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Widespread abnormality of the γ-aminobutyric acid-ergic system in Tourette syndrome

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Dysfunction of the γ-aminobutyric acid-ergic system in Tourette syndrome may conceivably underlie the symptoms of motor disinhibition presenting as tics and psychiatric manifestations, such as attention deficit hyperactivity disorder and obsessive–compulsive disorder. The purpose of this study was to identify a possible dysfunction of the γ-aminobutyric acid-ergic system in Tourette patients, especially involving the basal ganglia-thalamo-cortical circuits and the cerebellum. We studied 11 patients with Tourette syndrome and 11 healthy controls. Positron emission tomography procedure: after injection of 20 mCi of [^{11}C]flumazenil, dynamic emission images of the brain were acquired. Structural magnetic resonance imaging scans were obtained to provide an anatomical framework for the positron emission tomography data analysis. Images of binding potential were created using the two-step version of the simplified reference tissue model. The binding potential images then were spatially normalized, smoothed and compared between groups using statistical parametric mapping. We found decreased binding of $GABA_A$ receptors in Tourette patients bilaterally in the ventral striatum, globus pallidus, thalamus, amygdala and right insula. In addition, the $GABA_A$ receptor binding was increased in the bilateral substantia nigra, left periaqueductal grey, right posterior cingulate cortex and bilateral cerebellum. These results are consistent with the longstanding hypothesis that circuits involving the basal ganglia and thalamus are disinhibited in Tourette syndrome patients. In addition, the abnormalities

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in $GABA_A$ receptor binding in the insula and cerebellum appear particularly noteworthy based upon recent evidence implicating these structures in the generation of tics.

Keywords: Tourette syndrome; tics; $GABA_A$ receptors; flumazenil; PET

Abbreviations: BP_{ND} = binding potential; GABA = γ-aminobutyric acid; SN = substantia nigra

Introduction

Gilles de la Tourette syndrome is a complex neuropsychiatric disorder characterized by multiple motor and vocal tics, which are associated with behavioural and emotional disturbances including symptoms of attention deficit hyperactivity disorder, obsessive–compulsive disorder, anxiety and depression. In spite of these diverse and pronounced symptoms, the causes of Tourette syndrome remain elusive. For many years the 'dopaminergic' theory of Tourette syndrome (Butler *et al.*, 1979; Singer *et al.*, 1982), which postulated that hypersensitivity of dopamine receptors and/or hyperactivity of dopaminergic neurons underlay the pathophysiology of Tourette syndrome, was prevalent. Subsequently, the majority of neuroimaging studies concentrated on evaluating striatal dopaminergic systems using PET or single-photon emission computed tomography (SPECT). However, the results obtained in these studies were inconclusive (Singer *et al.*, 1992; Turjanski *et al.*, 1994; Wong *et al.*, 1997; Ernst *et al.*, 1999; Muller-Vahl *et al.*, 2000). Morphometric MRI studies showed abnormalities that included reduced caudate nucleus volume (Peterson *et al.*, 2003), abnormality of the corpus callosum (Moriarty *et al.*, 1997; Plessen *et al.*, 2004), enlargement of the left thalamus (Lee *et al.*, 2006), amygdala and hippocampus (Peterson *et al.*, 2007), and increased grey matter of the left mesencephalon.

Several previous functional neuroimaging studies reported metabolic or haemodynamic abnormalities within the basal ganglia, thalamus (Braun *et al.*, 1993; Peterson *et al.*, 1998; Baym *et al.*, 2008 a), insula and cerebellum (Bohlhalter *et al.* 2006; Lerner *et al.* 2007). These findings suggested that dysfunction involving striatal-pallidal-thalamic circuits could potentially contribute to the overactivity and disinhibition of the motor cortices seen in neuroimaging studies (Biswal *et al.*, 1998; Stern *et al.* 2000) and neurophysiological studies of Tourette syndrome (Ziemann *et al.*, 1997; Orth *et al.*, 2005). Therefore, we hypothesized that an abnormality in the function of these networks could be caused by pathological changes in γ-aminobutyric acid (GABA)-ergic receptors. We designed a PET study using the ligand [^{11}C]flumazenil to assess the involvement of the GABA-ergic system in Tourette syndrome pathology.

Materials and methods

Subjects

We studied 11 patients with Tourette syndrome and 11 normal volunteers. Patients' ages ranged from 19 to 38 years (1 female, 10 male) (Table 3); control subjects were age- and gender-matched to the Tourette syndrome subjects (Supplementary Table 1). All patients had normal neurological examinations except for their tics. Patients were diagnosed with Tourette syndrome based on the neurological exam and Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV). All patients were also evaluated with the Structured Clinical Interview for DSM-IV (SCID) to assess for possible comorbid psychiatric disorders. Four patients had obsessive–compulsive disorder, two had subthreshold obsessive–compulsive disorder, three had current attention deficit hyperactivity disorder and two had a remote history of attention deficit hyperactivity disorder (Table 3). Tic severity was quantified using the Yale Global Tic Severity Scale (Leckman *et al.*, 1989). The ratings and clinical evaluations were done while on usual medication. Due to constraints related to the PET scanning, only patients with mild-to-moderate tics and tics that did not interfere with the scanning procedure were included in the study. None of the patients was on any medication expected to affect the CNS for at least one week prior to imaging. During scanning some Tourette syndrome subjects had active tics; however, they were relatively mild and sporadic and did not interfere with scanning. The patients were continuously observed for occurrence of disruptive tic behaviour. The participants were instructed to relax, but were not asked specifically to suppress tics.

The study was approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke. All control subjects and patients gave written informed consent to participate.

Positron emission tomography procedure

PET scans were acquired with subjects at rest using a GE Advance scanner with septa retracted [35 contiguous slices; 4.25-mm plane separation; reconstructed 3D spatial resolution = 6–7 mm full-width at half-maximum (FWHM)]. A transmission scan was acquired to correct for attenuation. Following transmission scanning, a target dose of 20 mCi of high specific activity [^{11}C]flumazenil was injected and 60-min dynamic emission images of the brain were acquired. Subject motion correction during the PET acquisition was performed with a mutual-information registration of each scan time-frame to a standard frame before attenuation correction (using FLIRT software, FSL 3.2, Analysis Group, FMRIB, Oxford, UK) (Andersson *et al.*, 1995; Smith *et al.*, 1997). Based on the calculated motion, the transmission images were resliced and projected for final reconstruction and realignment. To provide an anatomical framework for analysis of the PET images, structural MRI scans, T_1-weighted pulse sequence were acquired. PET images were registered to each individual's MRI with a mutual information algorithm.

Data analysis

Image processing and analysis were performed on a Dell 5 Linux workstation (Round Rock, Texas, USA). Binding potential images were created using the two-step version of the simplified reference tissue

model (SRTM2) (Wu and Carson, 2002). The input kinetics for the reference tissue were derived from the pons (drawn on each individual's MR image), where the [^{11}C]flumazenil binding is predominantly accounted for by free and nonspecifically bound radiotracer (Millet *et al.*, 2002; Odano *et al.*, 2009). The binding potential (BP_{ND}) images (already transformed to MR space) were then spatially normalized to a standard PET template based on the Montreal Neurological Institute reference brain (Ashburner and Friston, 1999) and analysed using Statistical Parametric Mapping (SPM2) (Wellcome Department of Imaging Neuroscience, UCL, London, UK) implemented in Matlab. The normalized images of $2 \times 2 \times 2$ mm^3 voxels were smoothed with a 10-mm FWHM isotropic Gaussian kernel. We performed two types of analyses, namely with global normalization using proportional scaling (Table 1) and without global normalization of BP_{ND} values (Table 2); for both of these analyses height threshold was set $P = 0.05$ false discovery rate (FDR) corrected for multiple comparisons. The [^{11}C]flumazenil BP_{ND} values were compared between groups in a voxel-wise analysis using a two-sample *t*-test model. We performed also regression analyses using as regressors: (i) Yale Global Tic Severity Scale score; and (ii) age; the analyses were done using unscaled BP_{ND} values. Due to the small number of subjects in these analyses the height threshold was set at $P = 0.001$ uncorrected. The results of all analyses were converted into Talairach space (Talairach and Tournoux, 1988; Schmahmann *et al.*, 1999).

Results

In both analyses performed using BP_{ND} values with and without global normalization we found decreased binding of $GABA_A$ receptors in Tourette syndrome patients bilaterally in the ventral portions of the caudate nuclei, putamen, accumbens nuclei and globus pallidus (Fig. 1A, Tables 1 and 2). Decreased binding was also seen in the thalamus, right insula (Fig. 1B) and amygdala (Fig. 1C, Tables 1 and 2, Supplementary Fig. 1). There was increased binding of $GABA_A$ receptors in the bilateral substantia nigra (SN), left periaqueductal grey (Fig. 2A and B), right posterior cingulate cortex (Fig. 2B) and bilateral cerebellar dentate nuclei (Fig. 2C, Tables 1 and 2, Supplementary Fig. 1). The regression analysis that used the Yale Global Tic Severity Scale score (Supplementary Table 2) showed a positive correlation with the right postcentral gyrus-sensory cortex, and a negative correlation with the left frontal eye field (BA 8), right thalamus (ventral posterior lateral nucleus and ventral posterior medial nucleus), left thalamus (dorsomedial nucleus) and bilateral prefrontal cortex (BA 9). The regression analysis that used age of Tourette syndrome patients showed only positive correlation with the right cerebellar lobule VI (Supplementary Table 3).

Both analyses with and without global normalization showed essentially the same structures; however, there was an absence of some cortical areas in the analysis performed without global normalization including precuneus, cuneus, postcentral and occipital cortices, amygdala and hippocampus (Table 2).

Discussion

This is the first PET neuroimaging study to explore GABA-ergic abnormalities in Tourette syndrome patients. The most significant finding is that relative to healthy controls, the patients with Tourette syndrome showed decreased binding of $GABA_A$ receptors in the ventral striatum, globus pallidus, thalamus, amygdala and right insula, and increased binding in the bilateral SN, left periaqueductal grey, right posterior cingulate cortex and bilateral dentate nuclei of the cerebellum. The anatomical distribution of these changes implicates regions where abnormalities of structure or function have been reported in previous neuroimaging and histopathological studies in Tourette syndrome. This study was performed using two voxel-wise analysis approaches, namely with and without global normalization of BP_{ND} values. Both methods implicated essentially the same structures confirming validity of the results. The main difference was absence of amygdala and hippocampus and some cortical areas including precuneus, cuneus, postcentral and occipital cortices (Table 1), in the analysis that used unscaled binding potential.

Structures with decreased binding of $GABA_A$ receptors

Ventral caudate, putamen, nucleus accumbens and globus pallidus

According to the 'dopaminergic theory' of Tourette syndrome, the pathogenesis of the disorder results from an abnormality of striatal dopaminergic neurons and/or receptors. However, the striatum, comprised of the caudate, putamen and nucleus accumbens, also contains ~90–95% GABA-ergic neurons. The GABA-ergic neurons function as projection neurons and interneurons. The projection neurons, so-called 'medium spiny neurons', project from the striatum to the output nuclei of the basal ganglia (internal segment of globus pallidus and SN pars reticulata), whereas GABA-ergic interneurons form three classes distinguished by the presence of co-localizing proteins: (i) parvalbumin; (ii) calretinin and (iii) somatostatin (Kawaguchi *et al.*, 1995). Haber *et al.* (1986) found a significant decrease of dynorphin-like immunoreactivity in the globus pallidus of a Tourette syndrome patient suggesting an abnormality of the GABA-ergic striatopallidal pathway, whereas recent studies by Kalanithi *et al.* (2005) and Kataoka *et al.* (2010) showed a decreased number of GABA-ergic interneurons containing parvalbumin in the caudate and putamen and globus pallidus pars externa, accompanied by an increase of these interneurons in the globus pallidus pars interna of patients with Tourette syndrome. This finding was interpreted by Kalanithi *et al.* as being 'consistent with a developmental defect in tangential migration of some GABA-ergic neurons' from the medial ganglionic eminence to the striatum, cortex and hippocampus. An abnormal function of the ventral striatum was also found in several previous neuroimaging PET studies in Tourette syndrome. The ventral striatum was shown to have decreased metabolism in Tourette syndrome (Stoetter *et al.*, 1992) and tic-related increased activity (Baym *et al.*, 2008b). Other studies examining involvement of the dopaminergic system found increased dopamine release (Wong *et al.*, 2008), and increased binding of [^{11}C] dihydrotetrabenazine (DTBZ), a marker for type 2 vesicular monoamine transporter (VMAT2) (Albin *et al.*, 2003); this finding was interpreted as indicative of dopaminergic dysfunction.

Table 1 Brain areas with decreased and increased binding (BP_{ND}) of $GABA_A$ receptors in patients with Tourette syndrome (analysis used global normalization of BP_{ND})

Cluster size	Regions (Brodmann areas)	x	y	z	Z-value
	Brain areas with decreased BP_{ND}				
5702	Left nucleus accumbens, putamen, caudate nucleus	−8	10	−7	6.69
	Left inferior frontal gyrus (BA 47)				
	Right putamen, nucleus accumbens, caudate nucleus	18	8	−5	5.77
	Right insula (anterior and posterior)				
	Right transverse temporal gyrus (BA 41)				
	Right inferior frontal gyrus (BA 47)				
	Left and right thalamus (pulvinar, centromedian, dorsomedian nuclei)	−11	−25	14	5.77
131	Right precuneus (BA 7)	13	−58	53	4.29
181	Left amygdala and hippocampus	−18	−9	−22	3.97
765	Left postcentral gyrus (BA 1, 2, 3)	−34	−34	54	3.96
1136	Left superior occipital gyrus (BA 19)	−31	−75	29	3.87
	Right cuneus (BA 19)	8	−75	31	3.75
	Left medial occipital gyrus (BA 19)	−38	−75	12	3.36
222	Right amygdala and hippocampus	20	−11	−22	3.85
670	Left fusiform gyrus (BA 19)	−22	−60	−7	3.63
	Brain areas with increased BP_{ND}				
1953	Left and right SN and left periaqueductal grey	−3	−27	−10	4.83
256	Right posterior cingulate gyrus, sulcus calloso-marginalis (BA 31)	17	−13	41	4.20
1656	Left and right cerebellum, dentate nuclei	−8	−54	−29	4.10
		6	−56	−28	

Cluster size = number of voxels; *x*, *y*, *z* = stereotaxic coordinates in Talairach space; coordinates indicate the distance in millimetres from the origin (anterior commissure), with positive *x* indicating right, positive *y* indicating anterior and positive *z* indicating dorsal.

Table 2 Brain areas with decreased and increased binding (BP_{ND}) of $GABA_A$ receptors in patients with Tourette syndrome (analysis used unscaled BP_{ND})

Cluster size	Regions (Brodmann areas)	x	y	z	Z-value
	Brain areas with decreased BP_{ND}				
607	Left and right caudate nuclei	−18	−20	19	5.93
	Right and left thalamus (dorsomedian, midline, lateral dorsal, lateral posterior, ventral anterior, ventral lateral nucleus and pulvinar)	6	−13	13	5.14
59	Left caudate nucleus	−17	6	18	4.67
144	Right insula	40	−22	21	4.48
	Right transverse temporal gyrus (BA 41)				
148	Right caudate nucleus, nucleus accumbens	6	13	−1	4.35
	Left caudate nucleus, nucleus accumbens, putamen	−12	13	−7	4.32
10	Right putamen	20	12	−4	3.74
2	Right insula	34	12	10	3.64
	Brain areas with increased BP_{ND}				
1155	Left pons	−12	−23	−29	4.51
	Left and right SN	−6	−22	−9	4.50
	Left periaqueductal grey				
	Left and right red nuclei				
	Left and right subthalamic nuclei				
366	Left cerebellum, dentate nucleus, left lobules 4–5, 6, 8, 9	−10	−52	−23	3.87
93	Right posterior cingulate gyrus, sulcus calloso-marginalis (BA 31)	20	−10	44	3.86
3	Right cerebellum, dentate nucleus	10	−52	−23	3.35

Cluster size = number of voxels; *x*, *y*, *z* = stereotaxic coordinates in Talairach space.

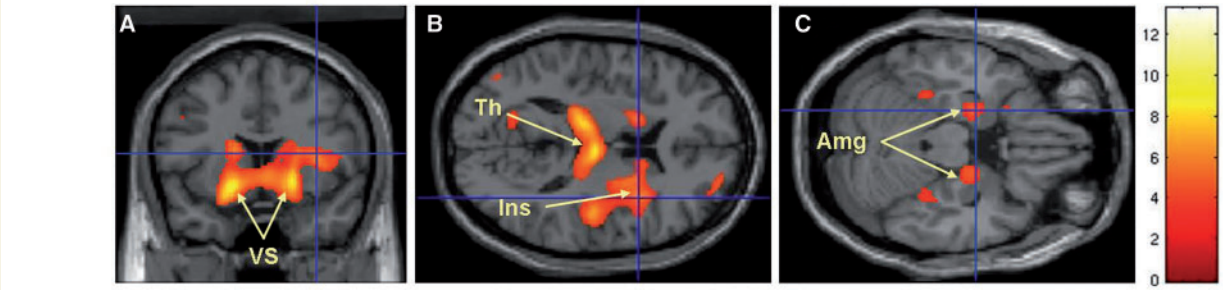

Figure 1 Brain areas with decreased binding of [^{11}C]flumazenil in Tourette syndrome patients versus control subjects: the most significant decreases were seen in the bilateral ventral striatum (VS), bilateral thalamus (Th), right insula (Ins) and bilateral amygdala (Amg).

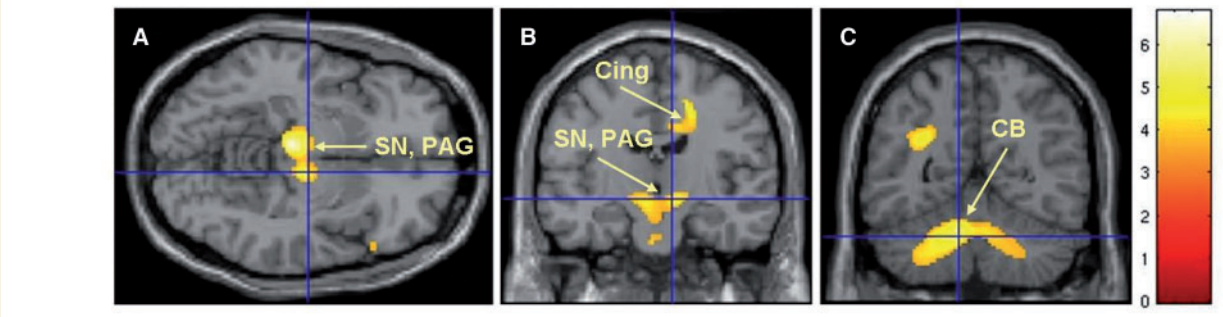

Figure 2 Brain areas with increased binding of [^{11}C]flumazenil in Tourette syndrome patients versus control subjects; the highest increases were noted in the bilateral SN, left periaqueductal grey (PAG), right posterior cingulate cortex (PCC) (Cing) and bilateral cerebellum, dentate nuclei (CB). The figures are from the analysis which used non-normalized BP_{ND} values as reported in Table 1 and $P < 0.05$, corrected for multiple comparisons.

Our study, which showed significant bilateral decrease of [^{11}C]flumazenil binding in the ventral aspect of the striatum and globus pallidus, further emphasizes involvement of the basal ganglia in Tourette syndrome; in particular, the limbic loop of the striatum (Alexander *et al.*, 1986; Voorn *et al.*, 2004), which is responsible for emotional and motivational processes known to be affected in Tourette syndrome.

Thalamus

Abnormalities of the thalamus in Tourette syndrome patients were observed not only in a number of functional neuroimaging studies (Peterson *et al.*, 1998; Stern *et al.*, 2000; Lerner *et al.*, 2007), but also recently in receptor binding and morphological studies. Gilbert *et al.* (2006) found significantly lower availability of D2 receptors in the mediodorsal nucleus of the thalamus in Tourette syndrome patients while Lee *et al.* (2006) observed increased left thalamic volumes in boys with Tourette syndrome.

Our study showed decreased binding of $GABA_A$ receptors in the left and right thalamus mainly in the pulvinar, centromedian and mediodorsal nuclei. The pulvinar is implicated in pain modulation, speech mechanisms and visual attention functions. The abnormality of the pulvinar conceivably may have clinical relevance and could be implicated in visuomotor integration deficits of Tourette syndrome patients (Schultz *et al.*, 1998). Also, the pulvinar was recently found to be involved in attention deficit hyperactivity disorder (Ferreira *et al.*, 2009), a disorder which frequently co-exists with Tourette syndrome. It is possible that the changes in the pulvinar could contribute to attention deficit hyperactivity disorder as well.

The mediodorsal nucleus has particularly prominent interconnections with the dorsolateral prefrontal cortex, which is a key area of executive functions and attentional focus. The mediodorsal nucleus is involved in planning, organization, attention, affective behaviour, memory and integration of visceral functions. Therefore, the abnormalities of two neurotransmitter systems in this nucleus, the dopaminergic D2 receptors (Gilbert *et al.*, 2006) and $GABA_A$ receptors could potentially explain impaired mediodorsal nucleus-related functions in Tourette syndrome patients, in particular attention and affective behaviour.

Additionally, regression analysis that used the Yale Global Tic Severity Scale score (Supplementary Table 2) showed negative correlation with BP_{ND} values of the right ventral posterior medial and lateral nuclei and left dorsomedial nucleus of the thalamus. The BP_{ND} values of the right somatosensory cortex, which is reciprocally connected to ventral posterior medial and lateral nuclei, showed positive correlation with Yale Global Tic Severity Scale score, whereas BP_{ND} values of the left frontal eye field (BA 8) and prefrontal cortex (BA 9) which are reciprocally connected to the dorsomedial nucleus, showed negative correlation. Involvement of frontal eye fields conceivably may be related to the ocular tics.

The centromedian nuclei (identified only with the analysis using global normalization) belong to the group of intralaminar nuclei

through which the cerebellum communicates with the striatum (Hoshi *et al.*, 2005). Recently shown involvement of the cerebellum in Tourette syndrome (Stern *et al.*, 2000; Bohlhalter *et al.*, 2006; Lerner *et al.*, 2007) could indicate dysfunction of the entire pathway involving the cerebellum, centromedian nucleus and striatum in Tourette syndrome.

The involvement of the thalamus in Tourette syndrome is further emphasized by efficacy of deep brain stimulation targeting thalamic nuclei of the centromedian–parafascicular complex used as a new treatment for tics (Visser-Vandewalle *et al.*, 2003; Maciunas *et al.*, 2007; Welter *et al.*, 2008; Porta *et al.*, 2009). The effectiveness of this approach could be explained by the interruption of major excitatory pathways from the cerebellum through the thalamus to the basal ganglia. It is possible that an abnormality of the thalamus reflects dysfunction of cerebellar and basal ganglia circuits; this dysfunction is then further projected to multiple cortical areas and detected in neuroimaging and electrophysiological studies frequently as disinhibition and overactivity.

Amygdala

Morphological abnormalities of the amygdala and hippocampus in Tourette syndrome patients have recently been reported by Peterson *et al.* (2007) in an MRI study and consisted of enlargement of the central and basolateral nuclei of amygdala and hippocampal dentate gyrus and sector CA3. A similar pattern of changes in the hippocampus was also observed in attention deficit hyperactivity disorder patients in an MRI study (Plessen *et al.*, 2006) suggesting that hippocampal abnormality in the dentate gyrus may contribute to attention deficit hyperactivity disorder co-morbidity in Tourette syndrome patients. Hippocampal and amygdala volumes were also abnormal in refractory obsessive–compulsive disorder patients (Atmaca *et al.*, 2008), implicating involvement of these structures also in obsessive–compulsive disorder co-morbidity. The decreased flumazenil binding in the amygdala and hippocampus (identified with the analysis using global normalization and unscaled BP_{ND} values at $P = 0.001$) in Tourette syndrome patients in our study may indicated contribution of amygdala to the attention deficit hyperactivity disorder component of Tourette syndrome and possibly also to obsessive–compulsive disorder and anxiety, as amygdala function has been implicated in both primary attention deficit hyperactivity disorder and primary obsessive–compulsive disorder (Davis, 1992; Breiter and Rauch, 1996; Szeszko *et al.*, 1999).

Structures with increased binding of GABA$_A$ receptors

Substantia nigra and periaqueductal grey

Involvement of the midbrain dopaminergic system and periaqueductal grey in Tourette syndrome was first proposed by Devinsky (Devinsky, 1983) and then subsequently confirmed with a MRI morphometric study by Garraux *et al.* (2006) and functional MRI study (Baym *et al.*, 2008b). Results of our study resemble the findings of the morphometric study by Garraux *et al.* (2006) with the involvement of the SN and periaqueductal grey and predominance of the changes on the left side.

The dopaminergic neurons of the SN pars compacta, which project throughout the striatum, receive potent GABA-ergic projections from the neostriatum, globus pallidus and SN pars reticulata (Paladini *et al.*, 1999). Therefore, altered function of GABA$_A$ receptors of SN pars compacta might have a profound effect on the function of dopaminergic neurons that project into the striatum. Dysfunction involving the GABA-ergic system of the SN pars reticulata, a major output structure of basal ganglia, would also have a significant impact, especially affecting, and possibly causing, disinhibition of thalamo-cortical networks and dopaminergic neurons of the SN pars compacta.

The abnormality of the GABA-ergic system in the SN shown in our study could potentially explain the gender difference in Tourette syndrome and predominance of male subjects. The SN pars reticulata has been shown to be one of the sexually dimorphic areas of the brain (Veliskova and Moshe, 2001). This differentiation occurring during the early development and maturation of GABA$_A$ receptor signaling (the switch from depolarizing to hyperpolarizing GABA-ergic currents) follows gender-specific patterns (Galanopoulou, 2008). Peterson *et al.* (1992) already pointed to the role of sex hormones, in particular androgens, and their influence on dimorphic brain structures in pathogenesis of Tourette syndrome. The androgenic hormones acting on the GABA$_A$ receptors' modulatory site could further contribute to the vulnerability of the GABA-ergic network in the SN and adversely affect its inhibitory action, causing emergence of tics. The exacerbation of Tourette syndrome by anabolic hormones has been reported by Leckman and Scahill (1990).

Cerebellum

The cerebellum has not been frequently implicated in the genesis of Tourette syndrome; however, some recent studies showed significant activation of the cerebellum during tic production (Stern *et al.*, 2000; Bohlhalter *et al.*, 2006; Lerner *et al.*, 2007). In our previous article, we suggested that the overactive cerebellum in Tourette syndrome could contribute to tic generation, in particular, through its influence on the putamen and caudate via the thalamic intralaminar nuclei (Hoshi *et al.*, 2005). The presence of an abnormality in the dentate nuclei which form the major output from the cerebellum appears to confirm cerebellar involvement in Tourette syndrome.

The regression analysis that used the age of the Tourette syndrome subjects (Supplementary Table 3) showed a positive correlation with the right cerebellar lobule VI. Cerebellar lobule VI is a part of the posterior lobe and has cognitive and affective functions as postulated by Schmahmann (2004), but it also contains some sensorimotor representation. Lobule VI was reported to be activated during orofacial movements (Dresel *et al.*, 2005), during language-related activity (Jansen *et al.*, 2005) and also during spatial, affective and working memory tasks (Stoodley and Schmahmann, 2010). Lobule VI was seen to be activated during tic release in a previous PET study (Lerner *et al.*, 2007), and an MRI volumetric study (Tobe *et al.*, 2010) showed changes in lobule VI that correlated with the severity of tics, particularly vocal tics.

In both analyses, several cortical areas showed abnormal binding of GABA$_A$ receptors, namely the limbic cortices (insula,

posterior cingulate), auditory cortex, area 41, frontal cortex area 47. However, only the analysis with global normalization showed abnormal binding in the somatosensory cortex, areas 1, 2 and 3, visual cortex area 19. All these cortical areas except for the posterior cingulate cortex showed decreased binding of $GABA_A$ receptors. Many of these areas have been shown to be involved in Tourette syndrome pathology.

Insula

A functional abnormality of the insula in Tourette syndrome patients was found in a number of neuroimaging studies; in perfusion studies (Stern *et al.*, 2000; Bohlhalter *et al.*, 2006; Lerner *et al.*, 2007), PET [^{18}F]fluorodeoxyglucose (FDG) study of metabolism (Stoetter *et al.*, 1992) and PET study of opiate binding (Weeks *et al.*, 1996). The insula, which serves as a cortical site for integrated interception where information about all bodily sensations converges (Craig, 2002), was also proposed to be responsible for controlling and suppressing natural urges (Lerner *et al.*, 2009). Therefore, disordered function of the insula conceivably might contribute to the premonitory urges of Tourette syndrome and difficulties with tic suppression, and probably also to the cognitive-behavioural disturbances associated with Tourette syndrome, especially, obsessive–compulsive disorder. Our study showed decreased binding of $GABA_A$ receptors in the right insula. This fact is particularly interesting, because the right insula was proposed to be involved in perception and processing of internal stimuli associated with stress responses and conveyed by the sympathetic nervous system (Craig, 2005). It is probable that in Tourette syndrome, the presence of tics and attempt to suppress them activates stress-related pathways.

Other issues

Possible reasons for altered $GABA_A$ receptor binding

The mechanism underlying the changes in flumazenil BP_{ND} in Tourette syndrome remains unclear, but conceivably may have multiple explanations. One proposed by Kalanithti *et al.* (2005) is a reduction of neurons arising from a 'developmental defect in tangential migration of some GABA-ergic neurons'. However, similar developmental defects might be more pervasive in Tourette syndrome and affect other brain areas as well. If so, this could explain the widespread changes in the BP_{ND} of $GABA_A$ receptors in our study.

Another possible reason for the changes of flumazenil BP_{ND} in Tourette syndrome is alteration in affinity of $GABA_A$ receptors, which might imply some structural changes within the receptor. A mutation of one or more of $GABA_A$ receptor subunits may potentially alter pharmacological properties of the receptor. Such mutations were shown to underlie a number of epilepsy syndromes (Noebels, 2003; Benarroch, 2007) and alter cortical excitability (Fedi *et al.*, 2008). There is also the possibility that flumazenil is sensitive to endogenous GABA levels (Frankle *et al.*, 2009).

Limitations

The results of this study probably reflect not only differences in the BP_{ND} of $GABA_A$ receptors between Tourette syndrome patients and control subjects but also the characteristic distribution of different $GABA_A$ receptors in the brain and their variable pharmacological profiles. This constitutes one of the limitations of this study that is related to the differential affinity of various $GABA_A$ subtypes for flumazenil. Some subunit combinations do not bind flumazenil, or bind it with lower affinity, in particular: $\alpha4$, $\alpha6$, $\gamma1$, δ, ε, ρ, θ (Bentue-Ferrer *et al.*, 1996; Barnard *et al.*, 1998; Sieghart and Sperk, 2002). This fact might have affected the results of the study because some brain structures implicated in Tourette syndrome might not have been visualized, due to the presence of subunits with low affinity for flumazenil. This is in particular the case of many thalamic nuclei and parts of the caudate and putamen, globus pallidus, subthalamic nucleus, cerebellar cortex and many brainstem nuclei (Dennis *et al.*, 1988; Zezula *et al.*, 1988; Kultas-Ilinsky *et al.*, 1998). The results of this PET study reflect only changes of $GABA_A$ receptors which bind flumazenil, therefore the present PET study could have missed potential abnormalities of some key structures involved in Tourette syndrome such as parts of basal ganglia, thalamus and cerebellum, which contain $GABA_A$ receptors subunits with low affinity for flumazenil such as $\alpha4$ and $\alpha6$. At this point, the picture of GABA-ergic involvement in Tourette syndrome is incomplete and imaging of other $GABA_A$ receptor subunits could bring important and complementary information.

Regarding potential impact of tics on radioligand uptake we do not think that tics occurring during the scanning would affect the results of the PET study as they were mild, occurring sporadically and of short duration. Also, the effect of medication, and comorbid illnesses could have influenced the findings of this study. However, the majority of Tourette syndrome patients were not taking any medication for years, and the four patients who were taking medication stopped generally 2 weeks before the study (Table 3). Regarding the influence of co-morbid illnesses, the majority of Tourette syndrome patients had attention deficit hyperactivity disorder and/or obsessive–compulsive disorder; however, these disorders form an integral component to the spectrum of phenotypic disturbances seen in the Tourette syndrome and examining the correlates of discrete symptoms would require a much larger sample size.

Conclusion

The abnormalities of GABA-ergic neurons found in so many functionally diverse structures might be responsible for motor, cognitive and affective dysregulation in Tourette syndrome and suggest a developmental pathogenesis for Tourette syndrome (Stern *et al.*, 2008). The complex set of functional domains affected in Tourette syndrome putatively involves developmental and/or genetic factors with superimposed plastic reorganization of neuronal circuits in different brain regions due to reactive changes down- and up-stream of the initial abnormality.

In view of such a prominent abnormality in flumazenil BP_{ND} in Tourette syndrome patients, it is conceivable that dysfunction involving the $GABA_A$ receptor system may play a major role in the pathophysiology of Tourette syndrome. The GABA-ergic system is the main inhibitory system in the CNS and GABA-ergic neurons are present in every brain structure, accounting in some

Table 3 Demographic data for patients with Tourette syndrome

Patient number	Age	Race	Sex	Socioeconomic status	YGTSS score	Y-BOCS age of onset; score	Medications: last 6 months; medication	Co-morbid disorders	Tics
1	31	W	M	College graduate/owns business	29 (19/10)		In the past: Haldol; last 6 months: none	None	Motor, vocal
2	21	W	M	College student	65[a] (35/30)	Full remission; 7 years of age (11/9)	None for last 2 years. In the past: Prozac, Celexa, Effexor, Depakote, Risperidol, Adderal (each for a few months); last 6 months: none	MDD recurrent, OCD, ADHD, panic with agoraphobia	Motor, vocal
3	38	W	M	College graduate/ administrative assistant	27 (17/10)		In the past: Haldol; last 6 months: none	None	Motor, vocal (in the past)
4	36	W	M	Middle school/ unemployed	35 (35/0)	Current; 9 years of age (10/10)	In the past for Tourette syndrome: Haldol, for ADHD/OCD: Ritalin, Clonidine, Paxil; last 6 months: none	MDD recurrent, OCD, ADHD, social phobia	Motor, vocal
5	32	W	M	High school/army-office work	44 (34/10)		In the past: Clonidine; last 6 months: Guafacine stopped 1 month prior the study	Alcohol abuse (in remission)	Motor, 1 vocal
6	24	W	M	High school/labourer	43[a] (33/10)		In the past: Zyprexa for 2 months; last 6 months: for Tourette syndrome: Clonidine 0.3, stopped 2 weeks prior to the study	Dysthymia, social phobia	Motor, 1 vocal
7	31	W	M	College graduate/ student	100[b]	Current; 9 years of age[c]	Last 6 months: none	OCD subthreshold, social phobia, history of marijuana use, stopped 1999	Motor, vocal
8	24	A	M	College graduate/ student	7 (7/0)		None, 20 mg Ritalin for exams in college; last 6 months: none	OCD, history of ADHD	Motor, vocal
9	19	W	M	College student	[c]		In the past: Adderal, Ritalin; last 6 months: for ADHD: Concerta 36 mg, stopped 10 days prior study	ADHD	Motor, vocal
10	23	W	M	College student	30 (20/10)	Full remission; 5 years of age (9/6)	In the past: Prozac, Zoloft; last 6 months: for OCD: Fluvoxamine 150 mg, stopped 7 days prior study	OCD, social phobia	Motor, vocal
11	20	W	F	College student	48[a] (48/0)	Full remission; 7 years of age (2/2)	In the past: Haldol, Klonopin, Risperidol, Prolixin, Desipramine; last 6 months: for mood disorder, Prozac, discontinued 3 months prior to study	Bipolar disorder II, OCD subthreshold did not meet full criteria for OCD, ADHD childhood, substance abuse (marijuana, stopped 10 months prior to study)	Motor, vocal

a The score for the worst week in life.
b Patient exaggerated the disorder, except for Subject 7 who submitted the score for the current symptoms.
c Forms/information not available.
Race column: W = White; A = Asian.
Values of Yale Global Tic Severity Scale are shown as a global number and then in parenthesis broken down to tic score/overall impairment score.
Values of the Y-BOCS Scale are provided for 'lifetime worst'.
Socioeconomic status is provided as education/profession.
ADHD = attention deficit hyperactivity disorder; MDD = major depressive disorder; OCD = obsessive–compulsive disorder; Y-BOCS = Yale-Brown obsessive compulsive scale; YGTSS = Yale Global Tic Severity Scale.

structures (e.g. striatum) for up to 95% of neurons. Additionally, this system plays an important role in the development of many brain structures. Recently, GABA-ergic interneurons were shown to play a key role in regulating cortical development including neuronal proliferation, migration and differentiation (Anderson *et al.*, 1999; Di Cristo, 2007). They were also found to have a critical role in the development of the striatum, cerebellum and hippocampus (Marin *et al.*, 2000; Pleasure *et al.*, 2000; Takayama, 2005; Huang *et al.*, 2007). Therefore, alteration of the GABA-ergic system can affect and alter function and morphology of many brain structures already implicated in Tourette syndrome, such as the cortex, striatum, hippocampus and cerebellum. However, in light of known abnormalities affecting other neurotransmitter systems [e.g. dopaminergic, serotonergic (Wong *et al.*, 2008), cholinergic (Kataoka *et al.*, 2010) and opioid], the role of $GABA_A$ receptor dysfunction in Tourette syndrome pathophysiology remains unclear, but merits further research. The alterations found in other neurotransmitter systems could represent compensatory changes due to a primary defect in the GABA-ergic system. We think that further studies of other neurotransmitter systems are necessary to delineate the final biological signature of Tourette syndrome; however, at this point we would like to propose to consider GABA-ergic morphological changes as detected by flumazenil binding as one of the biomarkers of Tourette syndrome.

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Supplementary material

Supplementary material is available at *Brain* online.

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