AN INVESTIGATION OF THE NEURAL MECHANISMS OF INTERVAL TIMING BEHAVIOUR

Lourdes Valencia Torres, BSc

Division of Psychiatry
School of Community Health Sciences
Faculty of Medicine
University of Nottingham

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LIST OF ABBREVIATIONS

%B	proportional selection of lever B
3-MT	3-methoxytyramine
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
8-OH-DPAT	8-hydroxy-2-(di-*n*-propylamino)tetralin
AADC	L-aromatic amino acid decarboxylase
ABC	avidin-biotin complex
Acb	nucleus accumbens
AcbC	nucleus accumbens core
AcbS	nucleus accumbens shell
ACh	acetylcholine
ADHD	Attention Deficit Hyperactivity Disorder
ANOVA	analysis of variance
AP-5	(2*R*)-amino-5-phosphonovaleric acid
AVP	arginine vasopressin
BEM	The Behavioural Economics of Choice and Interval Timing
BeT	Behavioural Theory of Timing
BR	burst ratio
BSA	bovine serum albumin
BW813U	(2[(E)-2-(3-chlorophenyl)ethenyl]-4,4,6-trimethyl-5,6-dihydro-1,3-oxazine)
cAMP	cyclic adenosine monophosphate
CGS-19755	selfotel

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ChAT	choline acetyltransferase
CLN	caudal linear nucleus
CNE	negative exponential function
COMT	catecol-O-methyltransferase
CR	conditioned response
CSTC	cortico-striato-thalamo-cortical
D_{28K}	calcium binding protein calbindin
DA	dopamine
d_A	delay to the smaller reinforcer
DAT	dopamine transporter
d_B	adjusting delay to the larger reinforcer
d_{B50}	indifference delay to the larger reinforcer
DFMO	α-difluoromethylornithine
DLCP	dorsolateral caudate putamen
DLPFC	dorsolateral prefrontal cortex
DMCP	dorsomedial caudate putamen
DOI	2,5-dimethoxy-4-iodoamphetamine
DOPAC	3,4-dihydroxyphenylacetic acid
DRL	differential reinforcement of low rates
DRN	dorsal raphe nucleus
DTPP	discrete-trials psychophysical procedure
EMQMCM	3-ethyl-2-methylquinolin-6-yl-(4-methoxycyclohexyl)methanone methanesulfonate
FI	fixed-interval
FIPP	fixed-interval peak procedure
fMRI	functional magnetic resonance

FOPP	free-operant psychophysical procedure
GABA	γ-aminobutyric acid
GPCR	G-protein-coupled receptor
GPe	globus pallidus external segment
GPi	globus pallidus internal segment
HVA	homovanillic acid
IEG	immediate/early gene
ILPFC	infralimbic prefrontal cortex
IRT	interresponse time
K	rate of delay discounting
L-DOPA	3, 4-dihydroxyphenylalanine
LeT	Learning to Time theory
MANOVA	multivariate analysis of variance
MAO	monoamine oxidase
MDL-100907	(±)2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol]
MDL-72222	topanyl 3,5-dichlorobenzoate
MHM	Multiplicative Hyperbolic Model of inter-temporal choice
MK-801	dizocilpine
MPFC	medial prefrontal cortex
MRN	median raphe nucleus
MTS	Multiple Time Scales model of habituation
NA	noradrenaline
NAT	noradrenaline transporter
NeuN	neurone-specific nuclear protein
NGS	normal goat serum

NMDA	N-methyl-D-aspartate
OPFC	orbital prefrontal cortex
PBS	phosphate buffered physiological saline
PkA	peak area
PkL	peak location
PLPFC	prelimbic cortex
Q	reinforcer size sensitivity parameter
R	operant response
S+	reinforcing stimulus
SBF	Striatal Beat Fequency
SERT	5-HT transporter
SET	Scalar Expectancy Theory
SHR	Spontaneously Hypertensive Rat
SKF-81297	6-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-hydrobromide
SKF-83566	8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol
SN	substantia nigra
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
SR-141716A	rimonabant
SSRI	selective 5-HT reuptake inhibitors
STN	subthalamic nucleus
T_{50}	indifference point
TH	tyrosine hydroxylase
THC	Δ^9-Tetrahydrorocannabinol
TMS	transcranial magnetic stimulation

TPH	tryptophan hydroxylase
TRD	temporal response differentiation task
US	unconditioned stimulus
VI	variable-interval
VMPFC	ventromedial prefrontal cortex
VTA	ventral tegmental area
WAY-100635	N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl) cyclohexanecarboxamide trihydrochloride
WIN-55,212-2	((R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone)

ABSTRACT

Timing behaviour plays an important role in the daily living of individuals from a great variety of species. For example, organisms must be able to discriminate between the durations of relevant events (temporal discrimination) and to regulate their own behaviour in time (temporal differentiation). The processes that allow animals to adjust their behaviour to the temporal regularities of the environment have been studied using different procedures which model the relationship between time and behaviour. A taxonomy of timing based on the subject's location in time with respect to the signalled duration has been proposed. When an organism judges the duration of an elapsed interval the timing is retrospective (e.g. interval bisection); when it responds during an elapsing duration the timing is immediate (e.g. fixed-interval peak procedure); and finally when it chooses between future delayed outcomes the timing is prospective (e.g. inter-temporal choice schedules). It has been proposed that the cortico-striato-thalamo-cortical (CSTC) circuits play a special role in interval timing and inter-temporal choice behaviour. This thesis examined whether performance of timing tasks by rats induces neuronal activation within the prefrontal cortex and corpus striatum, as revealed by Fos expression, and explored a new approach to analyzing performance in an inter-temporal choice schedule.

Chapter 1 describes the literature which forms the background of the project. It reviews interval timing and inter-temporal choice methodology and theory, the neurobiological substrates underlying both kinds of behaviour, and finally Fos expression, as a marker of neuronal activation.

Chapters 2-4 present experiments that examined whether, in intact rats, performance of different interval timing tasks was associated with neuronal activation in the dorsal striatum and prefrontal cortex, as revealed by expression of the Fos protein, the product of the immediate-early gene *c-fos* (Experiments 1-3).

Chapters 5-7 present experiments focused on some behavioural and neurobiological aspects of inter-temporal choice behaviour. One purpose of these experiments was to develop an abbreviated approach to estimate the rate of delay discounting (K) and reinforcer size sensitivity parameter (Q) based on the Multiplicative Hyperbolic Model of inter-temporal choice (MHM), using the adjusting-delay schedule. Additionally a novel way of quantifying transitional behaviour in the adjusting-delay schedule was presented based on analysis of the

power spectrum of cyclical changes in the adjusting delay, d_B (Experiment 4). This approach was used to analyze data obtained from rats performing on the adjusting-delay schedule under methodological manipulations (Experiment 5) and neurobiological interventions (Experiment 6).

Experiment 1 (Chapter 2) investigated whether, in intact rats, performance on the discrete-trials temporal discrimination task was associated with neuronal activation in the prefrontal cortex and corpus striatum, as revealed by enhanced Fos expression in these areas. Performance on temporal and light-intensity discrimination tasks was well described by a two-parameter logistic equation. The rats trained under the timing task showed increased Fos expression in the orbital prefrontal cortex (OPFC) and the nucleus accumbens (Acb) compared to the rats trained under the light-intensity discrimination task, indicating a substantial activation of these areas during the timing task. However, there was no evidence for involvement of the dorsal striatum in the performance of this task.

Experiment 2 (Chapter 3) examined whether performance on an interval-bisection task in the range of milliseconds showed increased Fos expression in the prefrontal cortex and corpus striatum compared to performance under a light-intensity bisection task. Performance on both bisection tasks conformed to the conventional logistic psychometric function. The rats trained under the timing task showed increased Fos expression in the OPFC, infralimbic and prelimbic cortex and Acb compared to the rats trained under the light-intensity bisection task. The results provided no evidence for an involvement of the dorsal striatum in the performance of this task.

Experiment 3 (Chapter 4) investigated whether performance on the fixed-interval peak procedure (FIPP) was associated with increased neuronal activity in the prefrontal cortex and corpus striatum, as revealed by Fos expression. The results showed that response rate during peak trials was characterized by a 'Gaussian plus ramp' function, with maximal responding (peak rate) occurring around the time of the reinforcement in the FI trials (peak time). Consistent with the results of Experiments 1 and 2, the concentration of Fos-positive neurones in the OPFC was greater in rats exposed to FIPP than in rats exposed to a VI schedule. However, the results did not provide any evidence for a specific involvement of the dorsal or ventral striatum in FIPP performance.

In Experiment 4 (Chapter 5), rats made repeated choices on an adjusting-delay schedule. Indifference delays, calculated from adjusting delays in the last 10 sessions, were shorter when the sizes of reinforcers were 14 and 25 µl of a 0.6 M sucrose solution than when they were 25 and 100 µl of the same solution. The ratio of the indifference delays (d_{50}) was significantly smaller than that predicted on the basis of an assumed linear relation between reinforcer size and instantaneous reinforcer value. Estimates of K and Q fell within the values reported previously. Adjusting delays in successive blocks of trials were analysed using the Fourier transform. The power spectra obtained from individual rats had a dominant frequency that corresponded to a period of oscillation of the adjusting delay between 30 and 100 trial blocks. Power in the dominant frequency band declined with extended training.

Experiment 5 (Chapter 6) examined the pattern of oscillation of d_B in an adjusting-delay schedule using the power spectrum analysis. The step-size in which the delay to the larger reinforcer (d_B) increased or decreased was tested across two conditions. In Condition 1, d_B increased or decreased (according to the rats' choice) by 20% from block n to block $n+1$. In Condition 2, the step size was 10%. The power spectrum analysis showed that the period of oscillation of the dominant frequency of the spectrum was significantly longer under Condition 2 than under Condition 1. There was a consistent trend for the power of oscillation to be higher in the initial segment of the experiment in both conditions.

Experiment 6 (Chapter 7) examined the effect of excitotoxic lesion of the core of the nucleus accumbens (AcbC) on K and Q in an adjusting delay schedule using the same protocol as Experiment 4. The effect of the lesion on the power spectrum parameters was also examined. The AcbC-lesioned group showed significantly lower values of d_{50} than the sham-lesioned group. The ratio of the indifference delays seen in both groups was substantially less than the value predicted on the basis of an assumed linear relation between reinforcer size and instantaneous reinforcer value. K was higher in the lesioned group than in the sham-lesioned group; Q was not affected by the AcbC lesion. Neither the spectral power within the dominant frequency band nor the period corresponding to the dominant frequency differed significantly between groups.

The final chapter (Chapter 8) summarizes the findings of the experiments, and discusses their implications for the putative role of the prefrontal cortex, and ventral and dorsal striatum in interval timing and inter-temporal choice, and for theoretical

models of these behaviours. The role of the dorsal striatum is questioned, while a possible role of the Acb in temporal processing is proposed. It is argued that an integrated model of interval timing and inter-temporal choice behaviour may require more than the processes of reinforcement and timing to account for both types of behaviour. Some possible directions for future research in this area are also discussed.

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 (2011) 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 (2011) 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 (2011) Nucleus accumbens and delay discounting in rats: evidence from a new quantitative protocol for analysing inter-temporal choice. *Psychopharmacology*, **in press**.

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[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.

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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*
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* 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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"What then is time? If no one asks me, I know what it is.
If I wish to explain it to him who asks, I do not know."

Saint Augustine of Hippo

This is by default the easiest section I have to write. No complicated theories to describe, no contradictory evidence to explain. The only thing it takes is to describe what I have been experiencing from the moment I started my PhD until today. I wish I had enough words to express my gratitude, admiration and respect to my supervisor Chris Bradshaw. As a scientist, Chris surprised me every single day with his deep knowledge about behaviour, neuroscience and pharmacology. I was amazed to see that there were very few questions that Chris could not answer. However, I was even more surprised to realize that he had the modesty to accept when something was out of his knowledge. His genius, dedication, but overall his humbleness served as an example. As a mentor, Chris was as a theatre director behind the stage, always overseeing and orchestrating the experimental work with great detail and passion. I truly enjoyed my weekly supervisions with him where we started discussing scientific dilemmas and closed the meetings talking about British or Mexican culture, traditions and history. During these meetings I got to know Chris better and was delighted to find out that he was not only a wonderful scientist/mentor, but he had an extraordinary human side. I know that I will eventually forget some of the scientific facts I learned in the last three years, but what I will never forget is the man that through his patience and kindness taught me that arrogance does not serve any purpose, and it is rather an obstacle. I feel honoured to have been able to work with him, and he has no idea of the positive impact he made in various aspects of my life. I could not ever thank him enough.

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CHAPTER 1

INTRODUCTION

1.1 Overview of the introduction

This thesis examines the neural mechanisms underlying the regulation of interval timing and inter-temporal choice behaviour, with special reference to the role of the cortico-striato-thalamo-cortical (CSTC) circuits.

The following section of the introduction (1.2) focuses on the methodology (operant conditioning schedules of reinforcement) used to study interval timing behaviour along with some relevant results in this area of research. In section 1.3, the most important theories of interval timing and inter-temporal choice are described. Next, in section 1.4, the neural underpinnings of timing and inter-temporal choice are reviewed. Finally, in section 1.5, the neuronal expression of Fos, the protein product of the early-immediate gene *c-fos* which is activated when neurones are active, is reviewed.

1.2 Interval timing methodology

Timing behaviour plays an important role in the daily living of individuals from a great variety of species. For example, animals must be able to discriminate between the durations of relevant events (temporal discrimination) and to regulate their own behaviour in time (temporal differentiation). The keystone of the study of animal timing behaviour comes from observations of Pavlovian and operant conditioning. Operant and Pavlovian conditioning share important similarities; for example, the changes in behaviour are related to some prior event in the organism's history. In the case of Pavlovian conditioning, stimulus 1 (S_1) is paired with stimulus 2 (S_2), which, after several pairings, evokes a conditioned response (CR); whereas in operant conditioning, an operant response (R) (e.g. lever pressing, key peck) leads to a reinforcing stimulus (S^+).

The first observations relating Pavlovian conditioning with timing were made by Pavlov himself, who observed an increment in the salivary response as an inverse function of the time elapsed between the presentation of the conditioned and unconditioned stimuli. Pavlov (1927) concluded: "… time has acquired the properties of the conditioned stimulus…". Ten years later, B.F. Skinner introduced the concept of

operant conditioning, which he described as "behaviour controlled by its consequences". In *The Behavior of Organisms"*, Skinner characterized time as "a single property of duration, comparable with intensity, wavelength and so on…" (Skinner 1938). Skinner's first contribution to the study of animal timing was the invention of reinforcement schedules. A reinforcement schedule is any procedure that delivers a reinforcer to a subject according to a specific rule (Staddon and Cerutti 2003). Fixed-interval (FI) schedules were particularly important in the development of animal timing research methodology, although Skinner's main interest was in the response rate which such schedules produced rather than the temporal pattern of responding. On FI schedules, a reinforcer is delivered following the first response that occurs after a fixed time interval has elapsed since the last reinforcement. Responding on an FI schedule is characterized by a "scalloped" pattern in which few responses occur at the beginning of the interval, and a high rate of responding appears in the latter half of the interval (Dews 1970).

The processes that allow animals to adjust their behaviour to the temporal regularities of the environment have been studied using different procedures which model the relationship between time and behaviour. Killeen and Fetterman (1988) proposed a taxonomy of timing based on the subject's location in time with respect to the interval being timed. The three types of timing described by these authors (retrospective, immediate and prospective timing) are described in the following sections.

1.2.1   Retrospective timing

In a retrospective timing schedule a subject's behaviour comes under the control of the duration of an elapsed interval. An example of this type of timing in the natural world is mate selection by some species of birds: the females of several bird species select their partners based on the duration of their song (Slabbekoorn and Smith 2002). With the aim of modelling this kind of phenomenon, several laboratory methods have been developed. The most extensively employed retrospective timing schedules are listed below.

1.2.1.1   Interval Bisection Procedure

In this procedure, subjects are trained to discriminate between two stimuli, one with a "short" signal duration (e.g., 2 s) and one with a "long" signal duration (e.g., 8 s), in a conditional discrimination procedure in which a response on lever A is reinforced

following the short stimulus, and a response on lever B following the long stimulus. Then, stimuli of intermediate durations (e.g., 3, 4, 5, 6 and 7 s) are presented (without reinforcement) and the relation between proportional choice of lever B (%B) and stimulus duration is determined. This relation generally takes the form of a sigmoid function in which the proportional selection of lever B (%B) increases as a function of the stimulus duration (t). The indifference point (T_{50}) or "Point of Subjective Equality" (PSE) is the value of t corresponding to %B=50, i.e. the duration at which the subject responds on lever B on 50% of the trials. The data are commonly analysed using the following two-parameter equation:

$$\%B = 100/(1+[t/T_{50}]^{\varepsilon}), \qquad (eq.1)$$

where ε is the slope of the function; ε has a negative value in the case of ascending sigmoid functions, flatter functions being associated with higher (i.e. less strongly negative) values of ε (Al-Zahrani et al. 1996). From the two parameters, T_{50} and ε, the difference limen and the Weber fraction (an index of discriminability) can be derived. The limen is defined as half the difference between T_{75} and T_{25} (T_{75} and T_{25} being the values of t corresponding to $\%B$=75% and $\%B$=25%), and the Weber fraction is the ratio of the limen to T_{50} (Church and Deluty 1977). Previous studies in animal research using this task have shown that the T_{50} lies close to the geometric mean of the standard signal durations (Chiang et al. 2000b; Church and Deluty 1977; Orduña et al. 2007). However, mixed results have been obtained in interval bisection with humans, some authors reporting that T_{50} lies close to the geometric mean (Allan and Gibbon 1991), and others that it lies closer to the arithmetic mean (Wearden 1991; Wearden and Ferrara 1993; 1995). In an attempt to reconcile these different results, Wearden et al. (1997) manipulated the stimulus spacing and the range of stimuli in an interval bisection task with humans. The results demonstrated that spacing the stimulus durations logarithmically resulted in a leftward shift of the psychophysical function and a non-significant decrease of the bisection point compared to the linear spacing. On the other hand, varying the range of values had very little effect on T_{50} which was located nearer to the arithmetic mean than the geometric mean. Wearden and Ferrera (1995) suggested that the location of T_{50} close to the arithmetic mean observed in human experiments is determined by the subject making explicit comparisons between the absolute difference

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between the stimulus duration, t, and the short and long standard stimuli. Subjects classify t as short if the absolute difference between t and the short standard stimulus is smaller than the difference between t and the long standard stimulus, and classify t as long in the reverse case.

The interval bisection task has been widely used to study temporal discrimination in humans and animals suffering from different pathological conditions or subjected to different neurobiological interventions. For example, Carroll et al. (2009) assessed the discrimination of durations in the millisecond and second ranges in patients with schizophrenia using two interval bisection tasks, and reported greater variability in the schizophrenic patients compared to the controls. Orduña et. al (2007) employed the interval bisection task using four conditions: 1-4 s, 2-8 s, 3-12 s and 4-16 s to test the time discrimination of Spontaneously Hypertensive Rats (SHR), an animal model of Attention Deficit Hyperactivy Disorder (ADHD), and concluded that the temporal behaviour of SHRs was not different from that of normal control rats. Furthermore, Ho et al. (1995) trained rats with lesions of their 5-hydroxytryptaminergic (5-HTergic) pathways on the interval bisection task using 2-s and 8-s stimuli, and reported that the lesioned rats' Weber fraction was not different from that of the control group, however the psychophysical function showed a displacement to the left. The effects of pharmacological interventions on performance on the interval bisection task are reviewed in greater detail in a later section (section 1.4)

1.2.1.2   Discrete-trials psychophysical procedure (DTPP)

In this schedule a stimulus (e.g. light or tone) is presented for a variable time, after which levers A and B are presented. A response on A is reinforced if the stimulus duration is shorter than a given value, whereas a response on B is reinforced when the stimulus duration is longer than that value. Timing is assessed from a psychometric function relating %B to time, t, measured from the start of the trial. Performance on this schedule is well described by the same two-parameter logistic function as that employed to analyze the data obtained under the interval bisection procedure (eq.1), from which T_{50} and the Weber fraction can be derived. Previous studies have demonstrated important differences between the T_{50} obtained under the DTPP and the interval bisection procedure (Hampson et al. 2010; see also Chapter 2). In the DTPP, the spacing of durations is usually linear. T_{50} has generally been found to lie close to the arithmetic

mean of the range of durations, and the psychometric function is symmetrical in linear coordinates (Asgari et al. 2005; Body et al. 2002a; Hampson et al. 2010). This is in contrast to the interval bisection task, where the spacing of stimulus durations is usually geometric. In this case, T_{50} lies close to the geometric mean of the range of stimulus durations and the psychometric function is symmetrical in semi-logarithmic coordinates (see previous section). In an early study of timing by pigeons, Stubbs (1968) used a similar task to the DTPP in which he employed 10 discrete durations: 1, 2, 3, ……., 10 s that varied in a non-systematic way from trial to trial. Short durations were from 1 to 5s, and long durations from 6 to 10s. Responding on a red key was reinforced if the duration was short, whereas responding on a green key was reinforced if the duration was long. Food reinforcement was obtained for every sixth correct choice response. Stubbs showed that T_{50} lay between 5 s and 6 s, being closer to the arithmetic mean than to the geometric mean.

1.2.1.3.   Temporal generalization

The temporal generalization task was first developed by Church and Gibbon (1982). Rats were exposed to periods of darkness of several durations. If the period of darkness had some standard duration (e.g. 4 s), the response was reinforced, whereas if the duration was longer or shorter than 4 s the lever-press was not reinforced. The temporal generalization gradient was obtained from the probability of responding plotted against stimulus duration. With this kind of task, it is generally observed that the generalization curve peaks at the standard duration (*T*); the probability of responding decreases symmetrically at shorter and longer durations, and the peak of the distribution increases proportionally with *T* (Church and Gibbon 1982).

1.2.1.4.   Conditional discrimination task

In this task, subjects are required to respond on one lever after a short stimulus, and another lever after a long stimulus. Correct responses result in the delivery of a reinforcement, whereas incorrect responses are usually followed by a time-out (Cevik 2003). Accuracy is measured in terms of percentage of correct responses. In addition, some studies have used a non-parametric measure of discriminability to analyze the

performance in this kind of schedule by employing the following equations derived from signal detection theory:

$$1) \quad A' = 1/2 + \frac{(H - FA)(1 + H - FA)}{4H(1 - FA)}$$

$$2) \quad B''d = \frac{(1 - H)(1 - FA) - HFA}{(1 - H)(1 - FA) + HFA},$$

where H ('hit') is the probability of choosing the response option associated with 'short' on a short-stimulus trial and FA (a 'false alarm') is the probability of responding on the lever associated with 'short' on a long-stimulus trial. An A' value of 1.0 describes a maximum accuracy and a value of 0.5 reflects chance-level performance. $B''d$ values that are less than 0 reflect a 'choose-short' bias, whereas values greater than 0 reflect a 'choose-long' bias (Harper et al. 2006).

This procedure has been used extensively in studies of temporal memory, in which delays are interposed between the durational stimulus and the opportunity to make a response. Spetch and Wilkie (1983) were the first to report a 'choose-short' effect in this task, the magnitude of which increased as a function of the post-stimulus delay. These authors proposed that durations are coded in an analogue fashion by animals (as opposed to categorical coding of durations as 'short' or 'long'), and that the 'choose-short' effect arises from 'subjective shortening' of durations in memory.

Al-Ruwaitea et al. (1997) extended the use of the conditional discrimination task to examine the types of error that may occur when delays are interposed between the stimulus and the opportunity to make a response. By introducing 'pre-stimuli' of varying durations before the stimulus whose duration was to be discriminated, Al-Ruwaitea et al. (1997) identified two types of discrimination error: 'confusional errors' in which the subject responded in a manner appropriate to the pre-stimulus rather than the stimulus, and 'summation errors', in which the subject appeared to concatenate the pre-stimulus and stimulus durations. Both types of error increased as a function of the delay interval.

1.2.2. *Immediate timing*

Animals' operant behaviour may come under temporal control during an ongoing interval. This type of timing is important in enabling animals to react appropriately in anticipation of a reinforcer becoming available.

1.2.2.1. Peak interval procedure

In this free-operant schedule (also known as the 'fixed-interval peak procedure', FIPP), standard fixed-interval (FI) trials are randomly alternated with extended peak trials. In the FI trials a discriminative stimulus is presented, and the first response after the designated time interval has elapsed is followed by the delivery of a reinforcer. Peak trials are usually three or four times longer than FI trials and the reinforcer is never delivered. Response rate during peak trials is usually characterized as a normal distribution with maximal responding (peak rate) occurring around the time of the reinforcement in the FI trials (peak time) (Roberts 1981). Performance under this schedule is analyzed using a modified Gaussian function ('Gaussian plus linear ramp' function: MacDonald and Meck 2005):

$$R = a \times e^{\left[-0.5 \times \left(\frac{t - t_{peak}}{b}\right)^2\right]} + \left[c + d \times (t - t_{peak})\right] \qquad \text{(eq. 2)}$$

where $(a + c)$ is the estimated peak response rate, t_{peak} is the peak time (location of the peak of the Gaussian component of the function), b represents the spread of the function (standard deviation of the Gaussian component); the right-hand term is a linear ramp of slope d and an ordinate value c at time $t = t_{peak}$. The Weber fraction is represented by the coefficient of variation of the Gaussian component of the function (b/t_{peak}) (Asgari et al. 2006b; Buhusi et al. 2005; MacDonald and Meck 2005). The peak interval procedure is one of the most frequently employed methods to study timing. Many of the theoretical approaches to interval timing have been developed through observations of the performance on the peak interval procedure (Gibbon 1977; Gibbon et al. 1984; Killeen and Fetterman 1988). An important modification of the traditional peak interval procedure is the introduction of a retention interval or "gap" during the presentation of the signal on peak trials (Roberts 1981). The design of the gap procedure was derived from the cognitive idea that interval timing is driven by an internal clock (Gibbon 1977;

Gibbon et al. 1984). A typical peak interval gap procedure consists of a mixture of FI trials, peak trials and peak trials with gaps. Interpretation of the change of the location of the peak time during gap trials has been controversial in recent years. A cognitive explanation of this phenomenon argues that if the change is equal to the duration of the gap, this means that the subject stopped its "internal clock" during the gap, and started timing again when the signal resumed. If the change in peak time is equal to the duration of the gap plus the duration of the signal prior to the gap, the subject is assumed to have reset its "internal clock". The "stop" or "reset" modes of operation depend on many factors, such as the species tested and the duration and location of the gap. However, several studies have found intermediate outcomes between those predicted by the "stop" and "reset" modes (Buhusi and Meck 2007; Orduña et al. 2008; Zentall and Kaiser 2005). Another interpretation, which avoids the postulation of an internal clock, is given in terms of the similarity of the gap presentation to events associated with the trial onset, in other words, the greater the similarity, the more likely it is that the outcome will be to "reset" (Staddon and Cerutti 2003).

1.2.2.2.   Free operant psychophysical procedure (FOPP)

In this schedule, reinforcement is provided in a series of trials under a variable-interval (VI) schedule. A discriminative stimulus starts the cycle; responses on lever A are intermittently reinforced during the first half of the trial whereas responses on lever B are intermittently reinforced during the second half of the trial (Killeen and Fetterman 1988). A typical pattern of responding consists of an increasing response rate on lever B and a declining response rate on lever A during the course of the trial. Temporal differentiation in this schedule is well described by the same two-parameter logistic function employed to analyze performance under the DTPP and interval bisection tasks (eq.1). This function relates the proportion of responding on lever B (%B) to time measured from the trial onset. The T_{50} and Weber fraction are derived in the same way as in retrospective timing schedules (see above). Another feature which is analyzed in this kind of task is the "switching" between response alternatives. For example, Al-Zahrani et al. (1996) studied the effect of destruction of the 5-HTergic pathways on rats' performance under the FOPP, and reported that the lesioned rats showed higher rates of switching between the two levers than the sham-lesioned rats, switching rate in both groups reaching a peak at a time that approximately corresponded to T_{50}. These authors

also reported that the T_{50} and Weber fraction of 5-HT depleted rats did not differ from those of sham-lesioned rats.

1.2.2.3. Differential Reinforcement of Low Rates (DRL) or Interreponse Time (IRT) Schedule

In this schedule the subject is required to withhold a response for a certain time, t, until the reinforcer is available. A response spaced by more than t s from the previous response is reinforced, whereas a response separated by less than t s from the previous response is not reinforced and restarts the IRT requirement from zero. This kind of schedule generates a bimodal IRT distribution with one mode at the shortest recorded IRT (burst responses) and another mode around the reinforceable IRT duration (t) (Wilson and Keller 1953). Performance on this task is typically analyzed by calculating the index of efficiency (reinforcers earned / total responses × 100). Although it is generally assumed that IRT performance depends on the ability of the organism to estimate time accurately, some authors have suggested the involvement of other processes which may influence performance in this kind of schedule (Orduña et al. 2009; Sanabria and Killeen 2008). In order to provide a quantitative measure of performance on IRT schedules, several analytic methods have been developed (Richards et al. 1993; Sanabria and Killeen 2008). For example, Richards and Seiden (1991) developed the Peak Deviation analysis which has proved to be very sensitive to pharmacological interventions (Jolly et al. 1999; Marek et al. 1993; Sabol et al. 1995). In this analysis, the IRT distribution is converted to a survival curve, and compared to its corresponding negative exponential function (CNE) which would result from a random distribution of responses with the same mean as the IRT distribution. The comparison provides three measures: peak area (PkA), peak location (PkL), and burst ratio (BR). The PkA is the proportion of the IRT duration that is not accounted for by the CNE. The PkL is the median IRT duration, that is, the IRT duration which bisects the PkA. The BR is calculated by dividing the obtained burst responses by the prediction of burst responses based on the CNE.

1.2.3. *Prospective timing*

Animals are generally very sensitive to the delay between emitting a response and

receiving a reinforcer. Laboratory tasks that involve this kind of timing generally take the form of *inter-temporal choice* schedules in which the subject chooses between reinforcers differing in magnitude and delay.

1.2.3.1. Adjusting-delay schedule

In the adjusting-delay schedule (Mazur and Coe 1987), the subject is faced with two alternatives: a "small" reinforcer after a fixed delay (d_A) or a "big" reinforcer after an adjustable delay (d_B). Repeated choice of the "big" reinforcer results in an increase in the value of d_B, whereas repeated choice of the "small" reinforcer decreases the value of d_B. The value of d_B increases and decreases as a result of the subject's fluctuating preference for the reinforcer B, eventually approaching a quasi-stable value of d_B, the 'indifference delay' (d_{B50}), which may be used to derive parameters that measure the subject's sensitivity to delay and reinforcer magnitude (Ho et al. 1999).

Most versions of the adjusting-delay schedule employ a fixed increment or decrement in d_B (e.g. 1 s) (e.g. Green et al. 2007; Mazur 1994, 1995, 1996). However, it has been argued that sensitivity to changes in delay of reinforcement probably conforms to Weber's law, as is the case with temporal discrimination in many types of timing schedule (Gibbon 1977; Killeen and Fetterman 1988). Weber's Law implies that proportional changes should be similarly discriminable across a broad range of delays, whereas a fixed increment would be less discriminable if the preceding value of d_B were, say, 30 s than if it were 2 s. This issue will be discussed further in later chapters of the thesis.

1.2.3.2. Progressive-delay schedule

The progressive-delay schedule allows the experimenter to program a series of delays during an experimental session or in different phases of the experiment. In the schedule described by Evenden and Ryan (1996), the subject chooses between lever A which provides a "small" reinforcer and lever B which provides a "big" reinforcer. At the beginning of the session both reinforcers are delivered immediately (i.e. $d_A = d_B = 0$), but after several trials a delay to the delivery of the "big" reinforcer (d_B) is imposed, which increases in successive blocks of trials, until at the end of the session there is a substantial time gap (e.g. 60 s) between the response and the delivery of the reinforcer

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(Evenden and Ryan 1996). Kheramin et al. (2002) modified this schedule and created a "multi-point inter-temporal choice" schedule. In each phase of the experiment, the delay to the "big" reinforcer increased progressively across trials allowing the d_{B50} to be estimated. The delay to the small reinforcer was manipulated across successive phases of the experiment. The theoretical advantage of this "multi-point inter-temporal choice schedule" is that a linear relation between d_{B50} and d_A can be obtained and parameters of the subjects' sensitivity to delay and size of reinforcement calculated (Ho et al. 1999). The theoretical basis of this approach is discussed in section 1.3.2.

1.2.3.3. Time-left procedure

In this procedure, subjects choose between two stimuli that signal different delays to reinforcement: a *standard* (*S*) stimulus associated with an FI schedule and a *comparison* stimulus (*C*) associated with a longer FI. The trial begins with *C* and then *S* is introduced at a certain time (*T*) into the trial, and the subjects can then choose between the two alternatives. Thus the subject chooses between the delay to reinforcement specified by the shorter FI (*S*), and the residual delay ('time left') associated with the longer FI (*C*). For example, Gibbon and Church (1981) exposed rats to choices between FI 30 s (*S*) and FI 60 s (*C*) with *T* set at 15 s, 30 s or 45 s. The rats were indifferent between the two levers when $T = 30$ s and their preference was symmetric with respect to indifference when $T = 15$ and 45 s. According to Gibbon and Church, this result indicates that subjective time is linearly rather than logarithmically related to physical time (see below for further discussion).

Different methods have been used to measure preference in the time-left procedure. Following Gibbon and Church (1981), most experiments have employed variable-interval (VI) schedules in the initial link of the trial, preference being assessed by the relative response rate in the two concurrent alternatives. However, Al-Ruwaitea et al. (1999a) introduced a discrete-trials procedure that more closely resembles the inter-temporal choice tasks described above. In Al-Ruwaitea et al.'s (1999a) procedure, each trial starts with the presentation of stimulus A which signals a fixed delay to reinforcer availability (T). At some point within this delay, *t* s after the trial onset, a second stimulus, B, is presented which signals a shorter delay to reinforcer availability (usually T/2), and the rat is then able to choose between the two alternatives. Thus choice of A leads to earlier reinforcer delivery when the 'time left', T-*t*, is shorter than T/2), whereas

choice of B leads to a shorter delay to reinforcement when T-*t* is longer than T/2).

1.3. Interval Timing and Inter-temporal choice theories

Interval timing and inter-temporal choice research have been two important themes in the study of operant behaviour. Although a close relationship between these two types of behaviour seems likely, a complete theory of operant performance which successfully integrates both has not yet been developed. While studies of interval timing have focused on the psychophysical representation of time and the mechanisms controlling the temporal regulation of behaviour, research on inter-temporal choice has focused on the delay and magnitude of reinforcement and how these two variables interact. In this section, interval timing and inter-temporal choice will be reviewed as separate entities.

1.3.1. *Interval timing theories*

Theories of timing have attempted to develop a comprehensive account of interval timing by humans and animals, which may be applicable to timing across a broad range of time intervals. Among the most influential theories we can mention the following.

1.3.1.1. Scalar Expectancy Theory (SET)

This theory explains timing behaviour based on the operation of a hypothetical internal clock, a memory system and a decision rule (Gibbon 1977). The clock has a pacemaker and an accumulator. The pacemaker is an oscillator that emits pulses at a high variable rate (λ). A switch operates between the pacemaker and the accumulator; if the switch is open no pulses are added to the accumulator, however when the switch is closed pulses enter the accumulator. The accumulator is reset to 0 at the beginning of each trial. When an important event occurs (e.g. delivery of a reinforcer) the switch opens and the value (i.e. the number of pulses that have occurred since the previous salient event) in the accumulator is multiplied by a random factor (k*) and transferred to the reference and working memories. Due to the fact that λ and k* are random variables, the value in the accumulator and the value in memory will be variable, even when the time interval has a constant duration. Each trial adds a new value to the memory, so that after several trials the memory will contain a distribution of values that represent the time when the

reinforcement is delivered. During an individual trial, the subject compares the current value in the accumulator with a sample taken from its reference memory, and applies a 'decision rule'. If the ratio between the accumulator value and the memory value crosses a threshold (Θ) a response is emitted (Gibbon et al. 1984). The timing model proposed by SET is summarized in Fig. 1.1.

SET derives its name from the scalar property observed in several timing procedures with different ranges of time (Church et al. 1994; Gibbon and Church 1990) The scalar property is analogous to Weber's law which proposes that the variability around some psychophysical measure is proportional to the absolute magnitude of that measure. The mathematical expression of the scalar property is the coefficient of variation (the ratio of the standard deviation to the mean), which remains constant across different durations. In an attempt to assess the generality of this principle, Lejeune and Wearden (1991) compared the data obtained under FI schedules using different animal species ranging from freshwater turtles to cats. These authors demonstrated that the coefficient of variation was constant over a fairly broad range of durations; however it increased at very long FI values. In addition, they also reported that the coefficient of variation varied from one species to another.
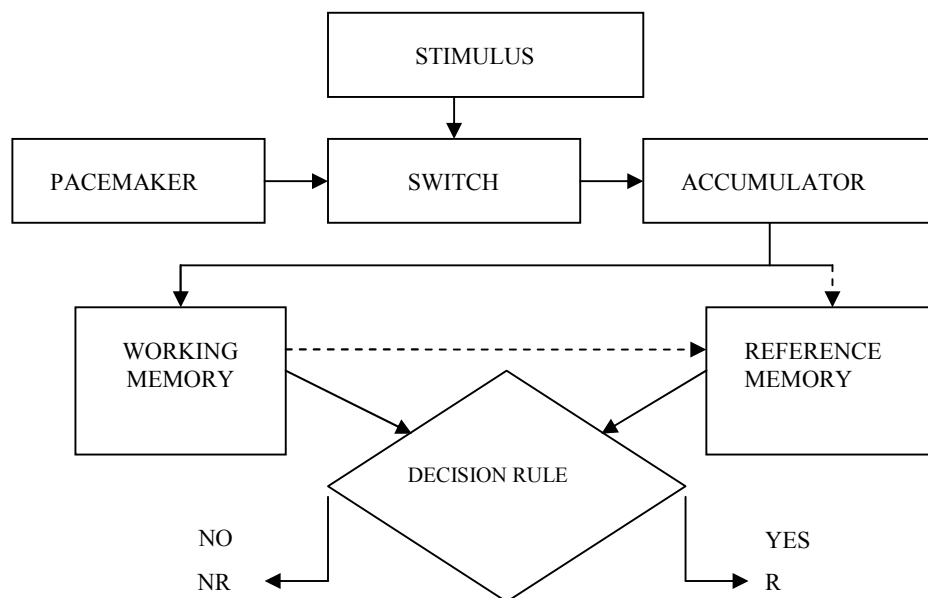
Fig. 1.1. Temporal information processing model proposed by SET. Pulses generated by the pacemaker are transferred, via the switch, to a counter ('accumulator'). The contents of the accumulator are transferred to working and reference memory. The updated contents of working memory are repeatedly compared with a sample drawn from reference memory, and when the two are 'approximately equal' (as determined by a decision threshold), a response is emitted. NR = no response; R = response.

An important consequence of the scalar property is the *superposability* of psychophysical functions (Dews 1970). For instance, if an animal is trained under the interval bisection procedure using a series of different long and short 'anchor' durations, and the time axis is re-scaled in proportion to the bisection point in each case, the obtained functions should exactly superpose (Gibbon 1977).

SET accounts for many results obtained using several timing procedures in animals and humans (e.g. temporal generalization, interval bisection, peak interval procedure) (for a review see Machado et al. 2009), and one of its major strengths lies in the scalar property itself. However recent research has demonstrated that the scalar property does not hold when very short durations are used (<100 ms) and the difficulty of the task is increased (Lejeune and Wearden 2006; Wearden and Lejeune 2008; Whitaker et al. 2008). For example, SET has not been able to predict the results obtained under a double bisection task where pigeons learn two temporal discriminations within the same session. In the procedure described by Keen and Machado (1999), there were two kinds of trial. In trial "1", two keys were presented simultaneously (red & green); the choice of a red key was reinforced if the preceding signal duration was 1 s, whereas the choice of the green key was reinforced if the preceding signal duration was 4 s. In trial "2", other keys were presented simultaneously (blue & yellow) the choice of the blue key was reinforced if the preceding signal duration was 4 s, whereas the choice of the yellow key was reinforced if the preceding signal duration was 16 s. Then, a signal duration (t) between 1 s and 16 s was presented with the choice between the green and the blue keys (both keys associated with 4 s). According to SET, pigeons' choice should be indifferent between the green and blue keys, because the signal duration t is compared with two samples that have identical distributions. However, the results showed that when the signal duration was 1 s, the pigeons preferred the blue key, whereas when the signal duration was 16 s, the pigeons preferred the green key. Keen and Machado (1999) explained this result by proposing that the pigeons' preference may be related to the alternative keys that are presented with the green and the blue key during training (the red key with the green key, and the yellow key with the blue key).

Despite the important contributions of SET, the idea of cognitive structures (e.g. internal clock) used to explain timing behaviour has been questioned in the last 10-15 years, as neurobiology has gained increasing relevance in this field (Ho et al. 2002; Meck 1996). Matell and Meck (2004) have proposed a hypothetical neurobiological substrate for the pacemaker, in the form of striatal medium spiny neurones which,

according to the authors' 'Beat Frequency' model, are able to detect coincident inputs from rapidly firing cortical and thalamic neurones. Matell and Meck's (2004) model is discussed in greater detail later in this chapter. In addition, behavioural alternatives to the cognitive/information-processing explanation of timing propounded by SET have been proposed by Killeen and Fetterman (1988: Behavioural Theory of Timing [BeT]) and Machado (1997: Learning to Time [LeT] model). These models are described in the following two sections.

1.3.1.2.   Behavioural Theory of Timing (BeT)

According to this theory (Killeen and Fetterman 1988), animals use adjunctive activities (behavioural states) as cues for the emission of the timed response. Each pulse of the pacemaker is associated with a behavioural state. The rate of the pacemaker depends on the reinforcement rate (arousal) in the experimental context. Reinforcement is assumed to strengthen sequences of behaviours, rather than individual responses, and the individual behaviours that make up a sequence are assumed to act as discriminative stimuli for the following response. For instance, a rat trained under an FI 30-s schedule may perform the following behavioural states: eat-drink-activity-groom-lever. Grooming is the last behaviour before the lever is pressed; thus it may serve as a discriminative stimulus for lever pressing.

BeT's assumption that interval timing relies upon conditioned association between operant timing responses and other 'behavioural states' suggests that the principles of associative learning, as exemplified by Pavlovian learning, may also apply to temporal learning.

Interestingly, BeT assumes that the occurrence of a response is related to the inter-pulse time, or period, of the pacemaker, which is determined by the average inter-reinforcer interval. In other words, the average inter-pulse time is a linear function of the average inter-reinforcer interval. Fetterman and Killeen (1991) investigated how inter-reinforcer time determines inter-pulse time by varying reinforcement probability in a temporal discrimination task. Subjects were trained to discriminate between a short duration (4 s) and a long duration (12 s). Probe trials were presented on half of the trials. Reinforcer probability was manipulated by reinforcing timing responses on 20% or 80% of the trials. It was expected that psychometric functions would shift to longer durations and the standard deviation would increase in the lower probability condition (20%). The

results confirmed the latter prediction by showing that there were larger standard deviations for the 20% condition than for the 80% condition. However, the predicted shift of T_{50} was not confirmed. In addition, Bizo and White (1994a,b) tested these assumptions of BeT using the FOPP (described in section 1.2.2.2). In this schedule, responses on a left key were reinforced for the first 25 s of a 50-s trial according to a VI schedule, and responses on the right key had no scheduled consequence. After 25 s had elapsed, responses on a right key were reinforced according to a VI schedule, and responses on the left key had no consequences. The VI schedules were manipulated across different conditions. The results showed that when the inter-reinforcer time decreased, the standard deviation of the psychometric function decreased (suggesting a reduction of the inter-pulse time), whereas an increase of the inter-reinforcer time had the opposite effect.

Despite the evidence for a linear relationship between adjunctive behaviour and reinforcement rate (Lejeune et al. 1998), some authors have pointed out that adjunctive behaviours are not always reliable or even observable (Lejeune 1980; Reid et al. 1993), and the exact relation between pacemaker activity and adjunctive behaviour is not clear (Lejeune et al. 2006). On the other hand, Fetterman et al. (1998) trained rats and pigeons to perform a retrospective timing task and reported that in a number of cases observed adjunctive behaviours were a better predictor of the choice response than was time per se. These authors argued that although the measurement of mediating behaviours may be imperfect, these serve as stronger observational evidence than unobservable cognitive mediation by accumulators and switches.

In summary, although BeT's prediction about the linear relation between adjunctive behaviours and reinforcement rate has generally been supported, attempts to identify the adjunctive behaviors specified by BeT have found mixed success, and some of BeT's predictions (for example, the predicted relation between reinforcement rate and T_{50}) have not been confirmed.

1.3.1.3.   Learning to Time model (LeT)

LeT (Machado 1997), like BeT, assumes that behavioural states serve as cues for responding. The states are activated serially and do not depend on the operation of a pacemaker. Each state is coupled with an operant response, and the degree of coupling decreases to 0 during extinction, and increases to 1 during reinforcement. States that are

strongly active when food is unavailable lose their coupling and do not support the operant response. On the other hand, states that are active when food is available increase their coupling and support the operant response. LeT proposes that after a significant biological event, a set of behavioural states is activated and the occurrence of an operant response depends on the level of "activation" of the state and the strength of its association with the operant response. Response rate is determined by a multiplicative rule that combines the levels of activation and association. Machado (1997) explains his model by comparing it to a cascade of water through connected compartments where: 1) the amount of water represents the degree of activation of a corresponding state, and 2) the activation of the states is determined by the size of the hole drilled at the bottom of each reservoir. For instance, during an FI schedule, the activation of a corresponding state (first reservoir in the cascade) immediately after reinforcer delivery is at its highest point, while coupling to the operant response is low because the reinforcer is never delivered in this state.

LeT, unlike SET, has been able to explain the results obtained during a double bisection task (described in section 1.3.1.1) where pigeons learn two interval bisection tasks simultaneously (e.g. 1 s versus 4 s, and 4 s versus 16 s), and then choose between the 4 s stimuli following sample stimuli of different durations. According to LeT, the subjects learn two rules during the performance of this task: (1) When the signal duration is 1 s, the animals learn through reinforcement to approach the key corresponding to the short duration (S_1), and to avoid the key corresponding to the long duration through extinction (L_1). (2) When the signal duration is 16 s the pigeon learns through reinforcement to approach the key corresponding to the long duration (L_2) and through extinction to avoid the key corresponding to the short duration (S_2). In the testing phase, when the signal duration is 1 s and the response choice is between L_1 and S_2, the choice is based on the second rule, because the S_1 key is absent. In consequence, the animal avoids the L_1 key and chooses the S_2 key instead. Thus LeT correctly predicts that pigeons should prefer S_2 to L_1, even though both stimuli were associated with the same duration during training – i.e. 4 s.

LeT not only makes predictions about patterns of temporal control, but also specifies how response rates should change during the passage of time. However, the results obtained in mixed FI schedules have proved to be the most challenging for this theory to explain (Lejeune et al. 2006; Macinnis et al. 2010; Whitaker et al. 2003). On a mixed FI schedule with two components, reinforcers become available either after the

shorter FI value or after the longer FI value. There are no distinguishing stimulus conditions associated with the two components, so the subject receives no indication of which FI is in force at the beginning of the interval. Considering a mixed FI 30 FI 120 schedule, LeT implies that in the short interval (FI 30) the states which are active at around 30 s strengthen their associative links; however the reinforcer delivery should have no effect on the associative links of states that are active at around 120 s. On one hand, during the long interval (FI 120) the states that were active around 30 s become active early in the interval and then extinguish, so their association to the operant response decreases during these intervals. On the other hand, the states which are active at the end of the long interval keep their association with the operant response because they are reinforced and, it is assumed, never extinguished. This suggests that response rates at the end of the long interval should be higher than at the end of the short interval. Whitaker et al. (2003) demonstrated that when animals were tested in a mixed FI FI schedule with a high ratio of the short and long components (e.g. FI 45 FI 300) the response rate was higher in the short FI than in the long FI, contrary to LeT's prediction.

Although LeT is able to explain the data obtained during acquisition by its central idea of conditioning and extinction of behavioural states, it has encountered the same inconsistency as BeT when trying to explain the variability of adjunctive behaviour sequences and the nature of the states (Lejeune et al. 2006).

1.3.1.4.   Multiple Oscillator Model of timing

This model is a development of SET (Church and Broadbent 1990). The internal clock is replaced by a set of multiple oscillators whose mean periods are arranged in a geometric progression (e.g. 200 ms, 400 ms, 800 ms, etc). The accumulator postulated by SET has been substituted by a set of "status indicators" which record the phase of a corresponding oscillator. The model assumes that animals have access to the half-phase of each oscillator (-1 or +1), so that the perceptual representation of time is a vector of unitary values (-1 or +1) for a series of oscillators. The time of the reinforcement is represented as a vector of status indicators recording the phase of each oscillator. A matrix in reference memory is updated every time reinforcement occurs and compared with the vector representing the current time; if this value is greater than the threshold a response will be emitted.

Wearden and Doherty (1995) examined this model by simulating performance under a peak-interval procedure and demonstrated that the Multiple Oscillator Model showed the same scalar property and pattern of responding as that generated by SET. In addition, it has been demonstrated that some specific intervals are timed more accurately than others, as would be expected if some intervals reside near an oscillator period (Church et al. 1998; Crystal et al. 1997). This idea is in agreement with the postulated existence of multiple oscillators that have selectivity for intervals within circumscribed ranges, rather than a single pacemaker-accumulator system.

One advantage of the Multiple Oscillator Model is that it provides a mechanism for representing time intervals covering a very wide range without burdening the system with an unwieldy quantity of information. For example, the information needed to encode an interval of, say, an hour, is no larger than that required to encode an interval of 100 ms, since each interval is represented by the vector of half-phases (Collyer et al. 1994).

1.3.1.5.   Multiple-Time-Scale model of habituation (MTS)

According to Staddon and Higa (1999), in a time discrimination task animals learn to discriminate between "memories of different ages and different strengths" (1999, p.229). These authors proposed that timing depends on the same mechanism as habituation. Habituation refers to the decrease of a reflex response to successive presentations of the stimulus. Habituation has an important property called "rate sensitivity" which refers to the fact that habituation (and recovery from habituation) is more rapid and complete when inter-stimulus intervals are short than when they are long. Staddon and Higa (1996) assume that increasing habituation during stimulus presentation is equivalent to increasing activation of a "short-term memory" and that the recovery from habituation corresponds to the decaying "trace" of the activated memory. In terms of timing, this means that animals associate the strength of the memory trace to responding. Habituation can be illustrated by a process in which response strength is the difference between a constant stimulus effect and a "leaky integrator". The simplest comparator mechanisms consist of a "leaky integrator" which is charged (V) by successive stimulus presentations. The response strength is determined by the difference between the stimulus input (S) and the memory, V. V increases with the stimulus presentation and then decays after the stimulus offset. V continues increasing if the next stimulus presentation occurs sooner,

and the difference between *V* and *S* decreases (decays exponentially); this is analogous to a habituating output. The decay output of this unit is fed to the next integrator, which has its own memory trace. The sum of all memory traces from each integrator generates an overall memory trace whose strength varies according to a time marker. For instance, in an FI schedule, the 'wait time', or post-reinforcement pause, is associated with a threshold value (e.g. reinforcement time); when the memory trace decays below that value a response is emitted.

MTS correctly predicts some aspects of performance on mixed FI FI schedules (Staddon and Higa, 1999). According to MTS, animals generally use reinforcement as a time marker. This leads to the prediction that in a mixed FI FI schedule, the duration of the post-reinforcement pause should be directly related to the immediately preceding reinforcement duration, because the level of memory trace activation increases with the duration of the time marker and would take longer to decay. Extant data on performance on these schedules are consistent with this prediction (Lowe et al. 1974; Staddon 1970).

Proportional timing and the conformity of timing performance to Weber's Law can also be explained by MTS. MTS predicts that memory traces will decay faster after frequent reinforcement, influencing the trace of the prevailing inter-stimulus interval. In other words, if the threshold is set a fixed distance from the value of the memory trace associated with reinforcement, the time following reinforcement at which the decay trace crosses the threshold will be a fixed fraction of the interval to be timed, as long as the memory trace strength is logarithmically related to time. MTS's assertion that subjective time is scaled logarithmically is in apparent conflict with the claim by proponents of SET that subjective time is linearly related to physical time (Gallistel 1999; Gibbon 1999). As discussed earlier, the finding of a psychometric function centred on the arithmetic mean in the time-left procedure has been taken as evidence for the putative linear scaling of subjective time (Gibbon and Church 1981). According to Gallistel (1999) and Gibbon (1999), MTS's failure to accommodate this finding is a major weakness of the model. Staddon et al. (1999), however, have argued that the time-left procedure is an inappropriate basis for assessing the nature of subjective time scales, because it is based on 'expectancy' of future reinforcement, in contrast to most other timing tasks, which are based on behavioural control by elapsed or ongoing time intervals. This controversy has not been resolved in the literature; however, the debate brings into focus the interesting question of whether prospective timing is qualitatively

different from retrospective and immediate timing (Killeen and Fetterman 1988), an issue that will be considered in later chapters of this thesis.

1.3.1.6   Packet Theory of Conditioning and Timing

This theory (Kirkpatrick 2002) derives its name from the observation that many operant behaviours (e.g. lever pressing, key pecking) occur in bouts (Killeen et al. 2009; Nevin and Baum 1980; Robinson et al. 2000). The two main postulates of this theory are: 1) responses occur in packets containing a random number of responses, and the mean number of responses in a packet, as well as the mean inter-response interval is unaffected by procedural variations, 2) the probability of a packet of responses occurring is determined by a *conditional expected time function* (see below) (Kirkpatrick and Church 2003). Bouts are treated as a basic unit of behaviour based on the finding that they are insensitive to procedural variations of interval durations and distributions (Kirkpatrick and Church 2003). The bouts are generated by a conditional expected time function which is defined as "the mean expected time until the next food delivery at *t* sec after an event" (Kirkpatrick and Church 2003; 2004). Packet theory proposes a model in which behaviour is controlled by the conditional expected time function, and it is based on three processes: perception, memory and decision. The perceptual module generates an expectation for each interval received during training. Expectations are constructed once the food is delivered and are stored in the memory which has the weighted sum of all input intervals. The function in memory is the conditional expected time function and will be different for different types of interval distributions. The decision process controls the responses occurring during an interval in anticipation of food delivery. Then, the conditional expected time function is transformed by reversing its direction and converting times into probabilities. The result is a probabilistic function which is inversely related to the conditional expected time function. The sum of probabilities in this function is the expected number of bouts per interval.

Kirkpatrick and Church (2000) trained rats under a tandem fixed-time (FT) random-time (RT) schedule that generated different conditional extected times to reinforcement (e.g. tandem FT 90 s RT 90 s and tandem FT 75 s RT 15 s). Kirkpatrick and Church reported that the response rate function was negatively related to the conditional expected time function, as predicted by Packet Theory. Although Packet Theory has accurately predicted the rate and form of responding in fixed, random and

tandem schedules (Kirkpatrick 2002), further investigation is needed to fit a wider range of procedures (e.g. peak procedure).

1.3.1.7   Striatal Beat Frequency Model (SBF)

SBF is a model that incorporates the general information processing components proposed by SET to their putative underlying neural structures. SBF proposes that the firing rates of neurones in the prefrontal cortex oscillate at different rates (5-15 Hz), and striatal spiny neurones decode this pattern of oscillation, serving as a coincidence detection mechanism. In this model, phasic dopaminergic (DAergic) activity initiates the interval timing system by "closing the switch", and later on, strengthens the synaptic connections which are active at the time of delivery of reinforcement. Tonic DAergic activity is proposed to modulate the speed of the "internal clock" by changing the frequency of cortical oscillations allowing anticipation of future events (see Fig. 1.2) (Matell and Meck 2004; Meck et al. 2008).

Matell et al. (2003) investigated the firing patterns of striatal and cortical neurones in rats performing a temporal generalization task. A multiple-duration FI procedure was employed. On the short trials, the first response after 10 s had elapsed was reinforced, whereas on the long trials the first response after 40 s had elapsed was reinforced. Electrodes were placed in the anterior dorsolateral striatum and the cingulate cortex, and electrical activity was recorded. The results showed, as expected, that the response distribution peaked twice, once at 10 s and then again at 40 s. A 10 s peak firing pattern was recorded in the striatal neurones, and coincided with the 10 s lever-pressing function, but not with the 40 s function. According to the authors, the fact that the neuronal activity pattern diverged from the temporal pattern of operant responding argues against the possibility that variations in motor behaviour were responsible for the peak function of neuronal firing. In addition, a subset of cortical neurones displayed peak-shaped firing rates that varied as a function of signal duration. The authors concluded that neurones in the dorsolateral striatum and anterior cingulate cortex encode specific signal durations as a direct function of their firing rate.

Information-processing model   **Striatal Beat Frequency Model**

Clock   ⟶   **Cortical and thalamic oscillations**

Integrator (accumulator)   ⟶   **Striatal spiny neurons**

Memory   ⟶   **Dopamine** modulates the cortico-striatal synaptic weight by being released upon the delivery of reinforcement

Decision stage   ⟶   **Striatal firing** integrated by the **basal ganglia output nuclei** sent through the **thalamus** to **the pre- and primary motor cortex.**

Fig. 1.2. Relationship between the striatal beat frequency (SBF) model and a general information processing model of timing (e.g. the scalar expectancy theory [SET]). The principal components of SET are shown on the left, and the corresponding neural processes postulated by the SBF model on the right .

Matell and Meck (2004) also carried out computer-simulation experiments which demonstrated that this hypothetical coincidence detection process is capable of generating timed output in the range of intervals commonly used in interval timing experiments with animals. Furthermore, there is evidence from functional brain imaging of human volunteers performing timing tasks that the striatum is active during the performance of such tasks (Dreher and Grafman 2002; Hinton and Meck 2004). Although SBF is innovative in terms of integrating the neural structures in the understanding of temporal control, there is a paucity of evidence from lesion studies with animals as to whether cortico-striatal mechanisms are crucial to the performance of interval timing tasks in animals.

1.3.1.8.   The Behavioral Economics of Choice and Interval Timing (BEM)

The aim of BEM is to integrate choice and interval timing into a single theory of operant performance. BEM is based on three assumptions (Jozefowiez et al. 2009):

1)   Animals have a logarithmic representation of time which follows Weber's Law.

2) For each value that subjective time can take in a particular situation, the animal associates a payoff for each response it can emit in that situation.

3) Payoff functions determine the probability of responding according to a maximizing response rule.

Consider the performance of an animal in FOPP (see section 1.2.2.2). In Bizo and White studies (1994a; 1994b; 1995a; 1995b) pigeons were given the option of responding on two keys. Responses on the right key were intermittently reinforced during the first half of a 50 s trial according to a VI schedule *(VI x)*, whereas responses on the left key were intermittently reinforced during the second half of the trial according to a VI schedule *(VI y)*. As expected, the proportion of responding on the left key was a sigmoid function of time from trial onset with the pigeons pecking the right key early in the trial before switching to the left key. When the reinforcement rates specified by the two VI schedules were equivalent ($x = y$), the indifference point, T_{50} was at 25 s into the trial. However, when $x < y$, T_{50} moved to the right and when $x > y$, it moved to the left. In other words, the animals stayed longer in, or switched earlier to, the schedule associated with the higher reinforcement rate. In order to test BEM's assumptions, Jozefowiez et al. (2009) applied the model to a simplified version of FOPP. In this version, the subject was presented with four stimulus durations which were equally spaced on a logarithmic scale: T_{11}, T_{12}, T_{21} and T_{22}. The animals were given a choice between two responses, b_1 and b_2. Response b_1 was reinforced during the two shorter durations T_{11} and T_{12} with probabilities p_{11} and p_{12}, whereas response b_2 was reinforced during the two longer durations T_{21} and T_{22} with probabilities p_{21} and p_{22}. Several simulations were run where the stimulus durations were held constant, but the reinforcement probabilities were changed (e.g. in the "bias b_1" condition, the reinforcement probability for b_1 after T_{11} and T_{12} was higher than for b_2 after stimulus durations T_{21} and T_{22}, and vice versa in the "bias b_2" condition). Manipulating the probability at a given point in the trial was equivalent to manipulating the VI in the Bizo and White studies (1994a; 1994b; 1995a; 1995b). The results showed that in the "bias b_1" condition the animals stayed longer on the side associated with the higher reinforcement probability, shifting the psychometric function to the right. Conversely, in the "bias b_2" condition the animal switched earlier to the side associated to the higher reinforcement probability, shifting the psychometric function to the left.

These results can be explained through the payoff functions. In the "bias b_1" condition, the higher reinforcement rate inflates the payoff function for b_1, whereas in the "bias b_2" the higher reinforcement rate inflates the payoff function for b_2. These changes shift the critical subjective value at the point where the two payoff functions are equal and determine when the animal switches from b_1 to b_2.

Then the model was applied to the original FOPP procedure employed by Bizo and White (1994a; 1994b; 1995a; 1995b), providing a good fit to the data (Jozefowiez et al., 2009). Similarly the model was able to account for the results obtained under the double-bisection procedure (Keen and Machado 1999; Machado and Pata 2005), a "metacognition" task based on interval bisection (Foote and Crystal 2007), matching (Herrnstein 1961) and the time-left procedure (Gibbon and Church 1981). Interestingly, BEM's simulations of performance in the time-left procedure were successful using identical parameters to those used by Gibbon and Church (1981) (see section 1.2.3.3), suggesting that these results can be explained even if time is represented logarithmically. However, BEM's simulations predicted that animals should switch to the time-left stimulus earlier than predicted by SET, a result found in all subsequent replications of the time-left procedure (Cerutti and Staddon 2004; Preston 1994). Although BEM has accounted for a wide range of data, it has not been able to account for the results obtained in concurrent FI and mixed FI schedules (Jozefowiez et al. 2005; 2006). The authors argue that "a full model of choice behaviour requires more than reinforcement and timing", and that the underlying assumption of BEM that animals are perfect optimizers is an over-simplification (Jozefowiez et al. 2009).

1.3.2. *Inter-temporal Choice Theories*

In an inter-temporal choice schedule, a subject chooses between reinforcers that differ with respect of their sizes and delays. Inter-temporal choice schedules have been widely used to study "impulsive" behaviour (Kheramin et al. 2004; Kheramin et al. 2002; Mobini et al. 2002; Mobini et al. 2000). "Impulsiveness" is defined as the failure to resist an impulse, drive or temptation to perform an act that is harmful for the person or others (APA 1994). The term impulsiveness has been applied to many different aspects of operant behaviour in humans and animals. For instance, the choice of smaller earlier reinforcers in preference to larger delayed reinforcers is just one of the many features that encompass impulsive behaviour (Evenden 1999; Ho et al., 1999; Dalley et al. 2008;

Basar et al. 2010). Specifically, "impulsive choice" is defined in the context of "intolerance of delay", and refers to the selection of small immediate gains in preference to larger delayed gains, or the selection of larger delayed losses in preference to smaller immediate losses (Ho et al. 1999). It has been generally observed that an organism will engage more readily in a behaviour which produces an immediate reinforcer than one that produces the same reinforcer after a delay (Ainslie 1974; Chung and Herrnstein 1967; Rachlin and Green 1972). Many animals tend to devalue or "discount" outcomes for which they have to wait. The function relating the length of delay to the value of the reinforcer is the *temporal discount function* (Monterosso and Ainslie 1999).

One common feature of inter-temporal choice models is that the value of the reinforcer is assumed to be a positive function of its size and an inverse function of its delay (Ainslie 1974; Chung and Herrnstein 1967; Ho et al. 1999; Mazur and Coe 1987). However, the decline in the value of the reinforcer as a function of its delay has been described in numerous ways (Green and Myerson 1996; Renner 1964). Economists proposed the following exponential function:

$$V = q.e^{-kd}$$
<div align="right">(eq. 3)</div>

where V is the hypothetical value of the reinforcer, q is its size, d is the delay and k an exponential decay parameter which is constant for any individual animal (Green and Myerson 1996). Exponential curves are percentage discount functions in which a reinforcer loses a fixed proportion of its value per unit of delay time. However, exponential functions do not predict preference reversal (see Fig. 1.3). For instance, Ainslie and Herrnstein (1981) trained pigeons to choose between 2- or 4-s access to grain with the larger reinforcer always presented 4 s later than the smaller. Then, the delay to the smaller rienforcer was varied from .01 to 12 s. The results showed that all pigeons reversed preference from the small-early to large-later reinforcement as the delay to the smaller reinforcer was progressively increased. In addition, Ainslie and Haendel (1983) showed that human subjects chose the larger and later of two alternative cash outcomes when both were distant, but changed to the smaller earlier one as they drew nearer. These findings, and others (Green and Estle 2003; Green et al. 1981; Bradshaw and Szabadi 1992; Wogar et al. 1992a), indicate that the delay function is more concave than an exponential curve. For a discount function to explain the preference reversal phenomenon, it must generate curves that cross one another as time

elapses (see Fig. 1.3). In other words, discounting is steeper at short delays and additional equal increments of delay produce progressively less additional discounting (Monterosso and Ainslie 1999). An alternative discounting function with the shape of a hyperbola was proposed by Ainslie (1974) based on Herrnstein's Matching Law. The Matching Law states that "an organism on a pair of concurrent variable interval schedules, each with its own rate, amount and delay of reinforcement, distributes responding in direct proportion to the rates and amounts and in inverse proportion to delays of reinforcement from the alternatives" (Chung and Herrnstein 1967; Herrnstein 1961):

$$\frac{B_1}{B_2} = \frac{R_1}{R_2} \cdot \frac{A_1}{A_2} \cdot \frac{D_2}{D_1}, \qquad \text{(eq. 4)}$$

where the subscripts 1 and 2 refer to the two response alternatives; B is the rate of emitting behaviour to the alternatives, and R is the rate, A the amount, and D the delay of reinforcement. The hyperbolic function relating behaviour to delay, implicit in Equation 4 (McDowell 1981), predicts a reversal of preference, given pairs of reinforcers which vary in both amount and delay. Thus as the opportunity to choose between the two alternatives draws near, preference may shift from the more delayed larger reinforcer to the more immediate smaller one (see Fig. 1.3).
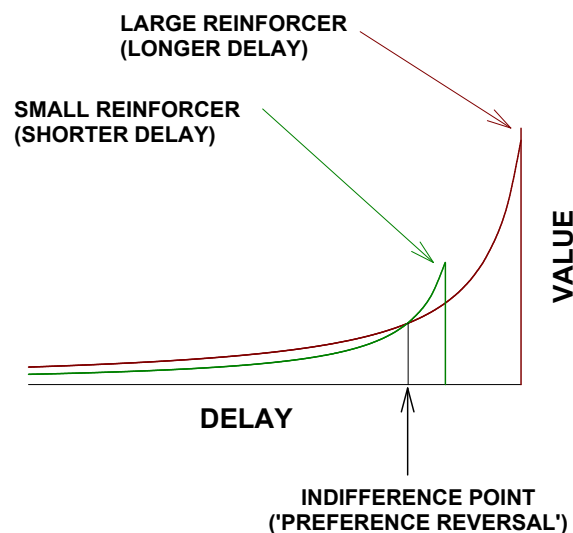
Fig 1.3. The hyperbolic discounting function accounts for the preference reversal phenomenon. The vertical lines indicate the amounts of two reinforcers. The curved lines show how the subjective value of each of these reinforcers changes hyperbolically as a function of the delay until its receipt (Ainslie & Herrnstein, 1981).

1.3.2.1.   Mazur's Hyperbolic Model of Inter-temporal Choice

According to Mazur's (1988; 1987) hyperbolic model of inter-temporal choice, reinforcer value (*V*) is a declining hyperbolic function of the delay interposed between the response and the primary reinforcer:

$$V = q \cdot \frac{1}{1 + K \cdot d} \qquad \text{(eq.5)}$$

where *q* is the size or magnitude of the reinforcer, *d* the delay associated with it and *K* is a parameter expressing the hyperbolic delay discounting rate. In the adjusting delay schedule (see also section 1.2.3.1) developed by Mazur (1988; 1987), the subject is confronted with two alternatives: a "small" reinforcer after a fixed delay (d_A) or a "big" reinforcer after an adjustable delay (d_B). Repeated choice of the "big" reinforcer results in an increase in the value of d_B, whereas repeated choice of the "small" reinforcer decreases the value of d_B. The value of d_B increases and decreases as a result of the subject's fluctuating preference for the reinforcer B, eventually approaching a quasi-stable value of d_B, the 'indifference delay' (d_{B50}). The indifference delay is the delay at which the subject chooses each alternative about equally often; it may be used to derive the parameter that measures the subject's sensitivity to delay, *K*.

Many studies have shown that the temporal discounting function is consistently hyperbolic and well described by eq. 5 (Green et al. 1994; Rodriguez and Logue 1988). However, it has been reported that the value of *K* varies across species (Hwang et al. 2009), and that different levels of deprivation produce different estimates of *K* (Bradshaw and Szabadi 1992; Ho et al. 1997). For example, pigeons discount the value of a delayed reinforcer more steeply than rats and monkeys; typical values of *K* for pigeons range between 0.3 and 2.24 s^{-1} (Green et al. 2004), whereas estimates of *K* for rats generally range between 0.05 and 0.3 (Kheramin et al. 2002, 2003, 2004; Bezzina et al. 2007, 2008a).

Rats maintained at 80% of their free-feeding body weight have lower values of *K* than rats maintained at 90% (Wogar et al. 1992). In addition, it has been demonstrated that the rate of discounting in humans decreases as a function of reinforcer size. This phenomenon is known as "the magnitude effect" (Green et al. 1997; Raineri and Rachlin

1993). Nevertheless, several studies have confirmed a lack of a magnitude effect in studies with non-human subjects, pointing out a species difference in this respect (Grace 1999; Green et al. 2004; Richards et al. 1997).

An important implication of eq. 5 is that if a reinforcer is delivered immediately (i.e. $d = 0$), the value of the reinforcer is proportional to its size. However, there is emerging evidence for a nonlinear relation between value and reinforcer size (Mazur and Biondi 2009; Rickard et al. 2009). This finding is taken into account by the following model.

1.3.2.2.   The Multiplicative Hyperbolic Model of Choice

This model is based on the idea that reinforcer value is determined by a series of hyperbolic functions each of which governs the influence of a particular characteristic of the reinforcer, such as size, delay and probability (Ho et al. 1999). It combines the following three postulates:

1) The value of an immediate reinforcer is an increasing hyperbolic function of its magnitude.
2) The value of a delayed reinforcer is a decreasing hyperbolic function of its delay.
3) The value of a probabilistic reinforcer is a decreasing hyperbolic function of the "odds-against" ratio, θ (where $\theta=[1/p]-1$)

$$V = \frac{1}{1+K \cdot d} \cdot \frac{1}{1+Q/q} \cdot \frac{1}{1+H \cdot \theta} \qquad (eq.6)$$

Thus, the value of the reinforcer is determined by independent hypebolic discount functions for size (q), delay (d) and the uncertainty (θ). Each discounting function is governed by its own stable discounting parameter: Q for the effect of reinforcer size upon value, K for the effect of delay and H for the effect of uncertainty. It is assumed that individual differences in these parameters influence preference in inter-temporal choice situations. For example, Fig. 1.4 shows theoretical delay discount functions for two organisms, one expressing a low value of K, and the other a high value of this parameter. In both cases, the subjects are confronted with a choice between a large

reinforcer of instantaneous value V_{iL}, and a smaller reinforcer of instantaneous value V_{iS}. The values of the two reinforcers, V_L and V_S, decline more steeply in the case of the "high-K" organism than in the case of the "low-K" organism, and if any fixed delay is imposed on the smaller reinforcer (d_S), the value of that reinforcer is less for the "high-K" organism than for the "low-K" organism. At the indifference point, where V_L is matched to V_S, the delay to the larger reinforcer (d_L) needs to be significantly shorter for the animal with the high K than for the animal with the low K. In other words, a relatively short delay imposed on the larger reinforcer may cause the subject with a high K to ignore the larger reinforcer in favour of the smaller more immediate one.
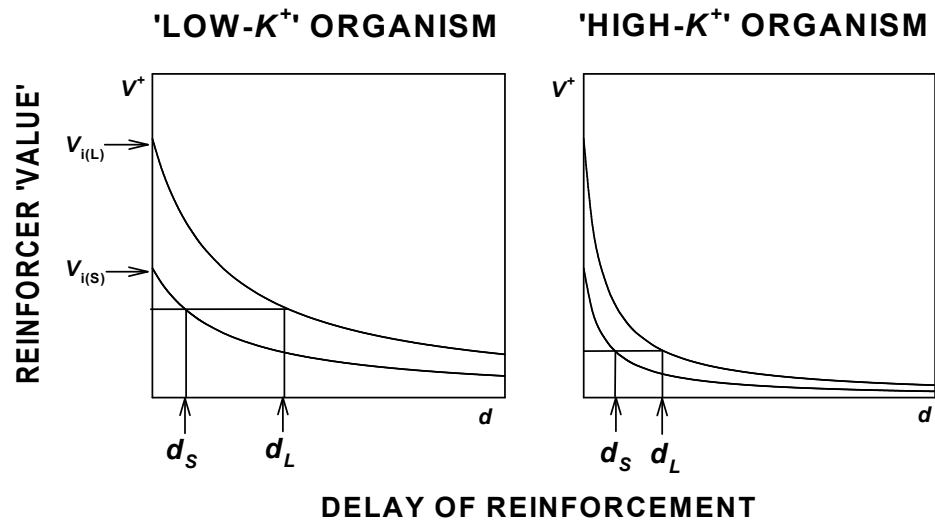
Fig. 1.4. Hyperbolic discounting functions for a large (L) and a small reinforcer (S) in organisms with low and high K. The left-hand graph shows the time discounting function for both reinforcers by a subject with a low K. The right-hand graph shows the corresponding discount functions for a subject with a high K. The horizontal line indicates the delay to the larger reinforcer which equates its value to that of the small reinforcer (figure reproduced from Ho et al. 1999).

As discussed above, in an inter-temporal choice situation a subject is faced with two alternatives, A and B, and the size, delay or probability is varied until the subject chooses the two alternatives equally often. In other words, the subject becomes indifferent between the two alternatives. It is assumed that under these conditions, the value of the two reinforcers is equal:

$$V_A = V_B \qquad \text{(eq. 7)}$$

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Assuming that uncertainty associated with the reinforcers is the same, the right-hand term of eq. 6 may be ignored. Substituting the remaining expressions of eq. 6 into eq. 7 yields the following relation:

$$\frac{1}{1+Q/q_A} \cdot \frac{1}{1+K \cdot d_A} = \frac{1}{1+Q/q_B} \cdot \frac{1}{1+K \cdot d_B} \qquad \text{(eq. 8)}$$

Rearranging the terms and solving for d_B yields the following linear relation between the indifference delay to the larger reinforcer, $d_{B(50)}$, and the delay to the smaller reinforcer, d_A (Ho et al. 1999):

$$d_{B(50)} = \frac{1}{K} \cdot \left[\frac{Q/q_A - Q/q_B}{1+Q/q_B} \right] + d_A \cdot \frac{1+Q/q_A}{1+Q/q_B} \qquad \text{(eq. 9)}$$

Eq. 9 can be used to examine the effect of neurobiological interventions on the processes of delay discounting (K) and sensitivity to reinforcer size (Q). For instance, if the sizes of two reinforcers are held constant and indifference delays are determined for a series of delays to reinforcer A, a change in the slope of eq. 9 induced by an intervention implies a change in Q. On the other hand, a change in the intercept without a concomitant change in the slope implies a change in K (Ho et al. 1999). The linear relation defined by eq. 9 is well supported by empirical data (Kheramin et al. 2002, 2003, 2004; Bezzina et al. 2007, 2008). For example, Fig. 1.5 shows data reported by Kheramin et al. (2002), comparing the inter-temporal choice behaviour of rats with a lesion on the orbital prefrontal cortex (OPFC) and sham-lesioned rats, analysed using eq. 9. The slope of the function was less steep for the sham-lesioned group than for the OPFC-lesioned group, whereas the intercept did not differ significantly between groups. The estimate of K derived from the group mean slopes and intercepts was higher for the OPFC-lesioned group. The authors concluded that the OPFC lesion produced two effects: 1) an increase in the rate of delay discounting and 2) an increased sensitivity to the reinforcer magnitude ratio.
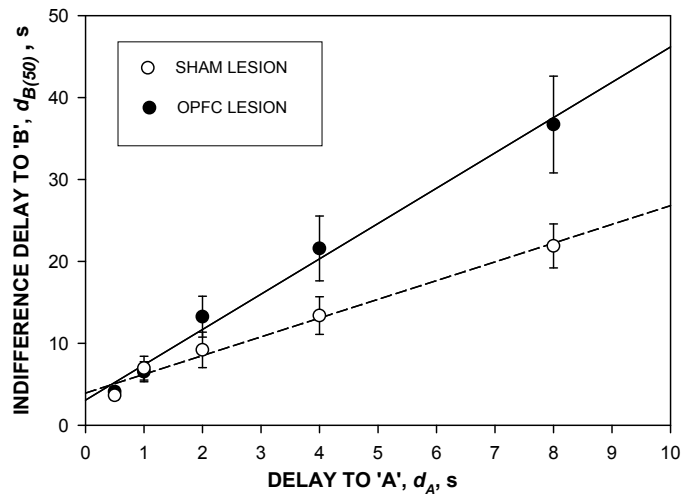
Fig 1.5. Linear indifference functions for OPFC-lesioned animals (filled symbols) and sham-lesioned rats (open symbols). Indifference delay for a larger reinforcer is plotted against delay to a smaller reinforcer. Note the steeper slope for the OPFC-lesioned group. Data from Kheramin et al. (2002)

1.3.2.3. Killeen's Trace Decay model of delay of reinforcement gradients

A recent review by Killeen (2011) argues that most theoretical treatments of delayed reinforcement seem to be founded on a tacit assumption that reinforcers operate 'backwards in time' to strengthen responses that have occurred before the moment of reinforcer delivery (as displayed graphically in the 'Ainslie diagram': Fig. 1.3). This is, at best, a convenient simplification which cannot be physiologically correct. A more appropriate way of conceptualizing the strengthening of operant responses by delayed reinforcement is to suppose that responses leave a 'memory trace' which decays in time; reinforcers act not on responses, but on their partially decayed traces. Killeen's model assumes (in common with most computational and psychological models of associative learning) that the process of decay follows an exponential time-course. When a reinforcer is delivered some time following the emission of an index response, the resulting strengthening effect on that index response is determined by competition between the partially decayed trace of the index response and the residual traces of other behavioural events that have occurred before and after the index response. One conceptual advantage of the model is that it does not require the presumption of a process of 'delay discounting', in the sense of delay-dependent reinforcer devaluation;

According to Killeen (2011), a delayed reinforcer does not have a lower 'value' than an immediate one, but its ability to strengthen an operant response is diminished due to the fact that it operates on a degraded memory trace of the response.

Killeen's (2011) model will not be examined in detail in this thesis, although its impications for some of the experimental results will be discussed in a later chapter. Killeen (2011) has shown that, in its present stage of development, the model can account for most of the phenomena that are accommodated by traditional hypberbolic models such as MHM, including preference reversal and linear indifference functions. However Killeen's model has not, as yet, generated any predictions that would allow experimental evaluation of its predictive validity in comparison with those of hyperbolic models.

1.4. Interval timing and inter-termporal choice behaviour – neurobiological substrates

Early research on interval timing focused on theories, and developed models that accounted for the results obtained with different timing procedures (see sections 1.3.1 and 1.3.2 above). However, research in the last two decades has emphasized the importance of the anatomical, physiological and pharmacological bases of interval timing. There is evidence that the basal ganglia, the prefrontal cortex and the cerebellum play important roles in interval timing and inter-temporal choice behaviour (see Cardinal 2006; Coull et al. 2010, for review). In addition, the ascending dopaminergic (DAergic) and 5-hydroxytryptaminergic (5-HTergic) pathways are believed to play crucial and differential roles in timing, as demonstrated by neuroimaging, neuropsychological and psychopharmacological investigations in humans, and lesion and pharmacological studies in animals. This section will briefly review the anatomy and general functioning of the neural substrates of interval timing; emphasis will be given to those structures and systems that are relevant to the experimental work described in later chapters.

1.4.1. *The central dopamine (DA) system*

1.4.1.1. Biochemistry

DA, along with noradrenaline (NA) and adrenaline, is a catecholamine neurotransmitter.

The DA molecule contains a catechol nucleus (a benzene ring with two adjacent hydroxyl substitutions) and an amine group on the side-chain. DA synthesis is considered to begin with tyrosine (an amino acid found in high concentrations in the plasma and brain). Tyrosine enters into the brain by an energy-dependent process in which it competes with other amino acids as a substrate for a transporter. Then the enzyme tyrosine hydroxylase (TH) converts the amino acid L-tyrosine into 3, 4-dihydroxyphenylalanine (L-DOPA). L-DOPA is subsequently metabolized by L-aromatic amino acid decarboxylase (AADC) to form DA (see Fig. 1.6).
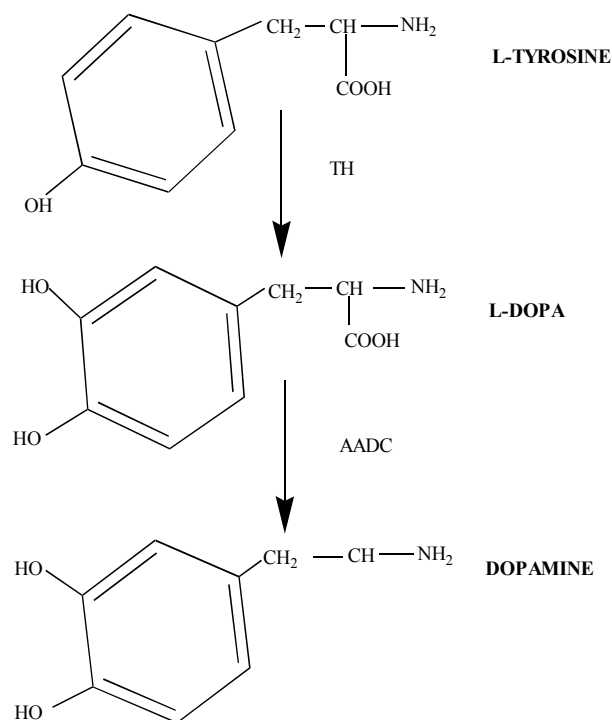
Fig. 1.6. Biosynthesis of dopamine (see text for abbreviations and details).

DA is stored in specialized subcellular vesicles. A specialized vesicular transporter is required for the dopamine formed in the cytosol to enter the vesicle. DA release operates through a calcium-dependent exocytotic process caused by the arrival of an action potential at the nerve terminal. In addition, DA can be released by a reversal of direction of transport through the membrane dopamine transporter (DAT) (an effect of some 'indirect agonists' such as amphetamine) (Butcher et al. 1988). DAergic function is regulated by the interaction of released catecholamine with specific DA *autoreceptors* located on the nerve terminal membrane. The autoreceptors are able to affect the synthesis and release of DA, and the firing rates of DAergic neurones (Cooper et al. 2002). Stimulation of *release-modulating* autoreceptors by synaptically released DA

inhibits further DA release. On the other hand, *synthesis-modulating* autoreceptors directly regulate DA synthesis (i.e. DA release decreases DA synthesis). Finally, *impulse-modulating* autoreceptors modulate the firing rates of DAergic neurones (Cooper et al. 2002).

Catecholamine catabolism involves two enzymes: monoamine oxidase (MAO), and catechol-O-methyltransferase (COMT). MAO transforms dopamine to a corresponding aldehyde which then can serve as a substrate for aldehyde dehydrogenase to produce 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC then diffuses out of the cells and can be either conjugated to glucuronides or transformed to homovanillic acid (HVA) by COMT. MAO is a mitochondrial enzyme located intraneuronally and in the liver. COMT is found only outside DAergic neurones. A proportion of the released dopamine is O-methylated by COMT to 3-methoxytyramine (3-MT) and then oxidized by MAO to form HVA (Mannisto et al. 1992; Wood and Altar 1988). Two forms of MAO have been identified: MAO_A which has high affinity for NA and 5-HT, but not DA, and MAO_B which has high affinity for *o*-phenylethylamines. COMT is a non-specific enzyme that transfers methyl groups from the donor S-adenosylmethionine to the *m*-hydroxy group of catechols. MAO and COMT inhibitors are used in the treatment of several neuropsychiatric disorders. For example, selegiline is a specific inhibitor of MAO_B that has been used as an initial treatment for Parkinson's disease. COMT inhibitors are also used in the treatment of Parkinson's by preventing the enzymatic inactivation of DOPA and prolonging its therapeutic action (Fernandez and Chen 2007).

DA inactivation is also a result of a reuptake process performed by two transporters: DAT and the noradrenaline transporter (NAT). Neither of these transporters is specific; both of them accumulate DA and NA. Interestingly, NAT has a higher affinity for DA than for NA. DAT is localized outside the synaptic junction, suggesting that the transporter accumulates and inactivates DA that has escaped from the synaptic cleft, and that diffusion is the initial process by which DA is removed from the synapse (Gainetdinov et al. 1998). DAT and NAT are important targets for many drugs. For instance, cocaine and amphetamine block transporters increasing extracellular levels of DA and NA (Cooper et al. 2002). Drugs that block catecholamine transporters and promote DA release (e.g. methyphenidate, amphetamine and atomoxetine) have been widely used to treat different psychiatric conditions such as Attention Deficit Hyperactivity Disorder (ADHD) (Brown et al. 2005).

1.4.1.2.   DA receptors

DA receptors are located in the pre- and post-synaptic cell, and are divided into two main classes: D_1-like receptors and D_2-like receptors. Both D_1-like receptors and D_2-like receptors are G-protein-coupled receptors (GPCRs); however, different G proteins and effectors are involved in their signalling pathways (Missale et al. 1988). D_1-like receptors include D_1 and D_5 receptors, and D_2-like receptors include D_2, D_3 and D_4 receptors. The main difference between D_1-like and D_2-like receptors is that D_1-like receptors activate adenylate cyclase whereas D_2-like receptors inhibit it. Additionally, D_1-like receptors have a larger molecular mass than D_2-like receptors (Lee et al. 2004).

Selective D_1-like receptor agonists include 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-hydrobromide (SKF-81297); selective antagonists include 8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SKF 83566). Selective antagonists of D_2-like receptors include 'conventional' antipsychotic drugs, including butyrophenones (e.g. haloperidol), and substituted benzamides (e.g. sulpiride). Selective D_2-like receptor agonists include quinpirole (Seeman and Van Tol 1994).

1.4.1.3.   Dopaminergic neuronal pathways

The principal DAergic neuronal systems that project to forebrain regions are as follows (Gray et al. 1995):

1) The nigrostriatal pathway arises from the substantia nigra (SN) and projects to the dorsal striatum. This pathway plays an important role in extrapyramidal motor control and degenerates in Parkinson's disease, causing the movement disorders characteristic of this condition.

2) The mesolimbic pathway arises from the ventral tegmental area (VTA) and projects to limbic areas (ventral striatum, nucleus accumbens, amygdala, olfactory tubercle, septal area and prefrontal cortex). This pathway is believed to play an important role in regulating the emotional and motivational functions that are subserved by limbic structures. There is also evidence that schizophrenia may be linked with dysfunction of the mesolimbic pathway (Gray et al. 1995).

3) The mesocortical pathway also arises from the VTA and projects to the frontal cortex. It is believed to play an important role in higher cognitive functions.

4) Additionally, DAergic neurones in the hypothalamus send axons to the median eminence to modulate the output of releasing factors by neuroendocrine cells.

1.4.1.4.   DA and interval timing and inter-temporal choice behaviour

Early studies showed that the indirect DA receptor agonist methamphetamine (a substance that enhances the release of DA, but has no specific agonist activity at the receptor itself), reduced the bisection point and displaced the psychometric function to the left in the interval bisection task, a retrospective timing task (Maricq and Church 1983; Maricq et al. 1981; Meck and Church 1983). However, more recent studies found that the closely related indirect agonist, d-amphetamine, had no effect on the bisection point, but increased the Weber fraction (McClure et al. 2005; Chiang et al. 2000a).

d-Amphetamine, methamphetamine and cocaine have been reported to have consistent effects in immediate timing tasks (Cheung et al. 2006; Matell et al. 2006; Meck 1996). These drugs reduce the indifference point (T_{50}) in FOPP, displacing the psychometric function to the left (Chiang et al. 2000a; Cheung et al. 2006). They also reduce the peak time and displace the Gaussian peak function to the left in the FIPP (Matell et al. 2006; Meck 1996).

It has been proposed that the effect of psychostimulants on these tasks is mediated by D_2 receptors (Meck 1996; 2005). However, several studies suggest the involvement of D_1 receptors (Cheung et al. 2006; Cheung et al. 2007a; Cheung et al. 2007c). For example, the D_1-like dopamine receptor agonist 6-chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine-hydrobromide (SKF-81297) reduced T_{50} in a dose-dependant manner in the FOPP procedure. This effect was reversed by the D_1-like receptor antagonist 8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol (SKF-83566), but not by the D_2-like receptor antagonist haloperidol (Cheung et al. 2007c). Similarly, d-amphetamine reduced T_{50} and this effect was reversed by SKF-83566, but not by haloperidol (Body et al. 2006; Cheung et al. 2006). Interestingly, quinpirole, a D_2-like dopamine receptor agonist, also reduced T_{50} in FOPP and the effect was attenuated by D_2-like dopamine receptor antagonists haloperidol, eticlopride and sulpiride, but not by SKF-83566 (Cheung et al. 2007b). Taken together, these results

suggest that the effects of d-amphetmaine and SKF-81297 on T_{50} in this kind of schedule are mediated by D_1-like receptors, while the effects seen with quinpirole involved D_2-like receptors (Cheung et al. 2007c). Moreover, it has also been reported that repeated administration of 2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT$_2$ receptor agonist, results in desensitization to DOI which confers cross-desensitization to d-amphetamine, but not to quinpirole in this same procedure (Cheung et al. 2007a). These observations suggest that two independent neuropharmacological mechanisms affect temporal differentiation, one involving the 5-HT$_{2A}$ and D_1 receptors, and other involving D_2 receptors (Body et al. 2006; Cheung et al. 2007b).

The results obtained employing the FIPP appear to differ from those obtained using FOPP. It has been generally found that D_2-like dopamine receptor antagonists induce a rightward displacement of the response rate function in the peak procedure (MacDonald and Meck 2005; Meck 1996). Nevertheless, some evidence suggests that D_2-like dopamine receptors play a minor role in performance on the FIPP when performance has reached a stable state (Cheng et al. 2007). It has been proposed that the differences between the pharmacological sensitivities of timing performance on FIPP and FOPP might reflect the involvement of different behavioural processes in these two procedures (Cheung et al. 2007c).

Performance on prospective timing (inter-temporal choice) procedures is also sensitive to DAergic manipulations. Cardinal et al. (2000) examined the effect of d-amphetamine on a progressive delay task when the delay to the larger reinforcer was signalled or unsignalled. The results showed that the indifference delay (d_{50}) was reduced when the delay to the larger reinforcer was unsignalled. On the other hand, d-amphetamine increased $d_{B(50)}$ when the delay to the larger reinforcer was signalled. The authors suggested that the cue-dependent effect of d-amphetamine was due to the potentiation of conditioned reinforcers which predicted the food delivery. They also suggested that d-amphetamine might affect the speed of a hypothetical internal clock hastening the within-session shift to the lever producing the immediate reward.

Richards et al. (1999) found that chronic administration of high doses of methamphetamine 22 hours before each test session promoted the preference for the smaller/sooner reward in an adjusting-magnitude schedule.

The effect of cocaine in an adjusting-delay schedule has also been examined. Cocaine-treated rats were found to have had shorter indifference delays (d_{50}) compared to untreated control animals (Logue et al. 1992).

These studies, along with others (for a review see Setlow et al. 2009), suggest that DA plays a key role in inter-temporal choice behaviour. However, it is unclear if the effects seen in these studies reflect changes in sensitivity to delay or size of reinforcement (Ho et al. 1999). As discussed earlier, most inter-temporal choice tasks entail reinforcers that differ with respect to both delay and size, making it difficult to determine whether the effect of an intervention on d_{50} reflects an effect of delay discounting or on sensitivity to reinforcer size. Quantitative analyses may be used to address this problem. For example, Kheramin et al. (2004) employed a "multi-point inter-temporal choice procedure" to study the effect of DA depletion of the OPFC, and concluded the lesion increased the rate of temporal discounting and also altered the rats' sensitivity to reinforcer magnitude.

1.4.2.   *The central 5-hydroxytryptamine (5-HT) system*

1.4.2.1.   Biochemistry

5-HT or serotonin is a monoamine with a two-ring indole structure that differentiates it from the single ring catecholamine (see Fig. 1.7). 5-HT is present throughout the body, especially in blood platelets and intestines. In the brain it is localized in the midline (raphe) nuclei of the pons and medulla. 5-HT is synthesized from tryptophan, which enters the 5-HTergic neurones, and is hydroxylated by tryptophan hydroxylase (TPH), giving rise to 5-hydroxytryptophan (5-HTP). Then 5-HTP is decarboxylated by L-aromatic amino acid decarboxylase (AADC) to form 5-HT (see Fig. 1.7).

Degradation by MAO is the primary metabolic pathway for 5-HT. 5-HT is mostly inactivated by MAO_A; however MAO_B inactivates 5-HT in the blood platelets (Sandler et al. 1981). Metabolism by MAO occurs in the cytosol of the neurone. The product of 5-HT degradation by MAO in almost all brain regions is 5-hydroxyindoleacetic acid (5HIAA) which is excreted in the urine (Sandler et al. 1981). An exception is the pineal gland, in which 5-HT is converted to melatonin (Mohammad-Zadeh et al. 2008).

5-HT is released upon neuronal depolarization into the synaptic cleft. Then it can bind to postsynaptic 5-HT receptors or 5-HT autoreceptors on the presynaptic membrane. Autoreceptors activation acts as a negative feedback against further release of 5-HT (Cerrito and Raiteri 1979).

The 5-HT transporter (SERT) is located in the presynaptic membrane and removes 5-HT from the synaptic cleft. 5-HT is transported to the presynaptic cell ('re-uptake'), recycled and stored again in vesicles. Selective 5-HT reuptake inhibitors (SSRI), which bind specifically to SERT increasing the availability of 5-HT at the synaptic junction for receptor binding, are widely used in the treatment of depression.

L-TRYPTOPHAN

TPH

5-HTP
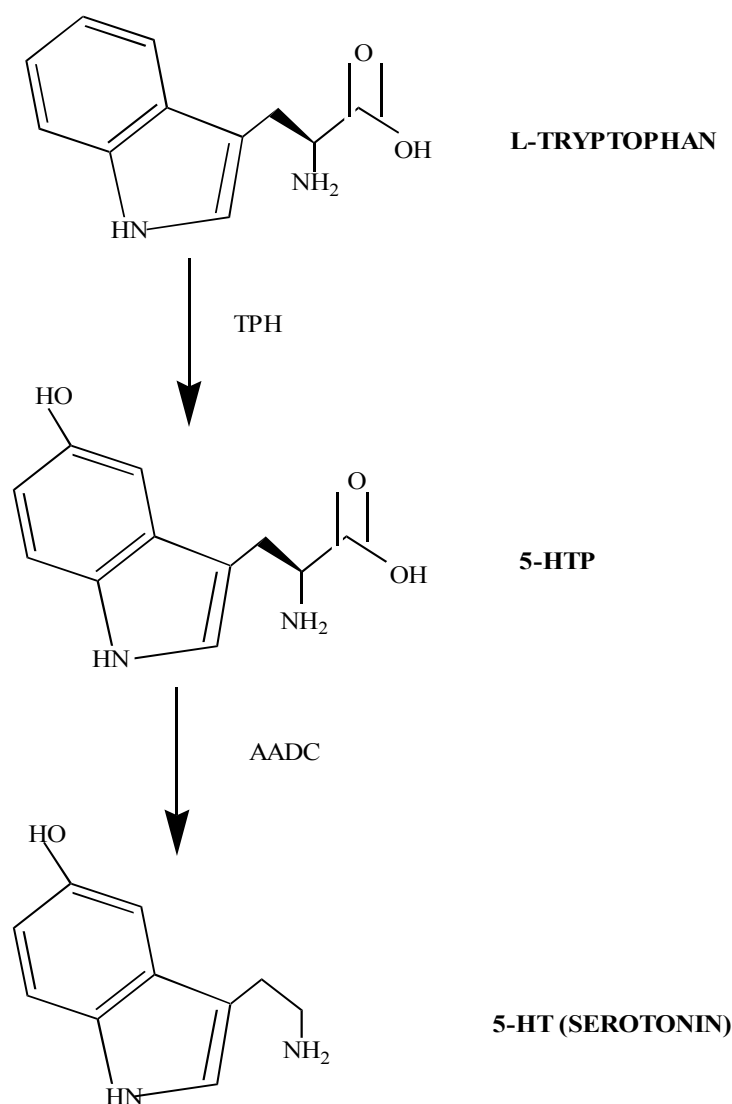
Fig. 1.7. Biosynthesis of 5-HT (see text for abbreviations and details).

AADC

5-HT (SEROTONIN)

1.4.2.2.   5-HT receptors

The task of unravelling the complexites of 5-HT's putative role in a variety of physiological and psychological processes arises, in part, from the imposing number of 5-HT receptors. Radioligand binding studies have enabled the characterization of seven

types of 5-HT receptor: $5\text{-}HT_1$, $5\text{-}HT_2$, $5\text{-}HT_3$, $5\text{-}HT_4$, $5\text{-}ht_5$, $5\text{-}HT_6$ and $5\text{-}HT_7$, many of which are in fact families of closely related receptor subtypes (see below). All of them are members of the G-protein coupled receptor (GPCR) 'super-family', except the $5\text{-}HT_3$ receptor, which is member of the ligand-gated ion channel 'super-family' (Mohammad-Zadeh et al. 2008). By convention, 5-HT receptors for which a physiological effector has been identified are designated $5\text{-}HT_n$, whereas those without a recognised effector are designated $5\text{-}ht_n$.

The $5\text{-}HT_1$ receptor family consists of five separate gene products ($5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1D}$, $5\text{-}ht_{1e}$ and $5\text{-}HT_{1F}$ receptors. All of these receptors inhibit adenylyl cyclase and thereby reduce cyclic adenosine monophosphate (cAMP) levels. Several of these receptors are also known to act as autoreceptors that regulate the excitability of 5-HTergic neurones and 5-HT release; for example, $5\text{-}HT_{1A}$ receptors are known to act as somatodendritic autoreceptors on 5-HTergic neurones as well serving a conventional post-synaptic receptor role in some structures (Stamford et al. 2000). $5\text{-}HT_1$ receptors have been implicated in a wide variety of functions. For instance, it is well known that $5\text{-}HT_{1A}$ receptor partial agonists are clinically useful anxiolytic drugs (Ramboz et al. 1998), and that $5\text{-}HT_{1F}$ receptor agonists exhibit anti-migraine effects through their action on the trigeminal nucleus (Shepheard et al. 1999).

The $5\text{-}HT_2$ receptor family consists of three receptor subtypes: $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$ and $5\text{-}HT_{2C}$. These receptors exhibit complex pharmacological profiles, operating through multiple signal transduction pathways. They have been implicated in a number of neuropsychiatric conditions (Barnes and Neumaier 2011).

The $5\text{-}HT_3$ receptor complex is a non-selective ion channel that mediates fast synaptic depolarizing neurotransmission. The $5\text{-}HT_3$ receptor is mainly located in the brainstem nuclei, including the chemoreceptor trigger zone. $5\text{-}HT_3$ receptor antagonists (e.g. ondansetron, tropisetron and palnosetron) have been widely used for their therapeutic effect in alleviating the nausea and vomiting associated with cancer chemo- and radiotherapy (Rojas et al. 2010; Rubenstein 2004). Activation of $5\text{-}HT_3$ receptors also modulates the release of various neurotransmitters including DA, γ-aminobutyric acid (GABA) and acetylcholine (Barnes et al. 1989; De Deurwaerdere et al. 1998; Diez-Ariza et al. 2002).

The $5\text{-}HT_4$ receptor is located in the basal ganglia including the globus pallidus, caudate nucleus, putamen, and nucleus accumbens, and also in the substantia nigra, hippocampus and cortex. It is positively coupled to adenylyl cyclase causing neuronal

excitation. This receptor is believed to have a role in learning and memory (Barnes and Neumaier 2011). In addition, 5-HT$_4$ receptor antagonists exhibit anxiolytic properties (Bockaert et al. 2004).

Although the 5-ht$_5$ receptor was discovered 20 years ago, its role remains poorly understood. However, two gene products are known: 5-ht$_{5a}$ and 5-ht$_{5b}$. There is evidence that 5-ht$_{5a}$ receptors inhibit adenylyl cyclase; they are located in the hypothalamus, raphe nuclei and locus coeruleus. Additionally it has been shown that 5-ht$_{5a}$ receptor knockout mice display enhanced exploratory behaviour in response to a novel environment (Grailhe et al. 1999).

5-HT$_6$ receptors activate adenylyl cyclase, and are expressed mainly in the striatum and hippocampus. Recent studies indicated that the 5-HT$_6$ receptor may be a fruitful target for the development of cognitive enhancing and weight reducing treatments (Mitchell and Neumaier 2005).

5HT$_7$ receptors also activate adenylyl cyclase. They are widely spread in the brain. Several atypical antipsychotic and antidepressant drugs have high affinity for this receptor. This receptor has also been implicated in circadian rhythmicity and vasodilatation (Barnes and Neumaier 2011).

1.4.2.3.   5-HTergic neuronal pathways

5-HTergic neuronal cell bodies are grouped in nine nuclei within the raphe (midline) area in the brainstem. These nuclei are divided into superior and inferior groups (Jacobs and Azmitia 1992). The inferior group consists of B1-B4 nuclei and is located between the caudal pons and the cervical spinal cord. The superior group is formed by four main nuclei: median raphe nucleus (MRN or B5 and B8), dorsal raphe nucleus (DRN or B6 and B7), caudal linear nucleus (CLN or B8), and area B9, and is located between the midbrain and the pons (Dahlstrom and Fuxe 1964).

Six main ascending 5-HTergic pathways from the midbrain raphe nuclei project to several areas including the hippocampus, the suprachiasmatic nucleus of the hypothalamus, the substantia nigra pars compacta, the amygdala, and the periventricular nucleus of the thalamus (Imai et al. 1986).

There is considerable overlap between the projection regions of the DRN and the MRN. 5-HT-containing neurones originating in the DRN project to the dorsal striatum, substantia nigra, VTA, amygdala and various parts of the cerebral cortex (Imai et al.

1986; Leger et al. 2001). 5-HTergic neurones projecting from the MRN innervate the parietal, occipital and frontal cortices, as well as the dorsal hippocampus and septum and amygdala (Kosofsky and Molliver 1987).

1.4.2.4.   5-HT and interval timing and inter-temporal choice behaviour

There is considerable amount of evidence that suggest an involvement of 5-HT in interval timing behaviour. However, most studies suggest that disruption of 5-HTergic function has qualitatively different effects on performance in different kinds of timing schedules (see Ho et al. 2002 for a review). For instance, in the case of the interval bisection task (a retrospective timing task) it has been shown that rats with lesions of their 5-HTergic pathways performing in the interval bisection task (a retrospective timing task), showed a leftward displacement of the psychophysical function and a reduction of T_{50}, but no difference in the Weber fraction compared to the sham-lesioned rats. However, the effect of the lesion on T_{50} was explained as a facilitatory effect of the lesion on the animals' tendency to move from the lever associated with the short stimulus to the lever associated with the long stimulus, since the introduction of a central nose-poke requirement between stimulus offset and the opportunity to make a lever-press response greatly attenuated the effect of the lesion on T_{50} (Ho et al. 1995). In support of this notion, Graham et al. (1994) demonstrated that when rats were trained to discriminate stimuli in the millisecond range (200 vs. 800 ms), which effectly precluded movement between the levers during stimulus presentation, the lesion had no effect on T_{50}.

In addition, it has been shown that the $5-HT_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) increases the Weber fraction without altering T_{50} in the interval bisection task and the DTTP (Body et al. 2002a; Chiang et al. 2000b). This effect is mediated by postsynaptic receptor populations, since it is not influenced by destruction of the ascending 5-HTergic pathway (Body et al. 2002a). Furthermore, the effect of quipazine, a non-selective 5-HT receptor agonist with high affinity for both $5-HT_3$ and $5-HT_{2A}$ receptors, produced a rightward displacement and flattening of the psychometric function, increasing T_{50} and the Weber fraction, in the performance of DTPP. This effect was not affected by co-administration of the selective $5-HT_3$ receptor antagonist topanyl 3,5-dichlorobenzoate (MDL-72222), but was completely abolished by co-administration of ketanserin, a $5-HT_{2A}$ receptor antagonist (Asgari et al. 2005). In

addition, the 5-HT$_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) also disrupted temporal discrimination and this effect was reversed by ketaserin and the highly selective 5-HT$_{2A}$ receptor antagonist ($\pm$)2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol] (MDL-100907) (Asgari et al. 2006a).

Taken together, these studies suggest that 5-HT$_3$ receptors do not influence temporal discrimination, and the effect described above is mainly mediated by 5-HT$_{2A}$ and 5-HT$_{1A}$ receptors (Asgari et al. 2006a; Asgari et al. 2005a).

The role of 5-HT has also been studied in immediate timing schedules. It has been shown that the chronic 5-HT depletion induced by intra-raphe injection of 5,7-dihydroxytryptamine does not alter the T$_{50}$ in FOPP. Interestingly, the lesion consistently increases the rate of switching between the two levers (Chiang et al. 1999) a phenomenon also seen in concurrent variable-interval schedules that do not entail explicit timing (Al-Ruwaitea et al. 1999b). The effect of 5-HTergic pharmacological manipulations has also been investigated in the performance of FOPP and FIPP. For instance, it has been demonstrated that acute treatment with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT reduces T$_{50}$ and the peak time, and this effect is blocked by the 5-HT$_{1A}$ receptor antagonist N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridyl) cyclohexanecarboxamide trihydrochloride (WAY-100635) (Asgari et al. 2006b; Body et al. 2002b). Equally, the 5-HT$_2$ receptor agonist DOI reduces T$_{50}$ and the peak time, and this effect is reversed by the 5-HT$_{2A}$ receptor antagonist ketanserin (Asgari et al. 2006b; Body et al. 2003). In addition, fenfluramine, a 5-HT releasing agent, reduces T$_{50}$ in FOPP and this effect is blocked by ketaserin, but not by WAY-100635 (Body et al. 2004). These effects were mainly mediated by postsynaptic 5-HT$_{2A}$ receptors since lesions of the 5-HTergic pathway abolished the effect of fenfluramine but failed to alter the effect of DOI or 8-OH-DPAT on timing performance (Body et al. 2001; Body et al. 2004). Similarly to the results obtained with temporal discrimination (Asgari et al. 2005a), 5-HT$_3$ receptors seem not to be involved in temporal differentiation and the effects of both DOI and quipazine on T$_{50}$ are mediated by 5-HT$_{2A}$ receptors (Body et al. 2005).

In addition, Body et al. (2009) provided some evidence for an interaction between the DAergic and 5-HTergic systems in the performance of FOPP. These authors demonstrated that d-amphetamine reduced T$_{50}$ only in intact animals, and had no effect in 5-HT depleted animals. They suggested that 5-HT may play a permissive role in the release of DA through 5-HT$_{2A}$ receptors.

Destruction of the 5-HT pathways also seems to affect performance on IRT schedules (Fletcher 1995; Wogar et al. 1992b; 1993a) by impeding the acquisition of temporal differentiation and disrupting temporal differentiation in well-trained animals. This effect reflects a more general deficit of 5-HT-lesioned animals' ability to learn to inhibit operant responses (Soubrie 1986). Interestingly, 5-HT-lesioned animals also exhibit a slower acquisition of the reduction of response rate in the latter segment of probe trials in FIPP (Morrissey et al. 1994; Al-Ruwaitea et al. 1997).

There is an increasing amount of evidence for an involvement of 5-HT in inter-temporal choice behaviour. Some studies have reported that the destruction of the ascending 5-HTergic pathways promoted the choice of the smaller and earlier of two reinforcers in discrete-trials lever-pressing tasks (Al-Ruwaitea et al. 1999a; Mobini et al. 2000; Wogar et al. 1993b).

In addition, there is some evidence that acute and chronic treatments with drugs that interact with the 5-HTergic system also affect inter-temporal choice, although there have also been reports of contradictory data. It has been reported that the 5-HT releasing agent d-fenfluramine promoted choice of the larger and more delayed of two reinforcers, whereas the $5-HT_{1A}$ receptor agonist 8-OH-DPAT had a biphasic effect, with lower doses increasing choice of the immediate reinforcer, and higher doses increasing choice of the delayed reinforcer (Poulos et al. 1996). Evenden and Ryan (1999) reported that 8-OH-DPAT reduced preference for the large reinforcer at the beginning of the session when the delay was short, and increased the preference at the end of the session when the delay was longest in an delayed reinforcement procedure (progressive delay schedule). Moreover, the $5-HT_2$ receptor agonist DOI increased preference for the smaller and more immediate of two reinforcers, whereas selective antagonists of $5-HT_{1A}$, $5-HT_2$ and $5-HT_3$ receptors had no effect. Wolff and Leander (2002) reported that chronic, but not acute, administration of the SSRIs fluoxetine, citalopram and paroxetine increased d_{B50} in pigeons performing on an adjusting-delay schedule, whereas neither acute nor chronic administration of the $5-HT_{1A}$ receptor agonist 8-OH-DPAT had any effect on d_{B50}. In a more recent study, Liu et al. (2004) reported the effects of acute and chronic treatment with buspirone, a non-selective $5-HT_{1A}$ agonist, on choice behaviour using a progressive delay schedule similar to the one used by Cardinal et al. (2000). The results showed that acute treatment with buspirone in animals without a history of chronic exposure to the drug increased the choice of the small immediate reinforcer, whereas chronic treatment had the contrary effect, increasing the choice of the larger delayed reinforcer. This latter

effect was blocked by the 5-HT$_{1A}$ receptor antagonist, WAY-100635. In vivo microdialysis in the ventral hippocampus, nucleus accumbens and medial prefrontal cortex indicated that acute buspirone reduced extracellular levels of 5-HT in the hippocampus irrespective of the chronicity of the treatment. On the other hand, acute treatment with buspirone reduced 5-HT levels in the prefrontal cortex. The authors suggested that chronic treatment with buspirone desensitized 5-HT$_{1A}$ autoreceptors and enhanced the activation of post-synaptic 5-HT$_{1A}$ receptors, leading to an increase in preference for the larger delayed reinforcer.

Interestingly, it has also been reported that the DAergic and 5-HTergic systems interact in the regulation of inter-temporal choice behaviour, as has been shown in retrospective and immediate timing behaviour (Cheung et al. 2007a; Cheung et al. 2007b; c). Robbins et al. (2010) reported that administration of d-amphetamine reduced impulsive choice and increased the choice for the larger delayed reinforcer in intact animals. This effect was blocked by the destruction of the 5-HTergic pathways. Moreover, the effects of d-amphetamine were blocked by the D$_1$/D$_2$ receptor antagonist α-flupenthixol, but only in the 5-HT-depleted animals, suggesting an interaction between the DAergic and 5-HTergic systems. In support of this notion, it has been demonstrated that mesolimbic DA depletion induced by intra-accumbens injection of 6-hydroxydopamine failed to block the "anti-impulsive" effect of d-amphetamine, and that the 5-HT$_{1A}$ receptor antagonist WAY-100635 successfully enhanced the effect of d-amphetamine in reducing impulsive choice (Winstanley et al. 2006).

Taking this evidence together (see Pattij and Vanderschuren 2008 for a review), it is clear that 5-HT plays a key role in the regulation of inter-temporal choice behaviour; however the exact mechanisms remain unclear. Pattij and Vanderschuren (2008) suggest that this could be due to "the complexity of the 5-HT system itself, the lack of availability of selective ligands for 5-HT receptor subtypes and interaction with other neurotransmitters." Furthermore, most of the studies reported to date fail to clarify whether the effects of these manipulations reflect changes in the animals' sensitivity of delay or sensitivity to reinforcer size. In an attempt to disentangle these two variables, Mobini et al. (2000) employed a "multi-point inter-temporal choice procedure" based on the multiplicative hyperbolic model of inter-temporal choice (Ho et al. 1999) (see section 1.3.2.2.) to examine the effect of central 5-HT depletion. The results showed that the lesion produced a parallel displacement of the linear indifference function, significantly reducing d$_{B50}$ and increasing the parameter of sensitivity to delay, *K*.

1.4.3. *Other neurotransmitter systems and interval timing*

Acetylcholine (ACh). ACh has also been implicated in interval timing. Meck and colleagues have proposed that drugs that target the central cholinergic pathways affect the "memory pattern" of interval timing performance (Meck 1996). This is evidenced by the gradual displacement of the timing functions that is observed only after several treatment sessions (Coull et al. 2010). This gradual change is predicted by SET, because the representations of the timed interval stored in reference memory are progressively overwritten with values generated with the new memory storage speed (Meck 1996). According to this interpretation, a chronic increase in the level of cholinergic function should produce a leftward shift of the timing function, whereas a decrease in the level of cholinergic function should produce a progressive rightward shift of the timing function (Meck 1996). It has been demonstrated that the inhibitor of choline acetyltransferase (ChAT), BW813U (2[(E)-2-(3-chlorophenyl)ethenyl]-4,4,6-trimethyl-5,6-dihydro-1,3-oxazine), increased the peak time in FIPP. Furthermore, when a 5-s gap was introduced into peak interval trials ('gap procedure': see section 1.2.2.1), control rats summed the signal duration before and after the gap, whereas rats treated with BW813U exhibited no retention of the signal duration prior the gap (Meck 1996). It has also been reported that repeated administration of pyrithiamine, an antimetabolite of thiamine, affects the peak time in a similar way to BW813U (Meck and Angell 1992). In addition, studies have been conducted to determine the relationship between ACh, age and timing. It has been shown that when rats aged between 10-13 months old received injections of arginine vasopressin (AVP), the age-related increase in the remembered time of reinforcement (rightward displacement of the peak time) was prevented (Meck et al. 1986). Moreover, chronic administration of physostigmine, a reversible cholinesterase inhibitor, produced a leftward shift of the timing function, whereas atropine, a muscarinic ACh receptor antagonist, produced a rightward shift of the psychophysical timing function in an interval bisection task (Meck 1983) and FIPP (Meck and Church 1987), and scopolamine, another muscarinic receptor antagonist, affected "short-term memory" in a delayed conditional time discrimination task (Berz et al. 1992).

In contrast to these results, some studies have failed to find an effect of cholinergic treatments on interval timing. Odum (2002) trained pigeons in a schedule similar to the interval bisection procedure. In one component of the schedule, when the

response key was blue, food was available after the houselight was presented for 5 s. In the other component, when the response key was green, food was available after the houselight was presented for 30 s. Food was not available for intermediate durations (10, 15, 20 and 25 s). The animals then received acute and chronic treatment with atropine or physostigmine. The results showed that acute administration of these drugs had little effect on the timing function and T_{50}, this being consistent with Meck's (1996) prediction based on SET. However, contrary to Meck's (1996) prediction there were no large or consistent effects on T_{50} when these drugs were administrated chronically, In support of this finding, Bourger et al (2004) reported that subchronic administration of metrifonate, a cholinesterase inhibitor, had no effect on the peak time or response rate distribution during performance of FIPP.

Noradrenaline. There has been no systematic exploration of the possible role of noradrenaline in interval timing. Al-Zahrani et al. (1998) observed that rats whose dorsal ascending noradrenergic pathways had been lesioned showed leftward-displaced psychometric functions in the FOPP, compared to sham-lesioned rats. This effect appeared to be a consequence of reduced responding on lever 'A' in the first half of the trial. The authors speculated that the lesion reduced the activating effect of lever presentation at the start of the trial.

Several studies have examined a possible role of noradrenergic mechanisms in inter-temporal choice. For instance, it has been demonstrated that the administration of atomoxetine, a noradreline re-uptake inhibitor, increased the preference for the large delayed reinforcer in a delayed reinforcement task (Robinson et al. 2008). Studies with human subjects have suggested that the noradrenergic system encodes features of uncertainty in a given context (Yu and Dayan, 2005), and it has been reported that when subjects are given the choice of simultaneous gambles differing in the magnitude of possible gains, losses, and probability of delivery, the administration of propanolol, a beta-adrenoceptor blocker, reduced the discrimination between the magnitude of losses when the probability of losing was high (Rogers et al. 2004).

Opiates. The effect of morphine, an opiate analgesic drug, has also been investigated in FIPP performance. It was reported that there was no effect on timing performance when morphine was administered alone, but when it was administrated with methamphetamine it produced a leftward shift in the peak time. This effect was

antagonized by the opiate receptor antagonist naloxone. On the other hand, morphine had no effect when administered together with haloperidol (Meck 1996). It has been suggested that opiate administration inhibits GABAergic neurotransmission and enhances DA activity in the nigro-striatal system (Meck 1996).

Glutamate. Fewer studies have been devoted to the role of the glutamate system and interval timing. Welzl et al (1991) reported that chronic intraventricular infusion of the competitive N-methyl-D-aspartate (NMDA) receptor antagonist (2*R*)-amino-5-phosphonovaleric acid (AP-5) in rats prevented the acquisition of accurate performance on an IRT schedule. In addition, rats treated with phencyclidine tended to show 'premature responding' in this schedule, pressing the lever earlier than control rats. These same authors studied the effects of the competitive NMDA receptor antagonist selfotel (CGS-19755) and the non-competitive antagonist dizocilpine (MK-801). Rats were trained on a delayed time discrimination procedure in which they were required to discriminate between a 2 and an 8 s light stimuli. The termination of either stimulus was followed by no delay or a delay of 2, 4, or 8 s. A nose-poke at the termination of the delay resulted in insertion of both levers. The results demonstrated that performance efficiency was affected by higher doses of selfotel and dizocilpine, and resulted in a decrement of the number of nose-pokes. The authors suggested that the effects of NMDA antagonists were due to the drug interfering with hippocampal and neocortical mechanisms which play a role in memory (Welzl et al. 1991).

Floresco et al (2008) investigated the role of glutamate in a delay discounting task. Administration of the non-competitive NMDA antagonist ketamine promoted the choice of the small immediate reinforcer and increased the apparent rate of delay discounting. Conversely, the metabotropic glutamate 1 receptor antagonist 3-ethyl-2-methylquinolin-6-yl-(4-methoxycyclohexyl)methanone methanesulfonate (EMQMCM), increased the preference for the larger reinforcer at longer delays in a delay discounting task similar to the one described by Cardinal et al. (2000) (Sukhotina et al. 2008). The discrepancy of these results might be explained by the complex pharmacology of ketamine, including its action at 5-HT$_2$ receptors and D$_2$ receptors (see Sukhotina et al. 2008).

Cannabinoids. Δ^9-Tetrahydrorocannabinol (THC) has also been shown to have an effect on time perception. In an early study by Altman (1977), rats were trained on an IRT schedule with a limited hold of 1 s (differential reinforcement of response duration –

DRRD). Administration of THC resulted in a premature release of the lever and increased the proportion of short IRTs. In a more recent study, Han and Robinson (2001) demonstrate that systemic administration of the cannabinoid receptor agonists WIN-55,212-2 ((R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone) and THC, and the cannabinoid receptor antagonist rimonabant (SR 141716A), affected the performance of rats on FIPP. Both agonists shortened the modal response time, whereas the antagonist had the contrary effect by increasing peak time.

1.4.4.   *Neuroanatomical structures implicated in interval timing and inter-temporal choice*

1.4.4.1.   The orbital prefrontal cortex (OPFC)

1.4.4.1.1.   Structure and functions of the OPFC

The OPFC consists of a large heterogeneous region that covers the ventromedial and ventral surfaces of the frontal lobes. It receives afferent projections from the mediodorsal nucleus of the thalamus, several regions of the cerebral cortex, and the monoaminergic nuclei of the brainstem (Fuster 1997). Its efferent projections include an excitatory projection to the ventral striatum. Connections between the OPFC, ventral striatum and thalamus constitute one of the major cortico-striato-thalamo-cortical (CSTC) circuits (see below, section 1.4.4.2).

Although the OPFC shows variation across species in the amount of granular and agranular cortex, similarities in the positions and connections of the orbital and medial areas indicates that the OPFC is comparable across species (Ongur and Price 2000; Uylings and van Eden 1990). The rat's OPFC is composed entirely of agranular cortex (Ongur and Price 2000; Ray and Price 1992).

Similar to monkeys and humans, visual, auditory, somatosensory, olfactory and gustatory inputs to the OPFC have been identified in rats (Conde et al. 1995; Reep et al. 1996). Studies performed with monkeys and rats have shown that cells in the OPFC respond to multisensory stimulation (Rolls et al. 1999), and these responses are related to the affective value of the stimulus and its sensory properties (Schoenbaum and Eichenbaum 1995). Schultz et al. (1998) have demonstrated that the OPFC is part of a

system along with limbic structures, the ventral striatum, and the midbrain dopamine cells that respond to reward associations of sensory stimuli. The OPFC has also been linked to visceral functions and to the autonomic nervous system via its direct connections with the hypothalamus and periaqueductal grey and with higher structures such as the amygdala and insular cortex which play a key role in autonomic regulation (Kaada et al. 1949; Neafsey 1990).

The OPFC contains the secondary taste cortex, and the secondary and tertiary olfactory cortical areas in which the reward value of taste and odour are represented (Rolls 2004). Furthermore the OPFC receives information about the sight of objects from the temporal lobe and the visual areas of the occipital cortex, which may enable it to encode the reward association of visual stimuli (Rolls 2004). Additionally, it has been proposed that other groups of neurones in the OPFC are part of a mechanism that enables very rapid reversal of behaviour by stimulus-reinforcement association re-learning when the association of stimuli with reinforcers is altered or reversed (Rolls 2004). In support of this notion, it has been reported that damage to the OPFC impairs learning and reversal of stimulus-reinforcement associations (Bohn et al. 2003a; b).

The OPFC also plays a role in social and emotional behaviour. There is evidence that monkeys with lesions in the OPFC show aggressive and aversive behaviour to threatening stimuli (Butter and Snyder 1972). Moreover, Butter and Snyder (1972) reported that animals with OPFC lesions lose their position in a social hierarchy. In the case of humans, Damasio et al. (1990) demonstrated that patients with OPFC damage gave deficient visceral responses to disturbing or exciting images and this was correlated with their inability to make appropriate decisions.

Such findings are consistent with the well-known neuropsychiatric consequences of damage to the OPFC, which include emotional and behavioural disinhibition and 'impulsive' decision making (Lishman 1998).

1.4.4.1.2.   Role of the OPFC in interval timing and inter-temporal choice behaviour

Several lines of research have provided evidence for the involvement of the OPFC in interval timing behaviour. For instance, brain imaging studies have demonstrated enhanced blood oxygenation level-dependent signals during the performance of several timing tasks (Hinton and Meck 2004; Lewis and Miall 2003; Pfeuty et al. 2005; Rubia et al. 2003). In addition, Mangels et al. (1998) reported that patients with OPFC damage

were impaired in a time discrimination task and showed a biased T_{50}. It has also been shown that repetitive transcranial stimulation of the right dorsolateral prefrontal cortex (rDLPFC) in humans produce underestimation of time intervals (Jones et al. 2004; Koch et al. 2009).

Research with animals has yielded evidence that is broadly consistent with a role for the prefrontal cortex in interval timing, although there is a paucity of evidence specifically linking the OPFC (as opposed to the prefrontal cortex in general, including the medial [MPFC] and dorsolateral [DLPFC] prefrontal cortices) to timing behaviour. Lesions of the prefrontal-caudate system impaired retention in a time discrimination task with cats (Rosenkilde and Divac 1976). Dietrich and Allen (1998) also reported that aspiration lesions of the MPFC slowed the acquisition of timing behaviour in FIPP and flattened the peak function in rats. Moreover, it has been reported that extensive lesions of the frontal cortex produced a rightward shift in the peak time (Meck 2006a; Olton 1989), and blocked the ability of dopaminergic drugs to alter the timing function (Meck 2006). In a more recent study, Kim et al. (2009) demonstrated that bilateral infusions of muscimol (a $GABA_A$ receptor agonist) into the MPFC impaired rats' performance in an interval bisection procedure. Positron emission tomography studies with monkeys indicated changes in the regional cerebral blood flow in the DLPFC during the performance of a time discrimination task (Onoe et al. 2001).

The OPFC has also been linked to inter-temporal choice behaviour. Specifically, it has been reported that lesions of the OPFC induced impulsive choice in humans and animals (Bechara et al. 1994; Bezzina et al. 2008a; Damasio 1995; Mobini et al. 2002). Impulsive choice has been defined as the tendency to choose small short-term gains in preference to larger delayed gains or larger delayed penalties in preference to smaller immediate penalties (see section 1.3.2.).

Mobini et al. (2002) reported that lesions of the OPFC induced by injections of the excitotoxin quinolinate promoted choice of the small immediate reinforcer, shortening the indifference delay, d_{B50}, in a progressive delay schedule where delay was varied across phases of the experiment. However, results apparently in conflict with Mobini et al.'s (2002) findings were reported by Winstanley et al. (2004), who observed an increased preference for the larger, more delayed reinforcer in OPFC-lesioned rats. Possible reasons for this discrepancy have been discussed in detail by Winstanley (2010), who concluded that multiple factors may be relevant, including the presence or

absence of intra-delay cues, the length of training under the inter-temporal choice task and the use of within-session or between-phase changes in delay.

Data obtained by Kheramin et al (2002) suggest that OPFC lesions may either promote or suppress "impulsive choice", depending on the relative delays and sizes of two reinforcers. An increasing tendency for OPFC-lesioned rats to show enhanced preference for the larger, more delayed reinforcer was seen as a function of the delay to the smaller, earlier reinforcer (d_A). Kheramin et al. (2002) suggested, based on the multiplicative hyperbolic model of inter-temporal choice (MHM: Ho et al. 1999), that the effect of the OPFC lesion reflected two actions: an increase in the rate of delay disounting (K), and an increase in the animals' sensitivity to the ratio of the sizes of the two reinforcers (Q).

OPFC-lesioned rats exposed to a progressive delay schedule where the probability and delay to reinforcer are both varied tend to be more risk-averse than normal rats when choosing between small certain reinforcers and large uncertain reinforcers (Kheramin et al. 2003). In other words, the OPFC lesion increased the value of the odds-discounting parameter (H) as well as that of the delay-discounting parameter, K (see section 1.3.2.2). The increases in these two discounting parameters may exert opposite influences on choice depending on the particular values of each feature of each reinforcer (Kheramin et al. 2003).

Kheramin et al. (2004) also examined the effects of dopamine depletion in the OPFC in a progressive delay schedule, and demonstrated that the value of $d_{B(50)}$ of the dopamine-depleted group was significantly longer than that of the sham-lesioned group under conditions of longer but not shorter delays to the smaller reinforcer (d_A). These authors suggested that the lesion induced faster delay discounting, but this effect was offset by the effect of lesion on the rats' sensitivity to the sizes of two reinforcers, the total effect being an apparent increase in "tolerance to delay" when the smaller reinforcer was subject to a delay.

The suggestion that OPFC lesions alter rats' sensitivity to the relative sizes of reinforcers has been supported by a study by Kheramin et al. (2005), based on a quantitative model of progressive-ratio schedule performance (see Killeen, 1994; Killeen et al. 2009). Kheramin et al. (2005) reported that a, a parameter which is believed to provide a quantitative measure of reinforcer value (Reilly 2003; Rickard et al. 2009), was significantly smaller in OPFC-lesioned rats than in sham-lesioned rats. Taken together, these results suggest that the OPFC lesion devalues positive reinforcers,

resulting in an increase in the *relative* value of the larger of two reinforcers in inter-temporal choice situations.

Bezzina et al. (2008a) investigated the effect of disconnecting the OPFC from the nucleus accumbens core (AcbC) in a progressive delay schedule. The results showed that the disconnection group had a higher value of *K*, but not of *Q*, than the sham-lesioned group. These results suggested that delay discounting is regulated by a mechanism which includes these two structures. Interestingly the OPFC-AcbC connections seem not to be involved in the sensitivity to reinforcer size. These authors proposed that while the OPFC integrates the information of several features of the reinforcer including size and delay, its role in delay discounting is more specifically related to its connections with the AcbC (Bezzina et al. 2008a). In another interesting study, da Costa Araújo et al. (2009) examined whether exposure to an inter-temporal choice schedule would induce neural activation in the OPFC and AcbC, as indicated by enhanced expression of the Fos protein. The results showed that exposure to an adjusting-delay schedule enhanced Fos expression in the OPFC and AcbC. The role of the AcbC in inter-temporal choice is discussed in greater detail in section 1.4.4.2.

Human studies have also provided evidence for the involvement of the OPFC in delay discounting. A study employing magnetic resonance imaging in the choice of hypothetical (imagined) rewards differing in magnitude and delays ranging from less than a day to 6 weeks showed that the lateral prefrontal and intraparietal cortical regions were activated independently of the delay, whereas the ventral striatum and the medial OPFC were activated with immediate rewards (McClure et al. 2004). Furthermore, humans with damage in the OPFC or the ventromedial prefrontal cortex (VMPFC), when performing in a gambling task tend to choose cards that give rewards with a moderate probability, but also carry a risk of heavy penalties (Bechara et al. 1994; Bechara et al. 1998; Damasio et al. 1990). Moreover, it has been reported that humans with OPFC damage show impaired performance on a task requiring them to choose between two possible rewards and to place bets on their choices; OPFC-lesioned subjects consistently failed to choose the optimal outcome (Rogers et al. 1999). In addition, Ernst et al. (2004) conducted a functional magnetic resonance (fMRI) study to examine the regional pattern of neural activations during a two-choice decision making task with probabilistic monetary gains, and reported that the OPFC was activated during the selection of high risk/reward options.

1.4.4.2   The Basal Ganglia

1.4.4.2.1.   Structure and functions of the basal ganglia

The basal ganglia consist of large subcortical structures comprising several interconnected nuclei in the telencephalon and diencephalon. The basal ganglia include the striatum (caudate nucleus, putamen, nucleus accumbens), the globus pallidus (internal segment or GPi; external segment or GPe; and ventral pallidum), and the subthalamic nucleus (STN). In addition, the midbrain substantia nigra (pars compacta or SNc and pars reticulata or SNr) are often included in definitions of the basal ganglia (Squire et al. 2008) (see Fig. 1.8).

For many years it was supposed that the principal, if not the only function of the basal ganglia was the control of movement ('extrapyramidal motor system'). However, it is now recognized that the basal ganglia are involved in a wide range of behavioural functions, including motivation, emotional behaviour, spatial perception, and various aspects of cognitive functioning (Squire et al. 2008). An important advance in understanding the multiple roles of the basal ganglia was the development of the concept of multiple circuits linking the basal ganglia, the cerebral cortex and the thalamus ('cortico-striato-thalamo-cortical [CSTC] circuits') that are organized in parallel, but remain functionally segregated from each other (Alexander and Crutcher 1990). It is believed that there are at least five such circuits, each of which engages different regions of the basal ganglia, thalamus and frontal lobes (Alexander and Crutcher 1990). The *motor circuit* is focused on the precentral motor fields, the *limbic circuit* on the anterior cingulate and medial orbitofrontal cortex, the *oculomotor circuit* on the frontal and supplementary eye fields, and two *prefrontal circuits* on the DLPFC and lateral OPFC respectively (Alexander et al. 1990). Each circuit includes a direct pathway to the output nuclei (GPi, SNr, and ventral pallidum), which arises from inhibitory striatal efferents that contain both GABA and substance P (Albin et al. 1989). When this pathway is activated, the thalamic stage of the circuit is disinhibited (see Fig. 1.8). There is also an indirect pathway which passes first to the GPe via striatal projection neurones that contain GABA and enkephalin, then from the GPe to the STN via a GABAergic pathway, and finally to the output nuclei via a glutamatergic projection from the STN (Alexander et al. 1990). The high spontaneous discharge of GPe neurones exerts an inhibitory influence on the STN, whereas the activation of the inhibitory

GABA/enkephalin projection from the striatum suppresses the activity of GPe neurones, disinhibiting the STN and thereby enhancing the excitatory drive to the output nuclei; this in turn enhances the inhibitory drive to their efferent targets within the thalamus (Alexander and Crutcher 1990) (see Fig. 1.8). Thus, the two striatal efferent systems of each circuit have opposite effects upon the basal ganglia output nuclei, and the thalamic targets of basal ganglia outflow.
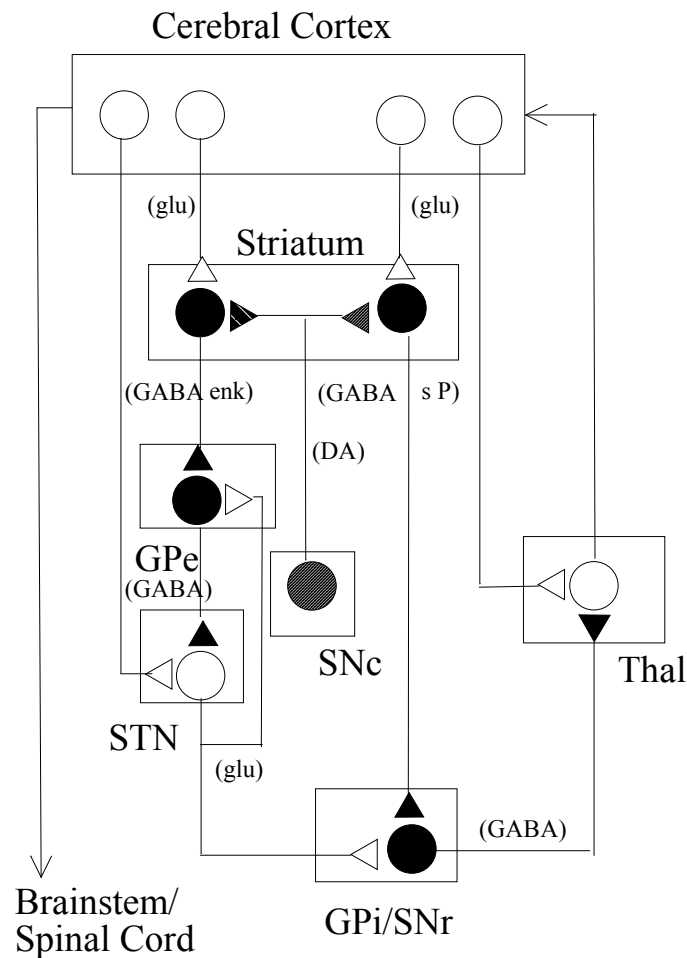
Fig. 1.8. Model of basal ganglia-thalamocortical circuitry. Inhibitory neurones are shown as filled symbols, excitatory neurones as open symbols. DA, dopamine; enk, enkephalin; GABA, γ-aminobutyric acid; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; glu, glutamate; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; s P, substance P; STN, subthalamic nucleus; Thal, thalamus (figure adapted from Alexander and Crutcher 1990)

1.4.4.2.2.   Role of the basal ganglia in interval timing and inter-temporal choice behaviour

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The basal ganglia have been proposed to play a key role in interval timing (Buhusi and Meck 2005; Coull et al. 2010; Matell and Meck 2000) and inter-temporal choice behaviour (Cardinal 2006; Winstanley 2010). With few exceptions, the dorsal striatum has been primarily implicated in interval timing in retrospective and immediate timing paradigms, whereas the ventral striatum has been mainly implicated in inter-temporal choice behaviour (i.e. prospective timing, as defined by Killeen and Fetterman 1988). In this section, the evidence pertaining to the dorsal and ventral portions of the striatum will be treated separately. It is important to note, however, that the notion that the dorsal and ventral striatum play distinct roles in interval timing and inter-temporal choice is by no means well established. To a considerable degree the notion of distinct roles for these two divisions of the striatum arises from the fact that workers with a particular interest in models of interval timing (e.g. Meck 2006b; Meck et al. 2008) have tended to focus on the dorsal striatum, whereas workers whose primary concern is delay discounting and models of choice (e.g. Cardinal 2006; Bezzina et al. 2007) have mainly concentrated on the putative role of the ventral striatum. One aim of the present project is to explore the links between these two lines of research.

The dorsal striatum

The dorsal striatum consists of the caudate nucleus and putamen. In rodents, these form a single structure, the caudate-putamen, with fibres of the internal capsule coursing through, whereas in primates these structures are separated by the internal capsule (Squire et al. 2008). The most numerous neurone type in the dorsal striatum is the medium spiny neurone. The dorsal striatum receives cortical input from the neocortex, in particular the sensory and motor cortical areas, and also receives a dense dopaminergic input from the substantia nigra (the nigro-striatal dopaminergic pathway) (Alexander and Crutcher 1990).

As reviewed in section 1.3.1.7, the dorsal striatum and its connections with the cerebral cortex play a key role in the SBF timing model proposed by Meck and colleagues (Meck et al. 2008). In this model, striatal medium spiny neurones are assumed to detect coincident activity of cortical neurones which are assumed to be synchronized at the onset of a trial, and to oscillate at a fixed frequency throughout the criterion interval (Meck et al. 2008). Empirical support for this notion derives from single-neurone recording studies in rats and computational simulations, which suggest

that striatal and cortical neurones may encode specific durations (see section 1.3.1.7) Additionally, a recent study using rhesus monkeys identified neurones in the OPFC and caudate nucleus that were specifically activated during the performance of a task involving temporal representation of a sequence of sensory and motor events (Jin et al. 2009).

Several electrophysiological studies in normal human subjects have demonstrated that the dorsal stiatum is activated during the performance of time discrimination tasks (Ferrandez et al. 2003; Lewis and Miall 2003; Nenadic et al. 2003), and other tasks that require integration of some stimulus dimension over a time interval (Nieder and Miller 2003).

Further support for the notion that the dorsal striatum is involved in interval timing derives from studies in rodents that have shown that lesions of the caudate-putamen impair timing performance in conventional timing schedules such as the FIPP (Meck 2006b).

Much of the evidence which links the basal ganglia with interval timing behaviour comes from clinical studies which have identified significant impairment of timing performance in patients with disorders that involve the CSTC circuits (Malapani et al. 2002; Malapani et al. 1998; Paulsen et al. 2004; Rammsayer 1990). For instance, it has been shown that patients with Parkinson's disease, in which the nigrostriatal dopaminergic projection degenerates, are unable to time two durations independently; the reproduced intervals for the two criterion durations migrate towards each other (Malapani et al. 1998). Interestingly, it has been found that this 'migration' effect is eliminated after stimulation of the subthalamic nucleus (Malapani et al. 1998). In addition, it has also been reported that patients suffering from Parkinson's disease showed impaired performance in time discrimination tasks (Harrington et al. 1998); their temporal discrimination exhibited the scalar property while they were medicated with L-dopa, but not when they were tested off medication (Malapani et al. 1998). Symptom-free individuals carrying the autosomal dominant gene for Huntington's disease (a neurodegenerative disease in which the caudate and putamen degenerate) who were approaching the age at which the disease was predicted to manifest itself were found to perform worse in a time discrimination task than control subjects or patients whose predicted time of disease onset was more than 12 years away (Paulsen et al. 2004). Moreover, an fMRI study demonstrated that patients approaching the predicted time of

onset of Huntington's disease showed reduced activation of the caudate-putamen during performance of a timing task compared to healthy controls subjects (Paulsen et al. 2004).

Although the majority of evidence relating inter-temporal choice behaviour and the striatum points towards a central role of the ventral striatum, specifically the nucleus accumbens core (AcbC) (see following subsection), some recent evidence suggests that the dorsal striatum may also contribute to performance of this kind of behaviour, at least in the case of primates. Hwang et al. (2009) trained two monkeys in an inter-temporal choice task in which they were required to choose between different amounts of juice available either immediately or after a delay. Single neurone activity was recorded in the caudate nucleus, putamen and ventral striatum during the performance of the task. Patterns of neuronal firing were identified that were related to the difference between the temporally discounted values of the two alternatives; these were encountered more frequently in the dorsal striatum than in the ventral striatum. A recent fMRI study (Pine et al. 2009) also implicated the dorsal striatum in performance of delay-discounting tasks in humans.

The ventral striatum

The nucleus accumbens (Acb) forms part of the ventral striatum along with the olfactory tubercle, ventral and medial parts of the caudate-putamen complex, as well as caudal areas of the caudate-putamen located dorsal to the amygdala (Meredith et al. 1996). The ventral striatum receives input from the olfactory cortex, the MPFC and OPFC, the hippocampus, the amygdala, and the thalamus. It also receives dopaminergic input from the ventral tegmental area (VTA), and sends a projection back to the VTA and the SNr (Squire et al. 2008)

The neuronal population of the Acb mainly consists of medium spiny neurones. Two types may be distinguished on the basis on their neurochemical characteristics; the first group contains GABA and substance P, and the second group GABA and enkephalin (Basar et al. 2010).

Within the Acb, there is a clear distinction between the shell and core subregions (AcbS and AcbC, respectively) (Zaborszky et al. 1985). The most well-established marker for the shell and core of the Acb in rats is the calcium binding protein calbindin-D_{28K} (Zahm and Brog 1992). Along with calbindin-D_{28K}, the mu-opioid receptors in the

human brain also show a differential distribution in the shell and core (Voorn et al. 1996).

The AcbC receives afferents from the dorsal prelimbic and anterior cingulate areas of the MPFC, the OPFC, the parahippocampal cortex, the caudal midline and rostral intralaminar thalamic nuclei, the anterior part of the basolateral amygdaloid nucleus, and the VTA (Brog et al. 1993). The AcbC sends fibres to the dorsal subcommisural part of the ventral pallidum, the medial part of the GPi and the dorsomedial part of the SNr (Heimer et al. 1991). The AcbS receives cortical inputs from the infralimbic and ventral prelimbic areas, the paraventricular thalamic nucleus, and the basolateral amygdaloid nucleus (Berendse and Groenewegen 1990; Wright et al. 1996). The output from the AcbS reaches the lateral hypothalamus, the VTA, the dorsal part of the SNc and the pedunculopontine tegmental nucleus (Basar et al. 2010).

One interesting difference between the regulation of the the outputs of the AcbC and AcbS is the lack of the suppressive role of the STN in the AcbS (Basar et al. 2010).

There is relatively little evidence that the Acb plays a significant role in interval timing in retrospective and immediate timing schedules In an early study, it was demonstrated that Acb-lesioned rats had the same peak time as control rats in the FIPP, but showed a lower maximal response rate (Meck 2006b). Hinton and Meck (1997a;b) argued that destruction of the Acb did not impair timing processes, but did disrupt the motivational aspect of operant responding in timing schedules. More recently it has been reported that AcbC lesions impaired the performance of rats in the FIPP, although this impairment was attributed to increased sensitivity to the omission of reinforcers in the peak trials rather than a deficit in time perception (Galtress and Kirkpatrick 2010).

A considerable body of evidence supports the idea that the Acb, in particular the AcbC, plays an important role in guiding inter-temporal choice. Cardinal et al. (2001) were the first to demonstrate that rats with lesions of the AcbC showed reduced preference for the larger, more delayed reinforcer, compared to sham-lesioned rats, in an inter-temporal choice task (Cardinal et al. 2001). Consistent with these results, da Costa Araújo et al. (2009) found that AcbC-lesioned rats showed shorter indifference delays in an adjusting-delay schedule. Additionally, AcbC-lesioned rats showed a reduced preference for delayed certain rewards when choosing between reinforcers of different probabilities (Pothuizen et al. 2005). These observations have been interpreted in terms of an increase in the rate of delay discounting induced by destruction of the AcbC (e.g. Cardinal 2006). Ostensibly contradictory evidence was provided by Acheson et al.

(2006), using an adjusting-magnitude schedule. These authors found that Acb-lesioned rats were less sensitive to within- and between-session changes in delay to reinforcement than sham-lesioned rats, and speculated that the tendency towards 'impulsive choice' in the AcbC-lesioned rats in Cardinal et al.'s (2001) experiment might be explained in terms of impaired learning about the within-session changes in delay that were entailed by the progressive-delay schedule, rather than accelerated delay-discounting.

Recent reviews by Winstanley (2010) and Basar et al. (2010) have drawn attention to multiple methodological differences between the studies of Cardinal et al. (2001) and Acheson et al. (2006). For example, the Acb lesion used by Acheson et al. (2006) incorporated the AcbS as well as the AcbC, whereas Cardinal et al.'s (2001) lesion was restricted to the AcbC. It seems unlikely, however, that this difference could account for the different results obtained, because Pothuizen et al. (2005) found no effect of AcbS lesions on inter-temporal choice. Differences in the behavioural methodology used in the two studies, for example the more restricted range of delays used by Acheson et al. compared to Cardinal et al., and the different schedules employed in the two studies, may be more pertinent.

A perennial difficulty in interpreting the effects of interventions on inter-temporal choice behaviour is the potential confounding of changes in delay discounting by altered sensitivity to reinforcer magnitude (see above, section 1.3.2.2). In an attempt to circumvent this problem, Bezzina et al. (2007) used a multi-phase design in which indifference delays to the larger reinforcer ($d_{B(50)}$) were determined for a range of delays to the smaller reinforcer (d_A). Bezzina et al. found that the AcbC-lesioned rats' linear indifference function (d_{B50} plotted against d_A) was displaced downwards in a parallel fashion compared to the function obtained from sham-lesion rats. According to Ho et al.'s (1999) multiplicative hyperbolic model of inter-temporal choice, such a parallel displacement of the indifference function is the hallmark of a selective increase in the rate of delay discounting, as expressed by the discounting parameter, K. Bezzina et al.'s (2007) results thus substantiate Cardinal et al.'s (2001) interpretation of their findings in terms of an effect of AcbC lesions on delay discounting. Interestingly, Bezzina et al. (2007) also found that AcbC-lesioned rats showed flatter preference functions (% selection of B plotted against d_B), consistent with Acheson et al.'s finding of reduced sensitivity to within-session changes in delay in their Acb-lesioned rats; however, a quantitative analysis of the preference functions was able to exclude the possibility that

this was responsible for the parallel displacement of the indifference function (see Bezzina et al. 2007).

A different approach was adopted by da Costa Araújo et al. (2010), who examined the expression of the Fos protein, a marker of neuronal activation (see below, section 1.5) in intact rats that were trained either under an adjusting-delay schedule that did not entail reinforcers of different sizes or under an adjusting-magnitude schedule that did not entail different delays to reinforcement. Fos expression in the AcbC was enhanced in rats exposed to the adjusting-delay schedule compared to rats trained under the adjusting-magnitude schedule and control rats, suggesting that the AcbC was specifically activated during performance of tasks that entail delay discounting.

1.4.4.3.　　　The subthalamic nucleus

As discussed above, the subthalamic nucleus (STN) is a key structure in the indirect loop of CSTC circuits, receiving inhibitory (GABAergic) input from the external pallidum and sending excitatory projections to the principal output structures of the basal ganglia – the internal segment of the pallidum and the substantia nigra pars reticulata (see section 1.4.4.2.1).

The STN's role in extrapyramidal motor control has been recognized for many years (see Parent and Hazrati 1995a, 1995b; Gerfen 2004). More recently it has been realized that the STN plays an important role in response inhibition and attention. For example, lesions of the STN enhance the tendency of animals to make premature responses in the 5-choice serial reaction time task (Baunez and Robbins 1997, Baunez et al. 2001; Aron and Poldrack 2006; Eagle et al. 2008) and DRL schedules (Uslaner and Robinson 2006).

STN lesions also disrupt performance in the FIPP (Wiener et al. 2008). However, this has been attributed to a general release of positively reinforced operant behaviour from learned inhibitory control, rather than a specific impairment of timing processes (Baunez and Robbins 1997; Phillips and Brown 2000 Wiener et al. 2008; for reviews, see Temel et al. 2005; Tan et al. 2006).

A growing body of evidence indicates that the STN may exert some limiting control over the incentive value of reinforcers. For example, Baunez et al. (2002) reported that lesions of the STN enhanced the induction of locomotion by conditioned stimuli that had been associated with food reward. STN lesions have also been found to

prolong responding on progressive-ratio schedules of food (Baunez et al. 2005; Bezzina et al. 2008b) and cocaine (Uslaner et al. 2005) reinforcement, and to facilitate sign-tracking behaviour conditioned with food or cocaine reinforcement (Uslaner et al. 2008).

The STN has been implicated in the regulation of inter-temporal choice behaviour. Lesions of the STN have been found to suppress preference for the smaller, earlier reinforcer in inter-temporal choice schedules (Winstanley et al. 2005; Uslaner and Robinson 2006).

Bezzina et al. (2009) recently examined the effect of STN lesions on inter-temporal choice using a protocol based on the multiplicative hyperbolic model of inter-temporal choice (MHM) (Ho et al. 1999). The lesioned rats showed flatter slopes of the linear indifference function than sham-lesioned control rats, indicating that the lesion reduced the value of Q, which in turn implies that the absolute instantaneous values of the reinforcers were higher in the STN-lesioned group than in the sham-lesioned group (Ho et al. 1999; Kheramin et al. 2005). This is consistent with the above-mentioned evidence indicating that destruction of the STN results in enhancement of the incentive values of outcomes associated with food reinforcement (Baunez et al. 2002; 2005; Uslaner and Robinson 2006; Uslaner et al. 2008; Bezzina et al. 2008b). Interestingly, Bezzina et al. (2009) found no evidence that the rate of delay discounting, K, was affected by destruction of the STN.

There are, however, some discrepancies in the literature on the effects of STN lesions on inter-temporal choice. Two studies reported enhanced preference for the larger, more delayed reinforcer following STN lesions (Winstanley et al. 2005; Uslaner and Robinson 2006), an effect that was not seen in Bezzina et al.'s (2009) experiment. Bezzina et al. (2009) enumerated several methodological differences between their experiment and the earlier studies which might have contributed to the differing results, one notable difference being the timing of the lesion. In Bezzina et al.'s experiment the lesion was inflicted before behavioural training, whereas Winstanley et al. (2005) and Uslaner and Robinson (2006) trained their subjects under the inter-temporal choice task before inflicting the lesion. Bezzina et al. (2009) suggested that reduction of the rate of delay discounting may be a transient effect of STN lesions, which dissipates during the extended training. In support of this suggestion, Bezzina et al. noted that Uslaner and Robinson (2006) only found enhanced preference for the larger delayed reinforcer for a brief period after destruction of the STN, although it remained possible to induce the effect by systemic treatment with d-amphetamine over a longer period.

In summary, although there is quite strong evidence for a role of the STN in response inhibition and the regulation of incentive value, its putative role in timing and delay discounting remains controversial.

1.4.4.4.   Hippocampus

The hippocampal formation, lying in the medial temporal lobe, constitutes one of the most ancient parts of the cerebral cortex. It comprises the 'hippocampus proper' together with adjacent structures, including the the hippocampal and dentate gyri. A major source of cortical afferent fibres is the entorhinal cortex which projects to the hippocampus via the 'perforant path'. Subcortical afferents include projections from the basolateral amygdala and septum, cholinergic fibres from the basal nucleus of Meynert and the diagonal band of Broca, dopaminergic fibres from the VTA, and 5-HTergic fibres from the median raphe nucleus (Amaral and Levenex 2006)

The hippocampus forms an important part of the Papez circuit. Its important role in memory has been suspected for many years. It is well known that hippocampal lesions result in memory impairment in humans (see Lishman 1998) and animals (Eichenbaum et al. 2007). Detailed analysis of the role of the hippocampus in associative learning has shown that damage to the hippocampus does not prevent the formation of Pavlovian associations involving discrete stimuli, but does impair learning of contextual associations (Winocur et al. 2007). The hippocampus also plays a key role in spatial memory and navigation (Morris et al. 1982).

Hippocampal lesions have been found to disrupt the acquisition and performance of timing tasks, including the FIPP (Meck et al. 1984; Olton et al. 1987; Meck 1988), DRL schedules (Costa et al. 2005), and the interval bisection task (Meck et al. 1984). Meck et al. (1987) reported that hippocampal lesions inflicted after the attainment of steady-state FIPP performance resulted in a gradually progressive leftward shift of the response rate function which persisted even after extended training. Meck et al. offered the following explanation for this observation in terms of SET. Due to the role of the hippocampus in working memory, damage to this structure may have resulted in a gradual build-up of faulty ('elongated') representations of the fixed interval in reference memory; this in turn may have resulted in a progressive increase in the likelihood of such a 'faulty' memory sample being selected in successive trials (see also Hinton and Meck 1997a; b; Coull et al. 2011). It should be noted, however, that some studies have

failed to detect any effect of hippocampal lesions on timing behaviour (Rawlins et al. 1983; Dietrich and Allen 1998).

Less is known about the effect of hippocampal lesions on inter-temporal choice. Cheung and Cardinal (2005) found that hippocampal lesions facilitated the learning of an instrumental task under conditions of delayed reinforcement. However, lesioned rats showed 'impulsive choice' in an inter-temporal choice schedule. It has yet to be determined whether the latter effect reflects an effect of the lesion on delay discounting or whether the effect may have been brought about by a change in sensitivity to reinforcer magnitude (see Cardinal 2006).

1.4.4.5.   Cerebellum

1.4.4.5.1.   Structure and functions of the cerebellum

The cerebellum is a structure located in the base of the skull, and it is present in all vertebrates, in most primitive pre-vertebrates up through primates. In reptiles, birds and mammals, the corpus cerebella constitutes the largest portion of the cerebellum and receives propioceptive, somatosensory, visual and auditory information, and projects to the tectum, the red nucleus and the cerebral cortex, via the thalamus (Squire et al. 2008). The cerebellar hemispheres receive input from the frontal, parietal, and visual cortices via the pontine nuclei and project to the motor and premotor cortices and anterior portions of the frontal lobe (Asanuma et al. 1983a, 1983b, 1983c; Schell and Strick 1984). The cerebellar cortex is formed by three layers and surrounds three pairs of deep cerebellar nuclei: the fastigius, the interpositus and the dentate (Squire et al. 2008). These structures along with the vestibular nuclei constitute the output structures of the cerebellum.

The cerebellum is no longer considered to control just voluntary movement, speech and equilibrium (Marr 1969). Considerable evidence suggests that this structure is critical also for thought, behaviour and emotion (Albus 1971; Marr 1969). Cerebellar lesions and stimulation experiments in animals have demonstrated that the cerebellum is related to classical conditioning, navigational skills, cognitive flexibility, predatory attack and aggression (Dum et al. 2002; Middleton and Strick 2001; Ohyama et al. 2003). Imaging studies with humans have shown that the cerebellum is activated during tasks that include verbal working memory, shifting attention, motor learning, sensory

processing and modulation of emotion (Decety et al. 1990; Frysinger et al. 1984; Kim et al. 1994; Leiner et al. 1993).

1.4.4.5.2.   The cerebellum and interval timing

Braitenberg (1967) was the first to propose that the cerebellum performs a pure timing function. This author proposed that a wave of activity propagated along a parallel fibre could be "tapped off" by successive Purkinje cells. Each tap would occur at an incremental delay after the onset of the wave and this delay could be used to time movements for up to 50 ms. More recent studies of the role of the cerebellum in classical conditioning have also emphasised its involvement in timing processes. For example, studies of eyeblink conditioning in which the conditioned response (CR) must be timed to occur just before the unconditioned stimulus (US) demonstrated that lesions of the cerebellum disrupt the CR (Gerwig et al. 2003; Thompson 1990). Ohyama et al (2006) demonstrated that behaviourally silent learning information is initially mediated by the cerebellar cortex, but that subsequent expression of timing behaviour depends upon the plasticity induced in the interpositus nucleus.

Additionally, patients with cerebellar lesions often display a breakdown of the timing and sequencing of muscular events (McNaughton et al. 2004; Timmann et al. 1999), and show increased variability in temporal production tasks, such as rhythmic tapping, or during the production of isolated movements with a specified target duration (Spencer et al. 2003). Generally, cerebellar timing deficits have been demonstrated for short, sub-second durations only (Harrington et al. 2004; Ivry 1996; Mangels et al. 1998). Moreover, transcranial magnetic stimulation (TMS) of the cerebellum impairs the timing of sub- but not supra-second durations (Fierro et al. 2007; Koch et al. 2007; Lee et al. 2007).

The effects of cerebellar lesions on timing in animals are more mixed and transient. For instance, Breukelaar and Dalrymple-Alford (1999) demonstrated that lesions of the cerebellar hemispheres produced deficits in a temporal discrimination task (interval bisection) in the range of milliseconds (200-800 ms) in rats. However performance in a temporal discrimination task in the range of seconds was unaffected. In addition, time estimation in the range of seconds measured by the temporal response differentiation task (TRD 10-14 s) and the differential reinforcement of low response rate task (DRL 10-14 s) was unaffected by α-difluoromethylornithine (DFMO) treatment

which causes cerebellar stunting in adult rats when administered during postnatal days (Ferguson et al. 2001). While the role of the interpositus nucleus is well supported in eye blink conditioning studies (Christian et al. 2004; Christian and Thompson 2003; Ohyama and Mauk 2001), lesions of this structure have been found to have only a transient effect on the Weber fraction and no effect on the acquisition of temporal discrimination in the range of seconds (Callu et al. 2009). Furthermore, lesions of the dentate nucleus produced a transient deficit in a temporal discrimination task in the range of milliseconds but not in the range of seconds (Clarke et al. 1996).

Some evidence has suggested that the role of the cerebellum in timing reflects a more general contribution to sensoriomotor and cognitive processes. For instance, it has been demonstrated that the cerebellum monitors and adjusts input from the cerebral cortex (Bower 1997; Parsons et al. 1997). Furthermore, cerebellar activity has been linked to event expectancy and may act by scaling output information to optimize operations of the cerebral cortex (Mauk et al. 2000). Under this view, Harrington et al. (2004) suggested that the temporal variability observed in patients with cerebellar lesions is due to the ineffective acquisition of the input to an intended temporal goal and coordinating it with an impaired motor-output system.

At the present time, there does not appear to be any evidence for a specific role of the cerebellum in the regulation of prospective timing in inter-temporal choice situations.

1.5.   Fos expression as an index of neuronal activation

Discoveries in the area of molecular biology had a major impact on neuroscience in the 1980s and 1990s. A major development was the discovery that induction of genes by neuronal activity could produce long-lasting changes in intraneuronal proteins which could provide highly sensitive markers of physiological processes (Herrera and Robertson 1996).

The first set of genes to be activated in response to neuronal stimulation is known as 'immediate/early genes' (IEGs). These IEGs encode transcription factors, which in turn alter the expression of other target genes, setting up a sequence of biochemical events that may eventually alter the physiological function of the neurone (Herrera and Robertson 1996). One IEG that has been widely used as a marker of neuronal activation is the proto-oncogene *c-fos* (cellular fos), which is found in neuronal nuclei. In most neurones, Fos levels are low under basal conditions, but neuronal firing results in an

increase in the production of Fos. Changes in Fos expression may therefore act as a biomarker for relatively short-term changes in neuronal activity induced by physiological or behavioural manipulations. Following a bout of neuronal activitation, there is a rapid induction of the *c-fos* gene. The gene's protein product, Fos, reaches its maximum expression 60 to 90 minutes after stimulation, and this level persists for 2 to 5 hours (Hoffman et al. 1993; Kovacs 2008).

The most widely used methods to detect Fos are based on immunohistochemical techniques. This approach enables individual neuronal nuclei containing the Fos protein to be visualized and quantified, and patterns of activation in different brain regions to be mapped (Bundzikova et al. 2007).

1.5.1   *Principles of immunohistochemistry*

Immunohistochemical techniques are based on the principle that antibodies for a specific substance, the antigen (neurotransmitter, protein, etc.), bind to it, allowing the detection of the antibody/antigen complex to be detected. Two methods are available for detecting antibody/antigen complexes. In the 'direct' method the antibody is labelled (for example with a fluorescent molecule), which is then detected (e.g. by fluorescence microscopy). In the 'indirect' method, a secondary antibody is used, which binds to the primary antibody, marking the site where primary antibody is bound to the antigen. The secondary antibody is usually labelled with a substance that generates a coloured reaction product, such as biotin. The indirect method has some advantage over the direct method in that it provides an additional amplification step and therefore tends to be more sensitive than the direct method (Bolam 1992; Junqueira 2003).

1.5.2   *Use of Fos expression in behavioural neuroscience*

Fos expression has become increasingly popular in behavioural studies with the aim of identifying neuronal activation associated with specific behavioural functions. Some examples of studies that have successfully employed Fos expression to explore the brain structures underlying particular behavioural functions are summarized below.

Moscarello et al. (2007, 2009) showed that food deprivation and food presentation produce distinct patterns of neuronal activation as indicated by Fos immunoreactivity in the MPFC, Acb (Moscarello et al. 2007) and amygdala (Moscarello

et al. 2009). Fos activation has been reported in the PFC and subcortical limbic brain areas when animals are exposed to an environment that has previously been associated with nicotine or chocolate (Schroeder et al. 2001) and in the amygdala during different stages of odour discriminating learning (Hess et al. 1997). Fos immunoreactivity has also been employed to identify brain areas responding to centrally acting drugs during drug-discrimination or drug reinforcement paradigms (e.g. cannabinoids: Rodriguez de Fonseca et al. 1997; methamphetamine: Nakajima et al. 2004), and also the brain regions that are active during the acquisition and memory consolidation of an operant task in mice (Bertaina-Anglade et al. 2000).

Recently, da Costa Araújo et al. (2010) used Fos expression to identify areas of neuronal activity following exposure to inter-temporal choice tasks. It was found that Fos expression was enhanced in both the OPFC and AcbC in response to exposure to a task which entailed choices between delayed reinforcers which did not differ in size, whereas only the OPFC was activated by a similar task in which rats chose between reinforcers that differed in size but not delay.

A persistent difficulty in behavioural studies employing Fos immunoreactivity is the need to control for the possibility that Fos expression may be induced by aspects of the behavioural task which may be unrelated to the behavioural process under investigation. It is important in such studies to control for such 'non-specific' factors as locomotor activity, food deprivation, discriminative stimulus presentation and reinforcer delivery, which may themselves induce Fos expression. In the present experiments, control groups of rats were employed that were exposed to conditions in which such factors as the motor requirements, deprivation conditions and reinforcement rate were matched, as closely as possible, to the behavioural task of interest.

1.6   Overview of the experiments

This thesis examined whether performance of timing tasks induces neuronal activation within the prefrontal cortex and corpus striatum, as revealed by Fos expression, and explored a new approach to analyzing performance in an inter-temporal choice schedule.

Chapters 2-4 present experiments that examined whether, in intact rats, performance of different interval timing tasks was associated with neuronal activation in the dorsal striatum and prefrontal cortex, as revealed by expression of the Fos protein, the product of the immediate-early gene *c-fos* (Experiments 1-3).

Chapters 5-7 present experiments focused on some behavioural and neurobiological aspects of inter-temporal choice behaviour. One purpose of these experiments was to develop an abbreviated approach to estimate the rate of delay discounting (K) and reinforcer size sensitivity parameter (Q) based on the Multiplicative Hyperbolic Model of inter-temporal choice (MHM), using the adjusting-delay schedule. Additionally a novel way of quantifying transitional behaviour in the adjusting-delay schedule was presented based on analysis of the power spectrum of cyclical changes in the adjusting delay, d_B (Experiment 4). This approach was used to analyze data obtained from rats performing on the adjusting-delay schedule under methodological manipulations (Experiment 5) and neurobiological interventions (Experiment 6).

CHAPTER 2

EXPERIMENT 1:

FOS EXPRESSION IN THE PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS AFTER EXPOSURE TO A DISCRETE-TRIALS TEMPORAL DISCRIMINATION TASK

2.1   Introduction

Temporal discrimination is revealed by retrospective timing tasks in which the subject is trained to emit different responses depending upon the durations of exteroceptive stimuli (Killeen and Fetterman 1988). An example of a retrospective timing task is the discrete-trials psychophysical procedure (DTPP) in which a light is presented for a variable time, after which levers A and B are presented. Responses on A are reinforced if the stimulus duration is shorter than a given value, whereas responses on B are reinforced when the stimulus duration is longer than that value. Performance in this schedule is well described by a two-parameter logistic function which is characterized by the indifference point, T_{50} (the time at which $\%B = 50$), and a slope parameter ε. These parameters may be used to derive the Weber fraction, an index of the precision of temporal discrimination (see section 1.2.1.2 for references).

According to one theory, the basic brain mechanisms of interval timing involve one of the cortico-striato-thalamo-cortical (CSTC) circuits which connect the cerebral cortex and the basal ganglia (see section 1.3.1.7 for references). This model proposes that the particular CSTC that is involved in regulating interval timing incorporates the prefrontal cortex and the dorsal striatum (Meck et al. 2008). Consistent with this proposal, it has been reported that there is an increase in the firing rate of some striatal and cortical neurones recorded in rats during performance of a temporal generalization task, showing a peak firing rate at the expected time of reinforcement (Matell et al. 2003). In addition, lesions of the striatum and the frontal cortex have been found to impair the performance of rats on the fixed-interval peak procedure (FIPP), an immediate timing schedule (Matell and Meck 2000; Meck 2006b). Moreover, evidence from functional brain imaging of human volunteers indicates that the striatum is active during the performance of timing tasks (Dreher and Grafman 2002; Hinton and Meck 2004).

The aim of the present experiment was to examine whether, in intact rats, performance of an interval timing task is associated with neuronal activitation in the dorsal striatum and prefrontal cortex, as revealed by expression of the Fos protein, the product of the immediate-early gene *c-fos* (see section 1.5 for references).

Most previous studies that have implicated the striatum in interval timing have employed immediate timing tasks such as the FIPP (Matell and Meck 2000; Meck 2006b). The present experiment employed a retrospective timing task: the DTPP. This

schedule has the potential advantage that the indices of timing are derived from single discriminative responses in discrete trials rather than from continuous free-operant responding. There is evidence that Fos expression may be induced by locomotor activity (Pesic et al. 2010; Webb et al. 2010); the use of a discrete-trials discrimination procedure should minimize the induction of Fos expression by high rates of operant responding.

Because Fos expression may also be induced by various aspects of the behavioural task not related to interval timing, for example, food deprivation and food consumption (Moscarello et al. 2007; 2009), control groups were trained under a light-intensity discrimination procedure (Hampson et al. 2010) which entailed the same food deprivation conditions, the same response requirements and the same overall reinforcement rate as the temporal discrimination task. The temporal and light-intensity discrimination tasks were also closely matched in terms of exposure to the light stimulus, the mean stimulus duration and the trial length.

2.2.   Materials and methods

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

2.2.1.   Subjects

Twenty-four female Wistar rats aged approximately 4 months and weighting 250–300 g at the start of the experiment were used. They were housed individually under a constant cycle of 12 h light and 12 h darkness (lights on at 0700–1900 h) and were maintained at 80% of their initial free-feeding body weights by providing a limited amount of standard rodent diet after each experimental session. Tap water was freely available in the home cage.

2.2.2.   Apparatus

The rats were trained in operant conditioning chambers of internal dimensions 20 cm × 23 cm × 22.5 cm (Campden Instruments Ltd., UK). One wall of the chamber contained a recess into which a motor-operated dispenser could deliver food pellets (TestDiet, MLab Rodent Tablet 45 mg; Sandown Scientific, UK). A frosted glass lamp-holder containing

a white light-emitting diode (LED; chromaticity co-ordinates: x=0.31, 31, y=0.32) was located 2.5 cm above the recess. The intensity of the light emitted by the LED was regulated by a computer-controlled variable current source; throughout this chapter 'light intensity' refers to luminance (cd m^{-2}) measured 5 cm directly in front of the light source using a Pentax Spotmeter V light meter. Apertures were situated 5 cm above and 2.5 cm on either side of the recess; motor-operated retractable levers could be inserted into the chamber through these apertures. The levers could be depressed by a force of approximately 0.2 N. The chamber was enclosed in a sound-attenuating chest; masking noise (approximately 80 dB[A]) was provided by a rotary fan. An Acorn 5000 microcomputer and interface unit (Paul Fray Ltd., UK), programmed in ARACHNID BASIC and located in an adjoining room, controlled the schedules and recorded the behavioural data.

2.2.3. *Behavioural training*

The rats were randomly allocated to two groups of twelve that were trained under the temporal and light-intensity discrimination procedures (see sections 2.2.3.1 and 2.2.3.2 below). At the start of the experiment, the food-deprivation regimen was started and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press the levers by providing reinforcers intermittently, in the absence of the levers, for three sessions (50 reinforcers per session), followed by three sessions of exposure to a discrete-trials continuous reinforcement schedule, in which the two levers were presented in random sequence. Thereafter, the rats underwent 50-minute training sessions on the discrete-trials discrimination tasks, as described below, 7 days a week at the same time each day during the light phase of the daily cycle (between 8.00 and 13.00 hours). The temporal and light-intensity discrimination procedures were the same as those described by Hampson et al. (2010).

2.2.3.1 Temporal discrimination procedure

Each session consisted of fifty trials, successive trials being initiated at 60-s intervals. Each trial started with the illumination of the lamp above the central reinforcer recess (intensity 22 cd m^{-2}). After a predetermined time, t, had elapsed (see below), the levers were inserted into the chamber. A single response on either lever resulted in withdrawal

of both levers and extinguishing of the light; the chamber remained in darkness until the start of the next trial. The duration of stimulus presentation, t, in each trial varied between 2.5 s and 47.5 s; ten values of t were employed: 2.5, 7.5, 12.5, 17.5, 22.5, 27.5, 32.5, 37.5, 42.5 and 47.5 s. If t was shorter than 25 s (the mean duration), a response on lever A resulted in reinforcer delivery, whereas a response on lever B did not. Conversely, if t was longer than 25 s, a response on lever B resulted in reinforcer delivery, whereas a response on lever A did not. If no response occurred within 5 s of lever insertion, the levers were withdrawn and the light was extinguished (this seldom occurred after the first few sessions of training). The positions of levers A and B (left versus right) were counterbalanced across subjects. In each session, there were 40 trials in which both levers were presented (four trials with each value of t, in pseudo-random sequence). The remaining trials were forced-choice trials in which only one lever was presented (lever A in five trials and lever B in the other five), the values of t occurring in a pseudo-random sequence.

2.2.3.2 Light-intensity discrimination procedure

The schedule was similar to that described above, except that the stimulus duration, t, was 25 s in each trial, but the intensity, i, varied between 3.6 and 128.5 cd m^{-2}; ten values of i were employed: 3.6, 5.7, 7.2, 11.3, 19.1, 28.9, 42.4, 62.0, 94.1, and 128.5 cd m^{-2}. If the stimulus intensity corresponded to any of the five lower values (i.e. if i <22 cd m^{-2}, the geometric mean of the intensity range), a response on lever A resulted in reinforcer delivery, whereas a response on lever B did not. Conversely, if i >22 cd m^{-2}, a response on lever B resulted in reinforcer delivery, whereas a response on lever A did not. The light-intensity discrimination procedure was thus closely matched to the temporal discrimination procedure in terms of the intensity of the light stimulus (geometric mean, 22 cd m^{-2}), stimulus duration (mean, 25 s) and trial length (successive trials were initiated at 60-s intervals).

Training under the schedules continued for 80 sessions.

2.2.4 *Immunohistochemistry*

Ninety minutes after the final session animals were transcardially perfused with phosphate buffered physiological saline (PBS) (0.1 M) followed by 4%

paraformaldehyde in PBS (formol PBS) under deep anaesthesia with sodium pentobarbitone. Brains were removed and fixed in formol PBS. After 4 hours, they were transferred to 30% sucrose solution for 48 hours. Forty-micrometer-thick coronal sections were cut on a freezing microtome. Free-floating sections were washed in PBS and then treated with 0.3% H_2O_2 in PBS for 30 min. Subsequently, the sections were treated with a blocking solution containing 3% normal goat serum (NGS) and 0.3% Triton-X in PBS, and incubated for 2 days at 4 °C with the primary antiserum [polyclonal anti-Fos protein raised in rabbit (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1:5000 dilution in PBS containing 3% NGS and 0.3% Triton-X]. This was followed by incubation with the secondary antibody, biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA; 1:600), for 2 h, and by incubation with peroxidase-conjugated avidin–biotin complex (Vector Laboratories) for 1 h. The reaction was developed with 3,3′-diaminobenzidine. The sections were mounted on chrome-gelatin-coated microscope slides and dehydrated in graded alcohols (70%, 80%, 90%, and 100%), cleared in xylene and coverslipped with DPX.

Fos-positive nuclei were identified by the dark reaction product confined to the nucleus and quantified from digital images of sections at a magnification of ×50 (ImageJ software; Wayne Rasband, National Institutes of Health, USA). The brain structures were outlined according to Paxinos and Watson's (1998) stereotaxic atlas. The areas analysed were the following: infralimbic (ILPFC), prelimbic (PLPFC) and orbital (OPFC) prefrontal cortex, the core of the nucleus accumbens (AcbC), the medial and lateral portions of the shell of the nucleus accumbens (AcbS) and the dorsomedial (DMCP) and dorsolateral (DLCP) caudate-putamen (Figure 2.4).

2.2.5 *Data analysis*

2.2.5.1 Temporal discrimination

The percentage of correct responding ('accurate A/B discrimination') was calculated in successive blocks of 10 sessions. In addition, for the quantitative analysis of the psychometric functions (*%B* responding plotted against stimulus duration, *t*), logistic functions were fitted to the data obtained during the last ten sessions of the experiment. The data were analysed using the following two-parameter equation: $\%B = 100/(1+[t/T_{50}]^\varepsilon)$, where T_{50} (indifference point) is the stimulus duration

corresponding to *%B*=50%, and ε is the slope of the function (ε has a negative value in the case of ascending sigmoid functions, flatter functions being associated with higher [i.e. less strongly negative] values of ε) (Al-Zahrani et al. 1996). The curve-fitting procedure yields estimates of the values of T_{50} and ε, from which the Weber fraction was determined as follows. The limen was defined as half the difference between T_{75} and T_{25} (T_{75} and T_{25} being the values of *t* corresponding to *%B*=75% and *%B*=25%), and the Weber fraction was calculated as the ratio of the limen to T_{50}. Goodness of fit of the functions was expressed as r^2.

2.2.5.2   Light-intensity discrimation

The percentage of correct responding ('accurate A/B discrimination') was calculated in successive blocks of 10 sessions. The quantitative analysis was essentially similar to that described above. The two-parameter logistic function fitted to the data from each treatment condition was *%B*=100/(1+[i/I_{50}]^ε), where I_{50} is the stimulus intensity corresponding to *%B*=50%. The Weber fraction was calculated in the same way as for the temporal discrimination data. Goodness of fit of the functions was expressed as r^2. Percent correct responding was compared between the two tasks by analysis of variance (group × session block, with repeated measures on the second factor). The logistic functions were fitted using SigmaPlot for Windows version 8.0.

2.2.5.3   Immunohistochemical data

Fos-positive cells were quantified as described above. The mean number of Fos-positive nuclei in each area was compared between groups by multivariate analysis of variance (MANOVA). Differences were considered statistically significant if $p{\leq}0.05$.

2.3   **Results**

Two subjects from the light-intensity discrimination group were removed from the analysis because they failed to acquire the discrimination within 60 days of training (<60% correct responding).

2.3.1.   *Behavioural data*

2.3.1.1.   Acquisition data

The acquisition data from the two groups are shown in Figure 2.1 Analysis of variance of the percent correct responding data indicated that the temporal discrimination was learnt more rapidly than the light-intensity discrimination task [group: $F(1,20) = 1.4$, NS; session block: $F(7,140) = 103.4$, $p < 0.001$; group × session block: $F(7,140) = 4.5$, $p < 0.001$]. However, the numbers of reinforcers earned in the final block of sessions did not differ significantly between the two groups [$t(20) = 1.2$, NS].
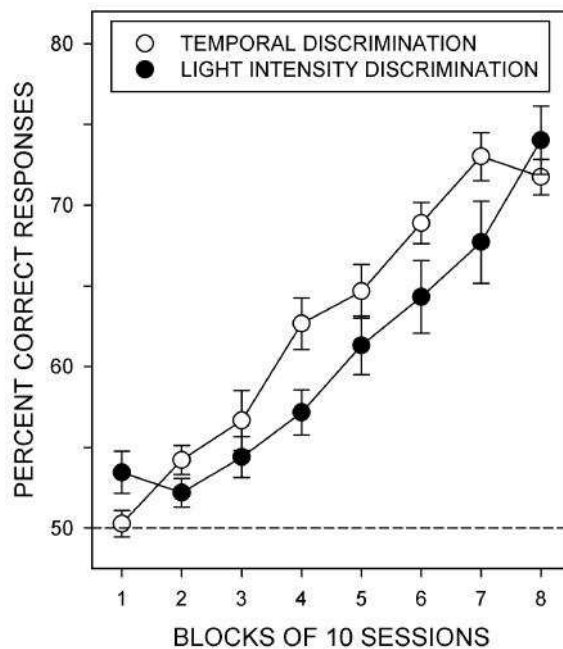
Figure 2.1 Acquisition of temporal and light-intensity discrimination. *Ordinate*: percent of trials in which a reinforcer was obtained; *abscissa*: blocks of ten sessions. Points are group mean data (± S.E.M.); empty circles, temporal discrimination group; filled circles, light-intensity discrimination group.

2.3.1.2.   Temporal discrimination

The psychometric functions are shown in Figure 2.2 (left-hand panel). The proportional choice of lever B (*%B*) increased progressively as a function of stimulus duration, *t*. Performance was well described by the two-parameter logistic function. The mean values of the parameters (± S.E.M.) and the goodness of fit of the functions (r^2) derived from the individual animals are shown in Table 2.1. The value of the indifference point, T_{50}, was close to the midpoint of the range of durations (27.5 s), and the Weber fraction

was approximately 0.62.

Table 2.1. Parameters of the logistic psychometric functions derived for individual rats trained under the temporal and light-intensity discrimination tasks (Experiment 1)

Task	Group mean ± S.E.M.			
	indifference point [a]	slope, ε	r^2	Weber fraction
Temporal discrimination	27.5 ± 2.3	-1.92 ± 0.27	0.90 ± 0.01	0.62 ± 0.08
Light intensity discrimination	20.6 ± 2.7	-1.15 ± 0.15	0.87 ± 0.04	1.76 ± 0.57

[a] T_{50} (s) in the temporal discrimination task; I_{50} (cd m^{-2}) in the light-intensity discrimination task (see text).

2.3.1.3.   Light-intensity discrimination

The psychometric functions are shown in Figure 2.2 (right-hand panel). The proportional choice of lever B *(%B)* increased progressively as a function of light intensity, *i*. Performance was well described by the two-parameter logistic function. The mean values of the parameters (± S.E.M.) and the goodness of fit of the functions are shown in Table 2.1. The value of the indifference point, I_{50}, was close to the geometric mean of the range of light intensities (20.6 cd m^{-2}), and the Weber fraction was approximately 1.76, this being significantly higher than that seen in the rats trained under the temporal discrimination task [$t(20) = 2.3$, $p < 0.05$].

2.3.2   Immunohistochemistry data

Figure 2.3 shows the group mean (± S.E.M.) numbers of Fos-positive cells in cortical and striatal areas of the rats trained under the two discrimination tasks. MANOVA revealed significant differences in the OPFC [$F(1, 20) = 4.8$, $p<0.05$] and the lateral AcbS [$F(1, 20) = 4.1$, $p = 0.05$] between the two groups, higher levels of Fos expression being seen in the rats trained under the temporal discrimination task than in those trained under the light-intensity discrimination task. Representative examples of coronal sections showing Fos expression in the OPFC and lateral AcbS of a rat from each experimental group are given in Figure 2.4.

TEMPORAL DISCRIMINATION

LIGHT INTENSITY DISCRIMINATION
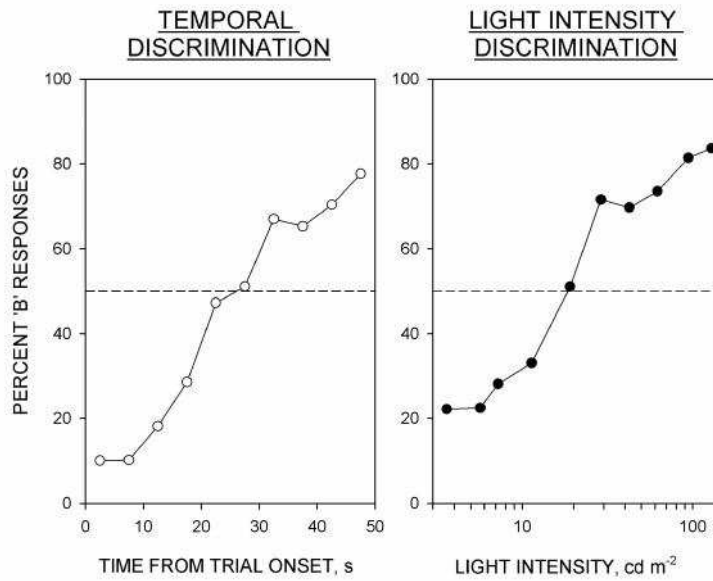
Figure 2.2. Psychometric functions. Left graph represents the relationship between proportional choice of lever B (*%B*) and stimulus duration (*t*, seconds) in the temporal discrimination procedure. Right graph represents the relationship between *%B* and light intensity (*i*, cd m^{-2}) in the light-intensity discrimination procedure. Points are group mean data in the last 10 sessions of the experiment.
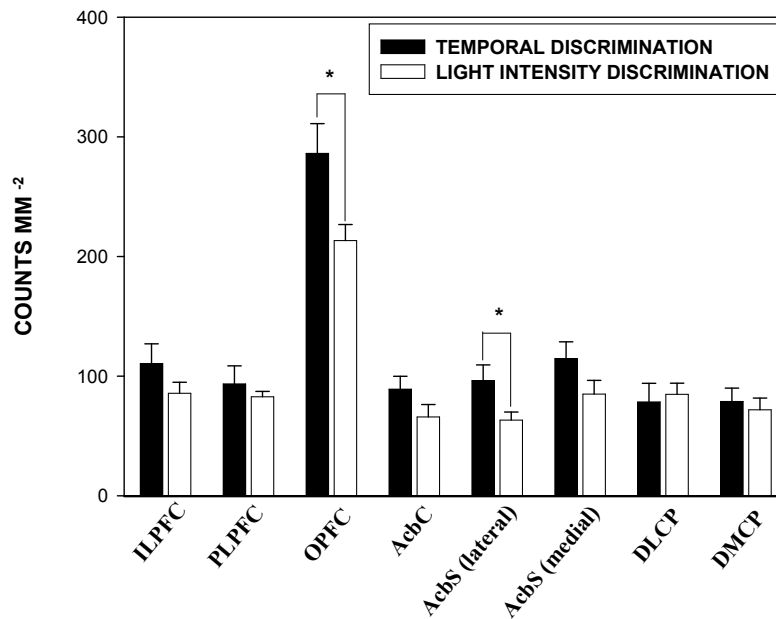
Figure 2.3. Concentration of Fos-positive units (counts.mm^{-2}) counted in the prefrontal cortex and corpus striatum: infralimbic (ILPFC), prelimbic (PLPFC) and orbital (OPFC) prefrontal cortex, the core of the nucleus accumbens (AcbC), the medial and lateral shell of the nucleus accumbens (AcbS) and the dorsomedial (DMCP) and dorsolateral (DLCP) caudate-putamen. Columns show the group mean data (+ S.E.M.) in each area for the rats trained under the temporal discrimination procedure (filled columns) and the light intensity discrimination procedure (empty columns). * Significant difference between the groups, $p \leq 0.05$.

81
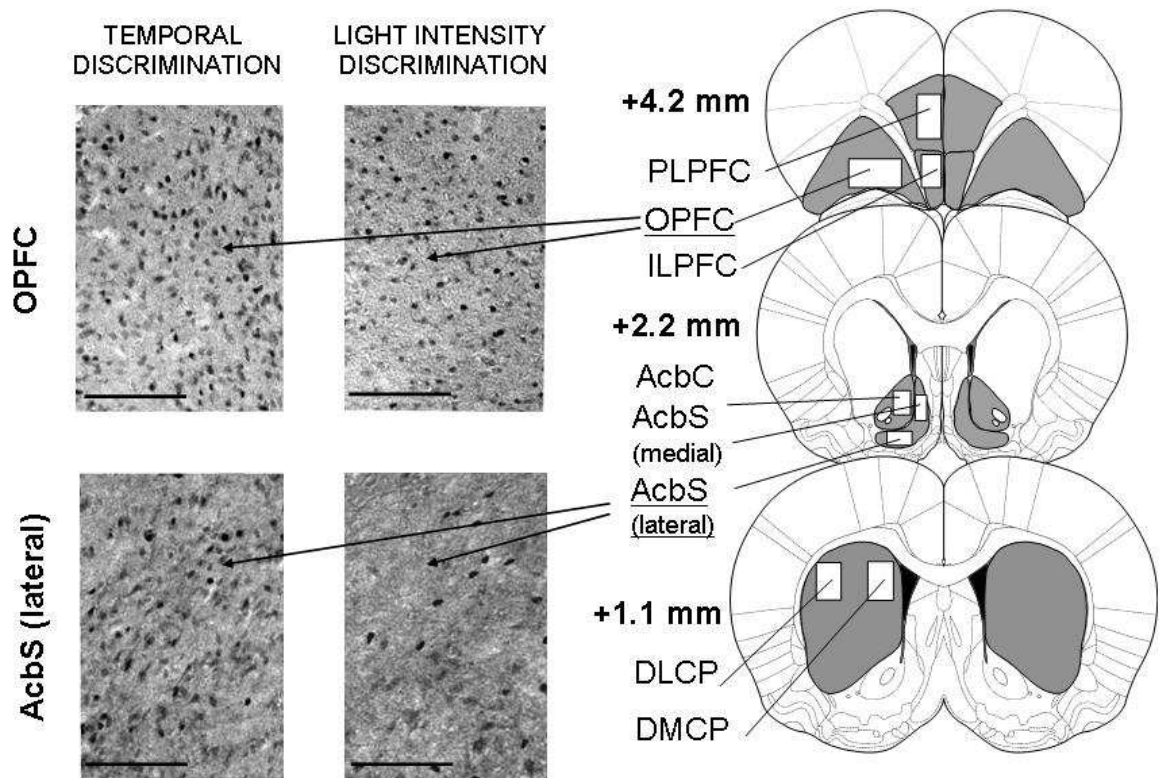
Figure 2.4. Left-hand panels: Examples of Fos expression in the OPFC (upper panels) and lateral AcbS (lower panels) in a representative rat from each group in Experiment 1. Note the greater number of Fos-positive units (dark points) in the samples taken form the rat trained under the temporal discrimination procedure compared to the rat exposed to the light-intensity discrimination procedure; bar, 50 µm. Right-hand panels: diagramatic representation of the areas selected for counting Fos-positive units (from Paxinos and Watson, 1998: see text for details).

2.4.   Discussion

The purpose of this experiment was to examine whether the performance of rats on temporal discrimination task is associated with increased neuronal activity in cortical and striatal areas, as revealed by Fos expression, in comparison with control groups trained under light-intensity discrimination task that were matched to the temporal discrimination task in terms of response requirement, trial length and exposure to the light stimulus.

The results of this experiment showed that the performance on the discrete-trials temporal discrimination task was similar to that reported in previous studies (Asgari et

al. 2006a; Asgari et al. 2005; Body et al. 2002a; Hampson et al. 2010). Proportional choice of lever B increased as a function of stimulus duration and was well described by a two-parameter logistic equation. Performance on the light-intensity discrimination task was well described by the same equation, as reported previously (Hampson et al. 2010). The two tasks apparently differed with respect to their 'difficulty', as indicated by the slower acquisition of accurate performance in the light-intensity discrimination task than in the temporal discrimination task. The Weber fraction was higher in the light-intensity discrimination task than in the temporal discrimination task, again suggesting that the former task was more difficult than the latter, although, as pointed out by Hampson et al. (2010), the Weber fractions obtained in the two tasks are not directly comparable due to the different spacing of the stimuli along the dimensions of interest (logarithmic versus linear). The spacing of the stimuli adopted in this experiment was based on previous observations which had shown that the psychometric function is approximately symmetrical in linear co-ordinates in the temporal discrimination task, the indifference point lying close to the arithmetic mean of the range of stimulus durations (Asgari et al. 2006a; Asgari et al. 2005; Body et al. 2002a; Hampson et al. 2010), whereas the psychometric function is symmetrical in semi-logarithmic co-ordinates in the light-intensity discrimination task, the indifference point lying near the geometric mean of the range of intensities (Hampson et al. 2010).

High levels of Fos expression were seen in the OPFC in rats exposed to both the temporal and light-intensity discrimination task. However the concentration of Fos-positive neurones (counts.mm^{-2}) was greater in the rats exposed to the temporal discrimination task, suggesting a greater activation of this area during performance of that task. Fos expression in the lateral AcbS was also higher in rats trained under the temporal discrimination task than in the rats trained under the light-intensity discrimination task.

The results of this experiment indicate that the temporal discrimination task induced more neuronal activation in the prefrontal cortex and ventral striatum than the light-intensity discrimination task. This suggests that these brain regions may play an important role in the processes of interval timing in retrospective timing tasks. The observed differences in Fos expression are unlikely to have been brought about by differences in reinforcer consumption, repetitive execution of the operant response, or overall exposure to the light stimulus, because these features were similar in the temporal and light-intensity discrimination tasks. Task 'difficulty' is potentially relevant,

because, as indicated above, the light-intensity discrimination task proved to be more challenging for the rats than the temporal discrimination task in both experiments. However, if differences in task difficulty had been responsible for the differences in Fos expression, one might have expected that the more difficult task would have produced more activation, and hence a higher level of Fos expression, than the easier one; yet the opposite was the case. This would seem to argue against a crucial role of task difficulty in the higher levels of Fos expression associated with temporal discrimination performance; however, further work comparing temporal discrimination with a variety of other discrimination tasks would seem to be warranted to address this issue.

The temporal and light-intensity discrimination tasks also differed with respect to the variability of the stimulus durations employed. A range of stimulus durations was employed in the temporal discrimination task, the duration being unpredictable in each trial, whereas the duration of stimulus presentation was the same in every trial in the light-intensity discrimination task. It is possible that in the latter case unmeasured food-anticipatory behaviours may have become conditioned to the appearance of the stimulus; however, such conditioning may have been less effective in the temporal discrimination task because the stimulus duration was variable (Balsam et al. 2009). The relationship between conditioned food-anticipatory behaviour and operant interval timing behaviour is uncertain, although emerging evidence indicates that the relationship is close. For example, conditioned anticipatory behaviour, like operant interval timing, is scalar in nature, and simultaneous timing of multiple intervals is possible in both cases (Antle and Silver 2009; Balsam et al. 2009). Further work is needed on the possible involvement of conditioned food-anticipatory behaviour in the tasks used in these experiments.

The finding that performance of the timing tasks was associated with neuronal activation in the prefrontal cortex was not unexpected, since it has previously been reported that lesions of the frontal cortex can disrupt the performance of rats on the FIPP, an immediate timing task (Meck 2006a). However, it must be emphasized that the prefrontal cortex is involved in a very wide range of behavioural functions, including behavioural inhibition (Eagle and Baunez 2010) and reversal learning (Churchwell et al. 2009; Dalley et al. 2004) and that it has been proposed that one function of the prefrontal cortex is to integrate the many processes that are involved in operant behaviour, assigning an overall value to each reinforcing outcome and thereby providing a basis for decision making in choice situations (Wallis 2007). Most timing schedules necessarily involve multiple behavioural processes, and it is unlikely that any single experiment will

be successful in isolating the processes of interval timing from the many other processes that may be subserved by the prefrontal cortex. The present experiment attempted to control for some of these processes, but the possibility cannot be excluded that the temporal and light-intensity discrimination tasks differed not only with respect to the presence or absence of temporal processing but also with respect to some other functions that are subserved by the prefrontal cortex. There may be no alternative to the laborious business of examining neuronal activation following exposure to a range of different timing schedules coupled with a range of different comparison procedures; the present experiment constituted an initial step in this endeavour.

Unexpectedly, Fos expression in the ventral striatum was higher in rats trained under the temporal discrimination task than in rats trained under the light-intensity discrimination task. Increased Fos expression was seen in the lateral AcbS. The AcbS has been proposed to play an important role in the mediation of feeding behaviour (Baldo et al. 2005; Hanlon et al. 2004). For example, it has been reported that stimulation of glutamate receptors in the AcbS elicits a powerful feeding response (Maldonado-Irizarry et al. 1995). In addition, microinjection of morphine into the AcbS increases food intake (Pecina and Berridge 2000). However, it is unlikely that the between-group differences in Fos expression in the present experiment were brought about by differences in feeding behaviour, since the light-intensity discrimination procedure was closely matched to the temporal discrimination procedure in terms of reinforcer delivery, and the groups were maintained under the same level of food deprivation. The present findings raise the possibility that the AcbS may make a specific contribution to the control of timing behaviour; further experiments are needed to investigate this possibility.

Previous studies have indicated that the dorsal striatum plays a critical role in interval timing (Matell and Meck 2004; Matell et al. 2003). For example, lesions of the dorsal striatum have been found to disrupt timing performance on the FIPP (Meck 2006b), and striatal neurones recorded during the performance of the same immediate timing task showed progressive increases in firing rate during the timing interval, which reached a peak at the expected time of reinforcement (Matell et al. 2003). The present results do not provide any evidence that the dorsal striatum was specifically activated during performance of the temporal discrimination task, since no differences in Fos expression were found in these areas between the temporal and light-intensity discrimination tasks. However, it should be noted that most of the studies that have

demonstrated a link between the dorsal striatum with interval timing behaviour have employed immediate timing tasks (Matell et al. 2003; Meck 2006b) whereas the task used in the present experiment was a retrospective (temporal discrimination) timing task. It is possible that performance on different timing tasks involves different neural systems. Some indirect evidence that this may be the case derives from previous experiments which have shown that systemic treatment with drugs acting at dopamine and 5-HT receptors can have qualitatively different effects on performance on immediate and retrospective timing schedules, suggesting that different behavioural and neural mechanisms may be involved in temporal differentiation and temporal discrimination (Asgari et al. 2006a; Asgari et al. 2005; Asgari et al. 2006b; Body et al. 2002a; Body et al. 2002b; Chiang et al. 2000a; Chiang et al. 2000b).

In summary, the goal of the present experiment was to investigate whether, in intact rats, performance on a retrospective timing task is associated with neuronal activation in the prefrontal cortex and corpus striatum, as revealed by enhanced Fos expression in these areas. The rats trained under the timing task showed increased Fos expression in the prefrontal cortex and nucleus accumbens compared to the rats trained under a light-intensity discrimination task, indicating a substantial activation of these areas during the timing task. However, the present experiment provides no evidence for an involvement of the dorsal striatum in the performance of this task. It is suggested that different neural mechanisms may be involved in temporal differentiation and temporal discrimination.

CHAPTER 3

EXPERIMENT 2:

FOS EXPRESSION IN THE PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS AFTER EXPOSURE TO A TEMPORAL BISECTION TASK

3.1 Introduction

The results from Experiment 1 showed that performance on the DTTP is associated with neuronal activation in the prefrontal cortex and nucleus accumbens, as revealed by enhanced Fos expression in these areas. There is evidence that rats performing retrospective timing tasks often position themselves close to the lever appropriate to the short stimulus at the start of the trial, and then migrate towards the lever appropriate to the long stimulus as the period of stimulus presentation progresses (Fetterman et al. 1998; Ho et al. 1995; Keen and Machado 1999). It is possible that systematic movement across the operant chamber occurred in the temporal discrimination task used in Experiment 1. Intra-trial movement was not recorded in these experiments, but it is possible that less movement was involved in performance of the light-intensity discrimination task, because although the light stimulus was present for 25 s in this task, discrimination of its intensity could have been made as soon as the stimulus was presented. This raises the possibility that the higher levels of Fos seen in the rats trained under the temporal discrimination task might have been caused, at least in part, by a greater amount of locomotor activity in these rats. Various methods have been adopted to suppress movement across the operant chamber during stimulus presentation in temporal discrimination tasks (Fetterman et al. 1998; Graham et al. 1994; Ho et al. 1995). For example, Graham et al. (1994) found that the use of millisecond-range stimuli (200 vs. 800 ms) in a temporal bisection task prevented intra-stimulus movement across the chamber, and thereby suppressed the bias towards the lever associated with the long stimulus which is shown by 5-hydroxytryptamine (5-HT)-depleted rats when longer stimuli are used (Ho et al. 1995; Morrissey et al. 1993). This approach was used in Experiment 2.

In the interval (or temporal) bisection procedure (Church and Deluty 1977), rats are first trained to respond on levers A and B following a short and a long presentation of a stimulus, and are then presented with a range of 'probe' stimuli of intermediate durations. Timing is assessed from a psychometric function relating proportional choice of B (%B) to the duration of the stimulus presentation (t). Performance is well described by a two-parameter logistic function which is characterized by the indifference point, T_{50} (the time at which %B = 50), and a slope parameter ε (see section 1.2.1.1). These parameters may be used to derive the Weber fraction, an index of the precision of temporal discrimination (Ho et al. 2002; Killeen and Fetterman 1988). The present

experiment examined whether, in intact rats, performance of a temporal bisection task in the range of milliseconds is associated with neuronal activation in the dorsal striatum and prefrontal cortex, as revealed by expression of the Fos protein.

3.2. Materials and methods

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

3.2.1 Subjects

Twenty-four female Wistar rats aged approximately 4 months and weighting 250–300 g at the start of the experiment were maintained under the same conditions as in Experiment 1.

3.2.2 Apparatus

The same apparatus was used as in Experiment 1.

3.2.3 Behavioural training

The rats were randomly allocated to two groups of twelve that were trained under the temporal and light-intensity bisection procedures (see below). Acclimatization to the food restriction condition and preliminary training to press the levers was carried out as in Experiment 1.

3.2.3.1. Temporal bisection task

The procedure was similar to that described by Graham et al. (1994). Phase 1 consisted of 70 sessions of temporal conditional discrimination training. Sessions consisted of 100 20-s trials in which a light stimulus (22 cd m^{-2}) was presented either for 200 ms or for 800 ms (the 'standard' stimuli: 50 trials in each case), after which levers A and B were inserted into the chamber. A response on either lever resulted in retraction of both levers; if no response occurred within 5 s of stimulus offset, the levers were retracted (this

seldom occurred except during the first few days of training). A response on lever A resulted in reinforcer delivery following a 200-ms stimulus presentation, whereas a response on lever B resulted in reinforcer delivery following an 800-ms stimulus presentation. Phase 2 consisted of 25 sessions of temporal bisection testing. Sessions were identical to those of Phase 1, except that 10% of the trials were probe trials in which stimuli of intermediate duration were presented (the 'probe' stimuli: 250, 320, 400, 500 or 640 ms; two trials in each case) and no reinforcers were delivered.

3.2.3.2. Light-intensity bisection task

The procedure was similar to that described above, except that the standard stimuli consisted of 400-ms presentations of a light whose intensity was either 1.8 or 257.0 cd m^{-2}, and the probe stimuli consisted of 400-ms presentations of a light whose intensity was 4.1, 9.4, 21.6, 49.3 or 112.5 cd m^{-2}.

3.2.4. Immunohistochemistry

The same protocol was used as in Experiment 1.

3.2.5 Data analysis

3.2.5.1 Behavioural data

The data from the acquisition phase were analysed in the same way as in Experiment 1. Logistic functions, fitted to the data from Phase 2 in which probe trials were included in the sessions, were also analysed as in Experiment 1 (see sections 2.2.5.1 and 2.2.5.2).

3.2.5.2 Immunohistochemical data

The data were analysed in the same way as in Experiment 1.

3.3. Results

One subject from the light-intensity discrimination group was removed from the analysis

because it failed to acquire the discrimination within the 70 days of training in Phase 1.

3.3.1 *Behavioural data*

3.3.1.1. Acquisition data

The acquisition data from the two groups are shown in Figure 3.1. Analysis of variance of the percent correct responding data indicated that the temporal discrimination was learnt more rapidly than the light-intensity discrimination [group: $F(1,21) = 7.9$, $p < 0.05$; session block: $F(8,168) = 152.6$, $p < 0.001$; group × session block: $F(8,168) = 10.8$, $p < 0.001$]. However, the numbers of reinforcers earned in the final block of sessions did not differ significantly between the two groups [$t(21) = 0.8$, NS].
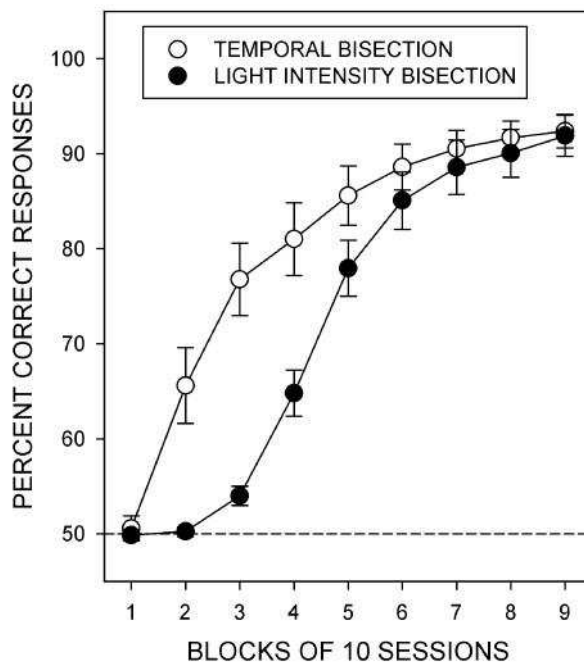
Figure 3.1 Acquisition of temporal and light-intensity discrimination. *Ordinate*: percent of standard trials in which a reinforcer was obtained; *abscissa*: blocks of ten sessions. Points are group mean data (± S.E.M.); empty circles, temporal bisection group; filled circles, light-intensity bisection group.

3.3.1.2. Temporal bisection

The psychometric functions are shown in Figure 3.2 (left-hand panel). The proportional choice of lever B (*%B*) increased progressively as a function of stimulus duration, *t*. Performance was well described by the two-parameter logistic function. The mean values of the parameters (± S.E.M.) and the goodness of fit of the functions (r^2) derived

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from the individual animals are shown in Table 2. The value of the indifference point, T_{50}, was somewhat higher than the geometric mean of the range of durations (400 ms), and the Weber fraction was approximately 0.26.
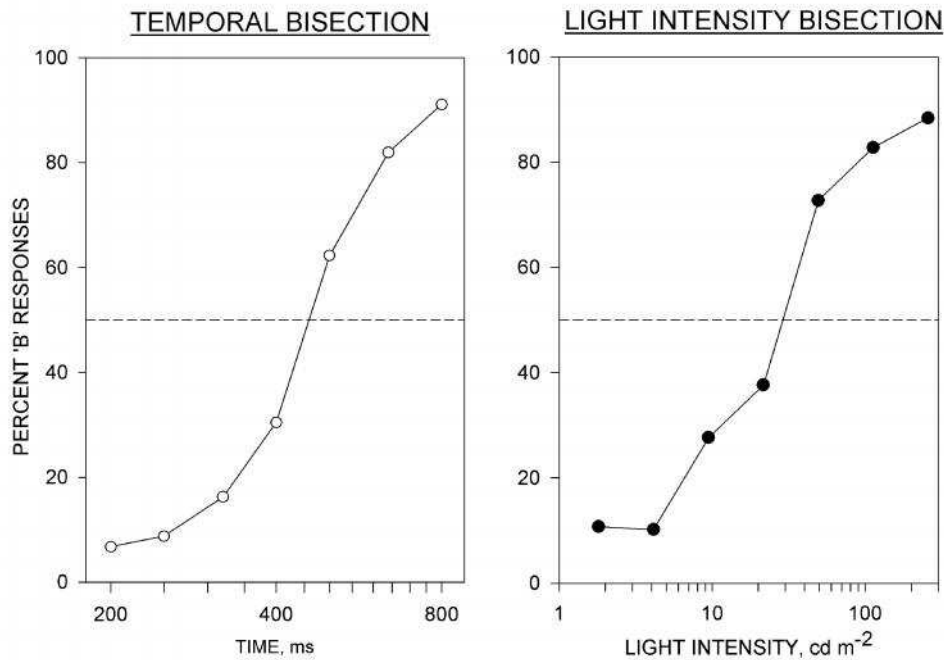
Figure 3.2. Psychometric functions. Left graph represents the relationship between proportional choice of lever B (*%B*) and stimulus duration (*t*, seconds) in the temporal bisection procedure. Right graph represents the relationship between *%B* and light intensity (*i*, cd m^{-2}) in the light-intensity bisection procedure. Points indicate group mean data in Phase 2 of the experiment.

Table 3.1. Parameters of the logistic psychometric functions derived for individual rats trained under the temporal and light-intensity bisection tasks

	Group mean ± S.E.M.			
Task	indifference point ^{a}	slope, ε	r^2	Weber fraction
Temporal bisection	458.45 ± 11.6	-5.17 ± 0.58	0.97 ± 0.01	0.26 ± 0.04
Light intensity bisection	27.6 ± 2.0	-1.43 ± 0.20	0.96 ± 0.01	1.27 ± 0.36

^{a} T_{50} (ms) in the temporal bisection task; I_{50} (cd m^{-2}) in the light-intensity bisection task (see text).

3.3.1.3.   Light-intensity bisection

The psychometric functions are shown in Figure 3.2 (right-hand panel). The proportional choice of lever B (*%B)* increased progressively as a function of light intensity, *i*. Performance was well described by the two-parameter logistic function. The mean values of the parameters (± S.E.M.) and the goodness of fit of the functions are shown in Table 2. The value of the indifference point, I_{50}, was somewhat higher than the geometric mean of the range of light intensities (21.6 cd m^{-2}), and the Weber fraction was approximately 1.27, this being significantly higher than that seen in the rats trained under the temporal discrimination task [$t(21) = 2.9, p < 0.01$].

3.3.2.   *Immunohistochemistry data*

Figure 3.3 shows the group mean (± S.E.M.) numbers of Fos-positive neurones in cortical and striatal areas of the rats trained under the two bisection tasks. MANOVA revealed significant differences in the OPFC [$F(1,20) = 4.8, p < 0.05$], the ILPLC [$F(1,20) = 4.8, p < 0.05$], the PLPFC [$F(1,20) = 4.8, p < 0.05$], the AcbC [$F(1,20) = 4.8, p < 0.05$], and the lateral AcbS [$F(1,20) = 4.1, p = 0.05$], between the two groups, higher levels of Fos expression being seen in the rats trained under the temporal discrimination task than in those trained under the light intensity discrimination task. Representative examples of coronal sections showing Fos expression in the OPFC, PLPFC, ILPFC, AcbC and the lateral AcbS of a rat from each experimental group are given in Figure 3.4.

3.4.   Discussion

The results of Experiment 2 showed that performance on the interval bisection task was similar to that reported previously (Church and Deluty 1977; Fetterman and Killeen 1991; Graham et al. 1994; Morrissey et al. 1993). Moreover, performance on the light-intensity discrimination task conformed to the conventional logistic psychometric function. As in Experiment 1, the light-intensity discrimination task appeared to be more 'difficult' than the temporal discrimination task, as indicated by the slower acquisition of accurate performance and the higher Weber fraction in the light-intensity discrimination task. In contrast to the temporal discrimination task used in Experiment 1, the spacing of
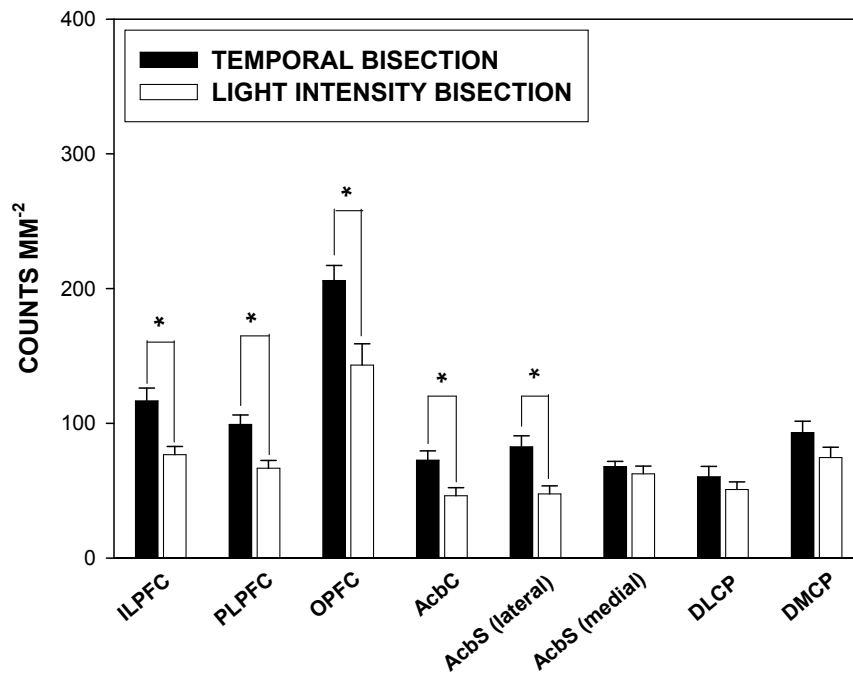
Figure 3.3 Concentration of Fos-positive units (counts.mm^{-2}) counted in the cortical and striatal regions. The conventions are the same as in Figure 2.3. Columns show the group mean data (+ S.E.M.) in each area for the rats trained under the temporal bisection procedure (filled columns) and the light intensity bisection procedure (empty columns). * Significant difference between the groups, $p \leq 0.05$.
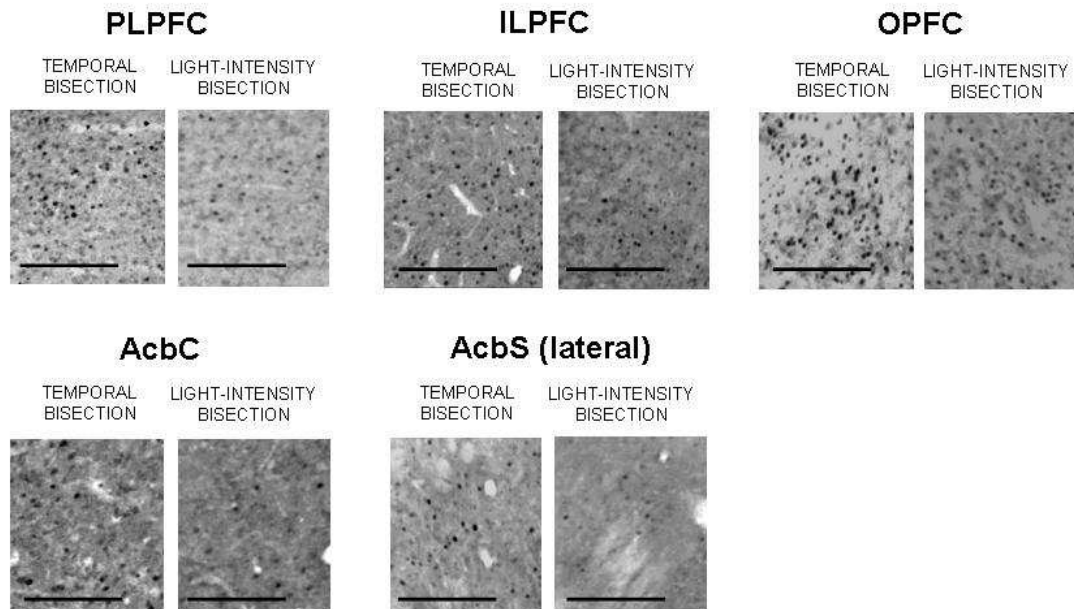
Figure 3.4. Examples of Fos expression in the OPFC, PLPFC, ILPFC, AcbC and lateral AcbS in a representative rat from each group. Note the greater number of Fos-positive units (dark points) in the samples taken form the rat trained under the temporal bisection procedure compared to the rat exposed to the light-intensity bisection procedure; bar, 50 μm.

the stimulus durations in the bisection task was geometric rather than arithmetic, in keeping with many previous findings that the psychometric function derived from this task is symmetrical in semi-logarithmic co-ordinates, the indifference point lying close to the geometric mean of the range of durations tested (e.g. Hampson et al. 2010; see section 2.4.).

As in Experiment 1, higher levels of Fos expression were seen in the prefrontal cortex and ventral striatum in the rats trained under the temporal bisection task than in those trained under the light-intensity bisection task. However the higher levels of Fos expression associated with temporal discrimination performance were somewhat more widespread in Experiment 2 than in Experiment 1. In the prefrontal cortex, the level of Fos expression was higher not only in the OPFC but also in the adjacent PLPFC and ILPFC, whereas in the ventral striatum the enhanced level of Fos expression was seen not only in the lateral AcbS but also in the adjacent AcbC.

Galtress and Kirkpatrick (2010) recently reported disruption of performance on the FIPP following lesions of the AcbC; however this effect was attributed to a specific impairment of sensitivity to the omission of reinforcers in the peak trials rather than to a deficit of interval timing processes per se, because the effect of the lesion on performance was restricted to the later portion of the peak function. This mechanism is unlikely to have been responsible for the present results, since discrete trials were used and the reinforcement rates were similar in the temporal and light-intensity bisection tasks. The present findings suggest that further exploration of a possible role of the ventral striatum, including the AcbC, in interval timing may be warranted, using a range of different timing schedules.

As discussed in the introduction of this chapter (see section 3.1.), the higher levels of Fos seen in Experiment 1 in the rats trained under the temporal discrimination (DTPP) task than in the control (light-intensity discrinmination) task might have been caused by a greater amount of locomotor activity in the rats trained under the DTPP. The present experiment attempted to minimize differences in locomotor activity between the temporal and light-intensity discrimination tasks. .It has previously been shown that the use of millisecond-range stimuli (200 vs. 800 ms) in a temporal bisection task prevented intra-stimulus movement across the chamber (Ho et al. 1995; Morrissey et al. 1993). This approach was used in Experiment 2. Performance of the temporal bisection task was associated with higher levels of Fos expression in the prefrontal cortex and nucleus

accumbens than performance of the light-intensity bisection task, arguing against a major role locomotor activity in the higher levels of Fos expression associated with temporal discrimination performance.

In agreement with the results of Experiment 1, the present results do not provide any evidence that the dorsal striatum was specifically activated during performance of the temporal discrimination tasks. No differences in Fos expression were found in these areas between the temporal and light-intensity discrimination task. Most of the studies that have demonstrated a link between the dorsal striatum and interval timing behaviour have employed immediate timing tasks (Matell et al. 2003; Meck 1996), whereas the tasks used in the present experiment was a retrospective timing task. It is possible that performance on different timing tasks involves different neural systems (see section 2.4 for references). The following experiment (Chapter 4) investigated the role of the cortico-thalamic circuits in an immediate timing task, the FIPP.

In conclusion, the rats trained under the timing task showed increased Fos expression in the prefrontal cortex and nucleus accumbens compared to the rats trained under the light-intensity discrimination task, indicating a substantial activation of these areas during the timing task. However, the results provide no evidence for an involvement of the dorsal striatum in the performance of this task. These results are in agreement with the results obtained in Experiment 1.

CHAPTER 4

EXPERIMENT 3:

FOS EXPRESSION IN THE PREFRONTAL CORTEX AFTER
EXPOSURE TO THE FIXED-INTERVAL PEAK PROCEDURE

4.1. Introduction

The results of Experiments 1 and 2 demonstrated a substantial neural activation of the prefrontal cortex and nucleus accumbens after exposure to retrospective timing tasks (DTPP and interval bisection). However they failed to provide any evidence for an involvement of the dorsal striatum in the performance of these tasks. This is apparently inconsistent with a considerable body of evidence that supports a pivotal role of the dorsal striatum in interval timing (see section 1.4.4.2.2). However, most of the studies that have demonstrated a role of the dorsal striatum in interval timing have used immediate timing schedules, in particular the fixed-interval peak procedure (FIPP) (see sections 1.3.1.7 and 1.4.5.2), whereas Experiments 1 and 2 used retrospective timing schedules. The aim of the present experiment was to examine the regional distribution of neuronal activation in the prefrontal cortex and corpus striatum in rats exposed to the FIPP.

In the FIPP, standard fixed-interval (FI) trials are randomly alternated with extended peak trials which are usually three or four times longer than FI trials. Reinforcers are never delivered in the peak trials. Response rate during peak trials is usually characterized as a modified Gaussian curve with maximal responding (peak rate) occurring around the time of the reinforcement in the FI trials (peak time) (see section 1.2.2.1 for review).

As previously stated in sections 2.1 and 3.1, Fos expression may be induced by various aspects of the behavioural task not related to interval timing, for example, food deprivation and food consumption (Moscarello et al. 2007; 2009). For this reason, a control group of rats was trained under a variable-interval schedule (VI) (Skinner 1958) which entailed the same food deprivation conditions, the same response requirements and the same overall reinforcement rate as the FIPP. In a VI schedule, the first response after a variable time has elapsed is reinforced; depending on the distribution of intervals in the VI sequence, a relatively constant rate of responding is maintained throughout each interval, indicating that temporal differentiation is not involved in this schedule (Catania and Reynolds 1968; Skinner 1958).

The present experiment examined whether, in intact rats, performance on the FIPP is associated with increased neuronal activity in the prefrontal cortex and corpus striatum, as revealed by Fos expression, a marker for neuronal activation (see section 1.5 for references). Of particular interest was the possibility that performance on the FIPP

might be associated with enhanced Fos expression in the dorsal striatum.

4.2.   Materials and methods

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

4.2.1   Subjects

Twenty-four female Wistar rats aged approximately 4 months and weighting 250–300 g at the start of the experiment were maintained under the same conditions as in Experiment 1.

4.2.2   Apparatus

The same apparatus was used as in Experiment 1. Only one lever was used in this experiment; this was the left-hand lever for half the rats and the right-hand lever for the other half.

4.2.3.   Behavioural training

The rats were randomly allocated to two groups of twelve that were trained under the FIPP or VI schedule (see below). Acclimatization to the food restriction condition and preliminary training to press the levers was carried out as in Experiment 1.

4.2.3.1.   Fixed-interval 30 s peak procedure (FIPP 30 s)

The rats underwent 50 min training sessions under the FIPP 30 s, 7 days a week, at the same time each day during the light phase of the daily cycle (between 8:00 h and 13:00 h). Each session consisted of 32 trials separated by 10-s intertrial intervals. Trials started with insertion of the lever into the chamber, and terminated with lever withdrawal. In fixed-interval trials (16 per session), reinforcement was delivered following the first response emitted after 30 s had elapsed since the onset of the trial. In

probe trials (16 per session), reinforcement was omitted, and the lever remained in the chamber for 120 s. The fixed-interval and probe trials occurred in a pseudo-random sequence with the constraint that no more than three trials of either type occurred in succession. Timing behaviour was assessed from performance in the probe trials (see section 4.2.5.1.).

4.2.3.2. Variable-interval schedule (VI 75 s)

Rats were exposed to a VI 75 s in which reinforcer availability varied randomly with respect to time within each trial, and entailed similar reinforcement and response rate to the FIPP 30 s. Experiments took place 7 days a week, at the same time each day during the light phase of the daily cycle (between 8:00 h and 13:00 h). Each session consisted of 32 trials separated by 10-s inter-trial intervals (16 30-s trials and 16 120-s trials). As in the FIPP, trials started with insertion of the lever into the chamber, and terminated with lever withdrawal. A constant-probability VI 75-s schedule was operative throughout all the trials, pausing only during the inter-trial intervals. The mean inter-reinforcer interval specified by the schedule (75 s) was chosen empirically in order to equate, as closely as possible, the overall rate of reinforcer delivery in the FIPP and the VI schedule.

4.2.4. Immunohistochemistry

The same protocol was used as in Experiment 1.

4.2.5 Data analysis

4.2.5.1. FIPP 30 s

Data obtained in the last ten training sessions were used in the analysis. Response rate was recorded in successive 2-s epochs of the probe trials. For each rat, mean response rate R was plotted against time measured from the onset of the trial, t. The following modified Gaussian function was fitted to each rat's data:

$$R = a \times e^{\left[-0.5 \times \left(\frac{t - t_{peak}}{b} \right)^2 \right]} + \left[c + d \times (t - t_{peak}) \right],$$

where $(a + c)$ is the estimated peak response rate, t_{peak} is the peak time (location of the peak of the Gaussian component of the function), b represents the spread of the function (standard deviation of the Gaussian component); the right-hand term is a linear ramp of slope d and an ordinate value c at time $t = t_{\text{peak}}$. This function has been found to provide an acceptable description of performance in the peak procedure (Buhusi et al. 2005; MacDonald and Meck 2005; Asgari et al. 2006). The following measures were derived for each rat for the last ten sessions: the peak time (t_{peak}), the peak response rate ($a + c$), and the Weber fraction (coefficient of variation of the Gaussian component of the function: b/t_{peak}). Goodness of fit of the fitted functions was expressed as r^2.

The mean numbers of responses and reinforcers per session were calculated for the last ten sessions, and were compared with the corresponding data from the control group (see below).

4.2.5.2.   VI 75 s

The mean numbers of responses and reinforcers obtained per session were calculated for the last 10 sessions, and were compared with the corresponding data obtained from the group trained under the FIPP using Student's t-test (criterion, $p < 0.05$).

4.2.5.3.   Immunohistochemistry data

The data were analysed in the same way as in Experiment 1.

4.3   Results

One subject in the VI 75 s group was removed from the analysis because it failed to develop stable response rates within trials. The analyses are therefore based on data from 12 rats trained under the FIPP and 11 rats trained under the VI schedule.

4.3.1   Behavioural data

4.3.1.1   FIPP 30 s

The group mean data obtained in the peak trials are shown in Fig 4.1 (filled circles). The peak of the response rate function occurred close to the time at which reinforcement became available in the FI trials.

Table 4.1 shows the group mean (±S.E.M.) values of the timing parameters derived from fitting the modified Gaussian function to the data from the individual rats. The peak time (t_{peak}) (26.1 s) was close to the scheduled reinforcement time (30 s) and the Weber fraction was approximately 0.76. Peak response rate was 38.7±9.7 and goodness of fit (r^2) was 0.895.
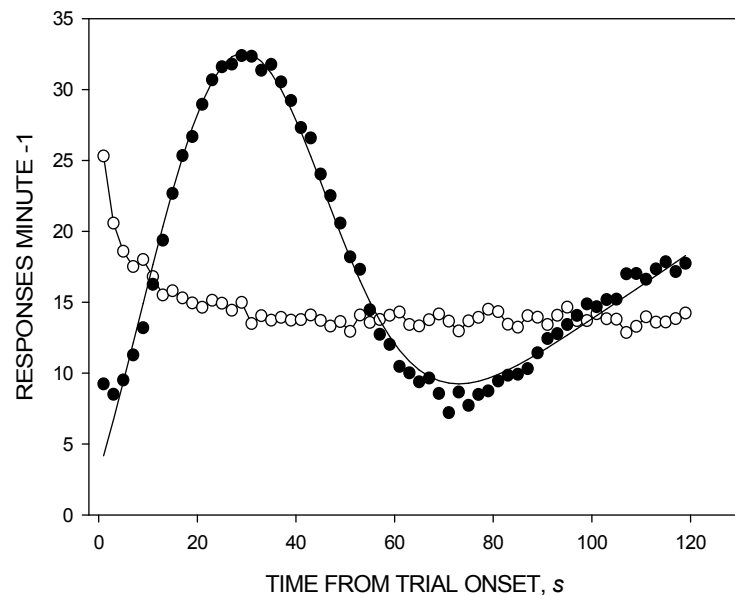
Figure 4.1 Comparison of performance on FIPP 30 s and VI 75 s Ordinate: absolute response rate (responses minute[-1]); abscissa: time from trial onset (s). Points are group mean data from successive 2-s time bins in the last ten sessions of the experiment: open circles, rats trained under VI 75 s; filled circles, rats trained under FIPP 30 s. The continuous curve is the best-fit 'modified Gaussian function' (see text). Note the absence of temporal differentiation of responding in rats trained under the VI schedule.

Table 4.1 The group mean (±S.E.M.) values of the timing parameters derived from fitting the modified Gaussian function to the data from the individual rats during the last ten sessions of training under the FIPP.

t_{peak} (s)	Weber fraction	r^2	SD of the Gaussian component	Peak response rate (responses min^{-1})
26.1±1.7	0.76 ±0.10	0.90 ± 0.02	18.7±0.9	38.7±9.7

4.3.1.2.   VI 75 s

Figure 4.1 (open circles) shows the response rates of the rats trained under the VI 75 s schedule. Response rate tended to be highest at the start of the trial, and then declined over approximately 8-10 s to a level that was maintained until the end of the trial.

4.3.1.3.   Comparison of performance under the FIPP and VI schedules

Table 4.2 shows the mean number of responses and reinforcers obtained during the last ten sessions. There were no significant differences in either the total number of responses or the total number of reinforcers between the two groups (*t*-test, *P*>0.1), indicating that the groups were well matched on these two measures.

Table 4.2 The mean number of responses and reinforcers (±S.E.M.) obtained during the last ten sessions.

	Schedule	
Performance measure	FIPP 30 s	VI 75 s
Total responses per session	1187.5 ± 225.4	952.7 ± 98.9
Total reinforcers per session	29.6 ± 0.1	30.0 ± 0.5

4.3.2   Immunohistochemistry data

Figure 4.3 shows the group mean (± S.E.M.) numbers of Fos-positive neurones in cortical and striatal areas of the rats trained under the FIPP 30 s and VI75 s schedules. MANOVA revealed a significant difference between the two groups in the OPFC

$[F(1,20) = 4.8, p < 0.05]$, higher levels of Fos expression being seen in the rats trained under FIPP 30 s than in those trained under VI 75 s. Representative examples of coronal sections showing Fos expression in the OPFC of a rat from each experimental group are given in Figure 4.4.

4.4 Discussion

The purpose of this experiment was to examine whether the performance of rats on the FIPP was associated with increased neuronal activity in cortical and striatal areas, as revealed by Fos expression, in comparison with a control group of rats trained under a
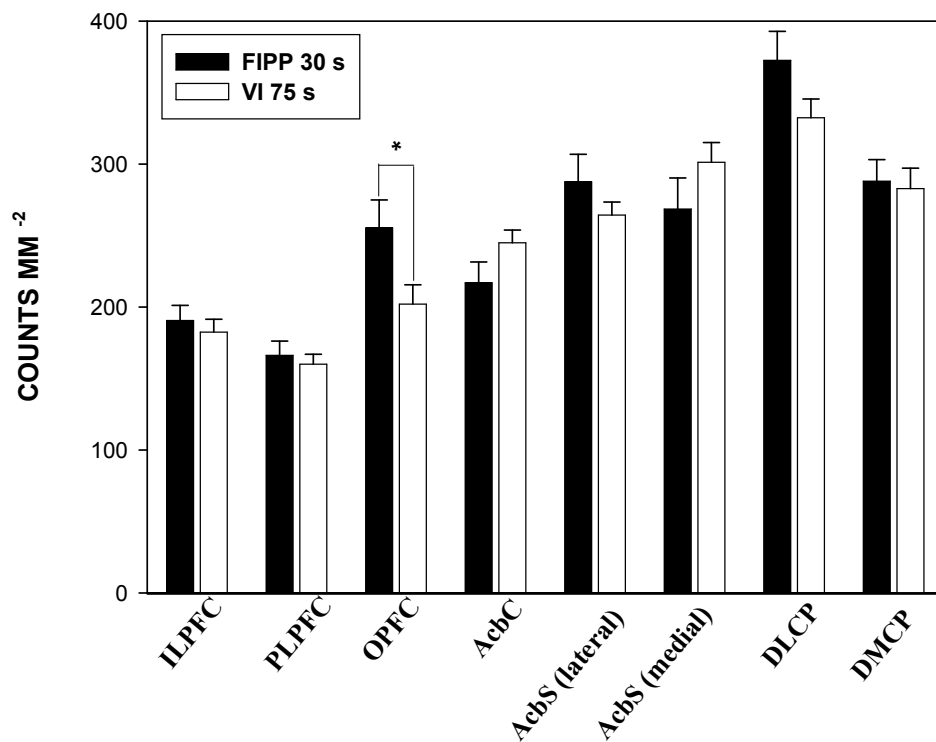
Figure 4.3. Concentration of Fos-positive units (counts.mm^{-2}) counted in the cortical and striatal regions. The conventions are the same as in Figure 2.3. Columns show the group mean data (+ S.E.M.) in each area for the rats trained under the FIPP (filled columns) and the VI (empty columns). * Significant difference between the groups, $p \leq 0.05$.

OPFC

FIPP 30 s   VI 75 s
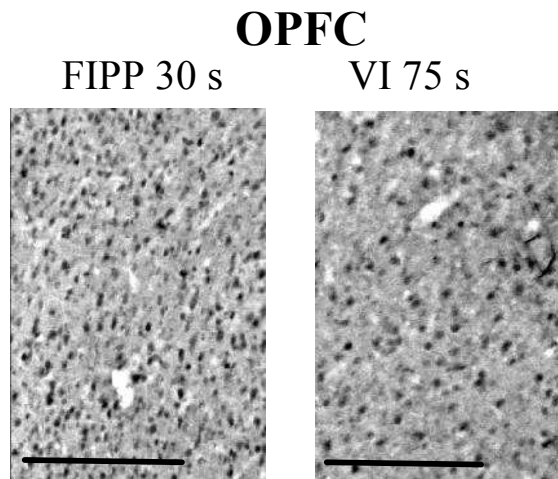
Figure 4.4. Examples of Fos expression in the OPFC in a representative rat from each group. Note the greater number of Fos-positive units (dark points) in the sample taken form the rat trained under FIPP compared to the rat exposed to VI; bar, 50 μm.

VI schedule that was matched to the FIPP in terms of food deprivation conditions, response requirements, and overall number of responses and reinforcers per session.

The results of this experiment showed that the performance on FIPP was similar to that reported in previous studies (Church and Broadbent 1990; Orduña et al. 2008; Roberts 1981; 1982). Responding increased to a maximum at about the scheduled reinforcement time and then decreased in a more or less symmetrical way. Performance under this schedule was well described by the 'modified Gaussian function' ('Gaussian plus ramp' function: MacDonald and Meck 2005). The peak time was around the time at which the reinforcer was delivered in the FI trials, and the Weber fraction was within the range of most of the reports in the literature (Roberts 1981).

Performance under the VI schedule was similar to that reported in previous studies (Catania and Reynolds 1968). A relatively constant rate of responding was maintained throughout each interval. Comparison of performance on FIPP and VI demonstrated a lack of temporal differentiation in the latter. In a VI schedule, a response at a given time after reinforcement is reinforced in some intervals, but not in others. The major difference between FI and VI schedules is that FI schedules provide reinforcement at a specific point along a temporal continuum, whereas a VI schedule provides reinforcement at unpredictable points (Catania and Reynolds 1968; Skinner 1958).

Consistent with the results obtained in Experiments 1 and 2, the concentration of Fos-positive neurones (counts.mm^{-2}) in the OPFC was greater in the rats exposed to FIPP than in the rats exposed to the VI schedule, suggesting a greater activation of this

area during the performance of the former task. This is consistent with the results of previous studies that have implicated the prefrontal cortex in temporal differentiation. For example, it has been reported that lesions of different subregions of the frontal cortex can disrupt the performance, or slow the acquisition of timing behaviour of rats on FIPP (Dietrich and Allen 1998; Meck 2006a, see also section 1.4.4). The observed differences in Fos expression between the two groups are unlikely to have been brought about by differences in reinforcer consumption or repetitive execution of the operant responses, since the control group trained under the VI schedule underwent the same food deprivation conditions as the group trained under the FIPP, and the mean number of responses emitted and reinforcers obtained did not differ between the two groups.

It has been reported that the dorsal striatum plays an important role in timing behaviour. Lesions of the dorsal striatum have been found to disrupt timing behaviour on the FIPP (Meck 2006b), and striatal neurones recorded during the performance of a temporal generalization task using a multiple-duration FI procedure showed progressive increases in the firing rate which reached a peak at the expected time of reinforcement (Matell et al. 2003). The present results, however, do not provide any evidence for a specific involvement of the dorsal striatum in FIPP performance, since no significant differences in the number of Fos-positive cells were found between the rats performing the timing task and the control group.

Experiments 1 and 2 showed that the concentration of Fos-positive neurones in the nucleus accumbens was significantly higher in the rats performing retrospective timing tasks than in the control groups trained under non-timing discrimination tasks. However, the present experiment did not find a significant difference between groups with respect to this area.

One conspicuous difference between the immunohistochemical results obtained in this experiment compared to the results of Experiments 1 and 2 is that the absolute numbers of Fos-positive neurones in the ILPFC and PLPFC, as well as the ventral and dorsal striatum, were considerably higher in the present experiment than in Experiments 1, and 2. One possible explanation is that the higher rates of responding generated by the FIPP and VI schedules, compared to schedules that employ discrete trials may have produced more activation in these areas, and hence a higher levels of Fos expression. In particular, it is well known that the striatum is involved in locomotion, and that neurological diseases affecting it induce movement disorders (Albin et al. 1989; DeLong 1990; Gerfen and Engber 1992). Liste et al. (1997) found that Fos expression in the

ventral and dorsal striatum of rats was enhanced following locomotion in a treadmill. It is possible that, in the present experiment, movement-induced Fos expression may have masked any increase in Fos expression induced by the temporal differentiation performance.

Most timing schedules involve multiple behavioural processes, and it is unlikely that one single experiment will isolate the temporal processes from other processes that may be controlled by different neuronal systems. It is clear that timing schedules differ from one another in a variety of respects, not only in the way temporal information is processed but also in the motor and perceptual requirements, and the intrinsic difficulty of learning the timing task. There is evidence that performance on different timing tasks involves different neural systems. For instance it has been reported that drugs acting at dopamine and 5-HT receptors can have qualitatively different effects on performance on immediate and retrospective timing schedules (Asgari et al. 2006a; Asgari et al. 2005; Asgari et al. 2006b; Body et al. 2002a; Body et al. 2002b; Chiang et al. 2000a; Chiang et al. 2000b). Taken together, the results of Experiments 1 and 2 and the present experiment suggest that the OPFC is engaged during timing performance in both retrospective and immediate timing schedules. Experiments 1 and 2 implicate the nucleus accumbens in temporal discrimination performance in retrospective schedules, whereas the present experiment did not provide supporting evidence for the involvement of the nucleus accumbens in temporal differentiation performance. None of the three experiments provided evidence supporting the involvement of the dorsal striatum in timing performance in either retrospective or immediate timing schedules. The negative evidence from these experiments should be treated with great caution, however, because it is not possible to exclude the possibility that small increments in Fos expression induced by timing performance may have been masked by larger increments induced by non-temporal aspects of schedule performance.

It may be of interest to consider the present results in the context of the putative role of the prefrontal cortex and ventral striatum in inter-temporal choice. Inter-temporal choice schedules have been regarded as a type of prospective timing task (Killeen and Fetterman 1988). Lesions of the OPFC have been found to disrupt inter-temporal choice behaviour (Cardinal et al. 2003; Kheramin et al. 2003; Kheramin et al. 2002; Mobini et al. 2002; Winstanley et al. 2004). Interpretation of these findings is complicated by the fact that most inter-temporal choice schedules entail choice between reinforcing outcomes that differ with respect to both the delays and the sizes of the primary

reinforcers, suggesting that these schedules involve the two processes of delay discounting and sensitivity to reinforcer magnitude (Ho et al. 1999). Quantitative studies based on a mathematical model of inter-temporal choice (Ho et al., 1999) have indicated that lesions of the OPFC disrupt both these processes (Kheramin et al. 2003; Kheramin et al. 2002). Moreover, it has recently been reported that Fos expression in the OPFC is elevated following performance of adjusting schedules based on choice between either delays or magnitudes of reinforcement (da Costa Araujo et al. 2010). The putative role of the ventral striatum in inter-temporal choice is controversial. Destruction of the AcbC has been found to alter inter-temporal choice in the direction of promoting preference for the smaller and earlier of two reinforcers (Acheson et al. 2006; Bezzina et al. 2007; Cardinal et al. 2001; da Costa Araujo et al. 2009), although the interpretation of these findings is unclear. In some cases the effect of AcbC lesions is attributable to alteration of the subjects' sensitivity to short-term changes in reinforcer delay or size (Acheson et al. 2006; Galtress and Kirkpatrick 2010). However, quantitative analysis of inter-temporal choice performance has indicated that, at least in some situations, destruction of the AcbC results in a selective increment in the rate of delay discounting (Bezzina et al. 2007; da Costa Araujo et al. 2009). Moreover it has recently been found that performance on an adjusting-delay schedule, but not an adjusting-magnitude schedule, is associated with increased Fos expression in the AcbC (da Costa Araujo et al. 2010). However, although the overlap between areas of neuronal activation in retrospective and immediate timing schedules, and inter-temporal choice schedules (Killeen and Fetterman 1988) is intriguing, it must be emphasized that the relationship between these types of adaptive behaviour is imperfectly understood (Gibbon et al. 1988; Killeen 2009). One of the goals of the present thesis is to examine the links between inter-temporal choice and timing behaviour. The following chapters will focus on some behavioural and neural features of inter-temporal choice. The question of whether interval timing and inter-temporal choice share a common basis will be considered in the General Discussion.

CHAPTER 5

EXPERIMENT 4:

TRANSITIONAL AND STEADY-STATE CHOICE BEHAVIOUR
UNDER AN ADJUSTING-DELAY SCHEDULE

5.1   Introduction

In an inter-temporal choice schedule, the subject chooses between reinforcers that differ with respect to their sizes and delays. For example, a subject may be confronted with two operanda, A and B; if it responds on A, a small reinforcer will be delivered after a short delay, whereas if it responds on B, a larger reinforcer will be delivered after a longer delay.

According to one model of inter-temporal choice behaviour (Mazur 1987; 2001; 2006), the reinforcing 'value' of each outcome is a declining hyperbolic function of the delay interposed between the response and the primary reinforcer.

$$V_A = q_A \cdot \frac{1}{1 + K.d_A} \quad ; \quad V_B = q_B \cdot \frac{1}{1 + K.d_B} , \qquad \text{[1a, 1b]}$$

where q_A and q_B are the sizes of the two primary reinforcers, d_A and d_B are the delays associated with each alternative, and K is a parameter expressing the rate of delay discounting (see section 1.3.2.1). It is assumed that when faced with a choice between A and B, the subject selects the outcome that has the higher overall value at the moment of choice. It should be noted that V refers not to the value of the primary reinforcer, but to the overall value of the conditioned reinforcing stimuli that are present at the moment of choice. Thus Equations 1a and 1b do not imply that the primary reinforcer itself is devalued as a function of delay (Mazur 1995).

Equations 1a and 1b imply direct proportionality between value and the size of a reinforcer. However, there is emerging evidence for a non-linear relation between value and reinforcer size (Mazur and Biondi 2009; Rickard et al. 2009), in keeping with the economic concept of diminishing marginal utility (Killeen 2009; Pine et al. 2009). Ho et al. (1999) proposed a modification of Equations 1a and 1b ($d_A = 0$) to take into account the possibility of such a non-linear relation ('multiplicative hyperbolic model' of inter-temporal choice [MHM]: see section 1.3.2.2). Ho et al. (1999) posited a hyperbolic relation, in which the effect of reinforcer magnitude on value is modulated by a single 'size-sensitivity parameter', Q:

$$V_A = \frac{1}{1 + Q/q_A} \cdot \frac{1}{1 + K.d_A} \quad ; \quad V_B = \frac{1}{1 + Q/q_B} \cdot \frac{1}{1 + K.d_B} . \qquad \text{[2a, 2b]}$$

By experimentally manipulating the delays and/or sizes of A and B, a point of indifference may be determined at which the subject shows no preference for either outcome. It is generally assumed that indifference implies that $V_A = V_B$. Equating the right-hand sides of Equations 2a and 2b and rearranging the terms yields the following linear relation between the 'indifference delay' to the larger reinforcer, $d_{B(50)}$, and the delay to the smaller reinforcer, d_A (Ho et al., 1999):

$$d_{B(50)} = \frac{1}{K} \cdot \left[\frac{Q/q_A - Q/q_B}{1 + Q/q_B} \right] + d_A \cdot \frac{1 + Q/q_A}{1 + Q/q_B}. \qquad [3]$$

This relation can be used to examine the effects of neurobiological interventions on the hypothetical processes of delay discounting and sensitivity to reinforcer size. For example, if the sizes of Reinforcers A and B (q_A and q_B) are held constant and indifference delays are determined for a series of delays to Reinforcer A (d_A), a change in the slope of Equation 3 induced by a cerebral lesion implies a change in Q (i.e. a change in sensitivity to reinforcer size), whereas a change in the intercept without a concomitant change in slope implies a change in K (i.e. a change in the rate of delay discounting) (Ho et al. 1999; Mazur 2006). Using this approach it has been found that excitotoxic and dopamine-depleting lesions of the orbital prefrontal cortex increase both Q and K (Kheramin et al. 2004; Kheramin et al. 2002), whereas destruction of the core of the nucleus accumbens or disconnection of the nucleus accumbens from the orbital prefrontal cortex produces a selective increase in K (Bezzina et al. 2008a; Bezzina et al. 2007). Equation 3 has also been applied successfully in experimental studies of inter-temporal choice in humans (Hinvest and Anderson 2010; Liang et al. 2010).

A significant practical difficulty with the application of Equation 3 is the length of time needed to collect sufficient data to fit the linear functions, because each value of $d_{B(50)}$ is derived from steady-state performance using a different value of d_A, which, in the case of animal subjects, may require 40-60 training sessions, and five or six $d_{B(50)}/d_A$ pairs are needed to obtain a reliable linear function (see, for example, Kheramin et al., 2002). Since neurobehavioural experiments involving cerebral lesions typically entail two or more groups of ten or more subjects, it is clear that this kind of experiment is very costly in terms of both time and money.

One purpose of this chapter is to describe an abbreviated approach to estimating Q and K based on Equation 3, which requires the determination of only two values of $d_{B(50)}$. The logic of the method is as follows. If the smaller of the two reinforcers is delivered immediately (i.e. $d_A \approx 0$), Equation 3 becomes

$$d_{B(50)} = \frac{1}{K} \cdot \left[\frac{Q/q_A - Q/q_B}{1 + Q/q_B} \right]. \qquad [3a]$$

Equation 3a is the limit of Equation 3 as $d_A \rightarrow 0$. If two indifference delays are determined using different pairs of reinforcer sizes (q_{A1}, q_{B1}, and q_{A2}, q_{B2}), then the ratio of the indifference delays is

$$\frac{d_{B(50)1}}{d_{B(50)2}} = \frac{1/q_{A1} - 1/q_{B1}}{1/q_{A2} - 1/q_{B2}} \cdot \frac{1 + Q/q_{B2}}{1 + Q/q_{B1}} \quad . \qquad [4]$$

If reinforcer sizes are selected such that $(1/q_{A1} - 1/q_{B1}) = (1/q_{A2} - 1/q_{B2})$, Equation 4 simplifies to

$$\frac{d_{B(50)1}}{d_{B(50)2}} = \frac{1 + Q/q_{B2}}{1 + Q/q_{B1}}, \quad \text{or} \quad Q = \frac{d_{B(50)1}/d_{B(50)2} - 1}{1/q_{B2} - (d_{B(50)1}/d_{B(50)2})/q_{B1}}. \qquad [4a]$$

Q may thus be estimated empirically from the ratio of the indifference delays, and this estimate of Q may be substituted into Equation 3a in order to derive an estimate of K for each value of $d_{B(50)}$.

The derivation of Equations 3 and 4 is based on an assumed hyperbolic relationship between value and reinforcer size, as postulated in Equations 2a and 2b (Ho et al., 1999). However a simpler version of Equation 4 may be derived from Equations 1a and 1b, which assume strict proportionality between value and reinforcer size. In this case, the ratio of two indifference delays is given by

$$\frac{d_{B(50)1}}{d_{B(50)2}} = \frac{q_{B1} - q_{A1}}{q_{A1}} \cdot \frac{q_{A2}}{q_{B2} - q_{A2}} \qquad [5]$$

Equation 5 contains no free parameters, and therefore yields a specific numerical prediction for the ratio of the two indifference delays. A second aim of this experiment was to examine whether the empirically obtained ratios of the indifference delays would be compatible with this prediction.

In the present experiment, indifference delays were determined using an adjusting-delay schedule (Mazur 1987). In this schedule the delay to the larger of two reinforcers varies in accordance with the subject's choice. For example, if, in a block of trials, the subject shows a preference for the larger reinforcer (B), the delay to that reinforcer is increased in the following block; conversely, if it shows a preference for the smaller reinforcer (A), the delay to Reinforcer B is reduced in the following block (see section 1.2.3.1). The principal dependent variable, the adjusting delay to the larger reinforcer (d_B), is seen to oscillate during an extended period of training, the amplitude of oscillation gradually declining as d_B approaches a quasi-stable value; this quasi-stable value of d_B is usually taken to represent the indifference delay, $d_{B(50)}$ (Mazur 1987; 1988). Adjusting-delay schedules have been used extensively in behaviour analytic studies of inter-temporal choice (e.g. Green et al. 2007; Mazur 1987; 1988; 1994; 1995; 1996; 2000; 2006; 2007), but less often in neurobehavioural experiments (da Costa Araujo et al. 2009; Mobini et al. 2000). A potential advantage of adjusting delay schedules in neurobehavioural investigations of inter-temporal choice is that in addition to generating quasi-stable indifference delays, the pattern of oscillation of the adjusting delay may also provide information about the effects of neurobiological interventions on the organism's adaptation to changing delays to reinforcement. An additional purpose of this chapter is to describe a novel way of quantifying transitional behaviour in the adjusting-delay schedule based on analysis of the power spectrum of cyclical changes in the adjusting delay.

5.2.   Materials and methods

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

5.2.1.   Subjects

Twelve female Wistar rats aged approximately 4 months and weighting 250–300 g at the

start of the experiment were used. They were housed individually under the same conditions as in Experiment 1 (see section 2.2.1.).

5.2.2. *Apparatus*

The rats were trained in standard operant conditioning chambers (CeNeS Ltd, Cambridge, UK) of internal dimensions 25 × 25 × 22 cm. One wall of the chamber contained a central recess covered by a hinged clear Perspex flap, into which a peristaltic pump could deliver a 0.6 M sucrose solution. Two apertures situated 5 cm above and 2.5 cm to either side of the recess allowed insertion of motorized retractable levers (CeNeS Ltd, Cambridge, UK) into the chamber. The levers could be depressed by a force of approximately 0.2 N. The 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.

5.2.3. *Behavioural training*

At the start of the experiment the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press two levers (A and B) for the sucrose reinforcer (50 μl, 0.6 M), and were exposed to a discrete-trials continuous reinforcement schedule in which the two levers were presented in random sequence for three sessions. Then they underwent daily 42-minute training sessions under the discrete-trials adjusting-delay schedule for the remainder of the experiment. Each experimental session consisted of seven blocks of four trials. The trials were 90 s in duration. The first two trials of each block were forced-choice trials in which each lever was presented alone in random sequence. The other two trials were free-choice trials in which both levers were presented. The beginning of each trial was signaled by illumination of the central light above the reinforcer recess. After 2.5 s the lever or levers (depending on the type of trial) were inserted into the chamber. When a lever press occurred, the lever(s) were withdrawn, the central light was extinguished, and the light located above the lever that had been depressed was illuminated. This light remained illuminated until the delivery of the

reinforcer, and was then extinguished. The chamber remained in darkness until the start of the following trial. If no lever press occurred within 5 s of the lever(s) being inserted, the lever(s) were retracted and the central light extinguished. (This seldom happened except during the first few training sessions.) A response on Lever A resulted in immediate delivery of the smaller reinforcer, of size q_A (i.e. $d_A \approx 0$). A response on Lever B initiated a delay d_B whose duration was increased or decreased systematically from one trial block to the next as a function of the choices in the prior block; at the end of this delay the larger reinforcer, of size q_B, was delivered. The positions of Levers A and B (left *vs.* right) were counterbalanced across subjects.

In each block of trials, the delay to the larger reinforcer, d_B, was determined by the rat's choices in the free-choice trials in the preceding block. If Lever A was chosen in both free-choice trials of block *n*, d_B was reduced by 20% in block *n*+1; if Lever B was chosen in both free-choice trials of block *n*, d_B was increased by 20% in block *n*+1; if Lever A and Lever B were each chosen in one free-choice trial in block *n*, d_B remained unchanged in block *n*+1. The value of d_B in the first block of each session was determined in the same way by the choices made in the final block of the previous session. Maximum and minimum values of d_B were set at 60 s and 0.75 s.

The experiment consisted of two Phases (I and II), the first lasting 100 sessions and the second 40 sessions. There were two experimental Conditions (1 and 2); for half the rats Condition 1 was in effect in Phase I and Condition 2 in Phase II; for the other rats the order of conditions was reversed. In Condition 1, the sizes of the two reinforcers (volume of 0.6 M sucrose solution) were $q_{A1} = 25$ µl and $q_{B1} = 100$ µl; in Condition 2, the sizes of the reinforcers were $q_{A2} = 14$ µl and $q_{B2} = 25$ µl. In the first block of the first session of each phase, d_B was set at 0.75 s. Phase I continued for 100 sessions and Phase II for 40 sessions.

Experimental sessions were carried out 7 days a week, at the same time each day, during the light phase of the daily cycle (between 0800 and 1400 hours).

5.2.4　　　　*Data analysis*

5.2.4.1　　　　Indifference delays and parameter estimation

For each rat, the mean value of d_B in the last ten sessions of each phase was taken as the indifference delay, $d_{B(50)}$. These data were analysed by a two-factor analysis of variance

(condition [1,2] × order of condition [1-first *vs* 2-first]) with repeated measures on the former factor. As this analysis showed no significant main effect of order and no significant order × condition interaction, the 'order' factor was ignored in all further treatment of the data. The ratio of the values of $d_{B(50)}$ obtained under the two conditions was calculated for each rat, and these values were used to calculate estimates of Q as described in the Introduction, using the following formula which comes from substitution into Equation 4a

$$Q = \frac{d_{B(50)1} / d_{B(50)2} - 1}{1/25 - (d_{B(50)1} / d_{B(50)2})/100} ,$$

100 and 25 being the sizes of Reinforcer B (volumes of 0.6 M sucrose, in μl) in the two conditions of the experiment (q_{B1} and q_{B2}, respectively). This estimate of Q was used to derive an estimate of K for each rat by substitution into Equation 3a.

The obtained ratios of the indifference delays were also compared with the ratio predicted on the basis of an assumed linear relation between reinforcer size and instantaneous value. Based on Equation 1, the indifference delay is

$$d_{B(50)} = \frac{1}{K} \cdot \frac{q_B - q_A}{q_A} .$$

Substituting the actual reinforcer sizes used in this experiment into this equation, the ratio of the indifference delays should be

$$\frac{d_{B(50)1}}{d_{B(50)2}} = \frac{100 - 25}{25} \cdot \frac{14}{25 - 14} = 3.81 .$$

(cf. Equation 5: Introduction). The obtained ratios were compared with this theoretical value using Student's *t*-test.

5.2.4.2   Transitional behaviour

In order to characterize the pattern of oscillation of d_B during the course of training, a

power spectrum analysis was carried out on the values of d_B obtained in each trial block during each phase of the experiment. The method is illustrated in Figure 5.1.
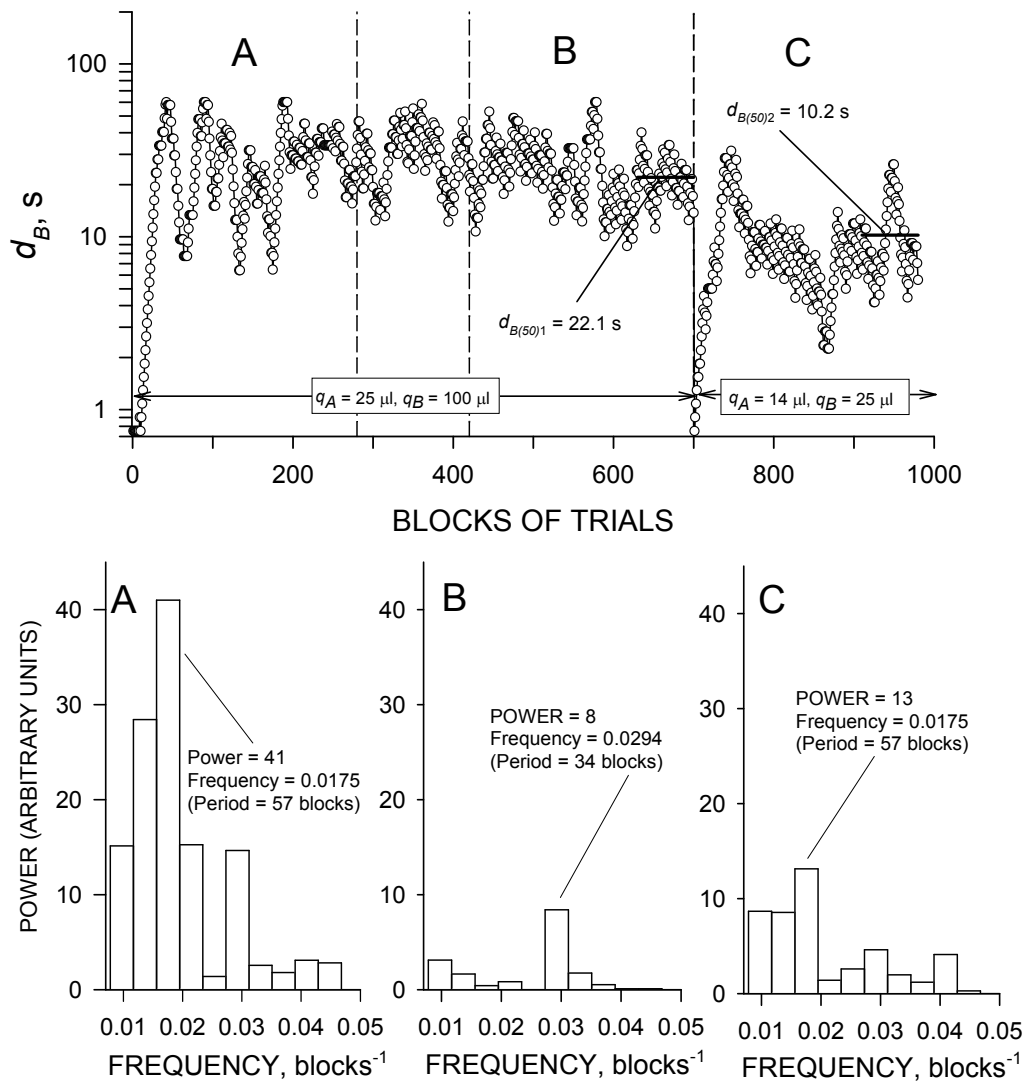
Fig. 5.1. Example of one rat's performance on the adjusting-delay schedule during the entire experiment, illustrating the methods of data analysis. *Upper graph:* Adjusting delay to the larger reinforcer (d_B, s) plotted against blocks of trials in the two phases of the experiment. In Phase I (trial blocks 1-700), the sizes of the reinforcers (q_A, q_B) were 25 and 100 μl of a 0.6 M sucrose solution; in Phase II they were 14 and 25 μl. The segments A, B, and C, demarcated by the broken lines, comprise the first (A) and final (B) 280 blocks of Phase I and the 280 blocks of Phase II (C) that were used in the Fourier transform analysis. The short horizontal lines indicate the mean values of d_B in the final 10 sessions (70 blocks) of the two phases ($d_{B(50)1}$ and $d_{B(50)2}$). *Lower panels:* Power spectra derived from Fourier transform analysis of the d_B data from segments A, B, and C (see above). Power is plotted against frequency (blocks^{-1}). The period of oscillation corresponding to the dominant frequency band, and the power within that band are shown for each segment.

Plots were obtained of log d_B *vs.* blocks of trials (Phase I, 700 blocks; Phase II, 280 blocks: see upper panel of Figure 5.1). These data, expressed as deviations from the mean value of d_B, were subjected to a Fourier transform (Spike-2, version 4.23: Cambridge Electronic Design, Ltd) in order to derive power spectra (power *vs* frequency: see lower panels of Figure 5.1). The reciprocal of the frequency is the cycle time (period) of oscillation of d_B, in blocks. The power of the dominant frequency of the spectrum within the frequency range of 0.01 (period = 100 blocks) and 0.04 (period = 25 blocks) and the length of the period corresponding to the dominant frequency were derived for each rat in each phase of the experiment (da Costa Araújo et al.,2009). Inspection of the data indicated that the amplitude of oscillation of d_B declined during the 100 sessions of Phase I. This impression was tested by comparing the power spectra derived from the first and final 280 blocks of trials of Phase I; comparisons were also made between the spectra derived from the final 280 blocks of Phase I and the 280 blocks that comprised Phase II.

5.3 Results

5.3.1 Indifference delays and parameter estimation

Figure 5.2 (left-hand panel) shows the values of $d_{B(50)}$ derived from the last ten sessions of each phase. In all but one of the twelve rats, the value of $d_{B(50)1}$ (Condition 1: $q_{A1} = 25$ µl, $q_{B1} = 100$ µl) was higher than that of $d_{B(50)2}$ (Condition 2: $q_{A2} = 14$ µl, $q_{B2} = 25$ µl). Analysis of variance indicated that there was a significant effect of condition [$F(1,10) = 28.3$, $p < 0.001$], but no significant effect of the order of conditions [$F(1,10) = 1.3$, NS] and no significant interaction [$F(1,10) = 2.7$, NS]. Accordingly, the data from all 12 rats were pooled in all subsequent analyses. Figure 5.2 (right-hand panel) shows the ratios of the two values of $d_{B(50)}$; the horizontal line indicates the ratio predicted on the basis of an assumed linear relation between reinforcer size and reinforcer value (3.81: see Data analysis section in the Method). The observed ratio (mean ± SEM: 2.34 ± 0.19) was significantly lower than the predicted ratio [$t(11) = 7.6$, $p < 0.001$].
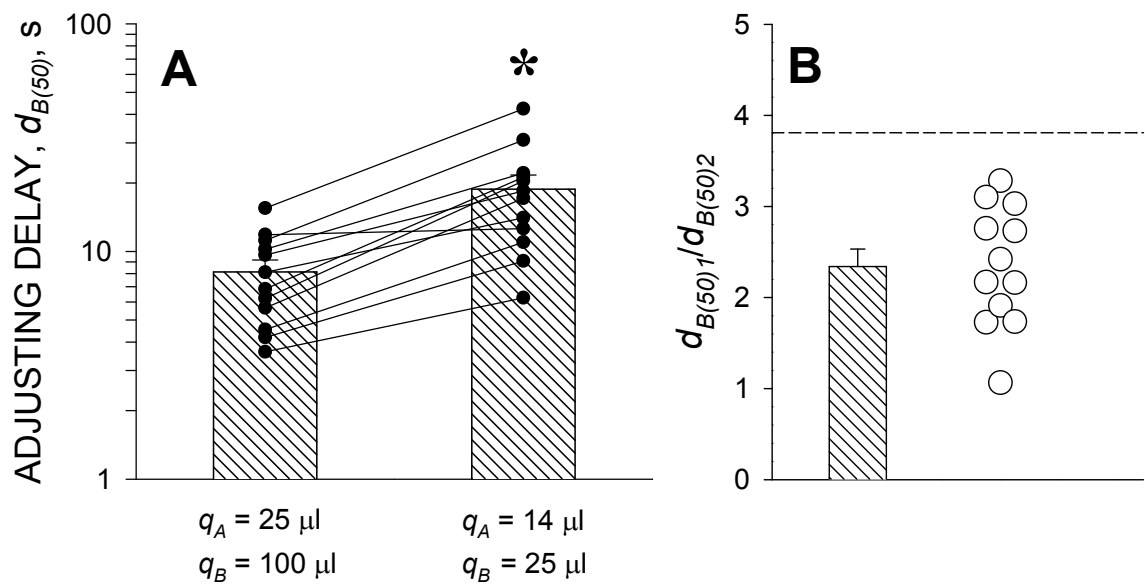
Fig. 5.2 Values of $d_{B(50)}$ (s) obtained under the two conditions. Columns are group mean data; connected points are data from individual rats. $d_{B(50)}$ was significantly longer under Condition 1 (reinforcer sizes: $q_A = 25$ μl, $q_B = 100$ μl) than under Condition 2 (reinforcer sizes: $q_A = 14$ μl, $q_B = 25$ μl). *B*. Ratio of the values of $d_{B(50)}$ obtained under the two conditions. Column shows the group mean ratio (+SEM); open circles show data from individual rats. Horizontal broken line shows the expected ratio based on the assumption that reinforcer value is linearly related to reinforcer size (see text).

The ratios of the $d_{B(50)}$s were used to compute estimates of the two parameters of Equation 2, *Q* and *K*. The results of this analysis are shown in Figure 5.3. There was one clear outlier in the case of both parameters, this being the rat that showed no difference between the $d_{B(50)}$s in the two conditions (see above). The parameter that expresses sensitivity to reinforcer size (*Q*) was derived by substitution of the ratio of the indifference delays into Equation 4a. The group mean value of *Q* (± SEM) was 113.8 ± 27.9 µl. Estimates of the delay discounting parameter (*K*) were derived by substituting each rat's estimated value of *Q* into Equation 3a. The group mean value (± SEM) was 0.082 ± 0.012 s^{-1}.

5.3.2   *Transitional behaviour*

In all twelve rats, the adjusting delay to the larger reinforcer, d_B, showed an oscillating pattern of change during the early stages of training, the amplitude of the oscillations tending to decline during extended training (see Figure 5.1 for an example).
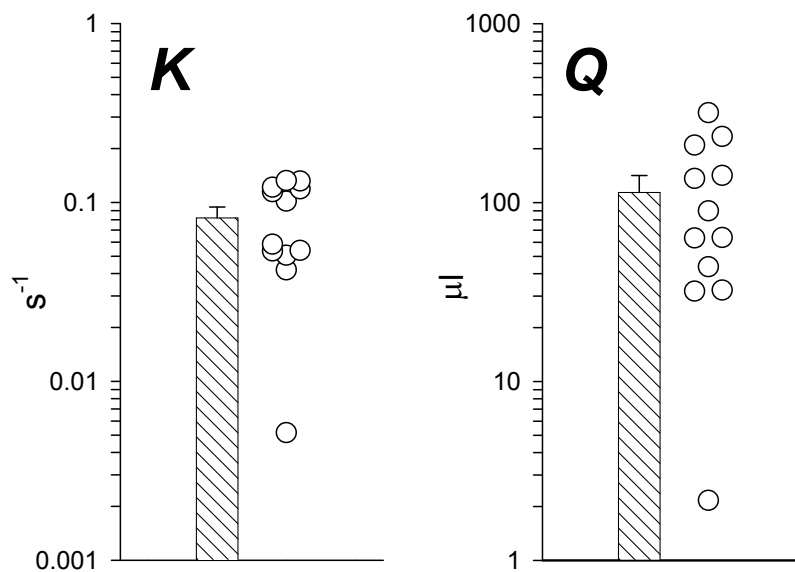
Fig. 5.3. Estimates of the parameters of Equation 2 expressing the rate of delay discounting, K (s^{-1}), and sensitivity to reinforcer size, Q (µl of the 0.6 M sucrose solution). Columns are group mean data (+SEM); open circles show estimates for individual rats. (In the case of both parameters, the outlier is the rat that produced the lowest ratio of the two values of $d_{B(50)}$: see Figure 5.2).

Power spectra were derived for each rat's data from three segments of training: the first and last 280 trial blocks of Phase I and the 280 blocks of Phase II. The power in the dominant frequency band and the period corresponding to the dominant frequency from each segment are shown in Figure 5.4 (upper panels: rats exposed to Condition 1 [q_{A1} = 25 µl, q_{B1} = 100 µl] in Phase I and Condition 2 [q_{A2} = 14 µl, q_{B2} = 25 µl] in Phase II; lower panels: rats exposed to the two conditions in the reverse order).

There was a consistent trend for the power in the dominant frequency to be higher on the initial segment of the first phase (Figure 5.4, left panels). Analysis of variance showed a significant main effect of segment [$F(2,20)$ = 29.7, $p < 0.001$], but no significant effect of group [$F(1,10)$ = 4.1, NS] and no significant interaction [$F(2,20)$ = 2.9, NS]. The power declined in the last segment of Phase I and in Phase II compared to the initial segment of Phase I (multiple comparisons with the Least Significant Difference test, $p<0.01$ in each case). The period corresponding to the dominant frequency (Figure 5.4, right panels) showed no significant effects of segment or group, and no significant interaction [all Fs < 1].
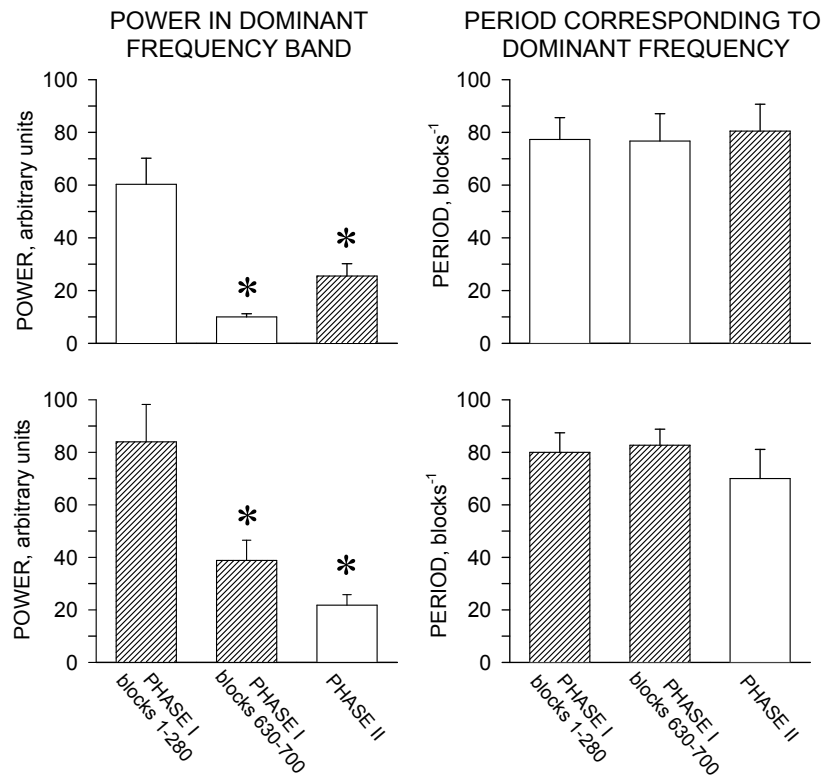
Fig. 5.4. Results of the power spectrum analysis. Comparison of power in the dominant frequency band and period of oscillation corresponding to the dominant frequency seen under Conditions 1 (open columns; reinforcer sizes: q_A = 25 µl, q_B = 100 µl) and 2 (shaded columns; reinforcer sizes: q_A = 14 µl, q_B = 25 µl). Columns show group mean data (+ SEM). In both groups power was significantly less in the final segment of Phase I and Phase II than in the initial segment of Phase I (* p <0.05). The period of oscilation did not vary significantly across the three segments.

Figure 5.5 shows the individual-subject data and the mean data from the rats in both groups in the terminal segment of Phase I and the segment comprising Phase II. Comparisons between the two conditions showed that power was significantly higher in Condition 2 than in Condition 1 [$t(11)$ = 3.7, p < 0.01], but there was no significant difference between period in the two conditions [t < 1].

5.4   Discussion

5.4.1   Indifference delays and parameter estimation

The quasi-stable adjusting delays seen during the last ten days of training under each condition were taken as indifference delays, $d_{B(50)}$. The value of $d_{B(50)}$ was higher in Condition 1, when the reinforcer sizes were 25 and 100 µl of the sucrose solution, than

in Condition 2, when they were 14 and 25 µl. This was an expected result, because $d_{B(50)}$ is assumed to depend on the relative instantaneous value of reinforcer B (relative to reinforcer A), which was higher under Condition 1 than under Condition 2 (cf. Equation 3).
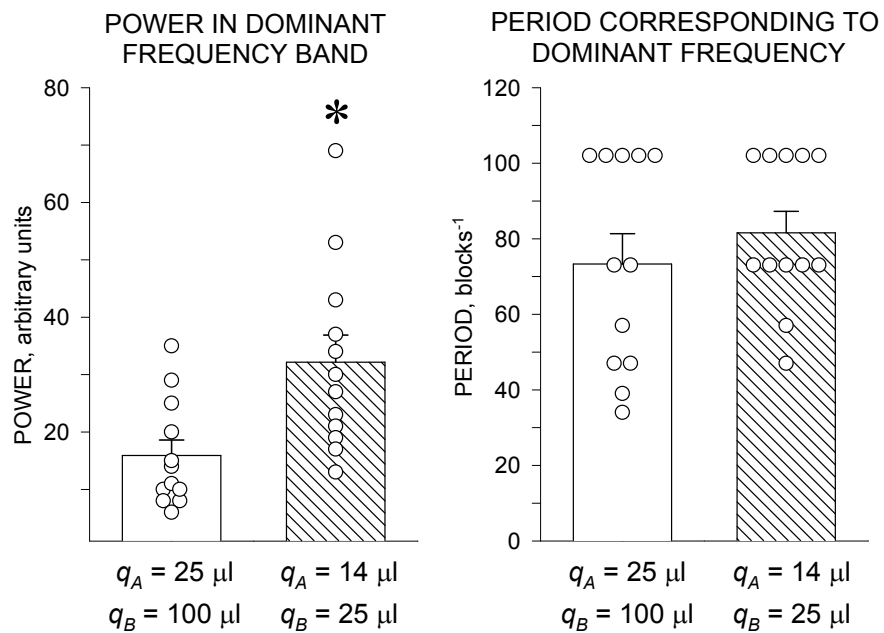
Fig. 5.5. Results of the power spectrum analysis. Comparison of power in the dominant frequency band and period of oscillation corresponding to the dominant frequency seen under Conditions 1 (open columns; reinforcer sizes: q_A = 25 µl, q_B = 100 µl) and 2 (shaded columns; reinforcer sizes: q_A = 14 µl, q_B = 25 µl). Columns show group mean data; open circles show data from individual rats. Power was significantly higher under Condition 2 than under Condition 1 (* p <0.05); there was no difference between the period of oscillation under the two conditions.

The ratio of the indifference delays, $d_{B(50)1}/d_{B(50)2}$, was compared with a predicted value of 3.81, which was calculated from the physical sizes of the reinforcers, based on the assumption that instantaneous reinforcer value is linearly related to reinforcer size (cf. Equation 1). The observed ratios were consistently smaller than 3.81, suggesting a non-linear relation between size and value. A non-linear relation is assumed by the model of inter-temporal choice proposed by Ho et al. (1999), which formed the basis of the method adopted here to derive numerical estimates of discounting parameters. According to this model, instantaneous reinforcer value is hyperbolically related to

reinforcer size, the relation being defined by a single free parameter, Q, which specifies the reinforcer size corresponding to the half-maximal value. Note that other non-linear size/value functions have recently been proposed, for example, by Killeen (2009) and Pine et al. (2009).

The present experiment employed two pairs of reinforcer sizes that allowed Q to be determined from the ratio of the two indifference delays (Equation 4a). It is of interest to compare the value of Q obtained using this approach (mean = 113.8 μl) with a value of Q derived using a different approach. Rickard et al. (2009) trained rats under a progressive-ratio schedule using a wide range of reinforcer sizes (different volumes of a 0.6 M sucrose solution). Response rates in successive ratios were analysed using an equation derived from Killeen's (1994) Mathematical Principles of Reinforcement. The 'specific activation' parameter of Killeen's model (a), which is presumed to reflect the incentive value of the reinforcer, was a monotonically increasing function of reinforcer volume. A hyperbolic function was fitted to the relation between a and reinforcer volume, from which it was determined that the value of Q was 158.9 μl. The fact that these radically different methods yielded numerical estimates of Q that were in same order of magnitude inspires some confidence in the general utility of this parameter as a descriptor of sensitivity to reinforcer size.

By substituting the estimates of Q into Equation 3, it was possible to derive estimates of the delay-discounting parameter, K. The mean value of K (0.082 s^{-1}) was similar to values of this parameter obtained in previous experiments with rats (approximately 0.1 s^{-1}: Green et al. 2004; Mazur 2007; Mazur and Biondi 2009).

5.4.2   Methodological considerations

The derivation of Equation 4 entails the simplifying assumption that when no delay is scheduled for Reinforcer A, $d_A = 0$. In fact, a brief delay necessarily occurs between the initiation of the reinforcer delivery and the subject's consumption of the reinforcer. Informal observation of rats trained under the present procedure suggests that this delay is in the order of half a second, and therefore it is unlikely that the approximation $d_A = 0$ significantly compromises the validity of Equation 4. Moreover, there is no measurable delay between the response on Lever A and the presentation of exteroceptive stimuli

associated with reinforcer delivery, which presumably acquire some conditioned reinforcing properties.

It must be pointed out that the algebraic substrate of the present method imposes some restrictions on the range of reinforcer sizes that may be employed. For example, in the present experiment we set $q_{B2} = q_{A1}$ (25 µl) and $q_{B1} = 4.q_{A1}$ (100 µl); the value of q_{A2} required in order to preserve the equality $(1/q_{A1} - 1/q_{B1}) = (1/q_{A2} - 1/q_{B2})$ was approximately 14 µl. Other reinforcer sizes might have been used, but the choice is not limitless. For example, instead of determining the required value of q_{A2} for given values of q_{B1}, q_{A1} and q_{B2}, the value of q_{A2} might have been pre-selected, and the required value of q_{B2} calculated accordingly. In this case, when $q_{B2} = q_{A1}$, the chosen value of q_{A2} would have had to have been less than $q_{B2}/2$, otherwise an appropriate value of q_{B1} could not have been found (e.g., if $q_{A2} = 25$, and $q_{B2} = q_{A1} = 50$, $q_{B1} = \infty$). Choosing convenient values of q_{A1}, q_{B1} and q_{B2} and calculating the required value of q_{A2} circumvents this limitation. Figure 5.6A shows the required values of q_{A2} for a range of values of q_{B2}, when q_{B1} is set at 100 and q_{A1} is set at 12.5, 25 or 50; the points indicate the values corresponding to the particular condition used in the present experiment, in which $q_{A1} = q_{B2}$. The figure shows that despite the limitation outlined above, the method can in principle accommodate a broad range of reinforcer sizes.

The equations also impose constraints on the range of $d_{B(50)}$ ratios that can generate meaningful values of the parameter Q. Inspection of Equation 4a shows that the range of 'allowable' ratios has a lower boundary of 1.0 and an upper boundary defined by q_{B1} and q_{B2}. If $(d_{B(50)1}/d_{B(50)2})/q_{B1} = 1/q_{B2}$, the recovered value of Q is ∞; higher $d_{B(50)}$ ratios yield negative values of Q, which are, of course, meaningless. The relation between Q and $d_{B(50)1}/d_{B(50)2}$ is illustrated in Figure 5.6B. Setting q_{B1} at 100 and $q_{B2}=25$ (the values used in the present experiment), meaningful values of Q require $d_{B(50)}$ ratios <4 (for $q_{B2}=50$ the upper limit is <2; for $q_{B2}=12.5$, it is <8). Within a substantial proportion of the range of 'allowable' ratios (approximately $1.5 - 3.5$ in the present instance), the relation between log Q and the $d_{B(50)}$ ratio is approximately linear. As shown in Figure 5.6B, the data from 11 of the 12 rats in this experiment fell within this band. The nature of the relation between the $d_{B(50)}$ ratio and Q dictates that small changes in the size of the ratio will tend to produce larger changes in Q at higher ratio sizes than at lower ratio sizes. This suggests that it may be appropriate for statistical tests on values of Q derived using the present method (for example in experiments examining the effect

of neurobiological interventions on this parameter) to be carried out on logarithmically transformed parameter values.
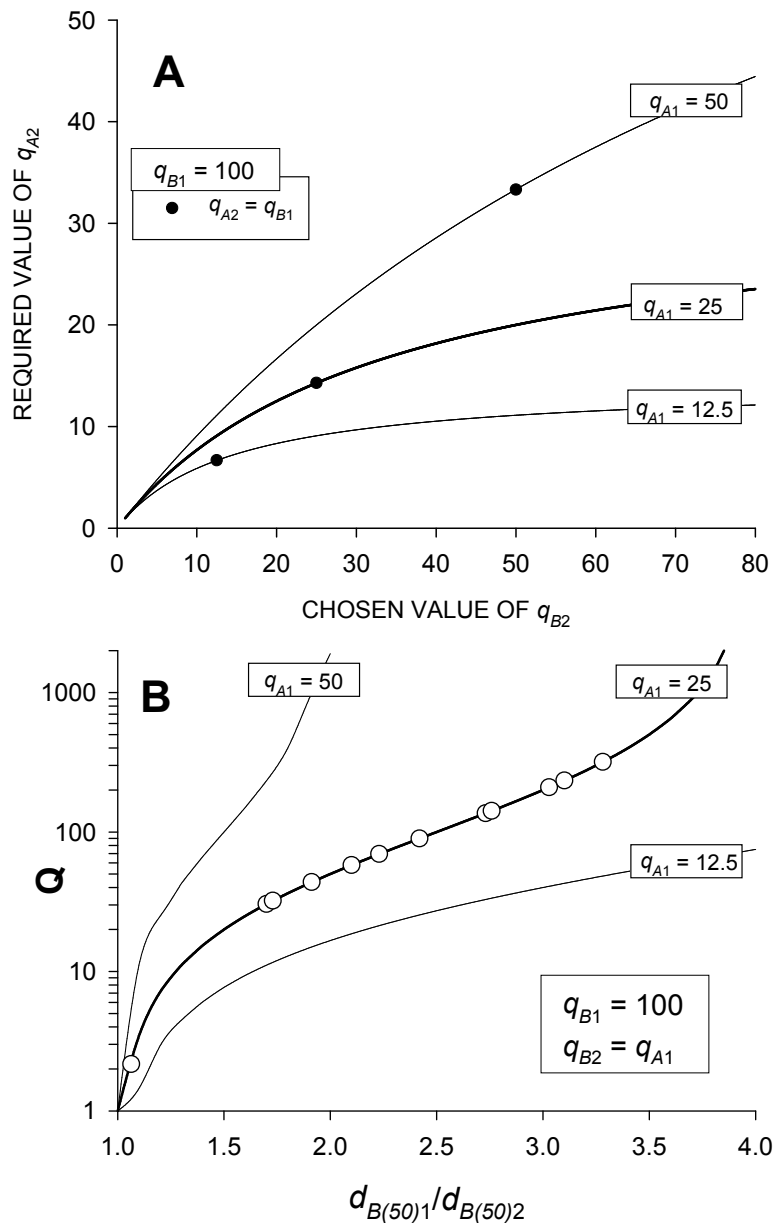
Fig. 5.6 . *A*. Relation between the required size of the smaller reinforcer in Condition 2 of the present method (q_{A2}) for a range of sizes of the larger reinforcer (q_{B2}). The three curves show the relation between q_{A2} and q_{B2} for three chosen sizes of the smaller reinforcer in Condition 1 ($q_{A1} = 12.5$, 25 and 50), the size of the larger reinforcer (q_{B1}) being set at 100 in each case. The thick curve corresponds to the value of q_{A1} used in this experiment (25 μl of a 0.6 M sucrose solution). The points indicate the required values of q_{A2} for the particular case of $q_{A1} = q_{B2}$, adopted in the present experiment; when $q_{A1} = q_{B2} = 25$, $q_{A2} \approx 14$. *B*. Relation between the recovered value of the size-sensitivity parameter Q and the ratio of the two indifference delays ($d_{B(50)1}/d_{B(50)2}$) in the present method. The curve shows the relation between log Q and $d_{B(50)1}/d_{B(50)2}$ in the case of the particular reinforcer sizes used in the present experiment ($q_{B1} = 100$ and $q_{B2} = q_{A1} = 25$). Meaningful values of Q are generated by ratios between 1 and 4. It can be seen that the relation is approximately linear between 1.5 and 3.5. Points show the data from the 12 subjects of the present experiment; data from all but one of the rats fall within that range. See text for further explanation.

It must be emphasized that the constraint on the range of 'allowable' $d_{B(50)}$ ratios is theoretical rather than methodological. In other words, it is not peculiar to the present application of Equation 4; rather it arises from the assumptions underlying Equations 2a and 2b, which form the basis of Ho et al.'s (1999) multiplicative hyperbolic model. A more stringent constraint is imposed by Equation 1, which does not incorporate a size-sensitivity parameter. When applied to the present protocol, Equation 1 specifies a $d_{B(50)}$ ratio of exactly 3.81. As discussed above, the present data do not comply with this specification. They are, however, compatible with the limits imposed by Equation 4, and are therefore compatible with the multiplicative hyperbolic model. It is possible, of course, that future applications of the present method may reveal empirical $d_{B(50)}$ ratios that are incompatible with Equation 4; such an occurrence would constitute a refutation of the underlying model.

5.4.3 *Transitional behaviour on the adjusting-delay schedule*

In agreement with previous reports (e.g. Mazur 1987; da Costa Araújo et al. 2009), the adjusting delay to Reinforcer B (d_B) showed cyclical changes which gradually declined in amplitude as training progressed. The fluctuation of d_B was charaterized by using the Fourier transform to derive a power spectrum of the frequency of oscillation. This analysis showed that the dominant frequency of the spectrum corresponded to a period of oscillation of approximately 80 trial blocks. A similar value for the period of oscillation was obtained in a recent experiment investigating the effect of lesions of the core of the nucleus accumbens on behaviour on the adjusting-delay schedule (da Costa Araújo et al. 2009). Since da Costa Araújo et al.'s (2009) experiment used qualitatively different reinforcers (food pellets) and different ratios of reinforcer sizes from those used in the present experiment, it seems that the period of oscillation of d_B may be relatively insensitive to reinforcer variables.

There was a consistent trend for the power of oscillation to be highest in the initial segment of the first phase of the experiment. The decline in power during Phase I presumably reflects some adaptation to the schedule contingencies during extended training (see below for further discussion). Interestingly, although there was a consistent

trend for power in the dominant frequency band to be higher under Condition 2 ($q_A = 14$ µl, $q_B = 25$ µl) than under Condition 1 ($q_A = 25$ µl, $q_B = 100$ µl), there was no overall difference between the power of oscillation seen in the second phase and that seen in the final segment of the first phase. This suggests that the gradual adaptation to the adjusting-delay contingencies was not disrupted by the change in reinforcer sizes at the start of the second phase.

As in previous experiments with adjusting-delay schedules (da Costa Araújo et al. 2009; Ho et al. 1997; Mobini et al. 2000; Wogar et al. 1992, 1993b), a proportional rather than fixed adjustment of d_B was used. The decision to use proportional adjustment was based on the assumption that sensitivity to changes in delay of reinforcement would conform to Weber's law, as is the case with temporal discrimination in many types of timing schedule (Gibbon 1977; Killeen & Fetterman 1988). Weber's Law implies that proportional changes should be similarly discriminable across a broad range of delays, whereas a fixed increment of, say, 1 s would be less discriminable if the preceding value of d_B were 30 s than if it were 2 s. However, fixed changes in d_B are commonly used (e.g. Green et al. 2007; Mazur 1994, 1995, 1996; Pietras et al. 2003), and it remains to be seen whether the use of different adjustment rules influences the pattern of oscillation of d_B revealed by the power spectrum.

5.4.4.   *Simulating behaviour on the adjusting-delay schedule*

Adjusting-delay schedules entail complex contingencies, and the processes underlying the oscillating pattern of changes in d_B remain conjectural at this time. A speculative model is described as a preliminary account of some of the processes that may be involved in the schedule used in this experiment.

Firstly, it is assumed that the value of each outcome is determined by Equations 2a and 2b. As there is no delay to Reinforcer A ($d_A = 0$), the value of A (V_A) depends only upon its size, q_A, and the size-sensitivity parameter, Q. The value of B (V_B), however, varies from trial block to trial block, due to the influence of d_B, modulated by the delay-discounting parameter, K. Next, it is postulated that the subject discriminates between V_A and V_B, and selects the outcome that has the higher value at the moment of choice. Thus, in any trial block in which $V_B > V_A$, the subject selects B, resulting in an increment in d_B in the following block; B will be selected repeatedly until $V_A > V_B$, at

which point the process will be reversed. Figure 5.7A shows how d_B would oscillate in the present protocol if behaviour were following this simple principle.

To make the model more realistic, it is assumed that rats' ability to discriminate reinforcer value is not perfect, and that standard psychophysical principles apply. In keeping with evidence from other delay-of-reinforcement paradigms (Gibbon and Fairhurst 1994), it is assumed that rats' discrimination of value depends on the ratio of two values, rather than the absolute difference between them. A simple logistic function centered on $V_B/V_A=1$ may be used to define the probability that B will be chosen: $p(B)=1/(1+[V_B/V_A]^s)$, where s defines the slope of the function. (The probability that A will be chosen is $p(A)=1-p(B)$.) Figure 5.7B shows the effect of introducing this element of variability into choice between the two outcomes.

Finally, it is supposed that rats' ability to discriminate reinforcer value improves with practice, and that the improvement takes the form of a progressive steepening of the psychophysical function that approaches an asymptote after extended training. The simulation uses an exponential learning function: $s = s_{max}.(1-e^{-n/c})$, where s_{max} is the asymptote, n is the ordinal position of the trial block, and c defines the rate at which s approaches its asymptotic value. This function is incorporated into the simulation shown in Figure 5.7C.

The model captures some features of behaviour on the schedule used in this experiment, although there is clearly room for improvement. Like the real rats, the model generates oscillation of d_B which declines in amplitude during the course of training. Entering the empirical values of Q and K into the model, it was found that d_B stabilized at values very close to the those seen in the two conditions of this experiment. Although transitional performance varied quite widely between simulations, the 'steady-state' values of $d_{B(50)}$ were quite consistent: using the parameters listed in the legend of Figure 5.7, 100 iterations of the simulation yielded mean values of $d_{B(50)}$ of 20.0 s (Condition 1: q_A=25 µl; q_B=100 µl) and 8.0 s (Condition 2: q_A=14 µl; q_B=25 µl), the coefficients of variation being 0.10 and 0.12, respectively. These values are reassuringly close to the group mean values shown in Figure 5.4 (18.8 s and 8.1 s).
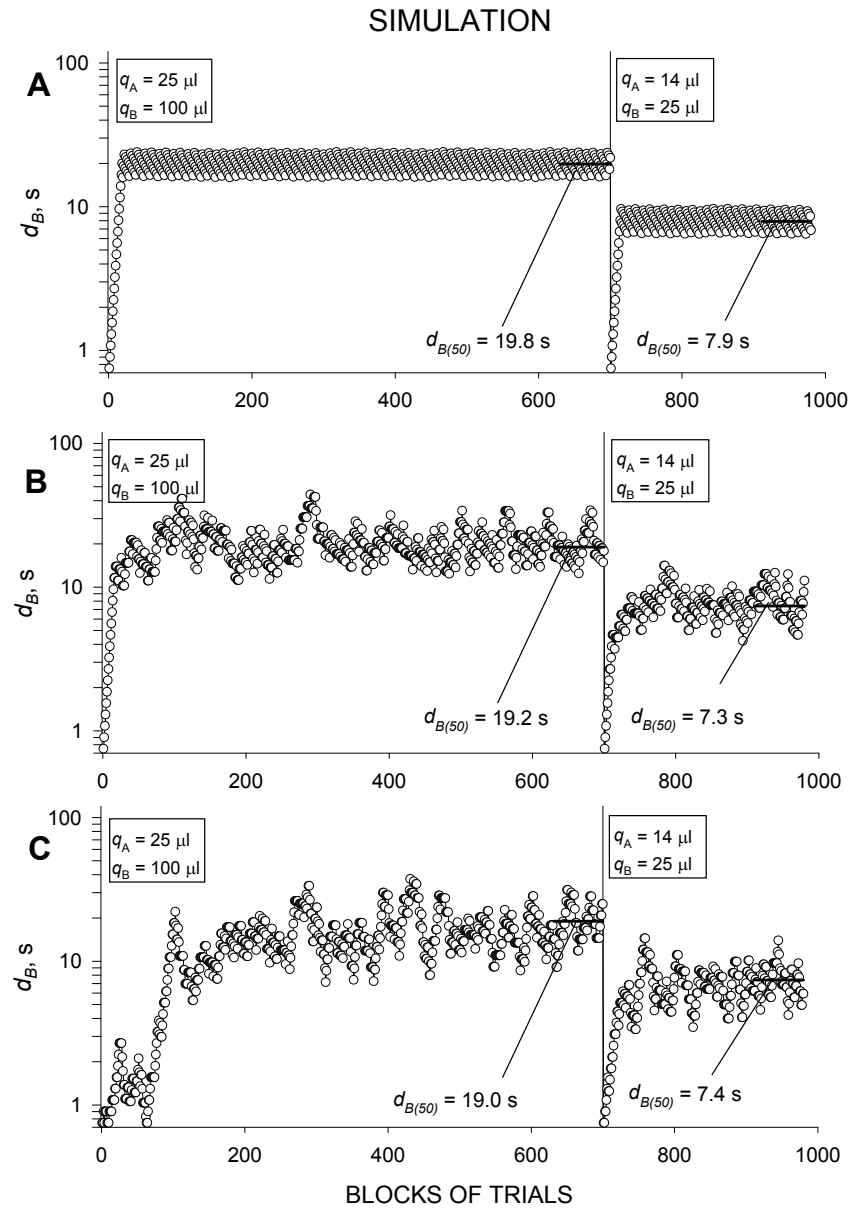
SIMULATION

Fig. 5.7 Simulation of behaviour under the adjusting-delay schedule, based on the model described in the text. *Ordinates:* adjusting delay to the larger reinforcer, d_B (s); *abscissae:* blocks of trials in the two phases of the experiment (cf. Figure 5.1). The group mean estimated values of Q and K (see Figure 5.5: $Q = 113.8$ μl, $K = 0.082$ s^{-1}) were used in the simulations. The reinforcer volumes shown in the insets correspond to those used in Conditions 1 and 2 of the experiment. The recovered values of $d_{B(50)}$ are the means of the last 70 data points of each phase. *A.* Simulation based on the assumption of perfect discrimination between the values of the two outcomes, the subject invariably selecting B when $V_B/V_A > 1$, and A when $V_B/V_A < 1$. *B.* Simulation incorporating variability of discrimination generated by a logistic 'psychometric' function in which the probability of selection of the larger reinforcer is $p(B)=1/(1+[V_B/V_A]^s)$; the slope of the function, s, was set at 2 in this simulation. *C.* Simulation incorporating the additional assumption that s increases during training, starting at 0 and approaching its asymptote s_{max} (2, in this simulation) according to the function $s = s_{max}.(1-e^{-n/c})$, where n is the number of trial blocks and c is a 'learning' parameter, which was set at 300 blocks in this simulation.

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The model was less successful in capturing the amplitude of oscillation of d_B in the early stages of training. In other words, the simulation tended to underestimate the power in the dominant frequency band during the initial part of Phase I. The reason for this is uncertain. Two possible explanations for the high-amplitude oscillations seen in this experiment are the following. (i) The rats may have had a tendency to perseverate; that is, under conditions of uncertainty, they may have tended to repeat the previous response, rather than selecting A or B randomly, as is assumed by the model. This would have had the effect of driving d_B towards the extremes of the range before the alternative response was selected. (ii) Alternatively or additionally, choices may have been based on outcomes obtained in an extended sequence of trial blocks, rather than being determined solely by the ratio of reinforcer values in the immediately preceding block, as is assumed in the present form of the model. Either or both of these hypothetical processes could be incorporated into the model. However, it may be premature to introduce additional parameters on an *ad hoc* basis, pending experimental exploration of the more basic aspects of the model.

5.4.5.   *Implications for behavioural neuroscience*

The neural mechanisms underlying inter-temporal choice have attracted considerable attention in recent years. A major incentive to research in this area has been the prospect of uncovering the biological bases of pathological 'impulsiveness' (Carroll et al. 2010; Sagvolden et al. 2005; Williams 2010; Yi et al. 2010). It is widely believed that a tendency to make impulsive choices arises from an abnormally high rate of delay discounting, and therefore discovery of the neural underpinnings of this process may lead to a greater understanding of the causes of pathological impulsiveness and, perhaps, to the development of more effective treatments for this disabling condition. A common tactic in this area of research is to examine the effect of a neurobiological intervention on preference for the larger of two reinforcers while progressively increasing the delay to that reinforcer. A leftward displacement of the resulting preference function (i.e., a reduction of the indifference delay) is often taken as an index of impulsiveness, which is not infrequently equated with an increase in the rate of delay discounting. Unfortunately, the logic of this approach is undermined if the indifference delay is determined by two parameters (for example, K and Q), either or both of which may be influenced by biological interventions. Leftward displacement of a single preference function provides

no clue as to whether the intervention in question has altered the rate of delay discounting or the sensitivity to reinforcer size (or both).

One way of overcoming this difficulty is to obtain several indifference delays corresponding to a range of delays to the smaller reinforcer (d_A), and thereby construct the linear indifference function defined by Equation 3. The slope and intercept of this function may then be used to infer changes in K and Q (Ho et al. 1999; Mazur 2006). Although this method has been used successfully in studies examining the effects of brain lesions on inter-temporal choice (see sections 1.3.3.2 and 5.1 for references), the method is very time consuming. The approach adopted in the present experiment, based, as it is, on only two indifference delays, considerably reduces the time needed to obtain estimates of K and Q. It may be noted that the present method based on Equation 4 is one of several ways in which K and Q might be estimated from two indifference delays. For example, in the case of choice between two reinforcers of sizes q_A and q_B, indifference delays $d_{B(50)1}$ and $d_{B(50)2}$ might be obtained for two values of d_A (d_{A1} and d_{A2}). According to the linear indifference function defined by Equation 3, K may be determined from the formula (slope-1)/intercept. With d_{A1} set at 0, the slope is defined by $(d_{B(50)2}-d_{B(50)1})/d_{A2}$, allowing K to be calculated from the formula $[\{(d_{B(50)2}-d_{B(50)1})/d_{A2}\}-1]/d_{B(50)1}$, and Q from the formula (1-slope)/(slope/q_B-1/q_A).

A detailed assessment of the reliability of the estimates of Q and K obtained using the present method remains a task for future research. It is likely that the estimates based on two data points will show greater variability than estimates derived by fitting linear indifference functions to a family of data points covering a broad range of indifference delays. However, in neurobehavioural investigations of inter-temporal choice, specifying the exact numerical values of the parameters is generally less important than determining whether a particular intervention affects one or other, or both, of the parameters in question (e.g. Kheramin et al. 2002; Bezzina et al. 2007). It may be noted that neurobiological interventions could provide a means of assessing the reliability and validity of the present approach. For example, experiments based on the linear indifference function defined by Equation 3 have indicated that destruction of the orbital prefrontal cortex results in increases in both K and Q, whereas destruction of the core of the nucleus accumbens has a selective effect on K (see sections 1.4.4.1.2 and 1.4.4.2.2). Confidence in the utility of Equation 4 would be greatly strengthened if the same effects of the lesions on the two parameters could be demonstrated using the present experimental protocol.

The present experiment used adjusting-delay schedules to obtain indifference delays. This is not an essential requirement. For example, progressive delay schedules (Evenden and Ryan 1996), which have been used extensively in neurobehavioural experiments (see Cardinal et al. 2003b; Winstanley 2010), are equally suitable. The adjusting-amount schedule (Richards et al. 1997) may be particularly advantageous, as it has been reported to generate stable choice behaviour within one or two sessions, as opposed to the many sessions needed to reach stability under adjusting-delay schedules (Richards et al. 1997; Green et al. 2007).

In summary, rats made repeated choices on an adjusting-delay schedule between a smaller reinforcer (A) that was delivered immediately after a response and a larger reinforcer (B) that was delivered after a delay which increased or decreased by 20% depending on the subject's choices in successive blocks of trials. Indifference delays, calculated from adjusting delays in the last 10 sessions of each phase, were shorter when the sizes of A and B were 14 and 25 µl of a 0.6 M sucrose solution than when they were 25 and 100 µl of the same solution. The ratio of the indifference delays was significantly smaller than that predicted on the basis of an assumed linear relation between reinforcer size and instantaneous reinforcer value, consistent with a previous proposal that this relation may be hyperbolic in form (Ho et al. 1999). Estimates of the rate of delay discounting based on the ratio of the two indifference delays (mean, 0.08 s^{-1}) were similar to values obtained previously using different inter-temporal choice protocols. Estimates of the size-sensitivity parameter (mean 113 µl) were similar to estimates recently derived from performance on progressive-ratio schedules. Adjusting delays in successive blocks of trials were analysed using the Fourier transform. The power spectrum obtained from individual rats had a dominant frequency that corresponded to a period of oscillation of the adjusting delay between 30 and 100 trial blocks (mean, 78). Power in the dominant frequency band was highest in the early sessions of the first phase and declined with extended training. It is suggested that this experimental protocol may have utility in neurobehavioural studies of inter-temporal choice.

CHAPTER 6

EXPERIMENT 5:

PERFORMANCE UNDER AN ADJUSTING DELAY SCHEDULE:
FURTHER OBSERVATIONS WITH A POWER SPECTRUM
ANALYSIS

6.1   Introduction

The previous chapter presented a novel way of quantifying transitional behaviour in the adjusting-delay schedule based on analysis of the power spectrum of cyclical changes in the adjusting delay. The results of Experiment 4 showed that the adjusting delay to the larger reinforcer (d_B) oscillated during an extended period of training, the amplitude of oscillation gradually declining as d_B approached a quasi-stable value. The power in the dominant frequency band was higher when reinforcers sizes were 14 µl and 25 µl than when they were 25 µl and 100 µl of a 0.6 m sucrose solution; however there was no significant difference when conditions were reversed. In addition, the period of oscillation of the dominant frequency (approximately 80 trial blocks) did not vary across conditions. These results suggested that the gradual adaptation to the adjusting-delay contingencies was not disrupted by the change in reinforcer sizes. Additionally, a previous study by da Costa Araújo et al. (2009) employing this same analysis showed no effect of AcbC lesions on either the power or the period of oscillation corresponding to the dominant frequency, suggesting that the lesion did not compromise the rats' ability to detect changes in delay to reinforcement.

In the adjusting delay schedule the delay to the larger of two reinforcers varies in accordance with the subject's choice (Mazur, 1987). This pattern arises because the subject continues to select the larger reinforcer until the progressive increase in delay to reinforcement eventually reduces the overall value of the larger reinforcer to an extent that it becomes less attractive than the smaller immediate reinforcer; repeated selection of the smaller reinforcer then reverses the process. The quantitative characteristic of this cyclical pattern conveys information about the rats' ability to detect short-term changes in delay to reinforcement: impairment of discrimination may be expected to produce oscillations of d_B that have high amplitude and/or a long period.

In the present experiment, the pattern of oscillation of d_B in an adjusting delay schedule was analyzed using a power spectrum analysis. Because the cyclical changes of d_B may reflect the ability of the rats to detect changes in delay to reinforcement from one trial block to the next, the step-size whereby d_B increased or decreases was manipulated across two conditions. In Condition 1, the delay to the larger reinforcer increased or decreased (according to the rats' choice) by 20% from block n to block $n+1$. In Condition 2, the delay to the larger reinforcer increased or decreased (according to the rats' choice) by 10% from block n to block $n+1$. Since relatively large changes in d_B may

be required in order for a change in reinforcer value to become detectable, manipulations of the step-size (10% *vs.* 20%) were predicted to change the period of the dominant frequency.

6.2. Materials and methods

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

6.2.1. Subjects

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

6.2.2 Apparatus

The same apparatus was used as in Experiment 4 (see section 5.2.2).

6.2.3. Behavioural training

At the start of the experiment the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. They were then trained to press two levers (A and B) for the sucrose reinforcer (50 μl, 0.6 M), and were exposed to a discrete-trials continuous reinforcement schedule in which the two levers were presented in random sequence for three sessions. Then they underwent daily 42-minute training sessions under the discrete-trials adjusting-delay schedule for the remainder of the experiment. The adjusting-delay schedule and the training protocol were the same as those used in Experiment 4 (see section 5.2.3). Each experimental session consisted of seven blocks of four trials. In each block of trials, the delay to the larger reinforcer, d_B, was determined by the rat's choices in the free-choice trials in the preceding block. There were two experimental conditions in which the step-size of d_B was manipulated. In Condition 1, if Lever A was chosen in both free-choice trials of block n, d_B was reduced by 20% in block $n+1$; if Lever B was chosen in both free-choice

trials of block n, d_B was increased by 20% in block $n+1$; if Lever A and Lever B were each chosen in one free-choice trial in block n, d_B remained unchanged in block $n+1$. In Condition 2, d_B was increased or decreased in steps of 10% rather than 20%. Under both conditions, the value of d_B in the first block of each session was determined in the same way by the choices made in the final block of the previous session. Maximum and minimum values of d_B were set at 60 s and 0.75 s.

The experiment consisted of two Phases (I and II), each lasting for 100 sessions. For half the rats Condition 1 was in effect in Phase I and Condition 2 in Phase II; for the other rats the order of conditions was reversed. In the first block of the first session of each phase, d_B was set at 0.75 s. The sizes of the reinforcers were the same for both conditions : $q_A = 25$ µl, $q_B = 100$ µl.

Experimental sessions were carried out 7 days a week, at the same time each day, during the light phase of the daily cycle (between 0800 and 1400 hours).

6.2.4 *Data analysis*

6.2.4.1 Indifference delays

For each rat, the mean value of d_B in the last ten sessions of each condition was taken as the indifference delay, $d_{B(50)}$. These data were analysed by a two-factor analysis of variance (order of condition × condition [step-size]) with repeated measures on the latter factor. As this analysis showed no significant main effect of order, the 'order' factor was ignored in all further treatment of the data. Accordingly, the data from all 8 rats were pooled in all subsequent analyses.

6.2.4.2 Power spectrum analysis

Plots were obtained of log d_B *vs.* blocks of trials (Phase I, 700 blocks; Phase II, 700 blocks). These data, expressed as deviations from the mean value of d_B, were subjected to a Fourier transform (Spike-2, version 4.23: Cambridge Electronic Design, Ltd) in order to derive power spectra (power *vs* frequency), as described in section 5.2.4.2. The reciprocal of the frequency is the cycle time (period) of oscillation of d_B, in blocks. The power of the dominant frequency of the spectrum within the frequency range of 0.01 (period = 100 blocks) and 0.04 (period = 25 blocks) and the length of the period

corresponding to the dominant frequency were derived for each rat in each phase of the experiment (da Costa Araújo et al., 2009). Power spectra derived in each phase was divided in three overlapping segments of 256 blocks (blocks 1-256, blocks 223-478 and blocks 445-700). The parameters of the power spectrum were compared by an analysis of variance (step-size × segment) with repeated measures on both factors.

6.3 Results

6.3.1 Indifference delays

Figure 6.1 shows the values of $d_{B(50)}$ derived from the last ten sessions of each phase. There was no significant difference between the value of $d_{B(50)1}$ (Condition 1: step-size 20%) and $d_{B(50)2}$ (Condition 2: step-size 10%). Analysis of variance indicated that there was no significant main effect of condition [$F(1,6) = 1.8$, NS] or order of conditions [$F<1$]; the interaction was of borderline significance [$F(1,6) = 6.3$, $p = 0.05$].
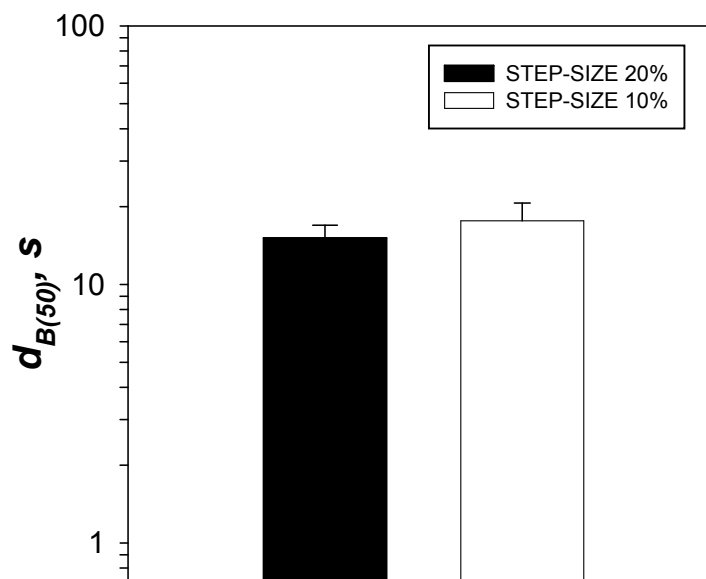
Fig 6.1 Values of $d_{B(50)}$ (s) obtained under the two conditions. Columns are group mean data; $d_{B(50)}$ was similar under the two conditions. Filled column is the group mean data of rats under Condition 1 (step-size 20%). Empty column is the group mean data of rats under Condition 2 (step-size 10%).

6.3.2 Transitional behaviour

Power spectra from each condition were divided in three segments of 256 blocks (see section 6.2.4.2). The power in the dominant frequency band and the period

corresponding to the dominant frequency from each segment are shown in Figure 6.2. In both groups of rats, power in the dominant frequency band declined with training (Fig. 6.2A). Analysis of variance (condition × segment) with repeated measures on the latter factor revealed an effect of segment [$F_{(2,14)} = 10.0$, $p < 0.005$), but no significant effect of condition [$F_{(1,7)} = 1.2$, NS] and no significant interaction [$F<1$]. The period corresponding to the dominant frequency (Figure 6.2B) showed a significant main effects of block [$F_{(2,14)} = 4.5$, $p < 0.05$] and condition [$F_{(1,7)} = 15.7$, $p < 0.01$], but no significant interaction [$F<1$].
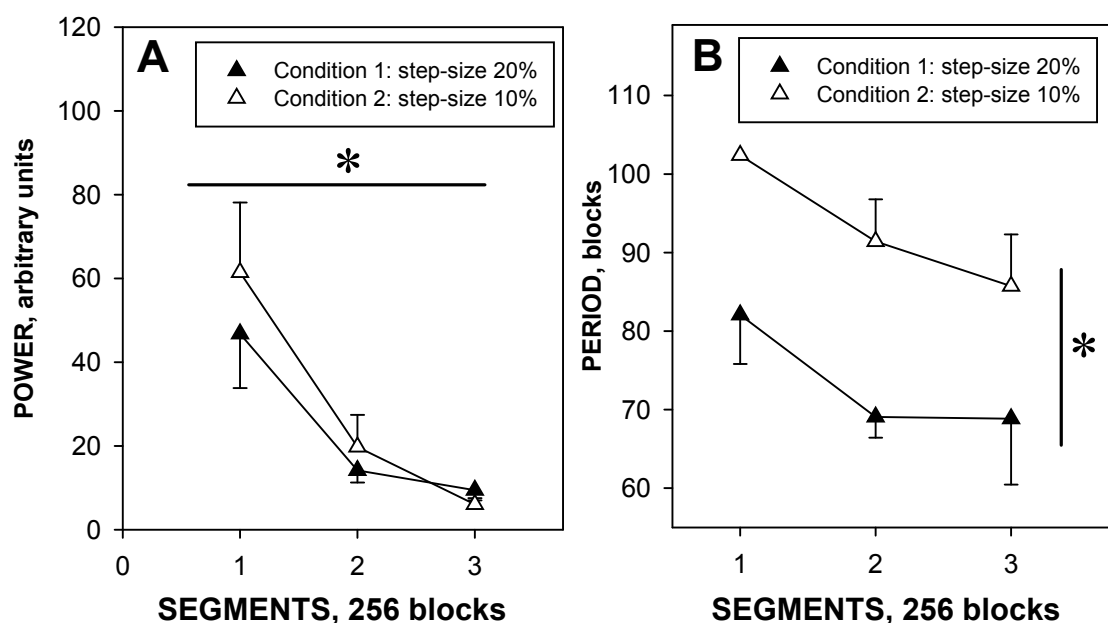
Fig. 6.2 Results of the power spectrum analysis. A. Comparison of power in the dominant frequency band in Condition 1 (filled triangles; step-size 20 %) and Condition 2 (empty triangles; step-size 10%). Points represent group mean data (±SEM). Power in the dominant frequency band declined significantly across segments (* P<0.05). B. Comparison of period of oscillation corresponding to the dominant frequency band in Conditions 1 and 2 (conventions as in A). The period was significantly longer under Condition 2 than under Condition 1 (* P<0.05) (see text for details).

6.4 Discussion

The quasi-stable adjusting delays seen during the last ten days of training under each condition were taken as indifference delays, $d_{B(50)}$. There was no significant difference in the $d_{B(50)}$ between Condition 1, where the step-size of the delay to the larger reinforcer

(d_B) was varied by 20%, and Condition 2, where it was varied by 10%. This was an expected result, because $d_{B(50)}$ is assumed to depend on the relative value of Reinforcer B, which was the same under both conditions (see section 5.1). Analysis of variance indicated a borderline interaction between condition (step-size) and order of condition. However inspection of the data segment by segment (three segments of 256 blocks each) did not reveal any significant difference between Condition 1 (step-size 20%) and Condition 2 (step-size 10%) (all paired-samples t-tests, $P>0.1$). For this reason, the data from all 8 rats were pooled in the analyses.

In the present experiment, the pattern of oscillation of d_B in the adjusting delay schedule was analyzed using a power spectrum analysis. Because the cyclical changes of d_B may reflect the ability of the rats to detect block-to-block changes in delay to reinforcement, the step-size whereby d_B was increased or decreased was varied across two conditions. In Condition 1, the delay to the larger reinforcer increased or decreased (according to the rats' choice) by 20% from block n to block $n+1$. In Condition 2, the delay to the larger reinforcer increased or decreased (according to the rats' choice) by 10%. As in Experiment 4 (see Chapter 5), the fluctuation of d_B was characterized using the Fourier transform to derive a power spectrum of the frequency of oscillation. This analysis showed that the period of oscillation corresponding to the dominant frequency of the spectrum was significantly longer under Condition 2 than under Condition 1. The value for the period of oscillation under Condition 1 (approximately 80 blocks) was similar to that obtained in Experiment 4 and a previous study (da Costa Araújo et al., 2009) which also employed a step-size of 20% for the adjustment of d_B.

The longer period of oscillation seen under Condition 2 than under Condition 1 was an expected result. According to the model presented in Chapter 5, the period of oscillation is influenced by the rats' ability to discriminate the values of the two reinforcing outcomes. Since a change in the value of B is assumed to be caused by a change in d_B, and since a greater number of blocks is required to produce a given change in d_B under Condition 2 (10% step-size) than under Condition 1 (20% step-size), it is to be expected that the period of oscillation (measured in blocks) would be longer under the former condition. In other words, the animals require more blocks in order to reverse their preference from B to A under Condition 2 than under Condition 1, and vice versa.

There was a consistent trend for the power of oscillation to be highest in the initial segment of the experiment in both conditions. The decline in power seen across segments presumably reflects some adaptation to the schedule contingencies during

extended training. This trend was also observed in Experiment 4 (see section 5.3.2).

The utility of analysing the power spectrum of oscillations of d_B requires further exploration. The method was used in a recent experiment examining the effect of destruction of the nucleus accumbens core on performance on an adjusting-delay schedule (da Costa Araújo et al., 2009). However, that study found no significant effect of the lesion on the power spectrum, and it remains to be seen whether the method will prove sensitive to other neurobiological interventions (see section 5.4 for discussion).

CHAPTER 7

EXPERIMENT 6:

NUCLEUS ACCUMBENS AND DELAY DISCOUNTING IN RATS:

EVIDENCE FROM A NEW QUANTITATIVE METHOD FOR

ANALYSING INTER-TEMPORAL CHOICE

7.1 Introduction

Inter-temporal choice has attracted much attention in recent years because of its purported relevance to impulsive behaviour in humans. Selection of a smaller earlier reinforcer in preference to a larger delayed reinforcer is often termed 'impulsive choice' (Ainslie 1974, 1975; Cardinal 2006; Evenden and Ryan 1999; Ho et al. 1999; Madden and Johnson 2010; Winstanley 2010). A predisposition to make impulsive choices is widely regarded as a key component of the multidimensional construct, 'impulsivity' (Dalley et al. 2008; Diergaarde et al. 2008; Evenden 1999) and this aspect of 'impulsivity' has been ascribed to an abnormally high rate of delay discounting (Dalley et al. 2008; Odum and Baumann 2010; Williams 2010; Yi et al. 2010).

In recent years, a considerable body of evidence has been accumulated about the neural substrate of inter-temporal choice. One structure that has been repeatedly implicated in delay discounting is the core of the nucleus accumbens (AcbC; for review, see section 1.4.4.2.2). Cardinal et al. (2001) first demonstrated that destruction of the AcbC disrupts inter-temporal choice, promoting the selection of small immediate reinforcers in preference to larger delayed reinforcers. These authors proposed that lesions of the AcbC induce an increase in the rate of delay discounting, resulting in a reduction of the value of delayed reinforcers. The finding of Cardinal et al. (2001) has been confirmed in a number of more recent experiments (Pothuizen et al. 2005; Bezzina et al. 2007, 2008; da Costa Araújo et al. 2009, 2010). Cardinal and Cheung (2005) and Cardinal and Howes (2005) showed that destruction of the AcbC did not alter the instantaneous value of reinforcers in choice schedules, suggesting that altered sensitivity to reinforcer size cannot account for the effect of AcbC lesions on inter-temporal choice (see below). Recently, da Costa Araújo et al. (2010) reported that performance of inter-temporal choice tasks was associated with an increase in neuronal activation (as indicated by enhanced expression of the nuclear protein Fos) in the AcbC, providing further support for the involvement of this structure in inter-temporal choice. Functional neuroimaging evidence in humans also provides some support for an involvement of the nucleus accumbens in inter-temporal choice (Kable and Glimcher 2007; McClure et al. 2004).

The aim of the present experiment was to examine the effect of lesions of the AcbC on the parameters Q and K using the method described in Chapter 5. Numerical estimates of Q and K, derived for individual subjects, were compared between sham-

lesioned and AcbC-lesioned rats. As in Experiment 4 (see Chapter 5), indifference delays were determined using an adjusting-delay schedule (Mazur 1987). A secondary aim of the present experiment was to re-examine the effect of AcbC lesions on the power spectrum of cyclical changes in the adjusting delay using different values of q_A and q_B from those used by da Costa Araújo et al. (2009, 2010).

7.2　　Materials and methods

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

7.2.1.　　Subjects

Forty-two female Wistar rats aged approximately 4 months and weighting 250–300 g at the start of the experiment were used. They were housed individually under the same conditions as in Experiment 1 (see section 2.2.1).

7.2.2.　　Surgery

The rats received lesions of the AcbC ($n = 18$) or sham lesions ($n = 24$). Anaesthesia was induced with isoflurane (4% in oxygen) and the rat positioned in a stereotaxic apparatus (David Kopf), with the upper incisor bar set 3.3 mm below the inter-aural line. Anaesthesia was maintained with 2% isoflurane in oxygen during surgery. A small hole was drilled in the skull over each hemisphere for microinjection of quinolinic acid into the AcbC. The following coordinates were used to locate the AcbC: AP +1.2, L ±1.8, V −7.1 (in millimetres, measured from bregma; Paxinos and Watson 1998). Injections were given via a 0.3-mm diameter cannula connected by a polyethylene tube to a 5-μl Hamilton syringe. In the case of the lesioned group, the cannula tip was lowered to the position of each site, and 0.5 μl of a 0.05 M solution of quinolinic acid (2,3-pyridinedicarboxylic acid) in phosphate-buffered 0.9% NaCl (pH 7.0) was injected at a rate of 0.1 μl per 15 s. The cannula was left in position for 3 min after completion of the injection in each site. In the case of the sham-lesioned group, the procedure was identical, except that the vehicle alone was injected.

7.2.3 Apparatus

The same apparatus was used as in Experiment 4 (see section 5.2.2).

7.2.4 Behavioural training

Two weeks after the surgery, the food deprivation regimen was introduced and the rats were gradually reduced to 80% of their free-feeding body weights. Training under the adjusting-delay schedule was carried out in the same way as in Experiment 4 (see section 5.2.3). The two conditions of the experiment entailed different reinforcer sizes. In Condition 1, the sizes of the two reinforcers (µl of a 0.6 M sucrose solution) were $q_A = 25$, $q_B = 100$; in Condition 2, the sizes were $q_A = 14$, $q_B = 25$. The order of presentation of the conditions in the two phases of the experiment was counterbalanced across subjects in each group. Phase I comprised 100 sessions, and Phase II 40 sessions.

7.2.5 Histology

At the end of the behavioural experiment, the rats were deeply anaesthetised with sodium pentobarbitone and perfused transcardially with 0.1 M phosphate-buffered saline (PBS), followed by 4% formol PBS. The brains were removed from the skull and fixed in formol PBS for 72 h. Coronal sections (40 µm) were taken through the nucleus accumbens region using a freezing microtome. Neurone-specific nuclear protein (NeuN) was labelled as described by Jongen-Relo and Feldon (2002) using the protocol described by Bezzina et al. (2007). Freshly sliced sections were rinsed in PBS and placed in 0.5% H_2O_2 in PBS for 30 min. After twice rinsing in PBS, they were 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 incubated for 48 h at 4°C with 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 (1:1,000 in 1% BSA and 0.3% Triton X-100 in PBS; Vector Laboratories). After further rinsing in PBS, they were placed for 2 h in avidin–biotin–horseradish peroxidase complex (1:200, ABC-Elite, Vector Laboratories) in PBS. After two further rinses 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 rinsing in PBS. The sections were floated on to chrome/gelatine-coated slides and mounted with DPX. The sections were examined microscopically and drawings of the area of the lesions were superimposed on the appropriate coronal sections in the stereotaxic atlas of Paxinos and Watson (1998).

7.2.6   Data analysis

7.2.6.1   Indifference delays and parameter estimation

The same procedure used as in Experiment 4 (see section 5.2.4.1).

7.2.6.2   Transitional behaviour

The same procedure used as in Experiment 4 (see section 5.2.4.2).

7.2.6.3.   Statistical tests

The values of d_{B50} were analyzed by a two-factor analysis of variance (condition [1, 2] x order of condition [1 – first vs. 2 – first] with repeated measures on the former factor. As this analysis showed no significant effect of order and no significan order x condition interaction, the 'order' factor was ignored in all further treatment of the data. Indifference delays obtained from each rat in each condition of the experiment were analysed by a two-factor analysis of variance (group × condition, with repeated measures on the latter factor), and the ratios of the indifference delays obtained in the two conditions were compared between groups using Student's t test. For each group, the ratios of the indifference delays were also compared with the theoretical value based on Eqs. 1a, 1b (see section 5.1) (3.81) using the t test (see above). Estimates of the parameters Q and K derived from the ratios of the indifference delays were compared between groups using the t test. Two indices were derived from the power spectrum of oscillations of d_B: the power of the dominant frequency of the spectrum within the frequency range of 0.01 (period = 100 blocks) and 0.05 (period = 20 blocks) and the length of the period corresponding to the dominant frequency. These measures were

analysed by a two-factor analysis of variance (group × condition, with repeated measures on the latter factor).

7.3 Results

7.3.1 Indifference delays and parameter estimation

Figure 7.1 (left-hand panel) shows the group mean values (+SEM) of $d_{B(50)}$ derived from the last ten sessions of each phase. Analysis of variance indicated that there were significant main effects of both group [$F(1,40) = 4.5 < 0.05$] and condition [$F(1,40) = 120.5, p < 0.001$], but no significant group x condition interaction [$F(1,40) = 1.0$, NS]. In both groups, the value of $d_{B(50)1}$ (condition 1: $q_{A1} = 25$ µl, $q_{B1} = 100$ µl) was higher than that of $d_{B(50)2}$ (condition 2: $q_{A2} = 14$ µl, $q_{B2} = 25$ µl). Under each condition, the value of $d_{B(50)}$ was lower in the AcbC-lesioned group than in the sham-lesioned group (multiple comparisons with the Least Significant Difference test, p<0.05 in each case). Figure 7.1 (right-hand panel) shows the group mean ratios of the two values of $d_{B(50)}$ (+SEM). There was no significant difference between the ratios obtained from the two groups [$t(40) = 1.0$, NS]. The horizontal line in the right-hand panel indicates the ratio predicted on the basis of an assumed linear relation between value and reinforcer size (3.81; see section 5.2.4.1). In both groups, the observed ratio was significantly lower than the predicted ratio [sham-lesioned group: $t(23) = 6.6, p < 0.001$; AcbC-lesioned group: $t(17) = 3.1, p < 0.01$].

The ratios of the $d_{B(50)}$s were used to compute estimates of the two parameters of Eqs. 2a, 2b (see section 5.1). The group mean values (+SEM) of the two parameters are shown in Fig. 7.2. Estimates of the delay discounting parameter (K) were significantly higher in the AcbC-lesioned group than in the sham-lesioned group [$t(40) = 2.3, p < 0.05$]. However, the estimated values of the parameter that expresses sensitivity to reinforcer size (Q) did not differ significantly between the two groups [$t(40) = 0.1$, NS].
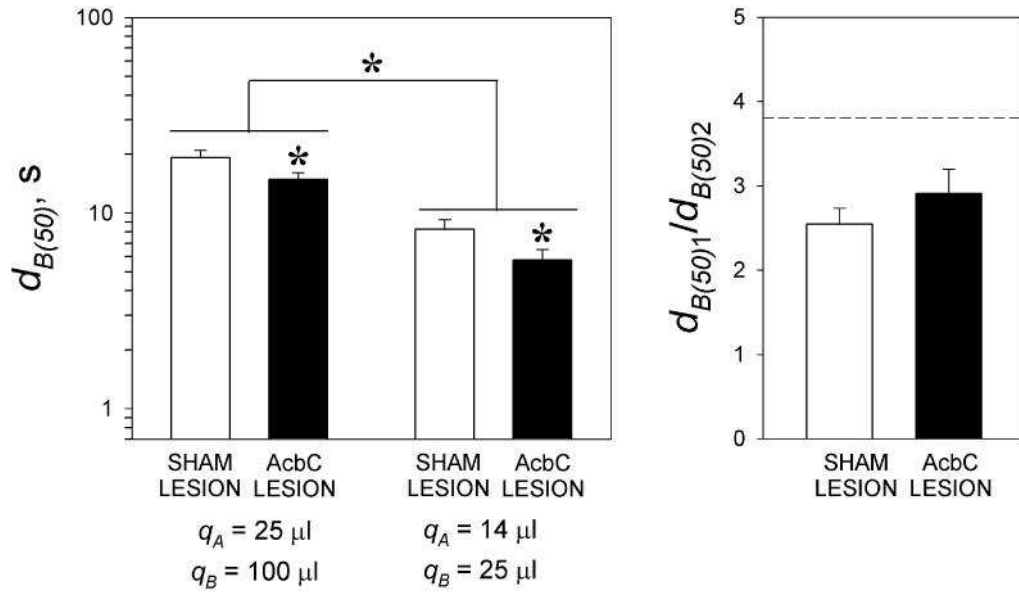
Fig. 7.1 *Left-hand panel*, Values of $d_{B(50)}$ (s) obtained under the two conditions. Columns are group mean data (+SEM) under condition 1 (reinforcer sizes: $q_A = 25$ μl, $q_B = 100$ μl) and condition 2 (reinforcer sizes: $q_A = 14$ μl, $q_B = 25$ μl): *white columns*, sham-lesioned group; *black columns*, AcbC-lesioned group. $d_{B(50)}$ was significantly longer under condition 1 than under condition 2 and significantly shorter in the AcbC-lesioned group than the sham-lesioned group under both conditions (*$p<0.05$). *Right-hand panel*, Ratio of the values of $d_{B(50)}$ obtained under the two conditions. The *horizontal broken line* shows the expected ratio based on the assumption that reinforcer value is linearly related to reinforcer size (see text).
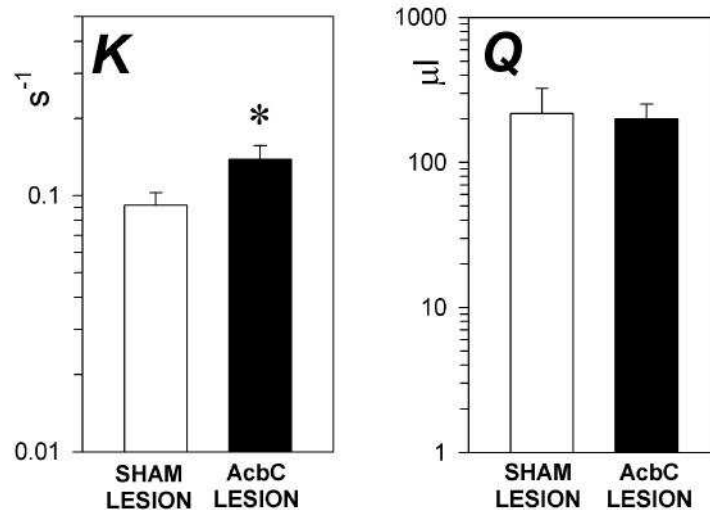
Fig 7.2 Estimates of the parameters of Eq.2 (see section 5.1) expressing the rate of delay discounting, K (per second), and sensitivity to reinforcer size, Q (microlitres of the 0.6 M sucrose solution). Columns are group mean data (+SEM); *white columns*, sham-lesioned group; *black columns*, AcbC-lesioned group. The value of K was significantly higher in the AcbC-lesioned group than in the sham-lesioned group (*$p<0.05$).

It occasionally happened that the value of d_B became 'stuck' at the lower extreme of the adjustable range (0.75 s) for an extended sequence of trial blocks. If a succession

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of more than four trial blocks in which d_B was 0.75 s occurred during the final ten sessions of either phase, the data from the sessions in question were replaced by data from sessions immediately preceding the last ten sessions. This criterion affected the data from two rats in the sham-lesioned group and three in AcbC-lesioned group. The analyses were repeated after elimination of the data from the rats whose adjusting delay (d_B) became 'stuck' at 0.75 s during the last ten sessions of either phase. Removal of these data did not alter the findings described above. There was no significant between-group difference in the $d_{B(50)}$ ratio [$t(35) = 1.1$, NS]. The estimated value of K was significantly lower in the AcbC-lesioned group than in the sham-lesioned group [$t(35) = 2.0$, $p < 0.05$], but there was no significant difference between the estimated values of Q in the two groups [$t(35)= 0.1$, NS].

The results were also reanalysed after removal of the data from those rats in the AcbC-lesioned group whose lesions extended into the ventromedial caudate–putamen. Removal of these data did not eliminate the effect of the lesion on K [$t(35) = 2.0$, $p < 0.05$]; as with the analysis of the complete data set, there was no significant between-group difference in the $d_{B(50)}$ ratios [$t(35) = 0.8$, NS] or the estimated values of Q [$t(35) = 0.2$, NS].

7.3.2   *Transitional behaviour*

The adjusting delay to the larger reinforcer, d_B, showed an oscillating pattern of change, the amplitude of the oscillations tending to decline during extended training. Power spectra were derived for each rat's data from the final 280 trial blocks under each condition using the Fourier transform. The group mean (+SEM) values of the period of oscillation corresponding to the dominant frequency band are shown in Fig. 7.3 (left-hand panel). Analysis of variance revealed no significant main effect of group [$F < 1$] or condition [$F(1,40) = 3.5$, NS] and no significant interaction [$F(1,40) = 1.2$, NS]. The group mean values (+SEM) of the spectral power within the dominant frequency band are shown in Fig. 7.3 (right-hand panel). In both groups, the power was greater under condition 2 ($q_{A2} = 14$ μl, $q_{B2} = 25$ μl) than under condition 1 ($q_{A1} = 25$ μl, $q_{B1} = 100$ μl). Analysis of variance revealed a significant main effect of condition [$F(1,40) = 11.2$, $p < 0.01$], but no significant main effect of group [$F < 1$] and no significant interaction [$F(1,40) = 1.4$, NS].

Examples of NeuN-stained coronal sections taken through the region of the AcbC from a sham-lesioned and an AcbC-lesioned rat are shown in the left-hand panels of Figure 7.4. The extent of the lesions is shown in the right-hand panel. There was extensive neuronal loss in the area of the AcbC of all the lesioned animals, with some minor neuronal loss in the ventromedial portion of the caudate–putamen in some animals; the mesial shell region of the nucleus accumbens was essentially spared.
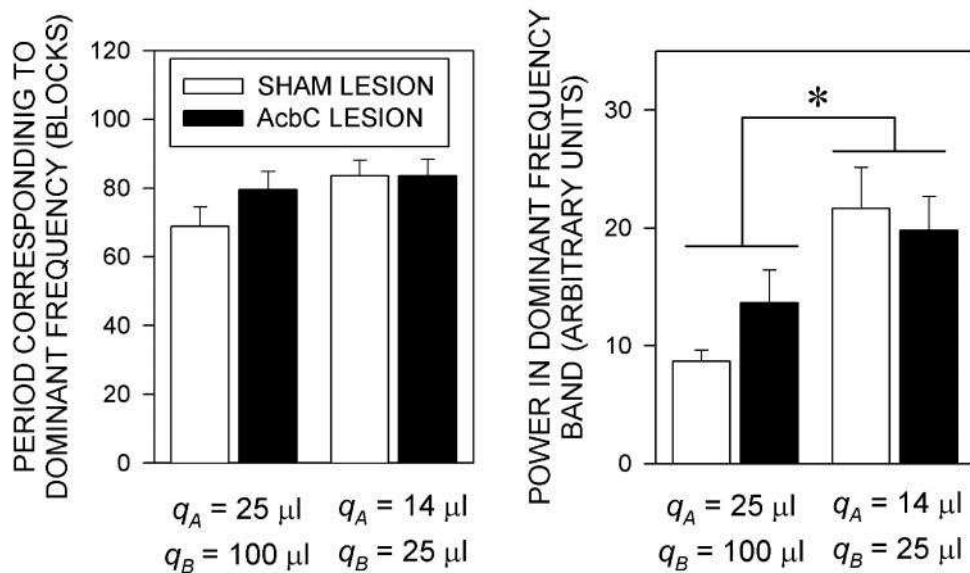
Fig. 7.3 Results of the power spectrum analysis. Comparison of power in the dominant frequency band and period of oscillation corresponding to the dominant frequency seen under conditions 1 (q_A = 25 μl, q_B = 100 μl) and 2 (reinforcer sizes: q_A = 14 μl, q_B = 25 μl). *Columns* show group mean data (+SEM): *white columns*, sham-lesioned group; *black columns*, AcbC-lesioned group. Power was significantly higher under condition 2 than condition 1 (**P<0.05*); there was no difference between the period of oscillation under the two conditions. There was no significant difference between the groups on either measure.
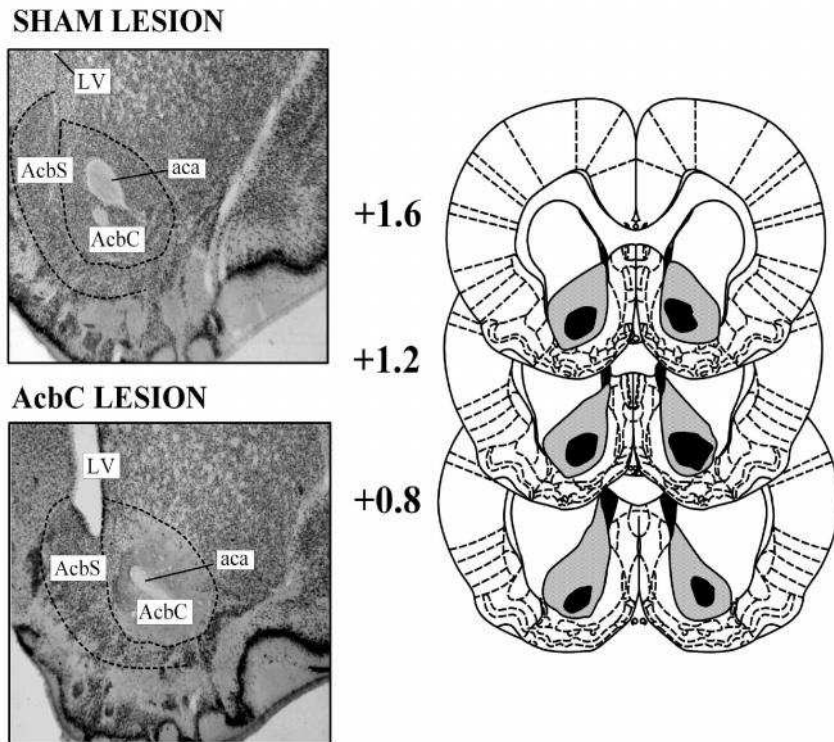
Fig. 7.4 *Left-hand panels*, Photomicrographs showing NeuN-stained coronal sections of the brains of a sham-lesioned rat (*upper panel*) and a AcbC-lesioned rat (*lower panel*). *LV* lateral ventricle, *AcbC* nucleus accumbens core, *AcbS* nucleus accumbens shell, *aca* anterior commissure. Note ventricular dilatation and neuronal loss in the AcbC of the lesioned brain. *Right-hand diagrams*, approximate area of destruction of the AcbC in the lesioned group. Drawings were made from the microscopic sections, superimposed on the relevant pages of Paxinos and Watson's (1998) stereotaxic atlas. Locations of the sections in the AP plane (millimetres anterior to bregma) are indicated on the *left*. The *black area* represents the smallest and the shaded area the largest lesion.

7.4   Discussion

Injections of quinolinic acid into the AcbC produced lesions of similar extent to those seen in previous experiments in which excitotoxins have been used to ablate this structure (Bowman and Brown 1998; Cardinal et al. 2001; Cardinal and Cheung 2005; Acheson et al. 2006; Bezzina et al. 2007, 2008a; da Costa Araújo 2009, 2010). The area of destruction was mainly restricted to the target structure, although some additional damage was inflicted on the ventromedial caudate–putamen in some rats.

The AcbC-lesioned group showed significantly lower values of $d_{B(50)}$ than the sham-lesioned group under both experimental conditions. This result, obtained with an adjusting-delay schedule, is consistent with previous findings made with adjusting-delay

(da Costa Araújo et al. 2009, 2010), progressive-delay (Cardinal et al. 2001; Pothuizen et al. 2005; Bezzina et al. 2007) and adjusting-magnitude (Acheson et al. 2006) schedules in showing that destruction of the AcbC disrupts inter-temporal choice behaviour, promoting choice of a smaller immediate reinforcer in preference to a larger delayed reinforcer (for review, see Basar et al. 2010*)*. This effect of the lesion has been attributed to an increase of the rate of delay discounting (see Cardinal et al. 2003; Dalley et al. 2008; Basar et al. 2010). The present findings are in agreement with this interpretation and also help exclude some competing explanations, which were not totally excluded in some earlier studies (see below).

The ratio of the indifference delays seen in both groups was substantially less than the value of 3.81 predicted by Eq. 5 (see section 5.1). This finding, which is in agreement with findings reported in Experiment 4, is inconsistent with the assumption of strict proportionality between the value of a reinforcer and its magnitude (cf. Eqs. 1a, 1b, see section 5.1) and supports the contention of Ho et al. (1999) that a 'size sensitivity' parameter is a necessary component of any quantitative model of inter-temporal choice (see also Killeen 2009). As pointed out by Ho et al. (1999*)* and Mazur (2006), the potential influence of neurobiological interventions on size sensitivity needs to be taken into account when interpreting the effect of such interventions on inter-temporal choice.

The approach adopted in this experiment, previously described in Experiment 4, enabled numerical estimates of the delay and size sensitivity parameters, K and Q, to be derived from two indifference delays. The logic of the method, based on the 'multiplicative hyperbolic model' of Ho et al. (1999), is illustrated in Fig. 7.5.
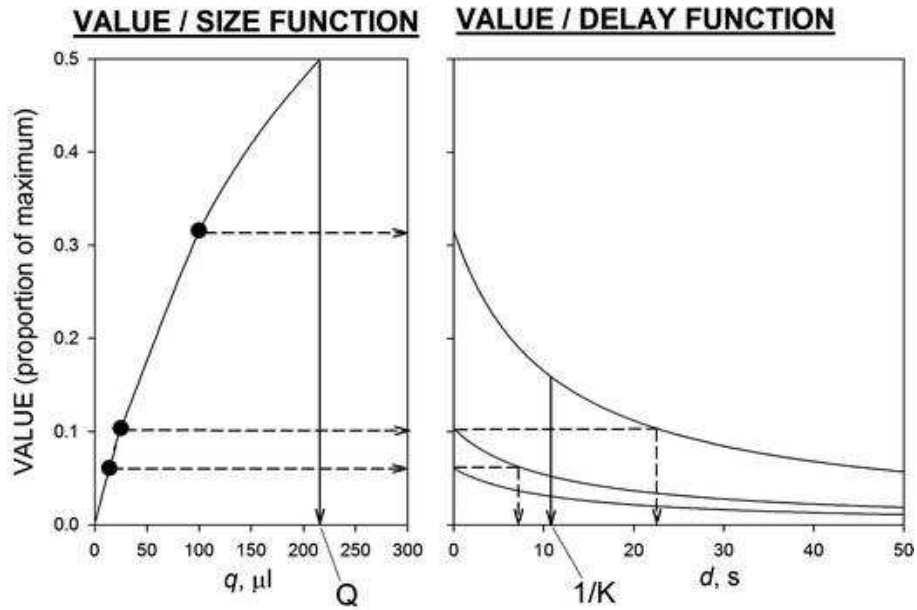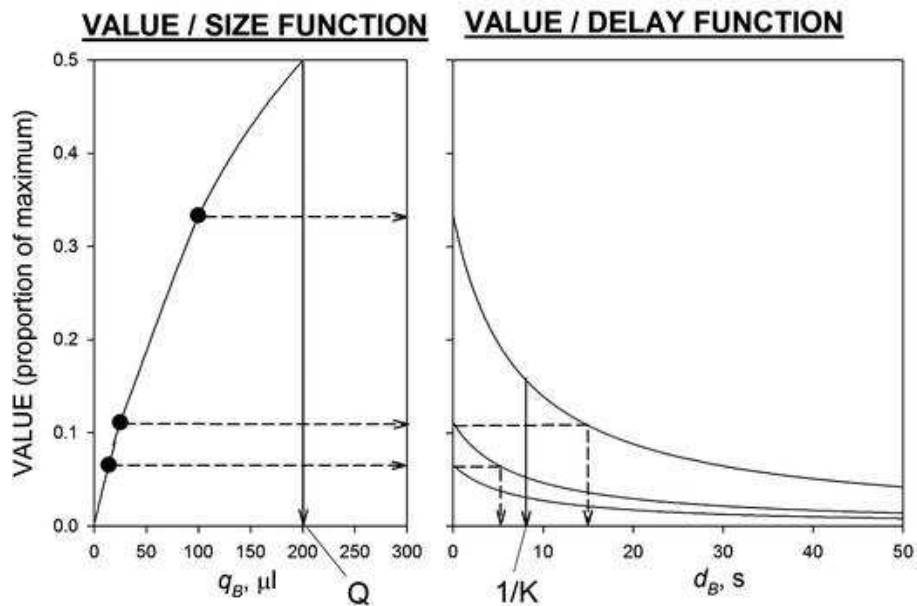
Fig. 7.5 Theoretical relationship between the size sensitivity and delay discounting parameters (Q and K) and the indifference delays derived under the two conditions of the experiment: sham-lesioned group (*upper graphs*) and AcbC-lesioned group (*lower graphs*). *Left-hand graphs*, Relation between reinforcer value and reinforcer size, q; the curves are defined by the size sensitivity term of Eqs. 2a, 2b (see section 5.1) using the mean estimates of Q derived for the sham-lesioned (*upper graph*) and AcbC-lesioned (*lower graph*) groups (the estimates of Q are indicated by the *drop down reference lines*). The *three points* show the instantaneous values of $q_{B(1)}$ (100 μl), $q_{A(1)}$ and $q_{B(2)}$ (25 μl), and $q_{A(2)}$ (14 μl). *Right-hand graphs*, Relation between reinforcer value and delay to reinforcement, d. The curves are defined by the delay discounting term of Eqs. 2a, 2b (see section 5.1) using the mean estimates of K derived for the sham-lesioned (*upper graph*) and AcbC-lesioned (*lower graph*) groups (the estimates of K are indicated by the *continuous drop down reference lines*); the y intercepts of the curves correspond to the instantaneous values of the three reinforcer sizes. The indifference delays, indicated by the *broken drop down lines*, are defined by the discounted values of the larger reinforcer that equate to the instantaneous values of the smaller reinforcer, as indicated by the *horizontal broken lines*. Note the lower value of K and the shorter indifference delays in the AcbC-lesioned group compared with the sham-lesioned group

The left-hand graphs show the postulated hyperbolic relation between instantaneous value and the size of a reinforcer; the right-hand graphs, the hyperbolic delay discounting function. The continuous vertical drop lines indicate the values of the two parameters (Q and $1/K$, respectively) derived for the sham-lesioned (upper graphs) and AcbC-lesioned (lower graphs) groups in the present experiment. The method requires three reinforcer sizes: in the present experiment, these were $q_{A(1)}$= 25 µl, $q_{B(1)}$=100 µl and $q_{A(2)}$=14 [note that $q_{B(2)}=q_{A(1)}$]. The points in the left-hand graphs show the instantaneous values corresponding to these reinforcer sizes, and the three curves in the right-hand graphs show the delay discounting functions for these instantaneous values. The indifference delay for condition 1 is defined by the discounted value of $q_{B(1)}$ that equates to the instantaneous value of $q_{A(1)}$ and the indifference delay for condition 2 by the discounted value of $q_{B(2)}$ that equates to the instantaneous value of $q_{A(2)}$; these indifference delays are indicated by the two broken drop lines in the right-hand graphs. It should be noted that although different reinforcer sizes might have been used, the choice was limited by the requirement that $(1/q_{A(1)} - 1/q_{B(1)}) = (1/q_{A(2)} - 1/q_{B(2)})$ (see section 5.1).

The present finding that the value of K was higher in the lesioned group than in the sham-lesioned group provides positive evidence for a lesion-induced increase in the rate of delay discounting. Moreover, the finding that the parameter Q was not affected by the AcbC lesion argues against the possibility that the effect of the lesion on inter-temporal choice reflects a change in sensitivity to the sizes of reinforcers. These findings obtained with adjusting-delay schedules using a novel quantitative protocol based on Eqs. 3a and 4 (see section 5.1) are in agreement with previous findings by Bezzina et al. (2007) using a different approach. These authors used linear indifference functions based on Eq. 3 (Ho et al. 1999) to demonstrate that lesions of the AcbC produced an increase in K with no significant effect on Q. Further evidence for a role of the AcbC in delay discounting derives from a study of Fos expression (a marker of neuronal activation) in intact rats performing inter-temporal choice tasks. da Costa Araújo et al. (2010) found that rats exposed to a task that entailed choices between delayed reinforcers which did not differ in magnitude (Mazur 1984) showed higher levels of Fos expression in the AcbC than rats trained under a control task that entailed choices between reinforcers that differed in magnitude but not in delay.

Another way in which interventions may influence inter-temporal choice performance, which may be mistaken for a change in the rate of delay discounting, is an

alteration of the subject's ability to detect short-term changes in delay of reinforcement (Acheson et al. 2006). Bezzina et al. (2007) analysed the preference functions of rats exposed to an inter-temporal choice task based on Evenden and Ryan's (1996) progressive-delay schedule and found that AcbC-lesioned rats showed somewhat flatter preference functions than sham-lesioned rats, consistent with the notion that AcbC lesions may have impaired rats' ability to detect within-session changes in delay (Acheson et al. 2006); however, Bezzina et al. pointed out that this could not account for the systematic reduction of $d_{B(50)}$ induced by the lesion. More recently, da Costa Araújo et al. (2009, 2010) analysed the cyclical changes in d_B in an adjusting-delay schedule using the Fourier transform and found no differences between the dominant frequency of the power spectrum of oscillations of d_B between AcbC-lesioned and sham-lesioned rats, suggesting that the lesion did not alter the rats' sensitivity to trial-by-trial changes in delay to reinforcement. The present finding that neither the spectral power within the dominant frequency band nor the period corresponding to the dominant frequency differed significantly between the AcbC- and sham-lesioned groups replicates the finding of da Costa Araújo et al. (2009, 2010) using pairs of reinforcer magnitudes different from those used in the previous study. The general features of the power spectra seen in both groups were similar to those reported in Experiment 4: the period corresponding to the dominant frequency was approximately 70–80 trial blocks under both conditions, and the power within the dominant frequency band tended to be higher under condition 2 (q_A=14 μl, q_B=25 μl) than under condition 1 (q_A=25 μl, q_B=100 μl).

The present results support the contention that the AcbC plays an important role in guiding inter-temporal choice. In particular, the present results, together with other recent findings with rats (Bezzina et al. 2007, 2008; da Costa Araújo et al. 2009, 2010), suggest that AcbC's role in guiding inter-temporal choice is specifically related to the hypothetical process of delay discounting. Moreover, recent functional neuroimaging studies with humans and single-unit recording studies with non-human primates have implicated the corpus striatum, including the AcbC or ventral striatum, in inter-temporal choice (e.g. Gregorios-Pippas et al. 2009; Hariri et al. 2006; Pine et al. 2009; Weber and Huettel 2008; Wittmann et al. 2007; Wittmann and Paulus 2008), although it remains uncertain whether, in primates, the striatum plays a specific role in delay discounting rather than a more general role in reinforcer valuation (Ballard and Knutson 2009; Kable and Glimcher 2009). The AcbC appears to function as one component of a complex circuit controlling choice behaviour. The OPFC constitutes an important component of

this circuit (see Cardinal 2006; Wallis 2007; Ballard and Knutson 2009; Kable and Glimcher 2009), which may integrate the influences of multiple dimensions of reinforcing stimuli (including magnitude and delay) to determine the overall value or utility of each rewarding outcome in a choice situation (Wallis 2007). Consistent with this notion is the finding that destruction of the OPFC in rats not only increased the rate of delay discounting, as measured by K, but also increased the size sensitivity parameter, Q, in the model of Ho et al. (1999) (Kheramin et al. 2002), suggesting that the lesion diminished the absolute values of reinforcers with a consequent increase in the ratio of the values of two reinforcers in an inter-temporal choice situation (see Kheramin et al. 2005).

The behavioural protocol used in the present experiment, similar to Experiment 4, was based on an algebraic derivation from the model of inter-temporal choice of Ho et al. (1999). A potential advantage of this protocol over the linear indifference function used in previous studies (e.g. Bezzina et al. 2007) is the relatively short training period required to obtain quantitative estimates of K and Q (140 sessions in the present study, as opposed to approximately 260 in studies based on the linear indifference function). Against this advantage is the potential drawback that parameter estimates based on two data points may turn out to be less reliable than estimates derived by fitting linear functions to a family of five or six points. Further work will be needed before the relative merits of the two methods can be properly assessed.

Although the derivation of quantitative estimates of K and Q in the present experiment was based specifically on the model of Ho et al. (1999), the principal finding that lesions of the AcbC reduced the indifference delays to the larger of two reinforcers is compatible with other models of inter-temporal choice. Killeen (2011) has recently described a model of inter-temporal choice based on the principle of exponential decay of memory traces. One advantage of this model is that it offers an explanation of delayed reinforcement that does not rely on the implausible notion of a retrograde influence of reinforcers on previously executed responses. The model generates 'hyperbola-like' delay-of-reinforcement gradients and a linear indifference function similar to that defined by Eq. 3. The delay discounting parameter, K, in hyperbolic discounting models has a close counterpart in the time constant of the decay of memory traces, τ, in Killeen's (2011) model. Thus, Killeen's model interprets the present finding of an AcbC lesion-induced increase of K as a reduction of the time constant, τ. This leads to the testable, but as yet untested, prediction that lesions of the AcbC should reduce τ in behavioural tasks

other than inter-temporal choice schedules; some tasks appropriate to addressing this prediction have been discussed by Killeen (2011).

CHAPTER 7

GENERAL DISCUSSION

8.1. Summary of the results

This thesis examined the neural mechanisms underlying the regulation of interval timing and inter-temporal choice behaviour, with special reference to role of the cortico-striato-thalamo-cortical (CSTC) circuits.

The first part of the thesis examined whether, in intact rats, performance of different interval timing tasks was associated with neuronal activation in the dorsal striatum and prefrontal cortex, as revealed by expression of the Fos protein, the product of the immediate-early gene *c-fos* (Experiments 1-3). Control groups of rats were employed that were exposed to conditions in which factors such as the motor requirements, deprivation conditions and reinforcement rate were matched, as closely as possible, to the behavioural task of interest.

The second part of the thesis focused on some behavioural and neurobiological aspects of inter-temporal choice behaviour. One purpose of this section was to describe an abbreviated approach to estimating Q and K based on Equation 3, which required the determination of only two values of $d_{B(50)}$ under the adjusting delay schedule (see section 5.1). Additionally a novel way of quantifying transitional behaviour in the adjusting-delay schedule was presented based on analysis of the power spectrum of cyclical changes in the adjusting delay (Experiment 4). This latter approach was used to analyze data obtained from rats performing on the adjusting delay schedule under methodological manipulations (Experiment 5) and neurobiological interventions (Experiment 6).

Experiment 1 investigated whether, in intact rats, performance on the discrete-trials temporal discrimination task was associated with neuronal activation in the prefrontal cortex and corpus striatum, as revealed by enhanced Fos expression in these areas. Performance on this schedule was similar to that reported in previous studies (Body et al. 2002a; Asgari et al. 2005; Asgari et al. 2006a; Hampson et al. 2010). Proportional choice of lever B increased as a function of stimulus duration and was well described by a two-parameter logistic equation. Performance on the light-intensity discrimination task was well described by the same equation, as reported previously (Hampson et al. 2010). The rats trained under the timing task showed increased Fos expression in the prefrontal cortex and nucleus accumbens, compared to the rats trained under light-intensity discrimination task, indicating a substantial activation of these areas during the timing task. However, the experiment provided no evidence for an

involvement of the dorsal striatum in the performance of this task.

The results of Experiment 2 showed that rats trained under an interval-bisection task in the range of milliseconds showed increased Fos expression in the prefrontal cortex and nucleus accumbens compared to the rats trained under a light-intensity bisection task, indicating a substantial activation of these areas during the timing task. Performance on the interval bisection task was similar to that reported previously (Church and Deluty 1977; Fetterman and Killeen 1991; Morrissey et al. 1993; Graham et al. 1994). It was argued that since the use of millisecond-range stimuli suppresses movement across the operant chamber during the period of stimulus presentation, it is unlikely that the higher levels of Fos expression seen in the rats performing the temporal discrimination task was caused by enhanced locomotor activity. As in Experiment 1, the results provided no evidence for an involvement of the dorsal striatum in the performance of the temporal discrimination task.

Experiment 3 investigated whether performance on the FIPP would result in increased neuronal activity in the prefrontal cortex and corpus striatum, as revealed by Fos expression. Performance on FIPP was similar to that reported in previous studies (Roberts 1981; 1982; Church and Broadbent 1990; Orduña et al. 2008). Consistent with the results of Experiments 1 and 2, the concentration of Fos-positive neurones (counts.mm^{-2}) in the OPFC was greater in the rats exposed to FIPP than in rats exposed to a VI schedule, suggesting a greater activation of this area during the performance of the former task. This is consistent with the results of previous studies that have implicated the prefrontal cortex in temporal differentiation (Dietrich and Allen 1998; Meck 2006a, see also section 1.4.4). The results, however, do not provide any evidence for a specific involvement of the dorsal striatum in FIPP performance.

Taken together, the results of Experiments 1, 2 and 3 suggested that the OPFC was engaged during timing performance in both retrospective and immediate timing schedules. Experiments 1 and 2 implicated the nucleus accumbens in temporal discrimination performance in retrospective schedules, whereas Experiment 3 did not provide supporting evidence for the involvement of the nucleus accumbens in temporal differentiation performance. None of the three experiments provided evidence supporting the involvement of the dorsal striatum in timing performance in either retrospective or immediate timing schedules.

In Experiment 4, rats made repeated choices on an adjusting-delay schedule. Indifference delays, calculated from adjusting delays in the last 10 sessions of each

phase, were shorter when the sizes of reinforcers were 14 and 25 µl of a 0.6 M sucrose solution than when they were 25 and 100 µl of the same solution. The ratio of the indifference delays was significantly smaller than that predicted on the basis of an assumed linear relation between reinforcer size and instantaneous reinforcer value, and consistent with a previous proposal that this relation may be hyperbolic in form (Ho et al., 1999). Estimates of the rate of delay discounting (K) based on two data points (mean, 0.08 s^{-1}) were similar to values obtained previously using different inter-temporal choice protocols (Green et al., 2004; Mazur, 2007; Mazur & Biondi, 2009). Estimates of the size-sensitivity parameter, Q (mean 113 µl) were similar to estimates recently derived from performance on progressive-ratio schedules (Rickard et al., 2009). Adjusting delays in successive blocks of trials were analysed using the Fourier transform. The power spectrum obtained from individual rats had a dominant frequency that corresponded to a period of oscillation of the adjusting delay between 30 and 100 trial blocks (mean, 78). Power in the dominant frequency band was highest in the early sessions of the first phase and declined with extended training.

Experiment 5 attempted to manipulate the pattern of oscillation of d_B in an adjusting delay schedule as revealed by the power spectrum analysis. Because it was assumed that the cyclical changes of d_B reflect the ability of the rats to detect block to block changes in delay to reinforcement, the step-size in which d_B increased or decreased was compared across two conditions. In Condition 1, the delay to the larger reinforcer increased or decreased (according to the rats' choice) by 20% from block n to block $n+1$. In Condition 2, the step size was 10%. Analysis of the power spectrum of the fluctuation of d_B showed that the period of oscillation coreresponding to the dominant frequency of the spectrum was significantly longer under Condition 2 (approximately 100 blocks) than under Condition 1 (approximately 80 blocks). The longer period of oscillation seen under Condition 2 was an expected result, since the period of the dominant frequency is measured in blocks, and relatively large changes in d_B may be required in order for a change in reinforcer value to become detectable. There was a consistent trend for the power of oscillation to be higher in the initial segment of the experiment in both conditions, a pattern that was also seen in Experiment 4. The decline in power seen across segments presumably reflects some adaptation to the schedule contingencies during extended training.

Finally, Experiment 6 examined the effect of lesions of the AcbC on the parameters Q and K using the method developed in Experiment 4. Numerical estimates

of Q and K, derived for individual subjects, were compared between sham-lesioned and AcbC-lesioned rats. As in Experiment 4, indifference delays were determined using an adjusting-delay schedule (Mazur 1987). A secondary aim of the present experiment was to examine the effect of AcbC lesions on the power spectrum of cyclical changes in the adjusting delay using different values of q_A and q_B. The AcbC-lesioned group showed significantly lower values of $d_{B(50)}$ than the sham-lesioned group under both experimental conditions. The ratio of the indifference delays seen in both groups was substantially less than the value predicted on the basis of the assumption of strict proportionality between the value of a reinforcer and its magnitude (cf. Eqs. 1a, 1b, see section 5.1). The fact that the value of K was higher in the lesioned group than in the sham-lesioned group provided positive evidence for a lesion-induced increase in the rate of delay discounting. The finding that the parameter Q was not affected by the AcbC lesion argued against the possibility that the effect of the lesion on inter-temporal choice reflected a change in sensitivity to the sizes of reinforcers. Additionally, neither the spectral power within the dominant frequency band nor the period corresponding to the dominant frequency differed significantly between the AcbC- and sham-lesioned groups, replicating the finding of da Costa Araújo et al. (2009, 2010) using pairs of reinforcer magnitudes different from those used in the previous study. The general features of the power spectra seen in both groups were similar to those seen in Experiment 4: the period corresponding to the dominant frequency was approximately 70–80 trial blocks under both conditions, and the power within the dominant frequency band tended to be higher under condition 2 (q_A=14 μl, q_B=25 μl) than under condition 1 (q_A=25 μl, q_B=100 μl).

8.2 Implications of the present results

8.2.1 *Implications of the present results for the neural substrates of interval timing*

As reviewed in section 1.3.1.7, it is believed that interval timing behaviour is regulated by striatal and cortical neurones (Matell and Meck 2004; Meck et al. 2008). In particular, it has been proposed that the dorsal striatum and its connections with the cerebral cortex play a key role in the SBF timing model proposed by Meck and colleagues (Meck et al. 2008). However, results from Experiments 1-3 provided only partial support for this notion. The results of Experiments 1 and 2 implicated the OPFC and the nucleus accumbens in the performance of retrospective timing tasks. Additionally, Experiment 3

showed a higher level of Fos expression in the OPFC of rats performing on the FIPP. None of the experiments implicated the dorsal striatum in the performance of retrospective or immediate timing tasks. Thus the present results question the involvement of the dorsal striatum in interval timing behaviour. Previous studies which have shown a link between interval timing and the dorsal striatum have employed FIPP (Matell and Meck 2004; Meck et al. 2008). FIPP has been classified as a free-operant immediate timing task (Killeen and Fetterman, 1988) and is usually characterized by a high rate of responding which increases to a maximum around the time of reinforcement in the FI trials (see section 1.2.2.1). It is possible that previous studies which have proposed the dorsal striatum as a key structure in interval timing behaviour (Matell and Meck 2004; Meck et al. 2008) had underestimated the effect of locomotor activity in the activation of this structure during the performance of FIPP. It is known that the striatum is involved in locomotion, and that neurological diseases affecting it induce movement disorders (Albin et al. 1989; DeLong 1990; Gerfen and Engber 1992). In this context, it is of interest to note a study by Liste et al. (1997) which demonstrated enhanced Fos expression in the mediodorsal and central striatum and nucleus accumbens, as well as in the dorsal region of the caudal striatum of rats exposed to treadmill running.

Comparison of the immunohistochemical results of Experiments 1 and 2, and Experiment 3 indicates that the absolute numbers of Fos-positive neurones in the ILPFC and PLPFC, as well as the ventral and dorsal striatum, were considerably higher in Experiment 3 than in Experiments 1, and 2. This raises the possibility that the higher rates of responding generated by the FIPP and VI schedules (Experiment 3), compared to schedules that employ discrete trials may have produced more activation in these areas, and hence a higher levels of Fos expression. It was also possible that movement-induced Fos expression may have masked any increase in Fos expression induced by the temporal differentiation performance.

In an attempt to minimize the effect of locomotor activity on Fos expression, a discrete-trial temporal discrimination task was employed in Experiment 1. Moreover, Experiment 2 used millisecond-range stimuli (200 vs. 800 ms) in a temporal bisection task to prevent intra-stimulus movement across the chamber. The results obtained in both experiments demonstrated that the nucleus accumbens is involved in the performance of retrospective timing. It is possible that the nucleus accumbens may make a specific contribution to the control of timing behaviour. It is of interest to consider these results in the context of the putative role of the ventral striatum in inter-temporal choice. Inter-

temporal choice schedules have been regarded as a type of prospective timing task (Killeen and Fetterman 1988). Quantitative analysis of inter-temporal choice performance has indicated that destruction of the AcbC results in a selective increment in the rate of delay discounting (Bezzina et al. 2007; da Costa Araújo et al. 2009). Moreover it has recently been found that performance of an adjusting-delay schedule is associated with increased Fos expression in the AcbC (da Costa Araújo et al. 2010). Further experiments using other timing tasks, as well as neurobiological interventions are needed, to explore the possibility of a specific role of the nucleus accumbens in retrospective and immediate timing.

A common result from Experiments 1-3 is the involvement of the OPFC in the performance of timing tasks. These results are compatible with previous studies which have demonstrated that lesions of the OPFC can disrupt the performance of rats on timing tasks (Dietrich and Allen 1998; Meck 2006a, see also section 1.4.4). Although a role of the OPFC in interval timing seems apparent, it has been proposed that one function of the prefrontal cortex is to integrate the many processes that are involved in operant behaviour, assigning an overall value to each reinforcing outcome and providing a basis for decision making in choice situations (Wallis 2007). Under this view, the OPFC plays a more general role in operant behaviour and possibly only an indirect role in interval timing. Most timing schedules necessarily involve multiple behavioural processes; further experiments are needed to isolate the processes of interval timing from the many other processes that may be subserved by the prefrontal cortex.

Finally, although the SBF model (Matell and Meck 2004; Meck et al. 2008) is innovative in terms of integrating the neural components of the CSTC circuits in the understanding of temporal control, the present results raise doubts about the purported crucial role of cortico-striatal mechanisms in interval timing performance. Moreover, the finding that different structures are activated during different timing tasks is not compatible with the idea of a unitary hypothetical biological "clock" proposed by SBF (Matell and Meck 2004; Meck et al. 2008).The notion of a unitary internal clock is also challenged by studies which have shown that that systemic treatment with drugs acting at dopamine and 5-HT receptors can have qualitatively different effects on performance on immediate and retrospective timing schedules, suggesting that different behavioural and neural mechanisms may be involved in temporal differentiation and temporal discrimination (see section 1.4.1.4 and 1.4.2.4 for references). Future timing theories that attempt to integrate behavioural and neurobiological evidence will need to address this

possibility.

8.2.2   Implications of the present results for the neural substrates of inter-temporal choice

As reviewed in section 1.3.2, in an inter-temporal choice schedule, a subject chooses between reinforcers that differ with respect of their sizes and delays. A common approach in this area of research is to examine the effect of a neurobiological intervention on preference for the larger of two reinforcers while progressively increasing the delay to that reinforcer. According to the multiplicative hyperbolic model of inter-temporal choice (MHM: Ho et al. 1999), the indifference delay is determined by two parameters, *K* and *Q*. Several indifference delays corresponding to a range of delays to the smaller reinforcer (d_A) are obtained, and then a linear indifference function defined by Equation 3 is constructed (see section 5.1). The slope and intercept of this function may then be used to infer changes in *K* and *Q* (Ho et al., 1999; Mazur, 2006). The approach adopted in Experiments 4 and 6 was based on only two indifference delays. The results obtained in Experiment 6 using this protocol are in agreement with previous findings by Bezzina et al. (2007) using the multi-point method described above. These authors demonstrated that lesions of the AcbC produced an increase in *K* with no significant effect on *Q*. An advantage of the abbreviated method over the linear indifference function used in Bezzina's et al. (2007) study is the relatively short training period required to obtain quantitative estimates of *K* and *Q*. However, the parameter estimates based on two data points may turn out to be less reliable than estimates derived by fitting linear functions to a family of five or six points. Further experiments will be needed to assess the advantages and disadvantages of these two methods.

The results of Experiment 6 are in agreement with evidence that supports the idea that the Acb, in particular the AcbC, plays an important role in guiding inter-temporal choice (Cardinal et al. 2001; Pothuizen et al. 2005; Cardinal 2006, da Costa Araújo et al. 2009; 2010;). These results have been interpreted in terms of an increase in the rate of delay discounting in Acb-lesioned rats (e.g. Cardinal 2006). However Acheson et al. (2006) trained animals under an adjusting-magnitude schedule and reported that Acb-lesioned rats were less sensitive to within- and between-session changes in delay to reinforcement than sham-lesioned rats. da Costa Araujo et al. (2010) suggested that analysis of the cyclical changes in d_B in an adjusting-delay schedule using the Fourier

transform may yield information about the sensitivity of rats to short-term changes in delay. This analysis was used in Experiment 6 to examine whether AcbC-lesioned animals were impaired in their ability to detect short-term changes in delay as suggested by Acheson et al. (2006). The result of this experiment demonstrated that neither the spectral power within the dominant frequency band nor the period corresponding to the dominant frequency differed significantly between the AcbC- and sham-lesioned groups suggesting that the lesion did not alter the rats' sensitivity to trial-by-trial changes in delay to reinforcement.

The power spectrum of oscillations provides a new approach to analyzing transitional behaviour under an adjusting-delay schedule. A verdict on the reliability of the method may have to await the results of future studies examining its sensitivity to a broader range of neurobiological interventions and behavioural manipulations.

8.2.3. *Prospects for a unified theory of interval timing and inter-temporal choice behaviour*

8.2.3.1 Behavioural processes and methodological considerations

As discussed in the Introduction (see section 1.2), Killeen and Fetterman (1988) proposed a taxonomy of timing schedules based on the subject's location in time with respect to the interval being timed. According to these authors, there are three types of timing schedules: retrospective, immediate and prospective. Although these different kinds of schedule entail different reinforcement contingencies, timing performance maintained under the three types of schedule share certain features. For instance, a considerable amount of evidence has shown that irrespective of the schedule used, timing behaviour conforms to Weber's Law (Gibbon 1977; 1999). This implies that psychometric functions obtained using different reference durations are superposable when rescaled to the empirical value of T_{50}. This is known as the scalar property of interval timing. However, it should be noted, that superposability applies only to psychometric functions obtained using the same timing schedule, and that the form of this function and the timing parameters differ somewhat between timing schedules (Grondin 2001). Additionally, some authors have pointed out differences in the sizes of the Weber fractions obtained with different timing tasks and suggested that different timing tasks entail different timing processes (Zeiler 1998; Grondin 2001; Bizo et al.

2006). Despite the importance of Weber's Law in interval timing theories, it should be emphasized that it is not a property of time perception only; rather it is a general property of perceptual processing across all modalities (Treisman 1964).

In retrospective and immediate timing tasks, the locus of the subject's timing behaviour in response to the changing reinforcement contingency in the schedule is taken as a measure of performance. This measure of central tendency differs between tasks. For instance, for tasks using a single operandum (e.g. FIPP), the locus is the time at which response rate is at its greatest. For choice tasks using two operanda (e.g. interval bisection) the locus is the time at which the subject chooses the two operanda equally (T_{50}). The variability around the locus is analyzed by calculating the coefficient of variation (standard deviation around the locus divided by the locus), a measure of the Weber Fraction which is regarded as an index of discriminability.

On the other hand, performance under prospective timing schedules (inter-temporal choice schedules) has generally been accounted for in terms of reinforcement value. However, this poses significant interpretative problems, because these schedules nearly always confound delay of reinforcement with some other parameter of reinforcement (usually reinforcer magnitude). For instance, according to Mazur's (1987; 1988) hyperbolic model of inter-temporal choice, reinforcer value (V) is a declining hyperbolic function of the delay interposed between the response and the primary reinforcer. An important implication of Mazur's model is that if a reinforcer is delivered immediately (i.e. $d = 0$), the value of the reinforcer is proportional to its size. However, there is emerging evidence for a nonlinear relation between value and reinforcer size (Mazur and Biondi 2009; Rickard et al. 2009). Ho et al. (1999) proposed a modification of Mazur's model to take into account the possibility of such a non-linear relation. These authors posited a hyperbolic relation, in which the effect of reinforcer magnitude on value is modulated by a single 'size-sensitivity parameter', Q (see section 1.3.2.2.).Thus, in Ho's et al. (1999) model the value of the reinforcer is determined by independent hyperbolic discount functions for size and delay. Each discounting function is governed by its own stable discounting parameter: Q for the effect of reinforcer size and K for the effect of delay.

As pointed out by Jozefowiez et al. (2009), research on choice has focused on the role of variables such as delay, frequency and magnitude, while research in timing has been analyzed from a psychophysical point of view and has emphasized the representation of time and information processing mechanisms. Some procedures to

study timing behaviour are choice procedures, and in some of them, reinforcement has been shown to influence psychometric properties of performance (Bizo and White, 1994a; 1994b; 1995a; 1995b). In an attempt to reconcile these approaches, Jozefowiez et al. (2009) developed the "Behavioural Economics of Choice and Interval Timing" model (BEM, see section 1.3.1.8). BEM shares some similarities with current models of interval timing and inter-temporal choice behaviour. Moreover, BEM integrates the concept of value ("payoffs") and the importance of reinforcement in timing behaviour. Firstly, BEM assumes that the representation of time follows Weber's Law, and that for each value that subjective time can take in a given situation, the organism associates a payoff for each response it can emit. The payoff functions determine the probability of emitting a response according to a maximizing rule. Although BEM has successfully explained the performance under wide range of procedures (FOPP, interval-bisection, double bisection, time-left procedure and matching), it has not yet been applied to performance under the adjusting-delay schedule (Mazur 1987). Extended training under the adjusting-delay schedule is associated with a period of cyclical change in d_B before a quasi-stable value is attained. This pattern may arise because the subject continues to select the larger reinforcer until the progressive increase in delay to reinforcement eventually reduces the overall value or "payoff" of the larger reinforcer to an extent that it becomes less attractive than the smaller immediate reinforcer; repeated selection of the smaller reinforcer then reverses the process, and so on. Although BEM, in its present form, does not consider the role of reinforcer magnitude in the performance observed under this schedule, it does emphasize the importance of reinforcement for both timing and inter-temporal choice behaviour. Future experiments are needed to establish whether BEM can account for the performance seen under inter-temporal choice schedules.

An integrated model of interval timing and inter-temporal choice behaviour may require more than reinforcement and timing to account for both processes. However, models such as BEM offer new alternatives over the cognitive models which generally disregard the possible interaction between reinforcement and timing. An alternative approach would be to consider interval timing and inter-temporal choice behaviour as completely separate entities. Although inter-temporal choice schedules have been considered as prospective timing schedules (Killeen and Fetterman 1988), theoretical accounts of inter-temporal choice, with their emphasis on the interacting influences of delay and magnitude of reinforcement, differ significantly from the theoretical and empirical view of interval timing behaviour. Much further research is needed to clarify

whether these two types of reinforcement schedule can be accounted for by a single theory.

8.2.3.2   Neural substrates

One important connection between inter-temporal choice and interval timing is the possibility that the same neural substrates may underlie both kinds of behaviour. A considerable amount of evidence has shown that the Acb plays an important role in guiding inter-temporal choice (see section 1.4.4.2.2 for references). Of particular interest are the studies which disentangle the effects of delay and magnitude, as K, the delay discounting parameter, implies the existence of a temporal process which allows the organism to perceive changes in delay of reinforcement. For instance, the results obtained in Experiment 6 and previous studies (see section 1.4.4.2.2 for references) have demonstrated that lesions of the AcbC increase K, but have no effect in Q. Consistent with these findings, the results of Experiments 1 and 2 demonstrated higher levels of Fos expression in the Acb, indicating activation of this area, during the performance of retrospective timing tasks. These results suggest a specific role of the nucleus accumbens in temporal processing. Further experiments using other timing tasks and neurobiological interventions are needed to explore this possibility.

As described in section 1.4.4.1.2, the OPFC has been linked to both inter-temporal choice and interval timing behaviour. Experiments based on MHM (Ho et al., 1999), have shown that lesions of the OPFC have a dual effect, increasing both K and Q. In this context, it is of interest to note a study by Bezzina et al. (2008) which investigated the effect of disconnecting the OPFC from the AcbC in a progressive delay schedule. The results showed that the disconnection group had a higher value of K but not of Q, than the sham-lesioned group. These authors suggested that delay discounting is regulated by a mechanism which includes both structures. Interestingly the OPFC-AcbC connections seemed not to be involved in the sensitivity to reinforcer size. It was suggested that while the OPFC integrates the information of several features of reinforcer including size and delay, its role in delay discounting is more specifically related to the AcbC.

Lesions of the OPFC also disrupt performance in retrospective and immediate timing tasks (see section 1.4.4.1.2 for references). Consistent with these findings, the results of Experiments 1-3 demonstrated higher levels of Fos expression in the OPFC in

animals performing retrospective and immediate timing task.

Taken together, these results suggest that the Acb is specifically related to delay discounting/temporal processing, and the OPFC plays a more general role in integrating several features of reinforcement. This is in agreement with a study by da Costa Araujo et al. (2010) in which exposure to the adjusting-delay schedule was associated with enhanced Fos expression in both the OPFC and AcbC, whereas exposure to the adjusting-magnitude schedule was associated with enhanced Fos expression in the OPFC but not the AcbC, compared to the control group. This supports the idea that one of the fuctions of the OPFC is to integrate the many processes that are involved in operant behaviour, assigning an overall value to each reinforcing outcome, thereby providing a basis for decision making in choice situations (Wallis 2007).

The Striatal Beat Frequency Model (SBF, Matell and Meck 2004; Meck et al. 2008) attempts to assign the general information processing components proposed by the Scalar Expectancy Theory (SET, Gibbon 1977) to putative underlying neural structures. SBF assumes the existence of a unitary mechanism or "internal clock" which underlies the ability of animals to regulate their own behaviour in time (temporal differentiation) and to discriminate between the durations of events (temporal discrimination). SBF implies that any neurobiological intervention will produce a qualitatively similar disruption of temporal differentiation and temporal discrimination. However, there is a considerable amount of evidence which suggest that temporal differentiation and temporal discrimination involve different behavioural and pharmacological mechanisms (see section 1.4.1.4 and 1.4.2.4 for references). For instance, d-amphetamine, 8-OH-DPAT and DOI induce leftward displacements of the timing functions in immediate timing schedules, but fail to do so in retrospective timing tasks (Chiang et al., 2000a; b; Asgari et al., 2006a; b; Body et al., 2006). These findings question the ability of models based on the concept of a unitary pacemaker that underlies all forms of interval timing behaviour to account for the findings of pharmacological studies.

Although SBF has taken an important innovative step by postulating an anatomical/physiological account of temporal control, there is a paucity of evidence from lesion studies with animals as to whether cortico-striatal mechanisms are crucial to the performance of interval timing tasks in animals. In particular, the present thesis provided no evidence for the putative role of the dorsal striatum in interval timing, and raised the possibility that the ventral striatum may play an important role in retrospective and immediate timing tasks, as it has been clearly established in the case of inter-

temporal choice behaviour.

8.3   *Future research*

The present set of experiments has raised a number of interesting questions that future work may help to address. The results of Experiments 1-3 suggested that the OPFC was engaged during timing performance in both retrospective and immediate timing schedules. Experiments 1 and 2 implicated the Acb in the performance of temporal discrimination schedules, whereas Experiment 3 did not provide supporting evidence for the involvement of the Acb in temporal differentiation. None of the three experiments provided evidence for an involvement of the dorsal striatum in timing performance. These experiments were based on measurement of the levels of Fos expression in different brain regions. Discrepancies between the present results and others (see section 1.3.1.7 for references) which suggested the dorsal striatum as an essential structure in interval timing behaviour may reflect the use of different biological techniques and timing schedules. Future studies using a wider range of biological techniques such as neurochemical lesions in the dorsal striatum and nucleus accumbens, and a variety of retrospective and immediate timing tasks, may elucidate whether these structures are involved in interval timing.

As discussed above, the results of Experiment 3 raise the possibility that the higher rates of responding generated by the FIPP and VI schedules, compared to schedules that employ discrete trials, may have produced more neuronal activation in these areas, and hence a higher levels of Fos expression. A study employing FOPP, which combines many features of DTPP with the use of free-operant schedules, may help to elucidate the effect of motor activity in the pattern of striatal Fos expression induced by interval timing schedules.

Bezzina et al. (2008) reported that rats that had undergone surgical disconnection of the OPFC from the AcbC showed higher values of *K*, but not of *Q*, than sham-lesioned rats. The authors suggested the OPFC-AcbC connections seem to be involved in delay discounting but not in sensitivity to reinforcer size. A study investigating the effect of this same disconnection lesion on performance on retrospective and immediate timing tasks could elucidate whether this pathway is specifically involved in temporal processing and provide a link between interval timing and inter-temporal choice.

One purpose of Experiments 4-6 was to describe a novel way of quantifying

transitional behaviour in the adjusting-delay schedule based on analysis of the power spectrum of cyclical changes in the adjusting delay. There is a considerable amount of evidence which suggests that lesions of the OPFC impairs the acquisition of new reinforcement contigencies in a continous delayed-non-matching-to sample task (Otto and Eichenbaum 1992), an olfactory discrimination task (Ferry et al. 2000; Schoenbaum et al. 2002), and conditioned responding after reinforcer devaluation (Gallagher et al. 1999). It has been suggested that inactivation of the OPFC in rats produces behavioural deficits which are strongly task-dependent. A study investigating the effect of PFC lesions on the parameters of the power spectrum could provide new insights into the role of the OPFC in the adaptation to changing contingencies in an adjusting-delay schedule, and test the utility of the Fourier transform-based analysis. Because the cyclical changes of d_B may reflect the ability of the rats to detect changes in delay to reinforcement from one trial block to the next, a lesion in the OPFC is predicted to change the period of the dominant frequency.

Adjusting-delay schedules entail complex contingencies, and the processes underlying the oscillating pattern of changes in d_B remain unclear at this time. A speculative model was described to account for some of the processes that may be involved in the performance under an adjusting-delay schedule (see section 5.4.3). In this model it was assumed that if there is no delay to Reinforcer A, ($d_A = 0$) the value of A (V_A) depends only upon its size, q_A, and the size-sensitivity parameter, Q. The value of B (V_B), however, varies from trial block to trial block, due to the influence of d_B, modulated by the delay-discounting parameter, K. Then, it was postulated that the subject discriminates between V_A and V_B, and selects the outcome that has the higher value at the moment of choice. It was assumed that rats' discrimination of value depends on the ratio of two values, rather than the absolute difference between them. A simple logistic function centered on $V_B/V_A=1$ was used to define the probability that B would be chosen. Finally, it was suggested that rats' ability to discriminate reinforcer value improved with practice, according to an exponential learning function (see Fig 5.7A, B and C). The model captures some features of behaviour on the schedule used in Experiment 4, although further experimentation using real data is needed to test the validity of the model. Additionally, further simulations varying the parameters of the model may elucidate the relationship between K and Q, and the postulated learning function.

Finally, Killeen (2011) recently presented a model of inter-temporal choice based on the principle of exponential decay of memory traces (see section 1.3.2.3). This model proposes that responses leave a 'memory trace' which decays in time; reinforcers act not on responses, but on their partially decayed traces. Killeen's model assumes that the process of decay follows an exponential time-course. When a reinforcer is delivered some time following the emission of an index response, the resulting strengthening effect on that index response is determined by competition between the partially decayed trace of the index response and the residual traces of other behavioural events that have occurred before and after the index response. Moreover, a delayed reinforcer does not have a lower 'value' than an immediate one, but its ability to strengthen an operant response is diminished due to the fact that it operates on a degraded memory trace of the response.

Killeen's model (2011) can account for most of the phenomena that are accommodated by traditional hypberbolic models, including preference reversal and linear indifference functions. Future experiments are needed to expand this model empirically and theoretically. Again, the model needs further testing in real data from rats performing under inter-temporal choice schedules.

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