

Attenuation of amygdala and frontal cortical responses to low blood glucose concentration in asymptomatic hypoglycemia in Type 1 diabetes: a new player in hypoglycemia unawareness?

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Abstract

Objective: Loss of ability to recognize hypoglycemia (hypoglycemia unawareness, HU) increases risk of severe hypoglycemia in insulin-treated diabetes 3-fold. We set out to investigate the cerebral correlates of 'unawareness' in Type 1 patients.

Research Design and Methods: Regional changes in brain glucose kinetics were measured using [¹⁸F]-fluorodeoxyglucose positron emission tomography (FDG-PET) in 13 men with Type 1 diabetes, 6 with hypoglycemia awareness (HA) and 7 with HU, at euglycemia (5mmol/l) and hypoglycemia (2.6mmol/l), in random order.

Results: Epinephrine responses to hypoglycemia were reduced in HU ($p < 0.0003$), as were symptoms. Statistical parametric mapping of FDG uptake using SPM2 at a statistical threshold of $p < 0.005$ showed increased FDG uptake in L amygdala in HA, but not in HU (Region of Interest analysis -0.40 ± 1.03 vs 3.66 ± 0.42 , $p = 0.007$) and robust increase in bilateral ventral striatum during hypoglycemia (RoI HU vs HA 3.52 ± 1.02 vs 6.1 ± 0.53 , $p = 0.054$). Further analysis at the statistical threshold of $p < 0.01$ showed bilateral attenuated activation of brain stem regions and less deactivation in lateral orbitofrontal cortex (OFC) in HU.

Conclusions: Ventral striatal, amygdala, brain stem and orbitofrontal responses to hypoglycemia indicate engagement of appetitive motivational networks, associated with integrated behavioral responses to hypoglycemia. Reduced responses in these networks in HU, particularly failure of amygdala and OFC responses, suggest habituation of higher behavioral responses to hypoglycemia as a basis for unawareness. New approaches may be needed to restore awareness effectively in practice.

Key words: Type 1 diabetes, hypoglycemia unawareness, cerebral metabolic rate, ventral striatum, amygdala.

Abbreviations: CMR_{glc} - cerebral metabolic rate for glucose; FDG [¹⁸F] - fluorodeoxyglucose; PET positron emission tomography

INTRODUCTION

Loss of awareness of hypoglycemia increases the risk of severe hypoglycemia during insulin therapy for diabetes three-fold.[1,2] Hypoglycemia unawareness (HU) is associated with impairments of the normal counterregulatory responses to hypoglycemia, in addition to loss of endogenous control of insulin secretion and the associated loss of the glucagon response to hypoglycaemia.[3] In HU, the catecholamine, growth hormone and cortisol responses to hypoglycemia are delayed and diminished,[4] as is the response of the sympathetic nervous system.[5] Counterregulatory failure and hypoglycaemia unawareness are induced and maintained by prior exposure to episodes of low blood glucose.[6-8] It is currently thought that the glucose sensing mechanisms, including in brain areas such as the hypothalamus, become adapted to hypoglycemia and respond less readily to later episodes. There is evidence that brain glucose transport may increase in response to hypoglycemia exposure.[9,10] Neuroimaging studies in man have failed to confirm this.[11] Indeed, using [11-C]methyl glucose PET, we found that a rise brain glucose uptake associated with subjective awareness of hypoglycemia failed to occur in the hypoglycemia unaware and suggested that unawareness involved failure of normal cortical responses to a falling blood glucose concentration.[12]

There are reports of restoration of hypoglycemia awareness by treatment strategies targetting avoidance of plasma glucose concentrations less than 3 mmol/l.[13-15] Despite the success of such strategies in studies, maintaining hypoglycemia avoidance and awareness long term is very difficult. Why this should be is not clear but the concept that adaptation of higher cerebral responses may lead to subjective 'unawareness' is worthy of further exploration. We hypothesised that the hypoglycemia unaware person is not unaware of an individual episode of hypoglycemia just because of a failure in primary glucose sensing, but because of failure to generate higher cerebral responses (and by extension, behavioral responses) to the hypoglycemic stimulus. Such mechanisms are described. For example, stress desensitisation is a simple form of learning

where exposure to a severe stressor results in attenuated responses to subsequent exposure to the same form of stressor.[16] Work on this form of learning has implicated mechanisms of regulation of the hypothalamic-pituitary-adrenal axis by amygdala and other basal ganglia networks.[17] If these processes occur in hypoglycemia unawareness, this may offer an alternative explanation for the phenomenon of unawareness itself, and for the relative resistance of unawareness to therapeutic intervention.

Functional neuroimaging using ¹⁸F-fluorodeoxyglucose (FDG) offers sensitive measures of regional brain metabolism, a proxy measure of neuronal activation, particularly suited to examining differences between conditions which have experimentally long time courses, such as hypoglycemia versus euglycemia. Glucose uptake and metabolism reflect relative activity in brain regional networks, and regional differences in FDG uptake are used as a marker for regional brain activation.[18] We previously conducted a study using FDG-PET during euglycemia and hypoglycemia in Type 1 diabetic men, with and without hypoglycemia unawareness.[19] The development of more sophisticated analytical techniques for FDG PET data has allowed us to re-examine those data, to address the questions of regional differences in cortical brain activation during hypoglycemia as a possible contributor to the syndrome of hypoglycemia unawareness.

METHODS

The subjects and study design have been reported previously.[19] In brief, 13 men with Type 1 diabetes with an unequivocal clinical history of either good subjective awareness of occasional hypoglycemia (HA) or severe hypoglycemia unawareness (HU) were recruited. HU was defined by one or more severe hypoglycemic episodes in the preceding year, self-reported asymptomatic hypoglycemic episodes and no less than 3 blood glucose measurements of <3 mmol/l without symptoms in a 2 week period of standardised home blood glucose monitoring. Aware subjects had to be free of all these features.[14,20] Subjects were studied on 2 occasions, not less than 3 weeks apart, in random order after an overnight fast, during which blood glucose

was controlled with intravenous insulin. In the morning, the left radial artery and the contralateral antecubital vein were cannulated. A primed continuous intravenous infusion of regular insulin (Human Actrapid, NovoNordisk, UK) was started, with a maintenance rate of 1.5 mU/kg body weight/min, and 20% glucose was infused to maintain arterial plasma glucose at 5mmol/l for 60min, during which time the subject was made comfortable in the scanner. Thereafter, arterial plasma glucose was either reduced to 2.6mmol/l over 40min by adjustment of the glucose infusion and then held there for 60min, or maintained at 5mmol/l throughout.[21] At 30min, 10min prior to achieving hypoglycemia in the hypoglycemia studies, a 10min transmission scan was taken. At 40min ~185MBq FDG was administered intravenously over 30sec and scanning then continued over the next 60 minutes. During scanning, arterial radioactivity was measured continuously in blood drawn past a scintillation detector, (Allogg, Stockholm) by a peristaltic pump (IVAC 572), with hand drawn samples taken every 5 min for immediate glucose analysis. Additional samples were taken for catecholamine measurement. Subjects were observed for sweating and asked whether they had been hypoglycaemic after each scan. On completion of the scan, plasma glucose concentration was restored where necessary and the subject was given a subcutaneous dose of regular insulin and a meal. The protocol was approved by the Ethical Committees of King's College and of St Thomas' Hospitals and all subjects gave written informed consent prior to study.

PET scans were performed on a CTI ECAT 951R PET camera (CTI/Siemens, Knoxville, TN), with an axial field of view of 10.8 cm and an intrinsic inplane spatial resolution of 6.5 mm (full width at half-maximum [FWHM]). All images were reconstructed by filtered back-projection and smoothed with a Hanning filter, so that the spatial resolution was 8.5 mm (FWHM) transaxially and axially. Reconstructed images were displayed in a matrix of 128x128x31 voxel format, each voxel measuring 2.0x2.0x3.43 mm. FDG uptake images were formed by summation of images acquired at 20-60 min postinjection.

Data analysis:

Quantification of Whole and Regional Brain Uptake:

The methods of quantifying glucose metabolism using FDG rely on the model developed by Sokolof et al, and use a 'lumped constant' (LC) to relate rates of deoxyglucose phosphorylation to rates of glucose metabolism.[22] As LC is not constant with changing arterial glucose levels,[23] absolute rates of glucose utilization in the brain cannot be calculated in hypoglycemia, during which FDG uptake will be expected to rise, in approximate inverse proportion to the change in blood glucose, because of decreased competition from native glucose with tracer. It is however feasible to assess any regional changes in brain glucose kinetics from FDG uptake as changes relative to the whole brain (or 'global') uptake. Thus, regional differences in FDG uptake across condition (euglycemia vs hypoglycemia) and/or group (aware vs unaware) were sought using an updated statistical parametric mapping (SPM2, www.fil.ion.ucl.ac.uk). PET images were co-registered to T1-weighted MRI images. The high resolution structural MRI images were spatially normalised into standard space (voxel size 2.0x2.0x2.0 mm) and these transformations applied to the low resolution PET images which were smoothed by a Gaussian kernel of 6mm. The transformed PET image intensities were normalised to remove effect of global differences and common regions compared statistically to identify regions where hypoglycemia and/or awareness status have significant effect.

The previously presented work[19] used an earlier version of the SPM software (SPM96). The new analysis is an improvement on the previous work mainly due to significant advances in the methods used in later versions of SPM. The present work uses the same models and study designs but a later version (SPM2) which uses much more robust registration, coregistration[24] and spatial normalisation techniques.[25] A summary of the improvements over SPM96 in later versions is available on the SPM website (www.fil.ion.ac.uk). Using these improved methods we expect a more rigorous identification of regional changes in FDG uptake.

Regions identified by SPM were defined anatomically using the Talairach atlas [26] and the database supplied with the SPM MASCO toolbox (<http://homepages.uni-tuebingen.de/matthias.reimold/mascoi/page2/page2.html>). Transforming coordinates in MNI space to Talairach space was done using the `mni2tal` function (<http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach>).

Further analysis on regional responses was made by extracting the tracer uptake values for the regions identified by SPM. The values for these regions are scaled to a whole brain value of 50, as is conventionally used with SPM. Regions of interest values were analysed using SPSS 14 (www.spss.com) and the main effects of and interactions between condition and group were compared using repeated measures ANOVA.

RESULTS

Glucose, Epinephrine & Whole Brain: Plasma glucose was held at 5mmol/l or reduced to 2.6mmol/l with a coefficient of variation of 0.03mmol/l and no significant difference between the 2 groups. As previously published, the brisk epinephrine response in the hypoglycemia aware was virtually absent in the unaware (peak values 0.77 ± 0.39 vs 7.52 ± 2.9 nmol/l, $p < 0.003$), and global FDG uptake, (which rose in both groups at hypoglycemia, as expected) was 20% lower in the unaware at either glucose level (euglycemia 2.018 ± 0.174 vs 2.592 ± 0.188 ; hypoglycemia 4.562 ± 0.312 vs 5.6190 ± 0.338 , dimensionless units, $p = 0.027$ for the effect of group (HA vs HU) in the repeated measures ANOVA comparing all four data sets (euglycemia and hypoglycemia in aware and unaware).

Regional effect of hypoglycemia: Statistical parametric mapping showed a significant effect of condition across both subject groups with an increase in FDG uptake with hypoglycemia in bilateral ventral striatum and occipital cortex (Fig 1A) at a statistical threshold of $p < 0.001$ and considering regions including >100 contiguous voxels. At thresholds of $p < 0.001$, there was a significant decrease of uptake, compared to global, in a large contiguous region (Figs 1B and 1C) including periventricular white matter and brain stem, as well as the bilateral OFC (Brodmann areas 45 & 47). Applying more stringent thresholds e.g.

$p < 0.0001$ allows the large contiguous region to be viewed as smaller separate clusters of left and right white matter and a cluster around the brainstem, including hypothalamus, mammillary bodies and parahippocampal gyrus (Brodmann areas 28, 34, 36).

Regional effect of awareness status (group) on responses to hypoglycemia (condition): At a less stringent statistical threshold ($p < 0.005$), a significant effect of hypoglycemia which differed according to awareness status, was identified, with increased FDG uptake in the aware compared to the unaware in the left amygdala, left ventral striate, occipital cortex and cerebellum (Fig 2A). Right amygdala was also revealed at an even less stringent threshold of $p < 0.05$. At $p < 0.005$, brainstem and parahippocampal gyrus revealed a fall in FDG uptake which was significantly less in the aware relative to the unaware (Fig 2A). At $p < 0.005$ there was also significant fall in FDG uptake in hypoglycemia in the aware subjects in the right orbitolateral frontal cortex (OFC, Brodmann areas 10 & 11) and right prefrontal cortex (PFC, Brodmann areas 45 & 46). FDG uptake remained unchanged or rose in the unaware (Fig 2B). Still less stringent thresholds of $p < 0.01$ revealed these responses bilaterally. Post-hoc analysis of the response in the white matter clusters did not reveal significant differences in response between the two subject groups ($p > 0.1$).

Region of Interest Analysis: As shown in Figure 3, the region of interest analysis confirmed that the hypoglycaemia evoked response from ventral striatum, amygdala and brainstem were reduced in the unaware, while the lateral orbitofrontal cortex response was greater. P values for the interaction of group and condition by repeated measures ANOVA were < 0.0001 and 0.0002 for amygdala and brain stem respectively and 0.0003 for orbito-frontal cortex.

The tables give the further analyses of the data in brain regions identified by SPM. Table 1 shows quantitative FDG uptake in the named regions corrected for global brain uptake in the aware and unaware groups individually and Table 2 shows the cluster sizes identified by the SPM with their anatomical location.

DISCUSSION

We have previously presented evidence that cerebral neuronal activation increases during symptomatic hypoglycemia and that this activation is reduced in hypoglycemia unawareness.[19] In our original analysis of the present data, we described a predictable approximate doubling in global brain FDG uptake during hypoglycemia in aware and unaware subjects, due to the effect of competition between FDG and glucose. The absolute measures were 20% less in the unaware than the aware subjects in each condition. Although in that analysis, we failed to detect a difference in the magnitude of the change with hypoglycemia between the aware and unaware, the study was small and may not have had the power to detect a difference in the face of the large effect of hypoglycemia *per se*. We did find a difference in FDG uptake in a brain region including the brainstem and hypothalamus, which showed reduced regional FDG uptake with hypoglycemia in the unaware.

In the present analysis comparing regional differences in FDG uptake with hypoglycemia between aware and unaware subjects with a more sophisticated analytic approach, we have found first that FDG uptake increases in all subjects during hypoglycemia in bilateral ventral striatum and occipital cortex. The ventral striatum is a key component of appetitive motivational networks, involved in the generation of food seeking behaviors, and strongly implicated in reward and reinforcement learning [27]. Simply put, increased activation of the ventral striatum by hypoglycemia indicates engagement of appetitive motivational networks subserving food seeking behaviours which will have survival benefit in motivating the person to seek food. As the analysis has corrected for global brain differences in FDG uptake, it may be that this effect is reduced in the unaware. However, it is clear that ventral striatum activation does occur in unaware subjects as well as aware.

The identification of a strong evoked response to hypoglycemia in the visual region of occipital cortex is unexpected, and may be related to increased visual activity under stress.

Brain areas that do show clear differences between the aware and unaware include the amygdala and also a network of brain

regions including the occipital cortex, cerebellum and brain stem, again extending into the area identified in the previous analysis around the hypothalamus. In these brain regions, the aware show significantly greater FDG uptake than the unaware. Activation of the amygdala is associated with fear and anxiety[28,29] and its activation in the aware subjects under conditions of hypoglycemia is compatible with the associated symptoms of distress and discomfort. Equally, the failure to activate the amygdala in hypoglycemia unawareness is compatible with absent activation of stress pathways and no perception of such stress. The failure of the amygdala response is associated with the lesser FDG uptake in the unaware at hypoglycemia in the hypothalamus; itself compatible with a failure of activation of the hypothalamic-pituitary adrenal axis and the absent or diminished counterregulatory responses to hypoglycemia in this group. The association between impaired stress hormone responses to hypoglycaemia in those with hypoglycaemia unawareness is well documented in the literature [e.g. 4, 14,15] and confirmed in our present study by the diminished epinephrine responses to the hypoglycemic challenge in the unaware. The failure of the epinephrine response is unlikely to be causing the neuroimaging changes as a different study design in healthy volunteers in which epinephrine levels were very different was not associated with changes in brain glucose metabolism.[11] The present data are compatible with a primary role for the amygdala in controlling the stress response to the hypoglycemic challenge. This also fits reported clinical experience of attempts to reverse hypoglycemia unawareness by manipulating the diabetes therapy with intent to avoid hypoglycemia. Failure to avoid hypoglycaemia risk has been described in an analysis of patient behaviors in the setting of problematic hypoglycaemia.[30] Our data are the first to present neuroimaging data that offers a potential explanation for this in terms of differential (reduced) activation of stress/distress pathways in the unaware.

In contrast to the above brain regions, the FDG uptake data suggest that the lateral orbitofrontal cortex is deactivated in the aware during hypoglycemia, and shows no change or even an increase in the unaware. These brain regions are activated in the perception of positively hedonic events

while deactivation is associated with negative unpleasant hedonic sensations.[31] Activity in this region has been shown to correlate with subjective pleasantness ratings of food stimuli.[32] The relatively activated status of this region in 'unaware' subjects suggests little modulation of hunger, desire for food, or subjective awareness of the dangerous state of hypoglycaemia.

Patients' reluctance to change potentially dangerous strategies to avoid high blood glucose has been ascribed to a taught fear of hyperglycemia-related complications. If this were the sole explanation, it should be relatively easy to re-educate patients in hypoglycemia avoidance, especially since fear of hypoglycemia itself is commonly expressed by people using insulin and their families.[33] The pattern of brain activation reported here offers a novel alternative explanation. The pattern of decreased activation in stress pathways (amygdala and hypothalamus) and intact activation of brain regions associated with motivation (ventral striatum) and reward perception (lateral orbitofrontal cortex) seen in the unaware subjects suggests that the experience of hypoglycaemia may be not only subjectively neutral, but subjectively *rewarding* to the person.[34] Similar patterns of brain activation are seen in response to substances with addictive potential which have highly rewarding subjective effects.[34,35]. This is a well-established concept in the animal literature [e.g.36,37] Such parallels between reward responses and hypoglycemia unawareness may predict clinical problems associated with reversing hypoglycemia unawareness by long-term hypoglycemia avoidance.

The observation of significantly decreased tracer uptake in large sections of white matter with hypoglycemia (in both groups) cannot be explained as related to neuronal activation, and deserves comment. Possible explanations could include a regional variation in the relationship between deoxyglucose phosphorylation and glucose metabolism. Sokoloff et al calculated a global brain value of the 'lumped constant' (LC) to directly relate the two.[22] However, regional calculations of LC have not found evidence for such a variation.[38] Damage to CNS and particularly white matter has been described following hypoglycemia and it has been found that mitochondrial activity in oligodendrocytes is reduced with falling

glucose levels.[39] Suda et al have documented a difference in local cerebral glucose utilization in white matter (corpus callosum) at hypoglycemia below $p < 0.01$ threshold in rat although this did not reach significance after multiple comparison corrections.[23] Significant increases of CMRglc in white matter have been found with rising glucose levels using methods other than FDG-PET.[40,41] This is compatible with a speculative model where white matter oligodendroglia are less able than gray matter neuronal-glia complexes to maintain CMRglc with falling blood sugar levels, leading to decreased measured metabolism. It should be noted however, that this phenomenon was not different between the two groups of patients included in this study and so does not account for the differences identified in this study.

Our study was focussed on the different regional brain activation responses to hypoglycaemia between hypoglycaemia aware and unaware Type 1 diabetic subjects. We cannot comment on the effects of diabetes *per se* on these responses, nor which of the awareness states more closely approximates to normal, although we assume that the asymptomatic state is the more pathological, with the deficits in the expected subjective and hormonal changes. The neuro-imaging literature suggests that global brain glucose uptake is not much affected by diabetes, [eg. 11,42,43] with only one study suggesting decreased brain glucose uptake in diabetic subjects with peripheral neuropathy [44] and another showing reduced glucose metabolic rate in Type 1 subjects with a lesser fall in hypoglycaemia than in the non-diabetic controls.[45]

The present analysis suggests that unawareness of hypoglycemia is associated with alterations in the cortical responses to the stimulus, as well as reduced endocrine responses. There is some maintenance of regional cerebral activation in response to hypoglycemia in brain areas relevant to motivation (ventral striatum), but at a lower level than in the aware and unequivocally reduced activation in regions involved in the generation of anxiety and stress responses (amygdala). In particular, brain regional networks subserving hedonic responses, de-activated in the aware, are relatively unaffected in the unaware, suggesting the experience of hypoglycaemia is not only not distressing, but may actually be weakly rewarding. Taken with clinical observations,

these observations suggest that habituation to recurrent hypoglycaemia involves differential involvement of distinct cortical mechanisms involved in learning, conditioning rather than, or in addition to, a primary alteration in hypothalamic glucose sensing. Based on these observations, it is reasonable to propose that these novel findings in hypoglycemia unawareness will require further investigation to demonstrate reversibility. If they are reversible, they may be exploited to devise novel treatments, both psychological as well as pharmacological, to reverse unawareness.

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References:

1. Gold AE, MacLead KM, Frier BM: Frequency of severe hypoglycemia in patients with type 1 diabetes mellitus with impaired awareness of hypoglycemia. *Diabetes Care* 17:697–703, 1994
2. Clarke W, Cox D, Gonder-Frederick L, Julian D, Schlundt d, Polonsky W. Reduced awareness of hypoglycaemia in IDDM adults: a prospective study of hypoglycaemia frequency and associated symptoms. *Diabetes*: 18: 517-522. 1995
3. Bolli G, DeFeo P, Campagnucci P et al., Abnormal glucose counterregulation in insulin-dependent diabetes mellitus. Interaction of anti-insulin antibodies and impaired glucagon secretion. *Diabetes*: 32; 134-141. 1983
4. Amiel SA, Sherwin RS, Simonson DC, Tamborlane WV. Effect of intensive insulin therapy on glycemic thresholds for counterregulatory hormone release. *Diabetes* 37:901-907. 1988
5. Ryder RJ, Owens DR, Hayes TM, Chatei MA, Bloom SR: Unawareness of hypoglycaemia and inadequate hypoglycaemic counter-regulation: no causal relationship with diabetic autonomic neuropathy. *BMJ* 301:783–787, 1990
6. Heller SR, Cryer PE. Reduced neuroendocrine and symptomatic responses to subsequent hypoglycaemia in non-diabetic humans. *Diabetes*; 40:223-226.1991
7. George E, Marques JL, Harris ND, Macdonald IA, Hardisty CA, Heller SR, Preservation of physiological responses to hypoglycemia 2 days after antecedent hypoglycemia in patients with IDDM, *Diabetes Care*, 20, 8, 1293-1298. 1997
8. Dagogo Jack S, Craft S, Cryer PE. Hypoglycemia associated autonomic failure in insulin dependent diabetes mellitus: Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia *J Clin Invest* 91; 819-828. 1993
9. Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C. Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci*; 91: 9352-9356. 1994
10. Boyle PJ, Kempers SF, O'Connor AM, Nagy RJ. Brain glucose uptake and unawareness of hypoglycemia in patients with insulin dependent diabetes mellitus. *New Engl J Med* 1995; 333; 1726-1731
11. Segel SA, Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Powers WJ, Cryer PE: Blood-to-brain glucose transport, cerebral glucose metabolism, and cerebral blood flow are not increased after hypoglycemia. *Diabetes* 50:1911–1917, 2001
12. Bingham EM, Dunn J, Smith D, Sutcliffe-Goulden J, Reed LJ, Marsden PK, Amiel SA. Differential changes in brain glucose metabolism in hypoglycaemia accompany loss of hypoglycaemia awareness in men with Type 1 diabetes mellitus. An [11C]-3-O-methyl-D-glucose PET study. *Diabetologia*;48:2080-2089. 2005
13. Cranston I, Lomas J, Maran A, Macdonald IA, Amiel SA Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes. *Lancet* 344: 283-87. 1994
14. Cryer Dagogo-Jack S, Rattarasarn C, Cryer PE. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes* 43: 1426–34. 1994
15. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, Lepore M, Annibale B, Ciofetta M, Bottini P, Porcellati F, Scionti L, Santeusano F, Brunetti P, Bolli GB. Meticulous prevention of hypoglycemia normalizes glycemic thresholds and magnitude of most of the neuroendocrine responses to, and symptoms of, and cognitive function during hypoglycemia in intensively-treated patients with short-term IDDM. *Diabetes* 1993; 42: 1683-1689.
16. Armario A, Valles A, Dal-Zotto S, Marquez C, Belda X. A single exposure to severe stressors causes long-term desensitisation of the physiological response to the homotypic stressor. *Stress*. 2004 Sep;7(3):157-72.
17. Armario A, Marti O, Valles A, Dal-Zotto S, Ons S. Long-term effects of a single exposure to immobilization on the hypothalamic-pituitary-adrenal axis: neurobiologic mechanisms. *Ann N Y Acad Sci*. 2004 Jun;1018:162-72.

18. Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE: Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)-2-fluoro-2-deoxy-D-glucose: validation of method. *Ann Neurol* 6:371–388, 1979
19. Cranston IC, Reed LJ, Marsden PK, Amiel SA. Changes in regional brain ¹⁸F-fluorodeoxyglucose uptake at hypoglycemia in Type 1 diabetic men associated with hypoglycemia unawareness and counterregulatory failure. *Diabetes*. 50: 2329-36. 2001
20. Maran A, Lomas J, Macdonald IA, Amiel SA: Lack of preservation of higher brain function during hypoglycaemia in patients with intensively treated insulin dependent diabetes mellitus. *Diabetologia* 38:1412–1418, 1995
21. Amiel SA, Simonson DC, Tamborlane WV, DeFronzo RA, Sherwin RS. Rate of glucose fall does not affect counterregulatory hormone responses to hypoglycaemia in normal and diabetic humans. *Diabetes* 36:518-522. 1987
22. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M. The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem*. 28:897-916. 1977
23. Suda S, Shinohara M, Miyaoka M, Lucigiani G, Kennedy C, Sokoloff L: The lumped constant of the deoxyglucose method in hypoglycaemia: effects of moderate hypoglycaemia on local cerebral glucose utilisation in the rat. *J Cereb Blood Flow Metab* 10:499–509, 1990
24. Ashburner J, Neelin P, Collins DL, Evans AC, Friston KJ Incorporating Prior Knowledge into Image Registration *NeuroImage* 6:344-352. 1997
25. Ashburner J, Friston KJ. Nonlinear Spatial Normalization using Basis Functions. *Human Brain Mapping*. 7:254-266. 1999
26. Talairach and Tournoux. Co-planar Stereotaxic Atlas of The Human Brain. 1988 Georg Thieme Verlag, Stuttgart. 1988
27. Kelley AE. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neuroscience and Biobehavioral Reviews* 2004; 27:765-776. 2004
28. Davis M, Whalen PJ The amygdala: vigilance and emotion. *Mol Psychiatry* 6: 13-34. 2001
29. Rauch SL, Shin LM, Wright CI Neuroimaging studies of amygdala function in anxiety disorders. *Ann NY Acad Sci* 985: 389-410. 2003
30. Cox D, Gonder-Frederick L, Ritterband L, Clarke W, Kovatchev B, Mazze R, Monk A, Weinger K, Zreibec J, Lee J. Factors that identify T1DM drivers at risk for future hypoglycaemia-related driving mishaps. *Diabetes* 2006; 55 suppl 1: (abstract 617).
31. Kringelbach ML. The human orbitofrontal cortex: linking reward to hedonic experience. *Nat Rev Neurosci*. 6:691-702. 2005
32. Kringelbach ML, O'Doherty J, Rolls ET, Andrews C. Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. *Cereb Cortex*. 2003 Oct;13(10):1064-71.
33. Gonder-Frederick LA, Fisher CD, Ritterband LM, Cox DJ, Hou L, DasGupta AA, Clarke WL. Predictors of fear of hypoglycemia in adolescents with type 1 diabetes and their parents. *Pediatr Diabetes*.7:215-22. 2006
34. Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry*. 2005;162:1403-13.
35. Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, Goodman JM, Kantor HL, Gastfriend DR, Riorden JP, Mathew RT, Rosen BR, Hyman SE (1997) Acute effects of cocaine on human brain activity and emotion. *Neuron* 19: 591-611.
36. Margules DL, Olds SJ. Identical "feeding" and "rewarding" systems in the lateral hypothalamus of rats. *Science*.;135:374-5 1962
37. Avena NM, Long KA, Hoebel BG, Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. *Physiol Behav* 84:359-62. 2005
38. Gjedde A, Wienhard K, Heiss WD, Kloster G, Diemer NH, Herholz K, Pawlik G. Comparative regional analysis of 2-fluorodeoxyglucose and methylglucose uptake in brain of four stroke patients. With special reference to the regional estimation of the lumped constant. *J Cereb Blood Flow Metab*. 1985;5:163-78.

39. Yan H, Rivkees SA. Hypoglycemia influences oligodendrocyte development and myelin formation. *Neuroreport*. 2006;17:55-9
40. Hasselbalch SG, Knudsen GM, Capaldo B, Postiglione A, Paulson OB. Blood-brain barrier transport and brain metabolism of glucose during acute hyperglycemia in humans. *J Clin Endocrinol Metab*. 2001;86:1986-90.
41. Blomqvist G, Grill V, Ingvar M, Widen L, Stone-Elander S. The effect of hyperglycaemia on regional cerebral glucose oxidation in humans studied with [1-11C]-D-glucose. *Acta Physiol Scand*. 1998;163:403-15.
42. Fanelli CG, Dence CS, Markham J, Videen TO, Paramore DS, Cryer PE, Powers WJ. Blood-to-brain glucose transport and cerebral glucose metabolism are not reduced in poorly controlled type 1 diabetes. *Diabetes*;47:1444-50. 1998
43. Brooks DJ, Gibbs JS, Sharp P, Herold S, Turton DR, Luthra SK, Kohner EM, Bloom SR, Jones T. Regional cerebral glucose transport in insulin-dependent diabetic patients studied using [11C]3-O-methyl-D-glucose and positron emission tomography. *J Cereb Blood Flow Metab*.;6(2):240-4) 1986
44. Ziegler D, Langen KJ, Herzog H, Kuwert T, Muhlen H, Feinendegen LE, Gries FA. Cerebral glucose metabolism in type 1 diabetic patients. *Diabet Med*;11:205-9. 1994
45. Gutniak M, Blomqvist G, Widen L, Stone-Elander S, Hamberger B, Grill V. D-[U-11C]glucose uptake and metabolism in the brain of insulin-dependent diabetic subjects. *Am J Physiol*.;258(5 Pt 1):E805-12. 1990

Table Legends

Table 1: Responses from clusters identified by SPM. Values are the quantitative measurement of tracer uptake and expressed relative to whole brain uptake, as percentages. The data are given as mean \pm SD for group at different blood glucose levels (5mmol/l and 2.6mmol/l) and mean change between hypoglycemic and euglycemic condition. * indicates significant difference of the effect of condition (hypoglycaemia) between the two groups (aware and unaware), $p < 0.05$ and ** indicates $p < 0.01$.

Table 2: Clusters of voxels reaching significance, $p < 0.001$ (uncorrected) and minimum cluster size (k) of 100 voxels (unless otherwise stated). T-value is peak t-statistic within cluster, p-value is the corrected p value for the cluster (not the voxel). Coordinates of the peak voxel are given in Talairach space. * threshold of $p < 0.0001$, $k > 100$; ** $p < 0.01$, $k > 100$.

Table 1 FDG uptake expressed relative to a global mean of 50 (arbitrary units), extent of increase or decrease,

	AWARE		UNAWARE		Aware	Unaware
	5	2.6	5	2.6	Hypo-Eu	Hypo-Eu
Ventral Striatum L **	85.22 1.57	93.61 1.15	85.63 4.52	90.28 4.20	8.39 1.57	4.65 2.18
Ventral striatum R	84.90 3.37	92.98 3.05	86.16 4.99	91.90 4.63	8.09 1.03	5.73 3.39
Occipital cortex **	96.28 6.61	107.37 9.17	93.76 4.46	98.21 3.77	11.09 3.78	4.45 2.66
Cerebellum L	63.36 3.95	57.92 9.89	63.92 4.43	60.59 5.67	-5.44 10.53	-3.33 8.29
Cerebellum R **	71.10 3.83	77.77 5.30	71.03 4.55	67.63 5.20	6.67 4.70	-3.39 3.37
Brainstem **	45.20 4.28	45.75 3.60	49.27 3.03	42.78 3.79	0.55 2.34	-6.48 2.38
Orbitofrontal cortex R **	82.38 8.60	74.25 10.99	80.84 4.41	82.47 3.30	-8.13 3.56	1.64 3.17
Amygdala L **	60.55 2.21	63.11 2.48	61.26 3.33	57.41 3.18	2.56 1.28	-3.85 2.35
White Matter L	45.80 4.66	39.01 3.87	46.54 1.82	38.79 1.49	-6.79 1.33	-7.75 2.12
White Matter R	42.75 3.16	36.09 3.58	44.20 2.45	36.10 2.70	-6.65 1.30	-8.10 1.89
Hypothalamus, Parahippocampal gyrus *	34.90 1.15	28.91 2.13	37.62 2.25	29.09 1.52	-5.93 2.09	-8.53 1.39

Table 2 Characteristics of clusters identified by SPM at statistical threshold of $p < 0.001$

	Cluster size	T (peak value in cluster)	P (corrected cluster level)	x	y	z
Increased uptake (across both groups)						
Ventral Striatum (left)	749	16.65	8.3e-7	-22	2	7
Ventral Striatum (right)	686	9.89	2.1e-6	18	12	3
Occipital Cortex	2008	8.94	1.5e-13	6	-81	17
Decreased uptake (across both groups)						
White Matter (WM)	20570	17.75	0	20	28	13
Left Inferior Frontal Gyrus	313	9.45	0.0013	-55	25	1
Right Inferior Frontal Gyrus	356	8.46	0.00057	59	17	-1
Right Anterior WM *	845	17.75	1.0e-14	20	28	13
Right periventricular WM *	2646	16.30	0	24	-31	38
Brainstem *	2427	16.27	0	4	-7	-16
Left periventricular WM *	4864	14.75	0	-16	-35	29
Left Inferior Frontal Gyrus *	119	9.45	0.00041	-55	25	1
Increase in Aware greater than Unaware						
Occipital Cortex	365	6.60	0.0004	-2	-93	3
Left Amygdala **	305	6.65	NS	-30	1	-15
Occipital Cortex **	1851	6.60	6.5e-5	-2	-93	3
Left Ventral Striatum **	196	5.54	NS	-24	0	7
Left Cerebellum **	191	5.24	NS	-46	-46	-21
Medial Cerebellum/ Brainstem **	2861	5.24	6.8e-7	6	-42	-18
Decrease in Aware greater than Unaware						
Brodmann Area 10, 11, 47 (R) **	1071	5.48	0.0041	36	32	-13
Brodmann Area 10, 45, 46 (R) **	414	4.37	NS	51	30	17
Brodmann Area 8, 9 (R) **	214	4.22	NS	24	23	41

Figure Legends

Figure 1. Cerebral responses to hypoglycemia. Voxels showing significant responses to hypoglycemia overlaid onto slices of averaged T1 MRI scan (152 subjects). A: Regions with significant increase of FDG uptake with hypoglycemia, relative to global uptake, across both groups. Significance threshold $p < 0.001$ (uncorrected), cluster size (k) > 100 . The regions shown are bilateral ventral striatum and occipital cortex. B: Regions with significant relative decrease of FDG uptake with hypoglycemia, across both groups ($p < 0.0001$, $k > 100$). Regions show periventricular white matter and a contiguous region including hypothalamus and parahippocampal gyrus.

Figure 2. Cerebral correlates of 'unawareness': A. Regions where aware group show relatively greater uptake with hypoglycemia than unaware (displayed on MRI slices, with $p < 0.01$, $k > 100$), showing amygdala, cerebellum and brainstem regions. B. Regions where aware group show relative lower uptake than unaware with hypoglycemia ($p < 0.01$, $k > 100$), showing right lateral orbitofrontal cortex

Figure 3. Box plots showing evoked responses with hypoglycemia (arbitrary units, relative to global mean of 50) by group in selected SPM defined clusters. The responses of the aware group are shown in open boxes and the unaware in shaded boxes. Outlying values are shown as open circles. Top: Responses in three regions are shown: left ventral striatum, right ventral striatum and amygdala, p values for condition*group interaction 0.005; 0.132 and < 0.0001 respectively. Bottom: As top with regional responses from clusters comprising right orbitofrontal cortex, brainstem and parahippocampal gyrus, p values for condition*group interaction 0.0003, 0.0002 and 0.02 respectively.

Figure 1

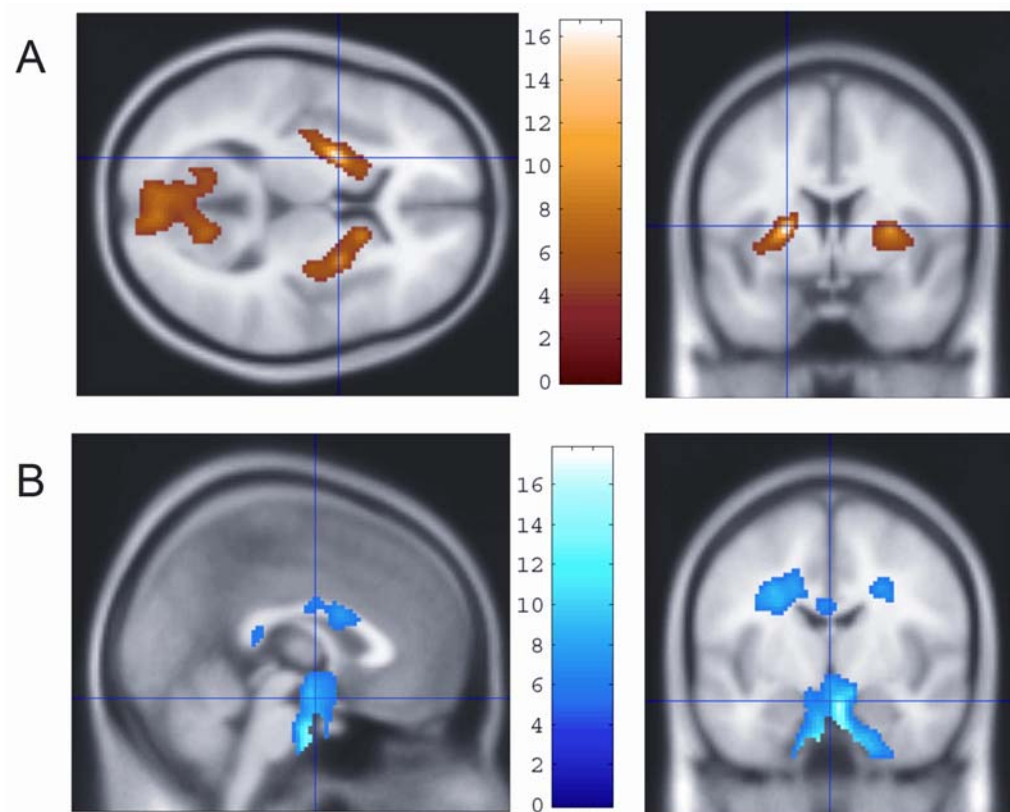


Figure 2

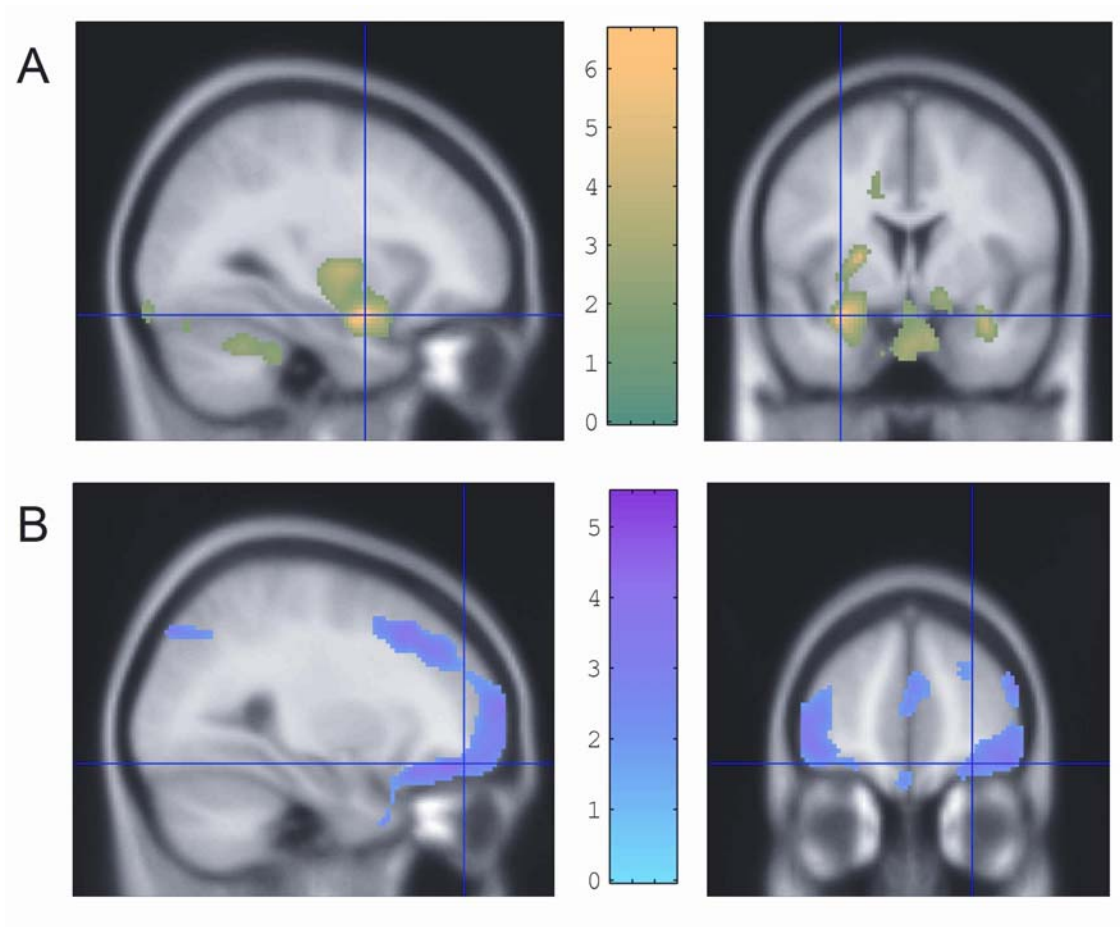


Figure 3

