its application to behavioural neuroscience.

Experiment 1 (Chapter 2) examined the effect of destruction of orexinergic neurones of the lateral hypothalamic area (LHA), which have been proposed to control reward processes and food intake. Orexinergic neurones were destroyed by intracerebral injection of a selective neurotoxin, the orexin-B-saporin conjugate (OxSap). OxSap-induced lesions had no effect on the parameter *a* and did not alter food intake. However, they did increase the response time parameter $\delta$, suggesting that the lesion had a motor debilitating effect.

Experiment 2 (Chapter 3) investigated the effect of disconnecting the LHA from the ventral tegmental area (VTA), a major area of projection of the orexinergic neurones. Functional disconnection was achieved by unilateral injection of OxSap into the LHA on one side and into the VTA on the contralateral side of the brain. The lesion had no effect

on *a* or any other of the motivational measures used, or on food intake. However $\delta$ was increased, suggesting that the lesion mainly affected motor functioning.

Since OxSap has a preferential destructive effect on neurones that express the orexin-2 (OX2) receptor, the possibility was considered that the putative role of orexins in regulating reinforcer value may be mediated by orexin-1 (OX1) receptors, rather than OX2 receptors. In order to explore this possibility, Experiment 3 (Chapter 4) examined the effect of acute functional disconnection of the LHA from the nucleus accumbens shell (AcbS), an area rich in OX1 receptors. Disconnection was achieved by unilateral OxSap-induced lesions of the LHA and infusion of the OX1 receptor antagonist, SB-334867-A (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl-urea hydrochloride) into the contralateral AcbS via indwelling intracerebral cannulae. The results showed a reduction of the activation parameter *a*, with no effect on any of the other parameters. These findings are consistent with the notion that OX1 receptors are involved in the control of the reinforcer value, whereas the OX2 receptors are more involved in the control of motor-related processes.

Experiment 4 (Chapter 5) examined the effect of cyproheptadine, a drug with 5-hydroxytryptamine ($5HT_{2A}$) and histamine ($H_1$) receptor blocking action. Cyproheptadine's effect on progressive-ratio schedule performance was compared with the effects of the 'atypical' antipsychotic drug clozapine, which shares many of cyproheptadine's pharmacological actions, and the 'conventional' antipsychotic haloperidol, whose principal action is antagonism of $D_2$-like dopamine receptors. In addition, the effects of two drugs with known effects on food intake, $\Delta^9$-tetrahydrocannabinol (THC) and chlordiazepoxide, were also examined. Cyproheptadine and clozapine increased both *a* and $\delta$. Haloperidol reduced *a* and increased $\delta$ and chlordiazepoxide increased *a*. Unexpectedly, THC had no effect on the parameters of MPR; this negative result was explored further in Experiment 6 (see below).

Experiment 5 (Chapter 6) examined the differential involvement of $D_1$-like and $D_2$-like dopamine receptors in the control of progressive-ratio schedule performance reinforced with a sucrose solution or corn oil. Performance maintained by both reinforcers conformed to the equation derived from MPR. Blockade of $D_2$-like receptors by haloperidol equally affected performance maintained by corn oil and sucrose, reducing *a*

and increasing $\delta$. However blockade of $D_1$-like receptors by SKF-83566 (bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrobromide) reduced both *a* and $\delta$ in rats trained with the sucrose reinforcer but had no effect on the rats reinforced with corn oil. This is consistent with the notion that $D_1$-like and $D_2$-like dopamine receptors exert differential influences on the values of different kinds of food reinforcer.

Experiment 6 (Chapter 7) examined the effect of THC on progressive-ratio schedule performance of rats reinforced with corn oil and sucrose. The rats were tested under food deprived and free feeding conditions. In addition, the effect of THC on food intake was assessed. The results confirmed that THC did not affect any of the parameters of MPR. When the animals were transferred from the food deprived to the free feeding condition they showed a reduction of *a* but no change of $\delta$. This is in agreement with the assumption of MPR that *a* and $\delta$ are independent parameters. Finally, there was a trend for THC to increase sucrose consumption and to reduce corn oil intake, suggesting that cannabinoid receptors may mediate different effects on the reinforcing values of these two foods.

Chapter 8 summarises the results of the experiments from the project, and discusses some of their implications. The implications of the findings of Experiments 1-3 for the role of orexinergic mechanisms in the regulation of reinforcer value and motor processes are discussed. The results of Experiments 4 and 5 are considered in the context of the putative involvement of dopamine receptors in reinforcement processes and the effects of conventional and atypical antipsychotics on motivated behaviour. The failure of THC to affect progressive-ratio schedule performance (Experiments 4 and 6) is discussed in the context of the relationship between reinforcer 'value' and food consumption. The general implications of these findings for behavioural pharmacology and MPR are considered. Finally, some futures lines of investigation are proposed.

## Declaration

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification in the University of Nottingham or any other university or learning institution.

## Publications[1]

Peer-reviewed papers

da COSTA ARAÚJO S, BODY S, VALENCIA-TORRES L. **OLARTE-SÁNCHEZ CM**, BAK VK, DEAKIN JFW, ANDERSON IM, BRADSHAW CM, SZABADI E (2010) Choice between reinforcer delays versus choice between reinforcer magnitudes: differential Fos expression in the orbital prefrontal cortex and nucleus accumbens core. *Behavioural Brain Research*, **213**, 269–277.

VALENCIA-TORRES L, da COSTA ARAÚJO S, **OLARTE-SÁNCHEZ CM**, BODY S, BRADSHAW CM, SZABADI E (2011) Transitional and steady-state choice behavior under an adjusting-delay schedule. *Journal of the Experimental Analysis of Behavior*, **95**, 57–74.

VALENCIA-TORRES L, **OLARTE-SÁNCHEZ CM**, BODY S, FONE KCF, BRADSHAW CM, SZABADI E (2011) Fos expression in the prefrontal cortex and nucleus accumbens following exposure to retrospective timing tasks. *Behavioral Neuroscience,* **125**, 202-214.

* **OLARTE-SÁNCHEZ CM**, VALENCIA-TORRES L, BODY S, CASSADAY,HJ, BRADSHAW CM, SZABADI E, GOUDIE AJ (2012) A clozapine-like effect of cyproheptadine on progressive-ratio schedule performance. *Journal of Psychopharmacology,* **in press.**

* **OLARTE-SÁNCHEZ CM**, VALENCIA-TORRES L, BODY,S, CASSADAY HJ, BRADSHAW CM, SZABADI E (2012) Effect of orexin-B-saporin induced lesions of the lateral hypothalamus on performance on a progressive-ratio schedule. *Journal of Psychopharmacology,* **in press.**

VALENCIA-TORRES L, **OLARTE-SÁNCHEZ CM**, da COSTA ARAÚJO S, BODY S, BRADSHAW CM, SZABADI E (2012) Nucleus accumbens and delay discounting in rats: evidence from a new quantitative protocol for analysing inter-temporal choice. *Psychopharmacology*, **in press**.

[1] Papers directly related to the work described in the thesis are indicated by an asterisk

Conference abstracts

VALENCIA-TORRES L, **OLARTE-SÁNCHEZ CM**, BODY S, FONE KFC, BRADSHAW CM, SZABADI E (2009) Lack of enhancement of fos expression in the dorsal striatum following performance of an interval timing task *Journal of Psychopharmacology* **23** A73.

* BODY S, HAMPSON CL, da COSTA ARAÚJO S, **OLARTE-SÁNCHEZ CM**, VALENCIA TORRES L, BRADSHAW CM, SZABADI E, GOUDIE AJ (2009) A clozapine-like effect of cyproheptadine on progressive ratio schedule performance. *Journal of Psychopharmacology* **23** A60

***** **OLARTE-SÁNCHEZ CM**, VALENCIA-TORRES V, BODY S, CASSADAY HJ, BRADSHAW CM, E. SZABADI (2010) Effect of destruction of lateral hypothalamic orexinergic neurones on progressive ratio schedule performance: evidence for an effect on motor performance *Journal of Psychopharmacology* **24** A65.

BRADSHAW CM, da COSTA ARAÚJO S, BODY S, VALENCIA-TORRES L, **OLARTE-SÁNCHEZ CM,** BAK VK, DEAKIN JFW, ANDERSON IM, SZABADI E (2010) Involvement of the orbital prefrontal cortex and nucleus accumbens core in inter-temporal choice: Evidence from FOS expression *Journal of Psychopharmacology* **24** A64

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# CHAPTER 1:

# GENERAL INTRODUCTION

## 1.1. Overview of thesis

The work described in this thesis was an attempt to use a quantitative model of schedule-controlled operant behaviour (Killeen 1994) to address some questions about the neurobiological mechanisms underlying the maintenance of voluntary behaviour by food reinforcement. Three experiments investigated the putative role of orexinergic neurones in reinforcement processes. A series of experiments examined the putative involvement of dopamine receptors in reinforcement processes, and compared the sensitivity of operant performance to disruption by 'conventional' and 'atypical' antipsychotics and cyproheptadine, a drug with 5-hydroxytryptamine (5-HT) and histamine receptor antagonist properties. A final experiment explored the sensitivity of operant performance to $\Delta^9$-tetrahydrocannabinol (THC). In each case, rats' performance on the progressive-ratio schedule of reinforcement was analysed using an equation derived from Killeen's (1994) model, and the effects of interventions (cerebral lesions, intracerebral drug treatment, or systemic drug treatment) on the parameters of the model were examined.

This introductory chapter reviews the background literature to the main topics of the thesis; topics that are related more particularly to individual experiments are reviewed in the introductory sections of the relevant chapters. In the next section the anatomy, pharmacology and principal behavioural functions of the orexinergic pathways are reviewed. This is followed by a brief account of the ascending dopaminergic pathways, and a selective review of evidence for the involvement of these pathways in positive reinforcement. This section also includes an overview of the effects of antipsychotic drugs (which are known to interact with dopaminergic mechanisms: King and Waddington 2004) on operant behaviour. The final major section of the Introduction summarizes Killeen's (1994) model, the *Mathematical Principles of Reinforcement (MPR)* model, focussing on the application of this model to performance maintained by progressive-ratio schedules.

## 1.2. Orexins

### *1.2.1. Anatomy*

Orexin A and B, also known as hypocretin 1 and 2, are neuropeptides (33 and 28 amino-

acids respectively) that were simultaneously discovered by two independent groups in 1998. Although the first name used was hypocretin, it was changed to orexin, from the Greek word orexis which means desire for food, due to the fact that the central administration of orexin produces an increment of food intake.

In the rat brain the orexin-containing neurones are principally localised symmetrically and bilaterally in the hypothalamus and adjacent regions. One major group of orexin-containing neurones is located in the lateral hypothalamic area (LHA). Other clusters of orexinergic neurones are found in the perifornical area of the hypothalamus (PFA), the posterior hypothalamus, the dorsomedial hypothalamic nucleus (DMH), the zona incerta, and the subincertal and subthalamic nuclei.

Both orexins are encoded by the same gene. They are derived from a common precursor, prepro-orexin (prepro-OX) (Sakurai et al. 1998), also called prehypocretin (de Lecea et al. 1998).

Although orexinergic neurones are few in number, approximately 50,000 in total in the human brain (Fronczek et al. 2005), these neurones project practically to the entire brain, including the cerebral cortex, olfactory bulb, hippocampus, basal ganglia, amygdala, thalamus, anterior and posterior hypothalamus and various brainstem regions (see below) (Date et al. 1999; de Lecea et al. 1998; Peyron et al. 1998(b)).

Orexinergic neurones of the LHA project to both the nuclei of origin and the projection fields of the mesolimbic dopaminergic pathways (ventral tegmental area [VTA] and the nucleus accumbens), which are widely believed to constitute a crucial component of the "motivational and reward system" (Harris and Aston-Jones 2006; Salamone and Correa 2002; Wise 2004(a); 2004(b)). Orexinergic neurones of the LHA and PFA project to noradrenergic neurones of the locus coeruleus, serotonergic neurones of the raphe nuclei, histaminergic neurones of the tuberomamillary nuclei and cholinergic neurones in the basal forebrain (Peyron et al. 1998(a); Peyron et al. 1998(b); Scammell and Winrow 2011; Yoshida et al. 2006). These regions have all been implicated in the regulation of behavioural arousal and the sleep/wakefulness cycle (see Scammell and Winrow 2011). The putative behavioural roles of these orexinergic projections are reviewed in Section 1.2.3.

The orexins bind to two G protein mediated receptors named Orexin receptor 1 (OX1 receptor) and Orexin receptor 2 (OX2 receptor). Orexin receptor mRNA is expressed broadly throughout the brain. Areas that contain a high level of OX1 receptor mRNA are the tenia tecta, the indusium griseum, the septohippocampal nucleus, the bed nucleus of the stria terminalis, the paraventricular thalamic nucleus, the CA1 and CA2 regions of the hippocampus, the amygdala, the raphe nuclei and locus coeruleus. OX2 receptors are expressed in high density in the olfactory tubercle, layer VI of the cerebral cortex, the shell of the nucleus accumbens, the paraventricular and central medial thalamic nuclei, the LHA, PFA and tuberomammillary nuclei of the hypothalamus, the subthalamic nucleus, the VTA, the anterior pretectal nucleus and the CA3 region of the hippocampus (Lu et al. 2000; Trivedi et al. 1998). It has also been shown that orexin A and OX1 receptor mRNA are expressed in peripheral tissues (Johren et al. 2001; Randeva et al. 2001). However as orexin expression in peripheral tissues is relatively low, it has been argued that it is unlikely that orexins exert significant peripheral effects (Scammell and Winrow 2011).

Orexinergic neurones themselves express orexin receptors (autoreceptors). For example, orexinergic neurones account for a substantial proportion of OX2 receptor expression in the LHA (Gerashchenko et al. 2001). However, other neuronal populations in the LHA also express OX2 receptors, suggesting that orexinergic transmission plays a significant role in the local intrahypothalamic circuitry in addition to its role in the efferent projection from this region. Hypothalamic neurones that express orexin receptors include melanocortin concentrating hormone (MCH)-containing, adenosine deaminase (ADA)-containing and histaminergic neurones (Blanco-Centurion et al. 2007; Frederick-Duus et al. 2007; Furlong and Carrive 2007; Gerashchenko et al. 2001).

### *1.2.2. Pharmacology*

As indicated above, there are two types of orexin receptor, OX1 and OX2 receptors. The two forms of orexin (orexin A and orexin B) have different affinity profiles for these receptors. Orexin A has a higher affinity than orexin B for OX1 receptors; however both orexins bind to OX2 receptors with approximately the same affinity (Sakurai et al. 1998). No selective synthetic OX1 and OX2 receptor agonists are available at the present time.

To study the orexin system, orexin A and B have been administered centrally (see Section 1.2.3). The neuropeptide Y receptor agonist, rat pancreatic polypeptide (RPP) has been found to induce Fos expression in orexinergic neurones (Campbell et al. 2003) and has also been used to stimulate the orexinergic system in behavioural studies (Borgland et al. 2009; Borgland et al. 2006; Harris et al. 2005; Jones et al. 2001; Sakurai et al. 1998; Thorpe et al. 2005a; Thorpe et al. 2006; Thorpe and Kotz 2005) (for discussion, see Section 1.2.3).

The first orexin receptor antagonist to be developed was SB-334867-A (1-(2-methylbenzoxazol-6-yl)-3-[1,5]naphthyridin-4-yl-urea hydrochloride). This is still the most selective and potent OX1 receptor antagonist available (Scammell and Winrow 2011; Smart et al. 2001). SB-334867-A binds to OX1 receptors with nanomolar affinity and inhibits the OX1 receptopr-mediated calcium response at similar concentrations. SB-334867-A also inhibits the OX2 receptor-mediated calcium response but only at substantially higher concentrations (Smart et al. 2001). Another OX1 receptor antagonist with similar affinity and selectivity is SB-408124 (Dugovic et al. 2009).

To disentangle the possible differential roles of the two orexin receptors, the specific OX2 receptor antagonist JNJ-10397049 (N-(2,4-dibromophenyl)-N'-[(4S,5S)-2,2-dimethyl-4-phenyl-1,3-dioxan-5-yl]-urea) ) was developed (Silveyra et al. 2007). Studies using this antagonist have indicated differential effects of OX1 and OX2 receptors in reward process and the promotion of wakefulness (Dugovic et al. 2009; Shoblock et al. 2010) ; see Section 1.2.3). Another orexin receptor antagonist is the dual OX1/OX2 receptor antagonist, almorexant (ASCT-078573), which has recently been used in clinical trials for the treatment of sleep disorders, including insomnia, and drug addiction (Brisbare-Roch et al. 2007; Roecker and Coleman 2008; Winrow et al. 2009).

To futher understand the behavioural functions of the orexinergic pathways, studies that entail the destruction of orexinergic cells or cells bearing the orexin receptors have been carried out on animals. A novel neurotoxin was developed by the conjugation of the ribosome-inactivating protein saporin (Sap) (Stirpe and Barbieri 1986; Stirpe et al. 1983) and the endogenous orexin receptor ligand orexin B. Since orexin B binds preferentially to OX2 receptors, the conjugate, known as OxSap (Advanced Targeted Systems, USA), has a selective neurotoxic action on neurones bearing these receptors. Since orexinergic

neurones themselves express OX2 receptors, OxSap has a powerful destructive action on these neurones, and when injected into the LHA produces a marked depletion of orexin-containing neurones (Gerashchenko et al. 2001). Although it is well documented that OxSap destroys orexinergic neurones in the LHA (Anaclet et al. 2009; Blanco-Centurion et al. 2007; Di Sebastiano et al. 2010a; Di Sebastiano et al. 2010b; Frederick-Duus et al. 2007; Furlong and Carrive 2007; Gerashchenko et al. 2003; Gerashchenko et al. 2001; Vetrivelan et al. 2009), it has been reported that OxSap also affects other, non-orexinergic, neurones which bear the OX2 receptor, including MCH, histaminergic and cholinergic neurones (Blanco-Centurion et al. 2007; Frederick-Duus et al. 2007; Furlong and Carrive 2007; Gerashchenko et al. 2001; Ocampo-Garces et al.). However, some recent studies did not find a significant loss of MCH-containing neurones when OxSap was injected in the LHA (Di Sebastiano et al. 2010a; Di Sebastiano et al. 2010b). It is thought that the specificity of OxSap is dose-dependent and that smaller doses seem to be more selective for orexinergic neurones (Anaclet et al. 2009; Gerashchenko et al. 2006; Vetrivelan et al. 2009) than higher doses (Furlong and Carrive 2007; Gerashchenko et al. 2006; Mistlberger et al. 2003).

### *1.2.3. Behavioural functions*

The orexins are proposed to be implicated in a broad variety of functions including feeding, reward processing, addictions, regulation of the sleep-wakefulness cycle, behavioural arousal and the distribution of muscle tone (de Lecea et al. 1998; de Lecea and Sutcliffe 1999; Piper et al. 2000; Sakurai 1999; 2002; 2005).

#### 1.2.3.1. Behavioural arousal and the sleep/wakefulness cycle

Central administration of orexins results in an increase of behavioural arousal, locomotor activity (John et al. 2000; Jones et al. 2001; Thorpe et al. 2005a; Thorpe et al. 2006; Thorpe and Kotz 2005; Thorpe et al. 2005b) and wakefulness (Bourgin et al. 2000; Eriksson et al. 2001; Espana et al. 2001; Piper et al. 2000). Futhermore the level of orexin in cerebrospinal fluid (CSF) is higher during the period of wakefulness, and this level decreases during sleep. This diurnal fluctuation of orexin levels has been found in different organisms including humans (Estabrooke et al. 2001; Grady et al. 2006; Mileykovskiy et al. 2005; Salomon et al. 2003; Yoshida et al. 2001; Zeitzer et al. 2003).

Narcoleptic patients have a deficit of orexin-containing neurones compared with non-narcoleptic humans (Peyron et al. 2000). Siegel (2004) suggested that disruption of the normal synthesis of orexins due to genetic alterations or degeneration of the orexinergic neurones or orexin receptors may cause narcolepsy in humans. This neurological disorder is characterized by uncontrollable somnolence during the day time and alteration of rapid eye movement (REM) sleep (Mignot 1998). This sleep alteration is characterized by a short REM sleep latency and a fast transition from waking state into REM sleep with a very short sleep onset period (Siegel 2004). Many, but not all, patients with narcolepsy experience cataplexy (brief periods of paralysis triggered by strong emotions) (Scammell and Winrow 2011). The majority of narcoleptic patients have undetectable or very little orexin A in their CSF (Nishino 2007; Nishino et al. 2000). These findings are in accordance with human post-mortem studies that showed a reduction of more than 80% of orexin-containing neurones in the hypothalamus (Peyron et al. 2000). All these studies are consistent with the proposal that the orexin system is involved in the pathogenesis of human narcolepsy (Harris and Aston-Jones 2006; Scammell and Winrow 2011).

Orexin has also been studied in relation to the sleep disorder narcolepsy. Rodents whose orexinergic pathways have been ablated and prepro-orexin knockout mice show symptoms that mimic those present in human narcolepsy. Similar symptoms were found in dogs and mice that lack the OX2 receptor (Chemelli et al. 1999; Hara et al. 2001; Lin et al. 1999; Willie et al. 2001). These findings led Lin et al (1999) to conclude that the cause of canine narcolepsy is a malfunctioning of the OX2 receptor and also that orexin is a major sleep modulating transmitter. However, there is some evidence that mice lacking both OX1 and OX2 receptors exhibit a more severe narcoleptic syndrome than those lacking only OX2 receptors, and that cataplexy occurs only in animals that lack both types of orexin receptor (Hondo et al. 2009; Scammell and Winrow 2011).

#### 1.2.3.2. Feeding and energy balance

Sakurai et al. (1998) were the first in inject orexin A and B intracerebroventricularly and found that both of these neuropeptides induced food intake; however, the effect of orexin A was stronger and lasted longer than that of orexin B. The effects of orexin administration are substantially less potent than those produced by other orexigenic

peptides such as neuropeptide Y (Edwards et al. 1999; Sakurai et al. 1998).

More recent studies have confirmed the increment in food intake induced by centrally administered orexin under different experimental conditions (e.g. free feeding vs food restricted) and in different areas in the brain (ventricles, nucleus accumbens and ventral tegmental area) (Farr et al. 2005; Nair et al. 2008; Thorpe et al. 2005a; Thorpe et al. 2006; Thorpe and Kotz 2005; Thorpe et al. 2005b). Orexin precursor (Prepro-OX) levels have been found to be augmented upon fasting and reduced after food consumption (Lopez et al. 2000). A role for orexinergic mechanisms in feeding is also suggested by the finding that systemic administration of the OX1 receptor antagonist SB-334867 (Haynes et al. 2002; Haynes et al. 2000) reverses the rise in food intake after the central administration of orexin A (Rodgers et al. 2000; Rodgers et al. 2001; Thorpe and Kotz 2005).

These findings indicate that the central administration of orexins induces an increase of food intake. However, the implications of this for the regulation of body weight remain unclear, since orexins have been implicated in the regulation of metabolic rate and locomotor activity, both of which have an impact on energy consumption (Kannan et al. 2007; Samson et al. 1999; Shirasaka et al. 1999). For example, some studies have found that daily central administration of orexin-A induced weight *loss* in rats (Novak and Levine 2009; Yamanaka et al. 1999). A further example of the complex interactions between orexinergic mechanisms, energy consumption and body weight is provided by a study using transgenic mice that lack orexinergic neurones (Hara et al. 2001). These mice developed obesity despite the fact that their food intake was less than that of wild type mice. The authors concluded that this result was due to a decrement in locomotor activity in the orexin-deficient mice, when compared to wild type mice, during the dark phase of the daily cycle, which resulted in reduced energy expenditure in the orexin-deficient mice (Hara et al. 2001). Espana et al. (2002) found that when orexin A was administered to rats during the dark phase of the cycle it did not produce an increase of food intake. The authors suggested that this lack of effect was due to the fact that rats are more active during the night. They also suggested that the orexin-A-induced increase in food intake that occured during the day was due to an increase of arousal (Espana et al. 2002).

Interestingly, there is evidence that the effect of orexins on energy expenditure is not

necessarily brought about by behavioural changes. Injection of orexin A into various hypothalamic nuclei, including the arcuate nucleus, the medial preoptic area, the dorsomedial nucleus and the LHA increases oxygen consumption and thermogenesis in anaesthetized as well as conscious animals (Novak et al. 2006; Wang et al. 2003). Summarising this literature, Teske et al. (2010) proposed that orexins directly stimulate energy expenditure as well as promoting behavioural activity which results in further energy expenditure.

#### 1.2.3.3. Reinforcement and drug addiction

Another area of investigation in relation to the orexinergic neurones of the LHA is their putative role in 'reward seeking' and addiction to drugs. There is evidence that orexins enhance neuronal activity in the mesolimbic dopaminergic projection from the VTA to the nucleus accumbens, which is believed to play a prominent role in reinforcement processes and drug addiction (Wise 1978; 2004(b)). For example, Borgland et al. (2006) found that locally administered orexins increased both the acute response of VTA neurones to glutamate and the longer-term functional plasticity (long-term potentiation) of these neurones induced by repeated stimulation by glutamate. Moorman and Aston-Jones (2007, unpublished experiments cited by Aston-Jones et al. 2009) found that orexins had an excitatory effect on VTA neurones, as well as enhancing the increase in firing of these neurones evoked by stimulation of the medial prefrontal cortex. Orexin receptors are present in considerable numbers in the nucleus accumbens, one of the main projection regions of the mesolimbic dopaminergic pathway (Martin et al. 2002; Trivedi et al. 1998); immunohistochemical studies have confirmed the presence of orexin-containing terminals in the nucleus accumbens, and retrograde tracing studies have demonstrated direct orexinergic projections from the LHA to the shell of the nucleus accumbens (Fadel et al. 2002; Peyron et al. 1998(b)).

Behavioural evidence consistent with a role of the LHA orexinergic system in reinforcement comes from a variety of sources. For example, injection of orexin A into the cerebral ventricles (Sakurai et al. 1998) or directly into the LHA (Dube et al. 1999; Sweet et al. 1999; Thorpe et al. 2006; Thorpe et al. 2005b) induces feeding in rodents. Consumption of high-energy diet has been found to be preferentially enhanced by orexin A (Clegg et al. 2002), and the OX1 receptor antagonist SB-334867 administered

systemically or into the ventral tegmental area (a prominent projection region of the LHA orexinergic neurones), has been found to attenuate spontaneous or opiate-induced consumption of high-fat diet (Choi et al. 2010; Zheng et al. 2007). More direct evidence for an involvement of orexins in reinforcement processes derives from the finding that conditioned place preference induced by food or opiate reinforcement is associated with activation of LHA orexinergic neurones, and that conditioned place preference can be attenuated by systemic administration of SB-334867 (Harris et al. 2005). Stimulation of these neurones by injection of the neuropeptide Y receptor agonist RPP into the LHA was found to reinstate a previously extinguished conditioned place preference for morphine, and this effect was also blocked by SB-334867 (Harris et al. 2005).

There have been several reports of the effect of manipulating orexinergic function on schedule-controlled operant behaviour. Most of these studies have employed the progressive-ratio schedule of reinforcement, in which the number of responses required in order to obtain a reinforcer (the response/reinforcer ratio) is progressively increased (Hodos 1961; Hodos and Kalman 1963). As the ratio is increased, response rate declines, until the subject eventually stops responding, The ratio at which responding ceases for some specified period defines the 'breakpoint'. Administration of orexin A into the third ventricle (Choi et al. 2010) or directly into the LHA (Thorpe et al. 2005) increased the breakpoint and facilitated food intake. Moreover, SB-334867 reduced the breakpoint when the reinforcer was cocaine or a highly palatable food (Borgland et al. 2009; Choi et al. 2010; Nair et al. 2008; Sharf et al. 2010a). Since the breakpoint is often used as an index of the subject's motivational state or the incentive value of reinforcers (Aberman et al. 1998; Barr and Phillips 1999; Bowman and Brown 1998; Cheeta et al. 1995; Hodos 1961; Richardson and Roberts 1996; Thorpe et al. 2005a), these findings are consistent with the notion that the orexinergic projection from the LHA forms part of the neural substrate of reinforcement.

Furthermore clinical evidence supports the involvement of the orexin system in addictions. For instance, patients suffering from narcolepsy are treated with psychostimulant drugs related to amphetamines (Nishino and Mignot 1997); however, they seldom become addicted to them (Akimoto et al. 1960; Guilleminault et al. 1974). As reviewed above, narcolepsy is associated with deficient orexinergic function. Similar results were found with rodents whose orexinergic neurones had been ablated; these

animals showed reduced susceptibility to morphine dependency compared to normal animals (Georgescu et al. 2003).

#### 1.2.3.4. Arousal and reinforcement: a 'dichotomy of orexinergic function'?

It has been proposed that the orexinergic neurones of the hypothalamus may be divided into two anatomically distinct groups which subserve different behavioural functions ('dichotomy of orexinergic function' Harris and Aston-Jones 2006). The first group corresponds to the orexinergic neurones of the LHA. This group is purported to be involved in the regulation of reward processing for both food and drugs. The second group of orexinergic neurones are those found in the PFA and DMH, which according to the 'dichotomy of orexinergic function' theory (Harris et al. 2005) react to stressors, and may be primarily responsible for the role of orexins in wakefulness and arousal. Lu et al. (2003) used 'stress' induced by foot-shock in order to re-establish extinguished drug seeking behaviour. This was associated with activation of the orexinergic neurones in the PFA and DMH but not in the LHA, consistent with the notion that the former group of orexinergic neurones subserves arousal, but not reinforcement processes (Harris et al. 2005). Similar findings have been reported when the stress was induced by restraint or cold-exposure, which increased Fos expression (a marker for neuronal activation: Hoffman et al., 1993) in PFA orexinergic neurones (Sakamoto et al. 2004; Winsky-Sommerer et al. 2004).

Further evidence for a role of PFA/DMH orexinergic neurones in arousal was provided by Estabrooke et al. (2001). These authors studied the activation of orexinergic neurones during wakefulness and sleep under different conditions (sleep deprivation, wakefulness induced with drugs, animals living in the dark, and animals maintained in a light/dark cycle). To measure the activation of orexinergic neurones, Eastabroke et al. used a double immunohistochemical labelling method for Fos and orexin, combined with physiological sleep recording. They found a positive correlation between Fos expression in the orexinergic neurones in the PFA and the percentage of time spent awake. Furthermore there was a greater change in Fos expression during the daytime in the PFA than in the LHA (Estabrooke et al. 2001).

However, there are some data that appear not to support the 'dichotomy of orexinergic

function' theory (Harris and Aston-Jones 2006). There is some evidence that not all reward seems to produce an increment of Fos expresion in LHA orexinergic neurones. This was reported in a experiment carried out by the authors of the 'dichotomy' theory with a conditioned place preference task using novel objects (Harris et al. 2005). In addition, several studies did not find significant differences in the activation of orexinergic neurones between LHA and DMH in stress studies with animals (Berridge et al. 1999; Espana et al. 2003). Finally in a study of pharmacologically-induced fat ingestion (Zheng et al. 2007), and in a reward study where animals were expecting chocololate or chow (Choi et al. 2010), there was an increment in the number of orexin-positive neurones in the PFA but not in the LHA.

A further problem for the 'dichotomy of orexinergic function' theory relates to the interpretation of some of the behavioural tests that have been used to assess reinforcement processes. It has been argued that some of the findings that have been attributed to the putative role of orexinergic mechanisms in reinforcement may also be interpreted in terms of their role in the control of muscle tone and motor output (Berridge et al. 2010; Siegel 2004; 2005) Thus, Berridge et al. (2010) have pointed out that increased levels of arousal induced by either aversive or appetitive stimuli may facilitate the reinstatement of conditioned place preference (Stewart 2000), and it is therefore possible that the effect of orexinergic manipulations on 'reward seeking' (Harris et al. 2005) may reflect reward-independent changes in arousal rather than changes in the value of positive reinforcers (Berridge et al. 2010).

To summarize, it can be said that although there is a consensus about the involvement of the orexinergic system in the regulation of sleep and wakefulness, the role of orexins in reinforcement processes remains controversial. This suggests that there is a need for further investigation of this aspect of orexinergic function.

## 1.3. The central dopaminergic system

### *1.3.1. Historical background*

The neurotransmitter dopamine (2-(3,4-dihydroxyphenyl)ethylamine) was first

synthesized by Barger and Ewens in 1910. However, it was thought that this monoamine was a mere mediator in the biosynthesis of the catecholamines noradrenaline and adrenaline. It was not until 1958 that Carlsson and colleagues demonstrated the existence of dopamine in the brain and provided the first evidence for the independence of dopamine as a neurotransmitter. These workers used an improved version of the fluorimetric method of Von Euler and Floding (1955) in combination with an ion-exchange chromatography technique. Although dopamine and noradrenaline were found to be present in equal concentrations in the central nervous system (CNS) (Carlsson et al. 1958), the anatomical distributions of the two catecholamines were different. For example, dopamine was found in the striatum in high concentrations, whereas there were very low concentrations of noradrenaline (Bertler and Rosengren 1959; Dahlstrom and Fuxe 1965). Later, the fluorescence histochemical method of Hillarp and Falk enabled dopamine to be detected in discrete neuronal pathways (Andén et al. 1966; Dahlstrom and Fuxe 1965). The first dopaminergic pathways to be discovered were the dopaminergic projections from the substantia nigra (A9 cell group) to the corpus striatum and from the VTA (A10 cell group) to the limbic areas of the brain.

During the half-century since its first recognition as a central neurotransmitter, dopamine has become in one of the most extensively studied neurotransmiters, due to its crucial importance in a wide variety of neurodegenerative and psychiatric disorders, including Parkinson's disease, schizophrenia, depression, drug addiction and attention deficit hyperactivity disorder (for reviews see: Bjorklund and Dunnett 2007; Grace et al. 2007; Iversen and Iversen 2007; Marsden 2006).

### *1.3.2. Anatomy of the dopaminergic pathways*

Classically the dopaminergic system has been divided into three principal pathways that are described below.

#### 1.3.2.1. The nigrostriatal dopaminergic pathway

This pathway projects from the substantia nigra pars compacta (SNc, the A9 group of monoamine-containing neurones) to the dorsal striatum. It plays a critical role in organising the functions of the basal ganglia. The principal components of the basal

ganglia are: the corpus striatum, which is subdivided into the caudate nucleus and the putamen, the globus pallidus, the substantia nigra (see below) and the subthalamic nucleus. This group of nuclei participates in the modulation of a broad variety of behaviours including learning, working memory and motor control.

The substantia nigra is divided into two cellular groups based on cytoarchitectonic and chemical characteristics. The first is the SNc, which is principally composed of dopaminergic neurones whose somata project to the striatum (Cebrian et al. 2005). The SNc is subdivided again into the dorsal and the ventral tiers (Haber and Fudge 1997; Prensa et al. 2009). The second group is the substantia nigra pars reticulata (SNr) which contains the cell bodies of γ-aminobutyric acid (GABA)-ergic neurones, innervating the pedunculopontine tegmental nucleus, thalamus and the superior colliculus (Halliday and Tork 1986; Olszewski and Baxter 1954; Poirier et al. 1983; Prensa et al. 2009). Together with the globus pallidus, the SNr provides the principal inhibitory output pathways of the basal ganglia (Gerfen 2004).

As discussed below (section 1.3.5.1) the nigrostriatal dopaminergic pathway plays a very important role in extrapyramidal motor control. Degeneration of this pathway underlies the movement disorder of Parkinson's disease, and blockade of dopamine receptors in the corpus striatum produces 'extrapyramidal motor symptoms' very similar to those observed in Parkinson's disease (Trevitt et al. 1997).

#### 1.3.2.2. The mesolimbic dopaminergic pathway

The mesolimbic dopaminergic pathway projects from the dopaminergic neurones in the midbrain ventral tegmental area (VTA: the A10 neuronal group) to the limbic system, particularly to the nucleus accumbens, the nuclei of the stria terminalis, some areas of the amygdala and hippocampus, lateral septal nuclei, the enthorinal cortex, the mesial and orbital frontal cortex and the anterior cingulate cortex. Some authors treat the subcortical and cortical branches of the pathway as separate entities ('mesolimbic' and 'mesocortical' dopaminergic pathways). However, there is no compelling evidence that the two branches arise from separate neuronal groups within the VTA (Gerfen 2004).

The mesolimbic/mesocortical dopaminergic projection has been implicated in a wide range of behavioural functions, including arousal, emotional behaviour, motivation and reinforcement functions (see sections 1.3.5.2 – 1.3.5.5). Carlsson (1974) was among the first to suggest that overactivity of the mesolimbic dopaminergic projection may be the pathophysiological basis of schizophrenia. The antipsychotic efficacy of $D_2$-like dopamine receptor antagonists has long been recognized (Seeman et al. 1976); more recently the selective blockade of dopamine receptors in limbic structures has been proposed as the basis of the superior clinical profile of some atypical antipsychotic drugs (Schoemaker et al. 1997) (see also section 1.3.4).

#### 1.3.2.3. The tubero-infundibular dopaminergic pathway

This pathway comprises dopamine-containing cells of the tuberal, arcuate and paraventricular nuclei of the hypothalamus that project to the intermediate lobe of the pituitary gland and median eminence. This system plays a very important role in the regulation of hormone release from the pituitary gland. For example, prolactin release from the anterior pituitary is maintained under inhibitory control by tuberoinfundibular dopaminergic neurones, the effect of the transmitter being mediated by $D_2$ dopamine receptors. As reviewed below (see section 1.3.4), the principal pharmacological action of conventional antipsychotic drugs is the antagonism of $D_2$ receptors. A known side-effect of these drugs is hyperprolactinemia, which can result in impotence and gynaecomastia in sensitive individuals (see Freeman et al 2000; Stahl 2002).

#### 1.3.2.4. Dopamine and the cortico-striato-thalamo-cortical circuits

There is widespread agreement that the cerebral cortex and the basal ganglia are connected by multiple ‘cortico-striato-thalamo-cortical (CSTC) circuits’ (Alexander et al. 1986, 1990). It is believed that these circuits are, to a large extent, both topographically and functionally separate from one another, although it is increasingly recognised that some interconnections do exist between them (Gerfen 2004; Haber 2003; Uylings et al. 2003). The basic principles of the CSTC circuits are illustrated by the ‘motor loop’, a diagram of which is shown in Figure 1.1.

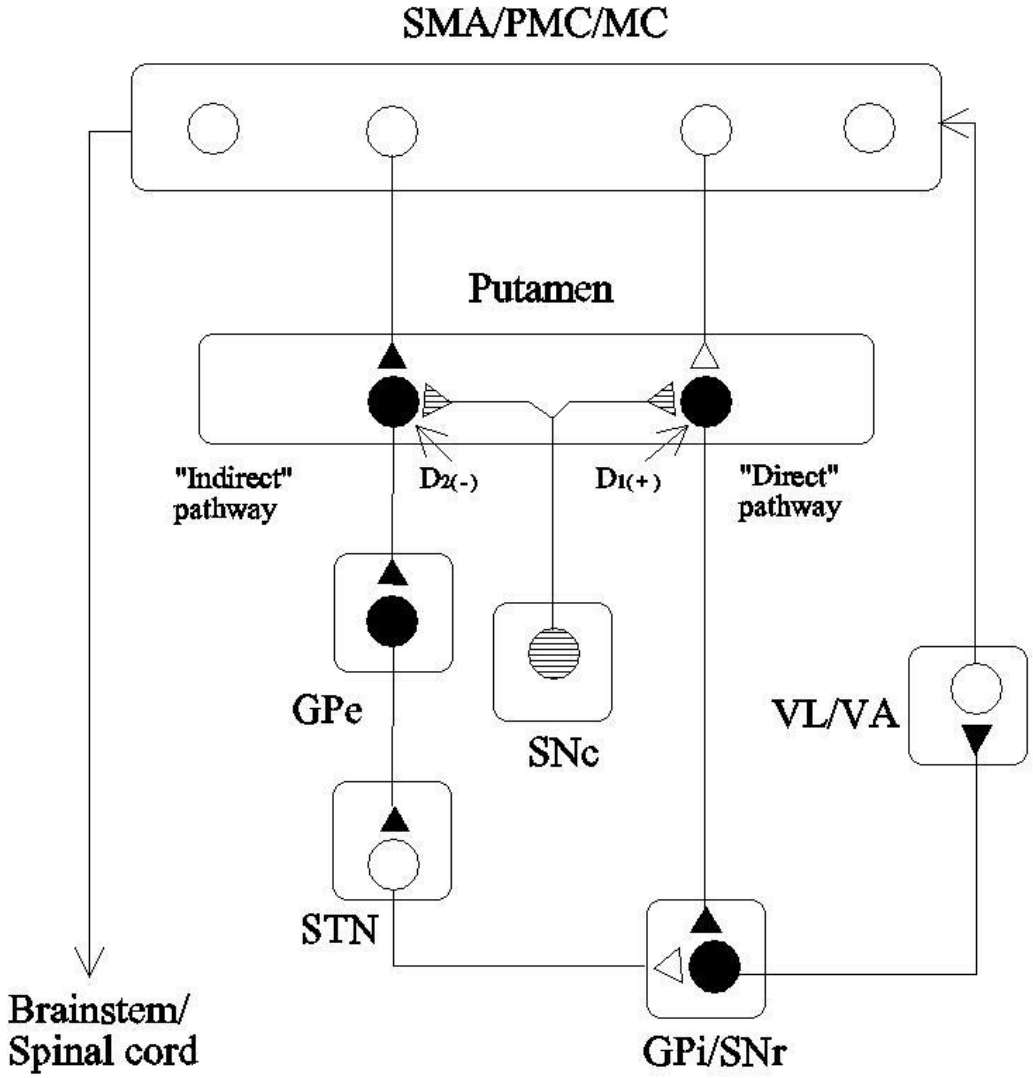


**Fig. 1.1.** Diagram of the principal neuronal pathways that comprise the 'motor loop' (Alexander et al. 1990). Filled circles are GABAergic neurones; unfilled circles glutamatergic neurones; hatched are dopaminergic neurones. Gpi, internal segment of the globus pallidus, GPe, external segment of the globus pallidus; PMC, premotor cortex; SMA, supplementary motor area; MC, motor cortex; STN, subthalamic nucleus; VL/VA, ventral lateral and ventral anterior SNr, substantia nigra pars reticulata ; SNc substantia nigra pars compacta. $D_1$-like dopamine receptors are purported to modulate activity in the 'direct' output pathway by depolarising the GABAergic neurones. $D_2$-like dopamine receptors modulate activity in the 'indirect' pathway by hyperpolarising these neurones. Therefore the release of dopamine in the putamen is proposed to have the overall effect of suppressing the inhibitory influence of the GPi and the SNr on the thalamic output to the motor cortex.

Excitatory (glutamatergic) cortifugal fibres project from the motor cortex to the caudate nucleus and putamen. These fibres make synaptic contact with medium spiny neurones, the most numerous type of neurone of the corpus striatum. These GABAergic neurones send inhibitory projections to the output nuclei of the basal ganglia (the internal segment of the pallidum [GPi] and the SNr) via a 'direct pathway' (GABAergic neurones of the caudate nucleus and putamen project directly to the output nuclei) and an 'indirect pathway' (GABAergic neurones of the caudate nucleus and putamen project to the external segment of the pallidum [GPe], which sends a GABAergic projection to the subthalamic nucleus, which in turn sends excitatory efferents to the GPi and SNr). The nigrostriatal dopaminergic fibres project to the striatal origins of both the direct and indirect output pathways (see Figure 1.1); it is believed that GABAergic neurones of the indirect pathway express inhibitory $D_2$-like dopamine receptors, whereas neurones of the direct pathway are believed to mainly express excitatory $D_1$-like receptors (Alexander et al. 1990; Gerfen 2004).

In addition to the 'motor loop', there is an oculomotor circuit that incorporates the frontal eye fields, and three 'limbic circuits' that connect the major regions of the prefrontal cortex to the ventral striatum. In primates, these prefrontal regions are the dorsolateral [DLPFC], medial [MPFC] and orbital [OPFC] prefrontal cortices). It is now generally accepted that rats possess functionally specialized prefrontal regions that are homologous with the OPFC, MPFC and DLPFC of primates; these are represented by the OPFC, the ventral MPFC (vMPFC, including the pre- and infralimbic cortices), and dorsal MPFC (dMPFC, including the dorsal anterior cingulate gyrus) (Uylings et al. 2003; Vogt et al. 2004). In the rat, these regions receive partially segregated input from the mediodorsal thalamus (Groenewegen and Witter 2004), and project to different parts of the striatum; the OPFC and vMPFC project mainly to the ventromedial striatum (ventral caudate-putamen and AcbC) (Palomero-Gallagher and Zillies 2004; Vogt et al. 2004) whereas there are connections between the dMPFC and the medial dorsal striatum (Gerfen 2004). Thus each prefrontal cortical region participates in a separate CSTC circuit. A particular feature of these 'limbic' CSTC circuits is the dual innervation of the cortical and striatal components of the circuits by dopaminergic afferents from the VTA (Bjorklund and Lindvall 1984; Tzschentke 2001). In general, the dopaminergic input is denser in the striatum than in the prefrontal cortex, and dopamine uptake appears to play a less

important role in the inactivation of dopamine in the cortex than in the striatum (see below, section 1.3.3).

It is widely believed that the various CSTC circuits subserve different behavioural functions. In man, damage to the DLPFC is commonly associated with cognitive dysexecutive symptoms, MPFC lesions with disturbances of motivation and emotion, and OPFC lesions with impulsive/disinhibited behaviour (Tekin and Cummings 2002). Behavioural specialization of CSTC circuits has also been demonstrated in the rat (Cardinal et al. 2002; Robbins and Everitt 2002; Uylings et al. 2003). For example, lesions of the medial and lateral striatum and its input from the dMPFC have been found to produce deficits in attentional performance (Christakou et al. 2001; Rogers and Hagan 2001); lesions of the vMPFC have been found to impair detection of instrumental contingencies (Balleine and Dickinson 1998); and lesions of the OPFC and AcbC can impair detection of changes of reinforcer value (Gallagher et al. 1999) and disrupt inter-temporal choice (Bezzina et al. 2008a; Bezzina et al. 2007; da Costa Araújo et al. 2009; Kheramin et al. 2002).

### *1.3.3. Synthesis and metabolism of dopamine*

The synthesis of dopamine (see Cooper et al 2003; Elsworth and Roth 1997) starts with the dietary amino-acid tyrosine. Tyrosine is accumulated in dopaminergic neurones by an energy-dependent uptake mechanism. In the dopaminergic neurones tyrosine is converted into 3,4-dihydroxyphenylalanine (L-DOPA) by the action of the enzyme tyrosine hydroxylase (TH), which is the rate-limiting step in the synthesis of dopamine (Nagatsu et al. 1964). L-DOPA is the immediate precursor of dopamine; conversion of L-DOPA to dopamine is brought about by the non-specific enzyme, L-aromatic amino acid decarboxylase. This process occurs so rapidly that levels of L-DOPA in the brain are virtually undetectable (see Cooper et al 2003). The production of dopamine can be increased by the administration of L-DOPA. In contrast, administration of tyrosine does not reliably increase the level of dopamine as, in normal circumstances, tyrosine concentrations are high in the brain and well above the dissociation constant of the enzyme TH; in other words, the enzymatic process that converts tyrosine to L-DOPA is

close to saturation under normal physiological conditions (see Cooper et al 2003; Elsworth and Roth, 1997; Squirrel et al 2001).

Once dopamine is synthesised, it is stored in vesicles, which protects it from enzymatic degradation in the cytosol. Endogenus mechanisms regulate the synthesis of dopamine by altering the rate of conversion of tyrosine to L-DOPA by the enzyme TH. Dopamine and other catecholamines act as end-products that directly inhibit TH. The rate of L-DOPA production by TH is also regulated by presynaptic dopamine autoreceptors which are activated by the release of dopamine from nerve terminals. (See Cooper et al 2003; Squirel et al 2008; Deutch et al 2004).

Synaptically released dopamine acts at a variety of post-synaptic receptors (see below). Inactivation of released dopamine is brought about principally by re-uptake into dopaminergic neurones via a high-affinity dopamine transporter (DAT) (Amara and Kuhar 1993; Gainetdinov and Caron 2003). DAT molecules are most abundant in the terminal membrane close to the synaptic cleft. However they are also found outside the synaptic cleft, which may indicate that DAT inactivates dopamine molecules that escape from the synaptic region. Psychostimulant drugs such cocaine or amphetamine bind to DAT, preventing dopamine transport and uptake (Mortensen and Amara 2003).

Once dopamine is released, it can be converted into three metabolites (see Cooper et al 2003). In the basal ganglia, the principal mode of inactivation consists of reuptake via DAT. Following reuptake, dopamine is either re-stored in vesicles or metabolised by the mitochondrial enzyme monoamine oxidase to dihydroxyphenylacetic acid (DOPAC). However, in some areas of the brain which contain relatively little DAT (e.g. the prefrontal cortex), dopamine is mainly metabolized extracellularly by catechol-O-methyltransferase (COMT) to form 3-methoxytyramine (3-MT). Both DOPAC and 3-MT are finally metabolised to homovanillic acid (HVA).

### *1.3.4. Dopamine receptor pharmacology*

The first indication of the presence of specific dopamine receptors in the central nervous system was the demonstration of the activation of adenylyl cyclase by dopamine, first in the pituitary and then in the brain. This was followed by the discovery of a common

binding site for dopamine and neuroleptic (antipsychotic) drugs including phenothiazines (e.g. chlorpromazine) and butyrophenones (e.g. haloperidol) (see Kebabian et al. 1972; Seeman et al. 1976). Although this site was first named the neuroleptic receptor, its name was changed to the dopamine receptor site (Kebabian and Calne 1979). Studies of the regional distribution of dopamine receptors showed that dopamine receptors occur both postsynaptically and presynaptically (autoreceptors located on dopaminergic neurones). At the same time, functional studies led to the classification of dopamine receptors based on whether or not dopamine receptor stimulation led to an increase in the production of cyclic adenosine monophosphate (cyclic AMP) by the enzyme adenylyl cyclase. Dopamine receptors coupled positively to adenylyl cyclase came to be known as $D_1$ receptors, and dopamine receptors whose stimulation either inhibited or not affect adenylyl cyclase as $D_2$ receptors. This classification was used until the new techniques of gene cloning were developed, which extended this classification to five subtypes ($D_1$ – $D_5$ receptors), which have been grouped into two 'subfamilies' of G-protein coupled (metabotropic) receptors, the $D_1$-like subfamily (including $D_1$ and $D_5$ receptors), and the $D_2$-like subfamily (including $D_2$, $D_3$ and $D_4$ receptors) (see Misale et al 1998; Cooper et al 2003; Alexander et al. 2009).

Understanding of the functions mediated by dopamine receptors has been greatly factilitated by the development of selective dopamine receptor agonist and antagonist drugs. It has been shown that dopamine receptors with the same pharmacological features may have different metabolic effects depending on the area of the brain in which they are expressed. For instance the $D_2$ receptors are found in the nucleus accumbens and the dorsal striatum; stimulation of these receptors has no effect on adenylyl cyclyse activity in the accumbens, whereas it inhibits the enzyme in the dorsal striatum.

#### 1.3.4.1. $D_1$ dopamine receptors

$D_1$ receptors are principally found in caudate nucleus, putamen, nucleus accumbens, olfactory tubercle and amygdala. The fact that this receptor has not been found in the substantia nigra or VTA may indicate that this receptor does not act as an autoreceptor (see Cooper et al 2003; Meador-Woodruff 1994). This receptor binds benzazepines with high affinity, including the full agonist SKF-81297 (6-chloro-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol) (Andersen and Jansen 1990; Arnt et al. 1992;

Izenwasser and Katz 1993) and the antagonists SKF-83566 (bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hydrobromide) and SCH-23390 (7-chloro-8-hydroxy-3-methyl-5-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) (Molloy et al. 1986; Vieira-Coelho and Soares-da-Silva 2000; Waddington 1986).

#### 1.3.4.2. $D_2$ dopamine receptors

These receptors are widely expressed throughout the brain. They are present in the substantia nigra, VTA, and hypothalamus. They are also highly expressed in the caudate nucleus, putamen, nucleus accumbens, olfactory tubercle, and various regions of the cerebral cortex (see Cooper et al 2003; Meador-Woodruff 1994). In the substantia nigra, VTA and hypothalamus, $D_2$ receptors are present on dopaminergic neurones of the nigrostriatal, mesolimbic and tuberoinfundibular dopaminergic pathways. Stimulation of these $D_2$ autoreceptors inhibits dopamine release.

$D_2$ receptors have a high affinity for 'conventional' antipsychotic (neuroleptic) drugs. These drugs, especially the butyrophenones (e.g. haloperidol and spiperone) and the benzamides (e.g. sulpiride, amisulpride, eticlopride and raclopride) are highly selective antagonists of the $D_2$-like receptor 'subfamily', but have similar affinities for different members of the 'subfamily'. Other 'conventional' antipsychotics, for example the phenothiazines (e.g. chlorpromazine) have more complex pharmacological profiles, although the ability to block $D_2$-like receptors is a prominent feature of all 'conventionl' antipsychotics (Lawler et al. 1999; Moreland et al. 2004; Patel et al. 1997; Perrault et al. 1997; Schotte et al. 1996; Seeman and Van Tol 1994; Sokoloff et al. 1990; Van Tol et al. 1991).

Selective agonists exist for $D_2$-like receptors, but none of these shows clear distinction between $D_2$, $D_3$ and $D_4$ receptors (see below). Among the most extensively studied of these agonists are quinpirole and bromocriptine (Seeman and Van Tol 1994; Sokoloff et al. 1990; Van Tol et al. 1991).

#### 1.3.4.3. $D_3$ dopamine receptors

$D_3$ receptors are principally found in the nucleus accumbens, in contrast to the dorsal

striatum, where $D_2$ receptors predominate over $D_3$ receptors. Other areas where $D_3$ receptors are expressed, but to a lesser extent, include the substantia nigra and VTA (where they may act as autoreceptors) (see Meador-Woodruff 1994). The $D_2$-like receptor agonists 7-OH-DPAT ((+)-7-hydroxy-2-aminopropylaminotetralin) shows some preference for $D_3$ over $D_2$ receptors (Mulder et al. 1987; Seeman and Van Tol 1994). Nafadotride is a $D_3$ receptor antagonist with a somewhat lower affinity for $D_2$ than $D_3$ receptors (Fliestra and Levant 1998; Pilla et al. 1999). Conventional antipsychotics show little selectivity for $D_2$ over $D_3$ receptors (Alexander et al. 2009).

#### 1.3.4.4. $D_4$ dopamine receptors

The $D_4$ receptors are the least abundantly expressed of all receptors in the $D_2$-like receptor subfamily (Jaber et al. 1996). $D_4$ receptors are expressed in the cortex, hypothalamus, amygdala, thalamus and cerebellum (Meador-Woodruff 1994). Most conventional antipsychotics are effective $D_4$ receptor antagonists, although sulpiride and eticlopride show a modest preference for $D_2$ and $D_3$ receptors over $D_4$ receptors (Seeman and Van Tol 1994). Some atypical antipsyhcotics (e.g. clozapine) have high affinity for the $D_4$ receptor, in contrast to benzamides (e.g. sulpiride). A relatively selective $D_4$ receptor antagonist is L-745870 (3-([4-(4-chlorophenyl)piperazin-1-yl]methyl)-1H-pyrrolo[2,3-b]pyridine), which is effective in some animal models of executive function (Zhang et al. 2004; Moustgaard et al. 2008). Sonepiprazole (U-101,387) is another potent $D_4$ receptor antagonist which has does not block amphetamine-induced locomotor activity, and does not produce the extrapyramidal effects caused by $D_2$ receptor antagonists (Merchant et al. 1996). A full potent agonist at $D_4$ receptors WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide), a drug better known for its potent action as a 5-$HT_{1A}$ receptor antagonist (Marona-Lewicka and Nichols 2009).

#### 1.3.4.5. $D_5$ dopamine receptors

These receptors are expressed in a few areas of the brain including the hippocampus, thalamus and hypothalamus (Meador-Woodruff 1994). At present there are few selective ligands available which allow studying the functional differences between $D_1$ and $D_5$ receptors, and few data are available at the present time about the specific properties of

the $D_5$ receptor (Giorgioni et al. 2008). There are no selective agonists for $D_5$ receptors, but 4-Chloro-7-methyl-5,6,7,8,9,14-hexahydrodibenz[*d,g*]azecin-3-ol has been reported to be a selective antagonist for $D_5$ receptors (Mohr et al. 2006).

#### 1.3.4.6. Antipsychotics

Henri Laborit was the first to use chlorpromazine as a tranquilizer agent for patients undergoing surgical procedures. Soon this drug and other phenothiazines, and later butyrophenones (e.g. haloperidol), were used to treat the positive symptoms associated with schizophrenia (Kane 1989). This group of drugs are known as the 'typical', 'conventional' or 'first generation' antipsychotics (see King and Waddington 2004). The high affinity of conventional antipsychotics for $D_2$-like receptors, and the similar order of potency of these drugs in receptor binding studies and in clinical practice (Seeman et al. 1976) is one of the cornerstones of the "dopamine theory" of schizophrenia which posits that schizophrtenia is associated with hyperactivity in the mesolimbic/mesocortical dopaminergic pathway, which may be corrected by blockade of $D_2$-like receptors in the cerebral cortex and subcortical limbic structures (see King and Waddington 2004).

A major shortcoming of typical antipsychotic drugs in clinical practice is their propensity to produce extrapyramidal side-effects. It is generally agreed that these side-effects arise from blockade of $D_2$-like receptors in the dorsal striatum, the target region of the nigrostriatal dopaminergic pathway (see Cunningham-Owens 1999). Another shortcoming of these drugs is that they are not effective in treating the negative symptoms of schizophrenia (see King and Waddington 2004). In contrast, the 'atypical' antipsychotics drugs, also known as second-generation antipsychotics (e.g. clozapine, quetiapine, olanzapine) are less liable to induce extrapyramidal side-effects, and are claimed to be more effective in treating the negative symptoms and cognitive deficits of schizophrenia (Corrigan et al. 2003; Müller-Spahn 2002).

The finding that 'atypical' antipsychotics have lower affinity for $D_2$-like receptors and also have high affinity for other types of receptor, including 5-hydroxytryptamine-2 (5-$HT_2$) receptors has led some authors to question the central role of $D_2$-like receptors in antipsychotic action (Meltzer et al. 1989), although other authors still regard antagonism of $D_2$-like receptors as the key common feature of all antipsychotic drugs (Kapur et al.

2000).

Several competing explanations have been proposed for the clinical efficacy of atypical antipsychotics. One theory proposes that effective antipsychotic action requires the dual action of blockade of $D_2$-like dopamine receptors and blockade of 5-$HT_{2A}$ and/or 5-$HT_{2C}$ receptors, a profile exemplified by clozapine (Meltzer et al. 1989; Meltzer 1995). Other writers have pointed to the differential effects of typical and atypical antipsychotics on different subtypes of $D_2$-like receptors: atypical antipsychotics (such as clozapine) act preferentially on $D_3$ and $D_4$ receptors, which are found mainly in the limbic system (see above), whereas the typical antipsychotics (such as haloperidol) act mainly at the $D_2$ receptors which are predominantly found in the caudate and putamen (see above) (see Cooper et al 2003; Kandel et al 2000; Mailman and Murthy 2010; Squirel et al 2008).

Yet another theory proposes that a final common pathway of the action of atypical antipsychotics is 5-$HT_{1A}$ receptor-mediated release of dopamine in the medial prefrontal cortex (Bantick et al. 2001; Ichikawa et al. 2001; Chou et al. 2003). However, although clozapine is known to act as a partial agonist at 5-$HT_{1A}$ receptors, not all atypical antipsychotic drugs share this action. Moreover, some behavioural effects of clozapine that had been attributed to 5-$HT_{1A}$ receptor stimulation have been found not to be antagonized by the 5-$HT_{1A}$ receptor antagonist WAY-100635 (see Zhang et al. 2005a, 2005b).

Aripiprazole has been described as belonging to a third generation of antipsychotics. Like clozapine, this drug has a prefential high affinity for $D_2$ and $D_3$ receptors and also $5HT_{1A}$, $5HT_{2A}$ and $5HT_{2B}$ receptors. However, in contrast to clozapine, aripiprazole acts as a partial agonist at $D_2$-like receptors (Lawler et al. 1999; Davies et al. 2004; DeLeon et al. 2004). It has been proposed that this property of aripiprazole enables it to ‘stabilize’ dopaminergic function, elevating hypo- and suppressing hyper-dopaminergic activity (Byars et al. 2002; Potkin et al. 2003). Although there is some debate about the mechanism of action, it seems to be effective on treating both positive and negative symptoms of schizophrenia (Mailman and Murthy 2010; Swainston Harrison and Perry 2004).

In summary, the pharmacological actions of ‘typical’ antipsychotics is well established;

these drugs are all antagonists of $D_2$-like dopamine receptors, and a strong case can be made for attributing both their therapeutic and their adverse effects to this action. In contrast, 'atypical' antipsychotics have complex and varied pharmacological profiles, and a unified theory of their mode of action remains elusive (see King and Waddington 2004; see Chapter 5 for further discussion).

### *1.3.5. The behavioural role of dopamine*

Dopamine is a neurotransmitter which is implicated in a wide number of behaviours, some of the most important of which are described below.

#### 1.3.5.1. Dopamine and motor function

One of the most important pieces of evidence for the involvement of dopamine in the control of motor functions came for the studies of Parkinson's disease in humans, which involves degeneration of the nigrostriatal dopaminergic pathway (see Robbins and Everitt 2002). Parkinson's disease is charaterized by a progressive depletion of dopaminergic neurones from the SNc. Patients suffering from Parkinson's disease experience muscular rigidity, tremor, difficulties with the initiation of movement and loss of postural reflexes (see Cooper et al 2003; Kandel et al 2000). The principal treatment modality for Parkinson's disease is systemic treatment with L-DOPA, the precursor of dopamine. Although the precise mechanism of L-DOPA's therapeutic efficacy is uncertain, it is generally agreed that it results in a restoration of striatal dopaminergic function (see Tintner and Jankovic 2002).

The involvement of the dopamine system in Parkinson's disease has also been modelled in animals. A widely employed model in early research in this area was the 'turning rodent' model, in which rats received unilateral injections of the selective neurotoxin 6-hydroxydopamine (6-OHDA) into the SNc or caudate nucleus (Ungerstedt 1971b; c). This results in unilateral degeneration of the nigrostriatal dopaminergic pathway. The lesion does not result in gross abnormality of spontaneous locomotor behaviour. However systemic treatment with a postsynaptically acting dopamine receptor agonist (e.g. apomorphine) causes the rat to rotate contraversively (i.e. 'away from' the lesioned side), due to asymmetrically enhanced receptor stimulation in the lesioned hemisphere

caused by receptor 'upregulation' following the loss of dopaminergic afferents in the striatum. In contrast, systemic treatment with an 'indirect agonist' (e.g. d-amphetamine), which releases endogenous dopamine from presynaptic terminals, produces ipsiversive rotational behaviour due to asymmetrically reduced receptor stimulation in the lesioned hemisphere caused by loss of dopaminergic terminals from the lesioned side (Blandini et al. 2008; Meredith and Kang 2006). Interestingly, although rats with unilateral dopamine depleting lesions generally show near-normal spontaneous locomotor behaviour, bilateral 6-OHDA lesions render rats severely hypokinetic and aphagic; death from starvation may ensue unless the rats are manually fed (Ungerstedt 1971 a,b,c).

In general, increased dopaminergic activity tends to produce behavioural hyperactivity whereas suppression of dopaminergic activity has the opposite effect. Very high doses of d-amphetamine and dopamine receptor agonists induce stereotyped behaviours (such as licking or self-chewing) whereas lower doses produce increments of locomotor behaviour (Jackson and Westlind-Danielsson 1994). Stereotyped behaviours are believed to be caused by overstimulation of dopamine receptors in the dorsal striatum, whereas hyperlocomotion is mediated by an increase of dopaminergic activity in the nucleus accumbens (Joyce and Iversen 1984; Kelley et al. 1989; Kelley et al. 1988; Kelly and Iversen 1976; Kelly et al. 1975; Koob et al. 1978; Solomon and Staton 1982).

Locomotor activity can be induced by the administration of both $D_1$-like (Desai et al. 2005; Nergardh et al. 2005) and $D_2$-like receptor agonists (Eilam and Szechtman 1989; Van Hartesveldt et al. 1992) and the opposite effects are produced by the administration of the respective antagonists (Hoffman and Beninger 1985; Jackson et al. 1989a; Jackson et al. 1989b; Jackson and Westlind-Danielsson 1994; Wanibuchi and Usuda 1990). The dopaminergic input to the 'motor loop' has been discussed earlier (section 1.3.2.4). As indicated above, striatal neurones of the 'direct' projection are thought mainly to express excitatory $D_1$ receptors; while neurones of the 'indirect' loop are believed mainly to express inhibitory $D_2$ receptors. Since the direct and indirect pathways are believed to exert opposing effects on the striatal output structures (SNr and GPi), the two receptor populations may be expected to exert summatory effects on overall striatal output. This may account for the qualitatively similar effects of $D_1$-like and $D_2$-like receptor agonists and antagonists on motor functions (see Gerfen 2004).

#### 1.3.5.2. Dopamine and arousal and the sleep/wakefulness cycle

Until recently, little attention was paid to dopamine in the regulation of the sleep/wakefulness cycle despite the fact that it was known that dopamine modulates sleep and waking (Monti and Monti 2007). In part, this was due to the fact that the firing rate of dopaminergic cells in the substantia nigra and VTA had been reported not to change with behavioural state (Hobson et al. 2000). Nevertheless Lu et al (2006) recently reported that in the periaquiductal gray area (which projects to areas related with sleep-wake control) there are 'wake-active' dopaminergic neurones. It was hypothesized that these dopaminergic neurones formed part of the monoaminergic ascending wake-promoting arousal system, along with the 5-HTergic, noradrenergic, orexinergic and histaminergic neurones of the dorsal raphé, locus coeruleus, lateral and/or perifornical hypothalamic areas, and tuberomammillary nucleus, respectively (Saper et al. 2005).

Dopamine concentration during rapid-eye-movement (REM) sleep and the waking state is higher in the accumbens and prefrontal cortex than during non-REM (slow wave, SWS) sleep (Lena et al. 2005). There is also evidence from studies with mice that show abnormally high dopamine levels during REM sleep. This abnormality can be reversed by $D_2$ receptor antagonists. Electrophysiological recordings from neurones of the VTA and SNc indicate that the mean firing rate varies little across the sleep/wakefulness cycle; however there are changes in the pattern of firing, characterized by increases in burst firing during wakefulness and REM sleep compared to SWS (Monti and Jantos 2008).

There is also clinical evidence for an important role of dopamine in sleep and wakefulness. Daytime sleepiness associated with narcolepsy can be treated with amphetamine and related drugs, one of whose actions is to promote dopamine release (Mitler et al. 1994). Furthermore, it is known that patients with Parkinson's disease and schizophrenia often have associated sleeping problems such as day-time sleepiness and a REM sleep disorder (Adler 2005; Gagnon et al. 2002; Miller et al. 1983; Tandon et al. 1992).

Behavioural findings in animals are also consistent with a role of dopamine in sleep. Pharmacological manipulation using $D_1$-like receptor agonists not only induces locomotor activity (see previous section), but also promotes waking and reduces both

REM and SWS in rats. This effect can be reversed by $D_1$-like receptor antagonists (Bo et al. 1988; Kropf and Kuschinsky 1993; Monti et al. 1990; Ongini et al. 1993; Ongini et al. 1985; Trampus et al. 1993; Trampus and Ongini 1990). $D_2$-like receptor agonists (quinpirole and bromocriptine) also induce SWS and REM sleep at low doses in rats, whereas high doses produce the opposite effect. Both these effects can be blocked by the $D_2$-like receptor antagonist haloperidol (Isaac and Berridge 2003; Monti et al. 1988; Monti et al. 1989; Ongini et al. 1993). The opposite effects of low and high doses of $D_2$-like receptor agonists may be explained by a preferential effect of lower doses at $D_2$-like autoreceptors on neurones of the VTA and SNc, the less sensitive post-synaptic receptors being stimulated only by higher doses (see Monti and Monti 2007).

In summary, although there is a strong body of evidence that shows that the dopamine system modulates the sleep/wakefulness cycle, the exact mechanism of this interaction is not fully understood at present (see Monti and Monti 2007; Saper et al. 2005; Schwartz and Roth 2008; Squire et al 2008).

#### 1.3.5.3. Dopamine and food intake

The homeostatic system in the hypothalamus responds to metabolic and satiation signals from the intestine. This system controls the amount of food intake in accordance with the energetic need of the organism (Abizaid et al. 2006a; Morton et al. 2006). However, in conditions where high caloric food is available and little effort is required to obtain it, this system is insufficient to explain the overcomsuption of food (Berthoud 2004). One growing idea is that a ‘reward system’, which evolved during times when food was scarce, takes control over the homeostatic system. This reward system is assumed to respond not only to food but also to the smell, taste, sight or any other sensory cue that predicts it (Palmiter 2007). It has long been suspected that the mesolimbic dopaminergic pathway, especially the projection from the VTA ro the nucleus accumbens, plays a pivotal role in this hypothetical ‘reward system’ (Kalivas and Volkow 2005; Stricker and Zigmond 1984; Volkow and Wise 2005; Wang et al. 2001; Wise 2006; Yamamoto 2006).

The first evidence that implicated the dopaminergic system in the regulation of food intake came from studies of the effects of centrally administered 6-hydroxydopamine (6-OHDA) (Ungerstedt 1971a). When administered intraventricularly or injected into the

substantia nigra, this neurotoxin reduced food intake and body weight (Ungerstedt 1971a; Zigmond and Stricker 1972). This led some authors to hypothesize that dopaminergic neurones in the midbrain specifically participate in the control of the motivation to eat (Volkow and Wise 2005; Wise 2004(b); 2006), and that the effects of dopamine depletion resembled the lateral hypothalamic syndrome (see Squire et al 2008). Further evidence for an involvement of the dopaminergic system in the regulation of food intake came from demonstrations that feeding induced by electrical stimulation of the lateral hypothalamus (Phillips and Nikaido 1975) or by food deprivation (Wise and Colle 1984; Wise et al. 1978b) could be blocked by systemic administration of the $D_2$-like receptor antagonist pimozide. More recent studies using genetically modified mice lacking the enzyme tyrosine hydroxylase, which converts tyrosine to DOPA (see section 1.3.3), showed that these animals suffered from adipsia and aphagia which, if untreated, led to premature death. However, injection of L-DOPA allowed the animals to survive (Palmiter 2007; Zhou and Palmiter 1995).

Early studies of the role of dopamine in food intake focused on the nigrostriatal pathways. It was noted that in Parkinson's disease, a disorder in which this pathway degenerates, there is frequently marked weight loss (Durrieu et al. 1992). However, it can be argued that this effect may be due to the associated cognitive and movement deficits associated with the condition (Bachmann and Trenkwalder 2006; Hodge and Butcher 1980). There are also some disagreements about the results of animal studies. It has long been known that lesions of the SNc in rodents produce body weight loss (Baez et al. 1977) and reduce food intake (Smith et al. 1972; Ungerstedt 1971b). However, more recent studies have shown that partial lesions of the SNc produce motor impairment but not feeding deficits or body weight loss (Pioli et al. 2008). Furthermore, animals lacking striatal dopamine will consume normal food if it is placed in the mouth (Cannon and Palmiter 2003). This led to the suggestion that the nigrostriatal pathway is needed for feeding-related behaviour, including goal-directed movement and food seeking behaviour, rather than food intake per se (Palmiter 2008).

More recent work has generally focused on the mesolimbic dopaminergic pathway. It has been suggested that the VTA is involved in food motivation (Papp and Bal 1986; 1987), drug self-administration (Corrigall et al. 1992), goal-directed behavior, motor control, and the recognition of rewarding stimuli (Narayanan et al. 2010), rather than feeding

behaviour itself.

Pharmacological manipulation of dopaminergic neurones in the VTA affects food intake (Ljungberg et al. 1992; MacDonald et al. 2004; Naleid et al. 2005). Furthermore, microdialysis experiments report alteration of dopamine levels in the nucleus accumbens when the LHA or VTA are stimulated or in response to food intake (Bassareo and Di Chiara 1999; Church et al. 1987; Hernandez and Hoebel 1988). Electrophysiological studies have shown that the mesolimbic dopaminergic system is activated during search for food (Schultz 1997; 2007) and in the presence of food reinforcers (Hyland et al. 2002); Romo and Schultz 1990; Schultz and Romo 1990). In addition dopaminergic neurones respond to unexpected and novel rewards (Ljungberg et al. 1992; Mirenowicz and Schultz 1994). All these data suggest that the mesolimbic dopaminergic system is more involved with food reward processes rather than food consumption itself (Narayanan et al. 2010).

The dopaminergic target areas have also been the object of study in relation with food intake. For instance accumbal dopamine depletion has no effect on feeding (Salamone et al. 1993; Weissenborn and Winn 1992); in contrast, it markedly affects food-reinforced lever pressing in operant behaviour tasks (Ishiwari et al. 2004; Roberts et al. 1977; Salamone et al. 2003). $D_1/D_2$ receptor antagonists injected in the nucleus accumbens affect locomotor behaviour but not total food consumption (Baldo et al. 2002). These data support the idea that the mesolimbic dopaminergic system is related with 'effort-related feeding behaviour' but not with free feeding (Salamone et al. 2007). In contrast, dopamine depletion in nigrostriatal projection regions, including the lateral striatum, decreases feeding (Salamone et al. 1993). Other dopaminergic projection areas, including the amygdala and prefrontal cortex, appear not to be crucial for the regulation of free feeding (see Narayanan et al 2010).

The dopaminergic system in the midbrain is also affected by circulating hormones which are involved in food intake and energy metabolism. These hormones include leptin, insulin and ghrelin.

Leptin is an anorexigenic hormone synthesised in the adipose tissue which circulates in the blood; its level is proportional to the fat content in the body (Palmiter 2007). Leptin

has a powerful action on dopaminergic neurones; when administered directly into the VTA, it decreases both food intake and the firing of dopaminergic neurones. Moreover leptin receptor 'knockdown' mice, which lack leptin receptors in the VTA, consumed more food but did not gain body weight in comparison to control mice (Hommel et al. 2006).

Insulin is an anorexigenic hormone produced in the pancreas which is crucial for the regulation of circulating glucose. Insulin levels are increased after food ingestion, promoting the use of glucose by the tissues (Palmiter 2007). There is evidence that relates insulin with the dopamine system. Insulin receptors are co-localized with insulin receptors on dopaminergic neurones of the VTA, and central insulin administration promotes dopamine uptake in the VTA. Behavioural studies have shown that intraventricularly administered insulin reverses the effect of food deprivation on operant performance rewarded by food or electrical brain stimulation, suggesting that insulin receptors may contribute to the regulation of central motivational processes (Figlewicz 2003; Figlewicz et al. 2003).

Ghrelin is an orexigenic peptide hormone which is produced in the stomach. Dopaminergic neurones of the VTA express ghrelin receptors (Abizaid et al. 2006) (see also Narayanan et al 2010; and Palmiter 2007). When administered into the VTA or nucleus accumbens ghrelin produces an increment of food intake (Abizaid et al. 2006; Naleid et al. 2005).

In summary, the evidence reviewed in this section clearly indicates that pharmacological and endocrinological manipulation of the mesolimbic dopaminergic pathway can alter food intake. Although it remains unclear exactly what behavioural processes underlie these effects, there is a growing body of evidence for dopaminergic regulation of motivation and/or reinforcement processes, rather than a direct control of ingestive behaviour (see Palmiter 2007; Narayanan 2010).

#### 1.3.5.4. Dopamine and reinforcement and drug addiction

Some clarification is needed in order to distinguish the different uses of the terms

'reward' and 'reinforcer'. For instance, Salamone et al (2007) defined reinforcement as follows: "*Positive reinforcement refers to a process by which a response is followed by the presentation of stimulus that typically is contingent upon that response; these events are followed by an increase in the probability of the occurrence of that response in the future".* Although from the Skinnerian point of view the term 'reinforcer' does not include any subjective or emotional connotation, its use in the literature generally implies a motivational component (Salamone and Correa 2002). The term 'reward' is often used interchangeably with 'reinforcement', although the former term usually carries connotations of emotions and feeling of pleasure (Everitt and Robbins 2005; Wang et al. 2009).

The notion that catecholaminergic neurotransmission may play a key role in the rewarding properties of food and other reinforcers has a long history. Initially, interest was focused on the noradrenergic system (Stein 1968). Later research placed greater emphasis on the dopaminergic system, although a contributory role of the noradrenergic system was supported by a substantial body of evidence (see Crow and Deakin 1985; Bradshaw and Szabadi 1989). The 'dopamine hypothesis of reward' (Wise 1978, 1982) arose from findings on the effects of 6-OHDA-induced lesions of the dopaminergic pathways (Fibiger 1978) and effects of neuroleptics (conventional antipsychotics) on operant learning and on operant performance maintained by positive reinforcers (Wise 1982). The dopamine hypothesis of reward (Wise 1978) claims that the dopaminergic system, in particular the dopaminergic input to the nucleus accumbens, actively participates in the rewarding properties of natural rewards such as water, food and sex. In addition this system is presumed to be activated by drugs of abuse (Wise 2004(b)). The hypothesis incorporates the more restricted 'anhedonia hypothesis of neuroleptic action' (Wise 1982), which proposes that neuroleptics reduce the rewarding impact of reinforcers by blocking $D_2$-like receptors.

The origin of these hypotheses can be traced to original experiments that showed that rats could be trained to work for brain stimulation reinforcement, in electrical self-stimulation paradigms, when certain areas of the brain were stimulated (Olds and Milner 1954). Olds and Milner (1954) indentified areas localized in the LHA and surrounding areas which would support self-stimulation; these areas came to be known as 'pleasure centres' (Olds 1956). The importance of dopamine for self-stimulation received support when the

dopamine system was targeted using antipsychotics. These drugs reduced electrical self-stimulation, whereas amphetamine increased self-stimulation (Olds and Travis 1960; Stein 1962). The effects of these drugs on food-reinforced behaviour parallelled their effects on operant behaviour maintained by electrical brain stimulation (Wise 1982).

The anhedonia hypothesis of neuroleptic action (Wise 1982) encountered controversy since it was first created. The detractors of the theory argued that the apparent 'anhedonia' effects mainly reflected motor deficits rather than reward related deficits (Ahlenius 1995; Freed and Zec 1982; Gramling et al. 1984; Koob 1982). Another, more recent, criticism of the anhedonia hypothesis is that neuroleptics produce a deficit in goal seeking or motivation to gain the reward (in other words, the 'willingness' of the animals to make an effort to obtain the reward), but not a devaluation of the receipt of the reward (Baldo and Kelley 2007; Berridge and Robinson 1998; Robinson et al. 2005; Salamone and Correa 2002).

The effect of lesions of the dopaminergic terminal fields in the nucleus accumbens has also proved controversial; some studies found that such lesions failed to disrupt operant behaviour (Salamone et al. 1997). Salamone et al. (2005) proposed that dopamine in the accumbens needs to be considered as a *modulator* of functions related with motivational behaviours, rather than a mediator of reward (Salamone et al. 2005). Among the behaviours that may be modulated by dopaminergic transmission in the nucleus accumbens are: motivation, the execution of effort, effort-related decision making and behavioural activation over time (see Salamone et al. 2005).

It has been suggested that dopaminergic mechanisms may play a specific role in conditioned reinforcement (Beninger and Phillips 1980; Robbins et al. 1983). The $D_2$-like receptor antagonist pimozide has been found to block the acquisition of conditioned reinforcement (Beninger and Phillips 1980), whereas the dopamine releasing agent *d*-amphetamine, administered either systemically (Robbins et al. 1983) or directly into the nucleus accumbens (Taylor and Robbins 1984), potentiates responding for a previously established conditioned reinforcer. Intra-accumbens injections of the $D_1$-like receptor agonist SKF-38393 (1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol) or the $D_2$-like receptor agonist quinpirole mimicked the effect of *d*-amphetamine (Wolterink et al.

1993), whereas intra-accumbens injections of 6-OHDA attenuated it (Taylor and Robbins 1986).

Manipulation of the dopaminergic system does not only affect operant behaviour maintained by 'natural' reinforcers; behaviour controlled by drug reinforcers is also affected. This has been widely regarded as evidence for a role of the dopamine system in addiction. For instance microdialysis experiments in rats showed that extracellular dopamine in the nucleus accumbens is increased after the administration of almost all drugs of abuse including ethanol, nicotine, amphetamine, opiates and cocaine (Carboni et al. 1989; Di Chiara et al. 2004; Di Chiara and Imperato 1988a; b; Imperato and Di Chiara 1986; Imperato et al. 1986). This effect was also observed in non-human primates and in functional brain imaging studies in humans (Bradberry et al. 2000; Di Chiara et al. 2004; Drevets et al. 1999). This increment of dopamine levels after drug administration is most apparent in the case of the accumbens shell (AcbS) in comparison with accumbens core (AcbC) (Di Chiara 2004). Studies using intracerebral drug administration also indicated that the accumbens shell is more sensitive than the accumbens core (Ikemoto 2003; Ikemoto et al. 1997; McBride et al. 1999).

More evidence for the involvement of the dopamine system in drug reinforcement came from pharmacological studies. Administration of dopamine receptor antagonists either systemically or centrally has been shown to decrease self-administration of cocaine or amphetamines (Koob 1992). Administration of opiates increases the firing of VTA neurones and increases the release of dopamine in the nucleus accumbens. However, 6-OHDA-induced lesions of the mesolimbic system or administration of dopamine receptor antagonists have been found not to affect heroin-self administration (Di Chiara and North 1992; Koob 1992). This may suggest that the reinforcing properties of opiates are mediated by both dopaminergic and non-dopaminergic mechanisms. Nicotine is an effective reinforcer in self-administration paradims. Nicotine is an agonist of nicotinic acetylcholine receptors. Administration of dopamine receptor antagonists and lesions of the mesolimbic dopaminergic system impaired nicotine self-administration, suggesting an interaction between dopaminergic and cholinergic mechanisms in the rewarding effect of nicotine (Picciotto et al. 1998; see also Squire 2008). Finally, alcohol self-administration can be attenuated by dopamine receptor antagonists; however, 6-OHDA

induced lesions of the nucleus accumbens did not alter responding for alcohol (Koob 1992; Koob and Le Moal 2001).

As indicated above, manipulation of the dopaminergic system can affect multiple aspects of operant behaviour (Choi et al. 2005; Kelley 2004; Kelley et al. 2005; Smith-Roe and Kelley 2000; Wise 2004(b)), including processes that operate in food-seeking and consumption, but which are deemed to be distinct from the 'pleasure' derived from primary reinforcers such as food (Everitt and Robbins 2005; Salamone and Correa 2002; Salamone et al. 2007; Salamone et al. 2005). For instance, lesions of the nigrostriatal projection induced by the selective neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) impaired the learning of a sequenced motor task (Matsumoto et al. 1999), an effect that has been attributed to a change in the organism's 'willingness' to expend effort in its quest for reinforcers (see Salomone 2007). Low doses of $D_2$-like receptor antagonists reduced lever pressing, but had less effect on nose-poking (Ettenberg et al. 1981), suggesting that dopamine receptor antagonism affects motor performance. In another experiment rats were trained under progressive-ratio and continuous reinforcement (fixed ratio [FR] 1) schedules; haloperidol (0.03 mg/kg) suppressed responding on the progressive-ratio schedule but not on the FR 1 schedule (Caul and Brindle 2001). Similarly, dopamine depletion in the nucleus accumbens reduced lever pressing under FR 5 but not under FR 1 (Ishiwari et al. 2004). Similar results have been obtained with other ratio schedules (Aberman and Salamone 1999; Salamone et al. 2001). All these findings have been taken to indicate that dopaminergic transmission in the accumbens is an important determinant of the amount of effort that an organism can exert; the fact that responding on FR 1, which entails relatively little effort to obtain a reinforcer, is unaffected by dopaminergic manipulations has been taken as evidence that dopaminergic mechanisms are not involved in motivational processes (Salamone et al. 2007). Although this argument appears intuitively reasonable, it may not fit comfortably with theoretical interpretations of the interactions of motor and motivational processes in ratio schedule performance (Killeen 1994); this issue will be examined in some detail in section 1.4.3.

Another approach to dissociating the effects of dopaminergic manipulation on motor and motivational processes makes use of quantitative analysis of operant behaviour. An example of this approach is the analysis of variable-interval schedule performance based

on Herrnstein's (1970) hyperbolic 'response strength' equation (see Bradshaw and Szabadi 1989). According to Herrnstein's equation, response rate is a hyperbolic function of reinforcement rate, defined by two free parameters, $R_{max}$, which represents the motor capacity of the organism, and $K_H$, which expresses reinforcer efficacy (lower value of this parameter indicate higher reinforcer efficacy) (Bradshaw and Szabadi 1989; the two parameters are designated $k$ and $r_0$ in Herrnstein's [1970] paper). Morley et al. (1984, 1987) found that the $D_2$-like receptor antagonist pimozide reduced $R_{max}$ but had no effect on $K_H$, suggesting that the drug affected motor rather than incentive processes. However, other workers found that pimozide and other dopamine receptor antagonists also affected $K_H$. (Heyman et al. 1987). The basis for this discrepancy has not been resolved.

Recently another mathematical model which may dissociate effects of drugs and brain manipulations on motor versus motivational processes is the 'Mathematical Principles of Reinforcement' model (MPR) (Killeen 1994). This model has been used in studies of systemic treatment with antipsychotics and other centrally acting drugs, and lesions of several brain structures including the nucleus accumbens (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; Ho et al. 2003; Kheramin et al. 2005; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b) This is the core theme of this thesis and will be discussed in detail in the experimental chapters of the thesis (see also section 1.4).

In summary, although there is compelling evidence for a role of dopamine in the control of behaviour by reinforcers, the view that dopaminergic neurotransmission is primarily responsible for the 'hedonic' properties of all reinforcers remains controversial. One of the difficulties that besets this area is that operant behaviour entails multiple behavioural mechanisms, and the interaction between these mechanisms is imperfectly understood (Berridge 1996; Di Chiara et al. 2004; Salamone and Correa 2002; Salamone et al. 1997).

#### 1.3.5.5. Dopamine and higher cognitive functions

Several methodologies have been developed to evaluate some of the aspects encompassed by the term 'cognitive functions' such as working memory, attention and executive functions, temporal judgement and decision making. A role for dopaminergic mechanisms has been proposed in some of these functions. In some cases, dopamine's

putative role has been attributed to its involvement in more basic behavioural processes that may be entailed in performance in complex 'cognitive' tasks.

For instance, a number of studies have examined the effect of manipulating the dopaminergic system on performance on the 5-choice serial reaction time task (5CSRTT: Robbins 2002). This task yields several behavioural measures which are thought to reflect different cognitive processes. Response accuracy is regarded as an index of 'attention'; the number of perseverative errors provides a measure of 'compulsivity'; the reaction time provides a measure of 'decision speed'; and the number of premature responses yields a measure of 'impulsivity' (Chudasama and Robbins 2006; Robbins 2002). It has been reported that $D_1$-like receptor partial agonist SKF-38393 injected into the medial prefrontal cortex improved the 'attentional' measure and the $D_1$-like receptor antagonist SCH-23390 decreased it (Granon et al. 2000). This effect was also replicated with a similar paradigm, the combined attention memory task (CAM) (Chudasama and Robbins 2004). In this paradigm the $D_1$-like receptor agonist SKF-81297 affected the 'attentional' measure (Chudasama and Robbins 2004).

The effect of manipulation of the dopaminergic system on 'decision making' has also been examined. This process is thought to be based on cost/benefit analysis (Salamone 1996; Salamone and Correa 2002; Salamone et al. 2007; Salamone et al. 1991). In one version of this kind of experiment, organisms are given a choice between a less preferred food (standard chow) at minimal cost, and a highly-preferred food at higher cost (FR5). In non-treatment situations rats generally obtained more of their food from responding on the more effortful FR5 alternative. $D_1$-like and $D_2$-like receptor antagonists and accumbal dopamine depletion shifted the rats' preferences from the high-cost to the low-cost reinforcer (Cousins et al. 1996). In another experiment using a T-maze task, one of the arms (A) was baited with 4 pellets of food which could only be obtained by climbing over a high barrier, whereas the other arm (B) had no barrier. Dopamine depleted and control rats climbed the barrier in arm A if no food was available in arm B. However when 2 pellets were presented in arm B and 4 pellets in arm A, the lesioned animals shifted their preference to B whereas the control rats continued to prefer arm A. The authors suggested that the mesolimbic dopaminergic system is a crucial pathway in the regulation of effort-related decision making (Cousins et al. 1996).

Dopaminergic manipulation has also been found to affect choice between outcomes that differ with respect to delay of reinforcer delivery ('intertemporal choice'). In this type of task, selection of a smaller immediate reinforcer in preference to a larger delayed reinforcer is often labelled 'impulsive choice' (Ainslie 1975). Cardinal et al. (2000) reported that preference for the larger delayed reinforcer was reduced by *d*-amphetamine when no intra-delay signals were presented, whereas the opposite effect was seen when the delay to the larger reinforcer was signalled by a light. According to Cardinal et al. (2000), facilitation of accumbal dopaminergic transmission induced by *d*-amphetamine promoted 'impulsiveness', and also enhanced the conditioned reinforcing effect of the intra-delay stimulus (see above, section 1.3.5.4). Destruction of the dopaminergic input to the OPFC has also been found to affect inter-temporal choice. Kheramin et al. (2004) found that rats with 6-OHDA-induced lesions of the OPFC tended to prefer the larger and more delayed of two reinforcing outcomes more than sham-lesioned rats; however the difference between the two groups depended critically on the presence of a delay to the smaller reinforcer. Analysis based on a quantitative model of inter-temporal choice (Ho et al. 1999) suggested that the lesion had a dual effect – increasing the rats' sensitivity to both the delay of reinforcement and the relative sizes of the reinforcers.

Further indirect evidence for the involvement of dopamine in cognitive processes come from human studies. For example, amphetamine-related drugs can improve the attentional deficits seen in attention-deficit/hyperactivity disorder (ADHD). This has led to the hypothesis that dopaminergic deficits in the prefrontal cortex are part of the cause of this disorder (see Sagvolden et al. 2005). The cognitive deficits experienced by schizophrenic patients and patients suffering from Parkinson's disease have also been attributed to dysfunction of the dopaminergic system (for review, see Braver and Todd 1999; Sawamoto et al. 2008; see also section 1.3.2.4).

## 1.4. Progressive-ratio schedules and the measurement of reinforcer value

### *1.4.1. Historical background*

A number of different approaches have been used to assess the 'efficacy' or 'value' of reinforcers, including methods based on Herrnstrin's (1970) quantitative law of effect

(see Heyman and Monaghan 1987; Bradshaw and Szabadi 1989; Stellar and Rice, 1989), 'place preference' paradigms (Carr et al. 1989), and the survival of responding following reinforcer 'devaluation' or extinction (Fouriozos et al. 1978; Pickens et al. 2005) (see Wise 1989 for a critique of some of these methods). One method that has been especially popular in behavioural pharmacological research is the progressive-ratio schedule of reinforcement. The progressive-ratio schedule was developed by William Hodos in 1961 as a method to study the motivational properties of reinforcers (Hodos 1961). At the time of its creation it was very innovative. One of the most widely used techniques to measure motivation at the time was the 'obstruction technique' (Moss 1924). This technique consisted in placing some obstacle (for instance an electrified grid) between the animal and the reinforcer (for instance food). In this situation the intensity of the electric current could be manipulated as well as others variables such as food deprivation level or reinforcer magnitude/quality. The principle behind this technique was that the electric current that an animal would be able to tolerate to obtain the reinforcer should correlate with motivational variables such as the food deprivation level (Moss 1924). The current intensity that stopped the animal from crossing the grid was called the 'breaking point' (Hodos1961). This technique presented several problems, especially those related with the establishment of reliable breaking points and the requirement of using aversive stimuli such as electric shock.

Hodos (1961) designed the progressive-ratio schedule to overcome some of the problems related with the obstruction technique. Hodos (1961) described the progressive-ratio as a method to measure reward strength by measuring the maximum number of responses that an animal will make in order to get a reinforcer. With this method a breakpoint could be obtained that was more sensitive to changes in reinforcer magnitude and deprivation level. In his first experiment, Hodos (1961) trained rats to press a lever on a progressive-ratio 2 (i.e., in order to obtain each successive reinforcer the rat had to increase the number of responses by 2 so the ratio progression was 2, 4, 6, 8, 10…). When the rat stopped responding for 15 minutes the session was terminated and the highest ratio completed was called the breakpoint. Hodos (1961) showed that the breakpoint was sensitive to reinforcer quality (different concentrations of condensed milk) and food deprivation level (i.e. no deprivation vs. 80% of their free feeding body weight). Hodos (1961) suggested that the progressive-ratio schedule would be a good method for assessing the efficacy of drug reinforcers in 'self-administration' paradigms.

In a second experiment (Hodos and Kalman 1963) two more variables were tested, namely the ratio step size and the volume of a sucrose reinforcer. The breakpoint increased as a function of the reinforcer volume. The breakpoint also increased as a function of step-size, although the number of reinforcers earned in a session declined (see Hodos and Kalman 1963).

Since its creation, the progressive-ratio schedule has been extensively used in assessing the efficacy of drugs and other reinforcers. For example it has been used in drug self-administration paradigms to measure the reinforcing properties of psychomotor stimulants (Depoortere et al. 1993; French et al. 1995; Li et al. 1994; McGregor et al. 1994; McGregor and Roberts 1993; 1995; Roberts 1989; Roberts et al. 1994; Roberts et al. 1989) and opiates (Roberts and Bennett 1993; Shaham and Stewart 1994), and electrical stimulation of different brain regions in 'self-stimulation' paradigms (Depoortere et al. 1999; Hodos 1965; Keesey and Goldstein 1968) (for an extended review see also Richardson and Roberts 1996; Arnold and Roberts 1997). However some problems related with the interpretation of the breakpoint when these drugs were administered are discussed below.

There are several ways of executing the progressive ratio schedule. For instance in its original form, the response/reinforcer ratio increased after each reinforcer delivery according to the arithmetic progression 2, 4, 6, 8,…. Other arithmetic progessions have been used, such as increments of 5 or 10 responses following each reinforcer. Some experimenters have preferred geometric progressions (e.g. 5, 10, 20, 40) (Hoffmeister 1979). A disadvantage of arithmetic progressions is that the increment becomes less and less discriminable as the schedule advances (for example, an increment of 10 responses marks a substantial change from a ratio of 10, but is probably indiscriminable when the preceding ratio is 200). Geometric progressions based on simple doubling of the response requirement have the disadvantage that the breakpoint is generally reached after a very small number of ratios. A compromise was suggested by Roberts and Richardson (1992), who used the exponential progression (1, 2, 4, 6, 9, 12, 15, 20, 25,…) derived from the formula $(5\times e^{0.2n})-5$, rounded to the nearest integer, where $n$ is the position in the sequence of ratios. The response increment requirement can also be increased at the start of each daily session (Griffiths et al. 1979; Griffiths et al. 1978; Hoffmeister 1979),

although the more common arrangement is for the response requirement to be increased after each reinforcer has been delivered (Bedford et al. 1978; Bezzina et al. 2008a; Bezzina et al. 2008b; den Boon et al. 2011; Hodos 1961; Kheramin et al. 2005; Mobini et al. 2000a; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). (How these different methodologies can affect the breakpoint is briefly discussed in section 1.4.2.)

### *1.4.2. The breakpoint and some of its shortcomings*

The great majority of behavioural pharmacological studies using the progressive-ratio schedule have relied on the breakpoint as the principal dependent variable, on the assumption that this provides a valid index of the 'efficacy' or 'value' of the reinforcer. However there has been increasing criticism of the use of this measure in recent years (Arnold and Roberts 1997; Killeen et al. 2009; Richardson and Roberts 1996; Rickard et al. 2009).

Perhaps the biggest problem related with this measure arises from the lack of a generally accepted methodology. For instance, there is no general agreement about the appropriate session duration, which varies from less than an hour to 18 hours in different studies (Arnold and Roberts 1997; Richardson and Roberts 1996). Neither is there agreement about the most appropriate ratio step size, despite the fact that this measure can affect the breakpoint (Hodos and Kalman 1963; see below). The kind of progression used (for instance, arithmetic or geometric) is another very important factor that is known to affect the breakpoint (Killeen et al. 2009). Furthermore, the breakpoint itself is arbitrary; there is no concensus about the amount of time that must elapse without any responding before the breakpoint is deemed to have been attained. For instance some authors have used a breakpoint criterion of 3 minutes (Dantzer 1976), others as long as 60 minutes (Roberts 1989). It has also been noted (Killeen et al. 2009) that the breakpoint is intrinsically unreliable because it is defined by a single data point (i.e. the ratio immediately preceding the criterion period of non-responding); no account is taken of responding that occurs in preceding ratios. Another problem with the breakpoint, which applies particularly to behavioural pharmacological experiments is that if the drug being tested alters the breakpoint, this will alter the length of the experimental session, which is clearly a problem when drugs with relatively short plama half-lives are being tested.

Because of these problems, some authors have attempted to produce a standard protocol for the progressive-ratio schedule. For example, Richardson and Roberts (1996) described an 'optimal' progressive ratio protocol to be used in cocaine self-administration paradigms, and also they discuss methodological factors (such as the type of ratio progression, the breakpoint criterion, etc) that need to be considered. Other authors have attempted to avoid some of the problems associated with variations of the session duration by using a standard session length and adopting the highest ratio completed within the time-limited experimental session, rather than the breakpoint, as the performance criterion (Aberman et al. 1998; Hamill et al. 1999; Ho et al. 2003; Weatherley et al. 2003).

In agreement with early observations of the sensitivity of the breakpoint to motivational variables (Hodos 1961; Hodos and Kalman 1963), Skjoldager et al. (1993) found that the breakpoint increased with the magnitude of the reinforcer and with the severity of food deprivation. In addition, they tested the effect of varying the height of the lever, and found that the breakpoint changed as a consequence of changes in this (presumed non-motivational) variable (Skjoldager et al. 1993). The sensitivity of the breakpoint to 'non-motivational' factors was noted by Aberman et al (1998) who argued that haloperidol's effect of reducing the breakpoint was probably due to a motor debilitating effect that prevented the rats from responding (Aberman et al. 1998).

Although the breakpoint does not reliably discriminate between motivational and motor influences of neurobiological interventions, quantitative analysis of response rates in successive ratios in the schedule may provide a basis for deriving separate indices of motor and motivational processes. Quantitative analyses of progressive-ratio schedule performance based on behavioural economic theory are discussed in the next section. Then, in section 1.4.4., the theory of schedule controlled behaviour developed by Killeen (1994), the Mathematical Principles of Reinforcement [MPR] model is described. MPR forms the theoretical substrate of the experimental work described in this thesis.

### *1.4.3. Behavioural economic analysis of progressive-ratio schedule*

Behavioural economics is a broad area of knowledge that was born as an opposition to the traditional neo-classical economic theory. According to classical theory the world is

inhabited by rational human beings intent on maximizing their access to goods ('homo economicus') (Thaler and Mullainathan 2008). In contrast, behavioural economists argue that this traditional economic approach ignores the important insights into human behaviour, including economic behaviour, that derive psychological studies (Thaler and Mullainathan 2008).

An important aspect of behavioural economics to which psychologists have contributed is the theory of 'decision making'. Principles derived from behavioural analytic approaches (using animals) have been used to develop models of decision making that have been successfully applied to improve understanding of human behavioural economics (Ainslie 1975; 2001; Bradshaw 2008; Logue 1988; Rachlin 2006). The fusion of behavioural and economic approaches has given rise to theories of the economic behaviour of small businesses, families and individuals operating with limited resources ('microeconomic theories') (Robin and Parkin 2001).

Another area of particular interest for behavioural economists, from the microeconomics perspective, is consumer demand (Hursh 1980; Rachlin et al. 1976). This special interest was due, among other reasons, to the analogy found between performance on schedules of reinforcement (for instance the progressive-ratio schedule) and demand curves (see Bradshaw 2008; Hursh and Silberberg 2008). Typically in a progressive-ratio schedule (Fig. 1.2 left hand panel) the response rate increases as a function of the ratio size until the subjects reach the maximum. After that the response rate decreases until eventually the subjects stop responding (see section 1.4). The progressive-ratio schedule has been extensively used in the field of behavioural economics to further understand motivated behaviours. The response rate function shown in the left-hand panel of Fig. 1.2 can be converted into a demand curve (Fig 1.2 right-hand panel), in which 'consumption' (i.e. the rate of reinforcement) is plotted against the 'price' (i.e. the response requirement or response/reinforcer ratio) in double-logarithmic coordinates. The demand curve illustrates the important economic concept of elasticity of demand. The relatively flat initial segment of the demand curve indicates that consumption is maintained at a high level despite the escalating price (i.e. demand is relatively 'inelastic'), while the steeper terminal segment shows that increases in price eventually lead to extinction of demand (Hursh and Silberberg 2008). When demand is totally inelastic, changes in price have no

effect on demand. In contrast, elastic demand implies that relatively small changes in price cause substantial changes in demand.

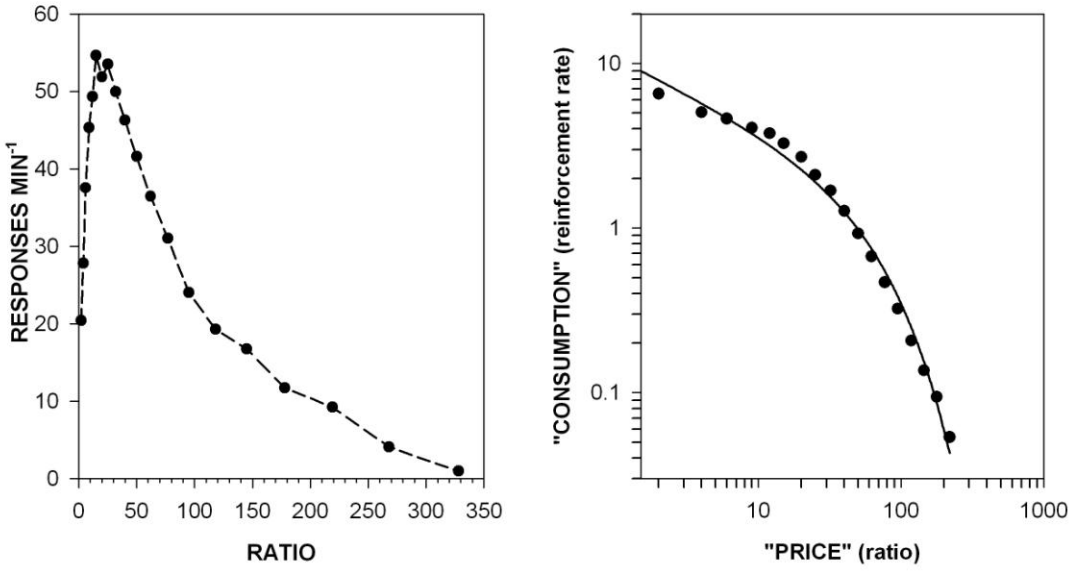


**Fig. 1.2**. Economic analysis of progressive ratio schedule performance. The left-hand graph shows mean rate of responding by 15 rats as a function of the number of responses (ratio) required to obtain a sucrose reinforcer; the right-hand graph shows the same data displayed as a demand function in which 'consumption' (reinforcement rate) is plotted against 'price' in double-logarithmic co-ordinates (Figure reproduced from Bradshaw 2008, showing unpublished data by J.F. Rickard, Z. Zhang, S. Body, C.M. Bradshaw and E. Szabadi).

Another important concept in behavioural economics is the substitutability of reinforcers (Rachlin et al. 1976). In particular it has been investigated how reinforcers can be interchanged and which are the variables that participate in this process. A very important variable is the availability of another reinforcer which is able to replace the first one. For instance, if coffee price goes very high the consumption of coffee may decrease and the consumption of tea may increase, as the latter maybe a good substitute for coffee. However, when the price of petrol is increased due to high tax, the consumption of petrol generally does not decline, probably because, for the majority of consumers, there is no satisfactory and readily available substitute to replace personal transport. This illustrates the close relationship between elasticity of demand and substitutability (Bradshaw 2008).

Some researches have applied these principles to the study of drug abuse in humans (see Johnson and Bickel 2006; Rodefer and Carol 1997). In contrast to what is popularly believed, the consumption of drugs of abuse is very sensitive to price change and the availability of other substitute drugs of abuse (Greenwald and Hursh 2006; Shahan et al. 2001). One model used in the study of drugs of abuse is the consumer demand model. This model proposes that the price (number of responses) needed in order to obtain a drug is an essential factor to predict the total amount of drug that would be consumed (Licata and Rowlett 2011). This is based on animal studies where increasing the price of food (e.g. increasing the number of responses under fixed-ratio schedules) reduced the total amount of food intake by animals and drug intake by humans (Bickel et al. 2000; Hursh et al. 1988; Hursh and Silberberg 2008).

An alternative way of conceptualizing the influence of the response requirement in ratio schedules is in terms of 'labour'. For example, in order to assess the role that responding plays in consumption, Allison (1983) developed the 'labour supply model' (Allison 1983; Allison and Boulter 1982). In this model the basic assumption is that consumption (for instance drug administration) declines as function of increasing effort to obtain the commodity (for instance increasing the response/reinforcer ratio). Moreover, this model also hypothesizes that when labour is too high, the relationship between consumption and labour will reverse and labour will decrease as a result of decreasing income (Allison 1983; Allison and Boulter 1982; Licata and Rowlett 2011).

Behavioural economic analysis of ratio schedule performance has been used extensively in behavioural pharmacology research. It is sometimes claimed that the elasticity of demand provides a quantitative measure of the 'intrinsic value' of reinforcers (Hursh and Silberberg 2008). For example, in a study of the effect of the cannabinoid receptor antagonist rimonabant in normal and genetically obese rats on ratio schedule responding for sucrose, Rasmussen et al. (2011) stated that "[the] demand equations quantify the value of a reinforcer by its sensitivity to price [ratio] increases". The authors found that rimonabant dose-dependently increased elasticity, and concluded that the drug had reduced the essential value of sucrose. However, there are problems with the use of elasticity as a measure of reinforcer value. For example, elasticity is influenced by the height of the initial segment of the demand function, which expresses the rate of consumption when the unit price is minimal (see Fig. 1.2). Various ways of normalizing

demand curves to take this into account have been recommended (Hursh and Silberberg 2008). However, the process of normalization has its own problems. Thus, Foster et al. (2009) recently reported data from hens responding for different wheat-based reinforcers, in which normalized demand curves yielded the *lowest* elasticity value associated with the food that was *least* preferred in free-choice tests.

Another shortcoming of demand curves for analysing progressive-ratio schedule performance is that, like the breakpoint, they do not provide a basis for identifying the effects of interventions on motor performance, since, in conventional demand curves, response rate is absorbed into the definition of 'consumption'. The potential for distinguishing between reinforcer value and motor influences on performance on progressive-ratio schedules is a major advantage of Killeen's (1994) model, described in the following section.

### *1.4.4. Killeen's 'Mathematical Principles of Reinforcement' (MPR) model*

As indicated above, the breakpoint, or alternatively the highest completed ratio, presents some problems, and these can be addressed by using Killen's mathematical model, *Mathematical Principles of Reinforcement* (Killeen 1994). In Killeen's model there are three factors which affect the control of behaviour by reinforcers. Firstly, reinforcers are purported to activate behaviour, and the duration of activation provides an index of the efficacy or 'incentive value' of the reinforcer. Secondly, the model recognizes that response rate is limited by biological constraints imposed, for example, by the motor system of the animal. Thirdly, it is proposed that schedules of reinforcement differ with respect to the degree to which they enable reinforcers to become 'coupled' to the index response. These three factors are represented by three parameters which feature in the fundamental equations of his theory.

Killeen (1994) proposed the following equation to describe response rate ($R$) in fixed-ratio schedules:

$$R = \frac{1-(1-\beta)^N}{\delta} - \frac{a}{N} \quad ; \qquad a, \delta > 0, \quad N \le a/\delta, \quad 0 < \beta < 1 \qquad [1]$$

where *N* is the response/reinforcer ratio. The parameter *a* (specific activation) defines the amount of time for which a reinforcer is able to activate behaviour; *β* ('currency parameter') represents the weight in short term memory assigned to the last response before the delivery of the reinforcer, and measures the efficiency with which a particular reinforcement schedule couples operant responses to reinforcers; *δ* is a parameter that expresses the minimum time that the animal requires to emit a response (i.e. it is the reciprocal of the theoretical maximum response rate).

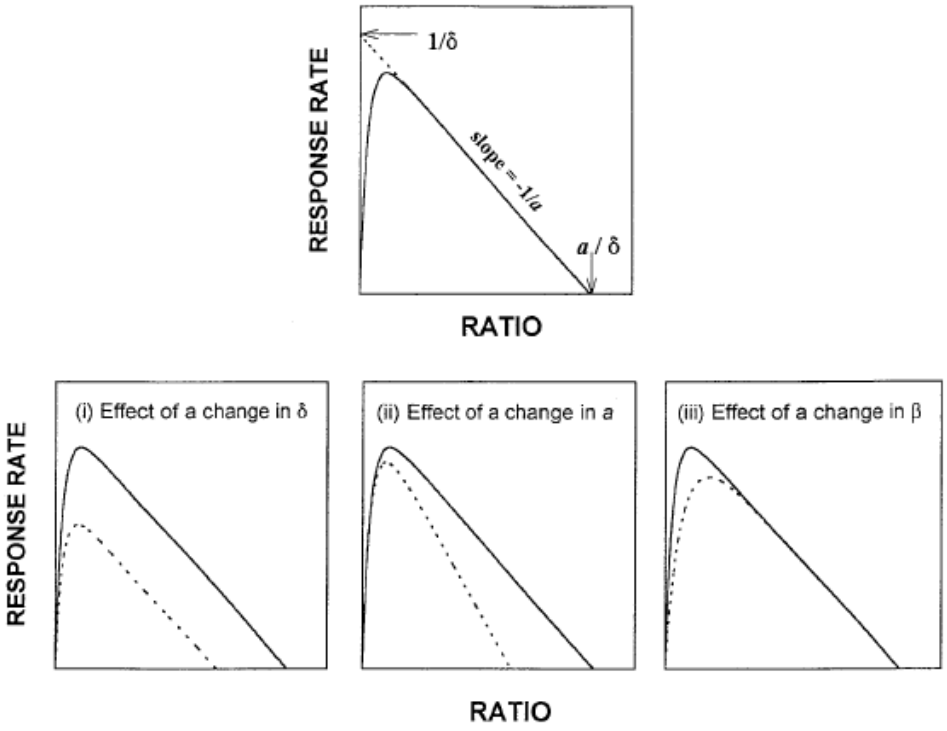


**Fig. 1.3** Theoretical response rate function (top graph) defined by Equation 1 (Killeen 1994). *Ordinate*: response rate (*R*); *abscissa*: response/reinforcer ratio (*N*). The graph exemplifies the measurement of the parameters *a* and *δ*: the projected point of intersection of the function with the ordinate is at $R=1/\delta$, and the slope of the descending limb of the function is $-1/a$; the point of intersection with the abscissa ('extinction ratio') is jointly determined by *δ* and *a* ($N=a/\delta$). The *three lower graphs* illustrate the hypothetical effects of a change in each of the three parameters of Equation 1; in each graph, the *continuous line* shows the baseline function, and the *dotted line* the changed function: (i) a change in *δ* alters the peak of the function and the extinction ratio; (ii) a change in *a* alters the slope of the descending limb of the function and the extinction ratio; (iii) a change in *β* alters the slope of the ascending limb of the function. See text for details. Figure adapted from Mobini et al. (2000).

Although Equation 1 was originally developed for fixed-ratio schedules (Killeen 1994), it is also able to describe accurately performance on progressive-ratio schedules (Bezzina et

al. 2008a; Bezzina et al. 2008b; Ho et al. 2003; Kheramin et al. 2005; Mobini et al. 2000a; Reilly 2003; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b; den Boon et al. 2011). Figure 1.3 illustrates response rates on ratio schedules as predicted by Equation 1.

Killeen's model has been used to detect the effects of magnitude and quality of reinforcers, brain lesions and systemic drug administration on motivational and motor-related processes (Bezzina et al. 2008b; Bezzina et al. 2008c; Covarrubias and Aparicio 2008; den Boon et al. 2011; Kheramin et al. 2005; Mobini et al. 2000; Reilly 2003; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b) (see section 1.4.5).

The relation between behavioural activation and incentive value derives from Killeen et al.'s (1978) studies which showed that unconditioned locomotor behaviour was triggered by reinforcers and that the duration of activation was in proportion with the magnitude and rate of reinforcement. Killeen et al. (1978) placed food deprived pigeons in an activity enclosure. Then they fed them once and recorded the behaviour for 30 minutes. They observed that the pigeons became active when the food was delivered and that this activation declined exponentially with time until the animals were settled (Killeen 1982; Killeen et al. 1978). When food was presented according to a periodic schedule, successive food deliveries produced a cumulative effect on the activation level which grew toward an asymptote. In addition they found that this cumulative effect became associated with the context in which the pigeons were fed (Killeen 1982; Killeen et al. 1978).

The specific activation parameter "*a*" has been proposed as a quantitative index of reinforcer efficacy (Killeen 1994; Killeen and Sitomer 2003). This suggestion has received some empirical support, as this parameter has been proved sensitive to manipulation of reinforcer size and quality (Bizo and Killeen 1997; Covarrubias and Aparicio 2008; Reilly 2003; Rickard et al. 2009). In this respect, *a* behaved similarly to $K_H$, the parameter of Herrnstein's (1970) equation which is also purported to express the incentive value of the reinforcer (Heyman and Monaghan 1987).

One of the purported advantages of applying Equation 1 to progressive-ratio schedule performance is that it provides a theoretical basis for distinguishing between the

influences of motor and motivational processes (Killeen 1994; Reilly 2003). Bizo and Killeen (1997) provided experimental support for this assumption. In an experiment with pigeons, two 'motivational' variables, the size of a reinforcer and the time that pigeons had access to a food hopper, were manipulated; *a* was affected by both manipulations (Bizo and Killeen 1997). In contrast, when the motor requirements of the operant task were manipulated (key-pecking versus treadle pressing), δ was higher in the case of the more effortful response.

In a recent experiment, Rickard et al (2009) reinforced rats with a range of volumes of a sucrose solution (6 – 300 µl). As expected, *a* increased as a function of reinforcer volume. This increment of *a* was accompanied by an increment of *δ*, which was attributed to longer post-reinforcement pauses caused by post-prandial behaviour associated with the larger reinforcer sizes (see below).

According to Equation 1 the breakpoint is predicted by the ratio of two parameters, *a/δ* (see Fig. 1.2). Therefore, an increase in the breakpoint can be produced either by a reduction in *δ* or by an increase in *a*, and vice versa. Consequently, it has been proposed that the parameter *a* of Equation 1 can provide a more reliable index than the breakpoint to measure motivation (Bezzina et al. 2008b; Bezzina et al. 2008c; den Boon et al. 2011; Kheramin et al. 2005; Mobini et al. 2000; Reilly 2003; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). More specifically, the parameter *a* has been proposed to be used to construct a quantitave scale to measure the incentive value of reinforcers (Reilly 2003).

Equation 1 was originally developed to describe performance on fixed- and variable-ratio schedules; however, it also provides a good description of overall response rates on progressive-ratio schedules (Bezzina et al. 2008b; Bezzina et al. 2008c; Covarrubias and Aparicio 2008; den Boon et al. 2011; Kheramin et al. 2005; Mobini et al. 2000; Reilly 2003; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). The application of Equation 1 to progressive-ratio schedule performance has been used successfully to detect the effects of brain lesions and drug administration on motivational and motor-related processes (Bezzina et al. 2008b; Bezzina et al. 2008c; Covarrubias and Aparicio 2008; den Boon et al. 2011; Kheramin et al. 2005; Mobini et al. 2000; Reilly 2003; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). A recent study of the effect

of reinforcer size on progressive ratio schedule performance (Rickard et al., 2009) revealed that while Equation 1 provided a good description of overall response rate, its fit to the 'running' response rate (response rate calculated after exclusion of the postreinforcement pause (Bizo et al., 2001)) was less satisfactory. Running response rate was, however, well described by a logistic function (see section 2.1).

$$R = R_i /(1+[N/b]^c) \qquad [2]$$

where $R_i$ is a parameter expressing the initial (maximum) response rate, $b$ expresses the rate of decay of the function, and the exponent $c$ modulates the curvature of the function. Rickard et al. (2009) found that $b$ was monotonically related to reinforcer size, while $R_i$ was unaffected by changes in reinforcer size.

The currency parameter $\beta$ derived from the MPR model has been less extensively studied in the field of behavioural pharmacology. Hence there are fewer empirical data to support the theoretical significance of this parameter. $\beta$ corresponds to the weight in short-term memory given to the most recent response (Killeen 1994). The weight given to individual responses decreases progressively as they become remote from the delivery of the reinforcer. It is assumed that this memory decay function conforms to the exponentially weighted moving average (Killeen 1981). According to Killeen (1994), $\beta$ is a weight without dimensions; if $\beta$ is close to 1 all the weight falls on the last response. However, when $\beta$ is close to 0 the weight is distributed across a longer sequence of responses. Based on a re-analysis of previously published data, Killeen (1994) estimated that a value of $\beta$ of 0.25 was typical of pigeons' keypeck responding on ratio schedules.

### *1.4.5. Application of MPR to behavioural pharmacology*

Despite more than 30 years of intensive research, there is no clear consensus about the putative role of the dopaminergic system in reward processes. Some authors claim that the manipulation of the mesolimbic dopaminergic system, for example by blockade of $D_2$-like dopamine receptors in the nucleus accumbens, degrades the incentive value of reinforcers. Other authors, however, claim that the effects of such manipulations on operant behaviour are due to motoric factors (see section 1.3.4.4; Salamone et al. 2007; Wise 2008). A similar controversy exists with respect to the orexinergic system.

However, it has been claimed that the orexinergic system may be involved in both motoric and motivational processes and that each of these processes is controlled by different neuronal sub-populations (Harris and Aston-Jones 2006; see section 1.2.3.4).

One of the principal objectives of the project described in this thesis is to explore the potential utility of MPR in examining the the roles of these neural systems in the control of motor and motivational processes. There is a small but growing literature on the application of MPR in behavioural neuroscience research. This literature is reviewed in the present section.

For over a decade MPR has been used to analyse the effects of acute systemic drug treatment on progressive-ratio schedule performance (Bezzina et al. 2008b; Bezzina et al. 2008c; den Boon et al. 2011; Kheramin et al. 2005; Mobini et al. 2000; Reilly 2003; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). For instance, there have been several studies examining the effects of conventional antipsychotics ($D_2$-like dopamine receptor antagonists) (den Boon et al. 2011; Mobini et al. 2000; Zhang et al. 2005a). In all these experiments, the $D_2$-like receptor antagonist haloperidol invariably reduced the breakpoint; analysis of the response rates using Equation 1 indicated that this was brought about not only by a reduction of the incentive value of food reinforcers (as defined by a reduction of the activation parameter $a$), but also by an impairment of motor capacity (as defined by an increment in the response time parameter $\delta$). The effect of haloperidol has been replicated with other $D_2$-like dopamine receptor antagonists, including *cis*-flupenthixol, raclopride and pimozide (Zhang et al. 2005a).

The psychostimulant and dopamine-releasing agent d-amphetamine has also been tested using MPR: Mobini et al. (2000) used the progressive-ratio schedule and Reilly (2003) a five-component multiple fixed-ratio schedule. Both studies found that d-amphetamine increased $\delta$. However, the two studies differed with respect to the observed effects on $a$. Mobini et al (2000) found a dual effect, lower doses of d-amphetamine (0.2mg $kg^{-1}$) reducing $a$ and higher doses increasing it (0.8mg $kg^{-1}$). In contrast, Reilly et al (2003) found an increase in $a$ across a broad range of doses, although a reduction of $a$ was seen with doses that were considerably higher than those used by Mobini et al. (2000) (3.2mg $kg^{-1}$). The basis of this discrepancy is unclear, although Reilly (2003) noted a number of methodological differences between the two studies, including different schedules (see

above), different reinforcers, and random (Reilly 2003) versus progressive (Mobini et al. 2000) presentation of the ratios.

Clozapine is an atypical antipsychotic which has a relatively low affinity for $D_2$-like dopamine receptors and high affinity for 5-$HT_{2A}$ and 5-$HT_{1A}$ receptors (see section 1.3.4.6). Several studies have examined clozapine's effect on the parameters of MPR (den Boon et al. 2011; Mobini et al. 2000; Zhang et al. 2005a; Zhang et al. 2005b). Clozapine has consistently been found to increase both *a* and *δ*. Although the breakpoint is increased with lower doses of clozapine, higher doses tend to reduce it. According to MPR, the breakpoint is jointly determined by *a* and *δ*. In the case of clozapine, the reduction of the breakpoint is attributable to the increase in δ, since *a* was increased by the drug. This is therefore an example of the breakpoint being reduced by the effect of a drug on 'motor' processes, and illustrates the difficulties that may arise when the breakpoint is used as an index of incentive value. The dual effect of clozapine on *a* and δ has been replicated with other atypical antipsychotics (quetiapine, olanzapine, ziprasidone: Zhang et al. 2005a). The qualitatively different profiles of effect of conventional and atypical antipsychotics on the parameters of Equation 1 has recently been confirmed by den Boon et al. (2011).

MPR was used by Ho et al (2003) to test the effect of the selective 5-$HT_{1A}$ receptor agonist 8-OH-DPAT. This drug was previously used in feeding experiments and the operant 'self-stimulation' (electrical brain stimulation reinforcement) paradigm. These studies showed both an increase of food intake and enhancement brain self-stimulation, effects which were attributed to an enhancement of motivation (Bendotti and Samanin 1986; Dourish et al. 1986; Montgomery and Grottick 1999; Montgomery et al. 1991). In agreement with this suggestion, Ho et al (2003) found that 8-OH-DPAT produced a dose dependent increment of *a*. There was also an increase of *δ*, suggesting that the increase of reinforcer value may have been accompanied by some impairment of motor functioning. Ho et al (2003) also found that the 5-$HT_{1A}$ receptor antagonist WAY-100635 had no effect on any of the parameters of MPR; however, when 8-OH-DPAT and WAY-100635 were administered together, the effect of 8-OH-DPAT was antagonized. Ho et al (2003) noted the similarity between the effects of 8-OH-DPAT and clozapine, and suggested that the motivation enhancing effect produced by clozapine might be mediated by 5-$HT_{1A}$ receptors (see below).

The hypothesis that clozapine's motivational effect was mediated by the $5HT_{1A}$ receptors was also investigated using MPR (Zhang et al. 2005b). In agreement with Ho et al.'s (2003) findings, clozapine and 8-OH-DPAT increased both *a* and δ. However, while WAY-100635 fully antagonized the effect of 8-OH-DPAT, it did not alter the effect of clozapine, indicating that clozapine's effect was not mediated by $5\text{-}HT_{1A}$ receptors.

MPR has also been used to analyse the effects of brain lesions on progressive-ratio schedule performance. Kheramin et al (2005) compared the performance of a group of rats whose orbital prefrontal cortex (OPFC) had been lesioned with quinolinic acid and another group that had received sham lesions with the vehicle (saline). After the animals recovered from surgery they were trained on the progressive-ratio schedule and the response rate data were used to fit Equation 1. The results showed that this lesion produced a reduction of the activation parameter *a*, suggesting that the lesion reduced the value of the food reinforcers. This result is consistent with previous findings by the same authors (Kheramin et al. 2002), based on an inter-temporal choice task, which indicated that lesions of the OPFC reduced the incentive value of food reinforcers.

Bezzina et al. (2008b) used MPR to analyse the effect of quinolinic acid-induced lesions of the nucleus accumbens core (AcbC). According to Wise (1982) the AcbC and its dopaminergic afferents are crucial to the regulation of the values of both natural rewards and drugs (see section 1.3.4.4). Bezzina et al. (2008b) found that the breakpoint was significantly lower in the lesioned group in comparison with the sham-lesioned group. Application of Equation 1 revealed that the activation parameter *a* was not altered in the lesioned group. However, the response time parameter $\delta$ was increased. In this experiment the magnitude of the reinforcers were manipulated. The value of *a* was higher in both groups when the reinforcer was two food pellets than when it was one pellet, consistent with Rickard et al.'s (2009) finding of a monotonic relation between reinforcer size and the value of *a*. Bezzina et al.'s (2008b) finding that the AcbC lesion did not affect *a* is consistent with the findings of Bezzina et al. (2007), based on an inter-temporal choice task, which indicated that lesions of this structure did not affect the incentive value of food reinforcers.

The findings reviewed in this section suggest that MPR may provide a useful framework

for analysing the effects of neurobiological interventions on reinforcement processes. Although only a limited range of interventions have been examined to date, the results obtained so far appear generally to support the claim that the application of Equation 1, derived from MPR, allows effects of interventions on performance that are mediated by changes in reinforcer value to be distinguished from effects that are mediated by changes in motor performance (Killeen 1994).

# CHAPTER 2

# EXPERIMENT 1: EFFECT OF OREXIN-B-SAPORIN INDUCED LESIONS OF THE LATERAL HYPOTHALAMUS ON PERFORMANCE ON A PROGRESSIVE-RATIO SCHEDULE

## 2.1. Introduction

The orexins are two neuropeptides which are synthesised in a small group of neurones localised in the hypothalamus and surrounding regions (see section 1.2.1). The orexin system has been implicated in several neurobehavioural functions, including reward processing and addictions (see section 1.2.3.4). According to the 'dichotomy of orexin function' hypothesis (Harris and Aston-Jones 2006), only the orexinergic neurones of the LHA are purported to be involved in reinforcement mechanisms (see section 1.2.3.4). Several experiments have studied the relationship between the orexin system and motivational processes using the progressive-ratio schedule of reinforcement (see section 1.2.3.3). Traditionally the breakpoint has been used as a measure of the subject's motivational state. However, as reviewed in section 1.4.1. the breakpoint is subject to several problems, that can be overcome by using Killeen's (1994) MPR model (see section 1.4.3).

According to MPR, the relationship between response rate (*R*) and ratio size (*N*) in ratio schedules can be described by the following equation:

$$R = \frac{1-(1-\beta)^N}{\delta} - \frac{N}{a} \qquad [1]$$

where δ ('response time') is the minimum time needed to execute a response, *a* ('specific activation') is the time for which a single reinforcer delivery is able to activate behaviour, and β ('currency'), which reflects the coupling of responses to reinforcers, expresses the weight given to the most recent response in the conditioning process (see section 1.4.3). A recent study of the effect of reinforcer size on progressive-ratio schedule performance (Rickard et al. 2009) revealed that while Equation 1 (see section 1.4.3.) provided a good description of overall response rate, its fit to the 'running' response rate (response rate calculated after exclusion of the post-reinforcement pause: Bizo et al 2001) was less satisfactory. Running response rate was, however, well described by the logistic function:

$$R = R_i/(1+[N/b]^c) \qquad [2]$$

where $R_i$ is a parameter expressing the initial (maximum) response rate, *b* expresses the rate of decay of the function, and the exponent *c* modulates the curvature of the function. Rickard et al. (2009) found that *b* was was monotonically related to reinforcer size, while $R_i$ was unaffected by changes in reinforcer size.

The present experiment used a combination of quantitative analysis of operant behaviour maintained by positive reinforcement with neurochemical lesions of orexinergic neurones in the LHA. The lesion was inflicted using the OxSap neurotoxin (see section 1.2.2). As the orexinergic system has been claimed to be involved in reward processing (see section 1.2.3.3), it was expected that the OxSap-lesioned group would show a lower value of the motivational parameter of Equation 1, *a* (specific activation), and the decay parameter, *b*, of Equation 2, compared to the sham-lesioned group, and a decrease in these parameters compared to their own pre-surgical performance. The effects of the lesion on locomotor behaviour, food consumption and body weight were also examined.

## 2.2. Methods

The experiments were carried out in accordance with UK Home Office regulations governing experiments on living animals.

### *2.2.1. Subjects*

Thirty experimentally naive female Wistar rats approximately 4 months old and weighing 250–300 g at the start of the experiment were used. They were housed under a constant cycle of 12 h light and 12 h darkness (light on 06:00 - 18:00), and were maintained at 80% of their initial free-feeding body weights throughout the operant behaviour experiment by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cages.

### *2.2.2. Surgery*

The rats were divided into two groups. The first group received bilateral lesions of the LHA with the orexin-B-saporin conjugate (OxSap: Advanced Targeted Systems, USA) (n=14). The control group was divided into two sub-groups which received either bilateral intra-LHA injections of unconjugated saporin (Advanced Targeted Systems, USA) (n=8), or (ii) bilateral intra-LHA injections of the vehicle solution alone (0.9% sodium chloride solution) (n=8). Anaesthesia was induced with isoflurane (4% in oxygen), and the animals were placed in a stereotaxic instrument (David Kopf), with the upper incisor bar set 3.3 mm below the inter-aural line. Isoflurane was kept at 2% in

oxygen during the surgical procedure. A small hole was drilled through the skull overlying each cerebral hemisphere for microinjection of the solutions into the LHA. The following coordinates were used to locate the LHA: AP -3.3, L ± 1.6, V -9.1, measured from bregma (Paxinos and Watson 1998).

Microinjections were administered with a 5 µl Hamilton syringe connected by a polyethylene tube to a 0.3-mm diameter cannula. The solution administered to the first group contained 15 ng OxSap dissolved in 0.3 µl of non-pyrogenic 0.9 % sodium chloride (Baxter Health Care, UK). (The dose used was based in preliminary studies carried out in this laboratory in which it was found that doses of 180 ng, 90 ng and 45 ng of OxSap produced substantial tissue damage in the LHA and surrounding areas. Furthermore these doses produced significant reductions of food intake and loss of body weight which necessitated euthanasia of most animals 2-3 weeks after surgery.) The cannula was slowly lowered to the target position, and the solution was injected during a 60-s period. The cannula was left in position for 3 minutes after completion of the injection, and was then withdrawn. The procedure for the other two groups was identical except that the injections were either 15 ng unconjugated saporin in 0.3 µl saline (n=8) or 0.3 µl of the saline solution (n=8).

### *2.2.3. Apparatus*

#### 2.2.3.1. Operant behaviour chambers

The rats were trained in custom built operant conditioning chambers of internal dimensions 20 cm × 23 cm × 22.5 cm. One wall of the chamber contained a recess into which a motor-operated dispenser could deliver 45-mg food pellets (TestDiet, MLab Rodent Tablet 45 mg; Sandown Scientific, UK). An aperture was situated 5 cm above and 2.5 cm to the left of the recess, through which a motor-operated retractable lever could be inserted into the chamber. The lever could be depressed by a force of approximately 0.2 N. The chamber was enclosed in a sound-attenuating chest; masking noise was provided by a rotary fan. An Acorn 5000 microcomputer and interface unit (Paul Fray Ltd.), programmed in ARACHNID BASIC and located in an adjoining room, controlled the schedules and recorded the behavioural data.

2.2.3.2. Locomotor behaviour chambers

Locomotor behaviour was recorded in transparent Perspex boxes of internal dimensions 42 cm × 32 cm × 29 cm, equipped with eight infrared photocell beams located at equal intervals along the long axis of the box, 4 cm above the floor. An Acorn microcomputer programmed in ARACHNID BASIC and located in an adjoining room recorded the data.

*2.2.4. Behavioural procedures*

2.2.4.1. Progressive-ratio schedule

Two weeks before the start of behavioural training, the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. Then they were trained to press the lever for a food-pellet reinforcer (45 mg) and were exposed to a fixed-ratio 1 schedule for 3 sessions, followed by fixed-ratio 5 for a further 3 sessions. Thereafter, they underwent daily training sessions under the progressive-ratio schedule. The progressive-ratio schedule was based on the following exponential progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, . . . , derived from the formula $[(5\times e^{0.2n})-5]$, rounded to the nearest integer, where $n$ is the position in the sequence of ratios (Roberts and Richardson 1992). Sessions took place at the same time each day during the light phase of the daily cycle (between 07:00 and 14:00) 7 days a week for the duration of the experiment. At the start of each session, the lever was inserted into the chamber; the session was terminated by withdrawal of the lever 50 min later. The rats were trained under the progressive-ratio schedule until the estimated parameters of Equation 1 (see *Data analysis*, section 2.2.6) had attained stability according to the following criterion. Each parameter was deemed to be stable when the cumulated change in the group mean value of the parameter over three successive blocks of 10 sessions was ≤ 10%. The value of parameter *a* increased steadily over 60 sessions and the formal criterion was not met until the 10$^{th}$ block. Surgery was carried out after 110 training sessions. Training continued for 40 sessions after surgery.

2.2.4.2. Locomotor behaviour

Each rat was tested at the same time of day, and under the same food restriction

conditions as in the operant behaviour experiment. Each session was 30 min in duration and the data were recorded in 6 successive epochs of 5 min. The tests took place 23 h after the last meal. The rats underwent the locomotor activity testing on 7 successive days: 5 habituation sessions and two recording sessions.

#### 2.2.4.3. Food intake

Food intake tests took place in the animal holding room. Each rat was placed in a cage similar to the home cage containing a pre-weighed dish containing approximately 25 g of the same 45-mg food pellets as were used in the operant behaviour experiment. The rats were left undisturbed for 60 min before being returned to their home cages. The dishes were re-weighed and the weight of food consumed was calculated. Three test sessions were carried out at intervals of 48 h while the rats were maintained under the same food deprivation conditions as in the operant behaviour experiment. In a second phase, the same procedure was carried out while the rats had free access to standard chow in their home cages. In this phase, the amount of chow consumed in 23 hours was also measured.

### *2.2.5. Immunohistochemistry*

At the end of the behavioural experiment, the rats were deeply anaesthetised with sodium pentobarbitone, and perfused transcardially with phosphate-buffered sodium chloride solution (PBS), followed by 10% formol PBS. The brains were removed from the skull and fixed in formol PBS for 3 days. Coronal sections (60-µm) were taken through the LHA region using a freezing microtome. Alternate sections were selected for labelling of neurone-specific nuclear protein (NeuN) and orexin.

NeuN was labelled as described by Jongen-Relo and Feldon (2002). The protocol followed was described by Bezzina et al. (2007). Three freshly sliced sections, taken at approximately 120-µm intervals between AP +3.0 and AP +3.6 were washed in 0.1 M PBS and placed in 0.5% $H_2O_2$ in PBS for 30 min at room temperature. Then they were washed twice in PBS and placed for 1 h in a blocking solution [10% normal horse serum (Vector Laboratories, Peterborough, UK), 1% bovine serum albumin (BSA, Sigma-Aldrich, Gillingham, UK) and 0.3% Triton X-100 (Sigma-Aldrich) in PBS]. They were then incubated for 48 h at 4°C in the primary antibody [monoclonal mouse anti-NeuN

serum (1:5,000, Chemicon, Chandlers Ford, UK) in 1% normal horse serum, 1% BSA and 0.3% Triton X-100 in PBS], washed twice in PBS, and incubated for 2 h at room temperature in biotinylated horse antimouse serum (Vector Laboratories; 1:1,000 in 1% BSA and 0.3% Triton X-100 in PBS). After two washes in PBS, they were placed for 2 h in avidin–biotin–horseradish peroxidase complex (1:200, ABC-Elite, Vector Laboratories) in PBS. Following two further washes in PBS, they were placed in a chromagen solution [0.05% diaminobenzidine (Sigma-Aldrich) and 0.01% $H_2O_2$ (Sigma-Aldrich)] for 5 min. The reaction was observed visually and stopped by washing in PBS. The sections were floated on to chrome-gelatine-coated slides and mounted with DPX.

The procedure for orexin staining was the same as that described above, except that the primary antibody was goat anti-orexin diluted 1:5,000 in PBS (Santa Cruz Biotechnology, USA) and the secondary antibody was horse anti-goat serum 1:1000.

NeuN-positive nuclei and orexin-positive neurones were counted blind from coded images of the sections using Image-J software (Wayne Rasband, National Institutes of Health, USA).

### *2.2.6. Data analysis*

The data from one rat belonging to the sham-lesioned group were discarded, because this rat failed to complete a sufficient number of ratios in each session to permit curve fitting analysis to be performed. A preliminary analysis comparing the immunohistochemical data and post-surgical performances of the two control sub-groups (i.e. the rats that received intra-LHA injections of unconjugated saporin and the saline vehicle) revealed no significant differences between the numbers of orexin-positive neurones or NeuN-positive nuclei or any of the performance measures, and therefore the data from the two sub-groups were pooled in all subsequent analyses. Analyses were thus based on the data from the OxSap-lesioned group (n=14) and the combined sham-lesioned group (n=15).

#### 2.2.6.1. Operant behaviour data

Data from the last block of 10 sessions before surgery and the four successive blocks of 10 sessions following surgery were used in the analyses.

(i) *Peak response rate.* Differences between the highest overall response rate (see below) attained during performance of the progressive-ratio schedule during the final pre-surgical block of sessions and the four post-surgical blocks were analysed by two-factor analysis of variance (group × block) with repeated measures on the latter factor. In the case of a significant main effect of group or a significant interaction term, comparisons were made between groups at each block using the least significance difference test.

(ii) *Highest completed ratio and breakpoint.* The breakpoint was defined as the last ratio to be completed before 5 min elapsed without any responding (Hodos 1961; Hodos and Kalman 1963). In most cases, this was identical to the highest ratio completed in the session. However, in some cases, this criterion was not met within the 50-min session. Therefore, the highest completed ratio and the breakpoint were analysed separately. The data were analysed in the same way as the peak response rate. Analysis of the highest completed ratio incorporated the data from all sessions, whereas analysis of the breakpoint entailed exclusion of data from sessions in which the formal breakpoint criterion was not attained.

(iii) *Overall response rate.* Overall response rate was calculated for each ratio using the total time taken to complete the ratio, including the post-reinforcement pause, measured from the end of the preceding reinforcer delivery until the emission of the last response of the ratio (Bizo and Killeen 1997). The first ratio (a single response) and any ratios that had not been completed at the end of the session were excluded from the analysis. The raw data from the final pre-surgical and the four post-surgical blocks of 10 sessions were analysed by three-factor analysis of variance (group × block × ratio) with repeated measures on the second and third factors. Equation 1 was fitted to the overall response rate data from each rat using an iterative least-squares method (SigmaPlot, Version 8.0), and the estimated values of the parameters β, δ and *a* were derived; goodness of fit was expressed as $r^2$, the proportion of the data variance accounted for by the equation. In agreement with previous findings (Bezzina et al. 2008b; Bezzina et al. 2008c; Ho et al. 2003; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b), examination of the data revealed that in some rats very low response rates were generated under the highest ratios, which did not conform to Equation 1. Therefore the equation was fitted to each rat's data after exclusion of these low rates using the following operational criterion (Mobini et al. 2000). Points were removed successively, starting

from the highest ratio completed, when the curve-fitting routine generated an abscissa intersection point ($a/\delta$) which lay to the left of the rightmost empirical datum point; such an intersection implies a negative predicted response rate, which is impossible empirically and specifically precluded by the model (section 1.4.3). A fit was accepted when the predicted response rates for all the surviving data points had positive values. This procedure seldom eliminated more than one datum point from the data sets derived from individual rats. A preliminary analysis of the estimates of the three parameters of Equation 1 ($a$, $\delta$ and $\beta$) in successive blocks of 10 sessions before surgery (analysis of variance: group × block) was carried out in order to check that there were no significant between-group differences in the pre-surgical data. Then the post-surgical changes of the estimates of the three parameters obtained during the final pre-surgical block of sessions and the four post-surgical blocks were analysed in the same way as the highest completed ratio and peak response rate.

(iv) *Running response rate.* Running rate was calculated by dividing the number of responses by the 'run-time' (i.e. the time taken to complete the ratio, excluding the post-reinforcement pause: Bizo et al. 2001). The data were analysed as described above. Equation 2 was fitted to the data from the individual rats and post-surgical changes in the parameters were analysed in the same way as the parameters of Equation 1.

Because the number of ratios completed within a session under a progressive-ratio schedule differs among individual subjects, analyses of variance of the raw response rates included only those ratios that were completed by at least 75% of the rats in each group in each phase of the experiment (ratios up to and including 62), missing values being filled using the value obtained in the highest ratio completed by the subject in question (Rickard et al. 2009). (Note that this limitation did not apply to the quantitative analysis of response rates using Equations 1 and 2, which entailed fitting functions to the data from individual rats.)

2.2.6.2. Locomotor activity data

Data were averaged across the two test sessions. The total numbers of beam breaks recorded in successive 5-min epochs were analysed by two-factor analysis of variance (group × epoch) with repeated measures on the latter factor.

2.2.6.3. Food intake data

Comparisons between groups were made using Student's *t*-test.

A significance criterion of $p \leq 0.05$ was adopted in all statistical analyses.

## 2.3. Results

### *2.3.1. Operant behaviour data*

2.3.1.1. Peak response rate

The mean (±SEM) post-/pre-surgical differences in peak response rate for the sham-lesioned and the OxSap-lesioned group are shown in Fig. 2.1 (left-hand panel). Analysis of variance revealed no significant main effect of group [$F(1,27) = 2.24$, *NS*] or block [$F<1$]. However there was a significant group × block interaction [$F(3,81) = 4.26$, $p<0.01$], reflecting a decrement of the maximum response rate in the OxSap-lesioned group in the last three blocks of sessions after surgery.

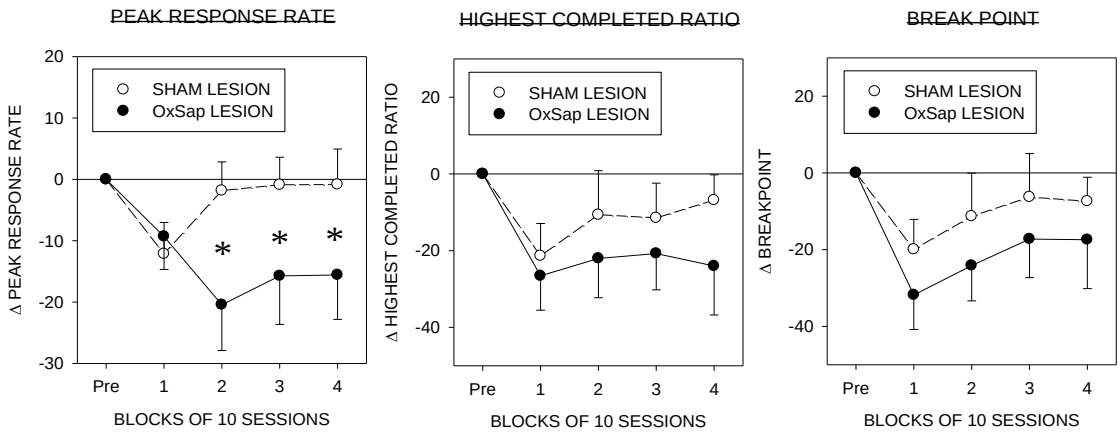


**Figure 2.1.** *Left-hand panel*. Changes in peak response rate following surgery in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups. *Ordinate*: change in the peak response rate (responses $min^{-1}$); *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p<0.05$. *Middle panel:* Changes in highest completed ratio; *right-hand panel:* Changes in break point*;* conventions as in left-hand panel.

2.3.1.2. Highest completed ratio and breakpoint

Both the highest completed ratio (Fig. 2.1, middle panel) and the breakpoint (Fig. 2.1, right-hand panel) were somewhat reduced after surgery, the reduction appearing slightly greater in the OxSap-lesioned group than in the sham-lesioned group. However, analysis of variance of the highest completed ratio revealed no significant main effect of group [$F<1$] or block [$F(3,81) = 1.05$, *NS*] and no significant interaction [$F<1$]. In the case of the breakpoint, there was a significant effect of block [$F(3,69) = 3.0$, $p<0.05$], but no significant effect of group and no significant interaction [$F$s<1].

2.3.1.3. Overall response rate

Fig. 2.2 shows the estimates of the parameters of Equation 1 in successive blocks of 10 sessions in the pre-surgical phase of the experiment. The parameter *a* showed a progressive increase during training, and the formal stability criterion (see above: *Method*) was not attained until the 10$^{th}$ block of sessions. Analysis of variance revealed a significant main effect of block [$F(10,270) = 10.9$, $p<0.001$], but no significant effect of group and no significant interaction [$F$s<1]; moreover there was no significant difference between the groups in the final pre-surgical block [$t(27) = 0.6$, *NS*]. The other two parameters ($\delta$ and $\beta$) remained relatively stable during the training phase, there being no significant differences between the two groups across the eleven blocks [all $F$s<1.2, *NS*] nor any significant difference in the final pre-surgical block [$\delta$: $t(27) = 0.8$, *NS*; β: $t(27) = 0.5$, *NS*].

Fig. 2.3 shows the group mean overall response rate data for the sham-lesioned (left hand panel) and OxSap-lesioned groups (right hand panel) in the last pre- and post-surgical blocks of sessions. In both groups, response rate increased rapidly to a peak, and then gradually declined as the response/reinforcer ratio was progressively increased. The maximum response rate was reduced in the Ox-Sap lesioned group following surgery. The fits of Equation 1 to the group mean data accounted for >90% of the total variance (*sham lesion*: pre-surgery, $r^2 = 0.93$; post-surgery, $r^2 = 0.90$; *OxSap lesion*: pre-surgery, $r^2 = 0.94$; post-surgery, $r^2 = 0.92$). There were significant main effects of block [$F(4,108) = 6.2$, $p<0.001$] and ratio [$F(11,297) = 20.1$, $p<0.001$], but not of group [$F<1$]. There were significant group × block [$F(4,108) = 4.5$, $p<0.01$] and block × ratio

[$F$(44,1188) = 1.9, $p$<0.01] interactions; the group × ratio and three-way interactions were not significant [$F$s<1].

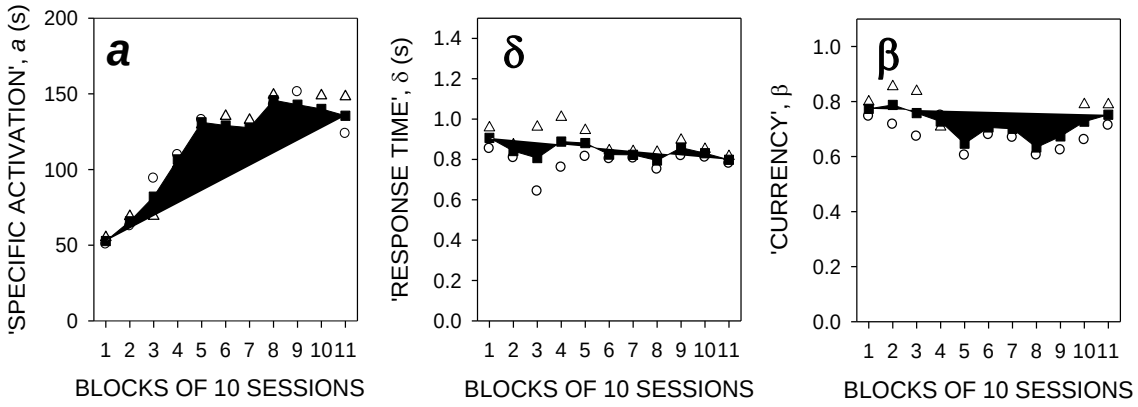


**Figure 2.2.** Values of the three parameters of Equation 1 (left-hand graph: *a*; middle graph: *δ*; right-hand graph: *β*) in successive blocks of 10 sessions during the pre-surgical training phase of the experiment. The filled symbols show the mean values for all rats; the open symbols show the mean values for the rats that were subsequently allocated to the sham-lesioned group (triangles) and the Ox-Sap lesioned group (circles).

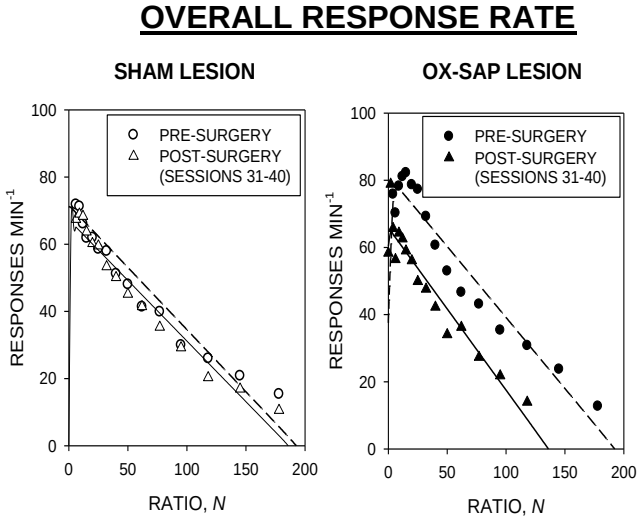


**Figure 2.3.** Overall response rates in successive ratios of the progressive-ratio schedule. *Left hand panel:* data from the sham-lesioned group; *right hand panel:* data from the OxSap-lesioned group. *Ordinates:* response rate (responses $min^{-1}$); *abscisae:* ratio, *N*. Points are group mean data. *Circles*: data from the last ten sessions before surgery; *triangles*: data from sessions 31-40 after surgery. The curves are fits of Equation 1 to the data (see text for details). Note the reduction of response rates in the OxSap-lesioned group, but not the sham-lesioned group, following surgery.

Fig. 2.4 shows the changes in the parameters of Equation 1 following surgery.

(i) *'Specific activation', a.*

There was no significant main effect of group [$F$<1] or block [$F$(3,81) = 1.1, *NS*], and no significant group × block interaction [$F$<1].

(ii) *'Response time', δ.*

There was a significant main effect of group [$F$(1,27) = 5.6, $p$<0.05], but not of block [$F$(3,81) = 2.1, *NS*]. There was a significant group × block interaction [$F$(3,81) = 2.3, $p$<0.05]. Multiple comparisons indicated that there was a significant increase in $\delta$ in the OxSap-lesioned group, compared to the sham-lesioned group in the second, third and fourth post-surgical blocks.

(iii) *'Currency', β.*

There was no significant main effect of group [$F$<1] or block [$F$(3,81) = 1.5, *NS*], and no significant interaction [$F$ (3,81) = 1.3, *NS*].

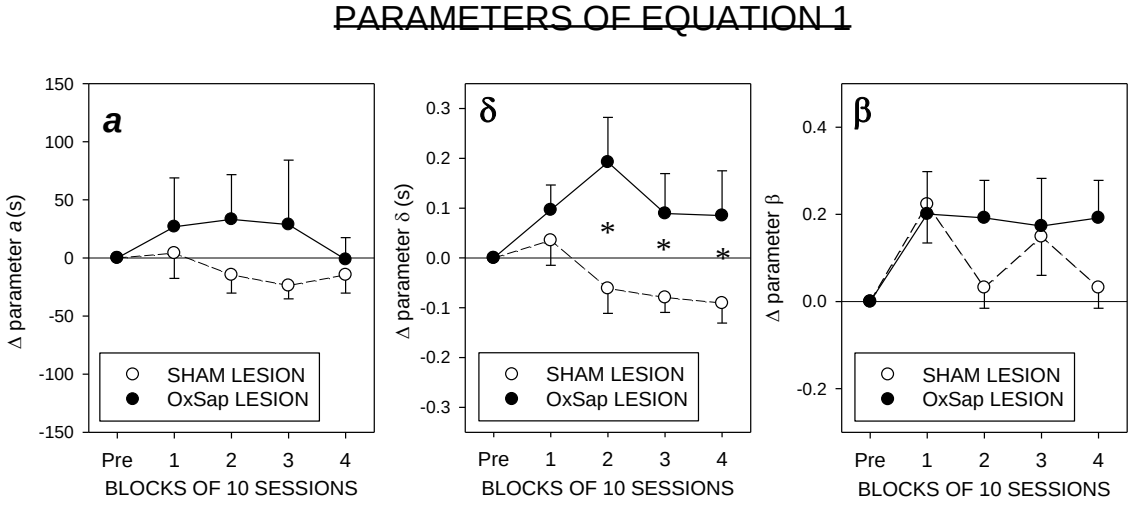


**Figure 2.4** Changes in the parameters of Equation 1 following surgery in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups. *Ordinates*: change in parameter value; *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p$<0.05. *Left-hand graph*: 'activation' parameter, *a* (s); *middle graph*: 'response time' parameter, $\delta$ (s); *right-hand graph*: 'currency' parameter, $\beta$.

2.3.1.4. Running response rate

Fig. 2.5 shows the group mean running response rate data for the sham-lesioned (left-hand panel) and OxSap-lesioned groups (right-hand panel) in the last pre- and post-surgical blocks of 10 sessions. In both groups, running rate declined monotonically as a function of the ratio requirement. Running rates were reduced following surgery in the OxSap-lesioned group. The fits of Equation 2 to the group mean data accounted for about 95% of the total variance. There were significant main effects of block [$F$ (4,108) = 7.2, $p$<0.001] and ratio [$F$(11,297) = 115.2, $p$<0.001], but not of group [$F$<1]. There was a significant group × block [$F$(4,108) = 10.0, $p$<0.01] interaction; the block × ratio [$F$(44,1188) = 1.2, *NS*], group × ratio [$F$<1] and three-way interactions [$F$<1] were not significant.

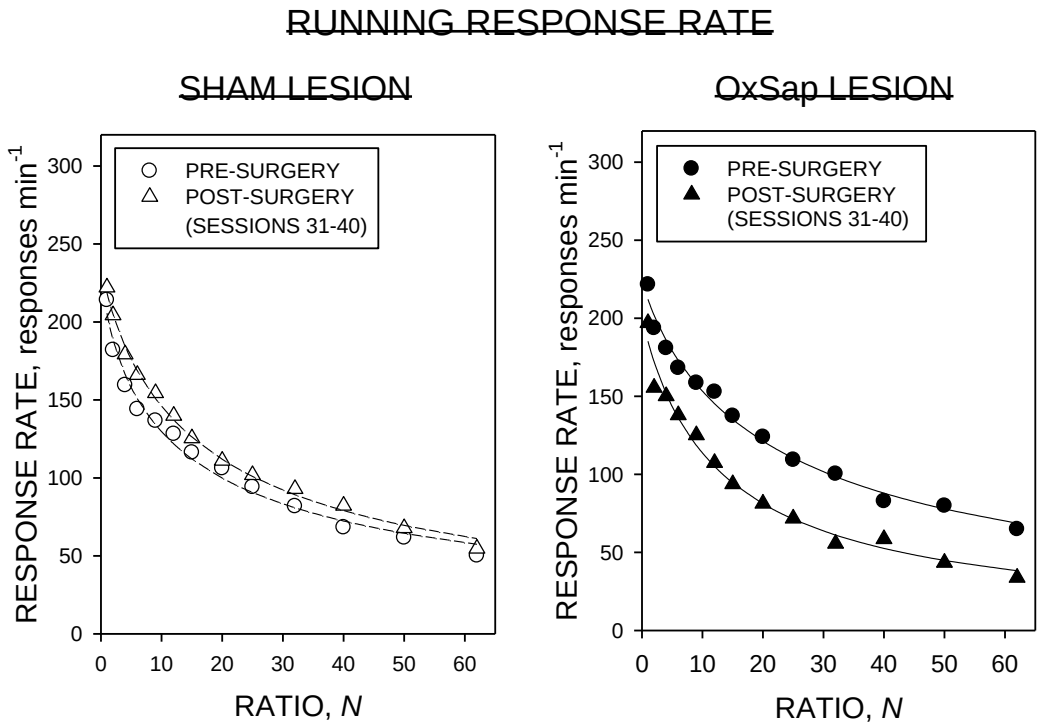


**Figure 2.5**. Running response rates in successive ratios of the progressive ratio schedule. *Left hand panel:* data from the sham-lesioned group; *right hand panel:* data from the OxSap-lesioned group. *Ordinates:* response rate (responses $min^{-1}$); *abscisae:* ratio, *N*. Points are group mean data . *Circles*: data from the last ten sessions before surgery; *triangles*: data from sessions 31-40 after surgery. Note the reduction of response rates in the OxSap-lesioned group, but not the sham-lesioned group, following surgery.

Fig. 2.6 shows the changes in the parameters of Equation 2 following surgery.

(i) *'Initial running rate', $R_i$.*

There was a significant main effect of group [$F(1,27) = 4.9$, $p<0.05$], reflecting a lower value of this parameter in the OxSap-lesioned group than in the sham-lesioned group. The main effect of block [$F<1$] and the interaction [$F(3,81) = 1.7$, *NS*], were not significant.

(ii) *'Decay parameter', b.*

There was no significant main effect of group [$F<1$] or block [$F<1$], and no significant interaction [$F(3,81) = 1.2$, *NS*].

(iii) *'Exponent', c.*

There was no significant main effect of group [$F(1,27) = 2.7$, *NS*] or block [$F<1$], and no significant interaction [$F<1$].

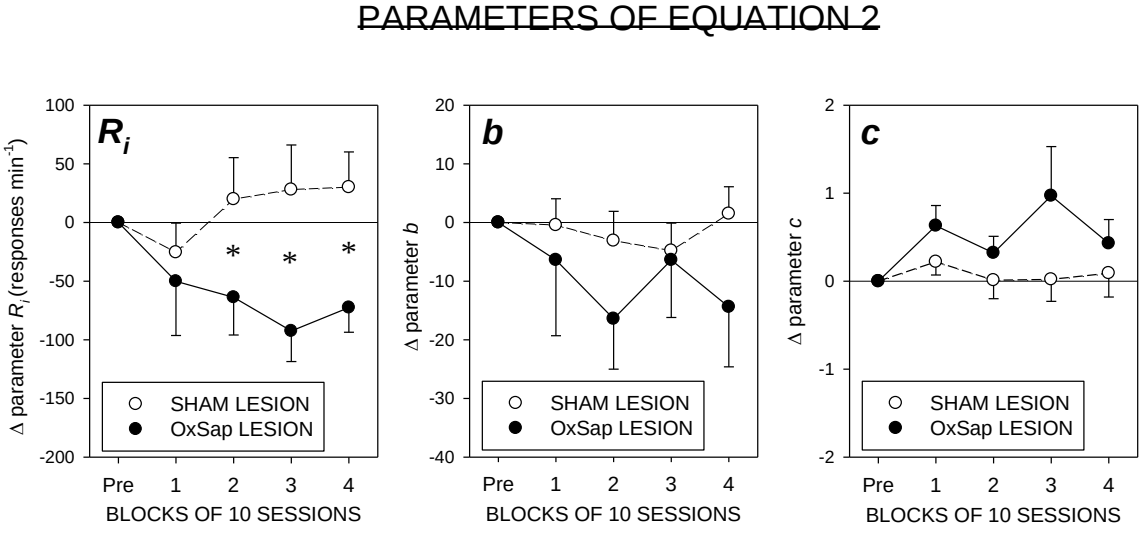


**Figure 2.6** Changes in the parameters of Equation 2 following surgery in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups. *Ordinates*: change in parameter value; *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p<0.05$. *Left-hand graph*: 'initial response rate' parameter, $R_i$ (responses min$^{-1}$); *middle graph*: 'decay' parameter, *b* (s); *right-hand graph*: 'exponent', *c*.

*2.3.2. Locomotor activity data*

Fig. 2.7 shows the locomotor activity data in successive 5-minute epochs of the 30-min test sessions. There was a significant main effect of epoch [$F(5,135) = 18.9$, $p<0.001$], reflecting the decline in activity after the first epoch. There was no significant main effect of group or group × epoch interaction [$F$s<1].

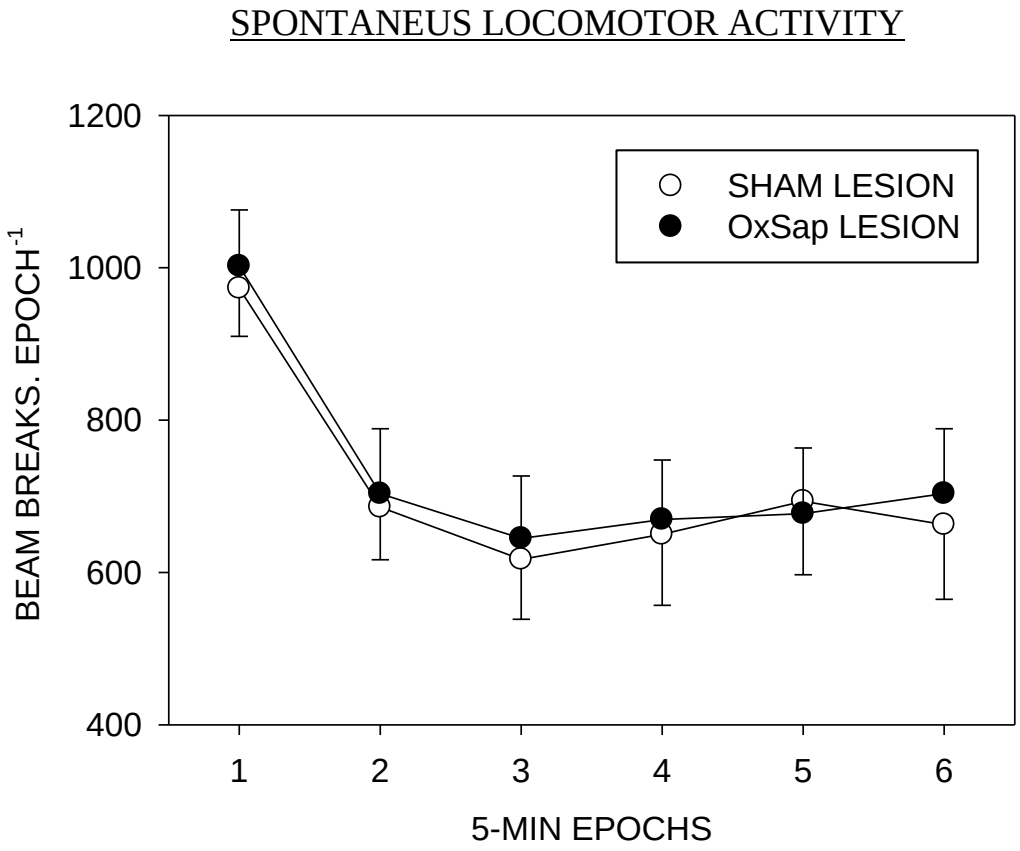


**Figure 2.7**. Locomotor activity in the sham-lesioned (open circles) and OxSap-lesioned (filled circles) groups in 30-minute sessions. *Ordinate*: total beam breaks per 5-min epoch; *abscissa*: epochs. Points are group mean data (± SEM).

*2.3.3. Food intake and body weight*

2.3.3.1. Feeding tests

Table 2.1 shows the total weights of 45-mg food pellets consumed during the 60-min tests under the food-restricted and free-feeding conditions. In neither case did food

consumption differ between the two groups [$t$<1 in each case]. Consumption of standard chow during the 23 hours between feeding tests under the free-feeding condition did not differ between the groups [$t(27) = 1.0$, *NS*].

2.3.1.2. Body weight

During the operant behaviour experiment, the rats' body weights were maintained at 80% of their initial free-feeding weights. There was no significant difference between the weights of the food rations needed to maintain the criterion weights in the two groups [group × blocks of sessions: all $F$s<1]. On return to the free-feeding condition, the body weights of both groups rapidly increased to their free-feeding levels; there was no significant difference between the two groups [$t(27) = 1.5$, *NS*; see Table 2.1].

**Table 2.1.** Results of food consumption tests and body weight

| | Sham-lesioned group | OxSap-lesioned group |
|---|---|---|
| *Consumption of food pellets (g per 60-min session)* | | |
| Food-restricted condition | 12.8 ± 0.4 | 13.2 ± 0.5 |
| Free-feeding condition | 5.6 ± 0.5 | 6.1 ± 0.4 |
| *Home-cage chow consumption (g per 23 h)* | | |
| Free-feeding condition | 22.4 ± 0.6 | 23.7 ± 1.1 |
| *Body weight (g)* | | |
| Free-feeding condition | 277.8 ± 2.6 | 283.0 ± 2.7 |

*2.3.4. Immunohistochemistry*

Fig. 2.8 shows representative photomicrographs of sections stained for orexin (upper panels) and NeuN (lower panels). OxSap resulted in a marked loss of orexin-positive neurones from the LHA with a smaller loss from the PFA. Numerical data are shown in

Fig. 2.9. There was a significant reduction of the number of orexin-positive neurones in the LHA [approximately 50% reduction: $t(27) = 3.7$, $p<0.001$], whereas the reduction fell short of statistical significance in the case of thePFA [$t(27) = 1.5$, *NS*]. There was a small reduction of the number of NeuN-positive nuclei in the LHA in the lesioned group [approximately 12% reduction: $t(27) = 1.9$, $p = 0.06$]; there was no significant difference between the two groups in the case of the PFA [$t<1$].

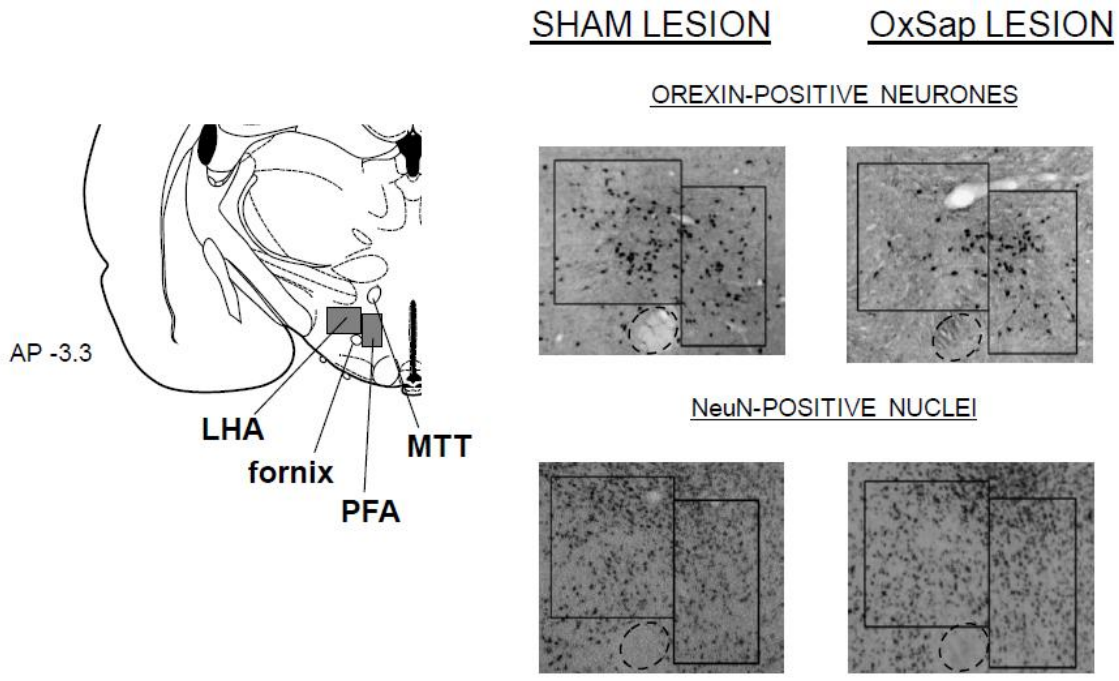


**Figure 2.8**. *Left-hand panel*. Diagram of coronal section of the rat brain at AP -3.3 mm, measured from bregma (from Paxinos and Watson 1998). The filled rectangles indicate the areas used for counting orexin-positive neurones and NeuN-positive nuclei. LHA, lateral hypothalamic area; PFA, perifornical hypothalamic area; MTT, mammillothalamic tract. Representative coronal sections through the hypothalamus of a sham-lesioned and an OxSap-lesioned rat are shown in the middle and right-hand panels; upper panels: sections stained for orexin; lower panels: sections stained for NeuN

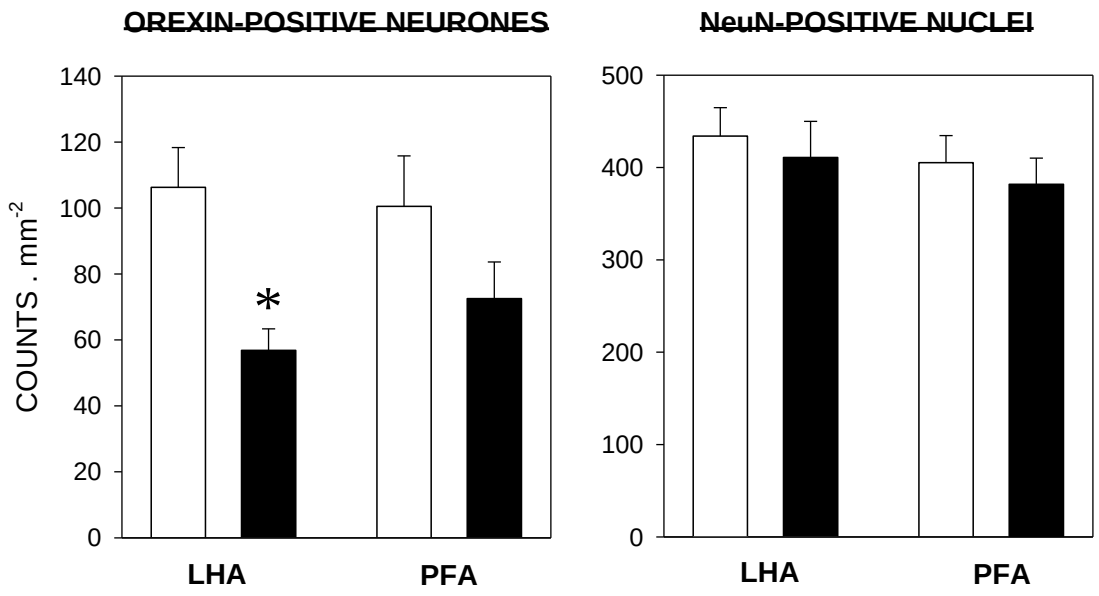


**Figure 9**. *Left-hand panel.* Density of orexin-positive neurones counted in the lateral hypothalamic area (LHA) and perifornical hypothalamic area (PFA). Ordinate: number of cells counted per $mm^2$. Columns show the group mean data (+SEM) for the sham-lesioned (white bars) and lesioned (black bars) groups. Significant difference from sham-lesioned group: * $p<0.05$. *Right-hand panel.* Density of NeuN-positive nuclei counted in the LHA and PFA (conventions as in the left-hand panel).

## 2.4. Discussion

Injection of the OxSap neurotoxin into the LHA produced a significant depletion of orexin-containing neurones from the LHA. The lesion was most pronounced in the LHA. Some loss of orexinergic neurones was evident in the perifornical region in some rats; however, this was not statistically significant in the group as a whole. The destruction of orexinergic neurones by local injection of OxSap into the LHA is consistent with previous reports (Anaclet et al. 2010; Blanco-Centurion et al. 2007; Di Sebastiano et al. 2011a; Di Sebastiano et al. 2010b; Frederick-Duus et al. 2007; Furlong and Carrive 2007; Gerashchenko et al. 2003; Gerashchenko et al. 2001; Vetrivelan et al. 2009).

It is well established that orexinergic neurones are susceptible to the neurotoxic effects of OxSap; however the relative vulnerability of other neurones bearing the OX2 receptor remains controversial (see also section 1.2.2.) In an early study employing OxSap, Gerashchenko et al. (2001) found that in addition to orexin-containing neurones, melanocortin concentrating hormone (MCH)-containing neurones were also destroyed by

OxSap, whereas neurones expressing *a*-melanocyte-stimulating hormone were resiliant. The susceptibility of MCH-containing neurones to destruction by OxSap has been confirmed in some subsequent studies (Furlong and Carrive 2007; Ocampo-Garces et al. 2011). However, there is evidence that MCH-containing neurones may be less vulnerable than orexin-containing neurones (Frederick-Duus et al. 2007), and indeed some authors found that injections of OxSap into the LHA which produced profound loss of orexinergic neurones had no significant effect on the numbers of MCH-containing neurones in this area (Di Sebastiano et al. 2011a; Di Sebastiano et al. 2010b). It is generally agreed that hypothalamic histaminergic neurones are sensitive to OxSap (Blanco-Centurion et al. 2007; Luo and Leung 2010), as are cholinergic neurones on the lateral dorsal tegmentum (Blanco-Centurion et al. 2004). Interestingly, noradrenergic neurones of the locus coeruleus have been found to be resistant to OxSap, probably reflecting the fact that these orexin-sensitive neurones express OX1 rather than OX2 receptors (Blanco-Centurion et al. 2004).

The relative selectivity of OxSap for OX2 receptor-bearing neurones appears to be dose-dependent. High doses of OxSap generally produce non-selective neuronal loss (Furlong and Carrive 2007; Gerashchenko et al. 2006; Mistlberger et al. 2003), whereas the effect of lower doses tends to be more selective (Anaclet et al. 2010; Gerashchenko et al. 2006; Vetrivelan et al. 2009). In the present experiment, the number of neurones destroyed by OxSap appears to have been a relatively small proportion of the total population of neurones in the affected region, as indicated by the modest reduction of the number of NeuN-positive nuclei seen in the OxSap-lesioned group compared to the sham-lesioned group (relative loss, 12%; $p$=0.06). This is consistent with the results of some previous studies which employed low doses of OxSap (Di Sebastiano et al. 2010a; Di Sebastiano et al. 2011b; Gerashchenko et al. 2006). Therefore, taking all these factors into account, it seems that while the lesion used in this experiment produced a substantial depletion of orexinergic neurones from the LHA and presumably induced a major disruption of orexinergic output from this area, it is not possible to quantify the contribution of a putative loss of other orexin (OX2) receptor-bearing neurones of the LHA to the behavioural effects of the lesion.

The performance of both the sham-lesioned and OxSap-lesioned groups on the progressive-ratio schedule was similar to that seen in many previous studies (see section

1.1.4; Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; Ho et al. 2003; Kheramin et al. 2005b; Killeen et al. 2009; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b).

It has been proposed that orexinergic neurones of the LHA play an important role in reinforcement processes (see sections 1.2.3.3 and 1.2.3.4: Cason et al. 2010; Harris and Aston-Jones 2006; Harris et al. 2005; Sharf et al. 2010b). It was therefore expected that destruction of orexinergic neurones of the LHA would suppress responding on this schedule. However, Killeen's (1994) theoretical analysis of reinforcement schedules indicates that suppression of responding on ratio schedules may be caused not only by a reduction of the activation parameter *a* but also by an increase of the parameter δ. Therefore a change in either or both of these parameters is expected to alter the traditional index of progressive-ratio schedule performance, the breakpoint (see section 1.4).

Additionally to the breakpoint, the highest completed ratio was also analysed (see section 2.2.6.1(ii): Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). Although both measures showed a tendency to be reduced following the OxSap lesion, the change was not statistically significant in either case.

Application of Equation 1 to the overall response rate data indicated that the OxSap lesion had no significant effect on *a*, suggesting that the lesion did not alter the intrinsic value of the reinforcer. This conclusion is supported by the analysis of the running rate data using Equation 2, which showed that the decay parameter, *b*, related to reinforcer value (Rickard et al. 2009), was not significantly altered.

On the other hand, the OxSap lesion produced a substantial increment in the 'response time' parameter, $\delta$, of Equation 1, a reduction of the 'initial response rate' parameter, $R_i$, of Equation 2, and a reduction of the peak response rate. Since $\delta$ specifies the minimum time required to execute an operant response, the increase of this parameter in the OxSap-lesioned group is consistent with an impairment of motor capacity. The reduction of $R_i$ is also consistent with this interpretation, and it is noteworthy that, unlike $\delta$, $R_i$ is not influenced by the duration of the post-reinforcement pause; therefore the reduction of this parameter cannot readily be attributed to subtle changes in post-prandial behaviour in

the lesioned group (see Bezzina *et al.*, 2008a, 2008b; Rickard *et al.*, 2009). $R_i$ has been found to be impervious to changes in reinforcer magnitude (Rickard et al. 2009), which further argues against the possibility that the effect of the lesion might have been caused by a change in reinforcer value.

An impairment of motor function induced by the OxSap lesion is consistent with previous reports that stimulation of orexinergic mechanisms increases, and suppression of orexinergic mechanisms reduces, postural muscle tone and motor output (Anaclet et al. 2010; Hara et al. 2001; Ida et al. 1999; Jones et al. 2001; Kiyashchenko et al. 2001; Torterolo et al. 2003; Wu et al. 2002).

The lack of effect of the lesion on the parameters of Equations 1 and 2 that are related to the incentive value of reinforcers is in apparent disagreement with previous findings indicating that the LHA orexinergic projection is critically involved in determining reinforcer efficacy. Evidence consistent with this proposition derives from a range of pharmacological manipulations, for example, systemic or central administration of orexin receptor antagonists (Borgland et al. 2009; Choi et al. 2010; Clegg et al. 2002; Harris and Aston-Jones 2006; Harris et al. 2005; Zheng et al. 2007), central administration of orexin or its precursor (Choi et al. 2010; Thorpe et al. 2005a; Thorpe and Kotz 2005), and a variety of behavioural paradigms (e.g., conditioned place preference: Harris *et al.*, 2005, 2007; Narita *et al.*, 2006; consumption of palatable diet: Borgland *et al.*, 2009; Clegg *et al.*, 2002; Sharf *et al.*, 2010; Zheng *et al.*, 2007; performance on progressive-ratio schedules: Borgland *et al.*, 2009; Choi *et al.*, 2010; Thorpe *et al.*, 2005). Several methodological factors may have contributed to the apparent discrepancy between the present findings and the results of previous experiments. Firstly, it may be noted that no previous investigation of the effect of manipulating orexinergic function on reinforcement processes has used the neurotoxin OxSap to destroy LHA orexinergic neurones. In the present experiment, OxSap injected into the LHA induced a 50% loss of orexin-containing neurones from this area. It is possible that a more extensive depletion may be required in order to reveal an effect on the incentive value of food reinforcers.

Another potentially important factor is the level of food deprivation. The subjects of this experiment were maintained at 80% of their free-feeding body weights, whereas most previous studies of the effect of manipulating orexinergic function on motivated

behaviour have been carried out on non-deprived animals or under milder deprivation conditions than that used in this experiment (Borgland et al. 2009; Cason et al. 2010; Harris and Aston-Jones 2006; Sharf et al. 2010b; Thorpe et al. 2005a). It is not clear how this might have influenced the results, although the control of food intake is known to be different in non-deprived and deprived states (Rowland et al. 2001). It is known that in food-deprived subjects various pathways in addition to those involving orexinergic mechanisms modulate food intake. For example, food deprivation increases immediate-early gene expression in neuropeptide-Y containing neurones of the arcuate nucleus (Cummings et al. 2001), and plasma ghrelin is elevated by food deprivation (Gerard et al. 1990; Meister 2007). Conceivably, the operation of non-orexinergic mechanisms might have compensated for an effect of orexin depletion on reinforcer value in the food-deprived animals used in the present study. In this context, it is of interest that Sharf et al. (2010) recently found that the performance of mice lacking the orexin precursor prepro-orexin (orexin -/-) on a progressive-ratio schedule did not differ from that of normal mice. Sharf et al. (2010) suggested that non-orexinergic mechanisms may have compensated for the lack of orexin in the genetically modified animals.

Yet another potentially relevant methodological factor is the length of training which the rats underwent before surgery. In most previous experiments in which the progressive-ratio schedule has been used to evaluate the effects of manipulating orexinergic function, the initial training period has been in the order of 5-15 sessions (Borgland et al. 2009; Choi et al. 2010; Thorpe et al. 2005a). Although responding on the progressive-ratio schedule is generally well established within a few days of training, several previous studies have found that many sessions are required in order to attain stability of the parameters of Equation 1 (Bezzina et al. 2008b; Bezzina et al. 2008c; Kheramin et al. 2005b; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). In the present experiment, attainment of the formal stability criterion based on the parameters of Equation 1 required approximately 100 training sessions; this was especially apparent in the case of the 'specific activation' parameter, *a* (see Figure 2.2). It is not clear how such protracted training might have affected the results of this experiment. There is evidence that after extended training learned behaviour tends to become 'habitual' and relatively insensitive to reinforcer devaluation or changes in motivational level (de Wit et al.; Dickinson 1985; Miles et al. 2003). It is possible that, in the present experiment, the rats' extensive experience with the progressive-ratio schedule reduced the impact of an

OxSap-induced decrease in reinforcer value on schedule performance. Further experiments may be warranted to examine the impact of prior training on the sensitivity of operant behaviour to manipulation of orexinergic function.

The 'currency' parameter, $\beta$, of Equation 1 did not differ significantly between the two groups. $\beta$ expresses the 'coupling' of responding to reinforcers, which is purported to be determined, in part, by the working memory limitations of the organism (Killeen 1994). An effect of the OxSap lesion on this parameter might therefore have been expected, in view of evidence indicating that manipulation of orexinergic function in the medial septum, hippocampus and dentate gyrus, whose orexinergic afferents emanate from the LHA, can alter mnemonic functions, including working memory (Akbari et al. 2008; Akbari et al. 2006; Smith and Pang 2005). However, it has yet to be demonstrated experimentally that the empirical value of $\beta$ derived from ratio schedule performance is sensitive to disruption of working memory.

There was no significant difference between the spontaneous locomotor activity of the OxSap-lesioned and sham-lesioned rats in this experiment. Previous studies have shown that acute central administration of orexins increases spontaneous locomotor behaviour, whereas orexin receptor antagonists reduce it (Jones et al. 2001; Thorpe and Kotz 2005). However, orexin deficient rodents have been found to show normal levels of locomotor activity (Sharf et al. 2010a) or altered activity levels that are restricted to a brief period at the start of the dark phase of the daily cycle (Hara et al. 2001). It is possible that alteration of locomotor behaviour is not sustained on a chronic basis in the absence of orexinergic function. In this experiment the rats underwent locomotor testing approximately six weeks after the OxSap lesion had been inflicted. It would be of interest to examine whether locomotor behaviour is suppressed at an earlier stage following this lesion.

Food intake was not affected by the OxSap lesion in this experiment. Previous reports have emphasised the preferential effect of orexinergic manipulations on the ingestion of especially palatable foods in non-deprived animals (Borgland et al. 2009; Farr et al. 2005; Nair et al. 2008; Sakurai et al. 1998; Thorpe et al. 2005a; Thorpe et al. 2006; Thorpe and Kotz 2005). The food pellets used in the food intake tests in the present experiment were the same as those used in the operant behaviour tests. Although these

pellets are more palatable than standard laboratory chow, it is possible that they were not sufficiently appetizing to reveal an effect of the lesion on food intake.

In conclusion, the results obtained in this experiment confirm that disruption of orexinergic mechanisms in the LHA results in a significant disruption of food-reinforced operant behaviour maintained under the progressive-ratio schedule. Quantitative analysis of performance based on Killeen's (1994) mathematical model of schedule-controlled behaviour (MPR) indicated that this disruption was not brought about by a change in the incentive value of the food reinforcer, but by a change in non-motivational processes that are encapsulated in the 'response-time' parameter, $\delta$, of Killeen's (1994) model. This is consistent with the suggestion that motor effects may play a more important role in the effects of manipulating orexinergic function on reinforced behaviour than has previously been recognised (Berridge et al. 2010; Siegel 2004; Siegel 2005b).

# CHAPTER 3

# EXPERIMENT 2: EFFECT OF OREXIN-B-SAPORIN-INDUCED DISCONNECTION OF THE LATERAL HYPOTHALAMUS FROM THE VENTRAL TEGMENTAL AREA ON PERFORMANCE ON A PROGRESSIVE-RATIO SCHEDULE

## 3.1. Introduction

As reviewed in Chapter 1 (section 1.2), the orexinergic projection from the hypothalamus is believed to play an important role in the modulation of reward processes, arousal and the regulation of the sleep/wakefulness cycle (see section 1.2.3). More specifically, there is a growing amount of evidence for the importance of the orexinergic pathway between the LHA and the VTA in reward processes, including both food and drug reinforcers (see section 1.2.3.3). For instance, the administration of orexin A directly into the VTA has been found to re-establish previously extinguished drug seeking behaviour (Harris et al. 2005). More recently, it has been reported that the disconnection of the LHA from the VTA disrupts drug seeking behavior (Harris et al. 2007). In this study of Harris et al. (2007), the LHA was unilaterally lesioned using the excitatory amino-acid N-methyl-D-aspartate (NMDA), and the OX1 receptor antagonist SB-334867 was microinjected in the contralateral or ipsilateral VTA. Since the LH-VTA pathway is principally ipsilateral, the contralateral treatment was assumed to produce an acute 'functional disconnection' of the LHA from the VTA (for other examples of 'funcitonal disconnections' see Bezzina et al. 2008a; Gaffan and Eacott 1995). Harris et al. (2007) found that a morphine-induced conditioned place preference (CPP) task was blocked in the disconnection group. The results led the authors to conclude that the orexinergic projections between LHA and VTA are important in drug reward processes.

As previously described, the progressive-ratio schedule (Hodos 1961; Hodos and Kalman 1963) has been used to evaluate the incentive value of reinforcers. However, there are several problems related with the traditional measure derived from this schedule, the breakpoint (see section 1.4.1). Some of these problems can be overcome by using Killeen's (1994) MPR model (see section 1.4.3). This model yields a quantitative index of reinforcer value (as denoted by changes in the parameter $a$) and dissociates effects of interventions on motor (expressed by the motor parameter $\delta$) and motivational processes (see section 1.4.3).

In the present experiment the putative role of the orexinergic projections between LHA and VTA in relation to reward or motor processes was investigated. Three groups of rats underwent surgical interventions where the LHA was chronically disconnected from the VTA or control procedures (unilateral lesions of the LHA and VTA, or sham lesions).

Lesions were induced by intracerebral injections of the selective neurotoxin OxSap (see sections 1.2.2 and 2.4). The effects of the interventions were evaluated using Killeen's (1994) MPR model (see section 1.4.3). Based on the putative role of the LHA/VTA orexinergic pathways in reward processes (Harris et al. 2007), it was predicted that this chronic VTA/LHA disconnection would produce a reduction of the incentive value parameter *a*. The disconnection lesion was not expected to change the response time parameter $\delta$ or the currency parameter $\beta$. In addition, food intake and body weight were compared between groups.

## 3.2. Methods

### *3.2.1. Subjects*

Forty four experimentally naive female Wistar rats approximately 4 months old and weighing 250–300 g at the start of the experiment were used. They were housed under the same conditions as in Experiment 1 (see section 2.2.1).

### *3.2.2. Surgery*

The rats were divided into three groups: disconnection (VTA lesion is contralateral to LHA lesion), unilateral (VTA lesion is ipsilateral to LHA lesion) and sham-lesioned groups. The first two groups received unilateral lesions of the LHA with the orexin-B-saporin conjugate (OxSap: Advanced Targeted Systems, USA). In addition to the LHA lesion, the disconnection group (n=16) received an OxSap injection in the contralateral VTA. The unilateral group (n=14) received an OxSap injection in the ipsilateral VTA. Finally, the sham-lesioned group (n=14) received injections of the vehicle solution (0.9% sodium chloride solution) instead of OxSap into the same target regions. The injection was administered to the left LHA in half rats in each group, and to the right LHA in the remaining rats. The coordinates for the LHA lesion were the same as in the previous experiment (see section 2.2.2). The coordinates for the VTA lesion were AP -5.3, L +/-0.9, V -8.4, measured from bregma. The quantity of OxSap injected into the LHA and VTA was 30 ng in 0.3 μl in each case. The rest of the surgical procedures were identical to those used in Experiment 1 (see section 2.2.2).

### *3.2.3. Apparatus*

The same operant conditioning chambers, control apparatus and software were used as in Experiment 1 (see section 2.4.1.1).

### *3.2.4. Behavioural procedures*

#### 3.2.4.1. Progressive ratio schedule

The behavioural training was carried out exactly in the same way as in Experiment 1 (see section 2.2.4.1).

#### 3.2.4.2. Food intake

This experiment was done in exactly the same way as in Experiment 1 (see section 2.2.4.3).

### *3.2.5. Immunohistochemistry*

The procedures for fixing the tissue, taking frozen sections and staining for orexin and NeuN were identical to those used in Experiment 1 (see section 2.4.1). In addition to the LHA sections, three sections were taken through the VTA for NeuN staining.

### *3.2.6. Data analysis*

The data from two rats were discarded (one from the disconnection group and the other from the unilateral group). These rats constituted 'outliers', as they had pre-surgical values of *a* over 900 seconds, well outside the range of values seen in the other rats. Analyses were thus based on the data from the disconnection (n=15), unilateral (n=13) and sham (n=14) lesioned groups. The data were analysed in the same way as in Experiment 1 (see section 2.2.6).

#### 3.2.6.1. Operant behaviour data

Data from the last block of 10 sessions before surgery and the four successive blocks of 10 sessions following surgery were used in the analyses. Behavioural measures (see below) were analysed by analysis of variance, followed, in the case of significant F-ratios, by post hoc comparisons of the two lesioned groups with the sham-lesioned group using Dunnett's test. The behavioural measures analysed were the same as those used in Experiment 1 (see section 2.2.6.1).

#### 3.2.6.2. Food intake data

The data were analysed in the same way as in Experiment 1 (see section 2.2.6.3).

## 3.3 Results

### *3.3.1. Operant behaviour data*

#### 3.3.1.1. Peak response rate

The mean (±SEM) post-/pre-surgical differences in peak response rate for the sham-lesioned, unilateral-lesioned and disconnection-lesioned groups are shown in Fig. 3.1 (left-hand panel). Analysis of variance revealed no significant main effects of group [$F(2,39) = 1.8$, *NS*]. However, there was a significant main effect of block [$F(3,117) = 12.4$, $p<0.001$] and group × block interaction [$F(6,117) = 2.6$, $p=0.05$]. Post hoc comparisons indicated a decrement of the peak response rate in the disconnection group in the second block of 10 sessions after surgery.

#### 3.3.1.2. Highest completed ratio and breakpoint

Both the highest completed ratio (Fig. 3.1, middle panel) and the breakpoint (Fig. 3.1, right-hand panel) were reduced in the first post-surgical block in all three groups. Analysis of variance of the highest completed ratio revealed no significant effect of

group [$F$<1]. However, there was a significant main effect of block [$F$(3,117) = 17.7 $p$< 0.001] and a significant block × group interaction [$F$(6,117) = 5.2 $p$<0.001]. In the case of the breakpoint, there was a significant effect of block [$F$(3,117) = 15.3 $p$<0.001] and a significant interaction [$F$(6,117) = 4.4 $p$< 0.001]. No significant main effect of group was found [$F$<1]. Comparison of the three groups at each level of block with one way analysis of variance followed with Dunnett's test did not reveal significant differences between the two lesioned groups and the sham-lesioned group.

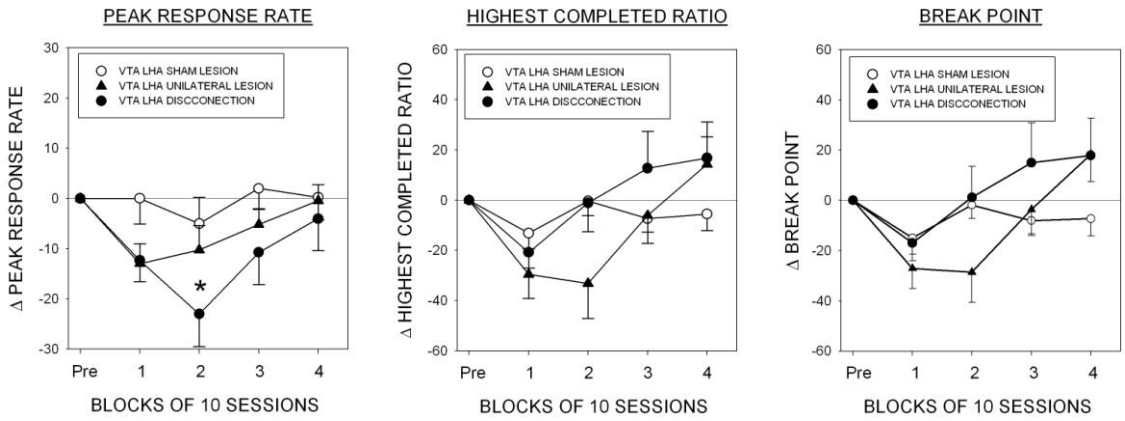


**Figure 3.1.** *Left-hand panel*. Changes in peak response rate following surgery in the sham (open circles), unilateral (filled triangles) and disconnection (filled circles) lesioned groups. *Ordinate*: change in the peak response rate (responses $min^{-1}$); *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between groups: * $p$<0.05. *Middle panel:* Changes in highest completed ratio; *right-hand panel:* Changes in break point*;* conventions as in left-hand panel.

3.3.1.3. Overall response rate

As in the previous experiment (see section 2.3.1.2), the parameters of Equation 1 reached stability after approximately 100 sessions. The same stability criterion was adopted for the present experiment.

The group mean data from the pre-surgical and third post-surgical block are shown in Fig. 3.2. In the three groups, response rate increased rapidly to a peak, and then gradually declined as the response/reinforcer ratio was progressively increased. The fits of

Equation 1 to the group mean data accounted for >90% of the total variance (*sham-lesioned group* : pre-surgery, $r^2$ = 0.96; post-surgery, $r^2$ = 0.93; *disconnection lesioned group*: pre-surgery, $r^2$ = 0.92; post-surgery, $r^2$ = 0.94; *unilateral lesioned group*: pre-surgery, $r^2$ = 0.92 post surgery, $r^2$ = 0.93). There was a significant main effect of block [$F$ (1,39) = 8.3, $p$<0.01] and ratio [$F$(17,663) = 208.7, $p$<.001], but not of group [$F$<1]. There was also a significant block × group interaction [$F$ (2,39) = 8.3, $p$<0.001] and group × ratio × block interaction [$F$(34,663) = 2.3, $p$<.001]. There were no significant ratio × group [$F$<1] or block × ratio [$F$<1] interactions.

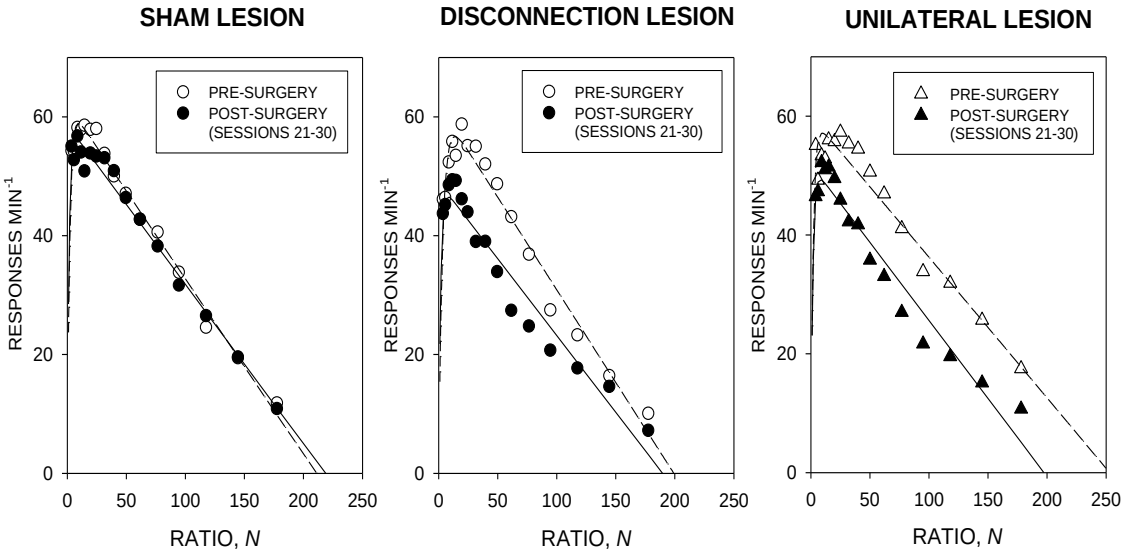


**Figure 3.2.** Overall response rates in successive ratios of the progressive-ratio schedule. *Left hand panel:* data from the sham-lesioned group; M*iddle panel:* data from the disconnection-lesioned group; *Right hand panel:* data from the unilateral-lesioned group *Ordinates:* response rate (responses min$^{-1}$); *abscisae:* ratio, *N*. Points are group mean data. *Open symbols*: data from the last ten sessions before surgery; *filled symbols*: data from sessions 21-30 after surgery. The curves are fits of Equation 1 to the data (see text for details).

Fig. 3.3. shows the changes in the parameters of Equation 1 following surgery.

(i) *'Specific activation', a*

There was no significant main effect of block [$F$(3,117) = 1.2, *NS*] or group [$F$<1] and no significant group × block interaction [$F$(6,117) = 1.2, *NS*]

(ii) *'Response time', δ*

There was an almost significant main effect of group [$F(2,39) = 3.1$, $p = 0.057$], but not of block [$F(3,117) = 1.5$, *NS*]. There was no significant group × block interaction [$F(6,117) = 1.0$, *NS*]. Multiple comparisons indicated that there was a significant increment of the motor parameter $\delta$ in the unilateral group in the first block and in the disconnection group in the third block compared with the sham-lesioned group.

(iii) *'Currency', $\beta$*

There was a significant main effect of block [$F(3,117) = 3.6$, $p<0.05$]. However, there was no significant main effect of group [$F<1$], and no significant interaction [$F<1$].

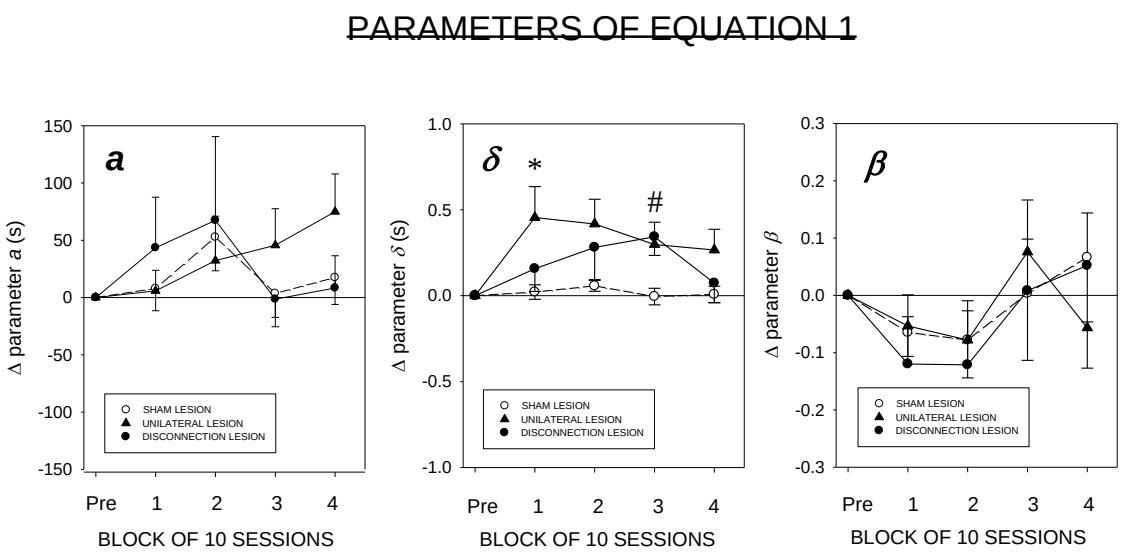


**Figure 3.3.** Changes in the parameters of Equation 1 following surgery in the sham-lesioned (open circles) unilateral-lesioned (filled triangles) and disconnection-lesioned (filled circles) groups. *Ordinates*: change in parameter value; *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant difference between unilateral- (*) or disconnection- (#) lesioned group and the sham-lesioned group: $p<0.05$. *Left-hand graph*: 'activation' parameter, *a* (s); *middle graph*: 'response time' parameter, $\delta$ (s); *right-hand graph*: 'currency' parameter, $\beta$.

3.3.1.4. Running response rate

Fig. 3.4. shows the group mean running response rate data for the sham- (left-hand panel) disconnection- (middle panel) and unilateral-lesioned groups (right-hand panel) in the

last pre- and post-surgical blocks of 10 sessions. In all three groups, running rate declined monotonically as a function of the ratio requirement. Running rates were reduced following surgery in both the disconnection- and unilateral-lesioned groups. The fit of Equation 2 to the group mean data accounted for over 95% of the total variance in each case. There was a significant main effect of ratio [$F$ (17,663) = 203.6, $p$<0.001] but not of block [$F$(1,39) = 2.9, NS] or group [$F$<1]. There was a significant group × block interaction [$F$(2,39) = 7.2, $p$<0.01], but neither the block × ratio [$F$<1] nor the group × ratio [$F$<1] interaction was significant. Finally, the three-way block × ratio × group interaction was significant [$F$(34,663) = 2.4, $p$<0.001].

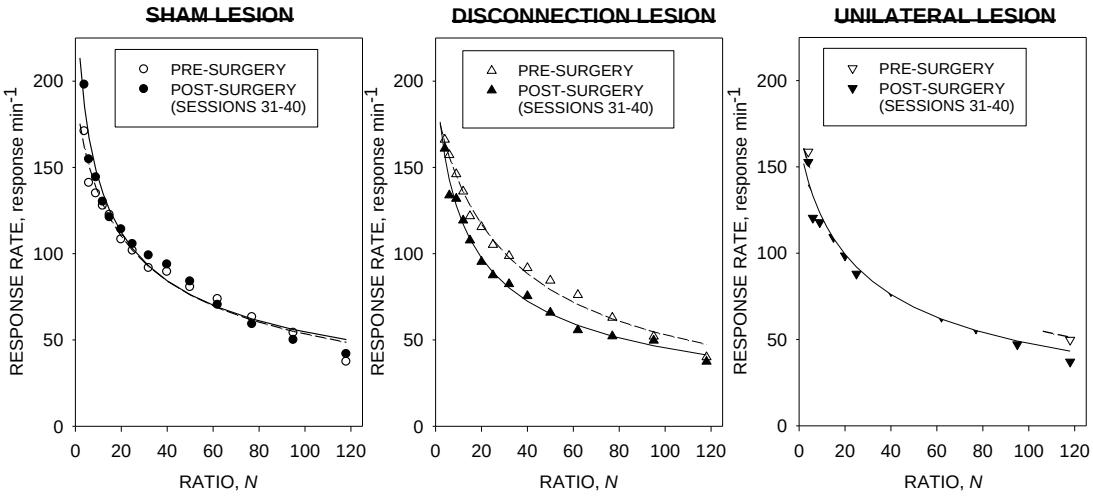


**Figure 3.4**. Running response rates in successive ratios of the progressive ratio schedule. *Left hand panel:* data from the sham-lesioned group; *middle panel:* data from the disconnection-lesioned group and *right hand panel* unilateral-lesioned group. *Ordinates:* response rate (responses $min^{-1}$); *abscise:* ratio, *N*. Points are group mean data. Open symbols: data from the last ten sessions before surgery; *filled symbols*: data from sessions 31-40 after surgery. Note the reduction of response rates in the disconnection and unilateral groups, but not the sham-lesioned group, following surgery.

Fig. 3.5. shows the changes in the parameters of Equation 2 following surgery.

(i) *'Initial running rate', $R_i$*

There was a significant main effect of group [$F$(2,39) = 7.6, $p$<0.01], reflecting a lower value of this parameter in the unilateral- and disconnection-lesioned groups in comparison with the sham-lesioned group after surgery. The main effect of block [$F$<1] and the group × block interaction [$F$(6,117) = 1.1, *NS*] were not significant. Post hoc

comparison with Dunnett's test revealed a significant decrement in this parameter in blocks 1, 3 and 4 after surgery in the disconnection- and unilateral-lesioned groups following the surgical procedure.

(ii) *'Decay parameter', b*

There was a significant main effect of block [$F(3,117) = 2.7$, $p=0.05$]. However, the main effect of group [$F<1$] and the block × group interaction [$F<1$] were not significant.

(iii) *'Exponent', c*

There was no significant main effect of group [$F<1$] or block [$F<1$], and no significant block × group interaction [$F(6,117) = 1.2$, NS].

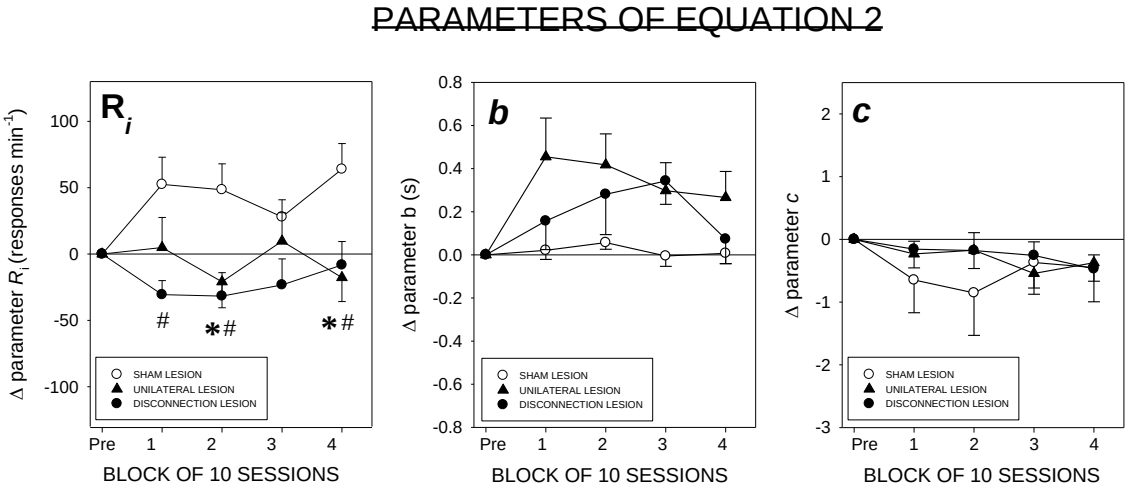


**Figure 3.5** Changes in the parameters of Equation 2 following surgery in the sham-lesioned (open circles) unilateral-lesioned (filled triangles) and disconnection-lesioned (filled circle) groups. *Ordinates*: change in parameter value; *abscissa*: blocks of 10 sessions. Points are differences between the values obtained in the four post-surgical blocks and the final pre-surgical block (Pre) (mean ± SEM). Significant differences between the sham- and unilateral-lesioned groups (*) and between the sham- and disconnection-lesioned groups (#): $p<0.05$. *Left-hand graph*: 'initial response rate' parameter, $R_i$ (responses $min^{-1}$); *middle graph*: 'decay' parameter, *b* (s); *right-hand graph*: 'exponent', *c*.

### 3.3.2. *Food intake and body weight*

#### 3.3.2.1. Feeding tests

Table 3.1. shows the total intake of 45 mg food pellets during 60-min test under food deprivation and free feeding conditions. ANOVA yielded a significant group effect [$F(2,39) = 5.8$, $p<0.01$] under food restricted conditions. Post hoc tests (least significant difference test) revealed higher consumption of food pellets in the unilateral-lesioned group in comparison with the disconnection- but not with the sham-lesioned group. There was also a significant higher consumption of pellets in the unilateral group [$F(2,39) = 5.3$, $p<0.01$] in comparison with both the sham-lesioned and the disconnection-lesioned groups under the free-feeding condition.

The total amount of chow intake during the free feeding condition did not show any significant differences among the groups [$F(2,39) = 1.5$, NS].

**Table 2.1.** Results of food consumption tests and body weight

| | sham-lesioned group | unilateral-lesioned group | disconnection-lesioned group |
|---|---|---|---|
| *Consumption of food pellets (g per 60-min session)* | | | |
| Food-restricted condition | 13.0 ± 0.5 | 14.0 ± 0.3*# | 12.2 ± 0.3 |
| Free-feeding condition | 4.5 ± 0.3 | 5.6 ± 0.2*# | 4.2 ± 0.4 |
| *Home-cage chow consumption (g per 23 h)* | | | |
| Free-feeding condition | 22.2 ± 0.3 | 23.7 ± 0.6 | 22.7 ± 0.8 |
| *Body weight (g)* | | | |
| Free-feeding condition | 291.1 ± 2.5 | 294.0 ± 3.1 | 286.0 ± 3.1 |

* significant difference from sham-lesioned group ($p<0.05$)

# significant difference from disconnection-lesioned group ($p<0.05$)

3.3.2.2. Body weight and food ration

As in the previous experiment (see section 2.3.3.2), the rats' body weights were maintained at 80% of their initial free-feeding body weights. The three groups' body weights were compared during the baseline and free-feeding conditions (Table 2.1). There were no significant time [$F<1$] or group effects [$F(2,39) = 1.4$, NS], and no significant group × time interaction [$F<1$]. A significantly higher amount of food ration was needed in the disconnection group in order to maintain the criterion weight during the food deprivation condition [$F(2,39) = 3.5$, $p<0.05$].

*3.3.3. Immunohistochemistry*

Fig. 3.6 shows representative photomicrographs of sections taken through the hypothalamus, stained for orexin (upper panels) and NeuN (middle panels), and sections taken through the VTA, stained for NeuN (lower panels). OxSap resulted in a marked loss of orexin-positive neurones from the LHA with a much smaller loss from the perifornical region. There was some loss of NeuN-positive units in the LHA, the PFA and the VTA.

Fig. 3.7 shows the numerical data for the counts of orexin-positive neurones in the LHA and PFA (left hand panel) and the NeuN-positive nuclei in the LHA, PFA and VTA (right hand panel). OxSap created a substantial reduction of the density of orexin-positive neurones in the LHA and a smaller reduction in the PFA. Analysis of variance revealed a significant reduction of the number of orexin-positive neurones in both OxSap-lesioned groups in the LHA [approximately 50%: $F(2,34) = 5.7$ $p<0.01$] when compared with the sham lesioned group. The effect of the lesion did not reach statistical significance in the case of the PFA [$F<1$].

OxSap produced a significant reduction [$F(2,37) = 14.8$, $p<0.01$] in the number of NeuN positive nuclei in the LHA in both the disconnection- and the unilateral-lesioned groups (approximately 40% and 30% respectively). In the case of the PFA the effect of OxSap was significant [$F(2,37)= 9.3$, $p<0.001$], but post hoc comparisons indicated that the only disconnection-lesioned group differed significantly from the sham-lesioned group. The number of NeuN positive nuclei was significantly reduced in the VTA [approximately

50%: $F(2,41) = 6.4$, $p<0.01$] in both the unilateral- and disconnection-lesioned groups when compared with the sham-lesioned group.

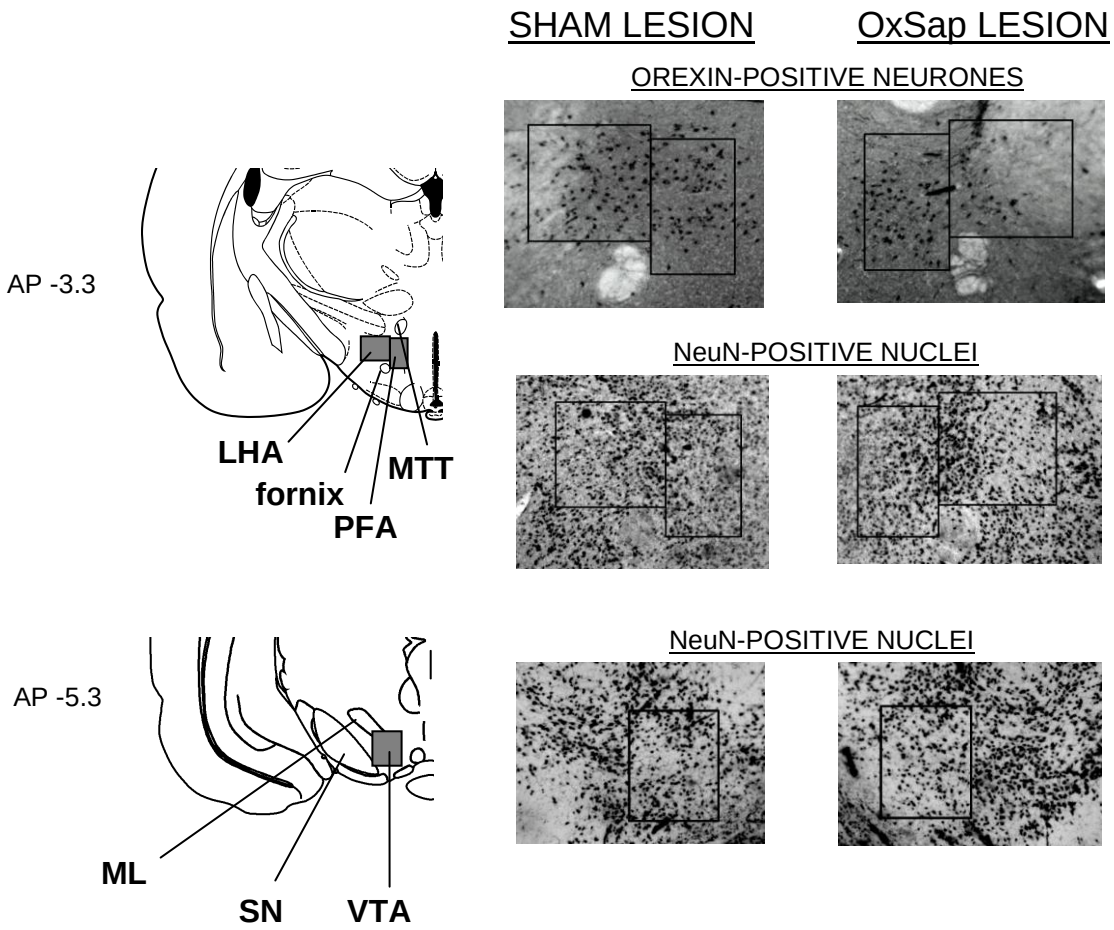


**Figure 3.6**. *Left-hand panels*. Diagrams of coronal sections of the rat brain at AP -3.3 and -5.3 mm, measured from bregma (from Paxinos and Watson 1998). The filled rectangles indicate the areas used for counting orexin-positive neurones and NeuN-positive nuclei in the hypothalamus (upper diagram) and the VTA (lower diagram). LHA, lateral hypothalamic area; PFA, perifornical hypothalamic area; MTT, mammillothalamic tract; ML, medial lemniscus; SN, substantia nigra; VTA, ventral tegmental area. *Middle and right-hand panels*. Representative coronal sections taken through sham-lesioned (middle panel) and OxSap-lesioned (right-hand panel) brains (each pair of sections is from the same rat); upper panels: sections through the hypothalamus stained for orexin; middle panels: sections through the hypothalamus stained for NeuN; lower panels: sections through the VTA stained for NeuN.

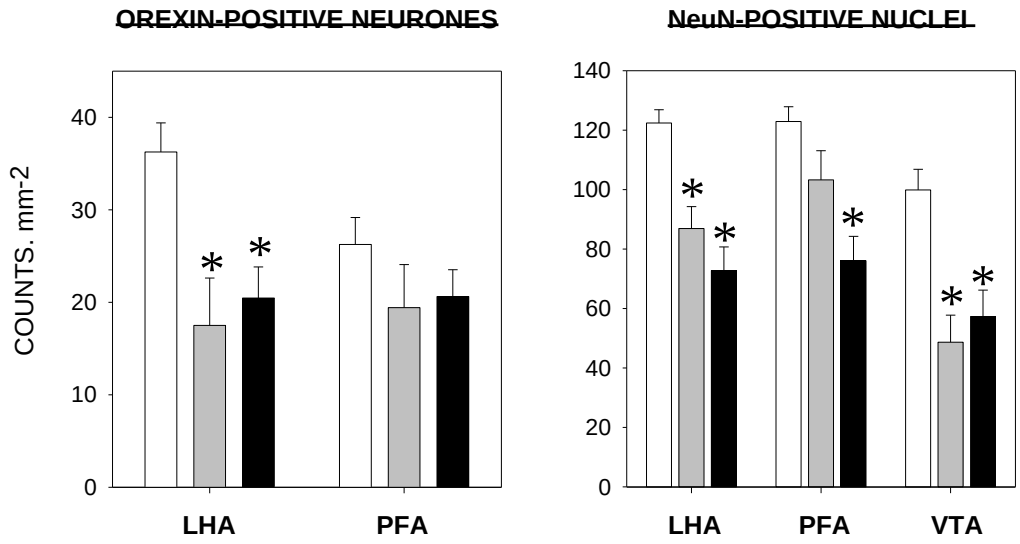


**Figure. 3.7**. *Left-hand panel.* Density of orexin-positive neurones counted in the lateral hypothalamic area (LHA) and perifornical hypothalamic area (PFA). Ordinate: number of cells counted per $mm^2$. Columns show the group mean data (+SEM) for the sham-lesioned (white bars), unilateral-lesioned (grey bars) and disconnection-lesioned (black bars) groups. Significant difference from sham-lesioned group: * $p<0.05$. *Right-hand panel.* Density of NeuN-positive nuclei counted in the LHA, PFA and VTA (ventral tegmental area; conventions as in the left-hand panel).

## 3.4. Discussion

Injection of OxSap into the LHA induced a significant reduction of orexin-containing neurones in both the unilateral- and disconnection-lesioned groups when compared with the sham-lesioned group. The adjacent PFA was also affected in both OxSap-lesioned groups, the effect reaching statistic significance in the case of the disconnection-lesioned group. The effective depletion of orexin-containing neurones using OxSap is in agreement with previous reports (Anaclet et al. 2010; Blanco-Centurion et al. 2007; Di Sebastiano et al. 2011a; Frederick-Duus et al. 2007; Furlong and Carrive 2007; Gerashchenko et al. 2003; Gerashchenko et al. 2001; Vetrivelan et al. 2009) and with the results of Experiment 1 (see section 2.3). As discussed in section 2.4, other neurones bearing the OX2 receptor are also susceptible to OxSap. To evaluate the extent of the lesion on other populations of neurones, the number of NeuN-positive nuclei was quantified in the LHA, PFA and VTA. The results showed that both OxSap-lesioned groups had a reduction of NeuN-positive nuclei in the LHA, PFA and VTA in

comparison with the sham-lesioned group. This reduction reached statistical significance in both the LHA and the VTA when compared with the sham-lesioned group.

The selectivity of OxSap is dose-dependent (see section 2.4). In this experiment the dose of OxSap was higher than in the previous experiment (30 ng vs 15 ng). The higher dose was adopted in this experiment in an attempt to produce a more profound depletion of orexinergic neurones in the target area. However, the effect of the higher dose on the density of orexin-containg neurones was very similar to that seen in Experiment 1 (approximately 50% depletion in both cases). The higher dose of OxSap did, however, result in a greater loss of NeuN-positive nuclei in the LHA in the present experiment (35% ), than in the previous experiment (12%). This result is in agreement with previous reports of dose-dependent selectivity of the effect of OxSap (Di Sebastiano et al. 2010a; Di Sebastiano et al. 2011b; Frederick-Duus et al. 2007; Gerashchenko et al. 2006).

It is not entirely clear why the higher dose of OxSap used in this experiment did not result in a more profound loss of orexin-containing neurones. One possibility is that a sub-population of orexinergic neurones in the LHA is relatively resistant to OxSap because they express OX1 receptors rather than OX2 receptors. OxSap is a conjugate of orexin-B and saporin; since orexin-B binds preferentially to OX2 receptors (Gerashchenko et al. 2001), any orexinergic neurones that do not express these receptors would be expected to be relatively resistant to the neurotoxin. It must be emphasised that this interpretation of the present findings is speculative, because there do not seem to be any published data on the proportion of hypothalamic neurones that exclusively express one or other of the two subtypes of orexin receptor.

Since OX2 receptors are expressed in the VTA (Lu et al. 2000; Trivedi et al. 1998) (see section 1.2.1), it was expected that OxSap would produce some neuronal loss in the VTA. This expection was confirmed by the reduction of NeuN-positive nuclei in the OxSap-treated hemisphere (see Fig. 3.7).

The performance of the disconnection-, unilateral- and sham-lesioned groups on the progressive-ratio schedule was similar to other studies (see section 1.4) (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al.

2003; Kheramin et al. 2005b; Killeen et al. 2009; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b).

As discussed earlier, the results of some previous studies have suggested that the orexinergic pathways emanating from the LHA may be involved in the regulation of reward value (see section 1.2.3.3 and 1.2.3.4) (Cason et al. 2010; Harris and Aston-Jones 2006; Harris et al. 2005; Harris et al. 2007; Sharf et al. 2010b). In particular, Harris et al. (2007) implicated the orexinergic projection from the LHA to the VTA in reinforcement processes. This experiment further investigated this hypothesis by disconnecting the orexinergic projection from the LHA to the VTA.

It was expected that the disconnection-lesioned group would show a decrement in the value of the activation parameter *a* (Equation 1), which has been proposed as a quantitative measure of reinforcer value (Reilly 2003; Rickard et al. 2009). Several previous studies have examined the effect of orexins or orexin receptor antagonists on progressive-ratio schedule performance, and it has been observed that these treatments have increased or decreased the breakpoint, respectively (Borgland et al. 2009; Choi et al. 2010; Nair et al. 2008; Sharf et al. 2010a). However, apart from the experiment described in Chapter 2, no previous studies have examined the effects of orexinergic treatments on the parameters of Equation 1. The present results show that *a* did not change significantly in any of the groups during the 4 blocks of 10 sessions after surgery. This finding is also supported from the analysis of the running rate (Equation 2). In this case, the decay parameter *b*, which has been shown to be sensitive to the magnitude of the reinforcer (Rickard et al. 2009), was not altered following the surgical intervention.

In addition, the traditional breakpoint and the highest completed ratio were also analysed. As can be seen from Fig. 3.1, there was a trend for both measures to be reduced in the second postsurgical block of 10 sessions. This apparent reduction could be explained as a postsurgical debilitating effect, due to the fact that in the following 3$^{rd}$ and 4$^{th}$ post-surgical blocks, the parameters recovered to similar values to those seen presurgically. It should also be noted that this reduction of the breakpoint and highest completed ratio was accompanied by a significant reduction of the peak response rate in the same block of sessions. In other words, the reduction of the breakpoint could have been due to motor rather than motivational factors. Furthermore, any conclusions based on the breakpoint,

which is based on a single data point, have to be taken cautiously, as there are several problems associated with this measure (see section 1.4). Moreover, methodological differences from previous studies, such as the food deprivation level, the length of training and the kind of reinforcers used, may have contributed to the lack of effect on the activation parameter (see section 2.4).

Although no reduction was found in either of the parameters that express the incentive value of food reinforcers *a* (Equation 1) or *b* (Equation 2), a significant effect was found on the two parameters which are related to motor performance, $\delta$ (Equation 1) and $R_i$ (Equation 2) (see section 2.1). The increment of $\delta$ in the disconnection-lesioned group is consistent with the previous experiment (see Chapter 2), where a bilateral lesion of orexinergic neurones in the LHA produced an increment of $\delta$ and a reduction of $R_i$. This increment of the response time parameter $\delta$ was significant in the third block of sessions in the disconnection group. The significant effect seen in the unilateral-lesioned group in the second block was unexpected. It is possible that it reflects a slower postsurgical recovery in this group, because the effect gradually subsided, and by block 4 the value of $\delta$ in this group had returned to a value similar to the presurgical value. Another possible explanation is that this group had slightly greater loss of orexin-containing neurones from the LHA, and NeuN-positive nuclei in the VTA, than the disconnection-lesioned group (see Fig 3.9).

Another factor that may have contributed to the lack of a clear dissociation between the disconnection-and unilateral-lesioned groups in this experiment is the presence of contralaterally projecting fibres from the LHA to the VTA. The logic of the disconnection lesion is based on the supposition that connections between the two component structures are exclusively unilateral (see Gaffan and Eacott 1995; Bezzina et al. 2008). However, Fadel et al. (2002) reported that although the predominant projections from the LHA to the VTA are ipsilateral, there is a small number of fibres that cross to the contralateral hemisphere. It is not clear whether these contralaterally projecting fibres make a significant contribution to the functional connection between the LHA and the VTA.

It should be noted that both $\delta$ (Equation 1) and $R_i$ (Equation 2) were affected by the OxSap lesion. As discussed earlier (see section 2.4), $\delta$ is based on overall response rates,

which incorporates the post-reinforcement pause. Therefore, the effect of the OxSap lesion on δ might, in theory, have been brought about by a selective effect on the post-reinforcement pause. However, this interpretation cannot account for the effect of the lesion on $R_i$, since this parameter is based on running response rates and is therefore not influenced by the post reinforcement pause. Furthermore, there is evidence that $R_i$ is not influenced by the magnitude of the reinforcer (Rickard et al. 2009). Therefore, the OxSap-induced decrement of this parameter is unlikely to have been affected by any change in reinforcer value, providing further circumstantial evidence that effect of the lesion on performance reflected a genuine alteration of motor performance.

Harris et al. (2007) disconnected the VTA acutely from the LHA by lesioning the LHA in one hemisphere and injecting the OX1 receptor antagonist SB-334867-A into the contralateral VTA (see section 3.1). They found a disruption of drug seeking behaviour in the conditioned place preference paradigm. There are many differences between the behavioural techniques used in Harris et al.'s experiment and the present one, which may have contributed to the differing results obtained. However, there are also differences between the disconnection procedures employed, which have potentially interesting implications for the role of orexinergic mechanisms in reinforcement processes. In Harris et al.'s (2007) experiment, N-methyl-D-aspartate (NMDA) was used to produce excitotoxic lesions of the LHA. Since NMDA receptors are present on most neurones, it is likely that the lesion was not restricted to neurones bearing OX2 receptors. The acute treatment with the OX1 receptor antagonist SB-334867-A, injected into the contralateral VTA, probably produced a quite specific interruption of synaptic transmission mediated by these receptors. Moreover, previous experiments that have purported to demonstrate an involvement of LHA orexinergic mechanisms in reward processes have mainly used treatments that preferentially target OX1 receptors (e.g. intracerebral treatment with orexin-A or OX1 receptor antagonists) (Borgland et al. 2009; Farr et al. 2005; James et al. 2011; Nair et al. 2008; Thorpe et al. 2005a; Thorpe et al. 2006; Thorpe and Kotz 2005; Thorpe et al. 2003; Thorpe et al. 2005b). In contrast, the use of OxSap in the present experiment probably had a preferential destructive effect on OX2 receptor-mediated transmission (see above). This raises the interesting possibility that OX2 receptor-mediated mechanisms play a predominant role in motor processes, whereas OX1 receptor-mediated mechanisms may be more important in the regulation of reinforcement processes. Indeed there is a growing body of evidence consistent with this

suggestion (Akanmu and Honda 2005; Chemelli et al. 1999; Hondo et al. 2009; Thompson and Borgland 2011). For instance Wang et al. (2009) trained rats to lever-press in a cocaine self-administration paradigm. They found that the injection of orexin A into the VTA produced an increment of cocaine-seeking behaviour This effect was blocked by an OX1 receptor antagonist. Furthermore this effect was not replicated when orexin B was injected into the VTA under the same conditions (Wang et al. 2009). In another cocaine self-administration experiment in rats, Smith et al. (2009) found that the OX1 receptor antagonist SB-334867-A, administered systemically, induced a dose-dependent decrement of cocaine-seeking behaviour. This effect was not found when the OX2 receptor antagonist 4PT (4-pyridylmethyl (S)-tert-leucyl 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) was injected. In addition, the same group tested these orexin receptor antagonists in a locomotor activity experiment. They found that 4PT but not SB-334867-A produced a significant reduction of the total distance travelled. Consistent with these findings, it has recently been reported that systemically-administered SB-334867-A reduces the breakpoint for highly salient positive reinforcers (Borgland et al. 2009), and that injection of this antagonist into the VTA attenuates cocaine seeking behaviour but has no effect on locomotor activity (James et al. 2011). All these experiments appear to support the hypothesis of differential involvement of the two types of orexin receptor in motivational and motor functions.

In conclusion, it can be said that this experiment demonstrated that OxSap-induced disconnection of the LHA from the VTA resulted in a significant alteration of food-reinforced operant behaviour under a progressive-ratio schedule of reinforcement. Quantitative analysis of behaviour based on Killeen's MPR (1994) and Equation 2 (Rickard et al. 2009) indicated that this alteration was caused by alteration of the response time parameter $\delta$ and the initial running rate parameter Ri. These results are consistent with previous experiments that have found motor output alterations following manipulation of orexinergic function. Consideration of recent literature suggests that the motor consequences of manipulating orexinergic function may be mediated mainly by OX2 receptors, whereas changes on reinforcer value may be mediated mainly by OX1 receptors. Further experiments investigating the effect of OX1 receptor antagonists on the parameters of Killeen's (1994) MPR and Rickard et al.'s (2009) equation could provide useful evidence for or against this suggestion. The experiment described in the following chapter is an initial effort in provide such evidence.

# CHAPTER 4

# EXPERIMENT 3: EFFECT OF 'ACUTE FUNCTIONAL DISCONNECTION' OF THE LATERAL HYPOTHALAMUS FROM THE NUCLEUS ACCUMBENS SHELL ON PERFORMANCE ON A PROGRESSIVE-RATIO SCHEDULE

## 4.1. Introduction

The orexinergic neurones of the LHA project to the nucleus accumbens (see section 1.2.1), which is an area that highly expresses OX1 and OX2 receptors (Martin et al. 2002). The nucleus accumbens is also a major projection region of the mesolimbic dopaminergic pathways that emanate from the VTA. Dopaminergic neurotransmission in the nucleus accumbens is believed to play an important role in the regulation of the reinforcing value of drugs of abuse and natural rewards such as water, food and sex (Hoebel 1997; Koob 1998; Koob and Nestler 1997; Salamone 2009; Salamone et al. 2005; Stratford and Kelley 1997; Wise 1998). Furthermore, the function of the dopaminergic system is believed to be modulated by the orexinergic system; for instance, VTA dopaminergic neurones are activated by orexin A (Martin et al. 2002; Nakamura et al. 2000), and the intra-VTA administration of orexin A has been found to elevate extracellular levels of dopamine in the AcbS and the prefrontal cortex (Narita et al. 2006; Narita et al. 2007; Vittoz et al. 2008).

Manipulation of the orexinergic system in the AcbS has produced conflicting results in different studies. On one hand, Baldo and Kelly (2001) found no effect on locomotor activity or food intake when they injected orexin A in the AcbS. On the other hand Thorpe and Kotz (2005) found an increment in both food intake and locomotor activity with intra-AcbS administration of orexin A. Unexpectedly, Thorpe and Kotz (2005) found that intra-AcbS injection of the OX1 receptor antagonist SB-334867-A produced an attenuation of food intake induced by orexin A, but had no effect on orexin A-induced locomotor activity. This led the authors to conclude that the effects of orexins on locomotion and feeding behavior may be meditated by different orexin receptors.

In the first experiment of this thesis, a major site of origin of the orexinergic projection, the LHA, was bilaterally lesioned with the neurotoxin OxSap (see Chapter 2). This was followed by an experiment where the site of origin of the mesolimbic dopaminergic pathway (the VTA) was disconnected from the LHA orexinergic neurones by unilateral injection of OxSap into the LHA and injection of OxSap into the VTA of the contralateral hemisphere (see Chapter 3). In both of theses experiments, a motor impairment was revealed, as expressed by an increment in the motor parameter $\delta$ derived

from Killeen's MPR (Killeen 1994) and $R_i$ (Rickard et al. 2009). In neither of these experiments was an effect on reward related measures found.

As reviewed in previous chapters, a number of studies have obtained evidence consistent with the hypothesis that disruption of the LHA orexinergic projection reduces the incentive value of positive reinforcers (for review, see Aston-Jones et al. 2009; see also section 1.2.3). A possible explantion for the apparent discrepancy between the results obtained in previous studies and those described in Chapters 2 and 3 is that in many previous studies that have reported effects on reinforcement processes, disruption of orexinergic function was achieved using the OX1 receptor antagonist SB-334867-A, whereas disruption of orexinergic function in the experiments described in Chapters 2 and 3 was achieved using the neurotoxin OxSap, a conjugate of orexin B and saporin. Although the selectivity of OxSap is not absolute (see section 1.2.2), it does show a greater destructive effect on neurones expressing the OX2 rceptor than on neurones that exclusively express the OX1 receptor, due to the fact that orexin-B has greater affinity for OX2 than OX1 receptors (Gerashchenko et al. 2001). Recently it has been suggested that the role of orexins in reinforcement processes may be mediated mainly by OX1 receptors, whereas their role in arousal and motor function may be mediated mainly by OX2 receptors (Hondo et al. 2009; Wang et al. 2009; Thompson and Borgland 2011). Therefore in the present experiment intracerebral injection of the OX1 receptor antagonist SB-334867-A was used to induce an acute disruption of orexinergic function of the AcbS.

In the present experiment the orexinergic neurones of the LHA were unilaterally lesioned with OxSap (see section 1.2.2) and SB-334867-A was microinjected into the ipsilateral or contralateral AcbS. The LHA-AcbS pathway is principally intra-hemispheric, the contralateral treatment was assumed to produce an acute 'functional disconnection' of the LHA from the AcbS (Bezzina et al. 2008a; Gaffan and Eacott 1995). The effect of this intervention was evaluated with Killeen's MPR model (1994) (see section 1.4.3). It was hypothesised that the 'disconnection' group would show a decrement in the 'incentive-value' parameter *a* (Killeen 1994), but would not differ from the control (ipsilaterally-treated) group with respect to the 'motor' parameter δ (Killeen 1994) (see section 1.4.3). Additionally locomotor activity was evaluated; no change was expected as a consequence of the treatment.

## 4.2. Methods

### *4.2.1. Subjects*

Twenty-six experimentally naive female Wistar rats approximately 4 months old and weighing 250–300 g at the start of the experiment were used. They were housed under the same conditions as in Experiment 1 (see section 2.2.1).

### *4.2.2. Surgery*

All the rats underwent two surgical procedures. In the first, each rat received a unilateral OxSap lesion of the LHA, the side of the lesion (left or right) being counterbalanced across subjects. This procedure was carried out in the same way as in Experiment 1 (see section 2.2.2). The only exception was that the dose of OxSap injected was 30 ng instead of 15 ng.

Approximately six weeks later the animals underwent a second surgical procedure for the microcannula implantation. The same anaesthetic and surgical preparation conditions were used as in Experiment 1 (see section 2.2.2). Bilateral 26-gauge guide cannula with a separation distance of 1.5 mm, mounted on a plastic pedestal (Bilaney Consultants Ltd, Sevenoaks, UK), were introduced into the brain via 1-mm holes drilled in the skull, symmetrically on either side of the midline. The assembly was lowered into position using the following stereotaxic coordinates to position the tips of the guide cannulae just above the medial shell: AP+1.3 mm, L±0.75 mm, V−4.7 mm, measured from bregma. (Paxinos and Watson, 1998). Three stainless steel anchor screws were placed in the skull, the cannula assembly was fixed to the skull with dental cement, and the wound was closed around the cannula assembly. Stylets were introduced into the guide cannulae, and the assembly was covered by a plastic screw cap. Fluid replacement (5 ml 0.9% saline solution, intraperitoneally) and wet mash diet were provided after the surgery. The rats were returned to the food-deprivation regimen and the daily training routine on the day following surgery.

### *4.2.3. Apparatus*

#### 4.2.3.1. Operant behaviour chambers

The same apparatus and software was used as in Experiment 1 (see section 2.2.3.1).

#### 4.2.3.2. Locomotor behaviour chambers

The same apparatus and software was used as in Experiment 1 (see section 2.2.3.2).

### *4.2.4. Behavioural procedures*

#### 4.2.4.1. Progressive ratio schedule

The behavioural training was carried out exactly in the same way as in Experiment 1 (see section 2.2.4.1).

#### 4.2.4.2. Locomotor activity

This experiment was done in the same way as in Experiment 1 (see section 2.2.4.2), the only exception being that the rats were placed in the locomotor activity boxes each day after the operant behaviour session, regardless of whether drug treatment had been administered.

### *4.2.5. Drug treatment*

One week after surgery, the rats were acclimatized to manual restraint and the intracerebral injection procedure over a number of sessions before starting the drug treatment regimen; the total number of training sessions that occurred before the start of the intracerebral injection regimen was >165. Intracerebral injections were given via bilateral 33-gauge injection cannulae which protruded 2 mm below the tip of the guide cannulae. Sterile SB-334867-A solution was injected into the AcbS on one side of the

brain and vehicle in the other side. The rats that received SB-334867-A in the AcbS that was contralateral to the LHA lesioned side were designated the 'disconnection' group (n=14), and the rats that received SB-334867-A into the same side as the lesion were designated the 'unilateral' group (n=12). The solutions were infused at a rate of 0.2 µl $min^{-1}$ via polyethylene tubes connected to 100-µl Hamilton syringes driven by a dual syringe pump (Linton Instrumentation, Diss, UK). The dose of SB-334867-A was based on previous studies (Thorpe and Kotz 2005): 6 ng in a volume of 0.5 µl (total injection time, 2.5 min). The injection cannulae remained in place for one minute after the completion of the injection to allow for diffusion within the tissue. The cannulae were then removed and the stylets replaced, and the rats were returned to their home cages for 12 min before being placed in the operant conditioning chambers. The experimental session began 15 minutes after completion of the injection. The data shown correspond to one injection per rat.

### *4.2.6. Immunohistochemistry and histology*

After completion of the behavioural experiment, the animals were deeply anaesthetized with pentobarbitone and perfused with phosphate buffered saline (PBS) followed by formol PBS using the same methods as in Experiment 1 (see section 2.2.5). The same immunohistochemical procedures as those used in Experiment 1 (see section 2.2.5) were used to prepare sections of brain tissue for the detection of orexin-positive neurones and NeuN-positive nuclei in the LHA.

Cannula placement was verified from coronal sections (60 µm) taken through the nucleus accumbens and stained using cresyl violet. These sections were mounted on gelatine-coated slides, dried overnight in formaldehyde vapour, and immersed in the following series of solutions: 95% ethanol (15 min), 70% ethanol (1 min), 50% ethanol (1 min), distilled water (2 min), 0.25% cresyl violet (2 min), distilled water (1 min), 50% ethanol (1 min), 70% ethanol (2 min), 95% ethanol (2 min), 100% ethanol (1 min), xylene (5 min). The slides were mounted with DPX and coverslipped. An investigator who was blind to the behavioural results performed the microscopic examination. Drawings of the locations of the cannula tips were superimposed on the appropriate pages of the stereotaxic atlas of Paxinos and Watson (1998).

### *4.2.7. Data analysis*

The data from 5 five rats were discarded (4 rats from the disconnection group and 1 from the unilateral group), because meaningful parameter estimates were not obtained in the drug-treatment session. Analysis were thus based on the data from the disconnection group (n=10) and unilateral group (n=11).

#### 4.2.7.1. Operant behaviour data

Data from the session in which SB-334867-A was injected into the AcbS were compared with the data obtained in the final 10-session block before drug treatment.

(i) *Peak response rate*

Peak response rate was analysed in the same way as in Experiment 1 (see section 2.2.6.1(i)), with the exception that comparisons were made between the intra-cerebral treatment session and the final 10-session pre-treatment block, as described above, rather than between the pre- and post-surgical data.

(ii) *Highest completed ratio and breakpoint*

These measures were analysed in the same way as in Experiment 1 (see section 2.2.6.1(ii)), with the same exception as above.

(iii) *Overall response rate*

Overall response rate was analysed in the same way as in Experiment 1 (see section 2.2.6.1(iii)) with the same exception as above.

(iv) *Running response rate*

Running response rate was analysed in the same way as in Experiment 1 (see section 2.2.6.1(iv)) with the same exception as above.

#### 4.2.7.2. Locomotor activity data.

The numbers of beam breaks in successive 5-minute epochs of the 30-min sessions were

analysed by three-factor analysis of variance : group [disconnection, unilateral] × treatment-day [SB-334867-A, no treatment] × epoch [1-6]) with repeated measures on the second and third factors.

## 4.3. Results

### *4.3.1. Operant behaviour data*

#### 4.3.1.1. Peak response rate

The mean (±SEM) post-/pre-treatment differences in peak response rate for the disconnection and the unilateral groups are shown in Fig. 4.1 (left-hand panel). Student's t-tests revealed no significant differences between the two groups [$t(19) = 1.1$, *NS*].

#### 4.3.1.2. Highest completed ratio and breakpoint

Although both groups showed slightly increased values of the highest completed ratio (Fig 4.1 middle panel) as a consequence of the drug treatment, this failed to reach statistical significance [$t(19) = 1.2$, *NS*]. The breakpoint increased in the unilateral group and decreased in the disconnection group (Fig 4.1 right-hand panel). However, these changes did not reach statistical significance [$t(19) = 1.3$, *NS*].

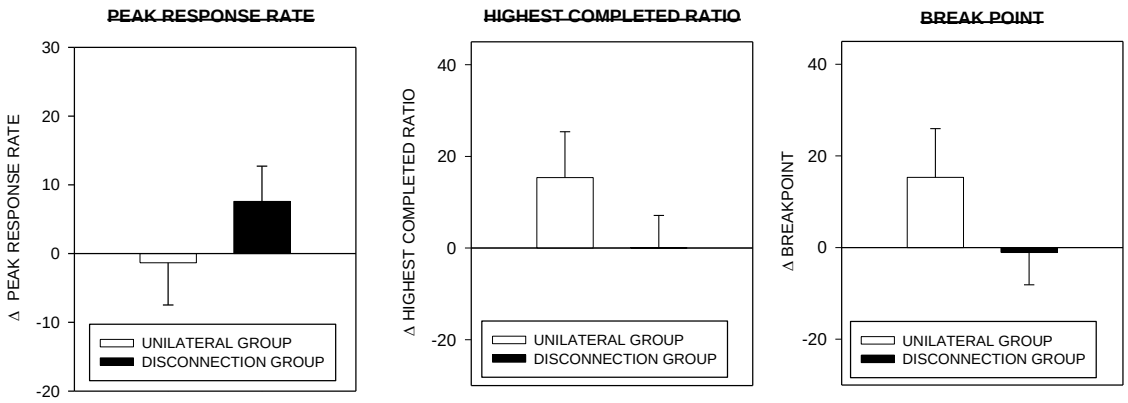


**Figure 4.1.** Changes in the peak response rate (*left panel*), highest completed ratio (*middle panel*) and breakpoint (*right panel*) following intra-AcbS SB-334867-A treatment in the unilateral (white bars) and the disconnection (black bars) groups. The data shown correspond to the differences (mean ± SEM) between the data obtained during the SB-334867-A treatment and the last 10 sessions before treatment commenced.

4.3.1.3. Overall response rate

Fig 4.2 shows the group mean overall response rate data for the unilateral (left-hand panel) and disconnection groups (right-hand panel) in the last pre-treatment block and on the treatment day. In both groups response rate increased to a peak, and then gradually declined as the response/reinforcer ratio was progressively increased. Analysis of the raw data revealed a significant main effect of ratio [$F(17,23) = 19.6$, $p<0.001$], but not of treatment [$F(1,19) = 2.5$, *NS*] or group [$F<1$]. There was a significant treatment × ratio interaction [$F(17,323) = 2.4$, $p<0.05$], but no significant ratio × group [$F(17,323) = 1.1$, *NS*], treatment × group [$F<1$] or treatment × ratio × group interaction [$F(17,323) = 1.2$, *NS*].

The fits of Equation 1 to the group mean data accounted for >84% of the total variance (unilateral group: pre-treatment, $r^2 = 0.97$; SB-334867-A treatment, $r^2 = 0.90$; disconnection group: pre treatment, $r^2 = 0.85$; SB-334867-A treatment, $r^2 = 0.84$). The activation parameter *a* was reduced in the disconnection group as a consequence of intra-AcbS SB-334867-A treatment (see below). This is reflected in the steeper descending limb of this group's curve in the treatment session compared to the no-treatment sessions.

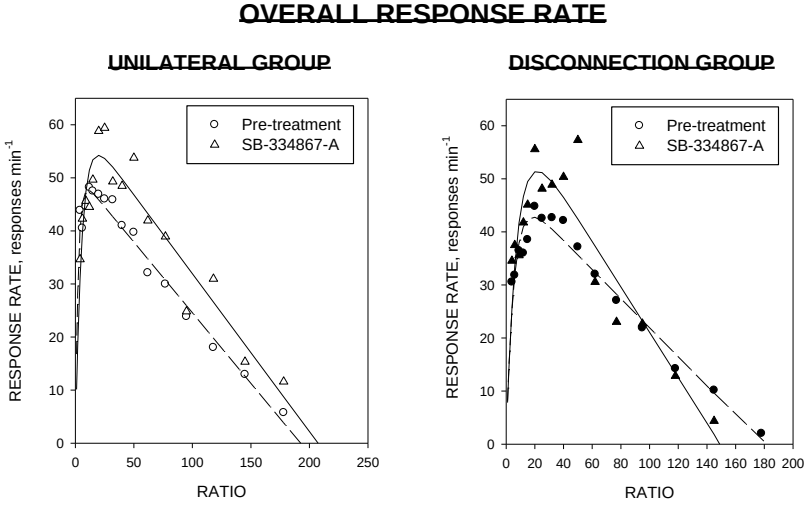


**Figure 4.2** Overall response rates in successive ratios of the progressive-ratio schedule. *Left hand panel:* data from the unilateral group; *right hand panel:* data from the disconnection group. *Ordinates:* response rate (responses $min^{-1}$); *abscisae:* ratio, *N*. Points are group mean data. *Circles*: data from the last ten sessions before drug treatment; *triangles*: data from intra AcbS SB-334867-A treatment. The curves are fits of Equation 1 to the data. Note the reduction in *a*, indicated by the steeper decline in response rate as a function of ratio size in the disconnection group following drug treatment.

Fig 4.3 shows the estimates of the parameters of Equation 1 in successive blocks of 10 sessions before the unilateral LHA lesion, before the implantation of the cannulae, and before the drug treatment session. The first surgical intervention was performed after 90 sessions when all the parameters were stable (see section 2.3.1.3). The two groups were compared before the intra-AcbS injections started, and no significant differences were found on *a* [t<1], $\delta$ [t<] or $\beta$ [t<1].

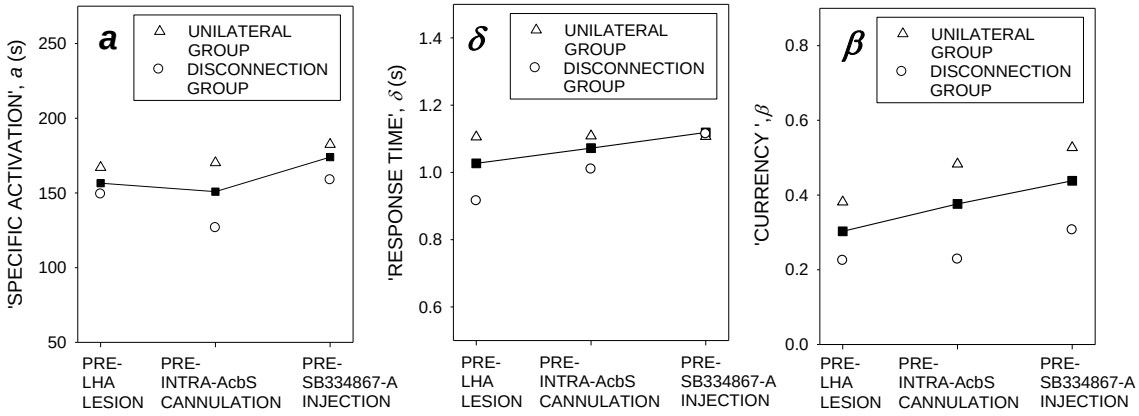


**Figure 4.3.** Values of parameters of Equation 1 (left-hand graphs: *a;* middle graph: $\delta$; right-hand graph: $\beta$) in the final block of 10 sessions before the unilateral LHA lesion, the block before the intracerebral cannulation, and the final block of sessions before intracerebral drug treatment The filled symbols show the mean values for all the rats; the open symbols show the mean values for the rats that were subsequently allocated to the unilateral group (triangles) and the disconnection group (circles).

Fig. 4.4 shows the differences the differences between the values of the parameters of Equation 1 obtained in the pre-treatment block and in the treatment session.

(i) *'Specific activation' a*

There was a significant reduction of the activation parameter *a* [$t(19) = 2.2$, $p < 0.05$] in the disconnection group following the AcbS SB-334867-A treatment, compared to the unilateral group.

(ii) *'Response time', $\delta$*

In both groups $\delta$ decreased after the drug treatment. However, this decrement did not

differ significantly [$t(19) = 1.2$, *NS*] between the disconnection and the unilateral groups.

(iii) *'Currency', β*

This parameter was reduced in both groups after the administration of the drug. However, this reduction did not differ significantly between groups [$t<1$].

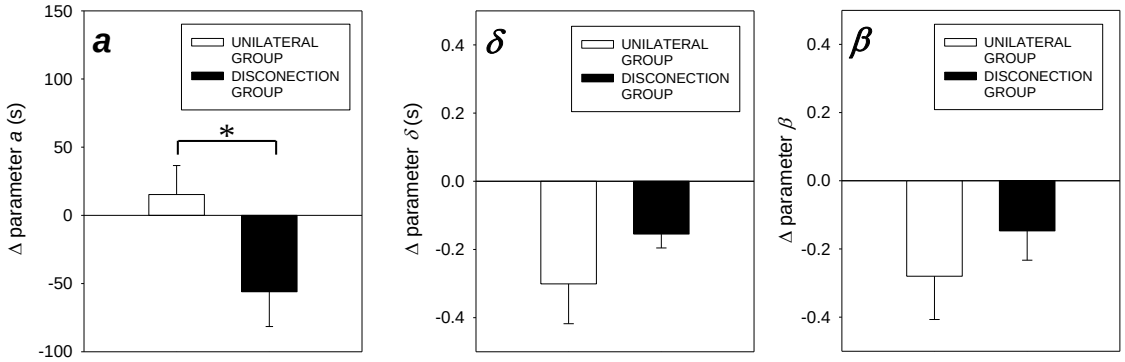


**Figure 4.4**. Changes in the parameters of Equation 1 following the intra AcbS SB-334867-A treatment in the unilateral (white bars) and disconnection (black bars) groups. *Ordinates*: differences between the values obtained in the pre-treatment blocks and on the treatment day (mean ± SEM). *Left-hand graph*: 'activation' parameter, *a* (s); *middle graph*: 'response time' parameter, *δ* (s); *right-hand graph*: 'currency' parameter, *β*. Intra-AcbS SB-334867-A treatment resulted in a significant reduction of *a* in the disconnection group, in comparison to the change seen in the unilateral group (difference between groups: * $p<0.05$).

4.3.1.4. Running response rate

Fig 4.5 shows the group mean running response rate data for the unilateral (left-hand panel) and the disconnection (right-hand panel) groups before SB-334867-A treatment and on the day of treatment. In both groups the running response rate was highest at the lowest ratios, decreasing monotonically as a function of increasing ratio size. Analysis of the raw data revealed no significant main effect of treatment [$F(1,19) = 3.1$, *NS*] or group [$F<1$], and no significant treatment × group [$F(1,19) = 2.4$, *NS*], treatment × ratio [$F<1$] or ratio × group [$F(11,209) = 1.6$, *NS*] interaction. There was a significant main effect of ratio [$F(11,209) = 57.0$, $p<0.001$], and the three-way treatment × ratio × group interaction was significant [$F(11,209) = 5.6$, $p<0.001$].

## RUNNING RESPONSE RATE

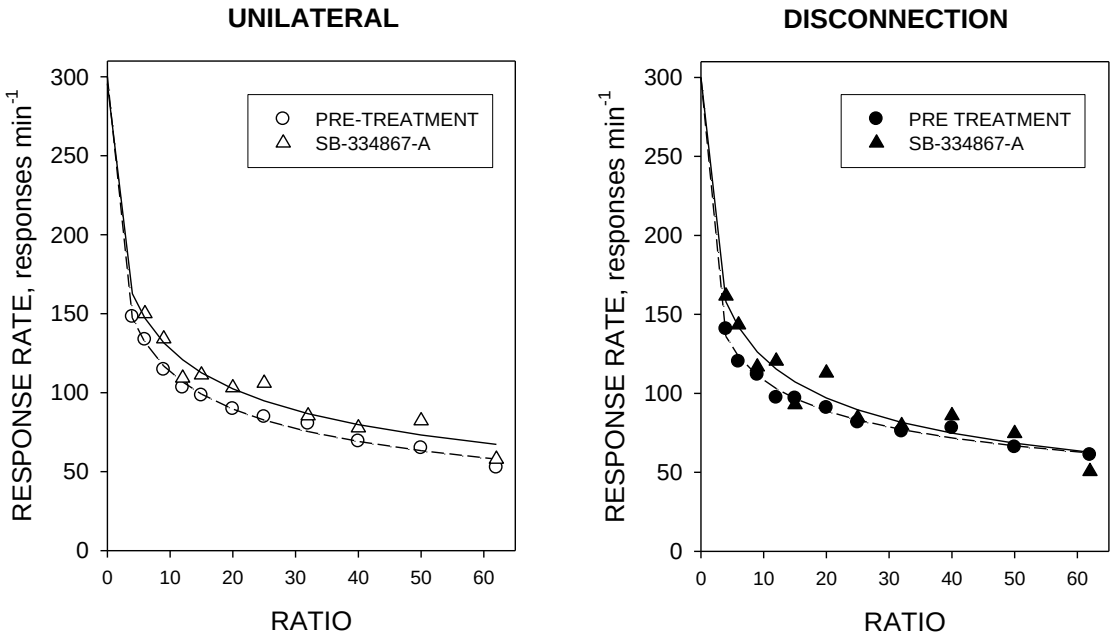


**Figure 4.5**. Running response rates in successive ratios of the progressive ratio schedule. *Left hand panel:* data from the unilateral group; *right hand panel:* data from the disconnection group. *Ordinates:* response rate (responses $min^{-1}$); *abscissae:* ratio, *N*. Points are group mean data. *Circles*: data from the last ten sessions before treatment; *triangles*: data from the session in which intra-AcbS injection of SB-334867-A was administered.

The fits of Equation 2 to the group mean data accounted for over 95% of the total variance. The effects of treatment on the parameters of Equation 2 are shown in Fig 4.6.

(i) *'Initial running rate', Ri*

There was a (non-significant) tendency for this parameter to be increased following treatment with SB-334867-A. There was no significant difference between the changes of this parameter seen in the two groups [$t<1$] (Fig. 4.6, left-hand graph).

(ii) *'Decay parameter', b*

This parameter showed a non-significant increase in the unilateral group, and a non-significant reduction in the disconnection group, following treatment with SB-334867-A. However the between-groups difference failed to reach statistical significance [$t(19) = 1.2$, *NS*] (Fig. 4.6, middle graph).

(iii) *'Exponent', c*

As was the case with the other parameters, there was no significant between-group difference in the change in this parameter [$t(19) = 1.4$, *NS*].

**PARAMERERS OF EQUATION 2**

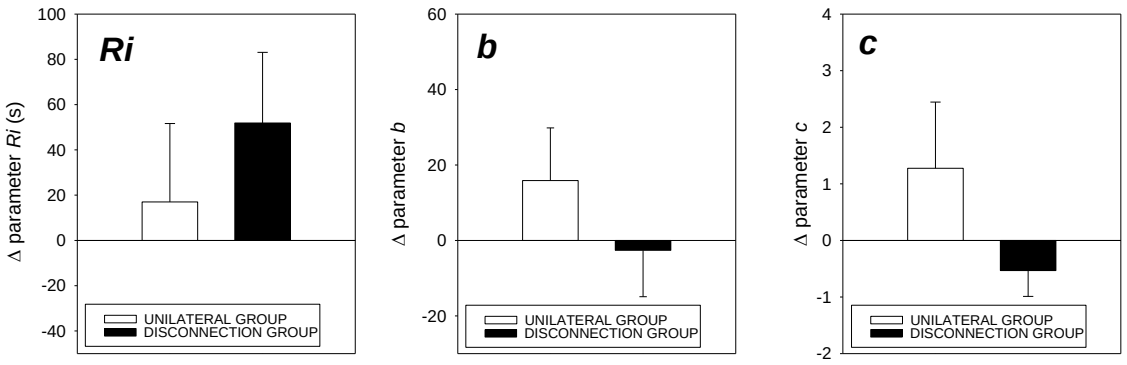


**Figure 4.6.** Changes in the parameters of Equation 2 following SB-334867-A treatment. Unilateral group (white bars) and disconnection group (black bars). *Ordinates*: change in parameter value; *abscissa*: differences between the values obtained during the treatment and the final pre-treatment block (mean ± SEM). *Left-hand graph*: 'initial response rate' parameter, $R_i$ (responses $min^{-1}$); *middle graph*: 'decay' parameter, *b* (s); *right-hand graph*: 'exponent', *c*.

### *4.3.2. Locomotor activity data*

Fig 4.7 shows the locomotor activity data in successive 5-minute epochs of the 30-minute test sessions on the days that the animals received the SB-334867-A treatment and no treatment. Analysis of variance revealed a significant main effect of treatment [$F(1,19) = 12.9$, $p=0.002$] and epoch [$F(5,95) = 11.0$, $p<0.001$], but not of group [$F(1,19) = 2.2$, *NS*]. The two-way treatment × group [$F$ (1,19), *NS*], treatment × epoch [$F<1$] and epoch × group [$F<1$] interactions and the three-way treatment × epoch × group interaction [$F<1$] were all non-significant.

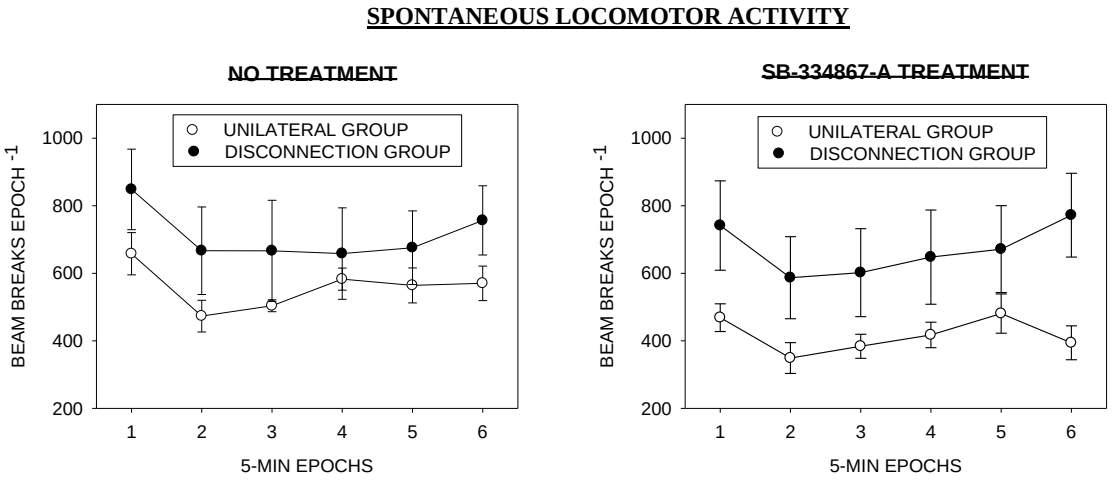


**Figure 4.7**. Locomotor activity in the unilateral (open circles) and disconnection (filled circles) groups in 30-minute sessions. *Left hand panel* corresponds to the no-treatment days and *right hand panel* to the SB-334867-A treatment day. *Ordinate*: total beam breaks per 5-min epoch; *abscissa*: epochs. Points are group mean data (± SEM).

### *4.3.3. Immunohistochemistry and histology*

Fig 4.8 shows representative photomicrographs of sections stained for orexin and NeuN in the OxSap and sham-lesioned hemispheres The numerical data for the densities of orexin-positive neurones and NeuN-positive nuclei detected in the LHA and PFA are shown in Fig. 4.9. OxSap resulted in a significant depletion of orexin-positive neurones from the LHA of the lesioned side [$t(25) = 5.5$, $p<0.001$] and PFA [$t(25) = 5.7$, $p<0.001$] in comparison with the sham-lesioned side. There was also a significant reduction of the the number of NeuN-positive nuclei in both the LHA [$t(43) = 8.5$, $p<0.001$] and the PFA [$t(43) = 8.5$, $p<0.001$].

Fig 4.10 is a representative photomicrograph showing the cannula placement. In each rat the tracks of the internal cannulae terminated in the medial area of the AcbS.

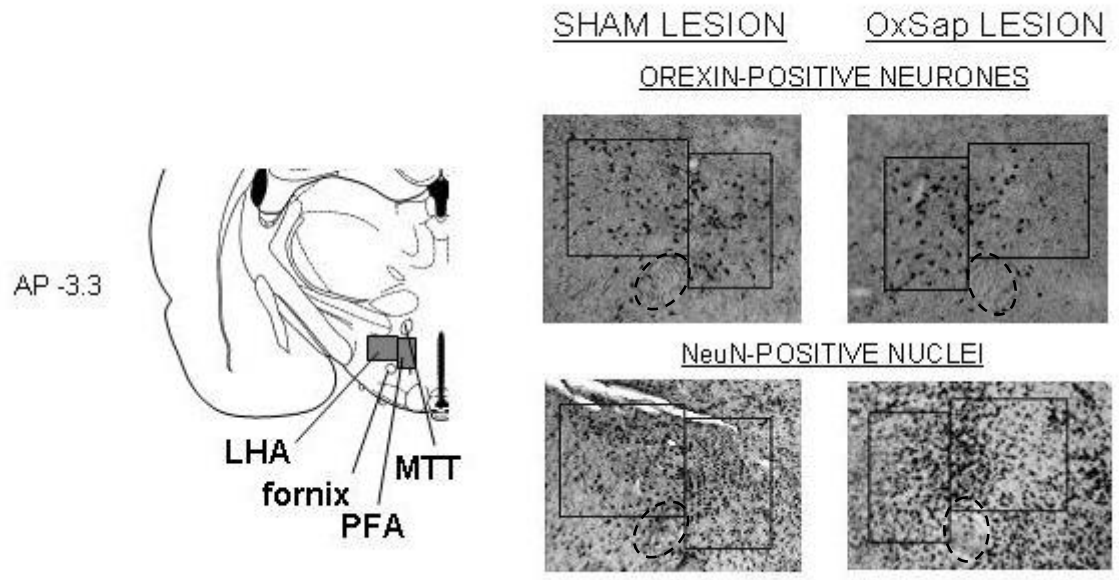


**Figure 4.8**. *Left-hand panel*. Diagram of coronal section of the rat brain at AP -3.3 mm, measured from bregma (from Paxinos and Watson 1998), The filled rectangles indicate the areas used for counting orexin-positive neurones and NeuN-positive nuclei in the hypothalamus. LHA, lateral hypothalamic area; PFA, perifornical hypothalamic area; MTT, mammillothalamic tract. *Middle and right-hand panels*. Representative coronal sections taken through sham-lesioned (middle panel) and OxSap-lesioned (right-hand panel) hemispheres; upper panels: sections through the hypothalamus stained for orexin; lower panels: sections through the hypothalamus stained for NeuN.

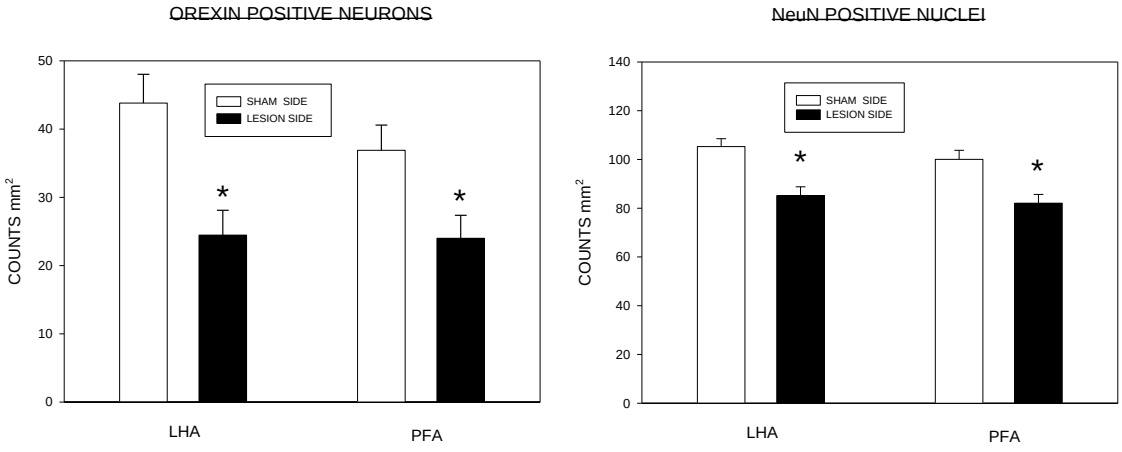


**Figure 4.9** *Left-hand panel.* Density of orexin-positive neurones counted in the lateral hypothalamic area (LHA) and perifornical hypothalamic area (PFA). *Ordinate:* number of cells counted per $mm^2$. Columns show the group mean data (+SEM) for the unilateral (white bars), and disconnection (black bars) groups. Significant difference between the two groups: * $p<0.05$. *Right-hand panel.* Density of NeuN-positive nuclei counted in the LHA and PFA (conventions as in the left-hand panel).

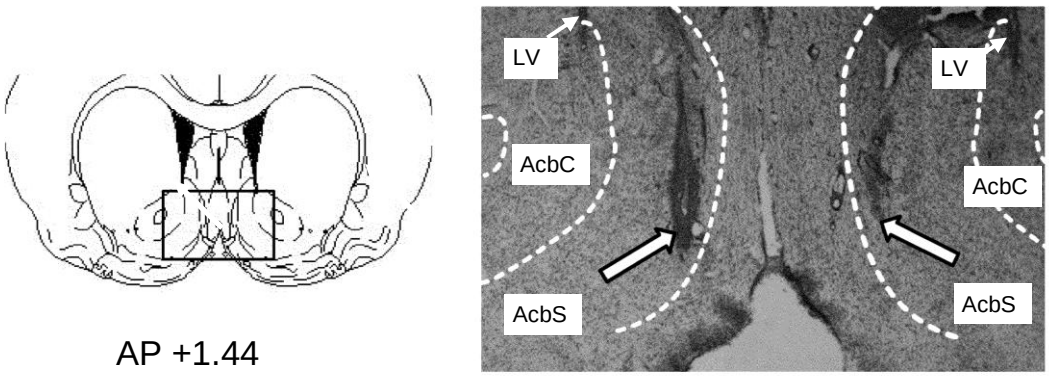


**Figure 4.10.** Placement of the bilateral cannulae in the nucleus accumbens shell. *Left-hand diagram*: map of coronal section of the rat brain 1.44 mm anterior to bregma (modified from Paxinos and Watson 1998); the rectangle indicates the area of the photomicrograph. *Right-hand panel*: Representative photomicrograph showing the cannula placement; the tracks of the internal cannulae (indicated by white arrows) terminate in the medial area of the AcbS. LV: lateral ventricle; AcbC: nucleus accumbens core; AcbS; nucleus accumbens shell.

## 4.4. Discussion

Unilateral OxSap injections into the LHA caused a significant reduction of the number of orexin-containing neurones in the lesioned side, when compared with the non-lesioned side, in both the disconnection and the unilateral groups. The lesion extended to the adjacent PFA, the reduction of orexin-containing neurones in the PFA reaching statistical significance when compared with the non-lesioned side. The successful depletion is in agreement with previous studies (Anaclet et al. 2010; Blanco-Centurion et al. 2007; Di Sebastiano et al. 2011a; Frederick-Duus et al. 2007; Furlong and Carrive 2007; Gerashchenko et al. 2003; Gerashchenko et al. 2001; Vetrivelan et al. 2009) and with the results of Experiments 1 and 2 (see sections 2.3.3 and 3.3.3). Other neurones containing the OX2 receptors are also vulnerable to effect of OxSap (see section 2.4). To evaluate the effect on other population of neurones affected by the Ox-Sap infusion, NeuN-positive nuclei were quantified in the LHA. The Ox-Sap lesioned side showed a significant reduction of the number of NeuN-positive nuclei in both the LHA and PFA. The extent of the depletion of orexin-containing neurones in the LHA in this experiment was similar to that seen in Experiments 1 and 2. The reduction of NeuN-positive nuclei was similar to that found in Experiment 2 (see section 2.4 and 3.4 for discussion).

The performance of the two groups on the progressive-ratio schedule was similar to that seen in previous studies (see section 2.4) (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al. 2003; Kheramin et al. 2005b; Killeen et al. 2009; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b) and in Experiments 1 and 2. Baseline performance was well described by Equation 1, and the unilateral OxSap lesion of the LHA and the stereotaxic placement of the indwelling guide cannulae had no significant effect on the parameters of the equation (see Fig. 4.3).

The principal aim of the experiment was to examine the effect of 'acute functional disconnection' of the orexinergic pathway from the LHA to the AcbS. As LHA-AcbS connections are predominantly ipsilateral (Stratford 2005), a contralateral disconnection method was used (Gaffan and Eacott 2995; Bezzina et al. 2008), in which the orexinergic projection emanating from the LHA on one side of the brain was permanently inactivated using the neurotoxin OxSap, and OX1 receptors in the AcbS on the other side were blocked acutely by intra-AcbS injection of the OX1 receptor antagonist SB-334867-A.

Injection of SB-334867-A into the AcbS affected performance on the progressive-ratio schedule. The motivational parameter *a* derived from Killeen's (1994) mathematical model was reduced by intra-AcbS injection of SB-334867-A in the disconnection group in comparison with the unilateral group. (A similar trend was seen in the case of the 'motivational parameter, *b*, derived from the running response rates [Equation 2; see Rickard et al. 2009]; however the reduction of this parameter in the disconnection group fell short of statistical significance.) The reduction of *a* is in accord with predictions based on the notion that functional connections between the LHA and the AcbS mediated by OX1 receptors may contribute to the regulation of the incentive value of food reinforcers (see section 4.1). Previous findings have implicated OX1 receptors in reinforcement processes (Hondo et al. 2009; Wang et al. 2009; Thompson and Borgland 2011), and there is abundant evidence for the importance of the AcbS in feeding behaviour and food reward (see Stratford and Kelley 1999; Baldo and Kelley 2007). The present results suggest that OX1 receptor-mediated neurotransmission in the AcbS may make a significant contribution to the role of LHA-AcbS connections in regulating reinforcer value.

As reviewed earlier (see section 1.2.3), central administration of orexin A increased the breakpoint and SB-334867-A injected into the AcbS decreased it (Borgland et al. 2009; Choi et al. 2010; Nair et al. 2008; Sharf et al. 2010a; Thorpe et al. 2005a). These effects on the breakpoint have been interpreted as changes in the motivational state of the organism as a consequence of manipulating orexinergic function. The present results also suggest that a decrement of motivated behaviour occured when SB-334867-A was centrally administered. However, despite the fact that this experiment found a modest reduction of the breakpoint (and the highest completed ratio), this was not statistically significant. Several factors mentioned before may account for these discrepancies, for example the type of reinforcer, the food deprivation level and the criterion used to define the breakpoint (see sections 1.4 and 2.4). An additional factor is the length of the experimental sessions. The sessions in this experiment lasted for 50 minutes, whereas in other studies sessions lasted for up to three hours (Borgland et al. 2009; Nair et al. 2008). It is possible that longer sessions might have revealed a reduction of the breakpoint in this experiment. However, as discussed earlier (section 1.4.1), very long sessions carry the disadvantage that the effects of acutely administered drugs may dissipate before the session finishes (see section 1.4).

The putative rewarding properties of the orexin system in the AcbS have been evaluated with feeding experiments, and have yielded contradictory results. On one hand Thorpe and Kotz (2005) showed that unilateral microinjections of orexin A increased food intake, and this effect was blocked by the administration of SB-334867-A. On the other hand Baldo and Kelly (2007) injected orexin A bilaterally and did not find any significant differences in food or water intake More evidence about the rewarding properties of the orexin system and their projections to the AcbS came from a study that showed that SB-334867-A reduced amphetamine-evoked dopamine release in the AcbS. This effect was not found when SB-334867-A was injected without amphetamine (Quarta et al. 2010). Sharf et al (2008) examined naloxone-induced morphine withdrawal symptoms in mice, and found that the administration of SB-334867-A decreased both withdrawal symptons and c-Fos expression in the AcbS (Sharf et al. 2008).

As expected, no significant changes were found in the response time parameter $\delta$ or in the peak response rate (two measures derived from the overall response rate). To exclude

the possibility that the post reinforcement pause (PRP) was masking any possible motor deficit, Equation 2 was applied to the running rate (see section 2.1). The results showed that $R_i$, was not significantly affected as a consequence of the disconnection treatment with SB-334867-A.

As predicted, the disconnection and the unilateral groups did not differ significantly during their performance in the spontaneous locomotor activity task. This is in agreement with previous reports of the effect of intra-AcbS-administered SB-334867-A (Thorpe and Kotz 2005). However, when orexin A has been injected in the AcbS it has caused contradictory results. For instance, Thorpe and Kotz (2005) found a significant increment in locomotor activity and Baldo and Kelley (2001) did not. The authors attributed these discrepancies to the doses used, which were considerably lower in Baldo and Kelley's experiment. The results from feeding and locomotor activity experiments led Thorpe and Kotz (2005) to conclude that motor processes are partially mediated by OX2 receptors and reward processes are modulated by OX1 receptors.

In the present experiment the orexinergic neurones in the LHA were depleted unilaterally and the OX1 receptor antagonist SB-334867-A was microinjected into the AcbS. This acute treatment in the AcbS resulted in a reduction in the motivational parameter *a* derived from Killeen's MPR (1994). As discussed before (see section 3.1) it is thought that the OX1 receptor is involved in reward-related processes and the OX2 receptor is more involved with motor processes (Borgland et al. 2009; Farr et al. 2005; James et al. 2011; Nair et al. 2008; Smith et al. 2009; Thorpe et al. 2005a; Thorpe et al. 2006; Thorpe and Kotz 2005; Thorpe et al. 2003; Thorpe et al. 2005b). The results from this experiment add more evidence in support of this theory, as the reduction on *a* occurred under the SB-334867-A-induced disconnection treatment and not under the unilateral treatment condition.

To summarise, it can be said that the acute orexinergic disconnection between the LHA and AcbS resulted in a significant alteration of food-reinforced operant behaviour under a progressive-ratio schedule of reinforcement. Quantitative analysis of behaviour based on Killeen's MPR (1994) indicated that this alteration was caused by a reduction of the incentive value of the reinforcer (as indicated by a reduction of the 'specific activation' parameter *a*). These results are in contrast with the results of Experiments 1 and 2 (see

Chapters 2 and 3), where a significant motor impairment was found (as indicated by an increase in the 'response time' parameter δ), without any apparent change in incentive value. It was suggested that in Experiments 1 and 2 the disruption of orexinergic function was mainly focused on OX2 receptor-mediated neurotransmission, whereas changes in reinforcer value seen in the present experiment may have been caused by disruption of OX1 receptor-mediated neurotransmission. More research is needed using MPR and manipulating the two subtypes of orexin receptor to add more evidence against or in favour of this hypothesis.

# CHAPTER 5

# EXPERIMENT 4: A CLOZAPINE-LIKE EFFECT OF CYPROHEPTADINE ON PROGRESSIVE-RATIO SCHEDULE PERFORMANCE

## 5.1 Introduction

As reviewed in Chapter 1 (section 1.3), it is generally agreed that 'conventional' antipsychotic drugs, including the phenothiazines and butyrophenones, are less effective in combating the negative symptoms of schizophrenia (anhedonia, avolition, flattened affect) than they are in suppressing positive psychotic symptoms (hallucinations, delusions). Indeed, there is evidence that conventional antipsychotics may have an adverse effect on motivation in man, as they are known to do in animals (King and Waddington 2004; Wise 1982; Wise 2006). In contrast, 'atypical' antipsychotics such as clozapine are less liable to induce negative symptoms, and in some cases may even help to alleviate these symptoms by enhancing motivation (Corrigan et al. 2003; Müller-Spahn 2002).

An adverse effect of conventional antipsychotics on motivation in animals has been recognized for many years (Wise 1982). For example, operant behaviour maintained by food or psychostimulant self-administration is suppressed by conventional antipsychotics, and it has been proposed that this effect is caused by a reduction of the incentive value of primary reinforcers (Wise 1982; Wise 2006). Less is known about the effects of atypical antipsychotics on motivation and reinforcement processes; however there is evidence that these drugs enhance appetite in rodents (Hartfield et al. 2003) as they do in humans (Meltzer et al. 2003), and that they may increase the incentive value of food in operant behaviour paradigms (Cilia et al. 2001; Mobini et al. 2000; Zhang et al. 2005a; Zhang et al. 2005b; see below).

The principal pharmacological action of conventional antipsychotics is the blockade of $D_2$ dopamine receptors (Kapur et al. 2000; Seeman et al. 1976), and it has been proposed that the adverse effects of these drugs on motivation reflects their ability to disrupt dopaminergic mechanisms in limbic structures (Wise 1982; Wise 2006; see section 1.3). Atypical antipsychotics, however, have complex and varied pharmacological profiles, and their principal mode of action remains in dispute. Clozapine, the most extensively studied drug of this class, has a relatively low affinity for $D_2$ dopamine receptors, but a high affinity for several subtypes of 5-hydroxytryptamine (5-HT) receptor, most notably 5-$HT_{2A}$ receptors (Bymaster et al. 1996). The combination of $D_2$ and 5-$HT_{2A}$ receptor antagonism has been proposed as the basis of clozapine's favourable therapeutic profile

(Ichikawa et al. 2001; Meltzer et al. 2003), leading to the suggestion that the combination of a conventional antipsychotic with a 5-$HT_2$ receptor antagonist might offer some therapeutic advantages over treatment with a conventional antipsychotic alone in the management of the negative symptoms of schizophrenia (Meltzer 1995). Clinical investigations of this proposal have yielded mixed results: some studies found that the 5-HT receptor antagonist cyproheptadine produced an improvement of negative symptomatology in schizophrenic patients (Akhondzadeh et al. 1999; Bacher et al. 1994; Silver et al. 1989), whereas others found no significant effect (Chaudhry et al. 2002; Lee et al. 1995; Silver et al. 1991).

There have been several preclinical studies comparing the behavioural effects of atypical antispychotics and 5-$HT_2$ receptor antagonists. Comparisons of clozapine and cyproheptadine are of particular interest because these drugs have similar profiles of affinity for different subtypes of 5-HT receptor, both drugs having particularly high affinity for 5-$HT_{2A}$ receptors (Bymaster et al. 1996; Goudie et al. 2007; Young et al. 2005). These drugs share many behavioural effects. Both drugs stimulate appetite and induce weight gain, have sedative effects, and reverse the suppressant effect of punishment on operant responding in rats (Hartfield et al. 2003; Ketelaars and Bruinvels 1989; Moore et al. 1992) Recently it has been found that the two drugs show generalization and mutual cross-tolerance in a drug discrimination paradigm (Goudie et al. 2007). The present experiment extended these observations by comparing the effects of clozapine and cyproheptadine on operant behaviour in rats, with the aim of establishing whether these drugs share the ability to enhance the incentive value of a food reinforcer. Killeen's MPR model (Killeen 1994) was used to assess the effects of these drugs on the incentive value of reinforcers (see section 1.4. and Chapters 2, 3 and 4).

Atypical antipsychotics have a profile of action on the parameters of Equation 1 (see sections 1.4 and 2.1) which distinguishes them from conventional antipsychotics. Clozapine and other atypical antipsychotics increase both $a$ and $\delta$ (Mobini et al. 2000; Zhang et al. 2005a; Zhang et al. 2005b) consistent with an increase in the incentive value of the reinforcer and an impairment of motor function. Conventional antipsychotics also increase $\delta$, but unlike the atypical antipsychotics they either have no effect on $a$ or reduce the value of this parameter (Mobini et al. 2000; Zhang et al. 2005a).

The present experiment compared the effects of cyproheptadine and clozapine on the parameters of Equations 1 and 2 (see section 2.1). Based on the similarity of these drugs' effects on appetite and the finding that these two drugs display behavioural cross-tolerance and generalization in drug discrimination studies (see above), it was predicted that cyproheptadine, like clozapine, would increase the values of $a$ and $\delta$. For comparison, the effects of the conventional antipsychotic haloperidol and two drugs with well documented appetite-enhancing effects, chlordiazepoxide (Berridge and Treit 1986; Freet et al. 2006) and $\Delta^9$-tetrahydrocannabinol (THC) (Abel 1975; Kirkham and Williams 2001; Koch 2001; Williams and Kirkham 2002), were also examined.

## 5.2 Methods

The experiment was carried out in accordance with UK Home Office regulations governing experiments on living animals.

### *5.2.1 Subjects*

Female Wistar rats approximately 4 months old and weighing 250–300 g at the start of the experiment were used. They were housed under the same conditions as in Experiment 1 (see section 2.2.1).

### *5.2.2 Apparatus*

The same operant conditioning chambers, control apparatus and software were used as in Experiment 1 (see section 2.2.3).

### *5.2.3. Behavioural procedures*

Training under the progressive-ratio schedule was carried out in the same way as in Experiment 1 (see section 2.2.4.1).

### *5.2.4 Drug treatment*

The drug treatment regimen started after 90 sessions of preliminary training under the progressive-ratio schedule. Injections of drugs were given on Tuesdays and Fridays, and injections of the vehicle alone on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays or Sundays. Different groups of rats were used to test the various drugs (see below); each rat was tested five times with each dose of the drug, the order of doses being counterbalanced across animals according to a Latin square design. Drugs were injected intraperitoneally (2.5 ml $kg^{-1}$; 25-gauge needle) 30 min before the start of the experimental session. Doses were calculated from the weights of the salts. Cyproheptadine hydrochloride (1 and 5 mg $kg^{-1}$; $n$ = 12) and chlordiazepoxide hydrochloride (3 and 10 mg $kg^{-1}$; $n$ = 12) were dissolved in sterile 0.9% sodium chloride solution. Clozapine (3.75 and 7.5 mg $kg^{-1}$; $n$ = 15) and haloperidol (0.05 and 0.1 mg $kg^{-1}$; $n$ = 11) were dissolved in 0.1 M tartaric acid, buffered to pH 5.5 and diluted with sterile 0.9% sodium chloride to give the desired concentration. $\Delta^9$-tetahydrocannabinol (THC, 1 and 3 mg $kg^{-1}$; $n$ = 12) was dissolved in a mixture of ethanol and Tween (1:1) and diluted with strerile water to give the desired concentration. Cyproheptadine and THC were obtained from Tocris Bioscience, Bristol, UK; clozapine, haloperidol and chlordiazepoxide were obtained from Sigma Chemical Company, Poole, UK. The doses of clozapine and haloperidol were chosen on the basis of previous findings of the effects of these drugs on progressive-ratio schedule performance (Mobini et al. 2000; Zhang et al. 2005a), the dose of cyproheptadine was chosen on the basis of previous findings with the drug discrimination paradigm (Goudie et al. 2007), and the doses of chlordiazepoxide and THC were chosen on the basis of their effects on feeding behaviour (Berridge and Treit 1986; Freet et al. 2006; Koch 2001; Williams and Kirkham 2002).

### *5.2.5 Data analysis*

The data obtained with each drug were analyzed separately. Only the data obtained from the sessions in which injections had been given were used in the analysis.

(i) *Peak response rate*

The highest overall response rate (see below) attained during performance on the progressive-ratio schedule was compared between treatments by repeated-measures one-

way analysis of variance, followed, in the case of a significant effect of treatment, by comparison of each dose of the drug with the vehicle-alone treatment using Dunnett's test.

(ii) *Highest completed ratio and breakpoint*

The highest completed ratio and breakpoint (see section 2.2.6.1) were compared between treatment conditions in the same way as the peak response rate.

(iii) *Overall response rate*

Overall response rate data were analysed by two-factor analysis of variance (treatment condition × ratio) with repeated measures on both factors. The parameters were derived and analysed as in Experiment 2 (see section 2.2.6.1).

(iv) *Running response rate*

The data were analysed as described above. All the calculations were carried in the same way as in Experiment 2 (see section 2.2.6.1)

## 5.3. Results

### *5.3.1 Cyproheptadine*

#### 5.3.1.1 Peak response rate

The mean (± SEM) data are shown in Table 5.1. There was a significant effect of treatment [$F(2,22) = 19.7$, $p<0.001$], peak response rate being significantly reduced by the higher dose of cyproheptadine.

#### 5.3.1.2. Highest completed ratio and breakpoint

The mean (± SEM) data are shown in Table 5.1. There was a significant effect of treatment [$F(2,22) = 10.7$, $p<0.01$], the ratio being significantly increased by both doses of the drug. The breakpoint was also affected [$F(2,22) = 4.0$, $p<0.05$], the effect of the higher dose being statistically significant.

**Table 5.1** Peak response rate, breakpoint and highest completed ratio under each treatment condition (mean ± SEM)

| Treatment | Peak response rate (responses $min^{-1}$) | Breakpoint (ratio) | Highest completed ratio |
|---|---|---|---|
| *Cyproheptadine* | | | |
| vehicle | 129.1 ± 5.2 | 116.8 ± 13.8 | 135.7 ± 16.9 |
| 1 mg $kg^{-1}$ | 132.0 ± 6.1 | 140.6 ± 16.0 | 186.3 ± 20.5* |
| 5 mg $kg^{-1}$ | 102.1 ± 6.6* | 189.0 ± 33.3* | 226.5 ± 29.3* |
| *Clozapine* | | | |
| vehicle | 92.4 ± 6.7 | 125.9 ± 10.7 | 134.5 ± 10.6 |
| 3.75 mg $kg^{-1}$ | 82.6 ± 7.7 | 135.5 ± 16.4 | 163.6 ± 19.8 |
| 7.5 mg $kg^{-1}$ | 70.4 ± 5.5* | 137.7 ± 16.8 | 159.8 ± 18.1 |
| *Haloperidol* | | | |
| vehicle | 108.1 ± 9.5 | 126.8 ± 18.9 | 208.0 ± 39.6 |
| 0.05 mg $kg^{-1}$ | 97.7 ± 7.1 | 59.8 ± 7.4* | 112.2 ± 27.0* |
| 0.1 mg $kg^{-1}$ | 78.8 ± 7.4* | 20.1 ± 2.5* | 28.7 ± 5.9* |
| *Chlordiazepoxide* | | | |
| vehicle | 126.0 ± 6.1 | 105.2 ± 13.5 | 120.4 ± 16.2 |
| 3 mg $kg^{-1}$ | 145.4 ± 8.7* | 151.4 ± 18.8* | 178.7 ± 20.8* |
| 10 mg $kg^{-1}$ | 127.5 ± 9.6 | 116.9 ± 15.3 | 161.3 ± 21.7* |
| *THC* | | | |
| vehicle | 134.5 ± 7.8 | 105.8 ± 14.3 | 127.4 ± 18.2 |
| 1 mg $kg^{-1}$ | 131.4 ± 7.4 | 112.5 ± 15.5 | 134.0 ± 19.9 |
| 3 mg $kg^{-1}$ | 133.6 ± 7.0 | 108.9 ± 13.1 | 127.4 ± 17.5 |

* Significance of difference from vehicle control, $p<0.05$ (see text for details)

5.3.1.3. Overall response rate

The group mean data under each treatment condition are shown in Fig. 5.1 (left-hand

panel). Response rate tended to be reduced in the case of lower ratios and increased in the case of higher ratios by the higher dose of cyproheptadine. Analysis of variance revealed significant main effects of treatment [$F(2,22) = 11.6$, $p<0.001$] and ratio [$F(15,165) = 16.1$, $p<0.001$] and a significant treatment × ratio interaction [$F(30,330) = 2.9$, $p<0.001$]. The fits of Equation 1 to the group mean data accounted for >85% of the total variance (vehicle: $r^2 = 0.98$; cyproheptadine 1 mg kg$^{-1}$: $r^2 = 0.95$; 5 mg kg$^{-1}$: $r^2 = 0.86$). The parameters of Equation 1 are shown in Table 5.2. Cyproheptadine significantly increased the 'specific activation' parameter, $a$ [$F(2,22) = 9.1$, $p<0.001$] and the 'response time' parameter, $\delta$ [$F(2,22) = 12.7$, $p<0.001$], the effect of the higher dose being significant in each case. There was no significant effect of cyproheptadine on the 'currency' parameter, $\beta$ [$F(2,22) = 1.3$, $p>0.2$].

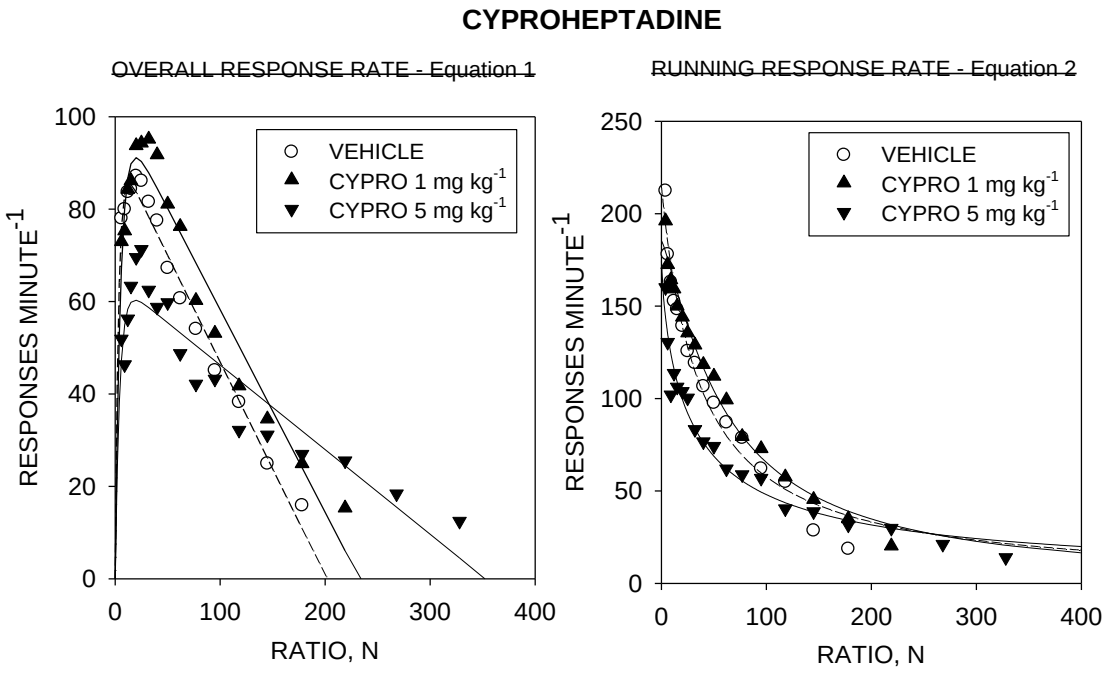


**Figure 5.1**. Effects of cyproheptadine (CYPRO) on progressive-ratio schedule performance. *Left-hand graph*: relation between overall response rate and the response/reinforcer ratio, *N*. Unfilled circles: vehicle-alone treatment; upright filled triangles: cyproheptadine 1 mg kg$^{-1}$; inverted filled triangles: cyproheptadine 5 mg kg$^{-1}$ (see inset). Curves are fits of Equation 1 to the data. *Right-hand graph*: relation between running response rate and *N*. The curves are fits of Equation 2 to the data; other conventions are as in the left-hand graph.

5.3.1.4. Running response rate

The group mean data under each treatment condition are shown in Fig. 5.1 (right-hand

**Table 5.2**. Parameters of Equation 1 (group mean data ± SEM)

| Treatment | *a* (s) | δ (s) | β |
|---|---|---|---|
| *Cyproheptadine* | | | |
| vehicle | 116.6 ± 12.2 | 0.69 ± 0.06 | 0.57 ± 0.09 |
| 1 mg $kg^{-1}$ | 151.0 ± 20.8 | 0.65 ± 0.08 | 0.45 ± 0.08 |
| 5 mg $kg^{-1}$ | 315.2 ± 64.3* | 0.88 ± 0.07* | 0.46 ± 0.08 |
| *Clozapine* | | | |
| vehicle | 174.7 ± 22.8 | 0.92 ± 0.09 | 0.37 ± 0.09 |
| 3.75 mg $kg^{-1}$ | 303.7 ± 39.3* | 1.19 ± 0.10 | 0.53 ± 0.10 |
| 7.5 mg $kg^{-1}$ | 470.9 ± 84.5* | 1.41 ± 0.24* | 0.57 ± 0.10* |
| *Haloperidol* | | | |
| vehicle | 252.7 ± 65.0 | 0.97 ± 0.10 | 0.36 ± 0.09 |
| 0.05 mg $kg^{-1}$ | 156.4 ± 53.6* | 1.11 ± 0.12 | 0.51 ± 0.09 |
| 0.1 mg $kg^{-1}$ | 40.2 ± 6.7* | 1.20 ± 0.16* | 0.63 ± 0.12 |
| *Chlordiazepoxide* | | | |
| vehicle | 101.6 ± 13.0 | 0.67 ± 0.06 | 0.42 ± 0.09 |
| 3 mg $kg^{-1}$ | 124.9 ± 15.1 | 0.56 ± 0.03* | 0.56 ± 0.07 |
| 10 mg $kg^{-1}$ | 135.6 ± 15.4* | 0.70 ± 0.06 | 0.67 ± 0.08 |
| *THC* | | | |
| vehicle | 106.9 ± 13.1 | 0.72 ± 0.11 | 0.57 ± 0.08 |
| 1 mg $kg^{-1}$ | 122.0 ± 16.6 | 0.68 ± 0.08 | 0.48 ± 0.07 |
| 3 mg $kg^{-1}$ | 101.7 ± 10.3 | 0.73 ± 0.13 | 0.52 ± 0.08 |

* Significance of difference from vehicle control, $P<0.05$ (see text for details)

panel). Running rate tended to be reduced in the case of lower ratios by both doses of cyproheptadine. Analysis of variance revealed significant main effects of treatment [$F(2,22) = 14.7$, $p<0.001$] and ratio [$F(15,165) = 81.3$, $p<0.001$] and a significant treatment × ratio interaction [$F(30,330) = 1.5$, $p<0.05$]. The fits of Equation 2 to the group mean data accounted for >90% of the total variance (vehicle: $r^2 = 0.97$; cyproheptadine 1 mg $kg^{-1}$: $r^2 = 0.98$; 5 mg $kg^{-1}$: $r^2 = 0.96$). The parameters of Equation 2

are shown in Table 5.3. Cyproheptadine significantly reduced the 'initial response rate' parameter, $R_i$ [$F(2,22) = 5.4$, $p<0.05$], the effect of the higher dose being statistically significant. There was no significant effect of cyproheptadine on the 'decay' parameter, $b$ [$F(2,22) = 2.4$, NS], or the 'exponent' parameter, $c$ [$F(2,22) = 1.8$, NS].

**Table 5.3**. Parameters of Equation 2 (group mean data ± SEM)

| Treatment | $R_i$ (responses min$^{-1}$) | $b$ | $c$ |
|---|---|---|---|
| *Cyproheptadine* | | | |
| vehicle | 222.0 ± 19.1 | 30.0 ± 13.1 | 1.43 ± 0.20 |
| 1 mg kg$^{-1}$ | 191.7 ± 15.9 | 66.9 ± 12.9 | 1.40 ± 0.11 |
| 5 mg kg$^{-1}$ | 151.9 ± 19.2* | 85.0 ± 27.0 | 2.47 ± 0.84 |
| *Clozapine* | | | |
| vehicle | 154.5 ± 10.8 | 59.4 ± 8.8 | 1.61 ± 0.21 |
| 3.75 mg kg$^{-1}$ | 138.7 ± 14.1 | 66.5 ± 12.3 | 1.40 ± 0.16 |
| 7.5 mg kg$^{-1}$ | 107.3 ± 11.4* | 72.3 ± 14.4 | 2.21 ± 0.72 |
| *Haloperidol* | | | |
| vehicle | 158.1 ± 15.9 | 59.5 ± 9.6 | 1.31 ± 0.12 |
| 0.05 mg kg$^{-1}$ | 148.1 ± 13.1 | 37.3 ± 9.4 | 1.78 ± 0.20 |
| 0.1 mg kg$^{-1}$ | 133.4 ± 17.4 | 14.5 ± 2.3* | 3.88 ± 0.75* |
| *Chlordiazepoxide* | | | |
| vehicle | 208.1 ± 20.2 | 51.6 ± 13.7 | 1.84 ± 0.40 |
| 3 mg kg$^{-1}$ | 210.7 ± 17.7 | 57.7 ± 10.8 | 1.44 ± 0.21 |
| 10 mg kg$^{-1}$ | 186.5 ± 15.0 | 53.4 ± 12.4 | 1.36 ± 0.15 |
| *THC* | | | |
| vehicle | 207.2 ± 20.1 | 48.2 ± 7.9 | 1.73 ± 0.19 |
| 1 mg kg$^{-1}$ | 201.8 ± 16.0 | 53.8 ± 11.4 | 1.82 ± 0.23 |
| 3 mg kg$^{-1}$ | 189.5 ± 9.5 | 57.0 ± 9.7 | 1.87 ± 0.11 |

* Significance of difference from vehicle control, $P<0.05$ (see text for details)

### *5.3.2. Clozapine*.

#### 5.3.2.1. Peak response rate

There was a significant effect of treatment [$F(2,28) = 10.2$, $p<0.001$], peak response rate being significantly reduced by the higher dose of clozapine (Table 5.1).

#### 5.3.2.2. Highest completed ratio and breakpoint

Clozapine had no significant effect on either measure [highest completed ratio: $F(2,28) = 2.6$, NS; breakpoint: $F<1$] (Table 5.1).

#### 5.3.2.3. Overall response rate

Clozapine tended to reduce overall response rate under the lower ratios and increase it under the higher ratios (Fig. 5.2, left-hand panel). Analysis of variance revealed significant main effects of treatment [$F(2,28) = 10.3$, $p<0.001$] and ratio [$F(15,210) = 7.3$, $p<0.001$] and a significant treatment × ratio interaction [$F(30,420) = 3.1$, $p<0.001$]. Equation 1 accounted for >85% of the total variance (vehicle: $r^2 = 0.88$; clozapine 3.75 mg $kg^{-1}$: $r^2 = 0.93$; 7.5mg $kg^{-1}$: $r^2 = 0.92$). Analysis of the parameters of Equation 1 (Table 5.2) showed that $a$ was increased by clozapine [$F(2,28) = 10.9$, $p<0.001$], the effects of both doses being statistically significant; δ was increased by clozapine [$F(2,28) = 3.4$, $p<0.05$], the effect of 7.5 mg $kg^{-1}$ being statistically significant; β was also increased by clozapine [$F(2,28) = 3.4$, $p<0.05$], the effect of 7.5 mg $kg^{-1}$ being statistically significant.

#### 5.3.2.4. Running response rate

The group mean data are shown in Fig. 5.2 (right-hand panel). The higher dose of clozapine reduced response rates in the case of lower ratios. Analysis of variance revealed significant main effects of treatment [$F(2,28) = 6.9$, $p<0.01$] and ratio [$F(15,210) = 74.8$, $p<0.001$] and a significant treatment × ratio interaction [$F(30,420) = 1.8$, $p<0.05$]. Equation 2 accounted for >90% of the total variance (vehicle: $r^2 = 0.98$; clozapine 3.75 mg $kg^{-1}$: $r^2 = 0.97$; 7.5mg $kg^{-1}$: $r^2 = 0.97$). Analysis of the parameters of

Equation 2 (Table 5.3) showed that clozapine significantly reduced $R_i$ [$F(2,22) = 5.2$, $p<0.05$], the effect of the higher dose being statistically significant. There was no significant effect of clozapine on $b$ [$F<1$] or $c$ [$F<1$].

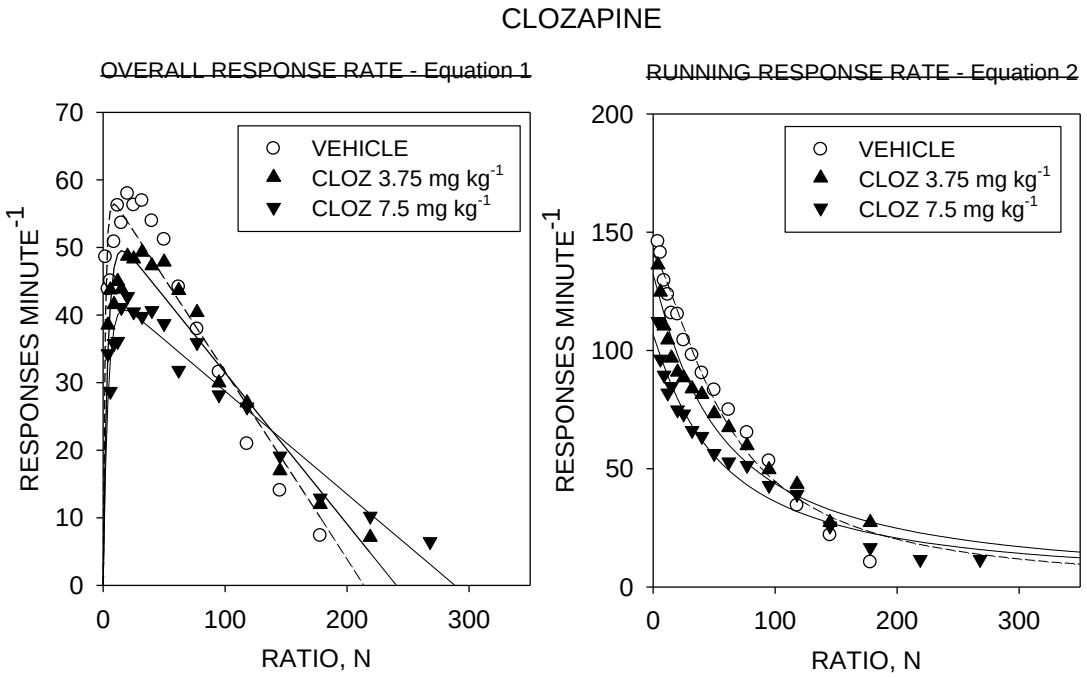


**Figure 5.2**. Effects of clozapine (CLOZ) on progressive-ratio schedule performance. Unfilled circles: vehicle-alone treatment; upright filled triangles: clozapine 3.75 mg $kg^{-1}$; inverted filled triangles: clozapine 7.5 mg $kg^{-1}$ (see inset). Other conventions are as in Fig.5.1.

### *5.3.3. Haloperidol*

#### 5.3.3.1. Peak response rate

There was a significant effect of treatment [$F(2,20) = 13.0$, $p<0.001$], peak response rate being significantly reduced by the higher dose of haloperidol (Table 5.1).

#### 5.3.3.2. Highest completed ratio and breakpoint

Haloperidol reduced the highest completed ratio [$F(2,20) = 23.4$, $p<0.001$], the effects of both doses being statistically significant. The breakpoint was also reduced [$F(2,20) =$

29.8, $p<0.001$], the effect of the both doses being statistically significant (Table 5.1).

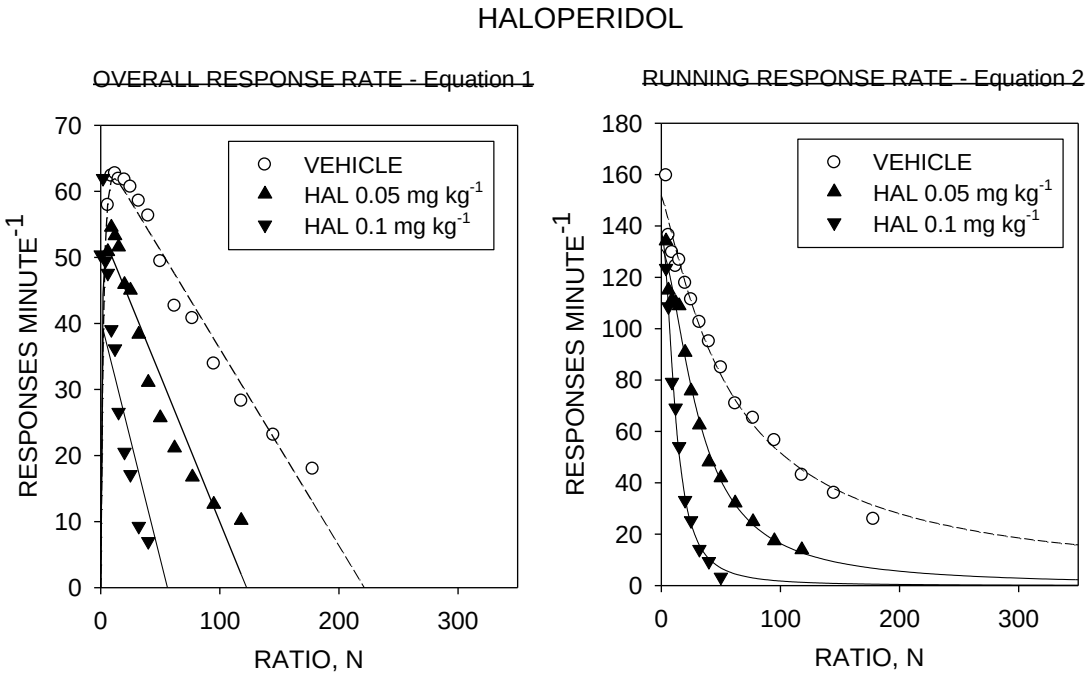


**Figure 5.3**. Effects of haloperidol (HAL) on progressive-ratio schedule performance. Unfilled circles: vehicle-alone treatment; upright filled triangles: haloperidol 0.05 mg $kg^{-1}$; inverted filled triangles: haloperidol 0.1 mg $kg^{-1}$ (see inset). Other conventions are as in Fig. 5.1.

5.3.3.3. Overall response rate

There was a dose-dependent suppression of overall response rate (Fig. 5.3, left-hand panel). There were significant main effects of treatment [$F(2,20) = 70.5$, $p<0.001$] and ratio [$F(11,110) = 14.4$, $p<0.001$] and a significant treatment × ratio interaction [$F(22,220) = 8.2$, $p<0.001$]. Equation 1 accounted for >90% of the total variance (vehicle: $r^2 = 0.97$; haloperidol 0.05 mg $kg^{-1}$: $r^2 = 0.93$; 0.1mg $kg^{-1}$: $r^2 = 0.92$). Analysis of the parameters of Equation 1 (Table 5.2) showed that haloperidol significantly reduced the value of $a$ [$F(2,20) = 9.2$, $P<0.001$] and increased the value of δ [$F(2,20) = 5.2$, $p<0.05$]; β was not significantly affected [$F(2,20) = 2.9$, $p>0.05$].

5.3.3.4. Running response rate

The group mean data are shown in Fig. 5.3 (right-hand panel). There was a dose dependent reduction of response rates. Analysis of variance revealed significant main

effects of treatment [$F(2,20) = 27.4$, $p<0.001$] and ratio [$F(11,110) = 52.0$, $p<0.001$]; the interaction term was not significant [$F(22,220) = 81.2$, NS]. Equation 2 accounted for >90% of the total variance (vehicle: $r^2 = 0.98$; haloperidol 0.05 mg $kg^{-1}$: $r^2 = 0.99$; 0.1mg $kg^{-1}$: $r^2 = 0.99$). Analysis of the parameters of Equation 2 (Table 5.2) showed that haloperidol did not significantly affect $R_i$ [$F(2,20) = 1.3$, NS]. There was a significant reduction of $b$ [$F(2,20) = 10.4$, $P<0.01$] and an increase of $c$ [$F(2,20) = 8.2$, $p<0.01$] produced by the higher dose.

### *5.3.4. Chlordiazepoxide*

#### 5.3.4.1 Peak response rate

There was a significant effect of treatment [$F(2,22) = 6.2$, $P<0.01$], peak response rate being significantly increased by the lower dose of chlordiazepoxide (Table 5.1).

#### 5.3.4.2. Highest completed ratio and breakpoint

Chlordiazepoxide increased the highest completed ratio [$F(2,22) = 7.7$, $p<0.001$], the effects of both doses being statistically significant. The breakpoint was also increased [$F(2,22) = 6.3$, $p<0.01$], the effect of the lower dose being statistically significant (Table 5.1).

#### 5.3.4.3. Overall response rate

Chlordiazepoxide tended to increase overall response rates, this being somewhat more apparent in the case of the smaller dose (3 mg $kg^{-1}$) (Fig. 5.4, left-hand panel). There were significant main effects of treatment [$F(2,22) = 11.6$, $p<0.001$] and ratio [$F(15,165) = 22.2$, $p<0.001$] and a significant treatment × ratio interaction [$F(30,330) = 1.8$, $p<0.01$]. Equation 1 accounted for >90% of the total variance (vehicle: $r^2 = 0.97$; chlordiazepoxide 3 mg $kg^{-1}$: $r^2 = 0.97$; 10 mg $kg^{-1}$: $r^2 = 0.97$). Analysis of the parameters of Equation 1 (Table 5.2) revealed a significant effect of treatment on $a$ [$F(2,22) = 5.2$, $p<0.05$], the increase produced by 10 mg $kg^{-1}$ being statistically significant. There was also a

significant effect on $\delta$ [$F(2,22) = 5.2$, $P<0.05$], reflecting a reduction of this parameter produced by the 3 mg kg$^{-1}$ dose. $\beta$ was not significantly affected [$F(2,22) = 3.1$, $P>0.05$].

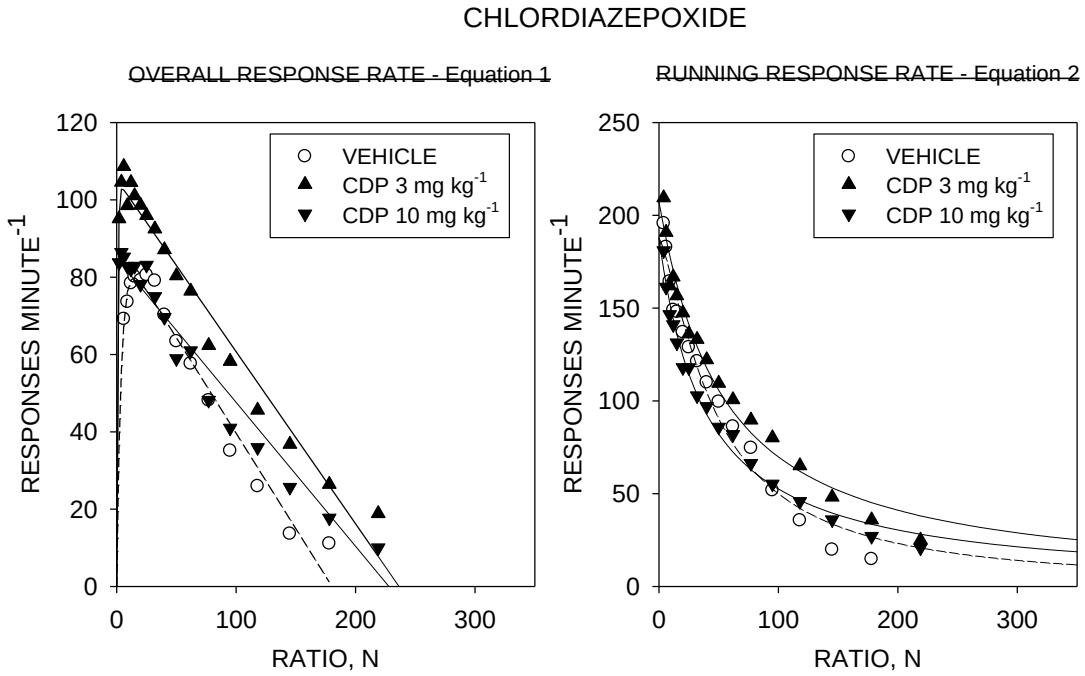


**Figure 5.4**. Effects of chlordiazepoxide (CDP) on progressive-ratio schedule performance. Unfilled circles: vehicle-alone treatment; upright filled triangles: chlordiadepoxide 3 mg kg$^{-1}$; inverted filled triangles: chlordiazepoxide 10 mg kg$^{-1}$ (see inset). Other conventions are as in Fig. 5.1.

### 5.3.4.4. Running response rate

The group mean data are shown in Fig. 5.4 (right-hand panel). The lower dose of chlordiazepoxide produced a modest increase in running response rate. There were significant main effects of treatment [$F(2,22) = 10.3$, $p<0.001$] and ratio [$F(15,165) = 66.6$, $p<0.001$]; the interaction was not significant [$F(30,330) = 1.1$, NS]. Equation 2 accounted for >90% of the total variance (vehicle: $r^2 = 0.97$; chlordiazepoxide 3 mg kg$^{-1}$: $r^2 = 0.98$; 10 mg kg$^{-1}$: $r^2 = 0.99$). Analysis of the parameters of Equation 2 (Table 5.3) showed that chlordiazepoxide had no significant effect on $R_i$ [$F<1$], $b$ [$F<1$] or $c$ [$F(2,22) = 1.3$, NS].

### *5.3.5. $\Delta^9$-tetrahydrocannabinol (THC)*

#### 5.3.5.1. Peak response rate

THC had no significant effect on the peak response rate [$F<1$] (Table 5.1).

#### 5.3.5.2. Highest completed ratio and breakpoint

THC had no significant effect on the highest completed ratio [$F(2,22) = 1.1$, NS] or the breakpoint [$F<1$].

#### 5.3.5.3. Overall response rate

THC had no apparent effect on overall response rate (Fig. 5.5, left-hand panel). Analysis of variance revealed a significant main effect of ratio [$F(15,165) = 15.5$, $p<0.001$], but no significant main effect of treatment [$F(2,22) = 2.6$, NS] and no significant interaction [$F<1$]. Equation 1 accounted for >90% of the total variance (vehicle: $r^2 = 0.97$; THC 1 mg kg$^{-1}$: $r^2 = 0.97$; 3 mg kg$^{-1}$: $r^2 = 0.97$). None of the parameters of Equation 1 was significantly affected by the drug [$a$: $F(2,22) = 2.2$, $p>0.05$; $\delta$: $F(2,22) = 1.6$, $p>0.1$; $\beta$: $F<1$] (Table 5.2).

#### 5.3.5.4. Running response rate

THC had no significant effect on running response rate (Fig. 5.5, right-hand panel). There was a significant main effect of ratio [$F(15,165) = 93.3$, $p<0.001$], but no significant main effect of treatment [$F<1$] and no significant interaction [$F(30,330) = 1.1$, NS]. Equation 2 accounted for >90% of the total variance (vehicle: $r^2 = 0.98$; THC 1 mg kg$^{-1}$: $r^2 = 0.98$; 3 mg kg$^{-1}$: $r^2 = 0.99$). None of the parameters of Equation 2 was significantly affected by THC [$R_i$, $b$, $c$: all $F$s<] (Table 5.3).

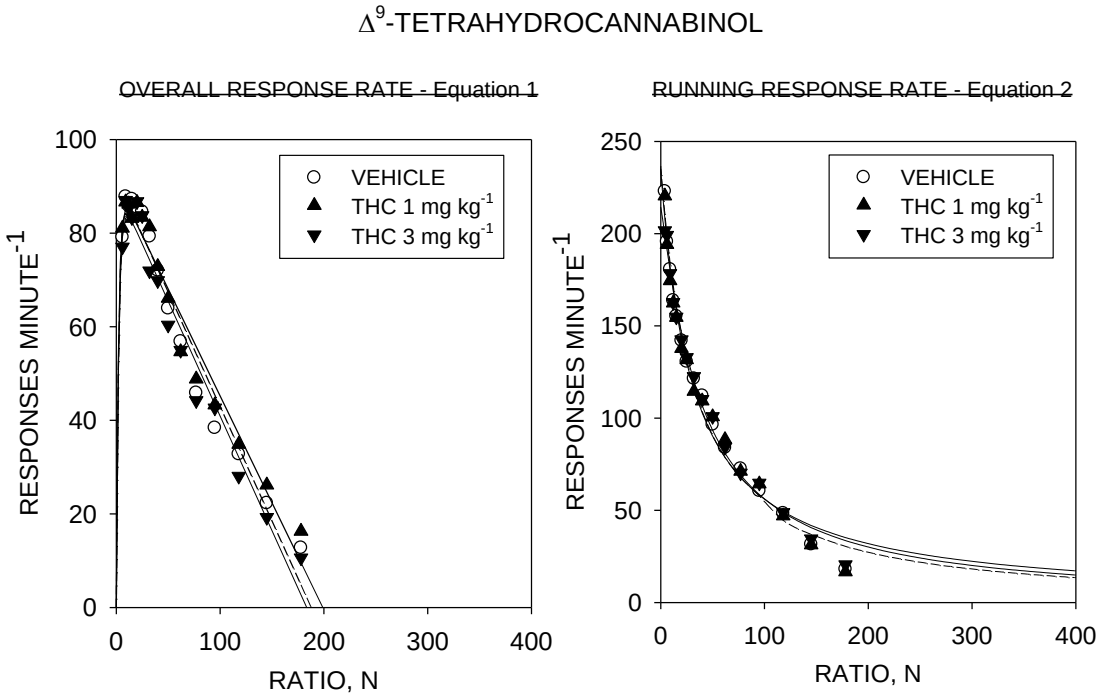


**Figure 5.5** Effects of $\Delta^9$-tetrahydrocannabinol (THC) on progressive-ratio schedule performance. Unfilled circles: vehicle-alone treatment; upright filled triangles: THC 1 mg $kg^{-1}$; inverted filled triangles: THC 3 mg $kg^{-1}$ (see inset). Other conventions are as in Fig. 5.1.

## 5.4. Discussion

Performance on the progressive-ratio schedule was similar to that seen in many previous studies (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al. 2003; Kheramin et al. 2005; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b) and in Experiments 2,3, and 4 (see section 2.4 for discussion).

Previous comparisons of the effects of conventional and atypical antipsychotics on progressive-ratio schedule performance found that the two classes of drugs exerted different patterns of effect on the parameters of Equation 1 (Mobini et al. 2000; Zhang et al. 2005a). Atypical antipsychotics increased the values of both the 'specific activation' parameter, *a*, and the 'response time' parameter, *δ*; in contrast, conventional antipsychotics increased δ, but either reduced or had no effect on *a*. The present results are consistent with these previous findings, in that clozapine increased both *a* and *δ*,

whereas haloperidol increased δ and reduced *a*.

The parameter *a* is believed to provide a quantitative measure of the efficacy or value of reinforcers (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Kheramin et al. 2005b; Killeen 1994; Killeen 2009; Killeen and Sitomer 2003; Reilly 2003; Rickard et al. 2009). Thus the opposite effects of clozapine and haloperidol on this parameter are consistent with the notion that clozapine increased and haloperidol reduced the incentive value of the food reinforcer. The decay parameter, *b*, of Equation 2 is also related to incentive value (Rickard et al. 2009). Clozapine tended to increase this parameter, although the effect was not statistically significant, whereas haloperidol significantly reduced it.

The apparent enhancement of the incentive value of food reinforcers by clozapine may be related to this drug's known appetite-enhancing effect (Comer et al. 1997; Goudie et al. 2007; Hartfield et al. 2003). The mechanism underlying this effect is uncertain. Clozapine has a complex receptor binding profile which includes notably high affinities for 5-$HT_{2A}$, histamine $H_1$ and muscarinic $M_1$ receptors (Bymaster et al. 1996); it has been suggested that the combined blockade of these receptors is responsible for the hyperphagic effects of this drug (Hartfield et al. 2003).

A reduction of the incentive value of food reinforcers by haloperidol is consistent with the well known 'anhedonia theory' which posits that conventional antipsychotics reduce the rewarding value of food and other reinforcers by blocking dopamine $D_2$ receptors in limbic structures (Wise 1982; Wise 2006). Evidence consistent with an antihedonic effect of conventional antipsychotics derives from a wide variety of behavioural approaches, including operant response rate measures (Heyman 1983; Morley et al. 1984; Salamone 1987; Salamone et al. 2001), conditioned reinforcement paradigms (Cador et al. 1991), and the rate of extinction of operant responses (Salamone 1986; Wise et al. 1978a). In some cases, these observations may be susceptible to an alternative interpretation in terms of a motor debilitating effect of the drugs (see Salamone et al. 1994, 1997). Quantitative analysis of schedule-controlled behaviour based on Killeen's (1994) MPR model enables these two processes to be separated mathematically. Previous applications of this method indicated a predominant effect of haloperidol on motor capacity (Mobini et al. 2000; Zhang et al. 2005a). However, the present results indicate that haloperidol

also produced a substantial reduction of the incentive value of the food reinforcer.

Since $\delta$ is believed to be inversely related to the motor capacity of the organism (Bezzina et al. 2008a; Bezzina et al. 2008b; Bizo and Killeen 1997; Kheramin et al. 2005; Killeen 1994; Killeen 2009; Killeen and Sitomer 2003; Reilly 2003; Rickard et al. 2009), the increase in this parameter produced by both clozapine and haloperidol is consistent with an adverse effect of both drugs on motor functioning. The parameter $R_i$ of Equation 2 may also be related to motor functioning, since it expresses the maximum rate of emission of trains of operant responses; however, unlike $\delta$, it is not influenced by the post-reinforcement pause, which may in part reflect post-prandial effects of the reinforcer (Rickard et al. 2009). Both clozapine and haloperidol tended to reduce this parameter, the effect being statistically significant only in the case of clozapine.

The apparent motor debilitating effects of clozapine and haloperidol are unlikely to reflect the same underlying process. In clozapine's case the effect may be related to sedation, a known side-effect of this drug which is generally attributed to the blockade of central $H_1$ receptors (King and Waddington 2004). Haloperidol's effect on the motor parameters of Equations 1 and 2 is more likely to reflect blockade of $D_2$ receptors in the basal ganglia, the basis of the ubiquitous extrapyrimidal motor side-effects of conventional antipsychotics (Cunningham Owens 1999).

Both clozapine and haloperidol produced modest increases in the value of the currency parameter, $\beta$, although this effect only reached statistical significance in the case of clozapine. The interpretation of this finding is unclear. Increases in this parameter have been found to occur following increases in reinforcer size (Bizo et al. 2001; Bezzina et al. 2008a; Rickard et al. 2009), possibly reflecting the propensity of larger reinforcers to induce more effective response-reinforcer coupling (Killeen 1994; Bizo et al. 2001; Rickard et al. 2009). This might suggest that clozapine increased the efficiency of response-reinforcer coupling in this experiment. However, it may be premature to attribute theoretical significance to the present finding, because previous experiments have not generally found reliable effects of clozapine and other antipsychotics on this parameter (Mobini et al. 2000; Zhang et al. 2005a, 2005b).

The main purpose of this experiment was to compare the effects of cyproheptadine and clozapine on progressive-ratio schedule performance. The results indicate that the two drugs had similar profiles of effect on the parameters of Equations 1 and 2. On the basis of the theoretical interpretation of these parameters discussed above, the present results are thus consistent with the notion that cyproheptadine, like clozapine, increased the incentive value of the food reinforcer and induced a degree of motor debilitation. An increase in the incentive value of food reinforcers by cyproheptadine may be related to the known appetite-enhancing effect of this drug, an effect that it shares with clozapine (Goudie et al. 2007; Hartfield et al. 2003). It has been proposed that the hyperphagic effects of both drugs reflect the combined blockade of central 5-$HT_{2A}$, $H_1$ and $M_1$ receptors (Goudie et al. 2007; Hartfield et al. 2003). The apparent motor debilitating effects of both drugs (increase in $\delta$ and reduction of $R_i$) may reflect sedation which may be attributable to $H_1$ receptor blockade in both cases.

The similar pharmacological profiles of cyproheptadine and clozapine has led to the suggestion that cyproheptadine may be of value in the management of schizophrenia, particularly in the case of patients for whom negative symptoms are especially troublesome. Although attempts to verify this proposal in clinical studies have so far yielded mixed results (see section 5.1), the finding that cyproheptadine, like clozapine, may enhance incentive reinforcer value suggests that further clinical studies may be worthwhile (see Goudie et al. 2007). It must be emphasized, however, that the present results were obtained exclusively with food reinforcers, and it remains to be established whether cyproheptadine's and clozapine's effects reflect interactions with general reward processes, rather than with mechanisms specific to food reinforcement. Further experimental work is needed in order to address this matter.

Combined treatment with cyproheptadine and haloperidol was not tested in these experiments. It would be of interest, in future experiments, to examine the effect of this treatment combination on different behavioural paradigms, in view of the suggestion that combined treatment with cyproheptadine and a conventional antipsychotic may confer some advantages in the management of schizophrenia (see above). However, it is questionable whether the progressive-ratio schedule would be the ideal model for assessing the utility of this treatment. The putative clinical value of this treatment approach resides in the combination of a drug that may alleviate anhedonia and other

negative symptoms (i.e cyproheptadine) and a drug with known ability to suppress positive symptoms (i.e. haloperidol). The quantitative analysis of progressive-ratio schedule performance adopted here provides a means of assessing the incentive value of positive reinforcers, and may therefore provide a useful measure of the propensity of drugs to alleviate negative symptoms; however it offers no insights into ability of drugs to suppress positive psychotic symptoms

Chlordiazepoxide's effect on the parameters of Equations 1 and 2 is consistent with an increase in the incentive value of the reinforcer (increase in *a* and *b*). However, unlike clozapine and cyproheptadine, its effect is not suggestive of motor impairment; indeed the lower dose actually reduced $\delta$ and increased $R_i$, suggesting a modest facilitation of operant responding. The increase in reinforcer value may be related to chlordiazepoxide's known ability to facilitate feeding in rodents (Berridge and Treit 1986; Freet et al. 2006) The mechanism underlying benzodiazepines' appetite-enhancing effect is uncertain. However, since the main pharmacological action of these drugs is to facilitate γ-aminobutyric acid (GABA)-mediated functions, and they do not interact directly with 5-$HT_{2A}$ or $H_1$ receptors (Cooper 2004), the mechanism proposed to account clozapine's and cyproheptadine's effects on food intake and incentive value is unlikely to be responsible for chlordiazepoxide's effects.

THC had no significant effect on performance on the progressive-ratio schedule. This negative result was unexpected in view of THC's known appetite-stimulating effect (Abel 1975; Williams and Kirkham 1999; Williams et al. 1998) and previous reports of its ability to increase the breakpoint in progressive-ratio schedules (Higgs et al. 2005). It is possible that the doses used in this experiment were inadequate to alter the incentive value of the food reinforcer. However, these doses were selected on the basis of previous studies that reported increases in food intake (Higgs et al. 2005; Jarrett et al. 2005). The effect of cannabinoids on food reinforcement is known to differ between different foodstuffs, being especially pronounced in the case of sweet foods (Ward and Dykstra 2005). The reinforcer pellets used in these experiments are more palatable and have a higher fat content than standard laboratory chow, but have no sucrose or other carbohydrate content (TestDiet published data). It may be of interest, therefore, to compare the effects of THC on the parameters of Equation 1 using progressive-ratio

schedules employing both fat and carbohydrohydrate reinforcers; this issue is addressed in the final experiment of the present project (see Chapter 7).

The present findings provide a further illustration of the implications of Killeen's (1994) MPR model for interpreting the effects of neuropharmacological interventions on progressive-ratio schedule performance (for review, see Killeen et al. 2009; Rickard et al. 2009). According to MPR, the traditional index of performance on this schedule (the breakpoint) is a hybrid measure, being jointly determined by the incentive value of the reinforcer, represented by $a$, and the motor limitations of the organism, represented by $\delta$. The estimated value of the breakpoint, derived by extrapolation of the descending limb of the response rate function, is defined as $a/\delta$. In the case of interventions that have little impact on motor performance, changes in the breakpoint may provide a reliable indication of changes of incentive value. However, an effect on motor performance may augment, attenuate or even completely override the impact of a change of incentive value on the breakpoint. In the present experiments, there was a general tendency for changes in $a$ to coincide with changes in the breakpoint. For example both doses of cyproheptadine and the lower dose of chlordiazepoxide increased $a$ and the breakpoint, whereas haloperidol produced dose-dependent reductions of both these measures. Clozapine, however, did not significantly alter the breakpoint, evidently because the effects of the drug on $a$ and $\delta$ exerted opposing influences on the breakpoint.

The breakpoint also presents practical difficulties for the behavioural pharmacologist. It is usual to describe the breakpoint as the response/reinforcer ratio at which responding ceases. However, the operational definition of the cessation of responding is arbitrary, and different criteria have been adopted by different workers (see Killeen et al. 2009). Moreover, allowing the experimental session to become extended for an indefinite period until the breakpoint is reached can result in test periods that differ in length between subjects and conditions, clearly a complicating factor when the effects of acute drug treatments are compared. In the present experiments, as in many previous studies (e.g. Aberman et al. 1998; Zhang et al. 2005a, 2005b), time-constrained sessions were used. Although this avoids the problem of variable session length, it results in the formal breakpoint criterion (in the present case, 5 minutes without responding) not being attained by all subjects in all sessions, and necessitates either the elimination of data from sessions in which the criterion was not reached or the use of some other measure, such as

the highest ratio completed within the time-constrained session. As argued elsewhere (Killeen et al. 2009; Rickard et al. 2009), the mathematical approach exemplified by MPR, by deriving parameter estimates for individual subjects, avoids many of the practical, as well as the theoretical difficulties associated with the use of the breakpoint.

In summary, the present results confirm the differing effects of haloperidol and clozapine on progressive-ratio schedule performance, and indicate that cyproheptadine's effect resembled that of clozapine. Quantitative analysis based on Killeen's (1994) theoretical model of schedule-controlled behaviour, MPR, indicated that both clozapine and cyproheptadine enhanced the incentive value of the food reinforcer and induced some degree of motor impairment. The results further confirm the utility of quantitative analysis of progressive-ratio schedule performance based on MPR for investigations of the effects of drugs on motivational processes (Killeen 2009; Rickard et al. 2009).

# CHAPTER 6

# EXPERIMENT 5: DIFFERENTIAL EFFECTS OF $D_1$ AND $D_2$ DOPAMINE RECEPTOR ANTAGONISTS ON PROGRESSIVE-RATIO SCHEDULE PERFORMANCE OF RATS REINFORCED WITH SUCROSE OR CORN OIL

## 6.1. Introduction

The rewarding properties of food have been extensively studied in animals and humans and it is thought that the sugar and fat content are primarily responsible for the rewarding effects of most foodstuffs (Levin and Dunn-Meynell 2002; Levine et al. 2003; Sclafani 2004). The overconsumption of food together with the lack of exercise are thought to be the major factors responsible for the world-wide increase of obesity. Although it is generally accepted that foods with high fat content are very rewarding for humans and animals (Drewnowski and Greenwood 1983; Mattes 1993; Takeda et al. 2000), only a few studies have analysed the reinforcing properties of fat (Hayward et al. 2002; Imaizumi et al. 2000; Imaizumi et al. 2001; Yoneda et al. 2007b). Understanding the neurobehavioral mechanisms that lead to the overconsumption of fat may contribute to the treatment of obesity. However, despite the importance of understanding how these mechanisms work, little is known about why animals and humans are so attracted to fat (Yoneda et al. 2007a).

The progressive-ratio schedule has been traditionally used to evaluate the efficacy of reinforcers under different experimental situations (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al. 2003; Hodos 1961; Hodos and Kalman 1963; Kheramin et al. 2005b; Killeen et al. 2009; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b). Hodos et al (1961) found that the breakpoint increased with the volume and concentration of a sweet solution, a finding that has been corroborated by subsequent experiments (Cheeta et al. 1995; Hodos and Kalman 1963; Naleid et al. 2008). Recently Rickard et al. (2009) carried out an experiment with rats reinforced with different volumes of a sucrose solution. Their results showed that the motivation to work for sucrose, as indicated by the parameter *a* in Killeen's (1994) MPR model, was directly proportional to the quantity of sucrose received (see section 1.4). Covarrubias and Aparicio (2008), in another experiment using progressive-ratio schedules, found higher breakpoints and a trend for *a* to be higher when rats were reinforced with saccharin in comparison with a sucrose reinforced condition. Other food reinforcers, including standard food pellets, which combined different kinds of nutrients and flavours, have been also used as reinforcers in progressive-ratio schedules (Bezzina et al. 2008b; Bezzina et al. 2008c; Borgland et al. 2009; Ho et al. 2003; Maccioni et al. 2008).

The advantage of using pure nutrients (e.g. sucrose solution or fat) is that the effects of pharmacological manipulation on operant performance may provide insights into the brain mechanisms involved in the rewarding properties of those particular nutrients. For example, it has been suggested that $D_1$ and $D_2$ dopamine receptors are differentially involved in regulating the rewarding qualities of carbohydrate and fatty foodstuffs (see below). It might therefore be expected that antagonism of these receptors would have different effects on the 'incentive value' (or 'specific activation') parameter, *a*, in MPR, when carbohydrates or fat are used as the reinforcer. This possibility was explored in the experiments described in this chapter.

As reviewed in section 1.3, the mesolimbic dopaminergic system has been proposed to regulate the rewarding properties of food and other reinforcers. Much of the evidence supporting this suggestion came from studies using conventional antipsychotic drugs (see section 1.3). For example, in the case of progressive-ratio schedules, it has been found that these drugs decreased both the breakpoint (Cheeta et al. 1995; Mobini et al. 2000; Zhang et al. 2005a) and the activation parameter *a* (Mobini et al. 2000; see also chapter 5) when the reinforcer was sucrose. The putative mechanism of action of conventional antipsychotics is the antagonism of $D_2$ dopamine receptors (Kapur et al. 2000; Seeman et al. 1976), and the anhedonic effects produced by these drugs are thought to be caused by the blockade of $D_2$ dopamine receptors in the limbic system (Wise 1982; Wise 2006) (see section 1.3).

However, although there have been numerous studies that have used sucrose-based reinforcers (see above), there have been relatively few studies that have used pure fat reinforcers in progressive-ratio schedules (Naleid et al. 2008; Ward and Dykstra 2005; Yoneda et al. 2007). Recently Naleid et al. (2008) tested the motivational properties of different concentrations of sucrose, oil or a mixture of the two on performance on progressive-ratio and fixed-ratio schedules. They found that the most effective reinforcers were sucrose solutions, and that the reinforcing efficacy of sucrose increased as a function of the concentration of the solution.

Yoneda et al. (2007b) examined the role of dopamine receptors in the reinforcing efficacy of corn oil in mice. They found that mice responded for corn oil under a

progressive-ratio schedule, and that the breakpoint was decreased by systemic treatment with the $D_2$ receptor antagonist sulpiride, but not by treatment with the $D_1$ receptor antagonist SCH-23390 (7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol). Further evidence in favour of the involvement of $D_2$ receptors in the rewarding properties of corn oil came from sham feeding experiments (Schneider et al. 1990; Schneider et al. 1986; Schneider et al. 1998; Weatherford et al. 1990; Weatherford et al. 1988). In this technique, animals are able to taste and swallow the foodstuff; however it is drained, via a gastric fistula, before reaching the intestine. Thus, the animals are exposed to the orosensory properties of food but the post-ingestive effects are minimized. Therefore, it is thought that the oromotor acts of licking and ingestion are due to the (sensory) reinforcing properties of food and not to the satisfaction of a 'hunger drive' (Weatherford et al. 1990). In Weatherford et al.'s (1990) experiment rats were sham fed with either corn oil or sucrose. The authors found that the $D_1$ receptor antagonist SCH-23390 reduced both sucrose and corn oil intake; however, while sucrose intake was reduced by SCH-23390 in a dose-dependent manner, corn oil intake was reduced only with the highest dose tested, which also produced a sedative effect. In contrast, the $D_2$ receptor antagonist raclopride was found to suppress both corn oil and sucrose sham feeding at doses that did not induce any sedative effect. These results suggest that $D_1$ dopamine receptor plays a less important role than $D_2$ dopamine receptors in the reinforcing effect of corn oil.

The role of $D_1$ dopamine receptors in the rewarding properties of corn oil have also been evaluated with the conditioned place preference test. Imaizumi et al. (2000) reported that acquisition of a place preference by corn oil intake was blocked by systemic treatment with either SCH-23390 (0.03 mg/kg) or haloperidol (0.1 mg/kg). The authors concluded that the positive reinforcing effects of corn oil were at least partially mediated by $D_1$ receptors (Imaizumi et al. 2000). In contrast, in the 'one bottle test', neither $D_1$ nor $D_2$ receptor antagonist drugs affected oil consumption, which led to Yoneda et al (2007a) to conclude that the dopamine system is involved in the reinforcing effect of corn oil but not on its consumption.

Although there is a general agreement about the involvement of the dopaminergic system in regulating the rewarding properties of reinforcers, it is not clear from the evidence outlined above which subtype(s) of dopamine receptors are involved in controlling the

reinforcing efficacy of sucrose or fat.

The first objective of this experiment was to test whether rats reinforced with corn oil would respond on the progressive-ratio schedule in a similar manner as they do with other reinforcers. In particular, it was of interest to establish whether meaningful data would be generated that could be used to fit Equations 1 and 2 for the derivation of estimates of the parameters of MPR. To address this objective, two groups of rats were trained on the progressive-ratio schedule to respond for either 25 µl of 100% (0.2 kcal) corn oil or 50 µl of a 0.6M (approximately 20%) sucrose solution (0.04 kcal).

The other objective of this experiment was to examine the effects of a $D_2$ receptor antagonist, haloperidol, and a $D_1$ receptor antagonist SKF-83566 on performance on the progressive-ratio schedule maintained by the two types of reinforcer, to see whether the effect of these drugs on Killeen's MPR model (1994) and Rickard et al.'s (2009) equation would differ between the two types of reinforcer. Based on the research outlined above (Yoneda et al. 2007b), it was predicted that in the group that received corn oil as the reinforcer, both motivational measures (*a* and *b*) would be reduced by the $D_2$ receptor antagonist, but not by the $D_1$ receptor antagonist. In contrast, it was expected that the sucrose-reinforced group would show a reduction of *a* and *b* when treated with either the $D_2$ or the $D_1$ receptor antagonist.

## 6.2. Methods

The experiments were carried out in accordance with the UK Home Office regulations governing experiments on living animals.

### *6.2.1. Subjects*

Twenty-four experimentally naive female Wistar rats approximately 4 months old and weighing 250–300 g at the start of the experiment were used. They were housed under the same conditions as in Experiment 1 (see section 2.2.1).

### *6.2.2. Apparatus*

The rats were trained in standard operant conditioning chambers (CeNeS Ltd, Cambridge, UK) of internal dimensions 25cm×25cm×22 cm. One wall of the chamber contained a central recess covered by a hinged clear Perspex (plexiglas) flap, into which a peristal peristaltic pump could deliver 50 µl of a 0.6 M (20%) sucrose solution or 25 µl of 100% corn oil. An aperture situated 5 cm above and 2.5 cm to one side of the recess (left for half the rats and right for the other half) allowed the insertion of a motorized retractable lever (CeNeS Ltd, Cambridge, UK) into the chamber. The lever could be depressed by a force of approximately 0.2 N. The operant chamber was enclosed in a sound-attenuating chest with additional masking noise generated by a rotary fan. No houselight was present during the sessions. An Acorn microcomputer programmed in Arachnid BASIC (CeNeS Ltd, Cambridge, UK), located in an adjoining room, controlled the schedules and recorded the behavioural data.

### *6.2.3. Progressive-ratio schedule*

The behavioural training procedure was carried out in the same way as in Experiment 1 (see section 2.2.4.1), with the exception that the session duration was 40 minutes rather than 50 minutes. Twelve rats were trained using the sucrose reinforcer, and twelve using the corn oil reinforcer.

### *6.2.4. Drug treatment*

The drug treatment regimen started after 120 sessions of preliminary training under the progressive-ratio schedule. Injections of drugs were given on Tuesdays and Fridays, and injections of the vehicle alone on Mondays and Thursdays; no injections were given on Wednesdays, Saturdays or Sundays. The same rats were used to test the two drugs and each drug was tested five times with each dose of the drug, the order of drugs and doses being counterbalanced across animals according to a Latin square design. Drugs were injected intraperitoneally (2.5 ml $kg^{-1}$; 25-gauge needle) 30 min before the start of the experimental session. Doses were calculated from the weights of the salts. Haloperidol (0.05 and 0.1 mg $kg^{-1}$) was dissolved in 0.1 M tartaric acid, buffered to pH 5.5 and diluted with sterile 0.9% sodium chloride to give the desired concentration. SKF-83566 (8-

bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hydrobromide) (0.015 and 0.03 mg $kg^{-1}$) was dissolved in 0.9% sodium chloride solution. Haloperidol was obtained from Sigma Chemical Company, Poole, UK; SKF-83566 was obtained from Tocris Bioscience, Bristol, UK. The doses of haloperidol were chosen on the basis of previous findings of the effects of this drug on progressive-ratio schedule performance (Mobini et al. 2000; Zhang et al. 2005a; see also Chapter 5). SKF-83566 has not been tested previously in this paradigm; the doses were chosen on the basis of recent findings of the effects of this drug on operant behaviour in free-operant timing schedules (Cheung et al. 2006; Cheung et al. 2007).

### *6.2.5. Data analysis*

The data from the two groups (sucrose reinforcer, corn oil reinforcer) were analysed as separate experiments. In each case, the same behavioural measures were used as in the previous experiment (see section 5.2.5). In the case of each measure, the values obtained in the presence of the two active doses of each drug were compared with the values obtained in the sessions in which the corresponding vehicle treatment was administered (see section 5.2.5).

## 6.3. Results

### *6.3.1. Sucrose reinforcement*

#### 6.3.1.1. Haloperidol

##### 6.3.1.1.1. Peak response rate

The group mean (± SEM) data are shown in Table 6.1. Haloperidol had no significant effect [$F(2,22) = 2.1$, NS].

##### 6.3.1.1.2. Highest completed ratio and breakpoint

The group mean (± SEM) data are shown in Table 6.1. In the case of the highest completed ratio, there was a significant effect of treatment [$F(2,22) = 8.7$, $p<0.05$], the

highest completed ratio being significantly reduced by both doses of haloperidol. In the case of the BP, there was also a significant treatment effect [$F(2,22) = 9.0$, $p<0.001$], the effect being significant only in the case of the higher dose.

**Table 6.1**. Progressive-ratio schedule performance: sucrose reinforcer. Effect of haloperidol on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| HALOPERIDOL | vehicle | haloperidol 0.05 mg $kg^{-1}$ | haloperidol 0.1 mg $kg^{-1}$ |
|---|---|---|---|
| *Highest completed ratio* | 130.2 ± 23.1 | 109.8 ± 17.3 * | 93.6 ± 18.8 * |
| *Breakpoint* | 122.8 ± 21.9 | 106.1 ± 16.8 | 88.6 ± 17.3 * |
| *Peak response rate* | 161.2 ± 15.0 | 157.3 ± 14.4 | 149.0 ± 10.7 |

* significant difference from vehicle-alone treatment: $p<0.05$

6.3.1.1.3. Overall response rate

Figure 6.1 (left-hand panel) shows the group mean overall response rate for each treatment condition. The response rate tended to be reduced by the higher dose of haloperidol. Haloperidol produced a dose-dependent reduction of response rate. Analysis of variance revealed significant main effects of treatment [$F(2,22) = 8.9$, $p<0.001$] and ratio [$F(14,154) = 43.1$, $p<0.001$] and a significant treatment × ratio interaction [$F(28,308) = 1.9$, $p<0.05$]. The fits of Equation 1 to the group mean data accounted for >90% of the total variance (vehicle, $r^2 = 0.92$; haloperidol 0.05mg $kg^{-1}$, $r^2 = 0.93$; haloperidol 0.1mg $kg^{-1}$, $r^2 = 0.90$). Table 6.2 shows the parameters of Equation 1. Both doses of haloperidol significantly reduced the activation parameter $a$ [$F(2,22) = 3.5$, $p<0.05$]. There was a significant effect of treatment on the response time parameter $\delta$ [$F(2,22) = 3.8$, $p<0.05$]; however, Dunnett's test showed that neither dose differed significantly from the vehicle-alone condition. The currency parameter $\beta$ [$F<1$] was not significantly affected.

6.3.1.1.4. Running response rate

Fig 6.1 (right-hand panel) shows the group mean data under each treatment condition.

Running response rate tended to be reduced by the higher dose of haloperidol. Analysis of variance yielded a significant main effect of ratio [$F(14,154) = 90.5$, $p<0.001$], and there was a borderline main effect of treatment [$F(2,22) = 3.0$, $p=0.06$]; the treatment × ratio interaction effect was not significant [$F(28,308) = 1.42$, NS]. The fits of Equation 2 to the group mean data accounted for >90% of the total variance (vehicle: $r^2 = 0.92$; haloperidol 0.05mg kg$^{-1}$: $r^2 = 0.95$; 0.1mg kg$^{-1}$ haloperidol 0.1 mg kg$^{-1}$: $r^2 = 0.93$). The parameters of Equation 2 are shown in Table 6.2. Neither dose of haloperidol significantly reduced *Ri* [$F(2,22) = 3.3$, NS]; however, the 'decay' parameter, *b*, was significantly reduced [$F(2,22) = 4.3$, $p<0.05$], the effect of the higher dose being statistically significant. The 'exponent' parameter *c* was not affected by the treatment [$F(2,22) = 1.7$, NS].

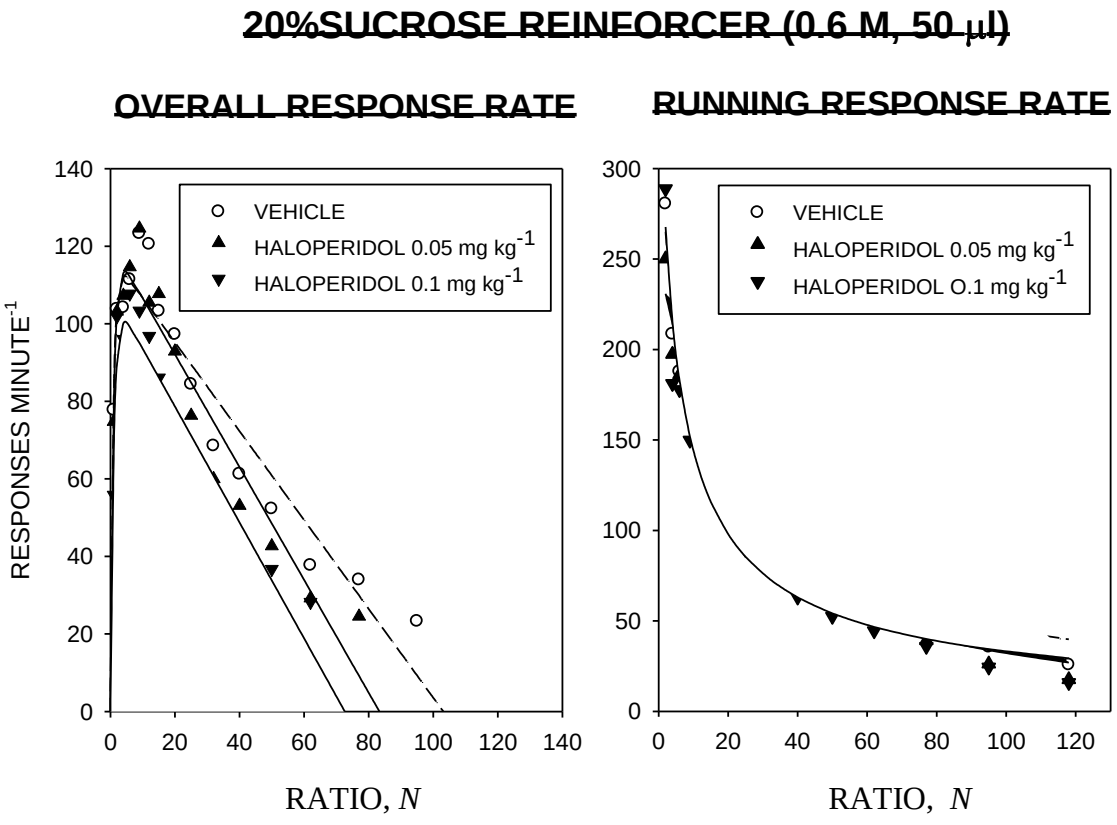


**Figure 6.1**. Effects of haloperidol on progressive-ratio schedule performance reinforced with sucrose. *Left-hand graph*: relation between overall response rate and the response/reinforcer ratio, *N* (see inset for treatment conditions). Curves are fits of Equation 1 to the data. *Right-hand graph*: relation between running response rate and *N*. The curves are fits of Equation 2 to the data; other conventions are as in the left-hand graph.

**Table 6.2**. Progressive-ratio schedule performance: sucrose reinforcer. Effect of haloperidol on the parameters of Equations 1 and 2 (group mean values ± SEM)

| HALOPERIDOL | vehicle | haloperidol 0.05 mg $kg^{-1}$ | haloperidol 0.1 mg $kg^{-1}$ |
|---|---|---|---|
| *Equation 1 (overall response rate)* | | | |
| *a* (s) | 59.2 ± 9.1 | 44.9 ± 6.3 * | 45.8 ± 8.9 * |
| $\delta$ (s) | 0.55 ± 0.06 | 0.52 ± 0.02 | 0.59 ± 0.06 |
| $\beta$ | 0.55 ± 0.06 | 0.63 ± 0.05 | 0.58 ± 0.06 |
| $r^2$ | 0.83 ± 0.03 | 0.81 ± 0.02 | 0.78 ± 0.03 |
| *Equation 2 (running response rate)* | | | |
| $R_i$ (responses $min^{-1}$) | 315.5 ± 24.4 | 274.5 ± 20.0 | 345.4 ± 18.0 |
| *b* | 25.2 ± 8.2 | 24.4 ± 6.2 | 11.5 ± 4.1 * |
| *c* | 1.4 ± 0.1 | 1.4 ± 0.1 | 1.1 ± 0.1 |
| $r^2$ | 0.92 ± 0.01 | 0.95 ± 0.01 | 0.93 ± 0.01 |

* significant difference from vehicle control ($P$ <0.05)

6.3.1.2. SKF-83566

6.3.1.2.1. Peak response rate

As can be seen from Table 6.3, the peak reponse rate was not affected by any either dose of SKF-83566 [$F(2,22) = 1.8$, NS].

6.3.1.2.2. Highest completed ratio and breakpoint

Table 6.3 shows the group mean (± SEM) data. There was a significant reduction of the highest completed ratio [$F(2,22) = 8.0$, $p<0.01$] and the breakpoint [$F(2,22) = 4.3$, $p<0.01$], the effect of the higher dose of SKF-83566 being significant in each case.

**Table 6.3**. Progressive-ratio schedule performance: sucrose reinforcer. Effect of SKF-83566 on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| SKF-83566 | vehicle | SKF-83566 0.15 mg $kg^{-1}$ | SKF-83566 0.03 mg $kg^{-1}$ |
|---|---|---|---|
| *Highest completed ratio* | 140.5 ± 23.6 | 129.9 ± 23.7 | 120.0 ± 19.6 * |
| *Breakpoint* | 137.3 ± 22.9 | 132.4 ± 22.0 | 116.0 ± 18.5 * |
| *Peak response rate* | 164.6 ± 17.6 | 164.6 ± 17.6 | 165.9 ± 15.5 |

* significant difference from vehicle control ($P$ <0.05)

6.3.1.2.3. Overall response rate

The group mean data (left-hand panel) for the two treatment conditions are shown in Figure 6.2. The higher dose of SKF-83566 produced an apparent reduction of response rate. However, analysis of the variance revealed a significant main effect of ratio [$F(15,165) = 40.3$, $p<0.001$] but not of treatment [$F(2,22) = 1.3$, NS], and there was no significant treatment × ratio interaction [$F<1$]. The fits of Equation 1 to the overall mean data accounted for >90% of the total variance (vehicle, $r^2 = 0.91$; SKF-83566, 0.015mg $kg^{-1}$, $r^2 = 0.91$; SKF-83566 0.03 mg $kg^{-1}$, $r^2 = 0.93$). Table 6.4 shows the parameters of Equation 1. Analysis of the variance revealed a borderline significant effect of treatment on the activation parameter, $a$ [$F(2,22) = 3.4$, $p=0.05$]; multiple comparisons indicated that the higher dose of SKF-835666 differed significantly from the vehicle condition. The response time parameter, $\delta$, was significantly affected [$F(2,22) = 6.8$, $p<0.01$], a significant reduction being produced by the higher dose. The currency parameter was not significantly affected by drug treatment [$F<1$].

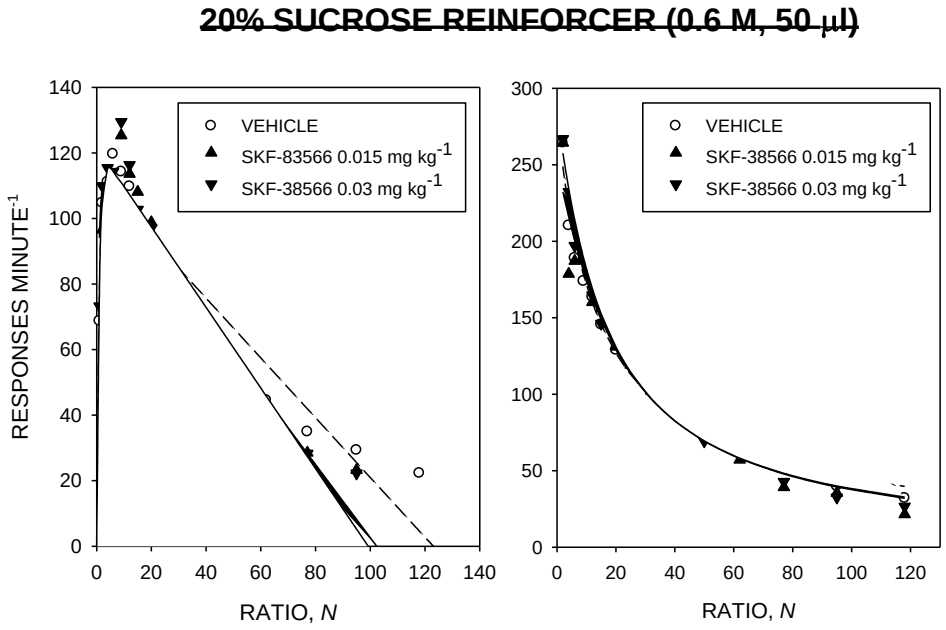


**Figure 6.2**. Effects of SKF-83566 on progressive-ratio schedule performance reinforced with sucrose. *Left-hand graph*: relation between overall response rate and the response/reinforcer ratio, *N* (see inset for treatment conditions). Curves are fits of Equation 1 to the data. *Right-hand graph*: relation between running response rate and *N*. The curves are fits of Equation 2 to the data; other conventions as in the left-hand graph.

**Table 6.4**. Progressive-ratio schedule performance: sucrose reinforcer. Effects of SKF-83566 on the parameters of Equations 1 and 2 (group mean values ± SEM)

| SKF-83566 | vehicle | SKF-83566 0.15 mg $kg^{-1}$ | SKF-83566 0.03 mg $kg^{-1}$ |
|---|---|---|---|
| *Equation 1 (overall response rate)* | | | |
| *a* (s) | 68.5 ± 10.1 | 60.8 ± 10.6 | 49.0 ± 7.4 * |
| $\delta$ (s) | 0.57 ± 0.06 | 0.55 ± 0.06 | 0.54 ± 0.06 * |
| $\beta$ | 0.63 ± 0.05 | 0.61 ± 0.06 | 0.58 ± 0.06 |
| $r^2$ | 0.83 ± 0.03 | 0.79 ± 0.03 | 0.83 ± 0.04 |
| *Equation 2 (running response rate)* | | | |
| $R_i$ (responses $min^{-1}$) | 312.6 ± 21.1 | 291.6 ± 21.5 | 311.8 ± 21.8 |
| *b* | 25.1 ± 7.6 | 25.8 ± 6.3 | 19.8 ± 5.2 |
| *c* | 1.1 ± 0.1 | 1.4 ± 0.2 | 1.3 ± 0.2 |
| $r^2$ | 0.93 ± 0.02 | 0.91 ± 0.02 | 0.91 ± 0.02 |

6.3.1.2.4. Running response rate.

Fig 6.2 (right-hand panel) shows the group mean data. Running rate was higher in the lowest ratios and declined monotonically as a function of ratio size. There was no significant effect of treatment [$F(2,22) = 3.0$, NS]; however the main effect of ratio [$F(14,154) = 76.5$, $p<0.001$] and the ratio × treatment interaction [$F(28,308) = 2.0$, $p<0.01$] were significant. The fits of Equation 2 to the group mean data accounted for over 90% of the total variance (vehicle, $r^2 = 0.93$; SKF-83566 0.015mg $kg^{-1}$, $r^2 = 0.91$; SKF-83566 0.03mg $kg^{-1}$, $r^2 = 0.91$). Table 6.4 shows the parameter values of Equation 2. None of the parameters of Equation 2 was significantly affected by SKF-83566 [*Ri*: $F<1$; *b*: $F<1$; *c*: $F(2,22) = 1.5$, NS].

*6.3.2. Corn oil reinforcement*

6.3.2.1. Haloperidol

6.3.2.1.1. Peak response rate

As can be seen from Table 6.5, the peak response rate was not significantly affected [$F(2,22) = 2.1$, NS].

6.3.2.1.2. Highest completed ratio and breakpoint

Both the highest completed ratio [$F(2,22) = 8.7$, $p<0.01$] and the breakpoint [$F(2,22) = 9.0$, $p<0.01$] were significantly affected by haloperidol, both measures being reduced by the higher dose of haloperidol (Table 6.5).

6.3.2.1.3. Overall response rate

Figure 6.3 (left-hand panel) shows the group mean data. The response rate was reduced by the higher treatment dose. Analysis of the variance revealed a significant main effect of treatment [$F(2,22) = 6.9$, $p<0.01$], ratio [$F(15,265) = 9.2$, $p<0.001$] and the treatment × ratio interaction [$F(30,330) = 2.9$, $p<0.01$]. The fits of Equation 1 to the group mean data accounted for 88% of the total variance (vehicle, $r^2 = 0.90$; haloperidol 0.05mg $kg^{-1}$,

$r^2$ = 0.90; haloperidol 0.1mg kg$^{-1}$, $r^2$ = 0.88). Table 6.6 shows the parameters of Equation 1. Analysis of the variance revealed a borderline significant effect on the activation parameter, *a* [$F(2,22) = 3.2$, $p$=0.06]; multiple comparisons indicated that *a* was significantly reduced by the higher dose of haloperidol. However the other two parameters, $\beta$ and $\delta$, were not significantly affected [$F$< 1 in both cases].

**Table 6.5**. Progressive-ratio schedule performance: corn oil reinforcer. Effects of haloperidol on the highest completed ratio, breakpoint and peak response rate (responses min$^{-1}$) (group mean values ± SEM).

| HALOPERIDOL | vehicle | haloperidol 0.05 mg kg$^{-1}$ | haloperidol0.1 mg kg$^{-1}$ |
|---|---|---|---|
| *Highest completed ratio* | 170.6 ± 44.6 | 160.5 ± 39.8 | 90.0 ± 24.8 * |
| *Breakpoint* | 170.6 ± 44.6 | 160.4 ± 39.8 | 88.7 ± 24.7 * |
| *Peak response rate* | 111.9 ± 11.0 | 113.9 ± 11.2 | 102.6 ± 10.3 |

* significant difference from vehicle condition: $p$<0.05

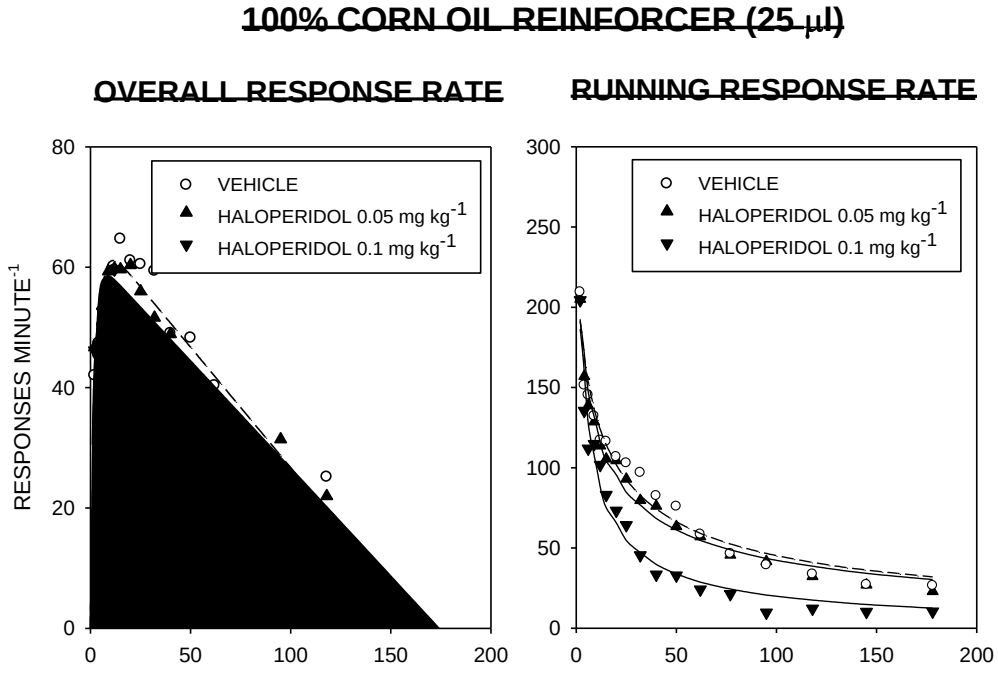


**Figure 6.3**. Effects of haloperidol on progressive-ratio schedule performance reinforced with corn oil. *Left-hand graph*: relation between overall response rate and response/reinforcer ratio, *N* (see inset for treatment conditions). Curves are fits of Equation 1 to the data. *Right-hand graph*: relation between running response rate and *N*. The curves are fits of Equation 2 to the data; other conventions as in the left-hand graph.

**Table 6.6**. Progressive-ratio schedule performance: corn oil reinforcer. Effects of haloperidol on the parameters of Equations 1 and 2 (group mean values ± SEM)

| HALOPERIDOL | vehicle | haloperidol 0.05 mg $kg^{-1}$ | haloperidol 0.1 mg $kg^{-1}$ |
|---|---|---|---|
| *Equation 1 (overall response rate)* | | | |
| *a* (s) | 285.0 ± 84.0 | 300.3 ± 81.4 | 215.7 ± 86.5 * |
| $\delta$ (s) | 0.95 ± 0.12 | 1.04 ± 0.15 | 1.09 ± 0.14 |
| $\beta$ | 0.37 ± 0.08 | 0.52 ± 0.11 | 0.45 ± 0.09 |
| $r^2$ | 0.79 ± 0.05 | 0.66 ± 0.03 | 0.66 ± 0.06 |
| *Equation 2 (running response rate)* | | | |
| $R_i$ (responses $min^{-1}$) | 256.7 ± 31.2 | 332.9 ± 38.1 | 304.0 ± 31.8 |
| *b* | 19.2 ± 4.8 | 12.3 ± 3.4 | 9.6 ± 2.7 |
| *c* | 1.5 ± 0.6 | 1.2 ± 0.3 | 1.1 ± 0.2 |
| $r^2$ | 0.84 ± 0.03 | 0.82 ± 0.03 | 0.87 ± 0.02 |

* significant difference from vehicle control ($p<0.05$)

6.3.2.1.4. Running response rate

The running response rates are shown in Figure 6.3 (right-hand panel). Running rate was reduced in the higher ratios by the higher dose of haloperidol. Analysis of the variance revealed a significant main effect of treatment [$F(2,22) = 5.9$, $p<0.01$], ratio [$F(14,154) = 31.2$, $p<0.001$] and a significant treatment × ratio interaction [$F(28,308) = 1.6$, $p<0.05$]. The fit of Equation 2 to the group mean data accounted for over 95% of the total variance (vehicle, $r^2 = 0.96$; haloperidol 0.05mg $kg^{-1}$, $r^2 = 0.98$; haloperidol 0.1 mg $kg^{-1}$, $r^2 = 0.96$). Table 6.6 shows the values of the parameters of Equation 2. Analysis of the variance showed that none of the parameters was significantly affected [*Ri*: $F(2,22) = 1.9$, NS; *b*: $F(2,22) = 2.6$, NS; *c*: $F<1$].

6.3.2.2. SKF-83566

6.3.2.2.1. Peak response rate

Table 6.7 shows the group mean data (± SEM) for the peak response rate. Analysis of variance revealed no significant effect of treatment [$F$<1].

6.3.2.2.2. Highest completed ratio and breakpoint

As can be seen from Table 6.7, neither the highest completed ratio [$F$<1] nor the breakpoint [$F$(2,22) = 1.4 NS] was significantly affected by SKF-83566.

**Table 6.7**. Progressive-ratio schedule performance: corn oil reinforcer. Effects of SKF-83566 on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| SKF-83566 | vehicle | SKF-83566 0.15 mg $kg^{-1}$ | SKF-83566 0.03 mg $kg^{-1}$ |
|---|---|---|---|
| *Highest completed ratio* | 170.6 ± 44.6 | 172.9 ± 45.8 | 157.6 ± 37.3 |
| *Breakpoint* | 174.5 ± 44.1 | 172.9 ± 45.8 | 156.7 ± 37.5 |
| *Peak response rate* | 113.6 ± 12.2 | 113.7 ± 13.4 | 109.4 ± 10.2 |

6.3.2.2.3. Overall response rate

Figure 6.4 (left-hand panel) shows the group mean data. The response rate was slightly reduced in the highest ratios with the higher dose of SKF-83566. Analysis of the variance revealed a significant main effect of ratio [$F$(14,165) = 7.5, $p$<0.001], however the main effect of treatment [$F$(2,22) = 1.0, NS] and the interaction [$F$(30,330) = 1.0, NS] were not statistically significant. The fits of Equation 1 to the group mean data accounted for >87% of the total variance (vehicle, $r^2$ = 0.87; SKF-83566 0.015 mg $kg^{-1}$, $r^2$ = 0.94; SKF-83566 0.03mg $kg^{-1}$, $r^2$ = 0.93). Table 6.8 shows the parameters of Equation 1. The

statistical analysis showed that none of the three parameters changed as a consequence of the SKF-83566 treatment [$a$: $F$<1; $\beta$: $F(2,22) = 1.5$, NS; $\delta$: $F(2,22) = 1.2$, NS].

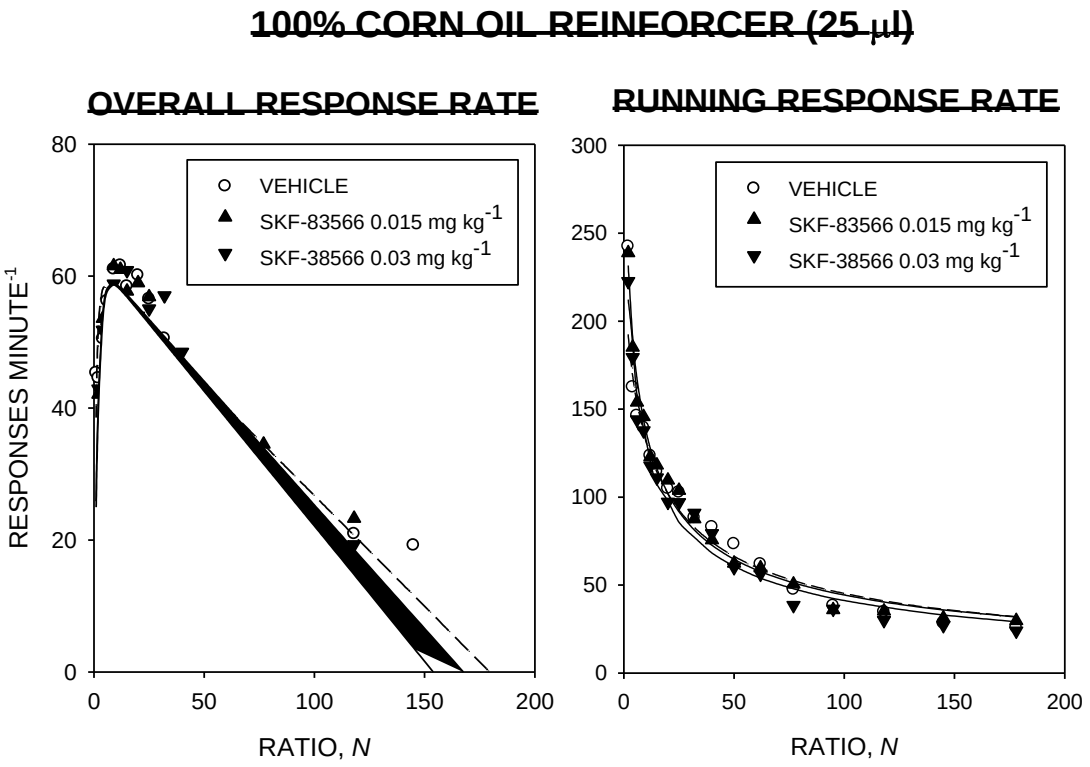


**Figure 6.4**. Effects of SKF-83566 on progressive-ratio schedule performance reinforced with corn oil. *Left-hand graph*: relation between overall response rate and the response/reinforcer ratio, *N* (see inset for treatment conditions). Curves are fits of Equation 1 to the data. *Right-hand graph*: relation between running response rate and *N*. The curves are fits of Equation 2 to the data; other conventions are as in the left-hand graph.

6.3.2.2.4. Running response rate

SKF-83566 had no significant effect on running response rate (Fig. 6.4, right-hand panel). There was a significant main effect of ratio [$F(14,154) = 41.8$, $p$<0.001], but no significant main effect of treatment [$F(2,22) = 1.8$, NS] and no significant interaction [$F$<1]. Equation 2 accounted for >95% of the total variance of the group mean data (vehicle, $r^2 = 0.97$; SKF-83566 0.015mg kg$^{-1}$, $r^2 = 0.99$; SKF-83566 0.03mg kg$^{-1}$, $r^2 = 0.98$). As can be seen from Table 6.8, none of the parameters of Equation 2 were

significantly affected by SKF-83566 [*Ri, b, c*: all Fs<1].

**Table 6.8**. Progressive-ratio schedule performance: corn oil reinforcer. Effects of SKF-83566 on the parameters of Equations 1 and 2 (group mean values ± SEM)

| SKF-83566 | vehicle | SKF-83566 0.15 mg $kg^{-1}$ | SKF-83566 0.03 mg $kg^{-1}$ |
|---|---|---|---|
| *Equation 1 (overall response rate)* | | | |
| *a* (s) | 311.6 ± 103.6 | 297.6 ± 95.0 | 275.4 ± 78.1 |
| $\delta$ (s) | 0.95 ± 0.12 | 1.04 ± 0.16 | 1.01 ± 0.13 |
| $\beta$ | 0.34 ± 0.09 | 0.41 ± 0.09 | 0.42 ± 0.10 |
| $r^2$ | 0.77 ± 0.04 | 0.69 ± 0.04 | 0.73 ± 0.05 |
| *Equation 2 (running response rate)* | | | |
| $R_i$ (responses $min^{-1}$) | 319.0 ± 29.8 | 326.5 ± 28.2 | 311.5 ± 28.2 |
| *b* | 15.0 ± 5.1 | 17.7 ± 6.6 | 14.6 ± 5.4 |
| *c* | 1.1 ± 0.2 | 1.2 ± 0.2 | 1.0 ± 0.2 |
| $r^2$ | 0.86 ± 0.03 | 0.86 ± 0.02 | 0.84 ± 0.03 |

## 6.4. Discussion

In this experiment one of the groups was reinforced with corn oil and the other with sucrose. The first objective of this experiment was to test whether the data derived from rats reinforced with corn oil could be adequately accommodated by Killeen's (1994) MPR model. The performance on the progressive-ratio schedule with both reinforcers was similar to that seen in previous studies using MPR (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al. 2003; Kheramin et al. 2005a; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b)

and in the other experiments described in this thesis (see section 2.4. for discussion). The performance maintained by the corn oil reinforcer appeared comparable to that maintained by the sucrose reinforcer used in this experiment. However quantitative comparison between the parameter values obtained using the two reinforcers would not be meaningful, because the sucrose and corn oil reinforcers used in these experiments were not matched according to any criteria such as volume, density or calorific content (see Chapter 7 for further discussion).

The effects of haloperidol on progressive-ratio schedule performance in the sucrose-reinforcement group are similar to previous studies using sucrose or food-pellet reinforcers (den Boon et al. 2011; Mobini et al. 2000; Zhang et al. 2005a) (see Chapter 5). In those studies, the application of Equation 1 to the overall response rate data revealed that $a$ was either reduced or not affected and $\delta$ was increased. In the present study $a$ was reduced and there was a trend for $\delta$ to be increased, although the latter effect fell short of statistical significance in the present experiment. Furthermore, in agreement with previous research on the effects of $D_2$ dopamine receptor antagonists, the highest completed ratio and the breakpoint were significantly reduced by haloperidol (den Boon et al. 2011; Mobini et al. 2000; Yoneda et al. 2007b; Zhang et al. 2005a). It is unclear why the effect of haloperidol on $\delta$ was less pronounced in this experiment than in previous studies, since the same same volume (50 µl) and concentration (0.6 M) of the sucrose solution and the same doses of haloperidol (0.05 and 0.1 mg $kg^{-1}$) were used as in previous studies (Mobini et al. 2000; Zhang et al. 2005a). The only obvious difference from previous studies is that the session time in the present experiment was shorter (40 min) than in Mobini et al. (2000) and Zhang et al. (2005a) (75 min and 50 min respectively). It is not clear how this could have influenced the effect of haloperidol on $\delta$. Further research is needed in order to address how the session duration may affect the parameters of Equation 1. The currency parameter $\beta$ was not affected by haloperidol; this parameter has not been affected consistently by haloperidol in previous experiments (see section 5.4 for discussion).

The running response rate data were analysed with Equation 2 (Rickard et al. 2009). Haloperidol reduced the 'decay parameter', $b$, which is purported to be related to the incentive value of reinforcers (Rickard et al. 2009). These data are in agreement with results of the previous experiment (see section 5.4), where haloperidol reduced this

parameter significantly. However, the finding that the 'initial response rate' parameter, *Ri*, was not affected is in disagreement with the previous experiment, where this parameter was significantly reduced. As mentioned previously in the context of $\delta$, which is conceptually related to *Ri* (Rickard et al. 2009), the shorter session time may have an impact on this parameter.

In agreement with the predictions outlined in the Introduction to this chapter (section 6.1), the $D_1$ receptor antagonist SKF-83566 reduced the activation parameter, *a*, in the sucrose reinforced group. Unexpectedly, the motor parameter $\delta$ was significantly reduced in a dose-dependent manner, indicating that the drug produced a facilitatory effect on responding on the progressive-ratio schedule. As expected, $\beta$ did not change as a consequence of the treatment. None of the parameters of Equation 2 was significantly affected by SKF-83566. However there was a trend for the 'decay parameter' *b* to be reduced by the drug, which is consistent with the significant decrease of the motivational parameter of Equation 1 (*a*). The breakpoint and highest completed ratio were reduced, which is also consistent with a reduction of the incentive value of sucrose reinforcers when the $D_1$ dopamine receptors are antagonized.

Another aim of this experiment was to examine the differential effect of the $D_1$ and $D_2$ dopamine receptor antagonists on the performance of rats reinforced with corn oil. In agreement with expectations (see above, section 6.1), the 'incentive value' parameter of Equation 1, *a*, was significantly reduced by the higher dose of haloperidol, and in addition the traditional measures of progressive-ratio schedule performance, the breakpoint and the highest completed ratio, were also reduced. This is in agreement with a study of progressive-ratio schedule performance by mice that were reinforced with corn oil. In this study the administration of the $D_2$ receptor antagonist sulpiride reduced the breakpoint (Yoneda et al. 2007b). Similarly to the sucrose-reinforced group (see above), the 'response time' parameter, $\delta$, was increased, although the effect of haloperidol on this parameter did not reach statistical significance, and the 'currency' parameter, $\beta$, was not affected by haloperidol. The application of Equation 2 to the running response rate data did not yield any significant results, although there was a trend for the 'decay parameter' *b* to be reduced, which is in accordance with the effect of the drug on *a*. Taken together, these results are consistent with the notion that blockade of $D_2$ dopamine receptors reduces the incentive value of oily/fatty reinforcers.

SKF-83566 had no significant effect on the performance of the corn oil reinforced group, although there was a non-significant trend for *a* to be reduced and for $\delta$ to be increased. This trend may be analogous to the finding of Weatherford et al. (1990) who found that a high dose of a $D_1$ receptor antagonist produced both a sedative effect and a reduction of the rewarding properties of corn oil.

More research is needed on the differential effects of $D_1$ and $D_2$ receptor antagonists on progressive-ratio schedule performance maintained by different reinforcers. Although the present results are suggestive of an involvement of both receptor subtypes in the reinforcing property of sucrose, and a preferential involvement of $D_2$ receptors in the reinforcing property of corn oil, it must be emphasised that only two doses of each drug were tested, and only one magnitude of each reinforcer was used. In future experiments it will be important not only to examine a range of compounds using a broader range of doses, but also to systematically manipulate the concentrations of both reinforcers. Another interesting question to be addressed in future experiments is whether selective $D_1$ and $D_2$ receptor agonists are able to enhance the values of sucrose and corn oil reinforcers, and if so whether the effects of the agonists can be selectively reversed by the appropriate antagonists.

To summarize, it can be said that corn oil is a potent reinforcer on progressive-ratio schedule performance. The quantitative analysis based on Killeen's (1994) theoretical model of schedule-controlled behaviour, MPR, indicated that both haloperidol and SKF-83566 reduced the incentive value of a sucrose reinforcer. Interestingly, while haloperidol tended to increase $\delta$, SKF-83566 reduced this parameter. This suggests that both $D_1$ and $D_2$ dopamine receptors may be involved in determining the incentive value of sucrose reinforcement, and that the two receptor subtypes may exert opposing influences on the motor aspects of progressive-ratio schedule performance. However, haloperidol but not SKF-83566, in the doses used in this experiment, affected performance maintained by the corn oil reinforcer, suggesting that, while $D_2$ dopamine receptors contribute to the regulation of the incentive value of this reinforcer, $D_1$ dopamine receptors may not play a significant role in this process. The results from this experiment yield more evidence about the utility of quantitative analysis of progressive-

ratio schedule performance based on MPR for investigations of the effects of drugs on motivational processes (Killeen 2009; Rickard et al. 2009).

# CHAPTER 7

# EXPERIMENT 6: EFFECTS OF $\Delta^9$-TETRAHYDROCANNABINOL ON PROGRESSIVE-RATIO SCHEDULE PERFORMANCE REINFORCED WITH SUCROSE OR CORN OIL UNDER FOOD DEPRIVATION AND FREE FEEDING CONDITIONS

## 7.1. Introduction

The psychoactive properties of the Marihuana plant (Cannabis sativa) have been known for more than 4000 years (Tucci et al. 2006). This plant has been used for centuries to induce appetite and more recently it has been used medicinally to treat the weight loss and cachexia caused by diseases such as AIDS (Di Marzo and Matias 2005). However, the principal compound, THC, responsible for the central properties of the Marihuana plant, was not discovered until the 1960's.

The discovery of THC led to increasing interest in the effects of the cannabinoids and their possible interaction with endogenous mechanisms of food intake regulation (Gaoni and Mechoulam 1964). These findings were followed by the discovery of the cannabinoid receptors ($CB_1$ and $CB_2$ receptors) and endogenous cannabinoids, including anandamide, 2-arachidonoylglycerol (2AG), noladin ether, N-arachidonoyl dopamine (NADA) and virodhamine, and the development of selective $CB_1$ receptor antagonists, including rimonabant (SR-141716) and surinabant (SR-147778) (Fride 2002; 2004).

A number of experiments have investigated the effect of THC on feeding behaviour in animals and humans. Williams et al. (1998) conducted an experiment where oral THC was administered to pre-fed rats. The results showed that the rats ate significantly more when THC was given than when the vehicle (saline solution) was administered. Similar results were found when the endogenous $CB_1$ receptor agonist anandamide was administered subcutaneously to rats (Williams and Kirkham 1999).

Several experiments have shown that THC preferentially increases the consumption of palatable food, especially the sweet variety (Foltin et al. 1988; Haney et al. 1999; Hollister 1971). For example, Foltin et al. (1988) reported that humans consumed more sweet items (candy bars) after the administration of THC. Similar results have been obtained with anandamide and 2AG, administered centrally and peripherally to rats (Hao et al. 2000; Jamshidi and Taylor 2001; Kirkham and Williams 2001; Williams and Kirkham 1999). For instance in a study using a 10% sucrose solution, total number of licks was augmented by THC and reduced by the $CB_1$ receptor antagonist rimonabant (Higgs et al. 2003). Although ingestion of sweet foods has been found to be especially sensitive to $CB_1$ receptor agonists, ingestion of other palatable foods is also affected. For

example, administration of THC has been found to increase the consumption of a high fat diet (Verty et al. 2011; Verty et al. 2004).

Higgs et al. (2005) examined the motivational properties of two $CB_1$ receptor agonists, THC and 1,1-dimethylheptyl-11-hydroxy-tetrahydrocannabinol (HU-210) using a progressive-ratio schedule. They found that THC but not HU-210 increased the breakpoint in the progressive-ratio schedule, and enhanced food intake in a free-feeding test. Higgs et al. used food pellets as reinforcers and a mixture of standard chow and condensed milk in the feeding test. Solinas and Goldberg (2005) also examined the effect of THC on progressive-ratio schedule performance. THC increased the breakpoint, an effect that was also produced by an increase in the severity of food deprivation. Both the THC-induced and the deprivation-induced increase in the breakpoint were reversed by the $CB_1$ receptor antagonist rimonabant (Solinas and Goldberg 2005). More recently, Maccioni et al. (2008) reported that the breakpoint was reduced by the CB1 receptor antagonist rimonabant in rats reinforced with a low-calorie chocolate beverage. It has also been shown that response rates of rats responding for 'normal food' (standard rat chow) on a fixed-ratio schedule are enhanced by a $CB_1$ receptor agonist, and this effect can be blocked by rimonabant (Freedland et al. 2000). These authors concluded that the use of highly palatable nutrients is not a necessary condition for revealing the anoretic effect of $CB_1$receptor antagonists.

In the experiment described in Chapter 5, THC was administered to rats that were responding for standard food pellets on a progressive-ratio schedule. None of the doses of THC administered (0.3, 1, 3 mg $kg^{-1}$) produced a change in the breakpoint. Progressive-ratio schedule performance was also examined using Killeen's MPR model (Killeen 1994) to analyse overall response rates and Rickard et al.'s (2009) equation to analyse running response rates. The results showed that none of the parameters that are purportedly related to the incentive value of food reinforcers was altered by THC. Various factors may have contributed to the lack of effect of THC in that experiment. It was noted that the food pellets used as the reinforcer in that experiment, although more palatable than normal rat chow, have no carbohydrate content. As reviewed above, many (but not all) previous studies have indicated that THC's orexigenic effect is most pronounced in the case of sweet foodstuffs.

Another possible factor could be the food deprivation level. In the experiment described in Chapter 5, the rats were maintained at 80% of their free-feeding body weights, whereas the majority of previous demonstrations of orexigenic effects of THC have employed mild food deprivation or free-feeding conditions. It has been reported that food deprivation increases the level of endocannabinoids and other orexigenic compounds such as neuropeptide-Y (NPY), melanin, orexins and ghrelin (Hanus et al. 2003; Lopez et al. 2000; Meister 2000; Sakurai et al. 1998). This raises the possibility that mechanisms induced by food deprivation might have over-ridden and masked the effect of THC on the motivational indices derived from progressive-ratio schedule performance in the previous experiment.

As discussed above, THC has been found to augment the intake of palatable food. However the term 'palatable' is ambiguous, and it is unclear whether the effect of THC on food intake is related to the fat, carbohydrate or other component of certain foodstuffs. In the experiments described in this chapter, 100% corn oil and 0.6 M sucrose solution (approx 20%) were used as reinforcers in a progressive-ratio schedule and the effect of THC on performance was evaluated with Killeen's (1994) MPR model. The effects of THC on performance on the progressive-ratio schedule was also examined under food-deprivation and free-feeding conditions. Finally, the effect of THC on 'free consumption' (i.e. not earned by operant responding) of the two reinforcers was also assessed.

## 7.2. Methods

The experiment was carried out in accordance with UK Home Office regulations governing experiments on living animals.

### *7.2.1. Subjects*

The animals from the previous experiment were used. Twelve rats underwent training under the progressive-ratio schedule using sucrose reinforcement (0.6 M, 50 μl); the remaining twelve were trained using pure corn oil (25 μl) as the reinforcer (see section 6.2.1). Under the *food deprivation condition*, the rats were maintained at 80% of their free-feeding body weights, as in previous experiments (see section 2.2.1); under the *free-feeding condition*, they had free access to standard laboratory rat chow in their home

cages (see below).

### *7.2.2. Apparatus*

The same operant conditioning chambers, control apparatus and software were used as in Experiment 5 (see section 6.2.2).

### *7.2.3. Behavioural procedures*

As the rats had previously undergone extensive training under the progressive-ratio schedule, no pre-training was necessary. Daily training under the progressive-ratio schedule was continued without interruption until the end of the operant behaviour experiment (see section 2.2.4.1).

Three experiments were carried out with each group:

(i) The effect of THC (0.3, 1 and 3 mg $kg^{-1}$) on progressive-ratio schedule performance was examined under conditions of food deprivation (the rats were maintained at 80% of their free-feeding body weights);

(ii) The effect of THC (1 mg $kg^{-1}$) on progressive-ratio schedule performance was examined in the absence of food restriction (the rats were allowed free access to food in their home cages);

(iii) The effect of THC (1 mg $kg^{-1}$) on consumption of the reinforcing substance (sucrose solution or corn oil) was measured during a 60-min period during which the substance was freely available (see section 2.2.4.3 for details of the testing procedure).

### *7.2.4. Drug treatment*

As the subjects had been used in the previous experiment with dopamine receptor antagonists (see Chapter 6), THC treatment started after a 10-day wash-out period. The drug injection protocol was the same as in previous experiment (see section 6.2.4). The

doses of THC and the drug preparation procedure were the same as in Experiment 4 (see section 5.2.4).

### *7.2.5. Data analysis*

The data from the two groups (sucrose reinforcer, oil reinforcer) were analysed separately, in the same way as in the previous experiment. The behavioural measures and statistical analyses were the same as in the previous experiment (see section 6.2.5.).

## 7.3. Results

### *7.3.1. Sucrose reinforcement*

#### 7.3.1.1. Effect of THC on progressive-ratio schedule performance maintained under the food deprivation condition

##### 7.3.1.1.1. Peak response rate

There was no significant effect of THC on peak response rate [$F<1$] (see Table 7.1).

##### 7.3.1.1.2. Highest completed ratio and breakpoint

THC had no significant effect on the highest completed ratio [$F<1$] or breakpoint [$F(3,33) = 1.1$, NS] (see Table 7.1)

**Table 7.1**. Progressive-ratio schedule performance: sucrose reinforcer, food deprivation condition. Effects of THC on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| THC | vehicle | THC 0.3 mg $kg^{-1}$ | THC 1 mg $kg^{-1}$ | THC 3 mg $kg^{-1}$ |
|---|---|---|---|---|
| *Highest completed ratio* | 170.8 ± 43.6 | 178.7 ± 44.0 | 176.3 ± 43.1 | 175.3 ± 43.2 |
| *Breakpoint* | 160.3 ± 43.6 | 172.7 ± 44.8 | 166.0 ± 43.9 | 175.3 ± 43.9 |
| *Peak response rate* | 144.8 ± 15.6 | 150.6 ± 21.6 | 160.9 ± 17.9 | 157.5 ± 19.6 |

7.3.1.1.3. Overall response rate

THC produced no significant effect on the overall response rate (Fig 7.1 left hand panel). Analysis of variance revealed a significant main effect of ratio [$F(14,154) = 25.8$, $p<0.001$], but no significant main effect of treatment [$F(3,33) = 2.3$, NS] and no significant interaction [$F<1$]. Equation 1 accounted for over 90% of the variance of the group mean data (vehicle: $r^2 = 0.95$; THC 0.3 mg kg$^{-1}$: $r^2 = 0.95$; THC 1 mg kg$^{-1}$: $r^2 = 0.93$: THC 3 mg kg$^{-1}$: $r^2 = 0.94$). Table 7.2 shows the parameters of Equation 1; none of the parameters was significantly affected by any of the doses of THC [$a$: $F<1$; $\beta$: $F<1$; $\delta$: $F(3,33) = 1.1$, NS].

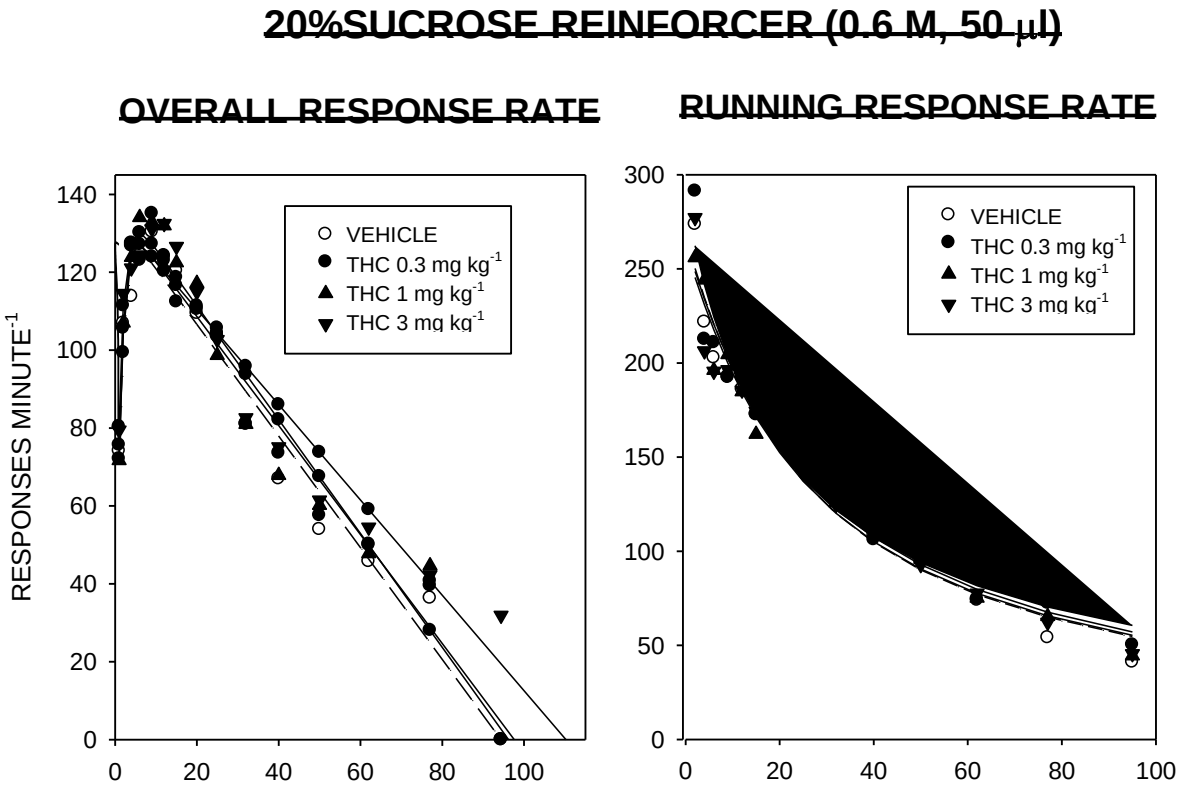


**Figure 7.1**. Effects of $\Delta^9$tetrahydrocannabinol (THC) on progressive-ratio schedule performance under the food deprivation condition. *Left-hand graph*: relation between overall response rate and the response/reinforcer ratio, *N*. Unfilled circles: vehicle-alone treatment; filled circles: THC 0.3 mg kg$^{-1}$; upright filled triangles: THC 1mg kg$^{-1}$; inverted filled triangles: THC 3mg kg$^{-1}$ (see inset). Curves are fits of Equation 1 to the data. *Right-hand graph*: relation between running response rate and *N*. The curves are fits of Equation 2 to the data; other conventions are as in the left-hand graph.

**Table 7.2** Progressive-ratio schedule performance: sucrose reinforcer, food deprivation condition. Effects of THC on the parameters of Equations 1 and 2 (group mean values ± SEM)

| THC | vehicle | THC 0.3 mg $kg^{-1}$ | THC 1 mg $kg^{-1}$ | THC 3 mg $kg^{-1}$ |
|---|---|---|---|---|
| *Equation 1 (overall response rate)* | | | | |
| *a* (s) | 85.2 ± 87.9 | 87.9 ± 23.6 | 83.4 ± 22.0 | 78.5 ± 22.6 |
| δ (s) | 0.50 ± 0.05 | 0.51 ± 0.06 | 0.48 ± 0.05 | 0.49 ± 0.06 |
| β | 0.64 ± 0.08 | 0.59 ± 0.07 | 0.52 ± 0.08 | 0.60 ± 0.08 |
| $r^2$ | 0.82 ± 0.05 | 0.91 ± 0.11 | 0.78 ± 0.05 | 0.80 ± 0.04 |
| *Equation 2 (running response rate)* | | | | |
| $R_i$ (resp. $min^{-1}$) | 238.3 ± 18.1 | 233.7 ± 20.5 | 245.0 ± 16.8 | 233.1 ± 27.8 |
| *b* | 39.1 ± 6.3 | 40.7 ± 6.6 | 43.3 ± 8.6 | 45.9 ± 9.1 |
| *c* | 1.6 ± 0.3 | 1.6 ± 0.2 | 1.6 ± 0.2 | 1.8 ± 0.3 |
| $r^2$ | 0.92 ± 0.01 | 0.90 ± 0.01 | 0.90 ± 0.1 | 0.91 ± 0.02 |

7.3.1.1.4. Running response rate

THC did not produce any significant effect on the running response rate (Fig 7.1 right hand panel). Analysis of the variance revealed a significant main effect of ratio [$F(13,143) = 43.3$, $p<0.001$], but no significant main effect of treatment [$F(3,33) = 1.1$, *NS*] or interaction [$F<1$]. Equation 2 counted over 95% of the variance of the group mean data (vehicle: $r^2 = 0.97$; THC 0.3 mg $kg^{-1}$: $r^2 = 0.98$; THC 1 mg $kg^{-1}$: $r^2 = 0.96$: THC 3 mg $kg^{-1}$: $r^2 = 0.96$). As can be seen for Table 7.2, none of the three parameters were significantly affected by THC [$R_i$: $F<1$; *b*: $F<1$; or *c*: $F(3,33) = 1.1$, NS].

7.3.1.2. Effect of THC on progressive-ratio schedule performance maintained under the free feeding condition

7.3.1.2.1. Peak response rate

THC had no effect in this measure [$F(1,11) = 1.9$, NS] (see Table 7.3).

7.3.1.2.2. Highest completed ratio and breakpoint

Neither of these two measures was significantly affected by THC [highest completed ratio: $F(1,11) = 2.3$, NS; breakpoint: $F<1$] (see Table 7.3).

**Table 7.3**. Progressive-ratio schedule performance: sucrose reinforcer, free feeding condition. Effects of THC on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| THC | vehicle | THC 1 mg $kg^{-1}$ |
|---|---|---|
| *Highest completed ratio* | 35.2 ± 4.5 | 32.9 ± 5.3 |
| *Breakpoint* | 32.2 ± 4.7 | 39.1 ± 5.6 |
| *Peak response rate* | 132.2 ± 11.1 | 140.5 ± 12.8 |

7.3.1.2.3. Overall response rate

Overall response rate was substantially lower under the free feeding condition than under the food deprivation condition (see section 7.3.1.3). Group mean overall response rates under the vehicle and THC 1 mg $kg^{-1}$ treatment conditions are shown in Fig. 7.2 (left hand panel). Analysis of variance showed a significant effect of ratio [$F(7,77) = 43.1$, $P<0.001$]; the main effect of treatment was of borderline significance [$F(1,11) = 4.6$, $p = 0.55$], but the interaction was not significant [$F(7,77) = 1.4$, NS]. Equation 1 accounted for over 90% of the total variance of the group mean data (vehicle: $r^2 = 0.95$; THC 1 mg $kg^{-1}$: $r^2 = 0.94$). The values of the three parameters of Equation 1, $a$, $\delta$ and $\beta$, are shown

in Table 7.3; analysis of variance showed that none of the parameters was significantly affected by THC [$F$<1 in all cases].

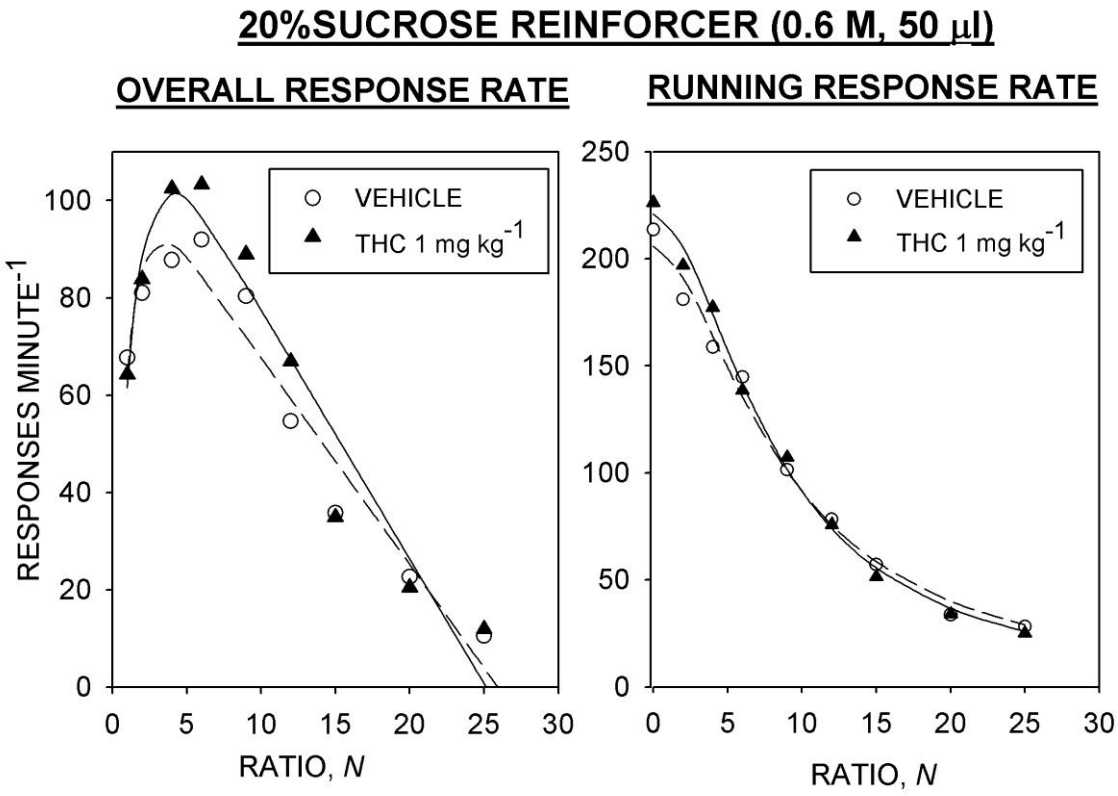


**Figure 7.2**. Effects of $\Delta^9$-tetrahydrocannabinol (THC) on progressive-ratio schedule performance under the free-feeding condition. Unfilled circles: vehicle-alone treatment; upright filled triangles: THC 1 mg kg$^{-1}$ (see inset). Other conventions are as in Fig.7.1

7.3.1.2.4. Running response rate

Fig 7.2 (right hand panel) shows the group mean data for the vehicle and THC 1 mg kg$^{-1}$ treatment conditions. THC had no effect on the running response rate. Analysis of variance indicated that the main effect of ratio was significant [$F(7,77) = 34.5$, $p$<0.001], however the main effect of treatment and the interaction were not significant [both $F$s<1]. Equation 1 accounted for 99% of the variance of the group mean data (vehicle: $r^2$ > 0.99; THC 1 mg kg$^{-1}$: $r^2$ > 0.99). Table 7.4 shows the parameters of Equation 2; none of the parameters was significantly affected by THC [*Ri*: $F$<1; *b*: $F$<1; *c*: $F(3,33) = 1.1$, NS].

**Table 7.4.** Progressive-ratio schedule performance: sucrose reinforcer, free feeding condition. Effects of THC on the parameters of Equations 1 and 2 (group mean values ± SEM)

| THC | vehicle | THC 1 mg $kg^{-1}$ |
|---|---|---|
| *Equation 1 (overall response rate)* | | |
| $a$ (s) | 12.1 ± 3.0 | 10.3 ± 1.5 |
| $\delta$ (s) | 0.47 ± 0.07 | 0.43 ± 0.04 |
| $\beta$ | 0.49 ± 0.06 | 0.49 ± 0.06 |
| $r^2$ | 0.86 ± 0.03 | 0.84 ± 0.04 |
| *Equation 2 (running response rate)* | | |
| $R_i$ (resp. $min^{-1}$) | 243.5 ± 19.1 | 242.1 ± 13.2 |
| $b$ | 10.9 ± 1.6 | 10.1 ± 1.1 |
| $c$ | 2.7 ± 0.4 | 2.8 ± 0.5 |
| $r^2$ | 0.95 ± 0.01 | 0.93 ± 0.02 |

7.3.1.3. Comparison of progressive-ratio schedule performance maintained under the food deprivation and free feeding conditions

As described above, each rat experienced training under the progressive-ratio schedule under both the the food deprivation and free feeding conditions, and the 1 mg $kg^{-1}$ dose of THC was tested under both conditions. The effects of food deprivation and THC 1 mg $kg^{-1}$ on each performance measure were analysed by analysis of variance (condition [food deprived, free feeding] × treatment [vehicle, THC 1 mg $kg^{-1}$]). For ease of comparison, the relevant data, extracted from Tables 7.1, 7.2, 7.3, and 7.4, are displayed in Table 7.5. Analysis of variance showed that, compared to the free-feeding condition, food deprivation produced an increase in the highest completed ratio [$F(1,11) = 9.9$, $p<0.01$], the breakpoint [$F(1,11) = 10.6$, $p<0.01$] and the peak response rate [$F(1,11) = 9.6$, $p<0.05$]. The deprivation condition was also associated with an increase of the

'activation' parameter (*a*) of Equation 1 [$F(1,11)$ 10.1, $p<0.01$] and the decay parameter (*b*) of Equation 2 [$F(1,11) = 19.2$, $p<0.001$]. None of the other parameters were significantly affected [deprivation condition, all $F$s(1,11) < 1.7, NS], and in no case was there a significant effect of THC [drug treatment factor: all $F$s(1,11) < 2.3, NS; deprivation × drug treatment interaction: all $F$s(1,11) < 1.6, NS].

**Table 7.5**. Effect of food deprivation condition and THC 1 mg kg$^{-1}$ on indices of performance on the progressive-ratio schedule

| | food deprivation | | free feeding | |
|---|---|---|---|---|
| | vehicle | THC 1 mg kg$^{-1}$ | vehicle | THC 1 mg kg$^{-1}$ |
| *Highest completed ratio* | 170.8 ± 43.6 | 166.0 ± 43.9 | 35.2 ± 4.7* | 39.1 ± 5.6* |
| *Breakpoint* | 160.3 ± 43.6 | 176.3 ± 43.1 | 32.1 ± 4.5* | 33.9 ± 5.3* |
| *Peak response rate (resp. min$^{-1}$)* | 171.7 ± 18.3 | 179.4 ± 21.0 | 132.1 ± 11.2* | 140.5 ± 12.8* |
| *Equation 1 (overall response rate)* | | | | |
| *a* (s) | 85.2 ± 87.9 | 83.4 ± 22.0 | 12.1 ± 3.0* | 10.3 ± 1.5* |
| $\delta$ (s) | 0.50 ± 0.05 | 0.48 ± 0.05 | 0.47 ± 0.07 | 0.43 ± 0.04 |
| $\beta$ | 0.64 ± 0.08 | 0.52 ± 0.08 | 0.49 ± 0.06 | 0.49 ± 0.06 |
| $r^2$ | 0.82 ± 0.05 | 0.78 ± 0.05 | 0.86 ± 0.03 | 0.84 ± 0.04 |
| *Equation 2 (running response rate)* | | | | |
| $R_i$ (resp. min$^{-1}$) | 283.3 ± 18.1 | 245.0 ± 16.8 | 243.5 ± 19.1 | 242.1 ± 13.2 |
| *b* | 39.1 ± 6.3 | 43.3 ± 8.6 | 10.9 ± 1.6* | 10.1 ± 1.1* |
| *c* | 1.6 ± 0.3 | 1.6 ± 0.2 | 2.7 ± 0.4 | 2.8 ± 0.5 |
| $r^2$ | 0.92 ± 0.01 | 0.90 ± 0.1 | 0.95 ± 0.01 | 0.93 ± 0.02 |

* Significant effect of the food deprivation condition ($p < 0.05$); no significant effect of the treatment condition and no significant interaction.

### *7.3.2. Corn oil reinforcement*

#### 7.3.2.1. Effect of THC on progressive-ratio schedule performance maintained under the food deprivation condition

##### 7.3.2.1.1. Peak response rate

The peak response was somewhat higher under the THC 1 mg $kg^{-1}$ treatment condition that under the other treatment conditions (see Table 7.6). However this increment did not reach statistical significance [$F(2,30) = 2.3$, $p = 0.09$] .

##### 7.3.2.1.2. Highest completed ratio and breakpoint

THC had no significant effect on the highest completed ratio [$F(3,30) = 1.8$, NS] or the breakpoint [$F(3,30) = 2.1$, NS] (see Table 7.6).

**Table 7.6**. Progressive-ratio schedule performance: corn oil reinforcer, food deprivation condition. Effects of THC on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| THC | vehicle | THC 0.3 mg $kg^{-1}$ | THC 1 mg $kg^{-1}$ | THC 3 mg $kg^{-1}$ |
|---|---|---|---|---|
| *Highest completed ratio* | 170.0 ± 49.8 | 154.9 ± 42.3 | 173.3 ± 52.4 | 169.6 ± 51.0 |
| *Breakpoint* | 172.5 ± 50.9 | 146.5 ± 38.4 | 172.9 ± 52.5 | 168.7 ± 51.2 |
| *Peak response rate* | 115.6 ± 13.4 | 116.1 ± 13.3 | 129.0 ± 15.8 | 116.2 ± 11.4 |

##### 7.3.2.1.3. Overall response rate

The group mean data are shown in Figure 7.3 (left hand panel). THC 1mg $kg^{-1}$ tended to produce an increase of the response rate. Analysis of variance yielded a significant effect of treatment [$F(3,33) = 4.3$, $p<0.05$] and ratio [$F(14,154) = 5.8$, $p<0.001$]. However, the treatment × ratio interaction was not significant [$F<1$]. Equation 1 accounted for over

80% of the variance of the group mean data (vehicle: $r^2$ = 0.92; THC 0.3 mg kg$^{-1}$: $r^2$ = 0.84; THC 1 mg kg$^{-1}$: $r^2$ = 0.87: THC 3 mg kg$^{-1}$: $r^2$ = 0.81). Analysis of variance revealed that none of the parameters of Equation 1 was significantly affected by THC [*a*: $F(3,30) = 2.1$, *NS*; $\beta$: $F<1$; $\delta$: $F<1$] (see Table 7.7).

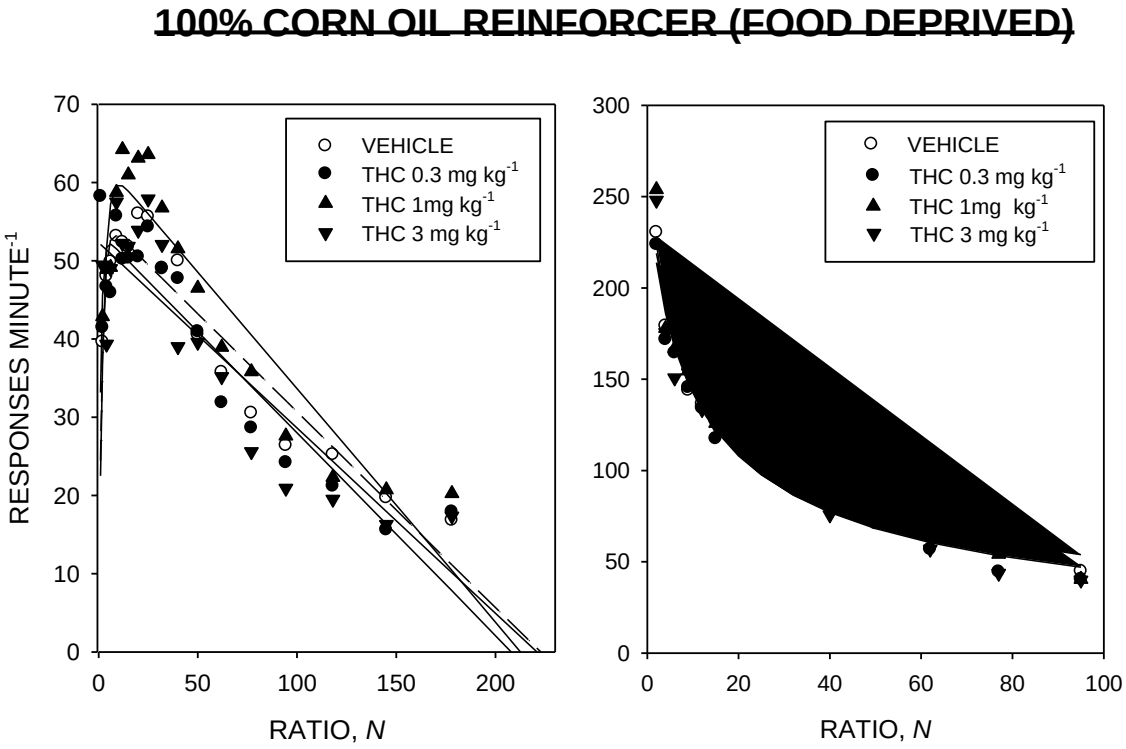


**Figure 7.3** Effects of $\Delta^9$-tetrahydrocannabinol (THC) on progressive-ratio schedule performance under food deprivation conditions. Unfilled circles: vehicle-alone treatment; filled circles: THC 0.3 mg kg$^{-1}$; upright filled triangles: THC 1mg kg$^{-1}$; inverted filled triangles: THC 3mg kg$^{-1}$ (see inset). Other conventions are as in Fig.7.1.

7.3.2.1.4. Running response rate

The group mean data are shown in Figure 7.3 (right-hand panel). THC did not have a significant effect on the running response rate. Analysis of variance showed a significant main effect of ratio [$F(13,130) = 27.9$, $p<0.001$] but the main effect of treatment [$F(3,30) = 1.3$, NS] and the interaction [$F<1$] were not significant. Equation 2 accounted for over 95% of the variance of the group mean data (vehicle: $r^2$ = 0.97; THC 0.3 mg kg$^{-1}$: $r^2$ = 0.98; THC 1 mg kg$^{-1}$: $r^2$ = 0.95: THC 3 mg kg$^{-1}$: $r^2$ = 0.96). Analysis of variance revealed that none of the parameters of Equation 2 was significantly affected by THC [*Ri*: $F(3,30) = 2.1$, NS; *b*: $F<1$; *c*: $F<1$] (see Table 7,7).

**Table 7.7.** Progressive-ratio schedule performance: corn oil reinforcer, food deprivation condition. Effects of THC on the parameters of Equations 1 and 2 (group mean values ± SEM)

| THC | vehicle | THC 0.3 mg $kg^{-1}$ | THC 1 mg $kg^{-1}$ | THC 3 mg $kg^{-1}$ |
|---|---|---|---|---|
| *Equation 1 (overall response rate)* | | | | |
| $a$ (s) | 328.3 ± 129.9 | 375.9 ± 158.3 | 405.1 ± 164.4 | 302.4 ± 133.8 |
| $\delta$ (s) | 0.98 ± 0.15 | 0.86 ± 0.15 | 0.97 ± 0.29 | 0.88 ± 0.12 |
| $\beta$ | 0.39 ± 0.11 | 0.30 ± 0.03 | 0.46 ± 0.13 | 0.40 ± 0.12 |
| $r^2$ | 0.71 ± 0.04 | 0.60 ± 0.07 | 0.63 ± 0.06 | 0.73 ± 0.03 |
| *Equation 2 (running response rate)* | | | | |
| $R_i$ (resp. $min^{-1}$) | 226.1 ± 22.9 | 237.9 ± 19.5 | 219.6 ± 24.6 | 257.3 ± 19.4 |
| $b$ | 48.7 ± 24.6 | 24.6 ± 6.4 | 49.6 ± 16.6 | 20.3 ± 5.6 |
| $c$ | 2.5 ± 0.8 | 1.3 ± 0.3 | 1.4 ± 0.2 | 2.1 ± 0.8 |
| $r^2$ | 0.84 ± 0.06 | 0.84 ± 0.05 | 0.77 ± 0.04 | 0.83 ± 0.03 |

7.3.2.2. Effect of THC on progressive-ratio schedule performance maintained under the free feeding condition

7.3.2.2.1. Peak response rate

As can be seen from Table 7.8, the peak response tended to increase under the THC 1 mg $kg^{-1}$ dose. However, this increment did not reach statistical significance [$F(1,10) = 2.4$, NS].

7.3.2.2.2. Highest completed ratio and breakpoint

Neither of the two variables was significantly affected by THC [highest completed ratio: $F(1,10) = 1.4$, NS; breakpoint: $F(1,10) = 1.3$, NS] (see Table 7.8.).

**Table 7.8**. Progressive-ratio schedule performance: corn oil reinforcer, free feeding condition. Effects of THC on the highest completed ratio, breakpoint and peak response rate (responses $min^{-1}$) (group mean values ± SEM)

| THC | vehicle | THC 1 mg $kg^{-1}$ |
|---|---|---|
| *Highest completed ratio* | 40.1 ± 9.8 | 33.1 ± 4.6 |
| *Breakpoint* | 37.9 ± 9.1 | 31.3 ± 4.6 |
| *Peak response rate* | 71.5 ± 7.0 | 79.7 ± 8.6 |

7.3.2.2.3. Overall response rate

The group mean data are shown in Figure 7.4. THC tended to increase the response rate in the lower ratios in comparison with the vehicle condition. Analysis of variance yielded a borderline significant main effect of treatment [$F(1,11) = 5.6$, $p=0.04$] and a significant effect of ratio [$F(7,77) = 6.3$, $p<0.001$]. However, the treatment × ratio interaction was not significant [$F<1$]. Equation 1 accounted for over 80% of the variance of the group mean data (vehicle: $r^2 = 0.91$; THC 1 mg $kg^{-1}$: $r^2 = 0.80$). The values of the parameters of Equation 1 are shown in Table 7.9. Analysis of the variance showed a non-significant trend for $a$ to be reduced by THC mg $kg^{-1}$ [$F(1,10) = 3.4$, $p=0.07$]. $\beta$ was significantly reduced by THC [$F(1,10) = 5.0$, $P<0.05$]. The response time parameter $\delta$ was not significantly affected by THC [$F(1,10) = 2.4$, NS].

7.3.2.2.4. Running response rate

The group mean data are shown in Figure 7.4 (right hand panel). Analysis of variance revealed that THC had no effect on the running response rate. There was a significant main effect of ratio [$F(7,70) = 27.9$, $p<0.001$]. However, the main effect of treatment [$F<1$] and the treatment × ratio interaction [$F<1$] were not statistically significant. Equation 2 accounted for over 99% of the variance of the group mean data (vehicle: $r^2 = 0.99$; THC 1 mg kg$^{-1}$: $r^2 = 0.99$). The parameters of Equation 2 are shown in table 7.9. None of the parameters was significantly affected by THC [$R_i$: $F<1$; $b$: [$F(1,10) = 1.5$, NS; $c$: $F<1$].

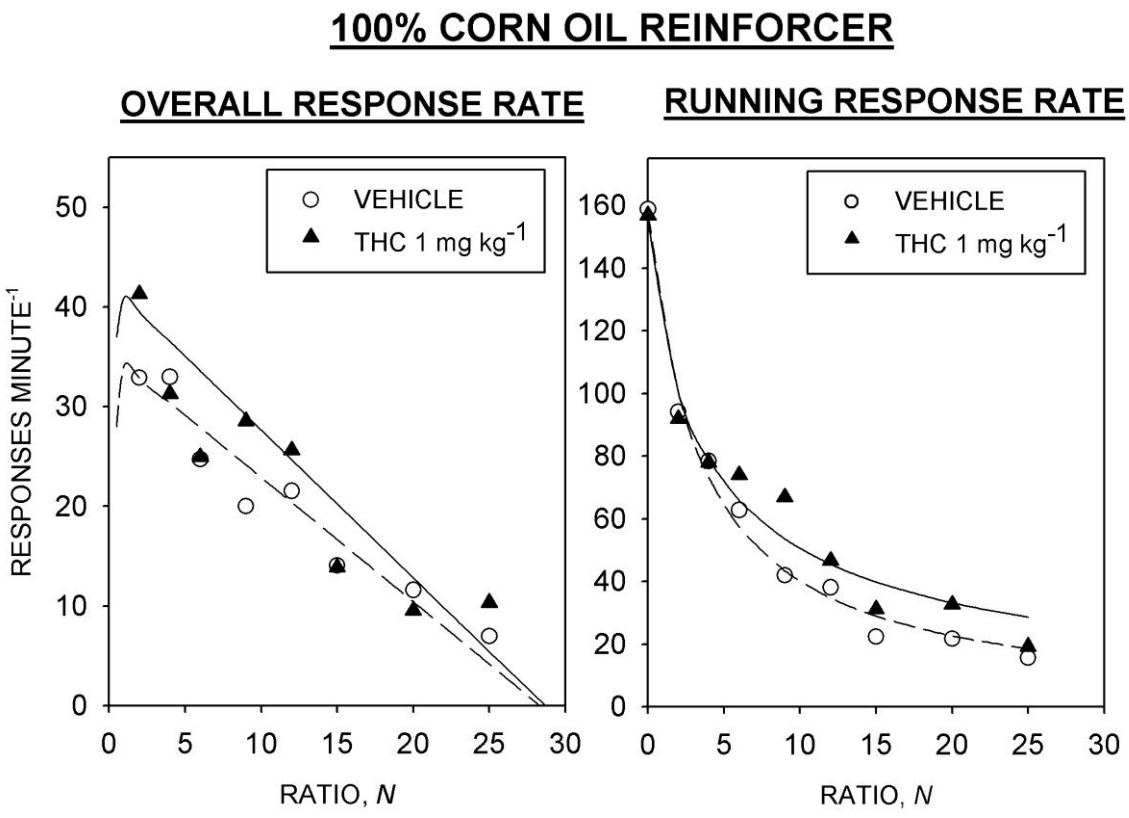


**Figure 7.4** Effects of THC on progressive-ratio schedule performance. Unfilled circles: vehicle-alone treatment; filled triangles: THC 1 mg kg$^{-1}$ (see inset). Other conventions are as in Fig.7.1

**Table 7.9.** Progressive-ratio schedule performance: corn oil reinforcer, free feeding condition. Effects of THC on the parameters of Equations 1 and 2 (group mean values ± SEM)

| THC | vehicle | THC 1 mg kg$^{-1}$ |
|---|---|---|
| *Equation 1 (overall response rate)* | | |
| $a$ (s) | 69.1 ± 26.6 | 34.4 ± 10.7 |
| $\delta$ (s) | 1.83 ± 0.63 | 1.17 ± 0.20 |
| $\beta$ | 0.64 ± 0.11 | 0.49 ± 0.11* |
| $r^2$ | 0.70 ± 0.05 | 0.65 ± 0.06 |
| *Equation 2 (running response rate)* | | |
| $R_i$ (resp. min$^{-1}$) | 220.0 ± 24.2 | 206.6 ± 29.8 |
| $b$ | 4.7 ± 1.3 | 5.9 ± 1.8 |
| $c$ | 5.5 ± 3.5 | 2.2 ± 0.4 |
| $r^2$ | 0.87 ± 0.07 | 0.85 ± 0.08 |

7.3.2.3. Comparison of progressive-ratio schedule performance maintained under the food deprivation and free feeding conditions

As described above, each rat experienced training under both the food deprivation and free feeding conditions, and the 1 mg kg$^{-1}$ dose of THC was tested under both conditions. The effects of food deprivation and THC 1 mg kg$^{-1}$ on each performance measure were analysed by analysis of variance (condition [food deprived, free feeding] × treatment [vehicle, THC 1 mg kg$^{-1}$]). For ease of comparison, the relevant data, extracted from Tables 7.6, 7.7, 7.8, and 7.9, are displayed in Table 7.10. Analysis of variance showed that, compared to the free-feeding condition, food deprivation produced an increase in the highest completed ratio [$F(1,11) = 11.3$, $p<0.01$], the breakpoint [$F(1,11) = 11.7$, $p<0.01$] and the peak response rate [$F(1,11) = 12.0$, $p<0.01$]. The deprivation condition was also associated with an increase of the 'activation' parameter ($a$) [$F(1,11) = 10.1$,

$p<0.01$] and the 'currency' parameter ($\beta$) [$F(1,11) = 10.0$, $p<0.01$] of Equation 1 and the decay parameter ($b$) of Equation 2 [$F(1,11) = 11.2$, $p<0.01$]. None of the other parameters was significantly affected [deprivation condition: all $F$s(1,11) < 1.7, NS], and in no case was there a significant effect of THC [drug treatment factor: all $F$s(1,11) < 4.8, NS; deprivation × drug treatment interaction: all $F$s(1,11) < 3.1, NS].

**Table 7.10**. Effect of food deprivation condition and THC 1 mg kg$^{-1}$ on indices of performance on the progressive-ratio schedule

| | food deprivation | | free feeding | |
|---|---|---|---|---|
| | vehicle | THC 1 mg kg$^{-1}$ | vehicle | THC 1 mg kg$^{-1}$ |
| *Highest completed ratio* | 170.0 ± 49.8 | 173.3 ± 52.4 | 40.1 ± 9.8* | 33.1 ± 4.6* |
| *Breakpoint* | 172.5 ± 50.9 | 172.9 ± 52.5 | 37.9 ± 9.1* | 31.3 ± 4.6* |
| *Peak response rate (resp. min$^{-1}$)* | 115.6 ± 13.4 | 129.0 ± 15.8 | 71.5 ± 7.0* | 79.7 ± 8.6* |
| *Equation 1 (overall response rate)* | | | | |
| $a$ (s) | 328.3 ± 129.9 | 405.1 ± 164.4 | 69.1 ± 26.6* | 34.4 ± 10.7* |
| $\delta$ (s) | 0.98 ± 0.15 | 0.97 ± 0.29 | 1.83 ± 0.63 | 1.17 ± 0.20 |
| $\beta$ | 0.39 ± 0.11 | 0.46 ± 0.13 | 0.64 ± 0.11* | 0.49 ± 0.11* |
| $r^2$ | 0.71 ± 0.04 | 0.63 ± 0.06 | 0.70 ± 0.05 | 0.65 ± 0.06 |
| *Equation 2 (running response rate)* | | | | |
| $R_i$ (resp. min$^{-1}$) | 226.1 ± 22.9 | 219.6 ± 24.6 | 220.0 ± 24.2 | 206.6 ± 29.8 |
| $b$ | 48.7 ± 24.6 | 49.6 ± 16.6 | 4.7 ± 1.3* | 5.9 ± 1.8* |
| $c$ | 2.5 ± 0.8 | 1.4 ± 0.2 | 5.5 ± 3.5 | 2.2 ± 0.4 |
| $r^2$ | 0.84 ± 0.06 | 0.77 ± 0.04 | 0.87 ± 0.07 | 0.85 ± 0.08 |

* Significant effect of the food restriction condition ($p < 0.05$); no significant effect of the treatment condition and no significant interaction

### 7.3.3. *Effect of THC on consumption of the sucrose solution and corn oil*

THC 1 mg $kg^{-1}$ produced a borderline increase in intake of the sucrose solution [$t(11) = 1.7$, $p=0.06$]. However, THC significantly reduced the amount of corn oil intake [$t(11) = 5.1$, $p<0.001$].

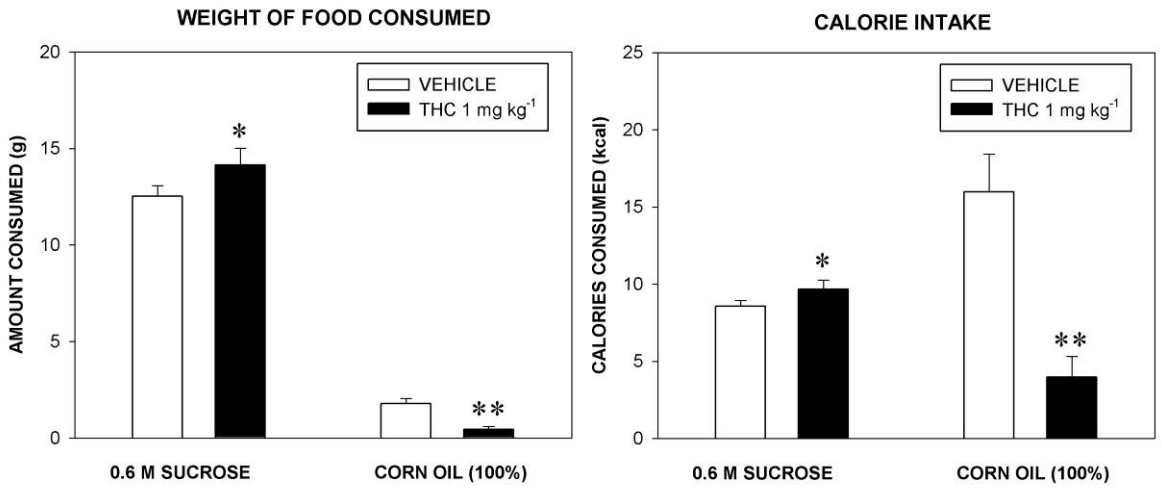


**Fig 7.5.** Left hand graph, effect of THC 1 mg $kg^{-1}$ on on intake (g) of the sucrose solution (0.6 M, 50 µl) and corn oil (100%) during 60-min test sessions. Unfilled bars: vehicle-alone treatment; filled bars: THC 1 mg $kg^{-1}$ (see inset). The right hand graph shows the same data, but the quantity of food is expressed as its calorific content rather than weight; other conventions are as in the left hand graph. Difference from the vehicle treatment: * $p=0.06$; ** $p<0.001$.

## 7.4. Discussion

The performance of the rats in this experiment was similar to that seen in previous studies (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al. 2003; Kheramin et al. 2005; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b) and in Experiments 1-5 of this thesis. As in Experiment 5, described in the previous chapter, corn oil proved to be an effective reinforcer in the progressive-ratio schedule.

Unexpectedly, none of the doses of THC had any significant effect on the parameters of Equations 1 and 2 (*a* and *b*, respectively) that are purported to measure the incentive value of reinforcers (Killeen 1994; Rickard et al. 2009), nor did they affect the traditional

measure derived from progressive-ratio schedule performance, the breakpoint. This lack of effect, which was apparent with both reinforcers, corn oil and sucrose, is consistent with the results obtained in Experiment 4 (see Chapter 5) obtained using food-pellet reinforcers. These results are in apparent disagreement with previous experiments in which THC has been found to increase the breakpoint in progressive-ratio schedules (Higgs et al. 2005; Solinas and Goldberg 2005). There are a number of methodological differences between the present experiment and the two previous studies that reported significant effects of THC on the breakpoint. In the present experiment the food restriction condition was more stringent (the rats were maintained at 80% of their free feeding body weights) than in those two experiments. Furthermore, the session duration in the present experiment was somewhat shorter (40 minutes), than in the previous experiments (approx 60 minutes) (see section 6.4 for discussion of this matter). Finally, the reinforcers used in the present experiment were different (0.6 M sucrose solution and 100% corn oil in the present experiment in comparison with pellets containing a mixture of nutrients in the previous studies by Higgs et al. (2005) and Solinas and Goldberg (2005). There is evidence that THC has a stronger effect on the rewarding value of palatable foods such as sweet (Foltin et al. 1988; Haney et al. 1999; Hollister 1971) or fatty foodstuffs (Verty et al. 2011; Verty et al. 2004). However as mentioned above (section 7.1) it is difficult to attribute the effect of THC (or other drugs) to a selective effect on sweet food when the reinforcers used contain a mixture of nutrients.

There were grounds for suspecting that the deprivation condition used in this experiment and in Experiment 4 might have masked the effect of THC on the indices of incentive value. For example, Williams et al. (1998) argued that the failure of some previous experiments to demonstrate a hyperphagic effect of THC was due to inappropriate test conditions. According to these authors, the optimal conditions to test the effect of THC on food intake include the use of non-food-deprived animals. Therefore, in the present experiment, the effect of THC 1 mg $kg^{-1}$ (the dose that has been used in most previous studies that have found an orexigenic effect of THC; e.g. Williams et al. 1998) was also tested under free-feeding conditions. As expected, response rates under the progressive ratio schedule decreased when the animals were transferred from the food deprivation condition to *ad libitum* feeding (see below). Unexpectedly, however, there was no effect of THC on the parameters *a* and *b* or on the breakpoint in the case of either reinforcer.

According to a recent report, THC exerted different locomotor effects depending on the diet to which the animals were exposed. Specifically, THC was found to induce hypomotility and cataplexy-like behaviour in rats maintained on a diet enriched with fat (Wiley et al. 2011). Informal observations of the rats did not indicate that THC had any such effect in the rats in the present experiment that received corn oil reinforcement, and there was no effect of THC on the motor indices of operant performance, $\delta$ and $R_i$. The corn oil intake that resulted from reinforcement under the progressive-ratio schedule in the present experiment (approximately 0.3-0.5 g per day) may have been insufficient to induce the effect noted by Wiley et al. (2011). However, it may be worth noting that the corn oil-reinforced group required a noticeably smaller daily food ration to maintain their body weights at the 80% target level than was the case with the sucrose-reinforced group, suggesting that the amount of corn oil earned in the daily experimental sessions made a substantial contribution to the total calorie intake of this group.

In another experiment using progressive-ratio schedules (Maccioni et al. 2008), rats maintained under *ad libitum* feeding were reinforced with a chocolate beverage. After they were trained, these rats underwent treatment with the $CB_1$ receptor antagonist, rimonabant. Rimonabant produced a significant reduction of the breakpoint. The authors concluded that $CB_1$ receptors were a crucial component of the system that regulates the rewarding and motivating properties of palatable food (Maccioni et al. 2008). The present experiment did not support this hypothesis. One possible argument could be that corn oil or sucrose on their own are not sufficiently palatable to generate an effect on reinforced operant behaviour when the cannabinoid system is manipulated, in contrast to chocolate, which contains a mixture of sucrose and fat. Further research is needed using reinforcers whose composition is fully known in order to further understand the effect of THC on the rewarding value of 'palatable food'.

Another aim of this experiment was to test whether the rats would respond on the progressive-ratio schedule even under free-feeding conditions. Interestingly, the rats not only responded on the schedule, but also the data derived from this condition could be used for fitting Killeen's (1994) equation in a similar way to experiments that employed conventional food deprivation conditions (Bezzina et al. 2008b; Bezzina et al. 2008c; Bizo and Killeen 1997; den Boon et al. 2011; Ho et al. 2003; Kheramin et al. 2005; Mobini et al. 2000; Rickard et al. 2009; Zhang et al. 2005a; Zhang et al. 2005b; also

Experiments 1,2,3,4 and 5 in this thesis). As expected, when the animals were transferred from the food deprived condition to the free-feeding condition, all the measures related to the incentive value of food reinforcers (*a* [Equation 1], *b* [Equation 2], and the breakpoint) were reduced (see Table 7.10). Furthermore, in agreement with Killeen's MPR (1994), the response-time parameter $\delta$ (which is supposed to be independent from *a*) was not altered. These findings are also supported by a decrement in the decay parameter *b* without any change in the maximum running response rate parameter $R_i$, derived from Equation 2 (Rickard et al. 2009). This last observation indicates that *b* and $R_i$ behave as indepent parameters. In this context it may be of interest to note that another quantitative model of operant performance, Herrnstein's (1970) hyperbolic 'response strength' equation, also reveals a selective effect of deprivation level on an index of reinforcer value, with no effect on an index of motor capacity (Bradshaw et al. 1983).

The free feeding condition was associated with somewhat higher values of the currency parameter, $\beta$, compared to the food deprivation condition. The reason for this effect, which was only apparent in the case of the corn oil reinforcer, is unclear. In MPR, this parameter expresses the weight given to the most recent response in the reinforcement process (Killeen 1994). It is possible that the corn oil reinforcer was less effective in controlling longer trains of responses under free feeding condition than under food deprivation conditions. However, this interpretation remains speculative, pending a more systematic examination of the sensitivity of β to manipulation of variables such as deprivation level, reinforcer size and reinforcer quality (for further discussion, see Bezzina et al. 2008a; Rickard et al. 2009).

As discussed in session 6.4 the parameters derived from behaviour maintained by the two reinforcers could not be compared quantitatively, as the two reinforcers were not matched in terms of any relevant variables, such as their calorific content, volume, etc. However, it may be of interest to compare the 'incentive value' parameter, *a*, obtained in this experiment with values of the parameter obtained by Rickard et al. (2009) for a range of volumes of a 0.6 M sucrose solution. In Figure 7.6, the value of *a* (s) is plotted against the calorific values of the reinforcers (kcal). It is apparent that the value of *a* derived from the sucrose-reinforcement group in the present experiment is similar to the value obtained by Rickard et al. (2009) using the same reinforcer (50µl of a 0.6 M sucrose

solution). The calorific of the corn oil reinforcer used in this experiment is similar to the calorific content of 250 µl of a 0.6 M sucrose solution. Comparison of the values of *a* for these two reinforcers suggests that the value obtained for corn oil (data from the present experiment) was somewhat higher than that obtained for 300 µl of the sucrose reinforcer (data from Rickard et al. 2009). It may also be noted that the between-rat variability in the parameter values was considerably higher in the case of the corn oil reinforcer. The apparently greater incentive value of the corn oil reinforcer than a calorifically equivalent sucrose reinforcer appears to be inconsistent with the claim that 'calorie-for-calorie', sucrose makes a greater contribution to the overall value of a food reinforcer than corn oil in progressive-ratio schedule-controlled operant behaviour (Naleid et al. 2008). It should noted, however, that Naleid et al.'s claim is based on experiments in which mixtures of sucrose and corn oil were used as the reinforcer, and the concentrations of

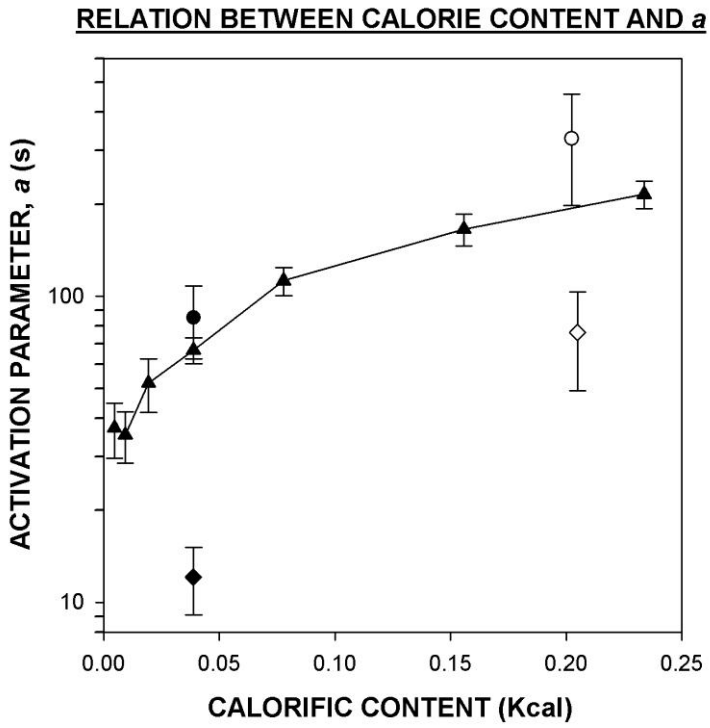


**Fig. 7.6.** Relation between the calorific content of reinforcers and the 'specific activation' parameter, *a* (Equation 1). Ordinate: value of *a* (s); abscissa: calorific content (kcal). Filled symbols: 0.6 M sucrose solution; open symbols: 100% corn oil. Connected filled triangles: data from Rickard et al. (2009) for a range of volumes of the sucrose solution; filled circle: data from present experiment using 50 µl of the sucrose solution. Unfilled circle: data from the present experiment using the corn oil reinforcer. Filled and unfilled lozenges: data from the free-feeding condition of the present experiment (filled lozenge: sucrose solution; unfilled lozenge: corn oil); all other points indicate data obtained under the food deprivation condition (rats maintained at 80% of free-feeding body weight). See text for further explanation.

both components were considerably lower than the concentrations of the pure substances used in the present experiment. Once again, it would seem that more research is needed before any definite conclusion can be reached about the relative efficacies of sucrose and corn oil as reinforcers. It should also be emphasized that calorific content is only one aspect of food reinforcement; sensory quality is likely to exert at least as great an influence on incentive value as energy content. In this context, it is of interest that Covarrubias and Aparicio (2008) have shown that saccharin pellets, with calorific value close to zero, generated a higher value of *a* and higher breakpoints than standard food pellets (Covarrubias and Aparicio 2008). This fascinating line of investigation needs to be further investigated in other to determine the way in which calories, nutritional content and flavours can affect the parameters of Equations 1 and 2 (Killeen 1994; Rickard et al. 2009).

The feeding experiment showed that there was a trend (not significant) for THC to increase the intake of the sucrose solution (Figure 7.5). These results are qualitatively in agreement with previous research (Williams et al. 1998). The fact that a single dose was used leaves open the possibility that a higher dose might have produced a significant increment of food intake, although it may be noted that the dose used in this experiment (1 mg $kg^{-1}$) has been reported to increase food intake in previous studies (e.g. Williams et al. 2008). Another potentially relevant factor is the time of the day when the rats were tested. In the present experiment, testing took place during the light phase of the daily cycle; however, Williams et al. (1998) noted that the effect of THC on food intake was more pronounced in the dark phase than the light phase. Perhaps the most surprising finding was the fact that THC significantly reduced the amount of corn oil intake. It can be seen from Figure 7.5 that under the vehicle treatment condition the rats consumed a much smaller quantity of corn oil than of the sucrose solution. However in terms of the calorific content, the rats that received corn oil consumed considerably more calories than the rats that received the sucrose solution.

In summary, the present results indicate than under food restriction conditions THC (0.3-3 mg $kg^{-1}$) had no significant effect on progressive-ratio schedule performance, with the concentrations of sucrose and corn oil used in this experiment. Moreover, removal of the food-restriction condition failed to unmask any significant effect of THC (1 mg $kg^{-1}$) on

performance. The results obtained from the feeding experiment indicate that THC may enhance sucrose intake when there is no operant response requirement; in contrast, corn oil intake was substantially reduced by THC. Furthermore, this experiment showed that the incentive value parameters $a$ and $b$, but not to the motor parameters $\delta$ and $R_i$, are sensitive to the food restriction condition. This adds more evidence for the utility of MPR (Equation 1) and Equation 2 as a basis for measuring the incentive value of food reinforcers. However the disparity between the effects of THC on progressive-ratio schedule performance and on unrestricted food intake indicates that caution is needed in the use of data from feeding tests to draw conclusions about the reinforcing value of foodstuffs. It seems that 'palatability' and 'rewarding value' should not be used as interchangeable terms.

# CHAPTER 8

# GENERAL DISCUSSION

## 8.1 Summary of results

The aim of the work described in this thesis was to answer some questions related to the neurobiological basis of the control of operant behaviour by food reinforcement. The main behavioural technique used in the research was the progressive-ratio schedule. The behavioural data generated by this schedule were analysed using a quantitative approach based on Killeen's (1994) Mathematical Principles of Reinforcement model (MPR). The ratio-schedule equation derived from MPR (Equation 1) was used to analyse the overall response rates in successive ratios of the schedule, and in addition, Rickard et al.'s (2009) logistic equation was used to analyse the running response rate data. The rationale for adopting this approach was that MPR provides a theoretical basis for discriminating between the effects of interventions on incentive and motor-related processes, which are represented by separate parameters in Equation 1 ($a$ and $\delta$, respectively). In this respect, analysis of performance based on MPR was deemed to be more informative than the traditional measure of performance on the progressive-ratio schedule, the breakpoint (Hodos 1961), which, according to MPR, confounds the influences of incentive and motor processes (Killeen et al. 2009; Rickard et al. 2009).

The first three experiments of this the project (Chapters 2-4) examined the effects of manipulating orexinergic function to assess the putative role of the orexinergic pathways in reward processes. According to the 'dichotomy of orexinergic function' hypothesis (Harris and Aston-Jones 2006), orexinergic neurones in the LHA are purported to play an important role in reward processes, whereas orexinergic neurones in the adjacent PFA are purported to control arousal and wakefulness. OxSap, a neurotoxin that (in small doses) selectively targets those neurones that bear OX2 receptors, was used to destroy orexinergic neurones of the LHA  In Experiment 1, bilateral lesions were inflicted in the LHA. Neither $a$ nor $\beta$ was significantly altered by the lesion. However, the response time parameter $\delta$ was significantly increased, which indicated that motor and not motivational processes were impaired by the lesion. Application of the logistic equation (Rickard et al 2009) to the running response rate data also indicated a motor deterioration, as shown by a reduction of the parameter $R_i$. The decay parameter $b$ and the exponent $c$ were not affected. Furthermore, feeding and locomotor activity experiment failed to show any differences between the sham-lesioned and OxSap-lesioned groups. The immunohistochemistry data showed selective depletion of orexin-containing neurones

from the LHA, consistent with the dose dependent selectivity of OxSap as a neurotoxin affecting OX2 receptor-bearing neurones. These data support the hypothesis that OX2 receptor-bearing neurones of the LHA are involved in the control of motor processes.

In Experiment 2, OxSap-induced lesions were used to sever the connections between the LHA and the VTA, one of the main projection areas of the orexinergic pathways. The results were similar to those of Experiment 1, in that the 'motor parameter' $\delta$ was increased in the disconnection-lesioned group, compared to the control group, whereas the other parameters were not significantly affected. Also in agreement with the previous experiment, $R_i$ was reduced in the disconnection group. Overall, the results suggest that orexinergic neurotransmission in the VTA mediated by OX2 receptors are involved in motor and arousal processes, but no evidence was obtained for a significant role of OX2 receptors in motivation-related processes.

In Experiment 3, the LHA was unilaterally lesioned with OxSap, following which the animals were implanted with guide cannulae targeting the AcbS. Then the rats received acute intra-AcbS injections of the OX1 receptor antagonist SB-334867-A ipsilaterally (unilateral group) or contralaterally (disconnection group). It was found that the 'acute disconnection' procedure reduced the incentive value of food reinforcer as indicated by a significant decrease in the parameter *a*. Treatment with SB-334867-A did not affect the motor parameter $\delta$ or the currency parameter $\beta$. The analysis derived from the running rate (Equation 2) revealed a trend for the decay parameter *b* to be increased whereas $R_i$ and *c* were not affected. The results from this experiment are consistent with the hypothesis of differential functions of the two orexin receptors, since blockade of OX1 receptor-mediated transmission in the AcbS reduced reinforcer value as defined by MPR.

Experiment 4 examined the effect of cyproheptadine on progressive-ratio schedule performance. Cyproheptadine is a non-specific 5-HT and histamine receptor antagonist that has been proposed as a potential adjunct to conventional antipsychotic treatment which might confer beneficial effects in the management of negative symptoms of schizophrenia (Goudie et al. 2007). The effect of cyproheptadine was compared with those of the conventional antipsychotic ($D_2$-like rdopamine receptor antagonist) haloperidol, the atypical antipsychotic clozapine, and two drugs with known orexinegenic effects, chlordiazepoxide and THC. The effects of haloperidol and

clozapine were similar to previous reports using MPR (e.g. Zhang et al. 2005a; den Boon et al. 2011). In addition clozapine and cyproheptadine showed similar patterns of effect on the parameters of the equations; that is, both drugs increased *a* and $\delta$ (Equation 1) and reduced $R_i$ (Equation 2). The fact that the breakpoint was not significantly increased by clozapine showed once more the utility of quantitative analysis of progressive-ratio schedule performance based on MPR (1994). Unexpectedly, THC had no effect on the parameters of MPR.

Experiment 5 examined the effects of blocking the $D_1$-like (SKF-83566) and $D_2$-like (haloperidol) dopamine receptors on progressive-ratio performance maintained by two different reinforcers, sucrose and corn oil. MPR revealed that the two antagonists had different effects on behaviour maintained by the two reinforcers. Haloperidol was able to reduce the 'incentive value' of both corn oil and sucrose, as indicated by a decrease of *a*. However the $D_1$-like receptor antagonist SKF-83566 reduced both *a* and $\delta$ in the case of sucrose but had no effect on the values of the parameters in the case of corn oil. These results are consistent with the notion that $D_1$-like and $D_2$-like dopamine receptors may be differentially involved in the regulation of the incentive value of different foods (Yoneda et al. 2007b).

The last experiment (Experiment 6, Chapter 7) examined the effect of THC on progressive-ratio schedule performance maintained by sucrose and corn oil under food-deprived and free-feeding conditions. No significant effects of THC on the parameters of the two equations were seen when THC was administered under either condition. However, consistent with the tenets of MPR, the 'activation' parameter was sensitive to food deprivation, higher values of *a* being seen when the rats were maintained under the food deprived condition than when they were given free access to food. Interestingly, in feeding tests, THC produced a (borderline significant) increase in sucrose consumption, and a substantial reduction of oil consumption.

## 8.2 Implications for the neurobiological substrate of reinforcement

### *8.2.1. Orexinergic mechanisms*

According to the 'dichotomy' theory of the orexinergic function, the orexinergic

neurones of the LHA are assumed to control the incentive or 'rewarding' properties of reinforcers, and those of the PFA are assumed to control motor and/or arousal functions (Harris and Aston-Jones 2006). The results from Experiments 1-3 did not provide unequivocal support for this hypothesis. In Experiment 1, bilateral destruction of orexinergic neurones in the LHA induced by the selective neurotoxin OxSap did not produce a decrement of any of the motivational indices (*a*, *b* or breakpoint), nor did it affect food intake. To some degree, the discrepancy between these findings and previously reported results may be ascribed to the use of different paradigms, different food restriction conditions and different reinforcers (see section 2.4). However, the fact that a 'motor performance' deficit was found in this experiment (an increment of $\delta$, and a decrement of $R_i$ and the peak response rate), strongly suggests that the LHA orexinergic neurones may make a more important contribution to the motor aspects of operant behaviour than has previously been recognised (see also Berridge et al. 2010; Siegel 2004; Siegel 2005b). In the following experiment, disconnection of the VTA from the orexinergic output of the LHA using OxSap had a very similar effect on performance to that seen in Experiment 1: the lesion produced an increase of $\delta$ and a reduction of $R_i$ and the peak response rate. This constitutes further evidence in favour of the notion that the LHA orexinergic projection contributes to the control of operant behaviour by regulating motor/arousal processes.

Although the results of Experiments 1 and 2 appear to be incompatible with a straightforward link between the LHA orexinergic neurones and the regulation of incentive value, as proposed by the 'dichotomy' theory, Experiment 3 provided preliminary evidence for a more circumscribed role of the LHA orexinergic projection in regulating incentive value. Since the neurotoxin used to lesion the orexinergic pathways shows some preference for neurones bearing the OX2 receptor rather than the OX1 receptor, the possibility that OX1 receptors in the target region may be mainly responsible for the proposed role of orexinergic mechanisms in incentive processes. The results of Experiment 3 indicated that unilateral OxSap lesions in the LHA coupled with blockade of OX1 receptors in the contralateral AcbS using the OX1 receptor antagonist SB-334867-A produced a selective reduction of the activation parameter, *a*, Interpretation of this finding is complicated by the fact that the LHA lesion was induced by OxSap, which selectively targets neurones that express OX2 receptors. At the present time it is not known whether there are separate sub-populations of orexinergic neurones

in the LHA that exclusively express either OX1 or OX2 receptors, although the existence of exclusively OX1 receptor-expressing orexinergic neurones might help to explain the findings that a low dose of OxSap only succeeded in destroying about 50% of orexin-containing neurones in the LHA (Experiment 1), and that this proportion was not increased when the dose of OxSap was doubled (Experiment 2).

In summary, the results of Experiments 1-3 lead to the idea that the putative 'dichotonmy' of the behavioural role of orexinergic function may be more related to the receptors involved than with the particular sub-region of the hypothalamus from which the orexinergic fibres arise. However this hypothesis needs further investigation before any definitive conclusion can be made.

### *8.2.2. Antipsychotics and dopaminergic mechanisms*

It is generally accepted that the principal pharmacological action of 'conventional' antipsychotics is antagonism of $D_2$-like dopamine receptors ($D_2$, $D_3$ and $D_4$ receptors). This action has been held responsible for both their therapeutic effects and their major side effects; blockade of $D_2$-like receptors in the limbic cortex and subcortical limbic structures may underlie their antipsychotic action, whereas antagonism of $D_2$-like receptors in the dorsal striatum is the basis of their extrapyramidal side-effects (see Cunningham-Owens 1999; King and Waddington 2004). Conventional antipsychotics are notoriously unsuccessful in alleviating the 'negative' symptoms of schizophrenia (e.g. anhedonia); indeed in some cases they may exacerbate them (see King and Waddington 2004). An antihedonic effect of $D_2$-like receptor antagonists is widely believed to underlie the suppressant effect of these drugs on operant behaviour maintained by food and drug reinforcement (Wise 1982). In contrast, atypical antipsychotics such as clozapine do not aggravate negative symptoms, and some authors claim that these symptoms may respond favourably to these drugs (Corrigan et al. 2003; Müller-Spahn 2002).

The mode of action of atypical antipsychotics remains in dispute, although several theories have been proposed (see section 1.3.4.6). The possibility that simultaneous blockade of $D_2$-like and 5-$HT_{2A}$ receptors may underlie clozapine's superior therapeutic profile (Meltzer 1995) led to the suggestion that the combination of a $D_2$-like receptor

antagonist (e.g. haloperidol) and a 5-$HT_{2A}$ receptor antagonist (e.g. cyproheptadine) might provide a satisfactory treatment regimen in schizophrenia (Goudie et al. 2007).

In Experiment 4, the effect of cyproheptadine on progressive-ratio schedule performance was compared with those of clozapine and haloperidol. Cyproheptadine's profile of effect on the parameters of MPR closely resembled that of clozapine: Both drugs increased *a* and *δ*, suggesting a dual effect of enhancing incentive value and impairing motor capacity. Since both drugs have quite complex pharmacologies, it is difficult to ascribe these effects to particular actions at the receptor level. However, as discussed in Chapter 5, histamine $H_1$ receptor antagonism may account for the motor debilitating (possibly sedative) effects of both drugs. The mechanism responsible for the apparent increase of incentive value by clozapine and cyproheptadine needs to be further investigated; one possibility is that it may be related to the appetite enhancing effects which have been attributed to the combined blockade of $5HT_{2A}$, $H_1$ and $M_1$ receptors (Goudie et al. 2007).

Haloperidol's effect on performance comprised a reduction of *a* and an increase of *δ*. As discussed above, both effects may be related to $D_2$-like receptor antagonism, Blockade of these receptors in the dorsal striatum may underlie the motor debilitating effect (increase in *δ*, reduction of $R_i$), whereas the reduction of *a* may reflect blockade of $D_2$-like receptors in limbic structures. The reduction of *a* is, of course, consistent with the basic tenet of the 'anhedonia' hypothesis (Wise 1982, 1986), that conventional antipsychotic drugs reduce the rewarding effect of positive reinforcers. However it should be noted that although this experiment is not alone in finding that haloperidol reduced the value of *a* (den Boon et al. 2011), some other studies based on MPR did not observe this effect (Mobini et al. 2000; Zhang et al. 2005a).

In Experiment 6, haloperidol and a $D_1$-like receptor antagonist SKF-83566 were used to test the hypothesis that the two 'subfamilies' of dopamine receptor exert different regulatory effects on the reinforcing property of sucrose and corn oil (Weatherford et al. 1990; Yoneda et al. 2007a). The results showed that haloperidol was able to reduce both *a* and *b* in both in the corn oil and sucrose reinforced group. However SKF-83566 reduced *a* in the sucrose-reinforced group, having no effect at all in the corn oil-reinforced group. This suggests that the incentive properties of different reinforcers could

be mediated by different receptors (see section 7.4 for discussion).

### *8.2.3. Cannabinoid receptors*

THC is a CB1 receptor agonist with is claimed to have powerful effects on food intake and food motivation. In Experiment 4, THC failed to produce an effect on the 'incentive value' parameters of Equations 1 and 2 across a wide range of doses (see Chapter 5). A possible explanation that was considered for this unexpected finding was the lack of carbohydrate content of the pellet reinforcers, since most previous studies that had demonstrated an orexigenic effect of THC used sweet foodstuffs (see Chapters 5 and 7). Therefore, in Experiment 6, the effect THC on progressive-ratio schedule performance was investigated again with two different reinforcers – sucrose and corn oil. When the rats were maintained under conventional food-restriction conditions (they were maintained at 80% of their free-feeding body weights), THC had no effect on any of the parameters of the two equations. Then the animals were tested again under free feeding conditions. The data from this condition conformed adequately to the two equations, with reduced values of *a* (Equation 1) and *b* (Equation 2), indicating a reduction of the incentive value of both reinforcers. Again, THC did not affect the parameters of the equations. Finally, a feeding test was carried out, in which THC was found to induce a (borderline significant) increase in the amount of sucrose solution consumed, and a significant reduction of corn oil intake. Therefore the results from this experiment provided no support the involvement of CB1 receptors in regulating the incentive value of sucrose or corn oil. The results also suggest that caution is needed in drawing conclusions about the effects of drugs on the 'incentive value' of foods in operant behaviour paradigms on the basis of their effects on food intake.

## 8.3 Implications for the Mathematical Principles of Reinforcement model

The progressive-ratio schedule has been a very popular tool in studying the effects of centrally acting drugs, neurobiological interventions and brain self-stimulation on 'motivational processes' (see Pin-Teng et al. 1996). The attraction of this tool no doubt derives from Hodos' (1961) original claim that the breakpoint provides a valid index of the 'efficacy' of the reinforcer. Nevertheless, as discussed earlier in this thesis (section 1.4.1), a number of authors have raised doubts about the reliability of the breakpoint as a

behavioural measure, and its validity as a measure of reinforcer efficacy or incentive value. Perhaps the greatest problem with the breakpoint is the discovery that it is sensitive to a number of variables that are believed to affect motor, rather than motivational, processes (see Arnold and Roberts 1997). MPR offers a means of overcoming many of the technical and theoretical problems that have been identified with the breakpoint. At a technical level, the use of the reponse rate function (response rate plotted against the response/reinforcer ratio) overcomes the weakness of reliance on a single data point to define the breakpoint. At a theoretical level, MPR provides an index of incentive value (the activation parameter, $a$) that is founded on a comprensive model of schedule-controlled operant behaviour, and, most importantly, this index is theoretical and empirically distinguishable from an index of 'motor capacity' (the response-time parameter, $\delta$) (Killeen 1994).

The equation used to describe progressive-ratio schedule performance (Equation 1) was originally developed for fixed-ratio schedules (Killeen 1994). It has been successfully used in progressive-ratio schedules, including a number of studies of the behavioural effects of drugs (see section 1.4.4). The results described in this thesis confirm the findings of previous experiments that changes in the breakpoint may be associated with a change in either $a$ or $\delta$ (or both), in keeping with the MPR's interpretation of the breakpoint as ratio of these two parameters ($a/\delta$) (Killeen 1994).

The work described in this thesis extends the application of MPR to a number of new situations. Equation 1 was found to provide a good description of progressive-ratio schedule performance maintained with a novel reinforcer, corn oil, as well as confirming previous work with other reinforcers (sucrose solutions and food pellets: e.g. den Boon et al. 2011; Rickard et al. 2009). In Experiment 6 rats were tested under food deprivation and free feeding conditions. The rats continued to respond on the progressive-ratio schedule under the free-feeding condition, and their response rates could be accommodated by Equation 1. When the rats were transferred to the free-feeding condition, the activation parameter $a$ decreased (as expected), whereas the response-time parameter $\delta$ was not affected. Therefore these results provide further support for the independence of the two parameters.

Despite its evident advantages over the breakpoint, the application of Equation 1 to

progressive-ratio schedule performance is not without problems. One difficulty is the systematic departure from linearity of the descending limb of the response rate function. This was first noted by Mobini et al. (2000), who recommended an operational criterion for removal of data points corresponding to very low response rates maintained by the highest ratios. Killeen et al. (2009) noted that the curvature of the descending limb is less marked in arithmetic progressive-ratio sequences than in exponential sequences, and suggested that arithmetic sequences might be preferable in order to avoid this deviation from the form defined by Equation 1, although they acknowledged that psychophysical considerations favour the use of exponential rather than arithmetic progressions (for discussion, see Rickard et al. 2009).

Killeen et al. (2009) described a modification of Equation 1 which takes into account the hypothetical effect of the 'reinforcing context' on progressive-ratio schedule performance. These authors proposed that 'contextual' effects may arise because the previous and upcoming ratios may distort the relationship between response rate and response/reinforce ratio (Baron and Derenne 2000). Killeen et al.'s modified equation incorporates a fourth parameter, *c*, which represents the 'context'. While the addition of this parameter enables better fits to empirical data to be achieved, work in this laboratory has found that *a* and *c* often interact in such a way that the two parameters respond inconsistently to interventions.

Another difficulty with the application of MPR to progressive-ratio schedule performance is the failure of Equation 1 to accommodate the data derived from the running response rates, although, according to Killeen (1994), the same equation should be applicable to both overall and running response rates. Rickard et al. (2009) found that running rates could be described by a logistic decay function (Equation 2), and also found a strong relationship between the activation parameter *a* (Equation 1) and the decay parameter *b* (Equation 2). One practical advantage of the use of running rates is that since running rates do not incorporate the post-reinforcement pause, any effect of an intervention on the post-reinforcement pause cannot be reflected in the recovered values of the parameters. This can be seen in the results of Experiment 4, where cyproheptadine, clozapine and haloperidol all increased the response-time parameter $\delta$ (Equation 1), but did not affect the maximum response rate parameter $R_i$ (Equation 2). This may suggest that the effect of the treatments on $\delta$ mainly reflects an increase in post-reinforcement

pausing, and that none of the treatments interfered with the rapid emission of trains of responses after the post-reinforcement pause. However, it is important to emphasise, as Rickard et al (2009) pointed out, that Equation 2 is entirely based on empirical description of the data and has no theoretical basis in MPR.

The lack of a unified model of progressive-ratio schedule performance that satisfactorily accounts for both overall and running response rates is a significant weakness of MPR in its present form. Ongoing theoretical work is attempting to rectify this shortcoming (Killeen 2011, personal communication).

## 8.4 Future directions

Although this project has provided some preliminary evidence about the different roles of the two subtypes of orexin receptor, OX1 and OX2 receptors, the former mediating motivational functions and the latter mediating arousal/motoric functions, this evidence is by no means conclusive. Therefore more experiments are needed. A significant problem that besets research in this area is the shortage of selective pharmacological tools. For example, the only available neurotoxin that shows any selectivity for the orexinergic system is OxSap, which preferentiually targets neurones that express OX2 receptors; there is no available toxin that shows preference for OX1 receptor-bearing neurones. As an alternative to OxSap, excitotoxins may be used to lesion the point of origin of the orexinergic pathways (e.g. Harris et al. 2007). While this approach is likely to be successful in destroying OX1 receptor-bearing neurones of the LHA, it would inevitably destroy other neuronal populations within the LHA.

Further studies are needed to answer some questions raised by Experiments 2 and 3. A disconnection study between the LHA and AcbS, but targeting OX2 rather than OX1 receptors in the AcbS, as in Experiment 3, could help to resolve the issue of whether OX2 receptor-mediated transmission is mainly responsible for the motor debilitating effects of destruction of the orexinergic projection. If such a disconnection lesion results in an increase of $\delta$ and a reduction of $R_i$, this would add further support to the hypotheses that the two populations of orexin receptors are differentially involved in motor and incentive processes. Another relevant experiment would be to disconnect LHA and VTA, targeting the OX1 receptor population in the VTA (for example using SB-334867-A),

rather than the OX2 receptor population as in Experiment 2.

The possibility that $D_1$-like and $D_2$-like receptors may make different contributions to regulating the reinforcing values of qualitatively different reinforcers (e.g. sucrose vs. corn oil) requires further investigation. The present preliminary observations with selective antagonists could be extended to include selective agonists of the two receptors followed by studies of agonist/antagonist interaction. The feasibility of such studies using MPR has been demonstrated previously (Ho et al. 2003).

The data shown in Fig 7.6 suggest that, calorie for calorie, corn oil may be more reinforcing than sucrose. This is another area that merits further investigation. Ho et al. (1999) proposed that the incentive value of a reinforcer is related to its magnitude according to a hyperbolic function, and Rickard et al. (2009) provided some evidence that the relationship between $a$ and reinfocrcer size could be used to characterize this relationship. This approach could be used to compare the efficacies of these two reinforcers.

The need for further development of MPR has been discussed above. One aspect of the model that has received very little attention is the coupling parameter $\beta$ (Killeen 1994). There have been no previous investigations specifically aimed at manipulating this parameter. This parameter is assumed to reflect, in part, the contingencies specified by the schedule of reinforcement which enable the index response to become 'coupled' to reinforcer delivery. Coupling is also assumed to be affected by the retention of traces of recently emitted responses in a short-term memory buffer (Bizo and Killeen 1997; Bizo et al. 2001; Killeen 1994; Killeen et al. 2009). It would be of interest to examine the effects of interventions that are known to affect working memory on this parameter.

In conclusion, the results obtained in this project offer encouragement for the application of quantitative models of operant behaviour to behavioural neuroscience. More specifically, they indicate that models such as MPR have the potential to differentiate between the effects of neurobiological interventions on different behavioural processes that may be difficult to disentangle using qualitative behavioural tests. Some possible directions for future research with MPR have been suggested, and the need for further development of MPR itself has been discussed.

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