Supplementary Online Materials

MATERIALS AND METHODS

Subjects

Six common marmosets (*Callithrix jacchus*) (1 female, 5 males) were housed in pairs and maintained on a 22-hour water deprivation regimen for 5 days per week. All monkeys were fed 20 g of MP.E1 primate diet (Special Diet Services; SDS) and two pieces of carrot after the daily behavioural testing session, when they also had 2 hours' access to water. At weekends, their diet was supplemented with fruit, rusk, eggs and marmoset jelly (SDS) and they had free access to water. All procedures were performed in accordance with the 1986 Animals (Scientific Procedures) Act.

Apparatus

Behavioral testing took place within a sound-attenuated box in a dark room. The animal sat in a Perspex transport box, one side of which was removed to reveal a colour visual display unit. The marmoset reached through an array of vertical metal bars to touch stimuli presented on the VDU. Responses were detected by an infra-red beam array attached to the screen. A reward of cooled banana milkshake was delivered to a centrally placed spout. Reward was dependent upon the marmoset licking the spout when reward was available; this was signalled by a 4 kHz tone played through loudspeakers situated on either side of the VDU. The test chamber was lit with a 3 W bulb. Stimuli presented on the VDU were 38 mm wide × 50 mm high and were generated on a computer, which also controlled and recorded responding.

Pre-Operative Training

All monkeys were trained on two 2-choice simple visual discriminations prior to surgery (D1 and D2). A response to the correct stimulus resulted in the incorrect stimulus disappearing from the

screen, and the correct stimulus remaining for the duration of a 5 s tone that signalled the availability of 5 s of reinforcement. Failure to collect the reward was scored as a missed trial. A response to the incorrect stimulus resulted in both stimuli disappearing from the screen and a 5 s timeout during which the houselight was extinguished. The intertrial interval was 3 s. Each monkey was presented with 30 trials per day, 5 days a week, until a criterion of 90% correct was attained, at which point the subject proceeded to the next stage. If a monkey showed a significant side bias (10 consecutive responses to one side), a rolling correction procedure was implemented automatically whereby the correct stimulus was presented on the non-preferred side until the monkey had made a total of 3 correct responses.

Surgical Procedure

Lesions of the serotonergic innervation of the PFC were made using 5,7-DHT (4.96 mM) in saline/0.1% L-ascorbic acid. To protect the noradrenaline and dopamine (DA) innervation, the noradrenaline uptake blocker nisoxetine (25 mM) and the DA uptake blocker GBR-12909 (1.0 mM) were also present in the solution.

Subjects were pre-medicated with ketamine hydrochloride (0.05 ml of a 100mg/ml solution, i.m.), anaesthetised with Saffan (alphaxalone 0.9% w/v, alphadolone acetate 0.3% w/v; 0.4 ml i.m.), and placed in a modified stereotaxic frame. Injections were made into 20 sites bilaterally within the PFC, using a 30-gauge cannula attached to a 10 µl Hamilton syringe, at 0.4 µl/20s. Coordinates and volumes used are described in Table S1. Sham surgery was identical except for the omission of the toxin from the infusion.

Behavioural procedure

Having reached criterion on the second discrimination (D2), 3 monkeys received 5,7-DHT lesions of the PFC and 3 monkeys received the sham control procedure. After 2 weeks recovery they

received the following series of discriminations with progression onto the next stage dependent on the attainment of 90% criterion in the previous session:

1) A retention test of the discrimination learned immediately prior to surgery (D2).
2) Acquisition of a third novel discrimination (D3)
3) A series of 4 reversals whereby for each reversal the previously incorrect stimulus became rewarded and the previously rewarded stimulus became incorrect.

Behavioral Measures

The main measure of the monkeys' ability to learn the visual discriminations was the total number of errors made prior to achieving criterion (excluding the criterion day of ≥ 90% correct) for each discrimination. Additional measures recorded for each trial were i) the latency to respond to the stimuli presented on the VDU (response latency), ii) the latency to collect the reward from the spout (lick latency) and iii) the left/right location of the response.

In order to characterise the type of errors that were made during reversal learning, sessions were classified as *perseverative* (where responding to the previously-correct stimulus was significantly above chance), *chance*, or *learning* (where responding to the newly-correct stimulus was at, or above chance respectively). Signal detection theory(see *1*) was used to establish subjects' ability to discriminate correct from incorrect stimuli independently of any side bias that might have been present. The discrimination measure d′ and the bias measure *c* were calculated and the normal cumulative distribution function (CDF) compared to the criterion values of a two-tailed *Z* test (each tail $p = 0.05$) to determine the classification of each session (perseveration, chance or learning). Sessions where $CDF(d') < 0.05$ were classified as *perseverative;* sessions where $CDF(d') > 0.95$ were classified as *learning,* and sessions where $0.05 \leq CDF(d') \leq 0.95$ were

classified as *chance.* Sessions where CDF(c) < 0.025 or CDF(c) > 0.975 were considered biased, but were not excluded as d′ is still a valid measure of discrimination.

Days on which subjects attained criterion were excluded, as were days in which Z(H) or Z(FA) were incalculable (in the rare event that a correction procedure resulted in all stimuli being presented on the same side for one session).

***In vivo* assessment of the serotonergic lesion of the PFC using microdialysis**

In order to determine the levels of extracellular 5-HT within the PFC of lesioned marmosets immediately after their performance of the series of reversals, *in vivo* microdialysis was employed. One subject from each group received striatal microdialysis and under the UK Home Office project licence at the time additional dialysis in the PFC was prohibited. Therefore we report data from the remaining 4 monkeys.

The concentric dialysis probes incorporated a 24-gauge thin-walled outer stainless steel barrel (40 mm length), an inner tubing of polyamide-coated silica glass (SGE, internal diameter 80 µm, outer diameter 110 µm) and a 1.0 mm active (exposed) length of dialysis membrane (diameter 220 µm). The probes were perfused with artificial cerebrospinal fluid (aCSF) at a flow rate of 1.0 µl/min using a Harvard microsyringe pump with 2.5 ml gas-tight syringes and a serial liquid switch. The composition of aCSF was as follows (in mM): NaCl 147; KCl 3; $CaCl_2$ 1.3; $MgCl_2$ 1; Na_2HPO_4 0.2; NaH_2PO_4 1.3 (pH 7.4). The aCSF also contained 1 µM of the selective 5-HT reuptake inhibitor citalopram to augment extracellular levels of 5-HT.

The probe was implanted acutely into the OFC at the stereotaxic coordinates of AP +17.75, LM –2, D +0.3 (from the skull base). Coordinates were adjusted *in situ* to ensure sufficient cortical depth. After a 3 hour post-implantation equilibration period, dialysates were collected every 20 min. Initially, four baseline determinations were taken, then a 10-min depolarising stimulus (75 mM K^+) and three further baseline determinations. Samples were stored in dry ice and then at –85^0C until analysis. Throughout the experiment the monkey was maintained under Saffan anaesthesia and its core temperature was monitored via a rectal probe. The microdialysis was performed between 3-10 months after the 5-HT lesion surgery.

The levels of 5-HT and the metabolite 5 hydroxy-indole-acetic acid (5-HIAA) in the dialysate samples were analysed using reversed phase high-performance liquid chromatography (HPLC) and electrochemical detection. Chilled 15 µl samples were separated on a C18 silica-based analytical column (1004.6 mm ODS3) using a mobile phase (13.6 g/l $KH_2PO_4.H_20$, 185 mg/l octane sulphonic acid and 18% methanol; pH 2.75) delivered at 22^0C and at 0.8 ml/min by a dual piston pump. Dialysate levels of 5-HT and 5-HIAA were quantified using a dual electrode analytical cell and electrochemical detector with electrode 1 set at -150 mV and electrode 2 set at 180 mV with reference to a palladium electrode. The resultant signal was integrated using Chromeleon software (version 6.20). The HPLC system was calibrated using standards containing known amounts of 5-HT (detection limit of 0.5 fmol).

Postmortem Assessment of the Serotonergic Lesion of the PFC

The specificity and extent of the 5,7-DHT lesion of the PFC was assessed using post-mortem tissue analysis of cortical and subcortical regions between 4-12 months after administration of 5,7-DHT(for full analytical details see *2*). 5-HIAA levels were consistent with effective 5-HT depletions (Table S2). Pilot data collected at 3 months post-surgery indicate that depletions seen in 5-HT, DA and NA are stable for the duration of the behavioural testing (Table S3) and that

additional subcortical areas within the forebrain are not affected by the lesion as indicated by analysis at 2 weeks post-surgery (Table S4).

Statistics

The behavioural results were subjected to ANOVA using the SPSS v.11.0 statistical package. ANOVA models are described in the form $A_2 \times (B_3 \times S)$, where A is a between-subjects factor with two levels and B is a within-subjects factor with three levels; S represents subjects. Where data did not display homogeneity of variance, appropriate transformations were employed (see *3*). *Post hoc* comparisons were made using simple main effects and tissue data were analysed using a Student's *t*-test.

References

1. N. A. Macmillan, C. D. Creelman, *Detection Theory: a user's guide* (Cambridge University Press, 1991).
2. A. C. Roberts, et al., *J. Neurosci.* **14**, 2531 (1994).
3. D. C. Howell, *Statistical Methods for Psychology* (Wadsworth, Belmont, California, ed. Fourth, 1997).
4. R. Dias, T. W. Robbins, A. C. Roberts, *Nature* **380**, 69 (1996).

Table S1. Stereotaxic coordinates (in mm) and injection volumes for the 5,7-DHT PFC lesion.

* AP locations were adjusted *in-situ* according to a cortical depth check (see *4 for details*).

** multiple injections were evenly spread between the cortical surface and the base of the skull at each site with an injection being at least 0.7 mm from the surface and base. The number of sites and coordinates used were adjusted *in-situ* according to the cortical depth.

AP*	LM (±)	volume (µl/site)	number of sites **
+17.0	0.75	3	3
+18.0	0.75	3	3
+18.0	1, 1.5, 2.5, 3.5, 4.5	2	2/3
+19.5	0.75, 1.5	3	3
+19.5	2.5, 3.5	2	2/3

Table S2. Mean levels of 5-HIAA (expressed as pmoles/mg wet tissue weight ± SEM) in the frontal cortex of the control and lesion groups, and the percentage depletions of 5-HIAA (± SEM) in marmosets with 5,7-DHT lesions of the frontal cortex. ***** mean scores of lesioned animals differ significantly from those of the control group: **OFC**, orbitofrontal cortex ($t_4 = 6.84$; $p = 0.002$); **LAT**, lateral granular PFC ($t_4 = 3.82$; $p = 0.019$); **MED**, medial PFC ($t_4 = 6.63$; $p = 0.003$); **M/PM**, primary motor and premotor cortex ($p > 0.05$); **DORSAL**, dorsal granular cortex ($t_4 = 3.29$; $p = 0.03$); **C1**, anterior cingulate cortex ($t_4 = 3.83$; $p = 0.019$); **C2**, mid-cingulate cortex ($p > 0.05$). In all cases n = 3 except for control C2 values where n = 2 due to data loss.

	5-HIAA (pmoles/mg)		
	Control	**Lesion**	**% depletion**
OFC	19.8 ± 1.7	7.1 ± 0.9	64.3 ± 4.7*****
LAT	10.0 ± 2.0	3.7 ± 0.3	63.4 ± 3.1*****
MED	19.5 ± 1.6	8.2 ± 0.8	57.8 ± 4.3*****
DORSAL	11.3 ± 1.9	5.5 ± 0.5	51.4 ± 4.0*****
M/PM	12.0 ± 2.4	7.3 ± 0.4	39.3 ± 3.6
C1	19.0 ± 1.8	9.7 ± 1.6	48.7 ± 8.3*****
C2	17.0 ± 2.5	8.9 ± 2.0	47.4 ± 11.6

Table S3. Levels of 5-HT, dopamine and noradrenaline (expressed as % depletion;'-' denotes % increase) in the frontal and cingulate cortex of a 5,7-DHT unilaterally lesioned marmoset at 3 months post-surgery. **OFC**, orbitofrontal cortex; **LAT**, lateral granular PFC; **MED**, medial PFC; **M/PM**, primary motor and premotor cortex; **DORSAL**, dorsal granular cortex; **C1**, anterior cingulate cortex; **C2**, mid-cingulate cortex.

	5-HT (% depletion)	**Dopamine (% depletion)**	**Noradrenaline (% depletion)**
OFC	80.0	27.6	26.2
LAT	87.9	34.2	36.1
MED	65.8	30.7	31.5
DORSAL	77.9	21.4	42.8
M/PM	55.9	28.2	27.6
C1	41.8	30.5	missing data
C2	31.4	-13.4	-7.2

Table S4. Levels of 5-HT and 5-HIAA expressed as % depletion in a 5,7-DHT unilaterally lesioned marmoset at 2 weeks post-surgery. **OFC**, orbitofrontal cortex; **LAT**, lateral granular PFC; **MED**, medial PFC; **M/PM**, primary motor and premotor cortex; **DORSAL**, dorsal granular cortex; **C1**, anterior cingulate cortex; **C2**, mid-cingulate cortex; **C3**, posterior cingulate cortex; **Ant Parietal**, anterior parietal cortex; **Post Parietal**, posterior parietal cortex; **Ant Caud**, anterior caudate; **Ant Put**. anterior putamen; **NAcc**, Nucleus Accumbens; **BF**, basal forebrain; **Temporal Pole**, temporal pole including amygdala and periamygdaloid cortex.

	5-HT (% depletion)	**5-HIAA (% depletion)**
OFC	80.1	62.1
LAT	70.7	58.6
MED	57.4	45.2
DORSAL	62.3	61.3
M/PM	48.5	36.4
C1	46.1	33.4
C2	28.2	20.6
C3	5.5	-8.4
Ant Parietal	32.1	15.4
Post Parietal	30.1	22.2
Ant Caud	-1.8	22.6
Ant Put	-1.9	-3.6
NAcc	21.6	-5.9
BF	-15.8	-12.7
Hypothalamus	9.3	16.2
Temporal pole	-6.8	6.1
Hippocampus	7.0	22.9