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Interrelation of Resting State Functional Connectivity, Striatal GABA Levels, and Cognitive Control Processes

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Abstract: Important issues for cognitive control are response selection processes, known to depend on fronto-striatal networks with recent evidence suggesting that striatal gamma-amino butyric acid (GABA) levels play an important role. Regional GABA concentrations have also been shown to modulate intrinsic connectivity, e.g. of the default mode network. However, the interrelation between striatal GABA levels, basal ganglia network (BGN) connectivity, and performance in cognitive control is elusive. In the current study, we measure striatal GABA levels using magnetic resonance spectroscopy (MRS) and resting state parameters using functional magnetic resonance imaging (fMRI). Resting state parameters include activity within the BGN, as determined by the low frequency power (LFP) within the network, and the functional connectivity between the BGN and somatomotor network (SMN). Specifically, we examine the interrelation between GABA, resting state parameters, and performance (i.e., accuracy) in conflict monitoring using a Simon task. Response control was affected by striatal GABA+ levels and activity within the BGN, especially when response selection was complicated by altered stimulus-response mappings. The data suggest that there are two mechanisms supporting response selection accuracy. One is related to resting state activity within the BGN and modulated by striatal GABA+ levels. The other is related to decreased cortico-striatal network connectivity, unrelated to the GABAergic system. The inclusion of all three factors (i.e., striatal GABA+ levels, activity within the BGN, and BGN-SMN network connectivity) explained a considerable amount of variance in task accuracy. Striatal neurobiochemical (GABA+) and parameters of the resting state BGN represent important modulators of response control. *Hum Brain Mapp 36:4383–4393, 2015.*

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INTRODUCTION

One important issue for cognitive control is response selection processes. Response selection processes are strongly demanded in situations where we have to execute responses against natural response tendencies [Keye et al., 2013]. Paradigms used to examine these processes present stimuli that usually have a relevant feature determining a response and an irrelevant feature that requires an alternative response. One example is the "Simon effect" [Simon and Small, 1969]. This effect refers to the fact that responses are faster and less error-prone when the task-irrelevant stimulus information corresponds to the location of the correct response. However, when the dimensions mismatch, responses are slowed and response errors are frequent [Keye et al., 2013]. In such conflicting situations the cognitive system is required to increase control [Botvinick et al., 2001] and for these processes it has been shown that a cognitive control network encompassing the anterior cingulate cortex (ACC) and lateral prefrontal regions is important [Botvinick et al., 2004].

Recently, it has been shown that different cognitive control functions seem to depend upon striatal concentrations of gamma-amino butyric acid (GABA) [Quetscher et al., in press; Yildiz et al., 2014]. It has been shown using magnetic resonance spectroscopy (MRS) that higher GABA concentrations are related to better response inhibition, response stopping, and switching processes [Quetscher et al., in press; Yildiz et al., 2014]. However, conflict monitoring processes, as another important instance of cognitive control functions, have until now not been examined. Yet, it is possible that conflict monitoring processes, as examined via the Simon task are affected by striatal GABA levels, because diseases affecting the basal ganglia, striatal GABA functions, and the ACC have been shown to affect conflict monitoring [Beste et al., 2008, 2012; Fielding et al., 2005; Willemssen et al., 2011; Wylie et al., 2010, 2012]. Also due to the supposed importance of GABAergic medium spiny neurons (MSNs) for response selection processes, as derived from theoretical basal ganglia models [Beste and Saft, 2015; Bar-Gad et al., 2003; Redgrave et al., 1999], it is possible that response selection processes under conflict are affected by striatal GABA levels.

As regards regional GABA concentrations, recent results suggest that GABA modulates intrinsic functional connectivity of specific networks [Duncan et al., 2014; Stagg et al., 2014]. Within the default mode network (DMN), for example, it has been shown that GABA concentrations in the posterior-medial cortex correlate negatively with functional connectivity within the DMN [Kapogiannis et al., 2013]. Similarly, it has been reported [Arrubla et al., 2014] that GABA concentrations in the posterior cingulate cortex are negatively correlated with the connection strength of putamen to the DMN. Interrelations between the DMN and GABA concentrations have also been reported for the ACC [Northoff et al., 2007; Shin et al., 2013] and hence functional neuroanatomical structures that are of importance for conflict monitoring functions. GABA levels therefore seem to modulate resting state functional connectivity. However, resting state networks have also been reported for the basal ganglia [Damoiseaux et al., 2008; Di Martino et al., 2008; Robinson et al., 2009] and hence for structures for which the GABA system has been shown to modulate some forms of cognitive control processes relevant to response selection and conflict monitoring. It is therefore possible that there is an interrelation between striatal GABA levels, resting state functional connectivity, and executive control functions. This interrelation has, however, until now not been tested.

In the current study, we examine the interrelation between striatal GABA levels, blood-oxygen-level dependent (BOLD) -related fluctuations in basal ganglia network (BGN), and performance in conflict monitoring using a Simon task. We do so by examining airplane pilot trainees (APTs) in comparison to healthy controls. APTs reflect an interesting "model" to examine neurobiological processes that are related to superior cognitive control mechanisms [Yildiz et al., 2014] and therefore offer the possibility to examine whether possible differences in performance levels are reflected at a neurofunctional level in terms of altered GABA levels and BOLD fluctuations. We hypothesize that better response selection during response conflict is related to higher striatal GABA levels, given recent reports from other cognitive tasks [Quetscher et al., in press; Yildiz et al., 2014] and theoretical accounts proposing an important role of striatal GABA levels in response control [Bar-Gad et al., 2003; Redgrave et al., 1999]. However, the Gratton effect [Gratton et al., 1992] is also important in conflict monitoring [Botvinick et al., 2001; Duthoo and Notebaert, 2012], which describes lower conflict effects after a trial in which also an incongruent stimulus-response mapping was evident, compared to the effect after a trial with congruent stimulus-response mapping. The Gratton effect thus describes the consequences of perceived conflict on subsequent action selection processes. If striatal GABA levels modulate conflict detection, it is possible that striatal GABA level modulate processes related to the consequences of conflict as well.

Abbreviations

BGN	basal ganglia network;
FNC	internetwork functional connectivity
GABA	gamma-amino butyric acid
LFP	low frequency power

We additionally hypothesize that performance will relate to altered network connectivity, but given the heterogeneous roles of the basal ganglia in motor control, it is unclear in which direction this will be. Here, we examine the functional connectivity between the BGN and somatomotor network (SMN). We decided on examining the SMN instead more decision-related networks, e.g. salience and attention networks, since we were more interested in the effect of the basal ganglia on the motor output rather than the effects of other networks on the basal ganglia. Although the relationship between the BGN and SMN is bidirectional, we suppose the influence of the BGN on the SMN, rather than the SMN's influence on the BGN, would be more relevant for task performance presented in the Simon task (including the Gratton effect). Finally, we look at low frequency power (LFP) within the BGN as a measure of local network BOLD-related activity and postulate that, as shown for motor [Fox et al., 2007], sensory [Haag et al., 2015], and executive control functions [Hao et al., 2013; Xu et al., 2014], local network BOLD fluctuations in the BGN would correlate with performance on the Simon task.

MATERIALS AND METHODS

Subjects

Twenty-two APTs (age 23.7; SD 2.5; range 20–30; five females) and 18 non-trainees (age 23.9; SD 2.5; range 20–30; five females) participated in this study. All participants were free of neurological symptoms, were unmedicated, and provided written informed consent. This study was approved by the Ethics Commission of the Ruhr-Universität Bochum and was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Simon Task

The Simon task was identical to previous work by our group [Stock and Beste, 2014; Stock et al., 2013]. The task was structured as follows: A white fixation cross and two horizontally aligned white frame boxes were continuously displayed in the center of a dark blue screen (1.1° distance between fixation cross and the inner border of the frames). Each trial began with the simultaneous presentation of a target stimulus (a yellow capital letter "A" or "B") and a noise stimulus (three white horizontal bars) in one of the two frames (target and noise stimuli were ~0.5° wide and 0.6° high). After 200 ms, the stimuli disappeared and the trial was ended by the first response. If the participants did not respond within the first 500 ms after the onset of the trial, a speed-up sign (containing the German word "Schneller!" which translates to "Faster!") was presented above the stimuli. In case no response was given, the trial automatically ended 1,700 ms after its onset and was coded as a "miss." The response–stimulus intervals (RSIs) varied randomly between 2,000 and 2,500 ms.

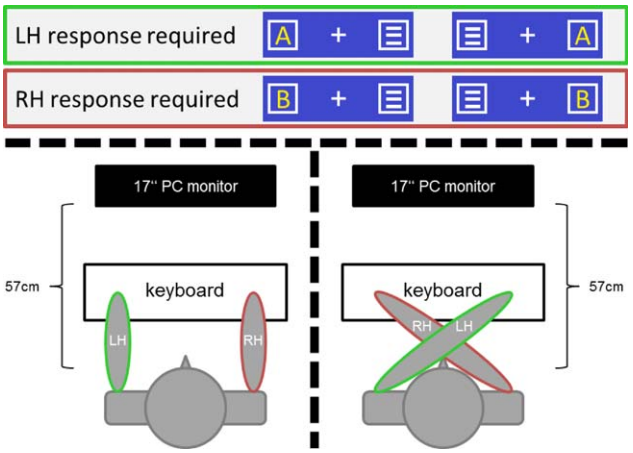

Figure 1.
The target stimuli (letters) could be located in either of the boxes as illustrated in the top rows. Letter A required a reaction of the left hand (respective box and limbs edged green) while letter B required a reaction of the right hand (respective box and limbs edged red). The parallel hands condition is shown in the bottom left part of the figure while the crossed hand condition is shown on the right side. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The experiment consisted of eight blocks, each comprising 160 trials. The four stimuli ("A" on the left side/"A" on the right side/"B" on the left side/"B" on the right side) were randomized and occurred equally often. For all blocks, participants were instructed to respond using the left index finger whenever the target stimulus was an "A" and to respond using the right index finger whenever the target stimulus was a "B" (in both cases irrespective of the target's location on the screen). All trials in which the target stimulus and the correct response button were located in the same hemifield (i.e., on the same side of the body) were classified as spatially congruent. Hence, all trials in which the stimulus and the button were located in opposing hemifields were classified as spatially incongruent. In Blocks 1, 3, 5, and 7 they were asked to respond with parallel hands while they were asked to cross their hands in Blocks 2, 4, 6, and 8 (i.e., placing the left arm above the right arm). The crossed-hands condition was included to further increase task difficulty to maximize possible performance (i.e., both accuracy and reaction time (RT)) differences between the examined groups. The experimental setup is outlined in Figure 1.

Data Acquisition

Participants were scanned using a Philips 3.0 T Achieva X scanner using a 32-channel head coil. The scanner was allowed at least 30 min of downtime to avoid gradient-induced field drifts [Lange et al., 2011] that could affect

GABA quantification [Harris et al., 2014]. All participants underwent one high-resolution structural T1-weighted scan (MPRAGE, Repetition Time (TR)/Echo Time (TE): 8.5/3.9 ms, voxel size (1 mm)^3 isotropic, field of view (FOV) 256 × 256 × 220 mm), followed by a MEGA-PRESS sequence with a separate water reference acquisition (see below), and finally a T2*-weighted resting state scan (Gradient-echo echo planar imaging (EPI), TR = 2,500 TE = 35 ms, Flip angle = 90°, FOV: 224 mm × 232 mm, 39 axial slices, slice thickness = 3 mm, no gap, 200 dynamic scans, 5 dummy scans, total acquisition time: 8 min 37 s).

MRS

A (3 × 3 × 2.5 cm^3) voxel was centered on the striatum using the fast T2-weighted structural reference image, acquired directly before MRS. Spectra were acquired using MEGA-PRESS, a GABA-sensitive editing sequence [Edden and Barker, 2007; Mescher et al., 1998], with the following parameters: TR/TE = 2,000/68 ms; a 15-ms editing pulse was applied either at 1.9 ppm (ON) or at 7.46 ppm (OFF); Segments of 16 ON followed by 16 OFF acquisitions (sampling rate 2,048 data points, spectral bandwidth 2 kHz) were interleaved 16 times, resulting in a total of 16 × 16 = 256 scans and a total acquisition time of 8.5 min per voxel. Spectra from both the left and right striatum were acquired to rule out potential laterality effects. Fat suppression was accomplished using outer volume suppression slabs and water suppression using VAPOR [Tkác et al., 1999]. Macromolecules were not suppressed and therefore those at the 1.72 ppm resonance were also partially inverted by the 1.9 ppm editing pulse. Since this signal is coupled to the 3.00 ppm resonance [Behar et al., 1994], those macromolecules would also have been affected by the editing pulse and therefore contribute to the difference spectra. Thus, GABA in this study refers to GABA+ macromolecules (GABA+). A total of 16 additional averages without water suppression were acquired, one at the beginning of each of the ON and OFF scan segments, and used as reference data for frequency and phase correction. A sample of a GABA+ spectrum is shown in Figure 2.

GABA+ and various metabolite concentrations were quantified using LCModel [Provencher, 1993] (version 6.2-0R), which fits in vivo MR spectra as a linear combination of single metabolite "basis spectra" (for details see Supporting Information Material by Dydak et al. [2011]. Specifically, GABA was quantified from the MEGA-PRESS difference spectra using basis spectra created using density matrix simulations. GABA fitting with LCModel was optimized by using a flexible baseline function to fit the confounding 3.0 ppm macromolecule peak [Dydak et al., 2011]. While this additional degree of freedom results in slightly larger %SDs, it provides a more accurate estimation for pure GABA [Dydak et al., 2011; Long et al., 2011]. Fits exceeding a 25% SD were excluded from further analysis. This threshold was chosen due to the flexible baseline approach and is well within accepted standards used in GABA-MRS studies [Marjańska et al., 2013; Silveri et al., 2013]. Additionally, the average %SD value for the GABA LCModel fits was 15.7 ± 3.5, and no GABA-edited spectra had to be excluded because of poor quality. All spectra had a linewidth of ≤10 Hz as determined by LCModel. To control for individual differences, GABA+ concentration was referenced to total creatine (tCr), which was obtained from fitting the averaged OFF spectra of the MEGA-PRESS acquisition, again using LCModel. The tCr peak was fit with low error ($tCr_{mmol/l} = 7.01 \pm 0.61$), supporting its usefulness as an internal reference. Since tCr is related to energy metabolism and, in the brain, neurons and glia have the highest metabolism, tCr also provided a partial volume correction. Therefore, no further correction using structural image segmentation was performed.

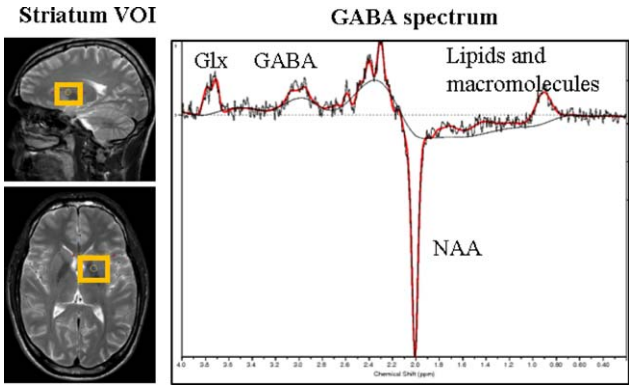

Figure 2.
Illustration of the placement of the volume of interests in the striatum including a representative example of the MEGA-PRESS edited GABA spectrum. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

fMRI Data Processing

Functional images were slice time corrected, realigned, normalized to the EPI template, and smoothed (6 mm full width at half maximum (FWHM) kernel) using SPM8. For the ICA, images were then entered into the GIFT Toolbox where they were intensity-normalized before data processing. For the ICA, data were reduced in two steps; the first step reduced each subject's dataset from 200 to 70 principle components and the second group level decomposition resulted in a user-defined 25 independent components to be used for further analyses. Infomax was chosen for the group ICA algorithm due to its robustness in a low-order dataset. This was run 20 times (using ICASSO) to improve the independent components' (IC) stability, which then was confirmed using the Iq measure of stability. Group ICs were visually inspected and then spatially sorted against the networks provided by the Stanford Resting State Network templates (http://findlab.stanford.edu/functional_ROIs.html). This was carried out using the "spatial

correlation" function provided by the GIFT toolbox. The IC with the highest spatial correlation with the basal ganglia and the sensorimotor templates were identified as the best representative of that resting state network and used for further analyses.

Group ICs were then back reconstructed using the GICA algorithm to create subject-specific component maps and time courses. An individual's back-reconstructed (br) map thus identifies those voxels that are both spatially and temporally most consistent with the group-identified IC. Components were not further scaled due to the preprocessing step of intensity normalization, which returns br maps in units of percent signal change. The higher the average component values, the stronger the intranetwork functional connectivity strength. Spectral analyses using MANCOVAN calculated the power of each frequency within the measured frequency band [(0–0.2 Hz, given a Nyquist frequency of 1/(TR/2)]. Individual time courses were log-transformed to obtain a normal distribution before statistical analyses. The sum of LFP (0.01–0.1 Hz) was taken to determine the strength of the "signal of interest" within each network. RSNs typically have high power in the low frequency range and low power in the high frequencies, characteristic of gray matter signal. Thus, the higher the power of the low frequencies (0.01–0.1 Hz), the stronger the neural component contributing to the BOLD fluctuations, and the more resting state activity there is within the network. Also using the MANCOVAN option within GIFT, the average BOLD signal within the BGN was temporally correlated with the BOLD signal within the SMN for each subject to determine the BGN–SMN internetwork connectivity (i.e., FNC).

Statistics

STATISTICA (StatSoft, Inc. version 10) and SPSS were used to analyze performance in the Simon task (RT and accuracy data), GABA+/tCr ratio (averaged between hemispheres), LFP within the BGN, and BGN–SMN internetwork functional connectivity (FNC). Partial correlation analyses investigated pair-wise relationships between task accuracy (number of correct responses in crossed-hands condition), GABA+/tCr levels, LFP of the BGN, and FNC between BGN and SMN, while controlling for (i.e., after the removal of the variance associated with) age and gender. All figures depict the pair-wise partial correlations after the removal of age and gender. Multiple regression analyses determined the combination of variables that explained the greatest amount of variance in task accuracy, as measured by the adjusted R^2. Results report the means ± SEM.

RESULTS

Behavioral Data

For the RT data, the mixed-effects ANOVA revealed a main effect "hand position" ($F(1,38) = 23.29$; $P < 0.001$; $\eta^2 = 0.380$) showing that RTs were faster when hands were positioned parallel to each other (398 ms ± 4.1) than when hands were crossed (406 ms ± 4.6). The main effect "congruency" ($F(1,38) = 201.39$; $P < 0.001$; $\eta^2 = 0.841$) showed that RTs were faster in congruent (385 ms ± 4.2) than in the incongruent condition (418 ms ± 4.7). However, there was no main effect "group" ($F(1,38) = 2.22$; $P > 0.15$) or any interaction (all $F < 1.4$; $P > 0.23$). Yet, results were different for the accuracy data.

For the accuracy data (response errors), the mixed-effects ANOVA also revealed a main effect main effect "hand position" ($F(1,38) = 22.60$; $P < 0.001$; $\eta^2 = 0.373$) showing that accuracy was higher when hands were positioned parallel to each other (185 ± 0.8) than when hands were crossed (181 ± 0.9). Similarly, accuracy was higher in the congruent (192 ± 1.1) than in the incongruent condition (174 ± 0.9) ($F(1,38) = 172.33$; $P < 0.001$; $\eta^2 = 0.819$). There was also a main effect "group" ($F(1,38) = 32.81$; $P < 0.001$; $\eta^2 = 0.463$) showing that accuracy was higher in Pilot trainees (188 ± 1.1) than in controls (178 ± 1.2). Interestingly, there was also an interaction "congruency × group" ($F(1,38) = 21.03$; $P < 0.001$; $\eta^2 = 0.356$). Post hoc independent samples t-tests show that there were no group difference in the congruent condition (Pilot trainees: 193 ± 0.8; controls: 190 ± 2.2; $t_{38} = 1.46$; $P > 0.15$), but in the incongruent condition (Pilot trainees: 182 ± 1.2; controls: 167 ± 1.5; $t_{38} = 7.81$; $P < 0.001$). Group differences were thus most pronounced in the more difficult (crossed hands) incongruent condition. There were generally no effects concerning the rate of missed responses (all $F < 0.5$; $P > 0.5$).

To examine the Gratton effect for the RT and error rate data we subtracted the Simon effect following correct incongruent trials (iI – iC) from the effect following correct congruent trials (cI – cC). In fact there was a group difference when error rates were used as parameter, such that control subjects had a more prominent Gratton effect in error than APTs, specifically in the parallel Simon task condition (controls: 7.00 ± 0.70, Pilot trainees: 5.05 ± 0.64; $F(1,38) = 4.19$, $P < 0.05$).

GABA+ Measures

GABA+ and tCr concentrations could be reliably measured and quantified in all participants. Total Cr levels (i.u.) did not differ between the groups (trainees: 5.94 ± 0.31; controls: 6.15 ± 0.12, t-test $P = 0.52$ ns), nor did corrected GABA+/tCr values (trainees: 0.22 ± 0.04; non-trainees: 0.23 ± 0.04; $t_{38} = -0.51$, $P = 0.61$ ns). The average GABA+/tCr level in the basal ganglia was positively correlated with correct responses in the crossed hands incompatible condition, both within the study cohort as a whole ($r = 0.40$, $P < 0.02$) as well as within trainee ($r = 0.60$, $P < 0.01$) and non-trainee ($r = 0.72$, $P < 0.01$) groups, separately. This correlation shows that higher GABA+/tCr values were associated with higher accuracy on the Simon task when response selection was most difficult (i.e., in

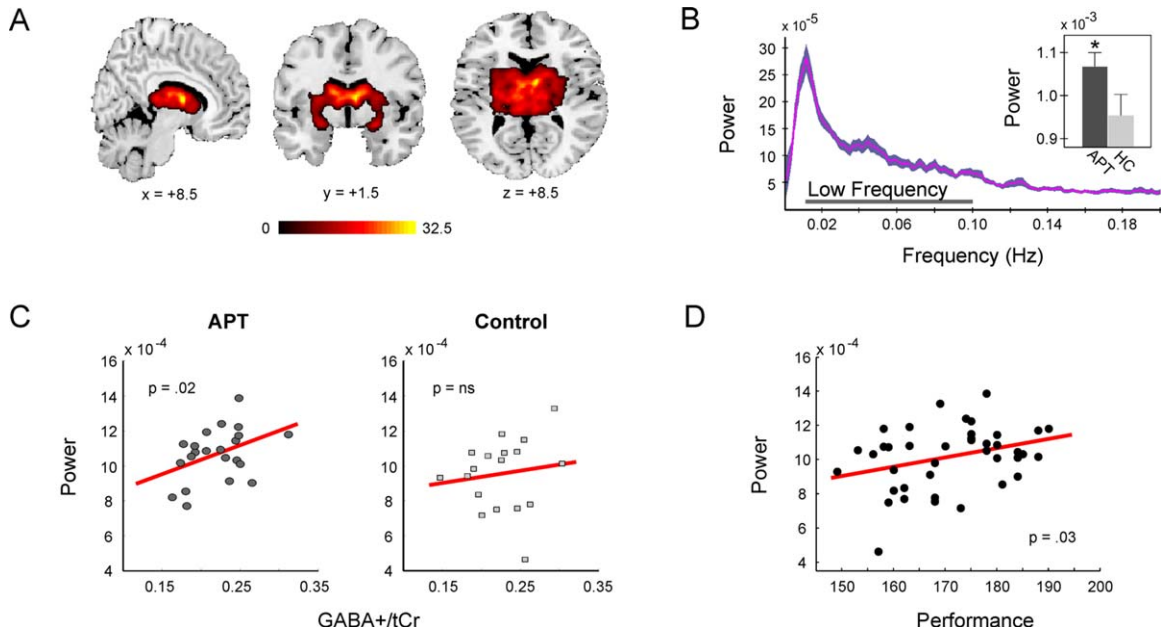

Figure 3.

The BGN (**A**) had elevated power **(**sum) of the low frequency range (0.01–0.1 Hz) in APTs (**B**, inset, "APT"). The sum of LFP was positively related to striatal GABA+/tCr in the APT group (**C**) and to Simon task performance **(**i.e. accuracy as measured by the number of correct responses) in the crossed hands condition within the group as a whole (**D**). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

crossed hands, incompatible condition). To control whether the correlations using GABA+/tCr were driven by the creatine (tCr) and not by GABA, we used the tCr concentration (i.u.) as obtained from the MEGA-PRESS spectra as additional regressor in the analyses. These analyses revealed no effect of tCr on the neurophysiological and the behavioral parameter (all $\beta < 0.045$; $t < 0.64$; $P > 0.7$). There was generally no effect of GABA+/tCr values on parameters of the Gratton effect (all $\beta < 0.055$; $t < 0.68$; $P > 0.6$).

fMRI Measures

Functional networks could be identified for both motor-related networks of interest, i.e. the BGN and SMN. Spatial sorting identified IC7 as having the highest spatial correlation with the basal ganglia template ($r = 0.41$; Fig. 3A), and included the bilateral putamen, caudate, globus pallidus, substantial nigra, subthalamic nucleus, and thalamus. This is consistent with previous reports of the BGN [Neta et al., 2015; Robinson et al., 2009]. IC15 was identified as the sensorimotor network ($r = 0.26$) and included primary motor and somatosensory cortices, as well as bilateral premotor and supplementary motor areas (SMA). Simon task accuracy was correlated with LFP and BGN-SMN FNC measures. In particular, higher accuracy in the crossed hands conditions was related to higher LFP of the basal ganglia (IC7) ($r = 0.35$, $P < 0.05$; Fig. 3D) as well as lower internetwork connectivity between the basal ganglia and SMNs ($r = -0.38$, $P < 0.02$; Fig. 4), over the study cohort. Task accuracy in the incongruent crossed hands condition was not significantly correlated with intrinsic functional connectivity within either the BGN ($P = 0.41$) or the SMN ($P = 0.64$).

Group differences were seen within the BGN network, where LFP was significantly higher in APTs as compared to the control group (APT: 0.0011 ± 0.00002, controls: 0.0010 ± 0.00005; $t = 2.03$, $P < 0.05$; Fig. 3B). No group

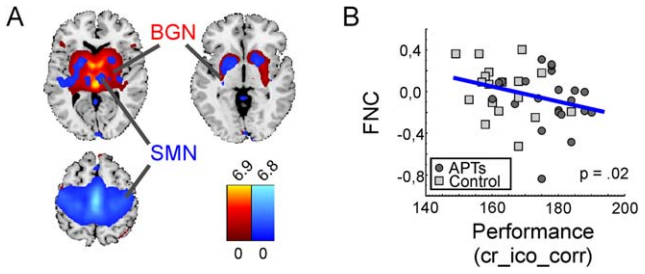

Figure 4.

Better Simon task accuracy was negatively correlated with functional connectivity between the BGN and the SMN. FNC = functional connectivity (between networks); cr_ico_corr = Number of correct responses (accuracy) on the crossed hands incongruent Simon task condition. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

differences were measured in functional connectivity within the BGN (APT: 10.21 ± 0.09, controls: 10.40 ± 0.11; $t = -1.39$, $P = 0.17$), within the SMN (APT: 6.74 ± 0.04, controls: 6.77 ± 0.07; $t = -0.35$, $P = 0.73$), or between the BGN and SMN (APT: -0.08 ± 0.06, controls: 0.02 ± 0.06; $t = -1.13$, $P = 0.26$).

However, using the Gratton effect as the dependent variable and GABA, LFP, and FNC as the three independent variables revealed no significant relationships ($P > 0.7$).

IC Measures and GABA+

Since GABA+/tCr was measured only in the basal ganglia, the identified BGN (IC7) was the only network investigated for its relationship to GABA+. While GABA+ did not significantly correlate with the intrinsic functional connectivity strength within the BGN ($P > 0.20$), GABA+/tCr was positively correlated with LFP of the BGN for the pilot trainee group ($r = 0.51$, $P < 0.05$; Fig. 3C), i.e., the more GABA+/tCr, the higher the BGN BOLD-related fluctuations, i.e. activity. The control group showed only a trend toward a relationship with GABA+/tCr levels in the basal ganglia ($P = 0.10$). GABA+/tCr levels were not associated with SMN–BGN internetwork functional connectivity ($P = 0.87$).

Best Predictors of Task Accuracy

Multiple regression analyses revealed that the highest amount of variance in task accuracy could be explained when the model included FNC (b: -0.43, $P < 0.01$), LFP (b: 0.34, $P < 0.02$), gender (-0.30, $P < 0.03$), and GABA+/tCr concentration (b: 0.30, $P < 0.04$), with the P-values representing the partial correlations. The full model explained 39% of the variance (adjusted R^2) and was highly significant ($F(4,35) = 7.20$, $P < 0.001$). Even in the absence of gender, MRS and fMRI parameters together could account for 32% of the variance in accuracy (adjusted $R^2 = 0.32$, $F(3,36) = 7.02$, $P < 0.001$). See Figure 5 for summary.

DISCUSSION

In this study, we investigated the interrelation of striatal GABA+ levels with executive control functions as examined via a modified Simon task. We also looked at resting state functional connectivity and activity (as assessed by the power of BOLD low frequency fluctuations) of the BGN and its relation to both, local GABA concentrations and task performance. This was done using a group comparison between APTs and controls. The behavioral data revealed typical Simon effects, i.e. higher error rates and RTs in the compatible than the incompatible condition and in the crossed vs. the uncrossed condition. The results further show that APTs were more accurate than controls in the task and this accuracy advantage shown in APTs was most pronounced in the most difficult incongruent condition.

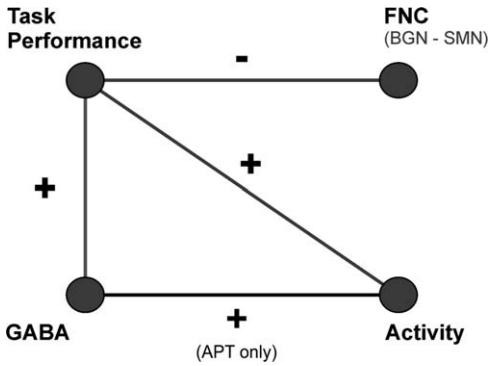

Figure 5.

Summary of the interrelationships between Simon task performance (i.e., accuracy as measured by the number of correct responses to the crossed hands condition) and striatal GABA+/tCr, striatal activity (i.e., LFP of the BGN), and functional connectivity between basal ganglia and SMNs (FNC). "(APT only)" denotes that the relationship between GABA and activity was only seen within the airplane pilot trainee (APT) group.

It was notably this crossed hands incompatible condition where correlations with striatal GABA+/tCr levels were obtained. It is possible that the lack of effects obtained for the parallel hands condition is due to a ceiling effect in performance, which reduced variance necessary to find significant correlations. Nevertheless, the results show that accuracy was higher, when the striatal GABA+/tCr content was higher. This effect is unbiased with respect to the tCr concentrations. The results suggest that higher striatal GABA+/tCr concentrations are related to better conflict monitoring and response selection processes. The striatal GABA-system has been suggested to play a major role in response selection processes [Gurney et al., 2004; Plenz, 2003; Redgrave et al., 1999]. This is because response selection at a striatal level has been conceptualized as function of MSNs [Bar-Gad et al., 2003] for which GABA is a key element. Striatal MSNs have been suggested to form a winner-takes-all network (WTA; i.e., meaning that the network of inhibitory connections between MSNs is assumed to inhibit neighboring neurons). This network architecture makes it possible to inhibit competing and conflicting response tendencies. It is possible that via such a mechanism the striatal GABA system supports increases in cognitive control. The higher striatal GABA+ concentrations may lead to a more efficient WTA network. This in turn may lead to a better inhibition of conflicting response tendencies and thus to a better accuracy in the task. The finding that this effect was only evident for the most difficult condition (i.e., crossed incongruent condition) either suggests that at lower complexity levels other factors, unrelated to GABAergic neural transmission modulate striatal response selection mechanisms, are important (e.g. dopamine), or (related) that at lower complexity levels the GABAergic system is not as much important as for higher

complexity levels because demands on response selection processes do not reach a critical level. The finding that the Gratton effect was not related to GABA levels suggest that GABA is only important to resolve the current amount of conflict in a given trials, but is not important for neural mechanisms mediating the effect of conflicts on subsequent response selection, which is reflected in the Gratton effect. Nevertheless, group differed in the Gratton effect with APTs showing a smaller Gratton effect than control. Given the superior overall performance in APTs this may suggest that response selection processes are more stable in APTs, while in controls response selection processes are more susceptive to modulations of response conflict. This may be the reason why the Gratton effect was larger in controls.

In addition to better conflict monitoring and response selection being related to higher striatal GABA+/tCr levels, better task accuracy was also related to a higher resting state activity level in the BGN, as measured by BOLD LFP. Similar results have previously been reported for higher resting state activity in motor areas [Fox et al., 2007]. It is possible that the higher resting state activity in the BGN leads to a higher "readiness" of this important striatal response selection network. This higher readiness of the BGN response selection network may be beneficial for response selection as processing resources may be permanently be pre-activated at a higher level. This higher pre-activity may make it easier for the BGN network to initiate response selection processes when these are strongly demanded. In line with this interpretation it has been shown that preparatory effects can augment performance during response selection in a Simon task [Strack et al., 2013]. It therefore seems that the higher BGN resting state activity levels may serve similar functions as the striatal GABA+/tCr level for response selection processes. As there was also no relation of resting state measures with the Gratton effect (as it is also shown for the GABA+/tCr level), it seems that the examined resting state measures are only important to resolve the current amount of conflict in a given trials, but is not important for neural mechanisms mediating the effect of conflicts on subsequent response selection. However, this may at least partly be due to the fact that striatal GABA+/tCr and BGN resting activity are related.

Our current findings that striatal GABA+/tCr promotes higher BGN resting activity are in line with data from pharmacological interventions in healthy participants, which have shown a similar effect of GABA agonists on resting state parameters. BOLD synchrony, for example, was increased in multiple networks during sedation with midazolam [Greicius et al., 2008; Kiviniemi et al., 2005], and zolpidem [Licata et al., 2013], with stimulant administration reducing resting state activity [Rack-Gomer et al., 2009]. Although pharmacological intervention cannot target just one region or network due to the systemic application, the aforementioned studies do suggest that GABA enhances local activity at rest. Our results on the basal GABAergic tone and local activity are in concordance with GABA-A interventions. We cannot, however, say whether this relates to the BOLD response to cognitive conflicts. Nonetheless, we show that the both resting BOLD activity and GABA have a direct relationship with performance. The fact that better conflict control was associated with both higher striatal GABA+/tCr and higher BGN activity, but that this activity was only positively associated with local GABA+/tCr in the high performers, suggests that multiple factors contribute to performance on cognitively demanding tasks. Nonetheless, striatal GABA+/tCr seems to be a common mechanism supporting performance on various cognitive control processes, as supported not only by the current data, but also previous data showing higher striatal GABA+/tCr being related to better response inhibition [Quetscher et al., in press; Yildiz et al., 2014] and more efficient action cascading [Yildiz et al., 2014]. The latter study additionally noted a positive correlation between striatal GABA+/tCr and EEG measures associated with pre-motor responses, specifically those related to cognitive conflict management. Our data support this finding, confirming the relevance of striatal GABA in conflict management and extending it to include a role in local resting activity, as measured by LFP, at least in high performers.

While local properties of the striatal network were related to conflict control, performance was further affected by connectivity between cortical and striatal networks. Specifically, we report that reduced BGN-SMN internetwork connectivity was associated with higher task accuracy. This may be a counterintuitive finding as cortico-striatal networks are important for action control, however we suspect that this likely due to the differential response the basal ganglia show to cognitive conflicts. Whereas other areas (e.g., ACC, SMA, and parietal regions [Liu et al., 2004; Wittfoth et al., 2008] are consistently activated by cognitive conflicts, the basal ganglia show both a delayed temporal response [Neta et al., 2015] and a contradictory decreased BOLD response following errors in performance [Wittfoth et al., 2009]. Our resting state data reflect a similar disconnect between the basal ganglia and cortical regions involved in motor performance (e.g., M1) and conflict monitoring (e.g., pre-SMA/SMA), and we postulate that this may allow the BGN to be less influenced by top-down mechanisms during a task, thereby allowing the network more stability. Parkinson's patients, for example, who show marked deficits in response selection, executive function, and control, also show increased cortico-striatal connectivity [Baudrexel et al., 2011; Fernández-Seara et al., 2015]. Thus, increased network connectivity is not necessarily associated with better performance in cognitive control and response selection. In particular for the Simon task, our data support the notion that reduced motor-related cortico-striatal connectivity may be one way high performers control conflicts and optimize response accuracy. Unlike the data supporting a

role of GABA+/tCr in BGN network BOLD activity level, our data show no evidence that striatal GABA+/tCr relates to cortico-striatal functional connectedness. This is consistent with pharmacological evidence that connectivity within resting state networks was increased by GABA-A modulators without altering connectivity between networks [Licata et al., 2013]. Thus the GABAergic system is related to the local BOLD activity, but is not necessarily related to internetwork connectivity.

A few limitations of this study are as follows. Importantly, while voxel placement was carefully executed for all subjects, the size of the MRS voxels used in the study prohibited the exclusive measurement of the basal ganglia. The contributions of external structures, e.g. the anterior thalamus and insular regions can therefore not be excluded, but should only contribute minimally in relation to the basal ganglia. Additionally, subjects were tested in a cross-sectional manner, limiting our conclusions to associations rather than causal interactions. While we cannot identify how high performers acquired the skill, we identify striatal activity and neurobiochemical mechanisms as likely playing a role. Since both APTs and control participants were healthy, the relationships we find in this study should be more generally applicable to the population, such that methods increasing striatal GABA+/tCr and local BOLD activity, as well as those reducing cortico-striatal connectivity, may be a target to support cognitive conflict performance. While effective connectivity analyses would shed light on causal relationships between imaging and behavioral measures, our study was not optimized for this, given the relatively long (2.5-s) TR. Therefore, it is still unclear whether targeting cortical or striatal regions would be most effective to modulate cognitive control. A further limitation is that, since we did not acquire task-evoked BOLD data, our conclusions cannot be extended to the brain's response during cognitive conflicts. It would be interesting, however, to see whether the relationship between GABA+/tCr, resting state BOLD parameters, and task accuracy extend to task-induced BOLD activity. Finally, the GABA+ signal measured in this study may still include residual signal from macromolecules not taken care of by our fitting strategy [Mullins et al., 2013].

In summary, our data suggest that there are two mechanisms supporting response selection performance. One is related to resting state BGN activity and modulated by striatal GABA+/tCr levels. The other is related to decreased cortico-striatal network connectivity, unrelated to the GABAergic system. The regression analysis (beta weights) shows that effect of network interconnectivity is even larger than the effect of BGN activity level thus making the level of functional connectedness an important modulator for task performance. The inclusion of all three factors (i.e., striatal GABA+/tCr levels, BGN resting state activity, and BGN network connectivity) in a regression model explained more than 30% of variance in task accuracy, suggesting that general neurobiochemical parameters (i.e., general GABA+/tCr level) and basic hemodynamic parameters (i.e., resting BOLD activity level and network interconnectedness) are important to consider when being interested in interindividual differences in cognitive control.

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