Attenuation of Amydgala 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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OBJECTIVE—Loss of ability to recognize hypoglycemia (hypoglycemia unawareness) increases risk of severe hypoglycemia threefold in insulin-treated diabetes. 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 [^{18}F]-fluorodeoxyglucose (FDG) positron emission tomography (PET), in 13 men with type 1 diabetes— 6 with hypoglycemia awareness and 7 with hypoglycemia unawareness—at euglycemia (5 mmol/l) and hypoglycemia (2.6 mmol/l), in random order.

RESULTS—Epinephrine responses to hypoglycemia were reduced in hypoglycemia unawareness ($P < 0.0003$), as were symptoms. Statistical parametric mapping (SPM) of FDG uptake using SPM2 at a statistical threshold of $P < 0.005$ showed increased FDG uptake in left amygdala in hypoglycemia awareness, but not in hypoglycemia unawareness (region of interest analysis -0.40 ± 1.03 vs. 3.66 ± 0.42, respectively; $P = 0.007$), and robust increase in bilateral ventral striatum during hypoglycemia (region of interest analysis hypoglycemia unawareness 3.52 ± 1.02 vs. awareness 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 in hypoglycemia unawareness.

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 hypoglycemia unawareness, particularly failure of amygdala and orbifrontal cortex 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. *Diabetes* **56:2766–2773, 2007**

Loss of awareness of hypoglycemia increases risk of severe hypoglycemia during insulin therapy for diabetes threefold (1,2). Hypoglycemia unawareness 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 hypoglycemia (3). In hypoglycemia unawareness, 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 hypoglycemia 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 those 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 humans have failed to confirm this (11). Indeed, [11-C]methyl glucose positron emission tomography (PET) found that a rise in brain glucose uptake associated with subjective awareness of hypoglycemia failed to occur in those with hypoglycemia unawareness and suggested that unawareness involved failure of normal cortical responses to falling blood glucose concentration (12).

There are reports of restoration of hypoglycemia awareness by treatment strategies targeting avoidance of plasma glucose concentrations <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 hypothesized that the individual with hypoglycemia unawareness 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 desensitization 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 ^{18}F-fluorodeoxyglucose (FDG) offers sensitive measures of regional brain metabolism, a proxy measure of neuronal activation, particularly suited to examining differences between conditions that 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 reexamine 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.

RESEARCH DESIGN AND METHODS

The subjects and study design have previously been reported (19). In brief, 13 men with type 1 diabetes with an unequivocal clinical history of either good subjective awareness of occasional hypoglycemia or severe hypoglycemia unawareness were recruited. Hypoglycemia unawareness was defined by one or more severe hypoglycemic episodes in the preceding year, self-reported asymptomatic hypoglycemic episodes, and no less than three blood glucose measurements of <3 mmol/l without symptoms in a 2-week period of standardized home blood glucose monitoring. Aware subjects had to be free of all these features (14,20). Subjects were studied on two 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; Novo Nordisk, Crawley, U.K.) was started, with a maintenance rate of 1.5 mU · kg body wt^{-1} · min^{-1}, and 20% glucose was infused to maintain arterial plasma glucose at 5 mmol/l for 60 min, during which time the subject was made comfortable in the scanner. Thereafter, arterial plasma glucose was either reduced to 2.6 mmol/l over 40 min by adjustment of the glucose infusion and then held there for 60 min or was maintained at 5 mmol/l throughout (21). At 30 min, 10 min before achieving hypoglycemia in the hypoglycemia studies, a 10-min transmission scan was taken. At 40 min, ~185 MBq FDG was administered intravenously over 30 s, and then scanning continued over the next 60 min. During scanning, arterial radioactivity was measured continuously in blood drawn past a scintillation detector (Allogg, Stockholm, Sweden) 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 hypoglycemic after each scan. On completion of the scan, plasma glucose concentration was restored where necessary and the subject given a subcutaneous dose of regular insulin and a meal. The protocol was approved by the ethical committees of King's College and St. Thomas' Hospitals, and all subjects gave written informed consent before the 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). All images were reconstructed by filtered back projection and smoothed with a Hanning filter so that the spatial resolution was 8.5 mm (full width at half maximum) transaxially and axially. Reconstructed images were displayed in a matrix of 128 × 128 × 31 voxel format, each voxel measuring 2.0 × 2.0 × 3.43 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. Methods of quantifying glucose metabolism using FDG rely on the model developed by Sokoloff et al. (22) and use a "lumped constant" to relate rates of deoxyglucose phosphorylation to rates of glucose metabolism. As a lumped constant 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 versus hypoglycemia) and/or group (aware versus unaware) were sought using updated statistical parametric mapping (SPM) software (SPM2 [www.fil.ion.ucl.ac.uk]). PET images were coregistered to T1-weighted magnetic resonance imaging (MRI) images. The high-resolution structural MRI images were spatially normalized into standard space (voxel size 2.0 × 2.0 × 2.0 mm), and these transformations were applied to the low-resolution PET images, which were smoothed by a Gaussian kernel of 6 mm. The transformed PET image intensities were normalized to remove any 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 normalization techniques (25). A summary of the improvements over SPM96 in later versions is available on the SPM Web site (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 analyzed 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, and whole brain. Plasma glucose was held at 5 mmol/l or reduced to 2.6 mmol/l, with a coefficient of variation of 0.03 mmol/l and no significant difference between the two groups. As previously published, the brisk epinephrine response in aware subjects was virtually absent in unaware subjects (peak values 0.77 ± 0.39 vs. 7.52 ± 2.9 nmol/l, respectively; $P < 0.003$), 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 [hypoglycemia awareness vs. unawareness] in the repeated-measures ANOVA comparing all four datasets [euglycemia and hypoglycemia in hypoglycemia awareness and unawareness]).

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 with that found globally, in a large contiguous region (Fig. 1B and C) including periventricular white matter and brainstem, as well as the bilateral orbitofrontal cortex (Brodmann areas 45 and 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, mammilary bodies, and parahippocampal gyrus (Brodmann areas 28, 34, and 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 that differed according to awareness status
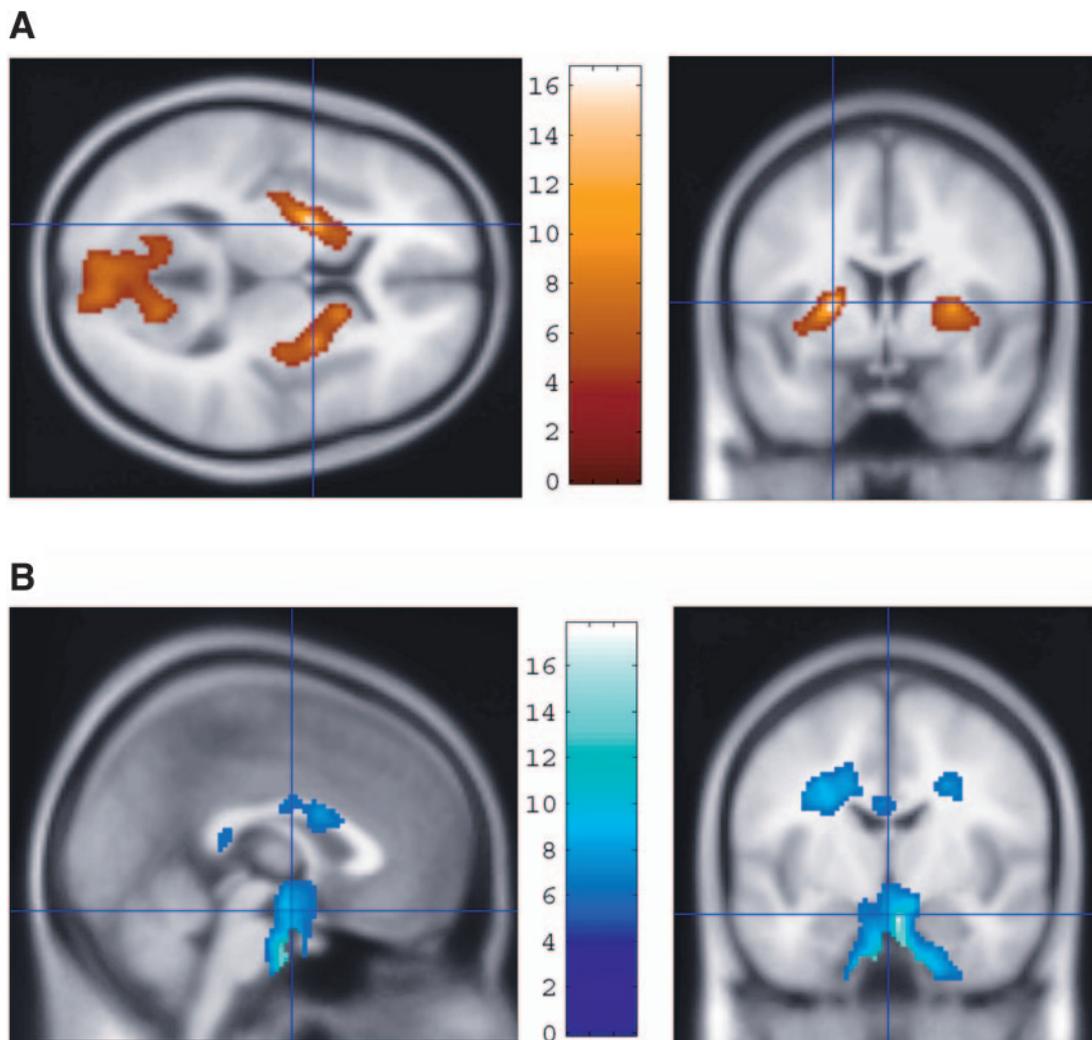
FIG. 1. Cerebral responses to hypoglycemia. Voxels showing significant responses to hypoglycemia overlaid onto slices of averaged T1 MRI scan (152 subjects). *A*: Regions with significant increase of FDG uptake with hypoglycemia, relative to global uptake, across both groups. Significance threshold $P < 0.001$ (uncorrected), cluster size >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 ($P < 0.0001$, cluster size 100). Regions show periventricular white matter and a contiguous region including hypothalamus and parahippocampal gyrus. (Please see http://dx.doi.org/10.2337/db07-0666 for a high-quality digital representation of this figure.)

was identified, with increased FDG uptake in the aware compared with the unaware in the left amygdala, left ventral striate, occipital cortex, and cerebellum (Fig. 2*A*). 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 that was significantly less in the aware relative to the unaware (Fig. 2*A*). At $P < 0.005$, there was also a significant fall in FDG uptake in hypoglycemia in the aware subjects in the right orbitolateral frontal cortex (Brodmann areas 10 and 11) and right prefrontal cortex (Brodmann areas 45 and 46). FDG uptake remained unchanged or rose in the unaware (Fig. 2*B*). 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 Fig. 3, the region of interest analysis confirmed that the hypoglycemia-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 brainstem, respectively, and 0.0003 for orbitofrontal cortex.

Tables 1 and 2 give 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 in the aware subjects in each condition. Although in that analysis, we

A

B
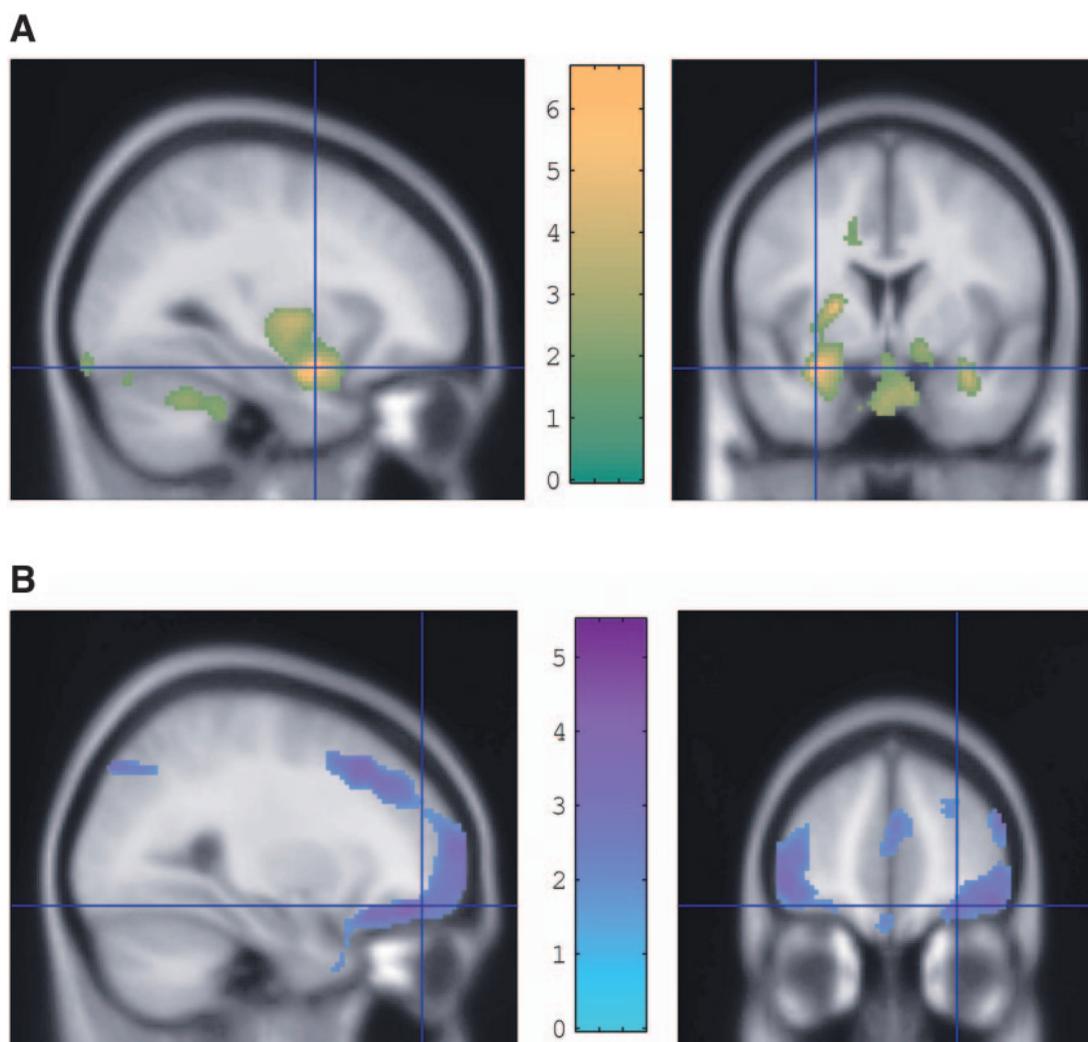
FIG. 2. Cerebral correlates of unawareness. *A*: Regions where aware subjects show relatively greater uptake with hypoglycemia than unaware subjects (displayed on MRI slices, with *P* < 0.01 and cluster size >100), showing amygdala, cerebellum, and brainstem regions. *B*: Regions where aware subjects show relatively lower uptake than unaware subjects with hypoglycemia (*P* < 0.01, cluster size >100), showing right lateral orbitalfrontal cortex. (Please see http://dx.doi.org/10.2337/db07-0666 for a high-quality digital representation of this figure.)

failed to detect a difference in the magnitude of the change with hypoglycemia between aware and unaware subjects, 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 unaware subjects.

In the present analysis, comparing regional differences in FDG uptake with hypoglycemia between aware and unaware subjects with a more sophisticated analytic approach, we 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 behaviors that will have a 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 unaware subjects. However, it is clear that ventral stria-

tum activation does occur in unaware as well as aware subjects.

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 aware and unaware subjects include the amygdala and also a network of brain regions including the occipital cortex, cerebellum, and brainstem, again extending into the area identified in the previous analysis around the hypothalamus. In these brain regions, aware subjects show significantly greater FDG uptake than that shown by unaware subjects. Activation of the amygdala is associated with fear and anxiety (28,29), and its activation in 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 unaware subjects at hypoglycemia in the hypothalamus, which is itself compatible with a failure of activation of the hypothalamic-pituitary-adrenal axis and the absent or diminished counterregulatory responses
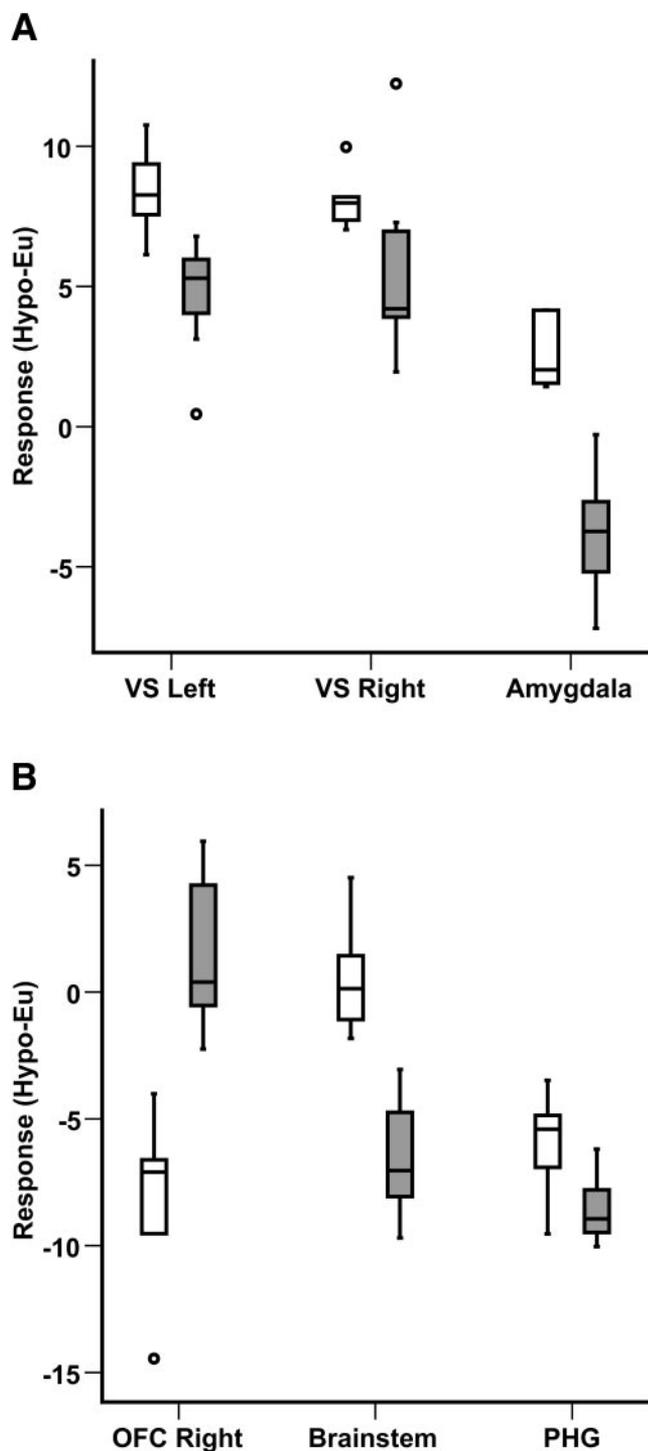
FIG. 3. Box plots showing evoked responses with hypoglycemia (arbitrary units, relative to global mean of 50) by group in selected SPM-defined clusters. *A*: Responses in three regions are shown: left ventral striatum (VS), right ventral striatum, and amygdala; *P* values for condition-by-group interaction 0.005, 0.132, and <0.0001, respectively. *B*: Regional responses from clusters comprising right orbitofrontal cortex (OFC), brainstem, and parahippocampal gyrus; *P* values for condition-by-group interaction 0.0003, 0.0002, and 0.02, respectively. □, Hypoglycemia awareness group; ■, hypoglycemia unawareness group; ○, outlying values.

to hypoglycemia in this group. The association between impaired stress hormone responses to hypoglycemia in those with hypoglycemia unawareness is well documented in the literature (4,14,15) and confirmed in our present study by the diminished epinephrine responses to the

hypoglycemic challenge in unaware subjects. The failure of the epinephrine response is unlikely to be causing the neuroimaging changes, as epinephrine responses in a different study design in healthy volunteers, in which epinephrine levels were very different, were 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 diabetes therapy with intent to avoid hypoglycemia. Failure to avoid hypoglycemia risk has been described in an analysis of patient behaviors in the setting of problematic hypoglycemia (30). Our data are the first to present neuroimaging data that offer a potential explanation for this in terms of differential (reduced) activation of stress/distress pathways in unaware subjects.

In contrast to the above brain regions, the FDG uptake data suggest that the lateral orbitofrontal cortex is deactivated in aware subjects during hypoglycemia and shows no change or even an increase in unaware subjects. 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 hypoglycemia.

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 reeducate 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 hypoglycemia may be not just subjectively neutral but, rather, subjectively rewarding to the person (34). Similar patterns of brain activation are seen in response to substances with addictive potential that have highly rewarding subjective effects (34,35). This is a well-established concept in the animal literature (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. (22) calculated a global brain value of the lumped constant to directly relate the two. However, regional calculations of lumped constant have not found evidence for such a variation (38). Damage to the central nervous system 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. (23)

TABLE 1
Responses from clusters identified by SPM

	Aware		Unaware		Aware	Unaware
					Hypoglycemic-euglycemic	Hypoglycemic-euglycemic
	5 mmol/l	2.6 mmol/l	5 mmol/l	2.6 mmol/l		
Left ventral striatum*	85.22 ± 1.57	93.61 ± 1.15	85.63 ± 4.52	90.28 ± 4.20	8.39 ± 1.57	4.65 ± 2.18
Right ventral striatum	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
Left cerebellum	63.36 ± 3.95	57.92 ± 9.89	63.92 ± 4.43	60.59 ± 5.67	−5.44 ± 10.53	−3.33 ± 8.29
Right cerebellum*	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
Right orbitofrontal cortex*	82.38 ± 8.60	74.25 ± 10.99	80.84 ± 4.41	82.47 ± 3.30	−8.13 ± 3.56	1.64 ± 3.17
Left amygdala*	60.55 ± 2.21	63.11 ± 2.48	61.26 ± 3.33	57.41 ± 3.18	2.56 ± 1.28	−3.85 ± 2.35
Left white matter	45.80 ± 4.66	39.01 ± 3.87	46.54 ± 1.82	38.79 ± 1.49	−6.79 ± 1.33	−7.75 ± 2.12
Right white matter	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

Data are means ± SD of tracer uptake expressed as percent of a global mean (50) at different blood glucose levels (5 and 2.6 mmol/l) and of change between hypoglycemic and euglycemic conditions. Significant difference of the effect of condition (hypoglycemia) between the two groups (aware and unaware subjects), *$P < 0.01$; †$P < 0.05$.

have documented a difference in local cerebral glucose utilization in white matter (corpus callosum) at hypoglycemia below $P < 0.01$ threshold in rats, although this did not reach significance after multiple-comparison corrections. Significant increases of cerebral metabolic rate for glucose 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-glial complexes to maintain cerebral metabolic rate for glucose with falling blood glucose 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 focused on the different regional brain activation responses to hypoglycemia between type 1 diabetic subjects with and without hypoglycemia awareness. We cannot comment on the effects of diabetes per se on these responses or 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.

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)						
Left ventral striatum	749	16.65	8.3e-7	−22	2	7
Right ventral striatum	686	9.89	2.1e-6	18	12	3
Occipital cortex	2,008	8.94	1.5e-13	6	−81	17
Decreased uptake (across both groups)						
White matter	20,570	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 white matter*	845	17.75	1.0e-14	20	28	13
Right periventricular white matter*	2,646	16.30	0	24	−31	38
Brainstem*	2,427	16.27	0	4	−7	−16
Left periventricular white matter*	4,864	14.75	0	−16	−35	29
Left inferior frontal gyrus*	119	9.45	0.00041	−55	25	1
Greater increase in aware than in unaware subjects						
Occipital cortex	365	6.60	0.0004	−2	−93	3
Left amygdala†	305	6.65	NS	−30	1	−15
Occipital cortex†	1,851	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†	2,861	5.24	6.8e-7	6	−42	−18
Greater decrease in aware than in unaware subjects						
Brodmann areas 10, 11, and 47 (right)†	1,071	5.48	0.0041	36	32	−13
Brodmann areas 10, 45, and 46 (right)†	414	4.37	NS	51	30	17
Brodmann areas 8 and 9 (right)†	214	4.22	NS	24	23	41

Clusters of voxels reaching significance, $P < 0.001$ (uncorrected) and minimum cluster size of 100 voxels (unless otherwise stated). T is peak t statistic within cluster; P is corrected for the cluster (not the voxel). Coordinates of the peak voxel are given in Talairach space. *Threshold of $P < 0.0001$, cluster size >100; †$P < 0.01$, cluster size >100. NS, not significant.

The neuroimaging literature suggests that global brain glucose uptake is not much affected by diabetes (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 diabetic subjects with a lesser fall in hypoglycemia than in nondiabetic control subjects (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 aware subjects, and unequivocally reduced activation in regions involved in the generation of anxiety and stress responses (amygdala). In particular, brain regional networks subserving hedonic responses, deactivated in aware subjects, are relatively unaffected in unaware subjects, suggesting that the experience of hypoglycemia is not only not distressing, but may actually be weakly rewarding. Taken with clinical observations, these observations suggest that habituation to recurrent hypoglycemia 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.

ACKNOWLEDGMENTS

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