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Increased resting hippocampal and basal ganglia perfusion in people at ultra high risk for psychosis: replication in a second cohort

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ABSTRACT

We recently reported that resting hippocampal, basal ganglia and midbrain perfusion is elevated in people at ultra-high risk (UHR) for psychosis. The present study sought to replicate our previous finding in an independent UHR cohort, and examined the relationship between resting perfusion in these regions, psychosis and depression symptoms, and traumatic experiences in childhood. Pseudo-Continuous Arterial Spin Labelling (p-CASL) imaging was used to measure resting cerebral blood flow (rCBF) in 77 UHR for psychosis individuals and 25 healthy volunteers in a case-control design. UHR participants were recruited from clinical early detection services at three sites in the South of England. Symptoms levels were assessed using the Comprehensive Assessment of At Risk Mental States (CAARMS), the Hamilton Depression Scale (HAM-D), and childhood trauma was assessed retrospectively using the Childhood Trauma Questionnaire (CTQ). Right hippocampal and basal ganglia rCBF was significantly increased in UHR subjects compared to controls, partially replicating our previous finding in an independent cohort. In UHR participants, positive symptoms were positively correlated with rCBF in the right pallidum. CTQ scores were positively correlated with rCBF values in the bilateral hippocampus and negatively associated with rCBF in the left prefrontal cortex. Elevated resting hippocampal and basal ganglia activity appears to be a consistent finding in individuals at high risk for psychosis, consistent with data from preclinical models of the disorder. The association with childhood trauma suggests that its influence on the risk of psychosis may be mediated through an effect on hippocampal function.

Keywords: Schizophrenia, Ultra high-risk, Cerebral blood flow, Childhood trauma

INTRODUCTION

Alterations in hippocampal anatomy and function are among the most robust biological findings in schizophrenia [1, 2], and have also been reported in people at ultra high risk (UHR) of developing psychosis [3-7]. These observations are consistent with preclinical models, which posit a key role for the hippocampus in the development of psychosis. Such models also suggest that resting hippocampal activity is increased prior to illness onset and linked to elevated activity in regions involved in dopamine signalling in the striatum and midbrain [8]. Resting cerebral activity in these regions can be assessed in vivo by measuring resting cerebral blood flow (rCBF), which is closely correlated with the level of local neural function due to neuro-vascular coupling [9, 10], and can be measured using a Magnetic Resonance Imaging technique called pseudo-Continuous Arterial Spin Labelling (p-CASL). In a previous study using this approach, we found that subjects at UHR for psychosis exhibited increased rCBF in the bilateral hippocampus/subiculum, basal ganglia and midbrain, relative to controls [11]. These data, along with independent findings using a different method for measuring cerebral perfusion [5], provided the first evidence that the increased resting activity evident in preclinical models of psychosis [12] was also evident in humans at high risk for psychosis.

However, initial findings in psychosis research have not always been replicated, and recently this has become a particular issue for neuroimaging studies because of concerns about image analysis methods [13, 14]. The present study sought to address this issue by aiming to replicate our previous finding of elevated hippocampal, basal ganglia and midbrain rCBF in UHR individuals. We repeated the study using the same neuroimaging methods in a second, and completely independent sample of UHR subjects and healthy controls. We tested the hypothesis that the UHR group would

again show elevated hippocampal, basal ganglia and midbrain rCBF relative to the controls. We then tested if elevated rCBF in these regions was associated with psychotic symptoms. Because depressive symptoms are also prevalent in about 40% of UHR subjects [15], and major depressive disorder is associated with alterations in hippocampal volume and function [16, 17], we also tested if elevated rCBF in the hippocampus was specific to psychosis, or was also associated with depressive symptoms scores.

We then sought to examine the relationship between rCBF in hippocampal, basal ganglia and midbrain regions and childhood trauma in UHR subjects. Childhood adversity is an important risk factor for psychosis [18] [19-21], and for other psychiatric disorders [22]. Exposure to environmental risk factors for psychosis may be especially influential during developmentally sensitive periods such as childhood [23]. However, the mechanisms through which environmental factors such as trauma in childhood alter brain development and increase risk for psychosis in adulthood remains unclear. One approach that can be used to address this issue is to examine the relationship between neuroimaging findings in adults and a measure of the extent to which they experienced trauma in childhood. A recent Positron Emission Tomography (PET) study employing this approach found that adversity in childhood was linked to elevated striatal dopamine function in adulthood [24]. However, whilst volumetric [25, 26] and functional neuroimaging studies [27] in adults with a history of childhood trauma report alterations in hippocampal and other regions, no studies have examined the relationship between rCBF and childhood trauma in an UHR cohort. Experimental studies in rodents have shown that peri-pubertal stress [28] can lead to alterations in striatal and cortical development and function [29, 30]. Based on these rodent studies and

findings in human subjects, we predict that in UHR subjects, childhood trauma will be associated with increased rCBF in hippocampal, basal ganglia and midbrain regions.

METHODS

Participants and Assessment

The study had National Research Ethics Service (NRES) approval and all participants gave written informed consent to participate. One hundred and two participants (25 healthy controls (CTRL) and 77 participants at UHR of psychosis) participated in the study. UHR subjects were recruited through clinical early detection services at three sites: OASIS (Outreach and Support in South London) [31], part of the South London and Maudsley NHS Trust; the West London Early Intervention Service, part of the West London Mental Health NHS trust; and CAMEO, part of the Cambridge and Peterborough NHS trust. All of the neuroimaging data were acquired at the Centre for Neuroimaging Sciences, King's College London. Diagnosis of the UHR state was made according to PACE criteria, using information acquired from the Comprehensive Assessment of At Risk Mental States (CAARMS [32]). Briefly, this required that participants had one or more of the following: a) attenuated psychotic symptoms (APS) b) brief limited intermittent psychotic symptoms (BLIP: a history of one or more episodes of frank psychotic symptoms that resolved spontaneously within 1 week in the past year) or c) a recent decline in function, together with either the presence of schizotypal personality disorder or a family history of psychosis in a first degree relative. All UHR participants met criteria for APS, 5 additionally met criteria for a BLIP and 2 for a recent decline in function/family history. Social and occupational functioning was measured using the GAF [33]

Eight of the UHR participants were being treated with low doses of antipsychotic medications (Quetiapine n=4, Olanzapine n=2, Risperidone n=2) and 19 with antidepressant medications (Mirtazapine = 3, Citalopram = 2, Sertraline = 9, Fluoxetine = 3, Amitriptyline = 1, Venlafaxine = 1). Healthy controls were recruited from the local community. Control participants with a history of psychiatric disorders or who were receiving prescription medications were excluded. None of the control subjects had a history of neurological illness, or met DSM-IV criteria for drug or alcohol dependence. All participants (in both groups) had an estimated pre-morbid IQ in the normal range (i.e. 80-110), as assessed using the National Adult Reading Scale (NART)[34]. Depression was assessed using the Hamilton Depression Scale (HAM-D) [35]. Hamilton Anxiety (HAM-A) [35] scores were also obtained for use as a covariate in statistical models (see below). Subjects were asked to provide information on tobacco (number of cigarettes per day) and cannabis use (0 = no use, 1 = experimental use, 2= occasional use, 3 = moderate use, 4 = heavy use). Subjects who met DSM-IV criteria for a substance use disorder were excluded. Childhood trauma was assessed using the Childhood Trauma Questionnaire (CTQ) [36]. This widely used instrument provides a retrospective measure of physical, emotional and sexual abuse that occurred before the age of 17 years. CTQ data were available in 38 UHR participants but not in CTRL.

p-CASL protocol and Image preprocessing

Arterial spin labelling allows the quantification of resting cerebral blood flow (rCBF) measures in units of ml/100g of tissue/per second. To optimise the sensitivity to regional tissue perfusion and neural activity, p-CASL images were acquired after a

long (1.5s) post-labelling delay, to ensure that the data reflected perfusion at the level of capillary micro-circulation, which is most closely associated with neural function [9]. p-CASL acquisition parameters and p-CASL image pre-processing procedures are detailed in the Supplementary Information document and elsewhere [11].

Statistical analysis

Analyses of demographic and global rCBF data were performed in SPSS version 22 using appropriate parametric and non-parametric tests. Statistical analyses of regional rCBF data were performed using Statistical Parametric Mapping Version 8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). We tested for significant group effects in rCBF quantities in CTRL and UHR using a region of interest (ROI) approach. ROIs were specified using coordinates from our previous rCBF study in a completely independent sample of UHR and CTRL subject (based on the contrast UHR > CTRLS [11]) (MNI coordinate system). ROIs were specified in the bilateral hippocampus/subiculum region (right ROI x, y, z = 20, -28, -8 and left ROI x, y, z = -22, -28, -8), the bilateral basal ganglia (right pallidum/putamen ROI x, y, z = 22, -12, -4, and the left pallidum/putamen ROI x, y, z = -18, -8, -4), and the left midbrain (ROI x, y, z = -10, -32, -18). Spheres (6mm) were then constructed to form a mask containing all ROIs. Statistical inferences were made at $p < 0.05$ with Family Wise Error (FEW) correction for multiple comparisons at the voxel-level after applying small volume correction (SVC). Regional (ROI) group effects were tested using independent t-tests in SPM-8 including nuisance covariates (see below). Mean global rCBF was extracted from each individual subject to assess global effects and an independent t-test was performed in SPSS. rCBF values (ml/100g/min) x10) were extracted from peak activations for use in the plots shown in figures 1 and 2 (for illustrative purposes and to check for outliers). As antipsychotic (AP) medication is

known to affect rCBF [37], additional analyses were conducted after UHR subjects receiving AP medication (n=8) had been excluded. To ensure group tests were conducted in the same way as our previous study [11] the following covariates were included in statistical models: age, gender, global rCBF, anxiety (HAM-A scores) and cigarettes per day.

To establish the effect of symptoms and childhood trauma scores on regional rCBF values, we used CAARMS positive symptom, HAM-D and CTQ scores (available in 38 UHR subjects) as regressors in separate statistical models. Cigarettes per day and cannabis use were included as covariates of no interest in the regression model as both have been reported to affect rCBF [38, 39]. Statistical inferences were made at $p < 0.05$ with FWE correction for multiple comparisons at the voxel-level after applying SVC. For completeness an exploratory whole brain analysis was also conducted to assess wider effects of symptoms and childhood trauma on rCBF. Significant results are reported at a FWE cluster level ($p < .05$) using a cluster detection threshold of $p < .001$ [14, 40] to reduce likelihood of false positive results.

RESULTS

Demographic, clinical and medication data

These data are summarised in Table 1. CTRL and UHR participants did not differ significantly in terms of age, gender, handedness, premorbid IQ or cigarettes smoked per day. However UHR participants were less educated and used more cannabis, and as would be expected, UHR participants had higher levels of anxiety and depression.

All of the UHR participants met the Attenuated Psychotic Symptoms criteria for inclusion in the study. A minority also met criteria for BLIPS (n=5) or the schizoptypy/familial risk criterion (n=2). The mean CTQ score for UHR participants was 56 meaning that as a group these UHR participants reported moderate to severe levels of childhood trauma [36].

TABLE 1 HERE

Global rCBF

Mean global rCBF (grey and white mater) did not differ significantly between the two groups (35.6 (s.d = 8.1) vs. 36.1 (s.d = 6.61) ml/100g/min respectively) ($t_{(101)} = 0.63$, $p = .94$).

FIGURE 1 HERE

Regions of Interest

Hippocampal/subiculum rCBF: Relative to the CTRL group, UHR participants showed increased rCBF in the right hippocampal ROI (hippocampal body extending to the subiculum/parahippocampal gyrus (x, y, z = 24, -24, -6; Z = 2.99; $K_E = 42$; $p_{FWE} = 0.021$; cohen's d = .62)) (Figure 1A). The group effect in the left hippocampal ROI (x, y, z = -30, -32, -4; Z = 2.14; $K_E = 15$; $p_{FWE} = 0.15$; cohen's d = .40) was non-significant. There were no hippocampal regions in which the UHR group showed reduced rCBF relative to the CTRL group. When the 8 UHR using antipsychotic

medication were removed from the model the result in the right hippocampal ROI remained significant (x, y, z = 24, -24 -6; Z = 3.00; K_E = 38; p_{FWE} = 0.024; cohen's d = .63).

Basal Ganglia rCBF: Relative to the CTRL group, UHR participants showed increased rCBF in the right basal ganglia ROI (in the pallidum/putamen (x, y, z = 22, -8, -2; Z = 2.85; K_E = 16; p_{FWE} = 0.03; cohen's d = .65)) (Figure 1B). The group effect in the left basal ganglia ROI (x, y, z = -22, -12, -6; Z = 1.69; K_E = 4; p_{FWE} = 0.25; cohen's d = .30) was non-significant. There were no basal ganglia regions in which the UHR group showed reduced rCBF relative to the CTRL group. When the 8 UHR using antipsychotic medication were removed from the model the result in the right basal ganglia ROI remained significant (x, y, z = 22, -8 -2; Z = 2.98; K_E = 38; p_{FWE} = 0.021; cohen's d = .68).

Midbrain ROI rCBF: There were no suprathreshold group effects within the midbrain ROI.

FIGURE 2 HERE

rCBF associations with symptoms and childhood trauma

CAARMS Positive symptoms: There was no association between CAARMS positive symptom scores and rCBF in the bilateral hippocampal or midbrain ROIs. There was a significant positive correlation between CAARMS positive scores in the right basal

ganglia ROI (globus pallidus/putamen (x, y, z = 28, -12, -4; Z=3.32: K_E = 29: p_{FWE} = .008) (Figure 2 A and D). Exploratory whole brain analysis was non-significant.

Depressive symptoms (HAM-D): There were no significant associations between HAM-D scores and rCBF in any ROI. Exploratory whole brain analysis was also non-significant.

Childhood Trauma and rCBF: ROI analysis revealed a positive association between CTQ scores and rCBF in right hippocampus/subiculum (right: x, y, z = 24, -30, -12; Z = 3.82; K_E = 59; p_{FWE} = 0.034) and the left parahippocampal gyrus extending to the thalamus (left: x, y, z = -18, -28, -4; Z = 3.00; K_E = 60; p_{FWE} = 0.021) (Figure 2 B and D). The association between CTQ scores and rCBF in basal ganglia and midbrain ROIs was non-significant. Whole brain analysis revealed that CTQ scores were negatively associated with rCBF in a large cluster spanning the left inferior frontal gyrus (x, y, z = -58, 18, 22; Z = 4.42; K_E = 308; p_{FWE} < 0.01) and superior/medial prefrontal cortex (x, y, z = -4, 6, 70; Z = 4.33; K_E = 209 ; p_{FWE} < .001).

FIGURE 3 HERE

DISCUSSION

The first aim of the present study was to replicate our previous finding of elevated hippocampal, basal ganglia and midbrain rCBF [11] in a larger, independent cohort of UHR individuals. We were unable to replicate our previous finding of elevated rCBF in the midbrain. Furthermore, elevated hippocampal and basal ganglia rCBF were not seen bilaterally, but were instead restricted to the right hemisphere. It is unclear why elevated midbrain and left hippocampal/basal ganglia rCBF were not observed in this

second cohort. Both cohorts presented with similar levels of UHR symptoms although the current group of UHR subjects were better matched to their control group in terms of IQ and cigarette smoking. However, elevated rCBF in the right hippocampus/subiculum and basal ganglia does appear to be a robust finding in UHR subjects (effect sizes in these regions were similar to those seen in our previous study i.e. in the small to medium range). This finding remained significant after excluding the minority of UHR participants taking antipsychotic medication, and was not attributable to a difference in global rCBF levels, which were not significantly different between groups. Elevated hippocampal rCBF is also consistent with evidence from studies using other MRI techniques reporting that UHR subjects show increased resting hippocampal perfusion [5, 41], reductions in hippocampal grey matter volume [3] and activation during cognitive tasks [7, 6]. Findings are also in line with data from preclinical models of psychosis that indicate that hippocampal neuronal activity is increased, leading to altered activity in striatal/basal ganglia regions involved in dopamine regulation [8]. Consistent with our previous study however, elevated hippocampal rCBF was not associated with levels of attenuated positive symptoms. Neither, in this second cohort, were hippocampal rCBF levels associated with depressive symptoms. Interestingly, rCBF levels in the right pallidum *were* associated with attenuated positive symptoms. The pallidum is part of the basal ganglia and a network of subcortical regions involved in the regulation of striatal dopamine function [8], which, has been shown to be aberrant in UHR subjects [42, 43].

We also aimed to investigate the relationship between rCBF levels and childhood trauma in UHR subjects. We found that CTQ scores in our UHR subjects were in the moderate to severe range [36], consistent with previous reports of increased levels of childhood trauma in UHR cohorts [24 20 19], and the well-established link between

childhood adversity and psychotic disorders in adulthood [18]. Within our UHR sample, CTQ scores were positively correlated with rCBF levels in the bilateral hippocampus extending to the thalamus and parahippocampal gyrus (left ROI). Whole brain analysis showed that CTQ scores were also negatively associated with rCBF in the left inferior and superior frontal gyrus. Previous neuroimaging studies in UHR subjects have reported alterations in rCBF [11 5], activation [6, 7, 44] and volume [3, 4] in hippocampal and prefrontal regions. However, surprisingly few studies have examined the relationship between neuroimaging measures in UHR subjects and a history of childhood trauma. The only previous study of this kind in subjects at UHR for psychosis reported that childhood adversity was linked to increased striatal dopamine synthesis capacity in adulthood, although this effect was evident across both UHR subjects and controls [24]. In patients with psychosis, one study described an association between childhood trauma and reduced prefrontal volume [45], but another failed to find an association between childhood trauma and hippocampal volume [46]. However the sample sizes in the studies to date have been relatively small; investigations involving larger samples are needed, particularly given the heterogeneity of the UHR category [47].

A recent meta analysis of volumetric imaging studies across psychiatric diagnoses found a robust relationship between a history of childhood trauma and reduced hippocampal and dorsolateral prefrontal volumes in adulthood [25]. It is possible that alterations in volume and function in hippocampal and prefrontal regions, due to childhood trauma, underlie vulnerability to a range of psychiatric disorders. Indeed, within UHR cohorts there are high levels of comorbidity, particularly with depression [15]. A previous perfusion study reported altered prefrontal and hippocampal rCBF in

patients with depression [53]. However, in the present study, we did not observe an association between rCBF levels and depressive symptoms.

Interestingly, the results of the present study show that elevated hippocampal rCBF, whilst associated with childhood trauma, was not directly related to levels of attenuated psychotic symptoms. It seems reasonable to speculate that elevated hippocampal rCBF in UHR subjects may be associated with a general psychiatric vulnerability. Accordingly, it is well established that the majority of UHR subjects do not go on to develop a psychotic disorder [48] and a significant proportion have additional clinical needs [49].

Mechanistically, interactions between the prefrontal cortex, hippocampus (and amygdala) are thought to be critical for normal emotional and stress regulation [50], and these regions have well-established roles in cognitive and mnemonic processing, which are known to be impaired across a range of psychiatric diagnoses. Hippocampal and prefrontal regions seem to be particularly susceptible to effects of environmental stressors, particularly in early life [25]. Adverse environmental experiences can lead to stress sensitisation and increased stress responsivity, which is thought to reflect disruption of hippocampal-prefrontal interactions [51].

Limitations

Although our sample was a good size, UHR and CTRL participants were not matched for education levels, cannabis use or anxiety levels. Whilst, this is not uncommon in case control studies comparing psychosis or psychosis risk populations to healthy controls we accounted for these group differences by including these factors in our analyses. Because CTQ data were not available from our healthy control participants, we could not assess whether the relationship between childhood trauma and

hippocampal rCBF that we identified is specific to UHR subjects. The relationship between childhood trauma and rCBF in healthy populations has not been examined before, but a recent meta-analysis found that childhood adversity was associated with reduced hippocampal volume in non-clinical and general population samples [26]. Further, CTQ scores were not available for all of the subjects in the UHR sample, and this may have limited our power to detect significant associations between childhood trauma and rCBF in other brain regions. Some participants were unwilling to complete a questionnaire on this sensitive topic, while others were unable to provide accurate or complete information, thus reducing the number of participants in which CTQ data were available. It is also worth noting that a recent study reported that young adults that retrospectively recalled having been being maltreated (i.e. using the CTQ) had a particularly elevated risk for psychopathology. However, when prospective informant-reports from caregivers and clinicians are used instead, the relationship between childhood trauma and later psychiatric problems appears to be less robust [54]. Nevertheless, the number of subjects in whom these data were available was comparable to that in previous studies of this type [24, 25]. Although most of our UHR subjects were medication-naïve, a minority (8 of 77) had been treated with low doses of antipsychotic drugs which could have altered both the severity of psychotic symptoms and rCBF [37]. However, the main findings remained significant after exclusion of these subjects. UHR subjects typically go on to have diverse clinical outcomes, with some developing psychotic or other Axis-I disorders, others having persistent attenuated symptoms, and some improving such that they no longer meet the inclusion criteria for the UHR state [52]. The UHR sample we studied remains to be followed up, at which point it will be possible to examine the relationship between baseline rCBF and these different outcomes.

Conclusions

Elevated resting activity in the right hippocampus and pallidum appears to be a consistent finding in people at UHR for psychosis. Increased rCBF in the hippocampus may be related to the severity of traumatic experiences in childhood.

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	UHR mean (n=77)	UHR sd	CTRL Mean (n=25)	CTRL sd	Statistics	*p*
Age (yrs)	22.6	3.64	23.9	2.85	t = 1.77	.09
NART IQ (estimated)	102.15	14.89	102.83	13.33	t = .20	.84
Years of Education	14.59	2.22	15.84	3.56	t =2 .13	.04
Cigarettes per day	6.28	8.18	3.72	5.50	t = -1.46	.14
Cannabis use (Median) [a]	2	--	1	--	Z = -1.91	.05
GAF	59.8	9.23	92.68	5.02	t = 15.24	<.001
Symptoms	58.61	11.70	92.40	5.11	t = 14.98	<.001
Disability	61.66	12.43	92.60	4.97	t = 14.93	<.001
CAARMS Total	42.17	21.96	--	--		
CAARMS Pos	10.08	4.32	--	--		
CAARMS Neg	4.97	4.11	--	--		
HAM-A	18.34	9.54	3.04	3.83	t = -7.79	<.001
HAM-D	16.88	10.35	1.33	2.93	t = - 6.73	<.001
CTQ [b]	56.00	8.10	--	--		
	N	**%**	**N**	**%**	**Statistics**	***p***
Past or Present MDD/Anxiety Disorder	24	31				
Antipsychotic Medication	8	10.3%	--	--		
Antidepressant Medication	19	24.6%				
Gender (Male)	44	57	13	52	0.66	0.72

Handedness (Right)	63	81	23	92	5.09	0.08

Table 1: Participant characteristics for UHR and CTRL groups. sd = standard deviation, NART = National Adult Reading Test, GAF = Global Assessment of Function, CAARMS = Comprehensive Assessment of At Risk Mental State. HAM-A = Hamilton Anxiety scale, HAM-D = Hamilton Depression Scale, MDD + Major Depressive disorder. [a] = data missing in 5 cases, [b] = data available in 38 UHR.

FIGURE LEGEND

FIGURE 1 A) *Coronal sections through the medial temporal lobe showing elevated rCBF in UHR relative to CTRL subjects (pFWE = .021) and scatter plot showing rCBF levels in each case.* **B)** *Coronal sections through basal ganglia regions showing elevated rCBF in UHR relative to CTRL subjects (pFWE =.03) and scatter plot showing rCBF levels in each case. rCBF levels are quantified in (ml/100g/sec) x 10.*

FIGURE 2 A) *Coronal sections and scatter plot, basal ganglia regions where rCBF is significantly correlated with CAARMS positive symptom scores (pFWE = .008).* ***B)*** *Coronal section and scatter plot, medial temporal lobe regions where rCBF is positively correlated with CTQ scores (pFWE = .024 (left) and .031 (right)).*

FIGURE 3. *Render and scatter plot, left prefrontal regions where rCBF is negatively correlated with CTQ scores (whole brain analysis) (pFWE <.001)*

SUPPLEMENTARY MATERIAL

TITLE: Increased resting hippocampal and basal ganglia perfusion in people at ultra high risk for psychosis: replication in a second cohort

RUNNING TITLE: Increased perfusion in adults at risk of psychosis

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METHODS

Neuroimaging protocol

Subjects were scanned with their eyes open using a General Electric Signa HDX 3.0T scanner, fitted with a receive only 8-channel phased array head coil at the Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience. For image registration both a high resolution T2-weighted Fast Spin Echo (FSE) image (0.468x0.468x4mm, TE=54.58ms, TR=4380ms, Flip angle 90deg, FoV=240) and a high-resolution T1-weighted Spoiled Gradient Recalled (SPGR) image (1.1x1.1x1.1mm, TE=2.848, TR=7.144ms, Flip angle=20deg, FoV=280) were acquired.

Resting Cerebral Blood Flow (rCBF) was measured using Continuous Arterial Spin Labelling (CASL) scans acquired with a 3D Fast Spin Echo (FSE) spiral multi-shot readout, following a post-labelling delay of 1.5s. This delay has been appropriate for investigations in participants of a similar age range as the ones included in this study.

The spiral acquisition used a short (4ms) TE, and 8 spiral-arms (interleaves) with 512 points in each arm. (FSE TE 32ms/TR = 5500ms; ETL = 64). Images were reconstructed to a 256^2 matrix, giving a final spatial resolution of 1x1 mm in plane. 60 slices of 3mm thickness were obtained. Three pairs of tagged-untagged images were collected. Background suppression included selective saturation of the image slab at 4.3s before acquisition, selective inversion 3s before acquisition and non-selective inversions at 1.5s, 764ms, 334ms and 84ms before imaging. This repeated inversion achieved successful suppression of the background static tissue signal, maximizing the sensitivity to blood perfusion.

Calibration images were collected with the same imaging sequence but with inversion recovery preparation instead of CASL. One sequence with saturation of 4.3s and then an inversion at 1650 ms before imaging was used to create a fluid suppressed image. A second sequence with saturation at 4.3s and then inversion at both 2408ms and 511ms was also acquired to create a fluid and white matter suppressed image. For both these sequences, the receiver gain was automatically lowered by 21 dB relative to the ASL sequence to avoid receiver saturation. These images were used to quantify blood flow in physiological units (ml blood/100gm tissue/min).

The sensitivity of the image to water was calibrated at each voxel [1-3]. When multi-channel coils are employed, the spatially non-uniform sensitivity complicates this calibration. Often the underlying tissue signal is used as an indicator of water sensitivity, but a water density in each voxel, or partition coefficient, must then be assumed. We observed that the signal intensity in the inversion-prepared fluid-suppressed image was relatively constant for different tissues. This is likely because

more complete recovery occurs for shorter T1 tissues, which tend to have lower water density. Using a neighborhood maximum algorithm to avoid regions with partial volume of suppressed fluid, a low-resolution sensitivity map was created. This map was calibrated for water sensitivity by assuming the tissue was white matter with a water concentration of 0.735 gm/ml [4] and a T1 of 900ms, and using the equations for inversion recovery signal attenuation. By assuming gray matter with a water concentration of 0.88 gm/ml and a T1 of 1150 there was only a 5% calibration difference. This calibration produced a sensitivity map, C, equal to the fully relaxed MRI signal intensity produced by 1gm of water per ml of brain tissue.

With this co-registered sensitivity map C, we calculated cerebral blood flow (CBF) using the equation:

Where ρ_b is 1.05g/ml (the density of brain tissue;[4], α is the labeling efficiency (assumed to be 95% for labeling times 75% for background suppression; [5], w is 1.5s (the post-labeling delay;[2], tl is 500ms (the labeling duration), $T1_a$ is 1.4 ms, ω_a 0.85 g/ml (the density of water in blood; [4], S_l and S_c are the signal intensities in the labeled and control images, respectively).

$$CBF = \frac{\rho_b\left(S_c - S_l\right)}{2\alpha C\,\omega_a T1_a \exp\left(-\frac{w}{T1_a}\right)\left(1-\exp\left(-\frac{tl}{T1_a}\right)\right)}$$

The whole ASL pulse sequence, including the acquisition of calibration images, was performed in 6:08min.

Image preprocessing

p-CASL images were processed using FMRIB Software Library (FSL) software applications (http://www.fmrib.ox.a.c.uk/fsl) [6]. For each participant, one Spoiled Gradient Recalled (SPGR) scan was used in the preprocessing steps in addition to the T2 images acquired at the time of both CASL images (baseline and follow-up), which ensured that the normalization parameters applied to each scan were identical for each individual. A multi-step approach was performed as follows:

(i) Extra-cerebral signal from the T2 scan was removed using the "Brain Extraction Tool" (BET) of FSL [7]. The skull stripped T2 volume and its corresponding binary mask were then coregistered to the rCBF map.

(ii) The coregistered binary mask was multiplied by the rCBF map to remove extra-cerebral signal from this scan. The skull stripped T2 and rCBF maps were then coregistered back to the space of the original T2 scan (returned to their original frame of reference).

(iii) The T2 scan was subsequently coregistered to each subjects structural (SPGR) scan, with the coregistration parameters applied to the corresponding rCBF maps and brain extracted T2 scans.

(iv) The SPGR was normalized to MNI space using a non-linear approach using FNIRT [8] (FMRIB Non-linear Image Registration Tool) and the transformation matrix was applied to the rCBF map and the T2 scans.

(v) All data were then smoothed using a 6 mm Gaussian Smoothing kernel.

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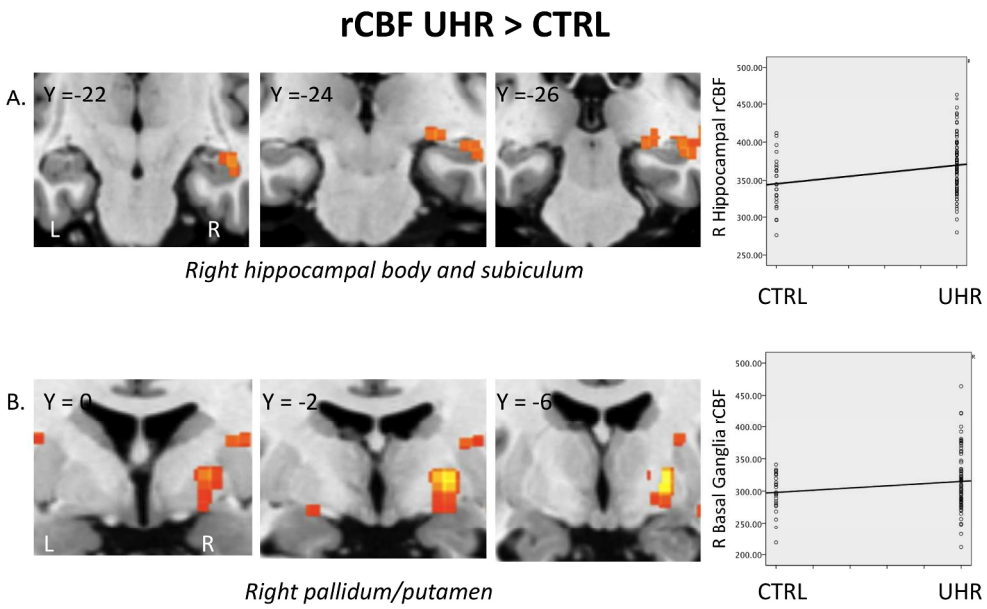

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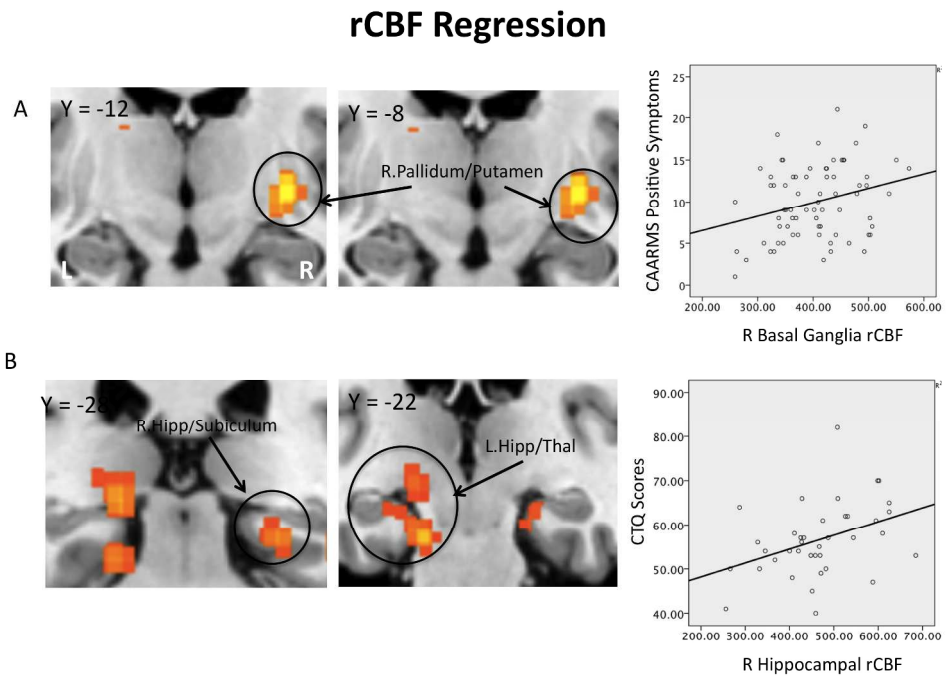

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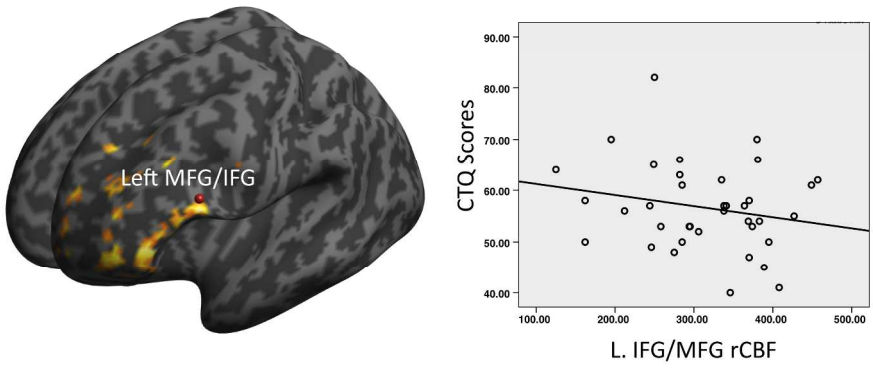

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