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Failure of Hippocampal Deactivation during Loss Events in Treatment-Resistant Depression

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Abstract

Major Depressive Disorder is characterised by anhedonia, cognitive biases, ruminations, hopelessness and increased anxiety. Blunted responses to rewards have been reported in a number of recent neuroimaging and behavioural studies of Major Depressive Disorder. In contrast, neural responses to aversive events remain an under-studied area. Whilst Selective Serotonergic Reuptake Inhibitors are often effective in treating Major Depressive Disorder, their mechanism of action remains unclear. Following a series of animal-model investigations of depressive illness and serotonergic function, Deakin and Graeff predicted that brain activity in Major Depressive Disorder patients is associated with an overactive dorsal raphe nucleus with overactive projections to the amygdala, periaqueductal grey and striatum, and an underactive median raphe nucleus with underactive projections to the hippocampus. Here we describe an instrumental loss-avoidance and win-gain reinforcement learning functional magnetic resonance imaging study with 40 highly treatment-resistant Major Depressive Disorder patients and never-depressed controls. The dorsal raphe nucleus/ periaqueductal grey region of the midbrain and hippocampus were found to be overactive in Major Depressive Disorder during unsuccessful loss-avoidance although the median raphe nucleus was not found to be underactive. Hippocampal overactivity was due to a failure to deactivate during loss events in comparison to controls, and hippocampal over-activity correlated with depression severity, self-report 'hopelessness' and anxiety. Deakin and Graeff argued that the median raphe nucleus normally acts to inhibit consolidation of aversive memories via the hippocampus and this system is underactive in Major Depressive Disorder, facilitating the development of ruminations, whilst the dorsal raphe nucleus system is engaged by distal cues predictive of threats and is overactive in Major Depressive

Disorder. During win events the striatum was underactive in Major Depressive Disorder. We tested individual patient consistency of these findings using within-study replication. Abnormal hippocampal activity correctly predicted individual patient diagnostic status in 97% (sensitivity 95%, specificity 100%) of subjects, and abnormal striatal activity predicted diagnostic status in 84% (sensitivity 79%, specificity 89%) of subjects. We conclude that the neuroimaging findings were largely consistent with Deaken and Graeff's predictions, abnormally increased hippocampal activity during loss events was an especially consistent abnormality, and brainstem serotonergic nuclei merit further study in depressive illness.

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Abbreviations: BDI: Beck Depression Inventory-II; BHS: Beck Hopelessness Scale;

DRN: Dorsal Raphe Nucleus; HAD-A: Hospital Anxiety and Depression – Anxiety

Scale; HAM-D: 17-item Hamilton Depression Rating Scale; MADRS: Montgomery-Åsberg Depression Rating Scale; MDD: Major Depressive Disorder; MGH-S: Massachusetts General Hospital staging method; MRN: Median Raphe Nucleus; PAG: periaqueductal grey; SPM: Statistical Parametric Mapping

SSRIs: Selective Serotonergic Reuptake Inhibitors; TRD: Treatment-resistant depression

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Introduction

In major depressive disorder (MDD), characteristic cognitive and attentional biases are directed towards aversively valenced information (associated with depressive ruminations, hopelessness, perceptions of helplessness, anxiety and irritability) and away from rewarding stimuli (anhedonia) (Beck, 1979). This is consistent with observations that neural responses to rewards are blunted and responses to aversive stimuli increased, with a recent systematic meta-analysis concluding that both abnormalities are reversed by antidepressant treatment (Ma, 2014). Previously we reported underactive striatal responses to rewarding events in MDD (Steele *et al.*, 2007; Kumar *et al.*, 2008; Gradin *et al.*, 2011) supported by a recent neuroimaging meta-analysis (Zhang *et al.*, 2013) and behavioural meta-analysis (Huys *et al.*, 2013).

In contrast to studies on rewards, there are far fewer studies on the neural responses to aversive stimuli in MDD and none to our knowledge on patients with treatment-resistant MDD using instrumental loss-avoidance learning paradigms. A few studies have reported responses to rewarding and aversive stimuli in depression (Knutson *et al.*, 2008; Stoy *et al.*, 2012; Ubl *et al.*, 2015) using a monetary incentive delay task where task difficulty is based on reaction times and reported differing abnormalities. Neural responses to aversive stimuli are particularly relevant given the link between aversive information processing and serotonergic function (Gray and McNaughton, 2000; Boureau and Dayan, 2011; Deakin, 2013) and the efficacy of Selective Serotonergic Reuptake Inhibitors (SSRIs) for many but not all patients.

Error commission is aversive (Hajcak and Foti, 2008) and it has been reported that depressed patients have an abnormal response to feedback of poor behavioural performance (Elliott *et al.*, 1997; Murphy *et al.*, 2003; Roiser *et al.*, 2012). Electrophysiological studies of behavioural errors in MDD have reported an abnormally increased Error Related Negativity (Chiu and Deldin, 2007; Holmes and Pizzagalli, 2010; Georgiadi *et al.*, 2011) and an abnormally increased Feedback Related Error signal which may be associated with enhanced avoidance learning (Cavanagh *et al.*, 2011). Whilst abnormal responses to rewarding events imply abnormal (Nestler and Carlezon, 2006) and blunted dopaminergic reward learning activity (Kumar *et al.*, 2008; Gradin *et al.*,

2011) although see (Huys *et al.*, 2013), the role of putative serotonergic abnormalities in MDD remains very unclear (Boureau and Dayan, 2011; Faulkner and Deakin, 2014).

Following a series of animal-model studies focusing on serotonergic function, Deakin and Graeff predicted that depressive illness involves an overactive dorsal raphe nucleus (DRN) with overactive projections to the amygdala (anxiety), periaqueductal grey (PAG; helplessness), and caudate/striatum (anhedonia), and an underactive median raphe nucleus (MRN) with underactive projections to the hippocampus (ruminations) (Deakin and Graeff, 1991; Deakin, 2013). The DRN-PAG-amygdala-striatum hypothesis is a particularly strong test of Deaken and Graeff's theory because such observations are highly unlikely according to a contrary theory of MDD as a 'serotonin deficiency' disorder (Deakin, 2013).

We chose loss-avoidance and reward-gain instrumental learning tasks because 'loss' is an aversive event prominently linked to MDD clinically (Beck, 2008) and blunted striatal reward-gain activity has been reported in many studies of depression (Pizzagalli *et al.*, 2009; Stoy *et al.*, 2012; Zhang *et al.*, 2013). Notably we focused only on unsuccessful loss-avoidance events, as loss events are experienced as aversive, in contrast to successful loss-avoidance which has similarities to a reward (Rolls, 1999). Whilst many studies on mood disorder involve recovered, previously ill patients or healthy subjects with transient mood induction, which may or may not reflect depressive illness, we chose to study treatment-resistant depressive illness given the remarkably high prevalence of chronic disability associated with mood disorder (Whiteford *et al.*, 2013). Whilst Deakin and Graeff made predictions about depression in general and not treatment-resistant illness in particular, we used Deakin and Graeff's framework to construct hypotheses about such patients, as this population may have more consistent abnormalities than treatment responsive patients.

The following three groups of hypotheses were tested: i) the DRN/PAG region of the midbrain, striatum and amygdala have abnormal activity in MDD (Deakin, 2013) during unsuccessful loss-avoidance events; ii) the hippocampus is abnormally increased in activity and the MRN region of the midbrain abnormally decreased in activity in MDD (Deakin, 2013) during unsuccessful loss avoidance; iii) the striatum (which includes the nucleus accumbens) is underactive in MDD during rewarding events (Kumar *et al.*, 2008;

Gradin *et al.*, 2011; Stoy *et al.*, 2012; Deakin, 2013). We also tested for correlations between loss and win event brain activity and ratings of illness severity and treatment-resistance for the patient group alone, to determine if any correlations were consistent with between groups (MDD versus control) abnormalities.

Standard fMRI analyses consist of testing the null hypothesis of no difference between patient and control groups. Despite this approach identifying disorder-related average group abnormalities, these are rarely consistent enough to make individual patient inferences. Therefore, to estimate the specificity of abnormal patterns of activation/deactivation for *individual* patients, we generated a predictive model and used within-study replication to form an unbiased estimate of consistency.

Materials and methods

The study was approved by the local Ethics Committee (the East of Scotland Research Ethics Service) and written informed consent was obtained from all volunteers. Forty one adults with treatment-resistant MDD and controls were recruited: twenty adults with treatment-resistant MDD from the Advanced Interventions Service in Dundee, a tertiary level UK-wide service specialising in treatment-resistant MDD receiving referrals from NHS consultant psychiatrists throughout the UK. Data from one control had to be excluded from the analysis due to a failure to perform the fMRI task meaning data from 40 subjects were included in the analyses. Diagnosis was made according to MINI PLUS (v5.0) criteria (Sheehan and Lecrubier, 1992). Each patient's clinical history was reviewed with respect the number of failed antidepressant treatment trials to quantify treatment-resistance in accordance with the Massachusetts General Hospital staging (MGH-S) method (Fava, 2003). Exclusion criteria were any potentially confounding diagnosis: including other primary psychiatric disorder, substance misuse or significant head injury. Twenty-one healthy controls with no lifetime history of MDD were recruited, mostly from partners, relatives and friends of patients and underwent psychiatric assessment using the MINI PLUS. None of the controls had a current or past psychiatric or neurological disorder and none were taking medication. Syndrome severity was assessed using the Montgomery-Åsberg Depression Rating Scale (Montgomery and Asberg, 1979), 17-item Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960),

Hospital Anxiety and Depression Rating – Anxiety Scale (HAD-A) (Zigmond and Snaith, 1983), Beck Depression Inventory-II (BDI) (Beck *et al.*, 1996) and Beck Hopelessness Scale (BHS) (Beck *et al.*, 1974). All MDD and control volunteers included in the analyses had a predicted pre-morbid Full Scale Intelligence Quotient above 106 as assessed by the National Adult Reading Test. Handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Apart from 2 left-handed subjects in the control group and 1 and 3 ambidextrous subjects in the control and patient groups respectively, all subjects were right-handed.

Image acquisition

For each participant functional whole-brain images were acquired using a 3T Siemens Magnetom TimTrio Syngo scanner using an echo-planar imaging sequence with the following parameters: TR = 2500 ms, TE = 30 ms, flip angle = 90°, FOV = 224 mm, matrix = 64 x 64, 37 slices, voxel size 3.5x3.5x3.5 mm. The first four blood-oxygen-level dependent volumes were discarded as standard due to transient effects.

Paradigm

The fMRI paradigm was a modified version of the Pessiglione task (Pessiglione *et al.*, 2006) which incorporated rewarding ('win'), neutral ('no change') and aversive ('loss') outcomes. One pair of fractal images was associated with each potential outcome type; win, loss or neutral. Associations between outcomes and fractal shapes were randomised across subjects. Win trials had the possible outcomes 'you win' or 'nothing' (no change) and loss trials had the possible outcomes 'you lost' or 'nothing'. A neutral condition with outcomes 'look' or 'nothing' was included whereby no change occurred regardless of choice. Participants were not informed that the win and loss fractal pairs had one option with a fixed 'high' (0.7) probability of winning or losing and the other option with a fixed 'low' probability (0.3). Subjects understood that the goal of the task was to maximise winning and minimise loosing 'vouchers' through trial and error but that no actual payments would be made. At the beginning of each trial subjects choose between a pair of fractal images, with the order of the fractal images randomly assigned to the left or right of the screen. Three seconds after the beginning of each trial the images were

replaced with a fixation cross; a small black "+" in the centre of a white background, then feedback of the outcome was given. The sequence and timing of win and loss pairs of fractal stimuli was optimised for detection of the signals of interest using 'optseq2' (http://surfer.nmr.mgh.harvard.edu/optseq/); during the inter-trial interval a fixation cross was displayed for a variable amount time (jitter) ranging between 3 and 13.75 seconds. A summary of the task is shown in Fig. 1.

Image pre-processing

All scans were visually inspected for artefacts (McRobbie *et al.*, 2010) and pre-processing done using SPM8 (http://www.fil.ion.ucl.ac.uk/spm). Images were realigned to the first image in each time series and co-registered to the SPM8 Montreal Neurological Institute echo planar imaging template. The average realigned co-registered image for each subject was used as a template to normalise each realigned and co-registered volume to the SPM8 echo planar imaging template image and smoothed.

Image analyses

Event-related random effects designs were used for analyses. The times of each category of feedback were modelled as truncated delta functions and convolved with the SPM8 canonical haemodynamic response function without time or dispersion derivatives. Vectors representing these events were entered into first level analyses for each subject and as usual, six motion realignment parameters calculated during realignment were entered as covariates of no interest to remove any residual motion related variance. Two images were generated during the first level analysis, a 'win' image as [(win − nothing) − (look − nothing)] and a 'loss' image as [(loss − nothing) − (look − nothing)].

The win and loss images for each subject were separately entered into second level analyses to test for within-group (one-group t-test) activations/deactivations and between-group differences (MDD versus controls, two-group t-tests). The patient group was investigated further by testing for correlations with syndrome severity (MADRS, HAM-D, HAD-A, BDI, BHS) and treatment resistance (MGH-S) with the object of determining whether any of the categorical between group (MDD versus control) differences for a given brain region were consistent with patient group-only correlations.

Significance was defined as $P < 0.01$ at a whole brain, Family-Wise Error corrected level, with simultaneous requirements for voxel threshold ($P < 0.05$) and minimum cluster extent (> 120 voxels) identified using a popular Monte-Carlo method (Slotnick *et al.*, 2003). All figures are shown at this threshold.

The DRN forms a rostrocaudal ventral midline column and is part of the PAG (Le Maitre *et al.*, 2013). The PAG activates in response to aversive stimuli (McNally *et al.*, 2011; Roy *et al.*, 2014) as do 'clocklike' serotonin neurones in the DRN (Schweimer and Ungless, 2010). The DRN projects to various regions such as the amygdala and striatum (Deakin, 2013) and the remainder of the PAG similarly also projects to the amygdala but indirectly via paths including the thalamus (McNally *et al.*, 2011). Whilst the DRN can be discriminated from the rest of the PAG using high resolution histological techniques, it was not considered practical to discriminate the DRN from the rest of the PAG given fMRI spatial resolution and similar responses to aversive stimuli. Our DRN region should therefore be viewed as a combination of the DRN and PAG. The DRN can be confused with the more inferior MRN so we followed recommended DRN identification criteria (Kranz *et al.*, 2012): to identify the DRN we required an activation locus to be included within a spherical region of interest centred at the level between the superior and inferior colliculi 6 mm in diameter (Fig. 2B) (Kranz *et al.*, 2012) and in addition required that this activation was in response to aversive (Schweimer and Ungless, 2010; Deakin, 2013) loss events. Kranz' corresponding definition for the MRN, to reliably delineate the MRN from the DRN, is a region of interest in the rostral pons (Kranz *et al.*, 2012). We did not identify significant activity in this region.

Calculation of specificity of findings for individuals

Conventional null hypothesis testing is important for identifying group level abnormalities but not ideal for testing the consistency of findings for individual patients. We therefore used a combination of 'feature selection' (automated identification of brain regions), machine learning (to optimally combine information from the selected brain regions) and within-study replication (cross-validation) to make unbiased inferences about individual patients (Johnston *et al.*, 2014). Using 'one left out cross validation' there were two stages to the calculation: 'training' of an optimal predictor using $n - 1$

subjects' images followed by testing using the 'left out' data set. Feature selection was only adjusted during training and consisted of varying the threshold on a t-test, a method which involved calculating a standard two-group t-test on each *training* dataset (omitting the testing data) using standard SPM routines (Johnston *et al.*, 2014). Machine learning was done using a Matlab (Mathworks Inc.) Support Vector Machine toolbox (Schwaighofer, 2001) with a Gaussian kernel. During the testing stage, accuracy, sensitivity and specificity were calculated for all subjects.

Results

Participants

Age and IQ did not differ significantly (t-test, $P > 0.1$) between MDD and control groups and gender was not significantly different (chi-square test). There were no significant differences in task performance between groups implying that neuroimaging differences cannot be attributed to a failure to perform the task. The average HAM-D, MADRS and BDI illness severity ratings in the TRD group (Table 1) indicated depression severity in the moderate range. The mean MGH-S score of 13.2 indicates these patients were highly treatment resistant with the score being comparable to a previous assessment of patients attending the specialist Advanced Interventions Service (15.5), and significantly greater than typical UK secondary care (CMHT) psychiatric (5.3) and primary care (0.5) treatment-resistance levels (Hazari *et al.*, 2013). Seventeen MDD participants were treated with one or more anti-depressant medications (venlafaxine (6), sertraline (3), trazodone (3), citalopram (2), fluoxetine (2), mirtazapine (2), isocarboxazid (1), L-tryptophan (1), phenelzine (1), and tranylcypromine (1); seven patients received anti-psychotic medications (quetiapine (6) and chlorpromazine (1)) and three lithium augmentation reflecting typical clinical practice with treatment-resistant MDD.

Loss trials

During loss events using one group t-tests, *controls* activated the bilateral insula (−40,18,−24), (38,16,−18) without significant activation in the DRN region of the midbrain, and deactivated a cluster including peaks at the bilateral hippocampus (34,−26,−10), (−32,−26,−10), subgenual anterior cingulate (−10,22,−10), (12,24,−12) and bilateral nucleus accumbens (−10,6,−10), (12,8,−14).

In contrast, *patients* activated the DRN/PAG region of the midbrain (0,−20,−2), bilateral insula (−42,18,−2), (38,18,8) and amygdala (−28,−6,−8), and deactivated a cluster including peaks at the bilateral subgenual anterior cingulate (−12,36,−10), (14,34,−6), bilateral nucleus accumbens (10,16,−10), (−8,14,−6) but not hippocampus.

Using two-group tests, during loss events MDD patients had increased activation within a cluster including peaks at the DRN/PAG region of the midbrain (0,−20,−2), hippocampus (−32,−28,−10), amygdala (−28,−6,−8) and insula (+/−46,−2,2), whereas controls had increased activity in the nucleus accumbens (10,16,−10). This is shown in Fig. 2 and Supplementary Table 1. Increasing the voxel significance threshold to $P < 0.001$ resulted in a distinct anatomical cluster within the hippocampus at (−32,−28,−10) with a cluster size of 576 voxels.

Win trials

During win events using one group t-tests, *controls* activated a cluster including peaks at the nucleus accumbens/inferior caudate (−2,8,−2), (−12,8,−8), (12,10,−10), subgenual anterior cingulate (−4,52,−8), posterior cingulate (−2,−16,40), (−6,−50,24) and deactivated lateral cortical regions (50,22,14), (−42,20,28), (−40,−60,48).

In contrast, *patients* activated fewer regions including the bilateral subgenual anterior cingulate (−14,58,0), (10,46,−2), bilateral insula (−48,4,−6), (50,0,0), bilateral amygdala (−28,2,−16), (26,0,−12), bilateral hippocampus (−30,−14,−10), (42,−16,−10) and deactivated the bilateral dorsolateral prefrontal cortex (44,16,26), (−58,20,26).

Using two-group t-tests, during win events controls had significantly increased activity in the subgenual anterior cingulate (−2,52,4), nucleus accumbens (−2,8,−2), bilateral posterior cingulate (2,−58,46), (−4,−50,18) and patients had significantly

increased activity in the bilateral insula (48,−8,−6), (−46,−2,−4). This is shown in Fig. 3 and Supplementary Table 2.

Correlations with illness severity scores

Linear regressions were done to test for variation in win and loss event related brain activity varying with symptom severity scores in the patient group alone, with particular attention to correlations which were consistent with between group results. In some cases (Supplementary Materials) between groups analyses identified large clusters of activity including several brain structures whereas the within patient group correlations identified more focal abnormalities. Identification of consistent abnormalities using different approaches increases the confidence in the results. Loss event images correlated significantly with several illness severity scores but win event images did not correlate significantly with any measure.

For loss events, the BHS 'hopelessness' score positively correlated with bilateral hippocampal (−34,−26,−18), (26,−28,−24) activations, BDI depression severity positively correlated with hippocampal (−36,−24,−10) and insula (−36,4,6) activations and HAD-A anxiety correlated positively with hippocampal (−34,−34,−12) and insula (36,6,−8) activations. This is shown in Fig. 4 and Supplementary Table 3.

Correlations with treatment resistance

Linear regressions were done to test for variation in win and loss event related brain activity varying with treatment resistance scores in the patient group alone. During loss events, activity in the bilateral hippocampus (−28,−16,−26), (30,−12,−26), amygdala (−32,6,−20) and insula (−50,10,−2) negatively correlated with MGH-S score. During win events, activity in a number of regions including the hippocampus (−28,−16,−24), (18,−16,−20), insula (−56,10,−14), medial frontal cortex (2,61,16) and thalamus (0,−14,14) negatively correlated with MGH-S score. Treatment resistance was therefore associated with blunted brain responses to both loss and win events. This is shown in Fig. 4 and Supplementary Table 3.

Consistency of loss and win event activation patterns

Feature selection, machine learning and within study replication were used to classify win and loss first level analysis images from MDD and control subjects. Win and loss images were analysed separately. Analysis using the win event images resulted in correctly predicting diagnostic status in 84% (sensitivity 79%, specificity 89%, $\chi^2 = 15.3$, $P < 0.0001$) of subjects. In comparison, loss event images resulted in predicting diagnosis correctly in 97% (sensitivity 95%, specificity 100%, $\chi^2 = 30.5$, $P << 0.0001$) of subjects, indicating greater consistency with loss event responses. A small number of brain regions were highlighted: the hippocampus (−27,−31,−2) was identified during the loss event calculation and the nucleus accumbens (−2,8,−2) plus subgenual anterior cingulate (−2,54,−7) during the win event classification.

Discussion

Deakin and Graeff predicted that the DRN is abnormally increased in activity and the striatum and amygdala have abnormal activity in depression (Deakin, 2013). Consistent with this, we found that during loss events, the DRN region (Kranz *et al.*, 2012) was significantly increased in activity in MDD, as was the amygdala, and patients deactivated the nucleus accumbens more than controls. In addition the nucleus accumbens was significantly and consistently decreased in activity during win events in MDD relative to controls replicating previous reports (Steele *et al.*, 2007; Kumar *et al.*, 2008; Gradin *et al.*, 2011).

Deakin and Graeff proposed that DRN projections act as an anticipatory anxiety system engaged by distal threats which facilitates learning about aversive stimuli by the amygdala, allowing an organism to move away from threatening stimuli by opposing dopamine incentive mechanisms, restraining unconditioned fight/flight (PAG) responses (Deakin, 2013). Concepts of perceived helplessness inform cognitive behavioural therapy and cognitive formulations of helplessness are predictors of depression in healthy humans (Alloy *et al.*, 1999). The learned helplessness animal model of depressive illness is associated with marked activation of DRN neurones (Maier and Watkins, 2005; Deakin, 2013), with large increases in extracellular 5HT from DRN projections to the amygdala

(Maier and Watkins, 2005). Amygdala overactivity has often been reported, particularly in response to aversive events, as noted in a review of studies on mood disorder (Roiser *et al.*, 2012). Deakin and Graeff hypothesised that the modulatory effects of DRN projections to the amygdala are mediated by 5HT2c receptors, supported by recent animal and human studies (Deakin, 2013). Notably, an acute effect of SSRIs is often to increase anxiety, whilst chronic administration desensitises/down regulates post synaptic 5HT2a/c receptors, whose activation by agonists increases anxiety-like behaviours (Van Oekelen *et al.*, 2003; Maier and Watkins, 2005).

Deakin and Graeff predicted that the hippocampus is abnormally increased in activity during aversive events and the MRN is abnormally decreased in activity (Deakin, 2013). Consistent with this we found that the hippocampus was abnormally increased in activity in MDD due to a failure to deactivate during loss events, with the magnitude of the abnormality correlating with depression severity, self-report hopelessness and anxiety in the MDD group alone. However we did not identify an underactive MRN region as anatomically defined (Kranz *et al.*, 2012). This might be due to the MRN having a more complex pattern of activity, reflecting the encoding of aversive expectation value (Amo *et al.*, 2014)

Deakin and Graeff proposed that MRN projections to the hippocampus mediate behavioural adaptation to repeated aversive stimuli, should anticipatory DRN mechanisms fail to prevent exposure (Deakin, 2013). Studies on animals indicated that 5HT1a receptors oppose the consolidation of aversive memories. It was argued that the MRN 'resilience' system normally functions to interrupt rehearsal of aversive memories and when this fails or is underactive, ruminations result with the elaboration of negative semantic self-knowledge (Deakin, 2013). In this context, it is possible that abnormal MRN function resulted in a failure to deactivate the hippocampus on loss events, and it is notable that the extent of the failure to deactivate the hippocampus correlated with depression, self-report 'hopelessness' and anxiety. Hopelessness about the future has long been considered a critical part of depressive illness empirically linked to suicidal behaviour (Beck *et al.*, 1990) and rumination is associated with increased hopelessness (Lavender and Watkins, 2004). A recent review concluded that the predominant weight of evidence supports the hypothesis that postsynaptic 5HT1a receptor binding/density is

reduced in the amygdala-hippocampal complex in depression (Savitz and Drevets, 2013). The hippocampus is often activated with the amygdala in response to fear inducing stimuli (Williams *et al.*, 2001; Surguladze *et al.*, 2003) and is associated with behavioural inhibition (Gray and McNaughton, 2000), acute administration of an SSRI to healthy subjects reduced hippocampal activation to fear-relevant stimuli (Harmer *et al.*, 2006) and treatment-responsive depressive illness was associated with decreased hippocampal activation to sad facial expressions (Fu *et al.*, 2004). A recent review concluded that chronic stress promotes depression via. monoamine changes and suppression of hippocampal neurogenesis (Mahar *et al.*, 2014), and a neuroimaging meta-analysis reported a reduced volume of the hippocampus and other emotion linked regions (Arnone *et al.*, 2012), making relatively increased hippocampal activity in MDD notable. Abnormal hippocampal activity in relation to loss events was particularly consistent in patients with treatment-resistant MDD. In contrast, blunted responses to win events were not as consistent.

Deakin noted that the hypothesis of an overactive DRN with effects on the amygdala and striatum is a strong test of the Deakin and Graeff theory of depression because its highly improbable according to a contrary view of depression as a serotonin deficiency disorder (Deakin, 2013). In choosing to study treatment-resistant MDD because of its remarkable associated disability (Whiteford *et al.*, 2013) we considered it unethical to discontinue medications as a patient's illness might worsen and not improve on reinstatement, as can be observed clinically. The neural responses to aversive and rewarding events in medicated treatment-resistant MDD were clearly abnormal, with the former increased and the latter decreased as reported in unmedicated patients, in contrast to antidepressant changes which occur in treatment-responsive illness (Ma, 2014). Notably, Deakin and Graeff predicted in 1991 that SSRIs would not be effective antidepressants because they should enhance activity in the DRN system and promote anxiety (Deakin, 2013). It is now known that whilst an initial increase in anxiety can indeed occur and may be linked to this mechanism, chronic antidepressant treatment can desensitise/down regulate DRN projection post synaptic 5HT2a/c receptors (Maier and Watkins, 2005).

The anterior insula is associated with emotional evaluation and is strongly activated during negative affect (Hester *et al.*, 2010; Palminteri *et al.*, 2012), anxiety and avoidance learning (Paulus and Stein, 2006; Samanez-Larkin *et al.*, 2008). Increased insula activation in response to aversive stimuli has been reported in young people at familial risk of depression (Samanez-Larkin *et al.*, 2008). We found significantly increased anterior insula activation in response to loss events in MDD which correlated positively with self-rated depression severity (BDI) and anxiety (HAD-A), and negatively with treatment-resistance (MGH-S). Therefore, in contrast to illness severity which correlated positively with abnormally increased activation during loss events, treatment-resistance was associated with blunted responses to loss and win events in the hippocampus, amygdala and insula but not DRN. If the therapeutic action of antidepressants is to reduce abnormally increased activation to aversive events and increase activation to reward events (Ma, 2014) this implies that the former but not the latter occurs in treatment-resistant illness, at least for the medial temporal lobe and insula. Notably though, there was not a significant correlation between treatment resistance and illness severity measures, implying a dissociation between hippocampal-insula brain activity and illness severity during loss events, which requires further investigation.

In summary, the neuroimaging results were consistent with a range of Deakin and Graeff's predictions for treatment-resistant MDD. Abnormally increased hippocampal activity during loss events was particularly consistent and associated with self-report depression, hopelessness and anxiety. Brainstem serotonergic nuclei abnormalities in MDD merit further study.

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Supplementary material

Supplementary material is available

Figures

Figure 1. Summary of the reward and loss-avoidance instrumental paradigm. Examples of (A) win pair, (B) control pair, (C) loss pair, counterbalanced across subjects.

Figure 2. Loss events. (A) DRN activation in patients, (B) DRN region of interest (Kranz *et al.*, 2012), (C) increased DRN activation in patients compared to controls, (D) increased bilateral hippocampal activity in patients compared to controls. (E) nucleus accumbens deactivation in patients, (F) increased nucleus accumbens deactivation compared to patients. All regions significant at $P < 0.01$ whole brain corrected.

Figure 3. Win events. (A) Activation in controls in response to win events, (B) significantly decreased activation in patients compared to controls during win events. All regions significant at $P < 0.01$ whole brain corrected.

Figure 4 Illness severity, treatment-resistance and consistent abnormalities. (A) Loss events - BHS 'hopelessness' positively correlating with hippocampus, (B) Loss events - BDI depression positively correlating with hippocampal and insula activity, (C) Loss events - HAD-A anxiety positively correlating with hippocampal activity, (D) Loss events - MGH-S treatment resistance negatively correlating with bilateral hippocampus and amygdala, (E) Win events - MGH-S treatment resistance negatively correlating with bilateral hippocampus, (F) Win events - MGH-S treatment resistance negatively correlating with medial frontal cortex and thalamus. All regions significant at $P < 0.01$ whole brain corrected. (G) Hippocampal region with consistently abnormal activity in patients during loss events, (H) nucleus accumbens and inferior medial frontal cortex regions with consistently blunted activity in patients during win events.

Table 1: Clinical and task performance descriptors for the patient and control groups. Variables are shown as mean (standard deviation). *chi-square test with other tests being t-tests.

	TRD	Controls	
Age	50.79 (10.6)	46.14 (13.97)	n.s.
IQ	122.58 (4.78)	116.95 (27.38)	n.s.
Female/Total*	15/19	15/21	n.s.
HAM-D	16.00 (5.72)	0.48 (0.93)	< 0.001
MADRS	22.05 (7.93)	0.48 (1.03)	< 0.001
BDI	32.42 (11.65)	0.43 (0.87)	< 0.001
HAD-A	11.26 (3.93)	1.62 (1.47)	< 0.001
BHS	14.05 (5.36)	1.43 (1.47)	< 0.001
MGH-S	13.24 (10.78)	N/A	N/A
Number of times won	33.58 (4.96)	34.90 (4.35)	n.s.
Number of times lost	29.05 (4.97)	29.10 (3.71)	n.s.
Total task score	4.53 (6.45)	5.81 (5.78)	n.s.

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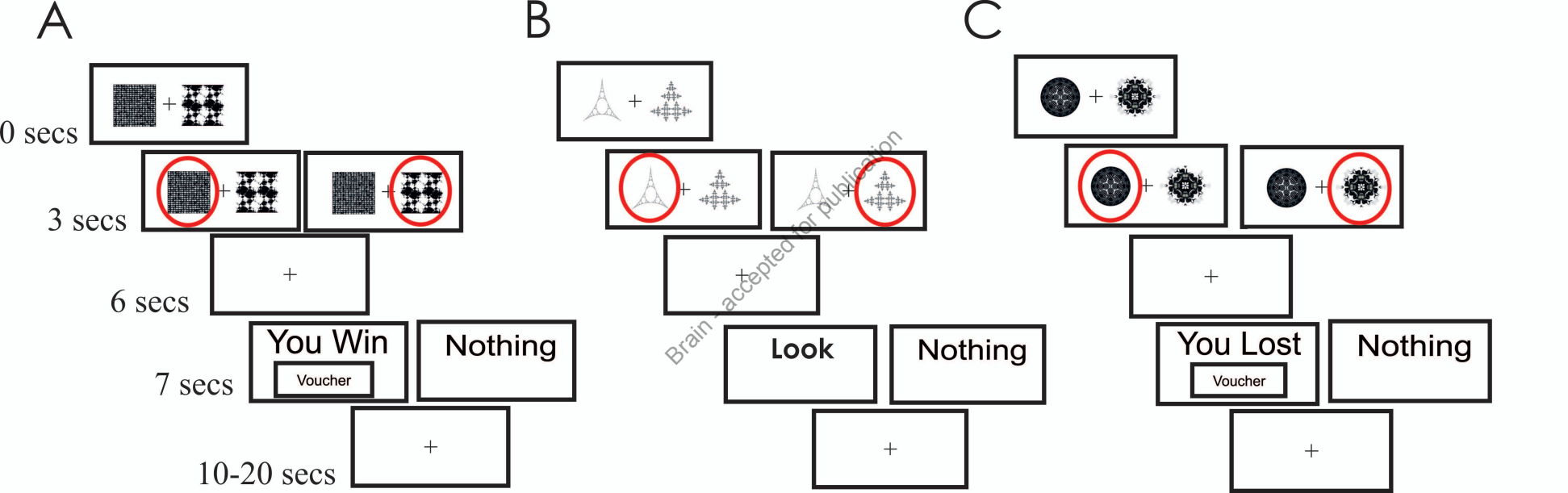

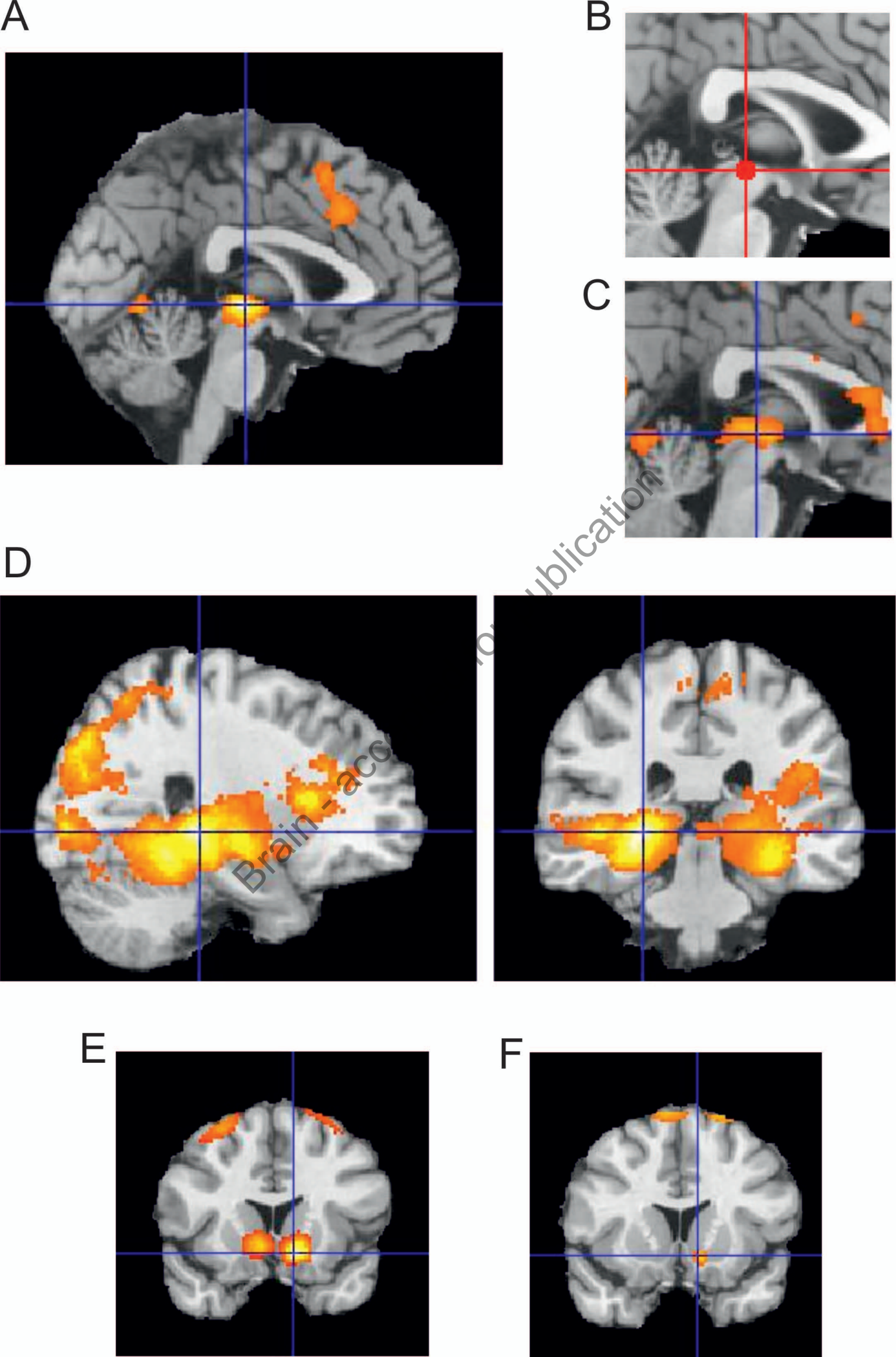
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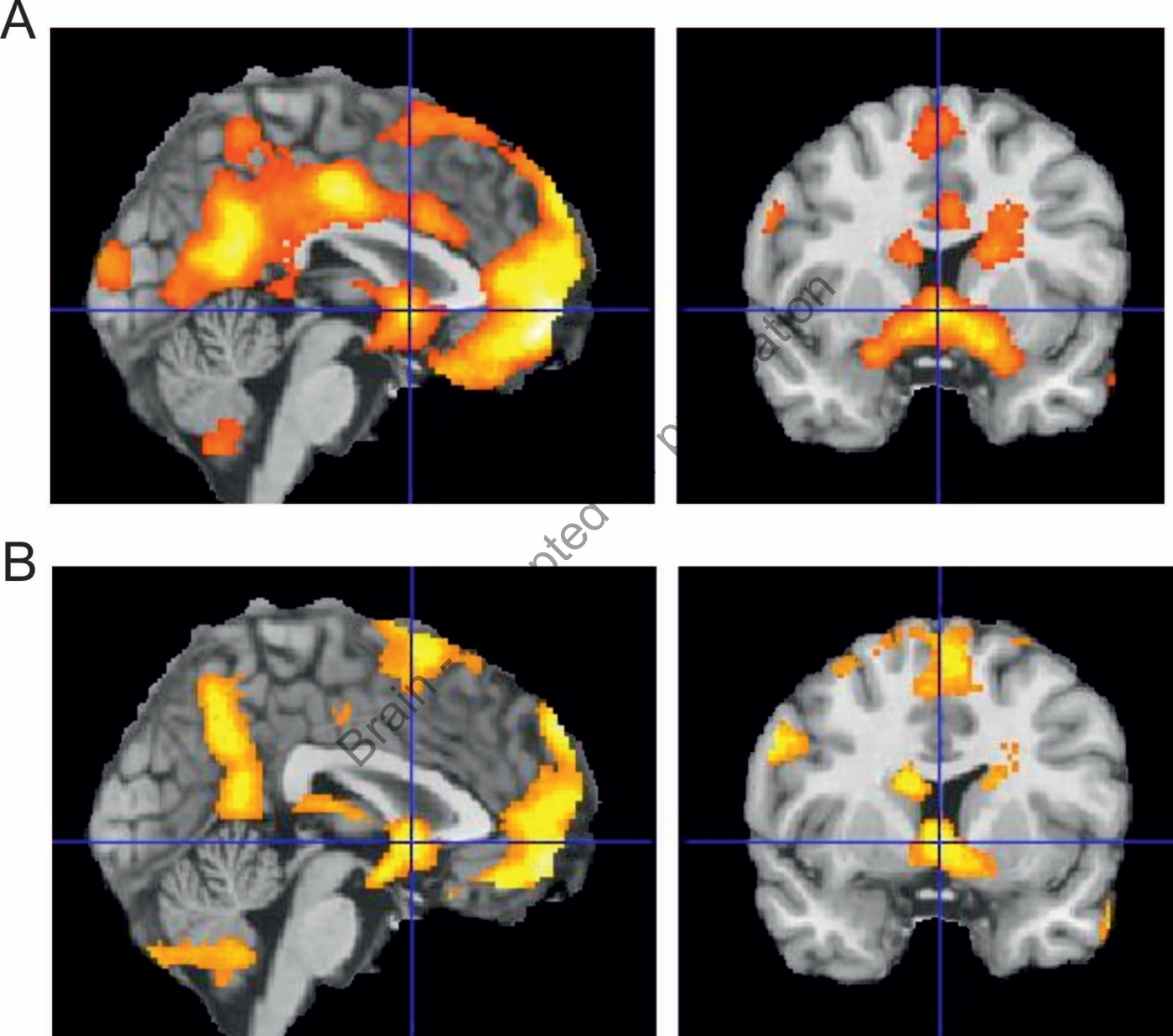
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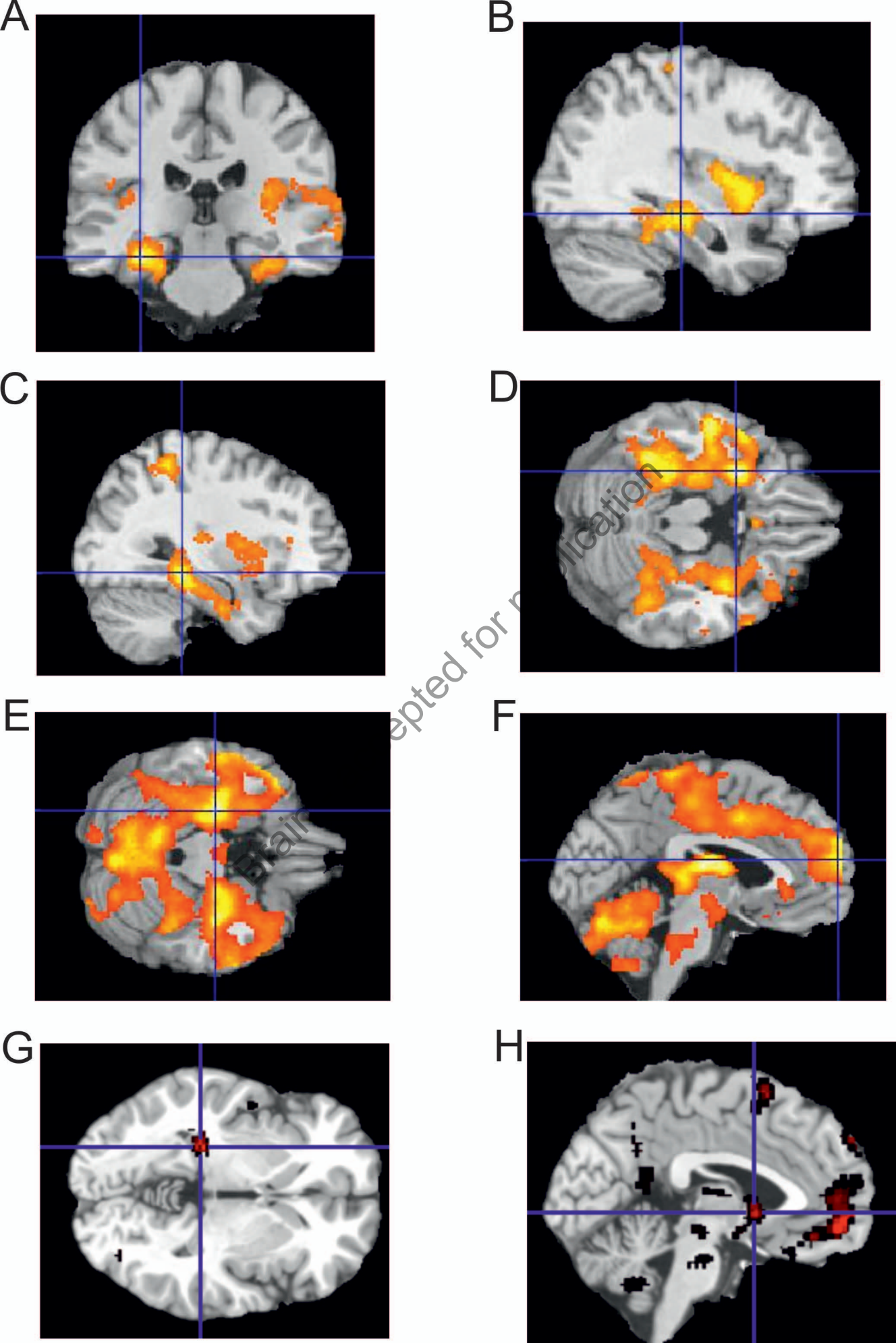
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Supplementary Material

Supplementary Table 1: Loss trials results (see Figure 2).

Within group:

Region	MNI coordinates			T-score	Resampled voxels per cluster
	x	*y*	*z*		
Controls (activations)					
Insula	38	16	−18	3.14	301
	−40	18	−24	2.72	188
Controls (deactivations)					
Cluster containing subgenual anterior cingulate, hippocampus and nucleus accumbens	−10	22	−10	6.09	71189
	12	24	−12	3.24	
	34	−26	−10	4.24	
	−32	−26	−10	3.96	
	−10	6	−10	5.91	
	12	8	−14	4.13	
Patients (activations)					
Insula	38	18	8	2.38	1010
Cluster containing insula and amygdala	−42	18	−2	3.74	6271
	−28	−6	−8	2.26	
DRN/PAG	0	−20	−2	4.79	1431
Patients (deactivations)					
Cluster containing subgenual anterior cingulate and nucleus accumbens	−12	36	−10	4.25	5056
	14	34	−6	3.76	
	10	16	−10	6.44	
	−8	14	−6	4.16	

Between group:

Region	MNI coordinates			T-score	Resampled voxels per cluster
	x	*y*	*z*		
Patients > Controls					
Cluster containing DRN/PAG, hippocampus, amygdala and insula	0	−20	−2	2.30	32651
	−32	−28	−10	3.34	
	−28	−6	−8	2.85	
	46	−2	2	3.37	
	−46	−2	2	3.45	
Controls > Patients					
Nucleus accumbens	10	16	−10	2.75	352

Supplementary Table 2: Win trials results (see Figure 3)

Within group:

Region	MNI coordinates			T-score	Resampled voxels per cluster
	x	*y*	*z*		
Controls (activations)					
Cluster containing nucleus accumbens/inferior caudate, subgenual anterior cingulate and posterior cingulate	−2	8	−2	3.97	41870
	−12	8	−8	3.65	
	12	10	−10	3.77	
	−4	52	−8	5.71	
	−2	−16	40	4.81	
	−6	−50	24	4.98	
Controls (deactivations)					
Lateral cortical regions	50	22	14	2.22	295
	−42	20	28	2.76	667
	−40	−60	48	3.14	1101
Patients (activations)					
Subgenual anterior cingulate	−14	58	0	3.38	178
	10	46	−2	2.46	670
Clusters containing insula, amygdala and hippocampus	50	0	0	3.20	1856
	26	0	−12	2.37	
	42	−16	−10	2.77	
	−48	4	−6	2.83	1239
	−28	2	−16	2.37	
	−30	−14	−10	2.86	
Patients (deactivations)					
Dorsolateral prefrontal cortex	44	16	26	2.88	873
	−58	20	26	4.54	1938

Between group:

Region	MNI coordinates			T-score	Resampled voxels per cluster
	x	*y*	*z*		
Patients > Controls					
Insula	48	−8	−6	3.17	294
	−46	−2	−4	2.24	442
Controls > Patients					
Cluster containing subgenual anterior cingulate and nucleus accumbens	−2	52	4	2.66	10871
	−2	8	−2	3.15	
Posterior cingulate	2	−58	46	2.86	4726
	−4	−50	18	2.91	

Supplementary Table 3: Patients' correlation results (see Figure 4).

Within group:

Region	MNI coordinates			T-score	Resampled voxels per cluster
	x	*y*	*z*		
BHS (positive correlations with loss events)					
Hippocampus	−34	−26	−18	4.82	1463
	26	−28	−24	3.25	830
BDI (positive correlations with loss events)					
Hippocampus	−36	−24	−10	2.85	2124
Insula	−36	4	6	2.66	485
HAD-A (positive correlations with loss events)					
Hippocampus	−34	−34	−12	4.15	4038
Insula	36	6	−8	2.91	3623
MGH-S (negative correlations with loss events)					
Cluster containing hippocampus, amygdala and insula	−28	−16	−26	3.60	29544
	30	−12	−26	2.70	
	−32	6	−20	4.65	
	−50	10	−2	2.90	
MGH-S (negative correlations with win events)					
Cluster containing hippocampus, insula, medial frontal cortex and thalamus	−28	−16	−24	5.81	55233
	18	−16	−20	5.33	
	−56	10	−14	4.86	
	2	61	16	4.52	
	0	−14	14	6.15	