Serotonin at the level of the amygdala and orbitofrontal cortex modulates distinct aspects of positive emotion in primates

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Abstract

Impaired top-down regulation of the amygdala, and its modulation by serotonin (5-HT), is strongly implicated in the dysregulation of negative emotion that characterizes a number of affective disorders. However, the contribution of these mechanisms to the regulation of positive emotion is not well understood. This study investigated the role of 5-HT within the amygdala and the orbitofrontal cortex (OFC), on the expression of appetitive Pavlovian conditioned emotional responses and their reversal in a primate, the common marmoset. Its effects were compared to those of the amygdala itself. Having developed conditioned autonomic and behavioural responses to an appetitive cue prior to surgery, marmosets with excitotoxic amygdala lesions failed to display such conditioned autonomic arousal at retention, but still displayed intact cue-directed conditioned behaviours. In contrast, 5,7-DHT infusions into the amygdala, reducing extracellular 5-HT levels, selectively enhanced the expression of appetitive conditioned behaviour at retention. Similar infusions into the OFC, producing marked reductions in post-mortem 5-HT tissue levels, had no overall effect on autonomic or behavioural responses, either at retention or during reversal learning, but caused an uncoupling of these responses, thereby fractionating emotional output. These data demonstrate the critical role of the amygdala in the expression of appetitive autonomic conditioning, and the region-selective contribution of 5-HT in the amygdala and OFC, respectively, to the expression of conditioned behaviour and the overall coordination of the emotional response. They provide insight into the neurochemical mechanisms underlying the regulation of positive emotional responses, advancing our understanding of the neural basis of pathologically dysregulated emotion.

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Introduction

Dysfunction in the orbitofrontal cortex (OFC) and amygdala has been implicated in a number of pathological conditions, including depression (Drevets, 2003, 2007), mania (Almeida et al, 2009), anxiety disorders (Etkin & Wager, 2007; Milad & Rauch, 2006), obsessive-compulsive disorder (Saxena & Rauch, 2000) and addiction (Childress et al, 1999; Grant et al, 1996). Research into the underlying causes of amygdala and OFC dysfunction has highlighted the importance of neuromodulatory systems including the ascending serotonin (5-HT) pathways. Pharmacological and genetic studies highlight the role of 5-HT in modulating emotional processing in the amygdala and prefrontal cortex of healthy people (Canli & Lesch, 2007; Cools et al, 2008) and patients with affective disorders (Harmer, 2008; Morilak & Frazer, 2004; Ressler & Nemeroff, 2000). The vast majority of these studies have focused on negative emotion. However, extensive research in animals has shown the vital role of the amygdala and OFC in positive affect (Balleine & Killcross, 2006; Everitt et al, 2003; Holland & Gallagher, 2004; Murray, 2007; Roberts et al, 2007) and recent findings have also highlighted the role of 5-HT in positive affect. Thus,
Peripheral administration of selective serotonin re-uptake inhibitors (SSRIs), has implicated 5-HT in the modulation of both these structures during negative and positive emotion in humans (McCabe et al. 2010; Norbury et al. 2009). In addition, the tonic activity of dorsal raphe neurons, which include those that release 5-HT, has been shown to correlate strongly with future rewarding outcomes, linking 5-HT with reward expectation (Bromberg-Martin et al. 2010). Despite this, little is known of the specific actions of 5-HT at the level of the OFC and amygdala on positive emotion.

Positive emotion is frequently studied in the laboratory using a Pavlovian conditioning procedure, whereby a previously neutral stimulus acquires emotional significance and induces a positive emotional state, through its association with reward. Integral components of this emotional state are the accompanying physiological and behavioural responses. The amygdala has been identified as a key region for the acquisition of behavioural responses in this paradigm (Gallagher et al. 1990; Groshek et al. 2005; Parkinson et al. 2000) and studies in our laboratory have shown that the expression of conditioned autonomic arousal accompanying learned appetitive behaviour is also critically dependent on the amygdala (Braesicke et al. 2005). While the expression of conditioned responses is not dependent upon an intact OFC, we have shown that the regulation of such anticipatory positive arousal in the event of unexpected reward omission, and the coordinated adaptation of Pavlovian autonomic and behavioural responses when stimulus-reward contingencies change, is dependent upon the OFC (Reekie et al. 2008).

To directly assess the contribution of 5-HT at the level of the OFC and amygdala in positive emotion, the present study compares the effects of selective 5-HT depletions within either the amygdala or OFC, using the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), on the expression and regulation of conditioned, appetitive autonomic and behavioural responses. For comparison purposes the effects of amygdala excitotoxic lesions were also investigated, as this particular version of the Pavlovian task in marmosets, with abstract auditory cues as the conditioned stimuli, had only been studied previously in the context of excitotoxic lesions of the OFC.

Materials and methods

Subjects

Sixteen common marmosets (Callithrix jacchus, five females, 11 males; average age 3.5 yr, range 2–6 yr) were pair-housed under temperature and humidity controlled conditions. [For details of diet see Supplementary Materials and methods (available online).] All procedures were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986, under project licence 80/2225. Following completion of this study, 11 of the marmosets (four out of five controls, three out of four 5,7-DHT amygdala-lesioned animals and all four 5,7-DHT OFC-lesioned animals) were tested on an incongruency discrimination task and the excitotoxic amygdala-lesioned marmosets (n=3) were tested on the acquisition of a mild aversive Pavlovian procedure, the results of which have been reported, respectively, in Man et al. (2010) and Mikheenko et al. (2010). A further four marmosets received unilateral 5,7-DHT amygdala lesions to investigate lesion time-course (see Supplementary Materials and methods).

Test apparatus

Behavioural testing took place within a test apparatus as previously described by Reekie et al. (2008; Supplementary Materials and methods).

Cardiovascular measurements

Blood pressure (BP) and heart rate (HR) changes were measured remotely using a PhysioTel Telemetry system (Data Sciences, USA) as previously described in Braesicke et al. (2005; Supplementary Materials and methods).

Surgical procedures

Implantation of telemetric devices

Prior to the start of the study marmosets received an implant of a radiotelemetry probe (Chronic Use TA11PA-C40 Implant, Data Sciences, USA) into the descending aorta under general anaesthesia, according to procedures described previously (Schnell & Wood, 1993; Supplementary Materials and methods). During testing, the probe transmitted BP data, which was recorded and analysed offline.

Brain lesions

General neurosurgery and recovery procedures were performed as described in Supplementary Materials and methods. Excitotoxic lesions and lesions of the serotoninergic innervation of the amygdala and OFC were performed as described in Table 1.

In-vivo microdialysis

Extracellular levels of 5-HT in the amygdala of two 5,7-DHT amygdala-lesioned and two sham-operated
control monkeys were measured by microdialysis, 8–10 months after lesion surgery. The marmosets were anaesthetized by isoflurane intubation (as detailed for brain lesions, Supplementary Materials and methods), and microdialysis probes were implanted acutely at the following stereotaxic coordinates based on the inter-aural plane: AP +9.3 mm, L = 5.6 mm, DV +4.0 mm, centred on the basal nucleus. Details of probes, artificial cerebrospinal fluid composition and dialysis protocol are described in the Supplementary Materials and methods.

### Table 1. Parameters for the amygdala and OFC lesions including stereotaxic coordinates of each injection (based on the inter-aural plane) and the injection volume

| Lesion area | Coordinates (mm) | Volume injected (µl) |
|-------------|------------------|---------------------|
|             | AP   | LM | V   | Quinolinic acid | 5,7-DHT<sup>b</sup> |
| Amygdala    | 9.3  | ±5.6 | 4.0 | 0.35 | 0.50 |
|             | 5.0  | ±2.5 | 0.7<sup>a</sup> | – | 0.40 |
| OFC         | 16.75| ±4.8 | 0.7<sup>a</sup> | – | 0.40 |
|             | 17.75| ±4.8 | 0.7<sup>a</sup> | – | 0.40 |
|             | 18.5 | ±4.0 | 0.7<sup>a</sup> | – | 0.60 |

OFC, Orbitofrontal cortex; AP, anteroposterior; LM, lateromedial; V, ventral.

Excitotoxic lesions of the amygdala (<i>n</i>=3) were made by infusing 0.35 µl of 0.12 M quinolinic acid (Sigma-Aldrich, Germany), in 0.01 M phosphate buffer (pH 7.0), bilaterally. Injections were made at a rate of 0.05 µl/20 s through a glass cannula attached to a 2 µl Hamilton syringe (Precision Sampling Co., USA). Immediately after injection, the cannula remained in position for a further 4 min, before being withdrawn slowly. Lesions of the serotonergic innervation of the OFC (<i>n</i>=4) and amygdala (<i>n</i>=4) were made following a procedure adapted from Clarke et al. (2007) using 4 µg/µl 5,7-DHT (creatinine sulphate salt; Sigma-Aldrich, Germany) in 0.1% l-ascorbic acid (Sigma-Aldrich, USA) in 0.9% saline, infused bilaterally. The sham-operated control group (<i>n</i>=5) received vehicle infusion into either the OFC (<i>n</i>=2) or amygdala (<i>n</i>=3).

* 0.7 mm above base of brain.

<sup>b</sup> The noradrenaline (NA) uptake blocker, nisoxetine (50 µM; Sigma-Aldrich, Germany), and the dopamine (DA) uptake blocker, 1-(2-(bis-(4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl) piperazine dihydrochloride (GBR 12909, 2.0 µM; Sigma-Aldrich, Germany), were administered concomitantly to protect NA and DA innervation, respectively.

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which could include a single CS− or multiple CS−, but only one CS+. A session started with the illumination of the house-light and CS were presented with a variable inter-trial interval (ITI) of 70–110 s. Immediately following CS+, the house-lights were turned off, the foodbox lights turned on, and both clear and opaque doors of one foodbox opened, allowing 120 s access to the marshmallow reward inside. After CS− presentation only the opaque door opened, allowing the animal to view an empty foodbox. The auditory CS continued to be played for the duration of the 120-s US period. Presentation of CS+ terminated the session on completion of the reward period. Animals received no more than 5 CS+ within a 2-wk period of 10 sessions. The pairing of sounds and outcomes was counter-balanced across subjects.

Training continued until subjects showed stable conditioning, as indicated by differential BP and behavioural responses to CS+ and CS− over approximately 1.5 wk (p<0.05). Subjects were then given two, one-trial probe sessions (probe tests 1 and 2) at least 1 wk apart, with additional re-conditioning trials in between.

**Probe test 1: CS termination with reward omission**

A single, 20-s CS+ was presented. This was followed by termination of CS+ and no presentation of reward (i.e. foodbox doors did not open).

**Probe test 2: unexpected reward with CS omission**

The US+ was presented alone without any prior or accompanying CS+. Stable conditioned responding across 1 wk, post-probe tests was required before surgery could take place (pre-surgery baseline phase). Subsequently, animals received one of the following: excitotoxic lesions of the amygdala, selective 5-HT depletion of the amygdala or OFC, or sham control surgery. After post-operative recovery, animals were re-tested on the same Pavlovian conditioning procedure until stable differential BP and behavioural responses, comparable to those of pre-surgery, had been achieved (retention phase). Probe tests 1 and 2 were then presented (the same as in pre-surgery).

**Reversal of reward contingencies**

On completion of the two probe tests, animals were given an additional week of Pavlovian training to allow BP and behavioural responses to re-stabilize. Thereafter, CS outcome contingencies were reversed, such that the previously rewarded CS+ became the CS− and was no longer rewarded, while the previously unrewarded CS− became the rewarded CS+ (reversal phase). All subjects received a predetermined sequence of sessions. They were maintained on reversal until they reached a performance criterion of significantly (p<0.05) greater conditioned behavioural and autonomic responses to CS+ than CS− across consecutive sessions, that included 6 CS+ presentations, or for a maximum of 100 sessions.

**Behavioural and cardiovascular analyses**

For the cardiovascular data, offline analyses involved the removal of abnormal spikes and outliers (typically BP values >400 mmHg or <0 mmHg), and then extraction of the minima and maxima of each heart-beat cycle to give systolic and diastolic events. Missing values of <0.4 s were filled with cubic spline interpolation, although longer disruptions were treated as missing values in the resulting dataset. For the 20-s CS period, the mean value for systolic and diastolic BP and HR was calculated and compared to mean baseline responding during the 20-s period immediately prior to the start of CS. Changes in systolic BP from baseline during the CS period was the primary measure for affective conditioned cardiovascular responses. For the US period, systolic and diastolic BP and HR was calculated over 60 s from when the animal first accessed the foodbox, compared to pre-CS baseline.

For behavioural data, all sessions were recorded onto DVD to be analysed post-testing. A researcher, blind to the experimental groups, scored all behaviours and latency measures.

**CS-directed behaviour.** The number of headjerks [HJs; CS-directed orienting responses consisting of a flick/snap of the head (Holland 1977; Reekie et al. 2008)] were counted during the 20-s CS period and subtracted from this was the number of HJs made during the immediately preceding 20-s baseline period.

**US-directed behaviour.** The number of looks (movements of the head) directed to the foodboxes was also scored during each CS and associated baseline period. In addition, at each US onset, the latencies to look, reach and eat (if US+) were measured. The total amount of food consumed was also recorded.

**Post-mortem neurochemical analysis**

Post-mortem neurochemical analysis was used to determine the specificity and extent of the 5,7-DHT lesion in the OFC and amygdala (see Supplementary Materials and methods).
Histological assessment of excitotoxic amygdala lesions

Animals with amygdala excitotoxic lesions were euthanased and their brains subsequently assessed for cell loss and gliosis (see Supplementary Materials and methods).

Statistical analysis

Data were analysed using SPSS for Windows (version 16.0, SPSS Inc., USA). Statistical significance was set at \( p < 0.05 \) and data were transformed as necessary if variances deviated significantly (as measured by Levene’s test of homogeneity).

Results

Post-mortem tissue levels of 5-HT and the other monoamines

Injections of 5,7-DHT into the OFC caused a significant (\( p < 0.05 \)) reduction of post-mortem 5-HT tissue levels in the OFC and a substantial, but non-significant, reduction in the neighbouring lateral prefrontal cortex (Fig. 2a, Table 2), when measured 9–11 months post-surgery. In contrast, there was considerable recovery of post-mortem tissue levels of 5-HT in the amygdala across the same time period following 5,7-DHT injections into the amygdala (Fig. 2a, b; Table 2). However, extracellular levels of 5-HT in the amygdala in the...
anaesthetized marmoset were considerably compromised at the time of behavioural testing (see below).

**In-vivo extracellular tissue levels of 5-HT and 5-HIAA**

Extracellular levels of 5-HT in both lesioned marmosets (Fig. 3a) were below the level of detection (2.0 fmol/20 min), in the baseline period and following K⁺ challenge, even though subsequent post-mortem analysis of tissue levels of 5-HT had shown full recovery in one of these animals (A3; Fig. 2b). Amygdala concentrations of 5-HIAA (a 5-HT metabolite) in the lesioned animals were also approximately half that observed in controls (Fig. 3b).

**Histological analysis**

Histological analysis of excitotoxic amygdala lesions (Fig. 4) revealed that all three animals sustained damage to the central, basal, accessory basal and medial nuclei bilaterally, although the damage was partial in extent and the most anterior aspects of the basal and accessory basal nuclei were spared. There was also, primarily unilateral, lesioning of the lateral nucleus. The cortical nucleus was intact in all subjects. One animal showed damage to the very anterior tip of the hippocampus, but otherwise there was no damage outside of the amygdala.

**Expression of conditioned and unconditioned cardiovascular and behavioural responses**

**Responses during the CS period**

All animals showed differential cardiovascular (Fig. 5a; Supplementary Table S1) and behavioural responses (Fig. 5b) to CS⁺ compared to CS⁻ prior to lesion surgery. Post-surgery, excitotoxic amygdala lesions significantly attenuated the expression of these conditioned BP responses, such that the responses to CS⁺ became indistinguishable from responses to CS⁻ (Fig. 5a). In contrast, these same animals continued to show intact CS-directed conditioned behaviour to CS⁺.
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Fig. 3. In-vivo extracellular tissue levels of 5-HT and 5-HIAA. In-vivo extracellular concentrations of (a) 5-HT and (b) 5-HIAA in the amygdala of two sham-operated controls and two animals with 5,7-DHT lesions of the amygdala, measured after completion of reversal learning. The values reflect mean concentrations of three 20-min baseline (basal) dialysate fractions (taken after a 3-h equilibration period) and the subsequent 20 min dialysate fraction during which 75 mm potassium (K\(^+\) challenge) was infused in the first 10 min. The dashed lines indicate the detection thresholds for 5-HT and 5-HIAA. Individual subject values for amygdala-lesioned animals are labelled (A2, A3) for ease of comparison with individual levels of post-mortem 5-HT depletion in Fig. 2b. Statistical analysis was not performed since n = 2.

(Fig. 5b). In contrast, 5,7-DHT amygdala lesions significantly enhanced the expression of CS-directed conditioned behaviour (Fig. 5b), which was accompanied by a somewhat (but non-significantly) augmented conditioned BP response (Fig. 5a). The conditioned BP (Fig. 5a) and behaviour (Fig. 5b) of 5,7-DHT OFC-lesioned animals did not differ from controls. Expression of conditioned responses was assessed within a month of surgery, when 5-HT depletions in the 5,7-DHT-lesioned animals would have been maximal (Fig. 2b).

A three-way ANOVA of systolic BP revealed a significant main effect of CS (F\(_{1,12}\) = 121.5, p < 0.001), a significant CS × group interaction (F\(_{3,12}\) = 5.8, p = 0.011) and an overall surgery × CS × group interaction (F\(_{3,12}\) = 12.07, p = 0.005). Further two-way ANOVAs (CS × group) demonstrated that pre-surgery, there was no difference between the groups (group: F\(_{3,12}\) = 2.1; group × CS: F\(_{3,12}\) = 1.7, n.s.), with all groups showing a clear conditioned rise in systolic BP to CS\(^+\) and not CS\(^-\). Post-surgery, however, a significant CS × group interaction (F\(_{3,12}\) = 22.3, p < 0.0001) was found. While there was a clear differentiation between CS\(^+\) and CS\(^-\) responses in the control group (t\(_4\) = 4.4, p = 0.002) and both 5,7-DHT-lesioned groups (amygdala: t\(_4\) = 15.4, p < 0.0001; OFC: t\(_4\) = 3.6, p = 0.01), the excitotoxic amygdala-lesioned monkeys showed no difference in their responses to the two stimuli (t\(_4\) = −0.12, n.s.). A similar pattern was seen for diastolic BP (see Supplementary Results). Log-transformed HR data was more variable with no significant main or interaction effects (surgery: F\(_{1,12}\) = 2.5; CS: F\(_{1,12}\) < 1; group: F\(_{3,12}\) = 2.4; surgery × CS × group interaction: F\(_{3,12}\) = 1.4, all n.s.).

A three-way ANOVA of CS-directed behaviour (HJs) showed a significant main effect of CS (F\(_{1,12}\) = 91.4, p < 0.001), a significant CS × surgery interaction (F\(_{1,12}\) = 14.8, p = 0.002) and significant surgery × CS × group interaction (F\(_{3,12}\) = 3.7, p = 0.04). Examination of CS\(^+\) only, revealed a significant group × surgery interaction (F\(_{3,12}\) = 5.6, p = 0.012), whereas no main or interaction effects for CS\(^-\) (all Fs ≤ 1, n.s.) were found.
Further post-hoc analysis for each group within CS\(^+\) revealed a significant effect of surgery for the 5,7-DHT-treated amygdala group only (\(F_{1,7}=7.8, p=0.03\), other groups all Fs <1, n.s.). Behavioural conditioning (i.e. greater number of HJs to CS\(^+\) compared to CS\(^-\)) was observed in all groups before and maintained by all groups after surgery, except those animals treated with 5,7-DHT in the amygdala, which displayed significantly elevated HJs to CS\(^+\), post-surgery (Fig. 5b).

Responses during the US period

Systolic and diastolic BP rose during the rewarded US period, over and above CS\(^+\) period, partly in response to the sight of the reward in the open box as well as during consumption. However, there were no differences between these measures pre- and post-surgery (see Supplementary Results).

Additional measures

Analysis of additional behavioural measures revealed a significant main effect of CS on the number of looks towards the foodbox (\(F_{1,12}=24.4, p<0.001\)) such that animals looked significantly more during CS\(^+\) compared to CS\(^-\) (Supplementary Table S2). There were, however, no effects of surgery, group or interaction effects (Fs <1, n.s.).

Reward omission and CS omission

There were no differences between the groups in their autonomic or behavioural responses during the two probe tests (Supplementary Results and Supplementary Tables S3 and S4).

Conditioned behavioural and autonomic responses following reversal of the contingencies

When the previously unrewarded stimulus became rewarded, and vice versa, all animals gradually reversed their conditioned responding, so that systolic BP and HJs became directed towards the new CS\(^+\) (criterion of learning: CS\(^+\) responses significantly greater than CS\(^-\) responses, \(p<0.05\), over a block of sessions including 6 CS\(^+\) presentations). There were no differences between the groups in the number of sessions taken to reach reversal criterion for either systolic BP (Table 3; \(F_{2,12}<1\), n.s.) or behaviour (Table 3; \(F_{2,12}<1\), n.s.). The final level of performance of the lesioned groups also did not differ from the control group (Table 3). Two-way ANOVAs of systolic BP and HJs at criterion demonstrated a significant main effect of CS for both measures (systolic BP: \(F_{1,18}=46.2, p<0.0001\); behaviour: \(F_{1,18}=55.9, p<0.0001\), but no effects of
Table 3. Reversal of conditioned systolic blood pressure (BP) and CS-directed behaviour

| Group               | Number of sessions to criterion | Systolic BP at criterion (mmHg) | Behaviour at criterion (no. HJs) |
|---------------------|---------------------------------|---------------------------------|---------------------------------|
|                     |       | CS+     | CS−     | CS+     | CS−     |
| Control             | 39.20 ± 10.86 | 4.22 ± 0.81 | 0.46 ± 0.76 | 4.17 ± 1.09 | 1.50 ± 0.53 |
| 5,7-DHT amygdala    | 52.50 ± 15.44 | 5.40 ± 0.45 | 0.02 ± 1.02 | 3.79 ± 0.50 | 1.00 ± 0.10 |
| 5,7-DHT OFC         | 64.00 ± 21.33 | 5.34 ± 2.01 | 1.24 ± 0.67 | 5.71 ± 1.64 | 2.31 ± 0.75 |

Number of sessions to reach criterion for reversal of conditioned systolic BP and CS-directed behavioural responses (headjerks, HJs), and conditioned systolic BP and behavioural responses at criterion (mean over a block of sessions including 6 CS+ presentations ± S.E.M.) for control (n = 5), 5,7-DHT amygdala (n = 4) and 5,7-DHT OFC-lesioned (n = 4) animals. As it was observed that post-reversal, many animals showed a delayed onset of the conditioned systolic BP responses during the 20 s CS period, their systolic BP responses at criterion were calculated as the mean of the last 10 s of the 20-s CS (control, n = 3; 5,7-DHT amygdala lesion, n = 3; 5,7-DHT OFC lesion, n = 2). Additionally, systolic BP responses calculated as the mean of the entire 20-s CS for all animals are given in parentheses.

Further analysis to determine the extent of the uncoupling of these two emotional response outputs in the 5,7-DHT OFC-lesioned monkeys revealed that uncoupling was also present at retention, despite the monkeys at this stage showing similar overall conditioned performance to the other two groups. It can be seen in Fig. 6b that the 5,7-DHT OFC-lesioned group showed significantly lower correlations than controls and 5,7-DHT amygdala-lesioned monkeys (one-way ANOVA: F11,12 = 5.01, p = 0.031). It should be noted that they also showed a significantly lower correlation than they had displayed pre-surgery, with their mean score post-surgery being less than the 95% lower confidence limits of their pre-surgery score (Fig. 6b).

**Discussion**

Excitotoxic lesions of the amygdala abolished, selectively, the expression of conditioned autonomic arousal in anticipation of reward, in agreement with previous findings (Braesicke et al. 2005). They left intact, CS-directed and US-directed conditioned behaviour, in the form of HJs and ‘looks’ towards the foodbox, respectively, during the auditory CS. In contrast, 5,7-DHT infusions into the amygdala significantly enhanced the expression of CS-directed conditioned behaviour. 5,7-DHT infusions into the OFC were without effect on the expression of conditioned responses, consistent with previous findings with excitotoxic OFC lesions (Reekie et al. 2008). However, they did cause an uncoupling of the behavioural and autonomic responses both at retention as well as during reversal learning. In contrast, neither 5,7-DHT infusions into the amygdala or the OFC affected the number of sessions it took for the conditioned responses to reverse. Thus, taken together, these data affirm the role of the amygdala in the expression of appetitive conditioned cardiovascular arousal, and...
demonstrate the selective role of 5-HT in the regulation of conditioned response expression (at the level of the amygdala), and coordination (at the level of the OFC).

**Amygdala, 5-HT and positive, emotionally salient stimuli**

The conditioned HJs displayed by marmosets, triggered by a diffuse auditory CS, appear to be highly similar to those described in rats and are thought to reflect enhanced attentional processing of the stimulus that has acquired emotional significance through learning (Holland, 1977). The present finding of intact expression of HJs, following post-training amygdala lesions in marmosets is entirely consistent with studies in rats which have shown that such CS-directed responses are only dependent upon the amygdala, and specifically the central nucleus, for their acquisition, but not subsequent expression (Gallagher et al. 1990; Groshek et al. 2005). The possibility that intact HJs in the present study were due to spared functioning in the lateral nucleus, which was unilaterally lesioned, is unlikely, as basolateral lesions of the amygdala have no effect on the acquisition, or expression, of CS-directed behaviour (Hatfield et al. 1996).

In contrast to excitotoxic amygdala lesions, selective 5-HT depletion in the amygdala resulted in significantly enhanced expression of CS-directed behaviour, and a similar (but non-significant) augmentation of conditioned cardiovascular arousal responses. To our knowledge, these data are the first to demonstrate the role of amygdala 5-HT in appetitive Pavlovian conditioning, since to date most investigations into the neurochemistry of this process have focused on dopamine (e.g. El-Amamy & Holland, 2007; Phillips & Hitchcott, 2009; Rosenkranz & Grace, 2002). Increased responsiveness to CS following a reduction in 5-HT levels in 5,7-DHT amygdala-lesioned marmosets is consistent with results from electrophysiological studies demonstrating that amygdala 5-HT inhibits the excitatory glutamatergic input to projection neurons (such as the sensory input from auditory areas) by activating inhibitory GABAergic interneurons (Rainnie, 1999; Stutzmann & LeDoux, 1999; Stutzmann et al. 1998). Thus, a reduction in tonic 5-HT induced by the 5,7-DHT lesion in the present study may have caused reduced inhibitory tone in the amygdala and therefore greater responsiveness to emotionally salient stimuli, i.e. the CS+.

The enhanced expression of CS-directed HJs in the 5,7-DHT amygdala-lesioned marmosets is somewhat similar to the enhanced approach responses of rats in

**Fig. 6.** Coupling of conditioned systolic blood pressure (BP) and CS-directed behavioural responses during reversal learning, retention and pre-surgery baseline. Mean correlation coefficients ($r$) for (a) systolic BP and CS-directed headjerks (HJs) at individual CS across reversal learning, and (b) at retention and pre-surgery baseline in control, 5,7-DHT amygdala and 5,7-DHT OFC groups. (a) Four out of five control subjects and all four 5,7-DHT amygdala-lesioned subjects showed a significant positive correlation between BP and HJs during reversal learning (significant correlation of individual monkeys indicated by filled symbols). In contrast, none of the four 5,7-DHT OFC-lesioned subjects displayed a significant correlation (indicated by unfilled symbols). Overall, the coupling of responses was significantly lower in the 5,7-DHT OFC-lesioned group compared to the other two groups (** $p=0.005$). (b) The coupling of responses in the 5,7-DHT OFC-lesioned group was also significantly lower during performance at retention (* $p=0.031$). Importantly, the groups did not differ from one another before surgery (baseline), as shown by their overlapping mean (solid lines) and 95% confidence intervals (dotted lines). Note that the mean of the correlations of the 5,7-DHT OFC-lesioned groups at retention, as well as the individual with the largest correlation (filled triangle), lay outside of the confidence intervals of their pre-surgery, baseline performance.
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an appetitive Pavlovian conditioning paradigm following intracerebroventricular infusions of 5,7-DHT (Winstanley et al. 2004). The findings differ in that the overall increase in approach responses in rats was not specific to CS+, unlike that in marmosets. Apart from the obvious difference in neuroanatomical selectivity between the two studies, the other major difference is that conditioning was trained pre- and post-surgery in marmosets and rats, respectively. While in marmosets the most salient stimulus post-surgery was the CS+, in rats both CS+ and CS− were equally salient, at the beginning of learning. Thus, the overall effects of 5-HT depletions within the amygdala may be to enhance responding towards the most salient stimuli.

Consistent with the role of 5-HT in appetitive conditioning are the recent findings that putative 5-HT neurons in the dorsal raphe respond during reward expectation (Bromberg-Martin et al. 2010; Nakamura et al. 2008). Whilst it would seem that a deficit in such 5-HT signalling in the amygdala would impair CS-directed behaviour, this may be more likely to occur during acquisition, rather than during expression of well-learned behaviour, as in the present study. In addition, alterations in the balance of 5-HT receptor signaling, i.e. 1A, 2A and 2C, following 5-HT depletion (Cahir et al. 2007; Yatham et al. 2001) may contribute to the observed effects, although such changes may have less of an impact immediately post-surgery when the 5-HT levels are very low.

Relating our findings in the primate to human studies warrants caution, as both the behavioural measures and the ways of manipulating 5-HT function employed in human research are different from those used in animals. Although enhanced processing of positive stimuli following manipulations of the 5-HT system in humans has been reported, both behaviourally (Browning et al. 2007; Harmer et al. 2003, 2004; Hayward et al. 2005; Murphy et al. 2006, 2009), and neurally (Kemp et al. 2004; Kerestes et al. 2009; Norbury et al. 2009; but see Canli et al. 2005; Demaree et al. 2009; Murphy et al. 2009) whether this is the result of enhanced or reduced 5-HT signalling, or other compensatory changes, is unclear.

OFC, 5-HT and uncoupling of the emotional responses

Previous studies have demonstrated a role for 5-HT at the level of the OFC in the regulation of reward-related actions, and specifically in reversal learning in both monkeys and rats (Clarke et al. 2004, 2005, 2007; Lapiz-Bluhm et al. 2009; Man et al. 2010; Walker et al. 2006, 2009; Winstanley et al. 2006). Together these studies have led to the proposal that 5-HT facilitates the OFC’s inhibitory, top-down control of responses orchestrated by subcortical regions, including the amygdala (Cools et al. 2008). We have previously shown that excitotoxic OFC lesions impair reversal learning and cause an uncoupling of behavioural and autonomic responses (Reekie et al. 2008). The present results extend these findings, implicating 5-HT within the OFC in the coordination of the emotional response. Thus, serotonergic dysfunction within the OFC caused an uncoupling of the autonomic and behavioural responses to appetitive stimuli, both during reversal learning and at retention. The finding that reversal learning, per se, was not affected by the 5,7-DHT OFC lesions is generally consistent with our previous studies investigating the effects of such lesions on an instrumental version of the discrimination reversal task. There, too, 5-HT depletions failed to affect performance on the first reversal of a series of reversals (Clarke et al. 2004, 2005, 2007). Very often, overall performance (errors to criterion) on subsequent reversals was also intact, the effect of the depletions being primarily an increase in perseverative responding to the previously rewarded stimulus (Clarke et al. 2005, 2007). Only when the intrinsic cell bodies of the OFC are destroyed is there an overall reversal impairment (Clarke et al. 2008; Reekie et al. 2008).

The finding of response uncoupling following OFC 5-HT depletions is of relevance to our understanding of emotion and, in particular, emotional dysregulation. Emotional states are the product of a variety of different response outputs, both physiological and behavioural, elicited by motivationally relevant external events. Somatic and autonomic interoceptive feedback arising from these response outputs has been proposed to contribute especially to subjective emotional experience or ‘feelings’ and to influence decision-making, effects dependent on the extent of the individual’s interoceptive awareness (Craig, 2002; Critchley, 2009; Critchley et al. 2004; Damasio, 2003; Dunn et al. 2010; Gray et al. 2007; Nicotra et al. 2006). Moreover, it has been reported that ambiguous peripheral signalling may contribute to the emotional and behavioural biases in schizophrenia, depression, autism and anxiety (Hirstein et al. 2001; Paulus & Stein, 2006; Williams et al. 2007), many of which are treated by drugs that target the 5-HT system. Thus, we propose that uncoupling of autonomic and behavioural components of emotion and thus dysregulation of interoceptive feedback, as a consequence of 5-HT dysfunction within the OFC, may have long
term effects on overall emotionality and adaptive decision-making.

**Additional considerations**

The differential effects of selective amygdala depletion within the amygdala and OFC highlight the distinct roles played by 5-HT in the two structures. However, post-lesion levels of 5-HT in the amygdala did show some recovery over the course of the study, unlike 5-HT levels in the OFC. While there was a large 5-HT depletion (~80%) just 2 wk after the lesion, the depletion was approximately 50% by 4 months and further recovery was evident at 11 months. Partial recovery could explain the lack of an effect of 5-HT amygdala depletion on reversal learning, since the latter took place between 1 and 7 months post-surgery. In contrast, it cannot explain the lack of an effect on response coordination at retention, as seen following OFC 5-HT depletion, since retention took place 2 wk post-surgery, when amygdala depletion was maximal, and indeed had caused an enhancement of conditioned behaviour towards the appetitive stimulus. In addition, serotonergic neurotransmission in these lesioned animals was still significantly compromised during reversal learning as shown by subsequent in-vivo microdialysis. This demonstrated that the basal extracellular levels of amygdala 5-HT were below detection threshold (2.0 fmol) and did not exceed the threshold following potassium challenge, although it must be noted that the microdialysis sampled a discrete amygdala region (probably in the basal or lateral nuclei) whereas the post-mortem analysis included the entire amygdala. Finally, these same lesioned animals went on to display differential effects to controls on a subsequent instrumental reward learning test (Man et al. 2010). However, further research in animals allowing selective manipulation of 5-HT receptor signalling in the amygdala, will be vital to understanding the underlying mechanism. In particular, it will be important to determine whether the behavioural effects following long-term changes in 5-HT levels induced by 5,7-DHT infusions could stem from adaptive changes in the function of specific 5-HT receptor subtypes within the amygdala, similar to the changes observed in other brain regions after prolonged alteration in 5-HT signalling (Cahir et al. 2007; Jennings et al. 2008).

In summary, we have demonstrated that 5-HT at the level of the primate amygdala and OFC modulates the expression and regulation, respectively, of conditioned Pavlovian responses in the appetitive setting. Thus, 5-HT modulates both positive and negative emotion, consistent with the effects of SSRIs on both positive and negative emotion in humans. It remains to be determined how 5-HT integrates emotional responding at these different levels of the control hierarchy.

**Note**

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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**Statement of Interest**

None.

**References**

Almeida JR, Versace A, Mechelli A, Hassel S, et al. (2009). Abnormal amygdala-prefrontal effective connectivity to happy faces differentiates bipolar from major depression. *Biological Psychiatry* 66, 451–459.

Balleine BW, Killcross S (2006). Parallel incentive processing: an integrated view of amygdala function. *Trends in Neurosciences* 29, 272–279.

Braesicke K, Parkinson JA, Reekie Y, Man MS, et al. (2005). Autonomic arousal in an appetitive context in primates: a behavioural and neural analysis. *European Journal of Neuroscience* 21, 1733–1740.

Bromberg-Martin ES, Hikosaka O, Nakamura K (2010). Coding of task reward value in the dorsal raphe nucleus. *Journal of Neuroscience* 30, 6262–6272.

Browning M, Reid C, Cowen PJ, Goodwin GM, et al. (2007). A single dose of citalopram increases fear recognition in healthy subjects. *Journal of Psychopharmacology* 21, 684–690.

Cahir M, Ardis T, Reynolds GP, Cooper SJ (2007). Acute and chronic tryptophan depletion differentially regulate central 5-HT1A and 5-HT2A receptor binding in the rat. *Psychopharmacology (Berlin)* 190, 497–506.
Canli T, Lech KP (2007). Long story short: the serotonin transporter in emotion regulation and social cognition. Nature Neuroscience 10, 1103–1109.

Canli T, Omura K, Haas BW, Fallgatter A, et al. (2005). Beyond affect: a role for genetic variation of the serotonin transporter in neural activation during a cognitive attention task. Proceedings of the National Academy of Sciences USA 102, 12224–12229.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, et al. (1999). Limbic activation during cue-induced cocaine craving. American Journal of Psychiatry 156, 11–18.

Clarke HF, Dalley JW, Crofts HS, Robbins TW, et al. (2004). Cognitive inflexibility after prefrontal serotonin depletion. Science 304, 878–880.

Clarke HF, Robbins TW, Roberts AC (2008). Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. Journal of Neuroscience 28, 10972–10982.

Clarke HF, Walker SC, Crofts HS, Dalley JW, et al. (2005). Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. Journal of Neuroscience 25, 532–538.

Clarke HF, Walker SC, Dalley JW, Robbins TW, et al. (2007). Cognitive inflexibility after prefrontal serotonin depletion is behaviorally and neurochemically specific. Cerebral Cortex 17, 18–27.

Cools R, Roberts AC, Robbins TW (2008). Serotonergic regulation of emotional and behavioural control processes. Trends in Cognitive Sciences 12, 31–40.

Craig AD (2002). How do you feel? Interoception: the sense of the physiological condition of the body. Nature Reviews Neuroscience 3, 655–666.

Critchley HD (2009). Psychophysiology of neural, cognitive and affective integration: fMRI and autonomic indicants. International Journal of Psychophysiology 73, 88–94.

Critchley HD, Wiens S, Rothsstein P, Ohman A, et al. (2004). Neural systems supporting interoceptive awareness. Nature Neuroscience 7, 189–195.

Damasio A (2003). Feelings of emotion and the self. Annals of the New York Academy of Sciences 1001, 253–261.

Demaree HA, Pu J, Jesberger J, Feeny N, et al. (2009). 5HTTLPR predicts left fusiform gyrus activation to positive emotional stimuli. Magnetic Resonance Imaging 27, 441–448.

Drevets WC (2003). Neuroimaging abnormalities in the amygdala in mood disorders. Annals of the New York Academy of Sciences 985, 420–444.

Drevets WC (2007). Orbitofrontal cortex function and structure in depression. Annals of the New York Academy of Sciences 1121, 499–527.

Dunn BD, Galton HC, Morgan R, Evans D, et al. (2010). Listening to your heart: how interoception shapes emotion experience and intuitive decision making. Psychological Science 21, 1835–1844.

El-Amamy H, Holland PC (2007). Dissociable effects of disconnecting amygdala central nucleus from the ventral tegmental area or substantia nigra on learned orienting and incentive motivation. European Journal of Neuroscience 25, 1557–1567.

Etkin A, Wager TD (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. American Journal of Psychiatry 164, 1476–1488.

Everitt BJ, Cardinal RN, Parkinson JA, Robbins TW (2003). Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. Annals of the New York Academy of Sciences 985, 233–250.

Gallagher M, Graham PW, Holland PC (1990). The amygdala central nucleus and appetitive Pavlovian conditioning; lesions impair one class of conditioned behavior. Journal of Neuroscience 10, 1906–1911.

Grant S, London ED, Newlin DB, Villemagne VL, et al. (1996). Activation of memory circuits during cue-elicited cocaine craving. Proceedings of the National Academy of Sciences USA 93, 12040–12045.

Gray MA, Harrison NA, Wiens S, Critchley HD (2007). Modulation of emotional appraisal by false physiological feedback during fMRI. PLoS One 2, e546.

Groshuk F, Kerfoot E, McKenna V, Polackwich AS, et al. (2005). Amygdala central nucleus function is necessary for learning, but not expression, of conditioned auditory orienting. Behavioral Neuroscience 119, 202–212.

Harmer CJ (2008). Serotonin and emotional processing: does it help explain antidepressant drug action? Neuropharmacology 55, 1023–1028.

Harmer CJ, Bhagwagar Z, Perrett DI, Vollm BA, et al. (2003). Acute SSRI administration affects the processing of social cues in healthy volunteers. Neuropsychopharmacology 28, 148–152.

Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM (2004). Increased positive vs. negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. American Journal of Psychiatry 161, 1256–1263.

Hatfield T, Han JS, Conley M, Gallagher M, et al. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. Journal of Neuroscience 16, 5256–5265.

Hayward G, Goodwin GM, Cowen PJ, Harmer CJ (2005). Low-dose tryptophan depletion in recovered depressed patients induces changes in cognitive processing without depressive symptoms. Biological Psychiatry 57, 517–524.

Hirstein W, Iversen P, Ramachandran VS (2001). Autonomic responses of autistic children to people and objects. Proceedings of the Royal Society of London, Series B: Biological Sciences 268, 1883–1888.

Holland PC (1977). Conditioned stimulus as a determinant of the form of the Pavlovian conditioned response. Journal of Experimental Psychology: Animal Behavior Processes 3, 77–104.

Holland PC, Gallagher M (2004). Amygdala-frontal interactions and reward expectancy. Current Opinion in Neurobiology 14, 148–155.
Jennings KA, Sheward WJ, Harmar AJ, Sharp T (2008). Evidence that genetic variation in 5-HT transporter expression is linked to changes in 5-HT2A receptor function. *Neuropsychopharmacology* 34, 776–783.

Kemp AH, Gray MA, Silberstein RB, Armstrong SM, et al. (2004). Augmentation of serotonin enhances pleasant and suppresses unpleasant cortical electrophysiological responses to visual emotional stimuli in humans. *Neuropsychopharmacology* 22, 1084–1096.

Kerestes R, Labuschanghe I, Croft RJ, O’Neill BV, et al. (2009). Evidence for modulation of facial emotional processing bias during emotional expression decoding by serotoninergic and noradrenergic antidepressants: an event-related potential (ERP) study. *Psychopharmacology* (Berlin) 202, 621–634.

Lapiz-Bluhm MD, Soto-Pina AE, Hensler JG, Morilak DA (2009). Chronic intermittent cold stress and serotonin depletion induce deficits of reversal learning in an attentional set-shifting test in rats. *Psychopharmacology* (Berlin) 202, 329–341.

Man MS, Dalley JW, Roberts AC (2010). Opposing effects of 5,7-DHT infusions into the orbitofrontal cortex and amygdala on flexible responding. *Cerebral Cortex* 20, 1668–1675.

McCabe C, Mishor Z, Cowen PJ, Harmer CJ (2010). Diminished neural processing of aversive and rewarding stimuli during selective serotonin reuptake inhibitor treatment. *Biological Psychiatry* 67, 439–445.

Mikheenko Y, Man MS, Braesicke K, Johns ME, et al. (2010). Autonomic, behavioral, and neural analyses of mild condition negative affect in marmosets. *Behavioral Neuroscience* 124, 192–203.

Milad MR, Rauch SL (2006). The orbitofrontal cortex and anxiety disorders. In: Zald DH (Ed.), *The Orbitofrontal Cortex* (pp. 523–543). New York: Oxford University Press.

Morilak DA, Frazer A (2004). Antidepressants and brain monoaminergic systems: a dimensional approach to understanding their behavioural effects in depression and anxiety disorders. *International Journal of Neuropsychopharmacology* 7, 193–218.

Murphy SE, Longhitano C, Ayres RE, Cowen PJ, et al. (2006). Tryptophan supplementation induces a positive bias in the processing of emotional material in healthy female volunteers. *Psychopharmacology* (Berlin) 187, 121–130.

Murphy SE, Norbury R, O’Sullivan U, Cowen PJ, et al. (2009). Effect of a single dose of citalopram on amygdala response to emotional faces. *British Journal of Psychiatry* 194, 535–540.

Murray EA (2007). The amygdala, reward and emotion. *Trends in Cognitive Sciences* 11, 489–497.

Nakamura K, Matsumoto M, Hikosaka O (2008). Reward-dependent modulation of neuronal activity in the primate dorsal raphe nucleus. *Journal of Neuroscience* 28, 5331–5343.

Nicotra A, Critchley HD, Mathias CJ, Dolan RJ (2006). Emotional and autonomic consequences of spinal cord injury explored using functional brain imaging. *Brain* 129, 718–728.

Norbury R, Taylor MJ, Selvaraj S, Murphy SE, et al. (2009). Short-term antidepressant treatment modulates amygdala response to happy faces. *Psychopharmacology* (Berlin) 206, 197–204.

Parkinson JA, Robbins TW, Everitt BJ (2000). Dissociative roles of the central and basolateral amygdala in appetitive emotional learning. *European Journal of Neuroscience* 12, 405–413.

Paulus MP, Stein MB (2006). An insular view of anxiety. *Biological Psychiatry* 60, 383–387.

Phillips GD, Hitchcott PK (2009). Blockade of the acquisition, but not expression, of associative learning by pre-session intra-amygdala R(+) 7-OH-DPAT. *Psychopharmacology* (Berlin) 203, 161–173.

Rainnie DG (1999). Serotonergic modulation of neurotransmission in the rat basolateral amygdala. *Journal of Neurophysiology* 82, 69–85.

Reekie YL, Braesicke K, Man MS, Roberts AC (2008). Uncoupling of behavioral and autonomic responses after lesions of the primate orbitofrontal cortex. *Proceedings of the National Academy of Sciences USA* 105, 9787–9792.

Ressler KJ, Nemeroff CB (2000). Role of serotoninergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depression and Anxiety* 12 (Suppl. 1), 2–19.

Roberts AC, Reekie Y, Braesicke K (2007). Synergistic and regulatory effects of orbitofrontal cortex on amygdala-dependent appetitive behavior. *Annals of the New York Academy of Sciences* 1121, 297–319.

Rosenkranz JA, Grace AA (2002). Dopamine-mediated modulation of odour-evoked amygdala potentials during pavlovian conditioning. *Nature* 417, 282–287.

Saxena S, Rauch SL (2000). Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. *Psychiatric Clinics of North America* 23, 563–586.

Schnell CR, Wood JM (1993). Measurement of blood pressure and heart rate by telemetry in conscious, unrestrained marmosets. *American Journal of Physiology* 264, H1509–H1516.

Stutzmman GE, LeDoux JE (1999). GABAergic antagonists block the inhibitory effects of serotonin in the lateral amygdala: a mechanism for modulation of sensory inputs related to fear conditioning. *Journal of Neuroscience* 19, RC8.

Stutzmann GE, McEwen BS, LeDoux JE (1998). Serotonin modulation of sensory inputs to the lateral amygdala: dependency on corticosterone. *Journal of Neuroscience* 18, 9529–9538.

Walker SC, Mikheenko YP, Argyle LD, Robbins TW, et al. (2006). Selective prefrontal serotonin depletion impairs acquisition of a detour-reaching task. *European Journal of Neuroscience* 23, 3119–3123.

Walker SC, Robbins TW, Roberts AC (2009). Differential contributions of dopamine and serotonin to orbitofrontal cortex function in the marmoset. *Cerebral Cortex* 19, 889–898.
Williams LM, Das P, Liddell BJ, Olivieri G, et al. (2007). Fronto-limbic and autonomic disjunctions to negative emotion distinguish schizophrenia subtypes. Psychiatry Research 155, 29–44.

Winstanley CA, Dalley JW, Theobald DE, Robbins TW (2004). Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. Neuropsychopharmacology 29, 1331–1343.

Yatham LN, Liddle PF, Shiah IS, Lam RW, et al. (2001). Effects of rapid tryptophan depletion on brain 5-HT(2) receptors: a PET study. British Journal of Psychiatry 178, 448–453.

Winstanley CA, Theobald DE, Dalley JW, Cardinal RN, et al. (2006). Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. Cerebral Cortex 16, 106–114.