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Double Dissociation between Serotonergic and Dopaminergic Modulation of Medial Prefrontal and Orbitofrontal Cortex during a Test of Impulsive Choice

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Dysregulation of the prefrontal cortex (PFC) has been implicated in impulse control disorders, including attention deficit hyperactivity disorder. A growing body of evidence suggests that impulsivity is non-unitary in nature, and recent data indicate that the ventral and dorsal regions of the PFC are differentially involved in distinct aspects of impulsive behaviour, findings which may reflect differences in the monoaminergic regulation of these regions. In the current experiment, levels of dopamine, serotonin and their metabolites were measured in the medial PFC ($n = 12$) and orbitofrontal cortex (OFC) ($n = 19$) of rats using *in vivo* microdialysis during the delay-discounting model of impulsive choice, where impulsivity is defined as selection of small immediate over larger delayed rewards. Yoked groups were also dialysed to control for instrumental responding and reward delivery. Significant increases in 5-hydroxytryptamine efflux were observed in the mPFC, but not in the OFC, during task performance but not under yoked control conditions. In the OFC, 3,4-di-hydroxy-phenylocetic acid (DOPAC) levels increased in animals performing the task but not in yoked animals, whereas mPFC DOPAC levels increased in all subjects. These data suggest a double dissociation between serotonergic and dopaminergic modulation of impulsive decision-making within distinct areas of frontal cortex.

Keywords: delay discounting, dopamine, impulsivity, *in vivo* microdialysis, serotonin

Introduction

Dysfunction within the prefrontal cortex (PFC) is associated with neuropsychiatric disorders, including those such as mania and attention deficit hyperactivity disorder (ADHD). However, it has recently been suggested that impulsivity is a non-unitary construct, and that different types of impulsivity may be underpinned by different biological mechanisms (e.g. Evenden, 1999). The PFC is not homogeneous, but is divided into distinct and functionally dissociable areas (Zilles and Wree, 1995; Goldman-Rakic, 1998; Dalley *et al.*, 2004). Studies of human volunteers and patients with brain damage suggest an important role for the orbitofrontal cortex (OFC), rather than the dorsolateral PFC, in impulsive decision-making, as determined through performance of gambling tasks (Damasio, 1994; Bechara *et al.*, 1999; Rogers *et al.*, 1999). In contrast, damage to regions such as the inferior frontal gyrus impair inhibitory control processes associated with impulsive action, i.e. the inability to withhold from making a motoric response (Aron *et al.*, 2003).

In rats, damage to medial prefrontal areas such as anterior cingulate and infralimbic cortex increase behavioural disinhibition as assessed by increases in the number of premature responses made in the five-choice serial reaction time task (5CSRT) (Muir *et al.*, 1996; Passetti *et al.*, 2002). In comparison, combined lesions of prelimbic plus infralimbic cortex, or anterior cingulate lesions, have little or no affect on a delay-discounting task, in which impulsive choice is defined as selection of a small immediate over a larger delayed reward (Cardinal *et al.*, 2001). However, both excitotoxic and dopaminergic lesions of the OFC decrease impulsive decision-making in this paradigm (Kheramin *et al.*, 2004; Winstanley *et al.*, 2004c), highlighting the importance of dopamine (DA) within this region in regulating this behaviour.

In contrast, *in vivo* microdialysis data indicate that 5-hydroxytryptamine (5-HT) rather than DA efflux in the medial PFC is correlated with levels of premature responding during a simplified version of the 5CSRT termed the 'one-choice' task (Dalley *et al*, 2002). Indeed, the neurochemical regulation of different aspects of impulsivity by the serotonergic and dopaminergic systems also appears to be dissociable. Administration of D-amphetamine, which elevates extracellular levels of DA and 5-HT in the brain and is used in the treatment of ADHD, increases premature responding in the 5CSRT (Harrison *et al.*, 1997), yet decreases impulsive choice in delay-discounting paradigms (Richards *et al.*, 1999; Winstanley *et al.*, 2003a). However, the ability of amphetamine to alter both these measures of impulsivity is at least partially dependent on intact serotonergic neurotransmission (Harrison *et al.*, 1997; Winstanley *et al.*, 2003a).

Furthermore, global decreases in serotonergic function increase impulsive action in various paradigms in both humans and rodents (e.g. Fletcher, 1995; Harrison *et al.*, 1997; Crean *et al.*, 2002), yet recent data indicate that such manipulations do not necessarily increase impulsive decision-making (Bizot *et al.*, 1999; Crean *et al.*, 2002; Winstanley *et al.*, 2003a, 2004b). However, administration of selective serotonergic agents have been found to alter levels of both impulsive action and impulsive choice in rats. In particular, higher doses of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) increases the number of premature responses made on the 5CSRT and also increases levels of impulsive choice (Carli and Samanin, 2000; Winstanley *et al.*, 2004a), an effect which appears to depend, at least in the case of impulsive decision-making, on the ability of post-synaptic 5-HT_{1A} receptors to alter dopamine release (Winstanley *et al.*, 2004a). In contrast, both systemic and intra-mPFC administration of the 5-HT_{2A} receptor antagonist M100907 have been shown to decrease premature responding (Higgins *et al.*, 2003; Winstanley *et al.*, 2003b, 2004d), yet antagonism of 5-HT_2 receptors does not appear to modify delay-discounting performance (Evenden, 1999).

To summarize, different frontal regions are differentially involved in impulsive choice and impulsive action, a distinction

that may relate to differences between the effects of serotonergic and dopaminergic manipulations on these behaviours. Therefore, the aims of this experiment were to investigate whether the OFC and mPFC are differentially regulated by 5-HT and DA during performance of a delay-discounting task through the application of *in vivo* microdialysis in behaving animals.

Materials and Methods

Subjects

Subjects were 72 male Lister Hooded rats (Charles River, Kent, UK), weighing 300–320 g at the start of each experiment and maintained at 85% of their free-feeding weight. Water was freely available. Animals were housed in groups of four under a reverse light cycle (lights on from 19.00 to 07.00 h). Testing took place between 08.00 and 19.00 h five to six days per week. Experiments were undertaken in accordance with the UK Animals (Scientific Procedures) Act 1986.

Experiment 1: Measuring Levels of DA, 5-HT and their Metabolites during Performance of a Delay-discounting Task

Behavioural Training

The behavioural apparatus and testing procedure have previously been described in detail (Cardinal *et al.*, 2000). Testing occurred in eight operant conditioning chambers (Med Associates Inc., St Albans, VT) fitted with two retractable levers located on either side of a food magazine into which 45 mg food pellets (Noyes dustless pellets, Sandown Scientific, Middlesex, UK) could be delivered, a magazine light and a houselight. Magazine entry was detected by an infrared photocell beam located across the entrance. Each chamber was encased in a sound-attenuating box and fan-ventilated. The apparatus was controlled by software written in Arachnid running on Acorn Archimedes Series computers (Cambridge, UK).

Subjects first learned to press levers for food reward under a fixed ratio FR1 schedule to a criterion of 50 presses in 30 min on each lever. Subsequently, subjects were trained to nosepoke in the magazine to trigger presentation of the levers in a simplified version of the full task. Every 40 s, a trial began with illumination of the houselight and the traylight. The subject was required to make a nose-poke response within 10 s to trigger presentation of a single lever. Responding on the lever within 10s led to illumination of the traylight and delivery of a single food pellet. The left and right levers were presented an equal number of times in the session with not more than two consecutive presentations of the same lever. Rats were trained to a criterion of at least 60 successful trials in 1 h.

Forty rats were then trained to perform a delay-discounting task (see Supplementary Fig. 1 for task schematic). Training continued over 35 sessions until stable baseline behaviour was achieved. Each session lasted 100 min, and consisted of five blocks of 12 trials. Each trial lasted 100 s regardless of the choice made by the subject, and each block began with two forced-choice trials. The onset of the houselight signalled the beginning of each trial, whereupon the rat had to nosepoke in the magazine to trigger lever presentation. An omission was scored if the rat failed to respond at the food magazine or subsequently on the levers within 10 s, and the box returned to the inter-trial interval (ITI) state with the houselight off until the next trial was scheduled to begin. The houselight was extinguished and the levers retracted once a response was made. Responding on one lever (lever A) always provided a small immediate reward of one pellet, the other (lever B) a large reward of four pellets. The location of levers A and B were counterbalanced between subjects. As the session progressed, the delay to the large reward increased in each block of trials from 0 to 10, 20, 40 and then 60 s. Onset of the traylight signalled food delivery, after which the box returned to the ITI state.

Surgery

Three animals were excluded following behavioural training as they showed a strong side bias, consistently responding on only one lever regardless of the reinforcement contingency in play. Subjects were matched for baseline performance and divided into two groups.

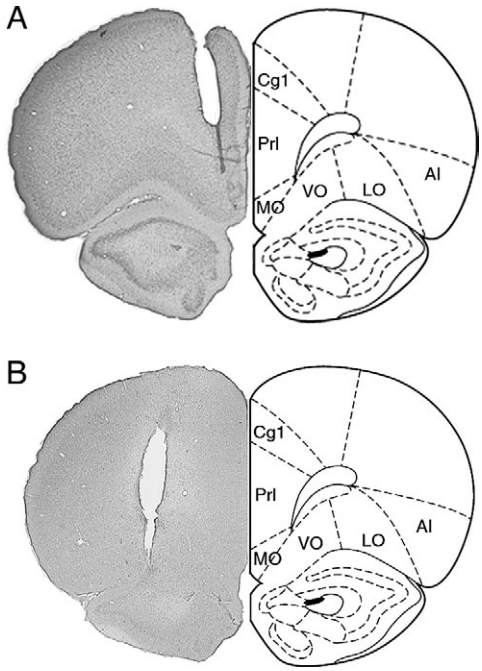

Figure 1. Representative locations of dialysis probes in the mPFC (*A*) and OFC (*B*).

A custom-made concentric design dialysis probe (for details, see Dalley *et al.*, 1998) was implanted into either the medial PFC ($n = 16$) or the ventrolateral OFC ($n = 21$) under ketamine (Ketaset, 100 mg/kg i.m.; Vet Drug, Bury St Edmunds, UK) and xylazine (Rompun, 10 mg/kg i.m.; Vet Drug) anesthesia using standard stereotaxic techniques. Probes were secured in place using dental cement and a bone screw. The active membrane (AN 69, Filtral 16; Hospal, UK) length was 2 mm for mPFC probes and 1 mm for OFC probes. All probes were primed with artificial cerebrospinal fluid (aCSF, in mM: NaCl, 147; KCl, 3; $MgCl_2$, 1; $CaCl_2$, 1.3; Na_2HPO_4, 1.3; $Na_2HPO_4.2H_2O$, 0.2; pH 7.4). The placements were made according to the following coordinates: mPFC AP +3.0 from bregma, L +1.2 from the midline, DV −4.0 from dura; OFC: AP +3.2, L +2.0, DV −5.5. The probes were inserted into the mPFC along a trajectory −12° off vertical, whereas probes were inserted into the OFC vertically. A small plastic tethering headpost (diameter × length = 4 × 10 mm) was also secured to the skull near lambda using four bone screws and dental cement. Animals were housed individually after surgery and allowed 48 h to recover prior to the start of the dialysis session, during which time they were fed 16 g of rat chow per day.

Dialysis Procedure

Animals were gently restrained to allow connection of the dialysis probes to the microperfusion pump and connection of the spring tether. The tether was connected to the subject by a small clamp screwed onto the headpost, and was fed vertically through a hole in the top of the operant box to a counterbalanced arm that was located centrally on the upper surface of the sound-attenuating chamber. The probe inlet was connected by a length of polyethylene tubing (i.d. = 0.28 mm, o.d. = 0.61 mm; Portex Ltd, Hythe, UK), housed inside the spring tether, to the low resistance port of a dual-channel liquid swivel (Instech, Stoelting, USA), which was serially connected to a 2.5 ml gas-tight syringe mounted on a microperfusion pump (Harvard, USA). The gas-tight syringe was loaded with aCSF which was perfused through the probe at a rate of 2 μl/min. In order to augment levels of 5-HT in the dialysate, the perfusate also contained a low concentration of citalopram (1 μM). The outlet tubing (FEP tubing, 1.2 μl/ 100 mm, Biotech Instruments Ltd, UK) was passed inside the spring tether to emerge below the rotational place of the liquid swivel. One hour after the animals had been placed in the operant chambers, a baseline sample was collected every 10 min for an hour. Following collection of these six baseline samples, the behavioural task was started and 10 further

samples were collected ever 10 min. Samples were stored on dry ice, and then at −80°C, before being analysed by high-performance liquid chromatography and electrochemical detection (HPLC–ECD).

Experiment 2: Measuring Levels of DA, 5-HT and their Metabolites in Subjects Yoked to Those Performing a Delay-discounting Task

Control conditions were incorporated into the overall experimental design to try to determine whether any changes in neurotransmitter level observed during the performance of the delay-discounting task were due to the making of delay-discounting judgements, from engaging in instrumental behaviour rewarded on a delay-dependent schedule or from simply receiving food reward. Eight rats with dialysis probes in the mPFC and eight with probes in the OFC, both impulsive and less-impulsive, were chosen from experiment 1 and formed the master group. The behavioural choice pattern recorded during the dialysis session of these rats were used as templates for the training of yoked control groups. The 'forced choice' (FC) yoked group was trained in the delay-discounting task as before, but only one lever was ever presented on each trial, and the probability that this lever produced a small immediate or large delayed reward was determined according to the choices the corresponding master rat made on the delay-discounting task during the dialysis session in experiment 1. Hence, these rats had to learn to respond for rewards which differed in size and in the delay to their delivery throughout the session in a similar way to animals which learned the delay discounting task, thus controlling for the effects of instrumental conditioning procedures inherent in the delay-discounting paradigm.

Another rat was yoked to each rat in the FC group, forming the 'free pellets' group (FP). These rats received the same food reward as the FC rats, and hence as the master rats, but they did not have to make an operant response to obtain it and no levers were ever presented to this group. These animals therefore formed an association between the context and the delivery of food reward, thus providing a control for the effects of aspects of Pavlovian conditioning intrinsic to the delay-discounting paradigm. During the experimental session where both groups of rats were dialysed (see above for details of surgery and dialysis procedures), the programme was set so that the reward offered or earned on each trial was matched exactly to the reward chosen by the animal in the master group. Rats were selected for the master group so that the average delivery of the large reward across delay and the variation in choice patterns between individuals was similar in both the yoked groups as compared to the whole cohort of animals which were dialysed during performance of the delay-discounting task.

HPLC Analysis with Electrochemical Detection (HPLC–ECD)

Levels of 5-HT, 5-HIAA, DA and DOPAC in dialysate samples were determined by HPLC–ECD as described previously in detail (Matthews *et al*., 2001; Dalley *et al*., 2002). Chromatographic data were acquired and processed using Chromeleon software (Dionex, Sunnyvale, USA).

Histological Verification of Probe Placements

Following the completion of behavioural testing, animals were terminally anaesthetized with sodium pentobarbitone (3 ml/kg i.*P*., Genus Express, Dunnington, UK) and perfused transcardially with 0.01 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. The brains were removed, post-fixed in 4% paraformaldehyde and transferred into 20% sucrose in 0.01 M PBS 24 h prior to being cut on a freezing Microtome. Coronal sections (60 μm) were stained with cresyl violet and the location of the placements verified using a stereotaxic atlas (Paxinos and Watson, 1998).

Data Analysis

In experiment 1, data were only available from 12 rats with probes in the mPFC as three probes failed and one animal did not perform the task during the dialysis session. Likewise, data were only available from 19 rats with probes in the OFC as one animal died perioperatively and one animal did not perform the task during the dialysis session. In order to allow valid comparisons between the data obtained from all three yoking conditions, levels of neurotransmitter or metabolite within the dialysate were expressed as a percentage of the levels obtained during baseline conditions. These data were analysed through an analysis of variance with sample ($baseline_1$, on-task $dialysis_{2\text{-}11}$) as the within-subjects factor and Condition (choice, forced choice and free pellets) as the between-subjects factor. Data from animals with probes in the mPFC and OFC were analysed separately. Behavioural measures were calculated and analysed as described previously (Winstanley *et al*, 2003a).

It was also of interest to determine whether there was any correlation between the levels of parent neurotransmitter or metabolite present in the dialysate and the level of impulsive choice shown. To this end, the average change from baseline across the task of 5-HT, 5-HIAA, DA and DOPAC and the total choice for the large reward across the session were subjected to bivariate correlational analysis to calculate the Pearson's product–moment correlation coefficient. The same analysis was also carried out using the absolute basal values of the neurotransmitters and metabolites. Due to the fact that multiple comparisons were being made, a more conservative level of significance was adopted ($P < 0.01$). The behavioural and neurochemical data were pooled across the session for this analysis, rather than attempting to associate changes in neurochemical efflux in particular samples with the level of impulsive choice shown at those timepoints, because such temporally dependent changes in neurotransmitter efflux may also reflect increased time spent engaged in delay-discounting behaviour or an increase in the difficulty of the judgements to be made, in addition to an increase or decrease in impulsive decision-making *per se* due to the stepwise increase in the delay to the large reward integral to the task structure.

Results

Verification of the Location of Dialysis Probes

Post-mortem histological analysis revealed that microdialysis probes were located in the mPFC or OFC accordingly. The location of the probes are represented in Figure 1.

Behaviour during In Vivo Microdialysis

As is typically found using this paradigm, a delay-dependent shift in choice of the large reward was observed [delay: $F(4,120) = 16.031$, $P < 0.001$, Fig. 2]. Subjects preferred the large reward when the delays were absent or short, but chose the smaller reward more frequently when the delay to the large reward was longer. This delay-dependent choice pattern was similar in animals with probes in both the mPFC and OFC [group: $F(1,30) < 1$, NS].

Subjects did omit some trials during the dialysis session, but the number of these omissions was comparable between groups and quite low overall [see Supplementary Table 1, group: $F(1,31) < 1$, NS], and did not affect the way in which choice of the large reward was calculated. The average latency to choose the levers was also very similar in both groups [see Supplementary Table 1, group: $F(1,31) < 1$, NS], as was the latency to collect the reward [see Supplementary Table 1, group: $F(1,31) < 1$, NS].

Animals in the forced choice group reliably responded for both the small immediate and larger delayed rewards during the dialysis session, regardless of the location of the dialysis probe, again omitting very few trials [see Supplementary Table 1, group: $F(1,15) < 1$, NS]. There were no differences in the latencies to respond on the lever presented, and to collect the associated reward between the two groups [response latency, group: $F(1,15) = 1.883$, NS; collection latency, group: $F(1,15) = 1.893$, NS]. Furthermore, no significant differences were found in these three variables between animals performing the delay-discounting and those in the forced choice group [condition: omissions, $F(1,46) < 1$, NS; choice latency, $F(1,46) < 1$, NS; collection latency, $F(1,46) < 1$, NS] or, overall, between those with dialysis probes in the mPFC versus OFC [group: omissions,

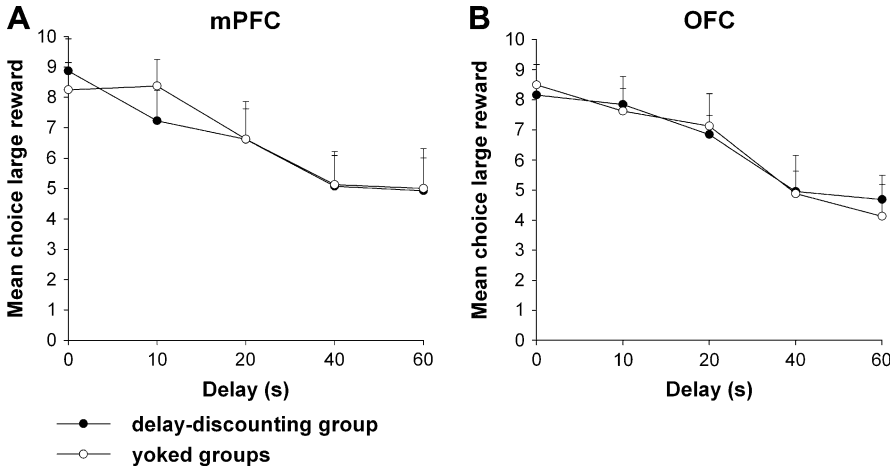

Figure 2. Choice pattern of subjects in animals performing the delay-discounting task and in the yoked groups. Although the subjects in the yoked groups were not actually choosing the large reward, the number of times the opportunity to earn the large reward was presented to the subjects (FC condition) or the large reward was delivered (FP condition) was matched to the behavioural group.

Table 1
Basal levels of 5-HT, 5-HIAA, DA and DOPAC in the mPFC and OFC of animals performing the delay-discounting task and yoked animals

	Delay-discounting		Forced choice		Free pellets	
	mPFC	OFC	mPFC	OFC	mPFC	OFC
5-HT	18.50 (2.62)	21.89 (2.25)	17.34 (1.59)	19.37 (4.53)	21.40 (4.41)	17.97 (8.98)
5-HIAA	269.34 (34.71)	202.51 (17.48)	367.56 (23.80)	188.95 (33.27)	345.04 (31.00)	203.10 (37.81)
DA	1.87 (0.77)		0.87 (0.26)		1.35 (0.39)	
DOPAC	41.99 (6.83)	22.00 (5.61)	43.77 (9.14)	27.64 (11.9)	40.45 (7.81)	29.96 (12.57)

Data shown are the mean fmol/sample (SEM). Note that the active membrane length of probes used in the mPFC and OFC were 2 and 1 mm respectively.

$F(1,46) < 1$, NS; choice latency, $F(1,46) < 1$, NS; collection latency, $F(1,46) < 1$, NS]. Subjects in the third group, passively yoked to the forced choice group, consumed all the food pellets that were delivered.

In Vivo Neurochemistry

Basal values of 5-HT, 5-HIAA, DA and DOPAC in the two frontal regions and different experimental conditions are given in Table 1.

5-HT

In the mPFC, levels of 5-HT increased during the task [sample: $F(10,260) = 4.714$, $P < 0.0001$, Fig. 3*A*]. However, there was also a main effect of condition [$F(1,26) = 5.482$, $P < 0.01$], indicating that this elevation was not uniform in all choice groups. In order to determine which groups were significantly different from each other, post-hoc Bonferroni-corrected multiple comparison tests, based on Student's *t*-statistic, were performed. 5-HT levels in the mPFC of animals which were freely choosing between response alternative, i.e. those performing the delay-discounting task, were significantly different from the two yoked groups (choice versus forced choice: $P < 0.046$; choice versus free pellets: $P < 0.023$), which did not differ from each other [forced choice versus free pellets: $F(1,22) < 1$, NS]. When data from the different conditions were analysed separately, a main effect of sample was only observed in the choice group [sample: $F(10,120) = 4.727$, $P < 0.003$]. In contrast, 5-HT efflux did not alter in the OFC during performance of the task [sample: $F(10,300) = 2.081$, NS, Fig. 3*B*], and there were no significant differences between any of the choice conditions [condition: $F(1,30) = 1.383$, NS].

5-HIAA

Levels of 5-HIAA did not alter significantly during task performance in either the mPFC [sample: $F(10,260) < 1$, NS] or the OFC [sample: $F(10,310) < 1$, NS; Fig. 3*C,D*]. Likewise, there were no differences in 5-HIAA efflux for any of the choice conditions in either brain region [mPFC condition: $F(1,26) < 1$, NS; OFC condition: $F(1,31) < 1$, NS], despite the robust change in 5-HT efflux seen in the mPFC. However, there is no simple relationship between extracellular levels of 5-HT and 5-HIAA, and previous studies have found that both increases in mPFC 5-HT efflux caused by administration of the selective 5-HT re-uptake inhibitors fenfluramine and citalopram and also decreases in 5-HT levels caused by administration of the 5-HT1A receptor agonist 8-OH-DPAT are not associated with similar changes in levels of 5-HIAA (Crespi *et al.*, 1990; Wong *et al.*, 1995; Arborelius *et al.*, 1996).

DA

In the mPFC, DA efflux increased during performance of the delay-discounting task [sample: $F(10,220) = 3.741$, $P < 0.007$, Fig. 4*A*]. Examining the data further, it appeared as if this increase was lower in the group actually engaged in task

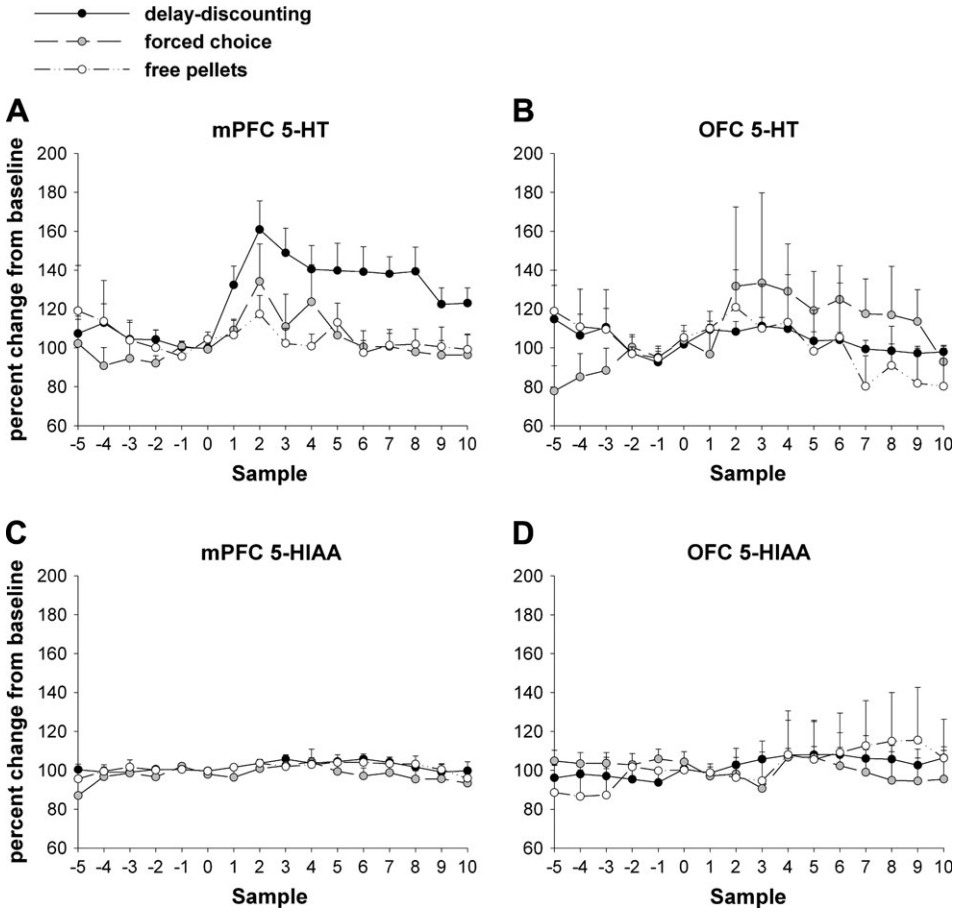

Figure 3. Levels of 5-HT and 5-HIAA in the mPFC and OFC of subject in all three experimental groups. Data are expressed as the percentage change from baseline levels ± SEM. Sample 1 was the first sample to be taken during performance of the behavioural task. Samples 1 and 2 were collected when the delay to the large reward was 0 s, samples 3 and 4 when the delay to the large reward was 10 s, samples 5 and 6 when the delay to the large reward was 20 s, samples 7 and 8 when the delay to the large reward was 40 s, and samples 9 and 10 when the delay to the large reward was 60 s.

performance compared to the yoked groups, but there was no significant difference between the different choice conditions [condition: $F(1,22) = 0.892$, NS]. It was not possible to measure DA reliably in the OFC, presumably because levels were too low for analytical detection by HPLC–ECD.

DOPAC

Levels of mPFC DOPAC also increased significantly during performance of the task in all three conditions [sample: $F(10,260) = 32.067$, $P < 0.0001$; condition: $F(1,26 = 1.455$, NS, Fig. 4*B*]. In the OFC, whilst there was no main effect of sample [$F(10,250) = 1.167$, NS], there was a main effect of condition [condition: $F(1,25) = 6.194$, $P < 0.007$, Fig. 4*C*]. When the data from the different groups were analysed further using post-hoc tests, DOPAC was significantly elevated in the choice group, which were actively engaged in the delay-discounting task [sample: $F(10,150) = 2.863$, $P < 0.025$], but not in the two yoked groups [forced choice, sample: $F(10,50) = 1.112$, NS; free pellets, sample: $F(10,70) = 0.696$, NS].

Correlational Analysis

Despite the changes observed during task performance, no correlations were observed between the average change from baseline of the neurotransmitters or metabolites, or the basal levels of these compounds, and the choice of the large reward made by animals performing the delay-discounting task, or likewise the number of times the large reward was earned (forced choice group) or delivered (free pellets group) in either of the two frontal areas (see Supplementary Tables 2 and 3).

Discussion

This is the first study in which *in vivo* microdialysis has been used to investigate the neurochemical correlates of impulsive decision-making. An increase in 5-HT efflux was observed in the mPFC, but not in the OFC, during performance of the task. This elevation in mPFC 5-HT was not observed in either of the two yoked groups, strongly suggesting that it is related to choice behaviour, rather than reflecting instrumental reward contingencies or reinforcement delivery. Likewise, DOPAC levels increased in the OFC of animals performing the task but not in yoked controls, whereas increases in DOPAC efflux were observed in the mPFC of all subjects, regardless of the reinforcement schedule in operation. These data suggest that DA utilization within the OFC is involved in mediating delay-discounting judgements, whereas DA utilization within the mPFC may be related to the earning or delivery of reward. Hence, the results presented here support and extend the hypothesis that the 5-HT and DA systems are implicated in the regulation of impulsive decision-making, and provide novel

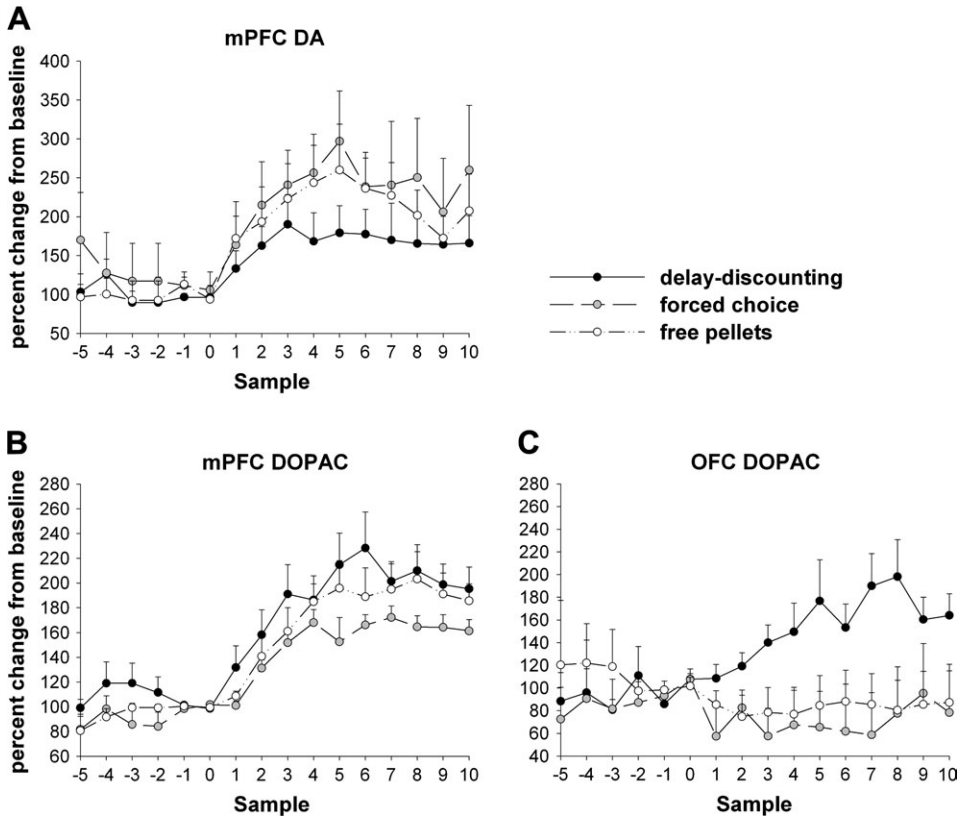

Figure 4. Levels of DA and DOPAC in the mPFC and DOPAC in the OFC of subject in all three experimental groups. Data are expressed as the percentage change from baseline levels ± SEM. Sample 1 was the first sample to be taken during performance of the behavioural task. Samples 1 and 2 were collected when the delay to the large reward was 0 s, samples 3 and 4 when the delay to the large reward was 10 s, samples 5 and 6 when the delay to the large reward was 20 s, samples 7 and 8 when the delay to the large reward was 40 s, and samples 9 and 10 when the delay to the large reward was 60 s.

evidence for a double dissociation between the functions of the DA and 5-HT systems within two distinct areas of the frontal cortex in the mediation of behaviour.

Functional differences between distinct frontal regions have been established using a variety of different approaches. Lesions to different areas of frontal cortex produce contrasting effects on numerous cognitive behavioural tasks, including the 5CSRT, autoshaping and reversal learning in rats (Muir *et al.*, 1996; Passetti *et al.*, 2002; Chudasama *et al.*, 2003; Chudasama and Robbins, 2003), and decision-making and working memory tests in humans and monkeys (e.g. Goldman-Rakic, 1998; Bechara *et al.*, 2000). It has been widely reported that dopamine levels in the medial wall of the prefrontal cortex are greater than in more ventrolateral regions (e.g. Berger *et al.*, 1976; Emson and Koob, 1978; Lindvall *et al.*, 1978; Tassin *et al.*, 1978; Descarries *et al.*, 1987) whereas 5-HT levels are higher in ventrolateral rather than medial prefrontal areas (Audet *et al.*, 1989). A similar pattern was observed in this study, as basal levels of DOPAC and DA were much lower in the OFC than in the mPFC, and vice versa for 5-HT and 5-HIAA (taking the active membrane length of the probes into consideration; see Table 1). In addition to the cytoarchitectural and anatomical distinctions delineating these areas, differential regulation of these regions by neurotransmitters such as 5-HT and DA may therefore also contribute to their different roles in the control of cognitive behaviour.

The increase in mPFC 5-HT levels observed during delay-discounting performance may reflect the processing of temporal information necessary for task performance. Lesions to the mPFC increase choice of the small reward when the delay to the large reward is short, yet decrease choice of the small reward when the delay to the large reward is long, a pattern of behaviour has been interpreted as a deficit in temporal judgement in that subjects are unsure as to how much time has elapsed within the session and therefore which reinforcement contingency is in play (Cardinal *et al.*, 2001). The 5-HT system has been heavily implicated in different aspects of the timing process, and although the precise regions where 5-HT is involved in regulating such behaviour are largely unknown (see Ho *et al.*, 2002), aspirative lesions of the mPFC have been shown to disrupt temporal discrimination in the peak interval procedure (Dietrich and Allen, 1998).

Dissociations between the roles of DA and 5-HT in the PFC have been observed previously using neurochemical lesions which target these different monoaminergic systems. For example, selectively decreasing levels of 5-HT within the frontal cortex impairs reversal learning in marmosets without altering the ability to shift attention within or between stimulus dimensions in order to identify the examplar associated with reward (Clarke *et al.*, 2004, 2005). However, decreasing DA within this region has the opposite pattern of effects (Roberts *et al.*, 1994; Crofts *et al.*, 2001). In contrast to 5-HT, levels of DA and DOPAC were significantly elevated within the mPFC in all three experimental conditions, and similar increases in DA and DOPAC levels have been reported during the 'one-choice' task (Dalley *et al.*, 2002). These data suggest that DA function may increase in this region in response to the earning or delivery of

reward, regardless of the type of Pavlovian or instrumental contingencies involved. Such observations are in keeping with a considerable body of literature positing a central role for the dopamine system in numerous reward-related processes (e.g. Wise and Rompre, 1989; Schultz, 1998), and more recent data demonstrating an increase in mPFC DA efflux following both appetitive and aversive conditioning procedures (Feenstra *et al.*, 2001; Mingote *et al.*, 2004). Deficits in processing information regarding reward may also contribute to the altered delay-discounting function demonstrated by mPFC-lesioned rats, in that subjects generalize their choice of the large reward across the delays because the schedule controlling reward delivery has minimal impact on choice. The increases in DA and 5-HT efflux observed within the mPFC during delay-discounting in this study may therefore signal the task contingencies or the passage of time, or a combination of both in a way which may inform response selection.

However, levels of DOPAC only increased in the OFC in animals performing the task but not in yoked controls, whereas OFC levels of 5-HT did not alter in any group. These data are indicative of a comparatively specialized role for dopaminergic innervation of this structure in delay-discounting judgements, potentially analogous to that suggested for 5-HT within the mPFC. Both excitotoxic and dopaminergic lesions of the OFC decrease impulsive choice in this delay-discounting paradigm (Kheramin *et al.*, 2004; Winstanley *et al.*, 2004c). Data from electrophysiological studies indicate that OFC neurons show specific reward-related activity that may be useful in enabling discrimination between responses yielding different rewards, and in updating the incentive value of response outcomes as they change (Schultz *et al.*, 1998; Schoenbaum *et al.*, 1999; Schultz *et al.*, 2000). The task-specific increase in DOPAC efflux observed in the OFC during delay-discounting performance supports the hypothesis that dopaminergic modulation of orbitofrontal function may be involved in these aspects of goal-directed action (Martin-Soelch *et al.*, 2001; Volkow *et al.*, 2002). It is also possible that the task-specific increases in OFC DOPAC and mPFC 5-HT levels observed reflect certain generalized decision-making processes rather than just impulsive choice. However, due to the lack of studies which have used *in vivo* microdialysis to measure neurotransmitter levels in these regions during the performance of complex behavioural tasks, the veracity of this hypothesis largely remains to be empirically determined.

It should be noted that changes in levels of DOPAC may also reflect alterations in noradrenergic activity. Levels of DOPAC have been shown to increase following stimulation of noradrenergic neurons (Nisenbaum *et al.*, 1991), and these neurons are also able to take up DA within cortical regions (Carboni *et al.*, 1990). Likewise, DA and noradrenaline (NA) can be co-released (Devoto *et al.*, 2003a,b), and an increase in both DA and NA has been observed in the PFC of food-restricted rats during feeding (Hernandez and Hoebel, 1990; Taber and Fibiger, 1997). Little is known about the role of NA in mediating the performance of delay-discounting tasks, and it did not prove possible in the current study to quantify NA levels in addition to those of DA, 5-HT and their metabolites. It has recently been shown that the NA re-uptake inhibitor atomoxetine, which increases levels of DA and NA in the prefrontal cortex (Bymaster *et al.*, 2002), can relieve the increase in impulsive behaviour associated with ADHD (for a review, see Simpson and Perry, 2003), suggesting that the role of NA in impulse control may be worthy of further investigation.

The increase in mPFC levels of 5-HT observed in this delay-discounting model of impulsive choice contrasts with the finding that 5-HT efflux did not alter during performance of the 'one-choice' test of motoric impulsivity (Dalley *et al.*, 2002). In addition, no significant correlations were observed between efflux of 5-HT and DA or their metabolites and the level of impulsive choice observed. In contrast, levels of motoric impulsivity, as measured by the number of premature responses made, are positively correlated with baseline levels of 5-HT efflux in the right mPFC (Dalley *et al.*, 2002). The fact that no relationship was observed in this study between impulsive choice and levels of prefrontal 5-HT or DA could be due to a lack of power, as fewer rats were used in this experiment (mPFC: $n = 13$; OFC: $n = 19$) compared to those used in the Dalley *et al.* study ($n = 31$). However, these data do provide preliminary evidence for a dissociation between the neurochemical regulation of these two different forms of impulsivity. Evidence from pharmacological studies also supports this suggestion. For example, D-amphetamine administration decreases impulsive choice yet increases impulsive action (Cole and Robbins, 1987, 1989; Richards *et al.*, 1999; Wade *et al.*, 2000; Winstanley *et al.*, 2003a). It appears that 5-HT and DA play very different, yet not unrelated, roles in modulating these various forms of impulsivity, the exact nature of which remains to be elucidated.

To summarize, through the application of *in vivo* microdialysis during performance of a delay-discounting task, it has proved possible to demonstrate a double dissociation between 5-HT and DA function within the mPFC and OFC. These data further implicate these monoamine systems in the control of impulsive choice, and suggest that their modulation of different regions of the frontal cortex, which themselves have been implicated in different aspects of cognition, may be functionally divergent. Furthermore, the data presented here using a test of impulsive choice contrast significantly with that obtained using a test of impulsive action, thus reinforcing the hypothesis that different aspects of impulsivity are underpinned by distinct neurobiological mechanisms.

Notes

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