Striatal GABA in Action Control
A combined MRS, EEG, and fMRI Approach

Inaugural-Dissertation
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
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<tr>
<td>APTs</td>
<td>airplane pilot trainees</td>
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<tr>
<td>BG</td>
<td>basal ganglia</td>
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<tr>
<td>BGN</td>
<td>basal ganglia network</td>
</tr>
<tr>
<td>BHC</td>
<td>benign hereditary chorea</td>
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<tr>
<td>BOLD</td>
<td>blood-oxygen-level dependent</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
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<td>cTBS</td>
<td>continuous theta burst stimulation</td>
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<td>DA</td>
<td>dopamine</td>
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<tr>
<td>dIPFC</td>
<td>dorsolateral prefrontal cortex</td>
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<td>EEG</td>
<td>electroencephalography</td>
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<td>EFs</td>
<td>executive functions</td>
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<td>ERP</td>
<td>event-related potential</td>
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<tr>
<td>FEF</td>
<td>frontal eye fields</td>
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<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>FSI</td>
<td>fast-spiking interneurons</td>
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<tr>
<td>GABA</td>
<td>γ-Aminobutyric acid</td>
</tr>
<tr>
<td>GABA_A</td>
<td>GABA_A receptor</td>
</tr>
<tr>
<td>GABA_B</td>
<td>GABA_B receptors</td>
</tr>
<tr>
<td>GAD</td>
<td>glutamic acid decarboxylase</td>
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<tr>
<td>GAT</td>
<td>GABA transporter</td>
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<tr>
<td>Glx</td>
<td>glutamate and glutamine</td>
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<td>Gpe</td>
<td>globus pallidus, internal segment</td>
</tr>
<tr>
<td>Gpi</td>
<td>globus pallidus, external segment</td>
</tr>
<tr>
<td>HD</td>
<td>Huntington’s disease</td>
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<tr>
<td>IPSC</td>
<td>inhibitory postsynaptic current</td>
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<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
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<tr>
<td>M1</td>
<td>primary motor cortex</td>
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<tr>
<td>MDD</td>
<td>major depressive disorder</td>
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<tr>
<td>mI</td>
<td>myo-inositol</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MRS</td>
<td>magnetic resonance spectroscopy</td>
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<tr>
<td>MSN</td>
<td>medium spiny neurons</td>
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<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>PLF</td>
<td>phase locking factor</td>
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<td>RT</td>
<td>response time</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>S1</td>
<td>primary somatosensory cortex</td>
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<tr>
<td>SCD</td>
<td>stop-change delay</td>
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<td>SCT</td>
<td>stop-change task</td>
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<tr>
<td>SMA</td>
<td>supplementary motor area</td>
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<td>SMN</td>
<td>somatomotor network</td>
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<tr>
<td>SN</td>
<td>substantia nigra</td>
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<tr>
<td>SNc</td>
<td>substantia nigra pars compacta</td>
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<tr>
<td>SNr</td>
<td>substantia nigra pars reticula</td>
</tr>
<tr>
<td>STN</td>
<td>subthalamic nucleus</td>
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<tr>
<td>TAN</td>
<td>tonically active neurons</td>
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<tr>
<td>tCho</td>
<td>total choline</td>
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<tr>
<td>tCr</td>
<td>total creatine</td>
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<tr>
<td>tDCS</td>
<td>transcranial direct current stimulation</td>
</tr>
<tr>
<td>TS</td>
<td>tourette syndrome</td>
</tr>
<tr>
<td>tVNS</td>
<td>transcutaneous vagus nerve stimulation</td>
</tr>
<tr>
<td>vIPFC</td>
<td>ventrolateral prefrontal cortex</td>
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<tr>
<td>WTA</td>
<td>winner-takes-all</td>
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Chapter 1

General Introduction
1.1 Theory of Executive Functions and Action Control

The ability to plan and execute goal directed behaviour, to choose the appropriate action in a constantly changing environment is vastly important for our everyday life. If this ability is impaired, it can affect all aspects of behaviour and lessen our capacity to live an independent, constructive, self-serving, and productive life (Lezak, 2004). Exactly how we are able to organize and control cognitive processes and more specifically how goal directed behaviour can arise from the activity of billions of single neurons, remains one of the most intriguing questions of cognitive neuroscience (Miller and Cohen, 2001; Miyake et al., 2000).

The control and management of cognitive processes is encompassed by the term executive functions (EFs), also called executive control or cognitive control (Diamond, 2013; Miller and Cohen, 2001; Powell and Voeller, 2004). Diamond (2013) defines EFs as “a family of top-down mental processes that are needed when you have to concentrate and pay attention, when going on automatic or relying on instinct or intuition would be ill-advised, insufficient, or impossible. EFs are described as a set of related but separate abilities, a concept referred to as the unity and diversity of EFs (Baddeley, 1996; Collette et al., 2005; Miyake et al., 2000).

Currently it is assumed that there are three core functions of EFs (Diamond, 2013; Friedman et al., 2008; Miyake et al., 2000):

- Inhibition: inhibiting dominant, automatic or prepotent responses.
- Updating or working memory: monitoring and updating of task relevant information
- Shifting or cognitive flexibility: switching between tasks or mental sets

These core functions then give rise to higher order EFs like reasoning, problem solving and planning (Collins and Koechlin, 2012; Diamond, 2013).

In the field of EFs the term action control describes a set of processes that involve the adaptation of an action based on an environmental input (Chmielewski and Beste, 2015). Depending on the received information, this adaptation can be a reaction, an adjustment, or the stopping of an ongoing action. Action control can be subdivided into categories that overlap with the core functions of EFs: response inhibition, conflict monitoring/error processing and response/task/set switching.

Exactly how we are able to organize and control cognitive processes and more specifically, how goal directed behaviour can arise from the activity of billions of single neurons, remains one of the most intriguing questions of cognitive neuroscience (Miller and Cohen, 2001; Miyake et al., 2000). The structure that is most often associated with EFs is the frontal lobe and in particular the prefrontal cortex (PFC; Alvarez and Emory, 2006; Collins and...
Koechlin, 2012; Duncan and Owen, 2000; Goldman-Rakic, 1995; Miyake et al., 2000; Stuss, 2011; Stuss and Alexander, 2000; Stuss and Knight, 2002). The term ‘frontal lobe functions’ has even been used by some researchers as a synonym for EFs although the actual picture is more complicated (D. T. Stuss & Alexander, 2000).

The PFC sends bias signals along neural pathways that guide the selection between behavioural alternatives in a given situation (Miller and Cohen, 2001). To understand how this selection is accomplished, and to gain insight into higher functions like action control, it is important to consider the role of cortical-subcortical circuitry (Alvarez and Emory, 2006; Parvizi, 2009). Of special importance for action control processes are the fronto-striatal circuits that are formed between the PFC and the basal ganglia (BG; Alexander et al., 1986; Beste et al., 2012a; Humphries et al., 2006).

1.2 The Anatomy of Action Control

1.2.1 The Basal Ganglia

The BG are a collection of subcortical nuclei located at the base of the forebrain, directly below the white matter. Great insight into the function of the BG has come from the study of Parkinson’s disease (PD) and Huntington’s disease (HD). Subsequently, the BG have long been associated with motor control (Albin et al., 1989; DeLong, 1990). However, more recent research showed that they also provide important contributions to cognitive and emotional processing (Steiner and Tseng, 2010). Accumulating evidence, mostly from neurocomputational studies shows action selection as the most important BG function (Gurney et al., 2004, 2001a, 2001b; Hikosaka, 2007; Humphries et al., 2006; Humphries and Gurney, 2002; Mink, 1996; Prescott et al., 2006; Redgrave et al., 1999). Moreover, it has been argued that the BG are a structure that is evolutionarily optimized to perform action selection (Bar-Gad et al., 2003; Mink, 1996; Plenz, 2003a; Redgrave et al., 2011, Figure 1. The basal ganglia seen in the frontal plane. This figure illustrates the anatomical position of the basal ganglia and the thalamus in the human brain. (see Brodal, 2010).
1999). Anatomically defined the BG consists of three primary structures globus pallidus (internal segment; GPi, external segment: GPe), caudate and putamen (see figure 1). The latter two together are referred to as the striatum. On a functional level the substantia nigra (SN; substantia nigra pars compacta: SNc, substantia nigra pars reticulata: SNr) and subthalamic nucleus (STN) are also counted as part of the BG (Brodal, 2010; Kolb and Whishaw, 2009).

1.2.2 Fronto-Striatal Circuits
The main pathways of the BG primarily form unidirectional, feed-forward loops with the cortex, that all follow the same basic structure (Bar-Gad et al., 2003; Brodal, 2010): Cortex → Striatum/STN → GPe → GPi/SNr → Thalamus → Cortex (Alexander et al., 1986; Brodal, 2010; Redgrave et al., 2011). Further insight into BG functioning comes from box and arrow models that describe multiple pathways (Albin et al., 1989; DeLong, 1990; Schroll and Hamker, 2013; see figure 2). Nuclei are modelled as boxes and connections between them as arrows. Depending on the neurotransmitter used, connections can increase (glutamate) or decrease (γ-Aminobutyric acid; GABA) firing in the target nuclei. In these models the cortex-BG-cortex loop contains a positive (direct pathway; striatum → GPi/SNr) and a negative feedback loop (indirect pathway; striatum → GPe → GPi/SNr) in competition with each other. Excitation in the direct pathway is thought to facilitate cortical activity whereas excitation of the indirect pathway leads to decreased cortical activity. Together the direct and indirect pathway act like a brake and release on BG output firing (Brodal, 2010; Gerfen and Bolam, 2010). Competition between the pathways could be the mechanism that provides the BG-cortex system with the ability to perform action selection (Leblois et al., 2006).

Box and arrow models were very successful in explaining the hypo-kinetic and hyper-kinetic movements in PD and HD respectively. However, box and arrow models give little insight into the functioning of healthy BG. Their main shortcoming is to overlook the complex firing patterns of BG neurons as well as the computations and interactions within BG.
nuclei (Bar-Gad et al., 2003). This weakness was overcome by more detailed BG network models that describe how an action in the BG is selected from multiple actions presented by the cortex (Mink, 1996). In many of these models this selection takes place within the striatum and the selected action is conveyed via the different pathways to the output nuclei of the BG (Bar-Gad et al., 2003; Gurney et al., 2004; Humphries et al., 2010; Plenz, 2003a; Redgrave et al., 1999). To understand how this selection in the striatum takes place, a more detailed look into striatal anatomy and function is required.

1.2.3 The Striatum

The striatum can functionally and anatomically be divided into the ventral striatum, including the nucleus accumbens, and the dorsal striatum consisting of caudate and putamen (Bar-Gad et al., 2003; Brodal, 2010; Middleton and Strick, 2000; Parent and Hazrati, 1995). Most afferents in the striatum are either glutamatergic projections from the cortex and intralaminar thalamic nuclei or dopaminergic projections from the SNc (dorsal striatum) and the ventral tegmental area (ventral striatum; Brodal, 2010; Gurney et al., 2004; Haber, 2003; Parent and Hazrati, 1995). Glutamatergic cortical input is the largest contingent and appears to be topographically organized by the cortical area of origin. The caudate nucleus primarily processes cognitive and emotional information from parietal, temporal, and frontal association areas including the PFC. This region is most strongly associated with goal-directed behaviour and action control processes (Brasted et al., 1999; Eagle and Robbins, 2003; Ito and Doya, 2015; Miyachi et al., 1997; Ragozzino, 2003; Ragozzino and Choi, 2004; Yin et al., 2005a, 2005b). The putamen receives somatotopically organized inputs from the primary somatosensory cortex (S1) and the primary motor cortex (M1) and is associated more with habitual behaviour (Adler et al., 2013; Miyachi et al., 1997; Yin et al., 2006, 2004). The ventral striatum, receives input from the anterior cingulate cortex (ACC) and other limbic areas and is thought to be involved in the regulation of mood, emotions, basic needs, and rewards (Alexander et al., 1986; Brodal, 2010; Cardinal et al., 2002; Redgrave et al., 2011).

Up to 95% of striatal neurons are GABAergic projection neurons with small/medium sized (12-20 μm) cell bodies and numerous spines on their dendrites (25-30 branches) called medium spiny neurons (MSN; Bolam et al., 2000; DiFiglia et al., 1976; Wilson and Groves, 1980). MSN dendritic spines with AMPA and NMDA receptors receive most of the glutamatergic cortical input (Bolam et al., 2000). The processing in MSN is influenced by the dopaminergic projections from the SNc and the ventral tegmental area (Bar-Gad et al., 2003; Brodal, 2010; Gurney et al., 2004; Haber et al., 2000; Steiner and Tseng, 2010). The modula-
tory dopamine (DA) effect seems to result from the convergence of glutamnergic and dopaminergic inputs at the dendritic spines of MSN. Most glutamnergic cortical efferents form synapses on the head of spines while dopaminergic efferents typically terminate at the neck of that spine. Based on their positioning, dopaminergic efferents can modulate the responsiveness of the postsynaptic spine to the glutamnergic input (Brodal, 2010; Smith and Bolam, 1990; Smith et al., 1994; Surmeier et al., 2007).

MSN can be subdivided into two main types. The first type contains substance P and expresses D1 receptors. Substance P containing MSN primarily project to GPi and SN. The second type contains encephalin, expresses D2 receptors (Gerfen et al., 1990; Gerfen and Bolam, 2010), and projects mainly to GPe (Parent and Hazrati, 1995). The two different types of MSN lie at the heart of the distinction of the direct and indirect pathway with D1 MSN in the direct pathway and D2 MSN in the indirect pathway. A third, less well characterized type of MSN exists that contains neurokinin B along with substance P and encephalin (Brodal, 2010; Smith et al., 1998). DA facilitates MSN excitability via D1 receptors and dampens MSN excitability via D2 (Bar-Gad et al., 2003; Brodal, 2010; Smith et al., 1998; Surmeier et al., 2007). Besides the projections to other BG nuclei, MSN form dense, local axon collaterals that synapse with other MSN (see figure 3; Somogyi et al., 1981; Wilson and Groves, 1980; Yung et al., 1996). The firing patterns of MSN are irregular and accompanied by membrane potential shifts. In vitro, spiking is only observed in the depolarized up-state (-60 to -40 mV) and not in the more hyperpolarized down-state (-90 to -40 mV; Mahon et al., 2006; Wilson and Groves, 1981).

3–5% of striatal neurons are made up of GABAergic, fast-spiking interneurons (FSI) that receive excitation form the cortex and are thought to subsequently inhibit MSN (Bolam et al., 2000; Brodal, 2010; Kawaguchi et al., 1995; Kita, 1993; Koós and Tepper, 1999; Plenz and Kitai, 1998; Tepper et al., 2004). In
addition to chemical synapses these interneurons form gap junctions with other FSI and exhibit electronic coupling (Kita et al., 1990; Koós and Tepper, 1999).

1-2% of striatal neurons are interneurons with a large cell body and smooth dendrites that contain acetylcholine. They are referred to as tonically active neurons (TANs) because of their spontaneous firing activity (3-10 Hz; Aosaki et al., 1995; Wilson et al., 1990). TAN receive glutamatergic projection from the intralaminar nuclei of the thalamus and GABAergic inputs from MSN axon collaterals. TAN mainly target MSN (Tepper and Bolam, 2004) and attenuate GABAergic inhibition of MSN (Koós and Tepper, 2002; Perez-Rosello et al., 2005)

1.3 Action Selection in the GABAergic Striatal Microcircuit

Even though the vast majority of synapses (80%) in the striatum are glutamatergic, almost all neurons in the striatum are GABAergic. Thus, the internal organization of the striatum is dominated by GABAergic transmission (Yamada et al., 1995). However, the functioning of GABA in the striatal microcircuit is complex and not yet understood in its entirety (Tepper and Plenz, 2007). The effect of GABAergic signalling depends on cell type and state, synapse location, receptor type, and the modulation by other transmitters. Therefore, only a brief introduction will be provided here. For a more extensive review please see Grillner & Graybiel (2006, Chapter 6 to 9) or Tepper et al. (2007).

1.3.1 A Short Overview of GABA

GABA is the main inhibitory transmitter and is present in almost every region in the central nervous system (CNS). Approximately 20% of synapses in the CNS are GABAergic with most GABAergic neurons being interneurons (Paredes and Agmo, 1992). GABA is synthesized from glutamic acid by the enzyme glutamic acid decarboxylase (GAD65 and GAD67) and pyridoxal phosphate as a cofactor (Brodal, 2010; Martin and Rimvall, 1993). High affinity GABA transporters (GAT) that are localized at neuronal membranes remove GABA from the extracellular space.

GABA acts via ionotropic GABA_A receptors (GABA_ARs) and metabotropic GABA_B receptors (GABA_BRs). GABA_ARs are ligand-activated chloride channels (Macdonald and Olsen, 1994). They consist of five distinct subunits (α1–α6, β1–β3, γ1–γ3, δ, ε, θ1–θ3, π, and ρ1–ρ3). The kinetics, pharmacological sensitivity, and subcellular location of the channel depend on the specific subunit composition (Fritschy and Brüning, 2003; Lüscher and Keller, 2004; Macdonald and Olsen, 1994; Vicini and Ortinski, 2004). The αβ2/3γ2 receptor combination is the most abundant in the brain and expressed in all parts of the BG (Benke et al.,
GABA<sub>A</sub>Rs that contain the γ2 subunit are primarily expressed postsynaptically and typically mediate precisely timed, phasic inhibition (Sigel and Steinmann, 2012). The opening of Cl<sup>-</sup> channels drives the membrane potential towards Cl<sup>-</sup> equilibrium (E<sub>CL</sub>, about -65 mV; Roth and Draguhn, 2012). γ2-GABA<sub>A</sub>R channel open quickly and induce a distinct, high-amplitude, rapidly decaying postsynaptic event (Brodal, 2010; Chen et al., 2005; Kong et al., 2004). The resting membrane potential of most neurons is less negative than the chloride reversal potential. Hence, activation of GABA<sub>A</sub>Rs usually results in hyperpolarization and inhibition of cell activity. α4/6β2/3δ-GABA<sub>A</sub>Rs, and those containing the α5 subunit, are primarily expressed extrasynaptically (Crestani et al., 2002; Semyanov et al., 2004). These receptors are persistently activated by extracellular GABA (Farrant and Nusser, 2005; Glykys and Mody, 2007; Semyanov et al., 2004) and are believed to mediate a more tonic, neuro-modulatory inhibition (Belelli et al., 2009).

GABA<sub>B</sub>Rs are metabotropic (G-protein coupled) transmembrane receptors and are expressed by almost all neurons and glial cells in the CNS. GABA<sub>B</sub>Rs consist of two principal subunits (GABA<sub>B1α</sub>/GABA<sub>B1β</sub> + GABA<sub>B2</sub>) and auxiliary units. GABA is bound by the GABA<sub>B1</sub> subunit and the GABA<sub>B2</sub> subunit conducts G-protein coupling and signalling (Emson, 2007). The exact kinetics and agonist potency of GABA<sub>B</sub>Rs probably depend on their auxiliary subunits (Gassmann and Bettler, 2012). Presynaptically GABA<sub>B</sub> autoreceptors control vesicular GABA release and GABA<sub>B</sub> heteroreceptors inhibit the release of other neurotransmitters by blocking CA<sup>2+</sup> channels. Postsynaptical GABA<sub>B</sub>Rs mostly act by activating inwardly rectifying K<sup>+</sup> channels which causes a slow inhibitory postsynaptic current (IPSC) peaking and decaying much slower than GABA<sub>A</sub> mediated IPSCs (Bettler et al., 2004; Chebib and Johnston, 1999).

### 1.3.2 GABA in the Striatal Microcircuit

In the striatum GABA<sub>B</sub> heteroreceptors are primarily found presynaptically on glutamatergic axon terminals where they regulate glutamatergic inputs to the striatum (Lacey et al., 2005). Presynaptic GABA<sub>B</sub> autoreceptors are present on the terminals of spiny projection neurons and striatal GABAergic interneurons (Emson, 2007).

The most essential signalling in the striatum, however, is via GABA<sub>A</sub>Rs. A diverse mixture of subunits is expressed with a majority of α2, α4 and β3 (Goetz et al., 2007). Most GABA<sub>A</sub>Rs are found postsynaptically (Fujiyama et al., 2002). MSN are under tonic GABAergic inhibition (Kita, 1996; Nisenbaum et al., 1992a) and blocking of GABA<sub>A</sub>Rs more than triples MSN firing (Darbin and Wichmann, 2008; Mallet et al., 2005; Nisenbaum et al.,

2004).
GABA\textsubscript{A}Rs on MSN mediate the two most important types of inhibition in the striatum: feedback and feedforward inhibition.

Feedback inhibition occurs between MSN. Although MSN form dense local axon collaterals terminating on other MSN, these projections have been found to be low amplitude and exhibit a high failure rate (Tunstall et al., 2002). The strength of synapses between MSN suggests that they can only modulate each other’s outputs and regulate spike timing but not directly inhibit each other (Czubayko and Plenz, 2002; Jaeger et al., 1994; Tepper et al., 2004; Tunstall et al., 2002). The exact function of this weak feedback inhibition in MSN is not yet fully understood (Kong et al., 2004). The sources of the strong GABA-mediated response in MSN are in fact the fast-spiking GABAergic interneurons. FSI provide a strong feedforward inhibition to MSN (Kita, 1993; Koós and Tepper, 1999; Plenz and Kitai, 1998; Tepper et al., 2004) and are a key factor in controlling MSN firing (Plenz, 2003a). Even though MSN receive less synapses from FSI than from other MSN, their inhibitory effect is much stronger (Wilson, 2007). FSI innervate MSN close to the soma (Kita et al., 1990) which allows them to produce large inhibitory postsynaptic potentials (IPSPs) with a low failure rate (Koós and Tepper, 1999; Plenz and Kitai, 1998). In fact, the inhibition from a single FSI is strong enough to inhibit or delay action potential firing in MSN (Koós and Tepper, 1999). FSI receive and respond to cortical input faster than MSN (Kawaguchi, 1993) and can in turn exert fast inhibitory action on MSN (Planert et al., 2010).

A peculiarity of MSN is that GABAergic inputs are only hyperpolarizing close to spike threshold (~ -45 mV; Ade et al., 2008; Bracci and Panzeri, 2006) due to the highly polarized resting membrane potential of MSN (~ -80 to -90 mV; Wilson and Kawaguchi, 1996). At resting membrane state GABAergic input to MSN is depolarizing (Ade et al., 2008; Bracci and Panzeri, 2006). This depolarization alone does not excite MSN because the maximum depolarization lies at chloride ion equilibrium which is below the spike threshold (Kita, 1996; Mercuri et al., 1991; Tunstall et al., 2002). However, GABA might act by facilitating excitatory synaptic inputs (Gulledge and Stuart, 2003). Caused by strong bursts of glutamatergic input from the cortex, MSN shift from the hyperpolarized down-state to the depolarized up-state, just below spike threshold (Calabresi et al., 2000; Wickens and Wilson, 1998). If a GABAergic postsynaptic potential happens shortly before the burst of cortical input, this increases the probability of the MSN reaching firing threshold at the beginning of the Up-state (Bracci and Panzeri, 2006). Near action potential threshold GABAergic inputs might control the timing of postsynaptic action potentials by delaying or preventing action potential through brief hyperpolarization (Koós and Tepper, 1999; Plenz, 2003a).
GABA_ARs in the striatum are also expressed extrasynaptically. Here, they generate a persistent current which could be hyperpolarizing or depolarizing in MSN due to their bimodal membrane potential. In any case, the tonic current mediates a shunting inhibition that makes it less likely to generate an action potential; the tonic conductance decreases the membrane resistance which in turn makes the neuron less sensitive to sharper changes in voltage and the effect of excitatory input at the synapse is attenuated (Lee and Maguire, 2014). This persistent inhibition may allow for the regulation of network excitability and information processing (Scimemi et al., 2005; Semyanov et al., 2004, 2003). Studies in mice showed that in adult animals this tonic GABA current is up to six times greater in D1 MSN (Ade et al., 2008; Janssen et al., 2011; Santhakumar et al., 2010). This is corroborated by D1 MSN’s decreased excitability and more hyperpolarized membrane potential at rest compared to D2 MSN (Gertler et al., 2008; Kreitzer and Malenka, 2007).

Besides the vesicular and extracellular GABA that is involved in transmission, a third major pool of GABA is cytoplasmic GABA. It is uniformly distributed throughout the neuron and hypothesized to be an intermediated in the energy metabolism of GABAAergic cells (Martin and Rimvall, 1993).

1.3.3 Response Selection in the Striatal Network

Because of its vast GABAAergic connectivity and absence of layers, the striatum has been viewed as a lateral inhibitory network that implements a winner-takes-all (WTA) mechanism. Different options are fed into the striatum and try to inhibit each other via GABAAergic interconnections. The best, or strongest, response option that has inhibited all competing responses is the ‘winner’. Only a single neuron or cluster is left active and the winner is conveyed to the output layer of the BG to be carried out (Bar-Gad et al., 2003; Plenz, 2003a). Action selection in the striatum via a WTA network is implemented in many neurocomputational BG models (Bar-Gad et al., 2003; Gurney et al., 2004, 2001a, 2001b; Humphries et al., 2006; Humphries and Gurney, 2002; Plenz, 2003a; Redgrave et al., 1999; Wickens, 1990)(Bar-Gad et al., 2003). In biologically constrained computational models the WTA mechanism is modelled as the actions of MSN (Gurney et al., 2004; Plenz, 2003a; Redgrave et al., 1999).

The concept of the striatum as a lateral inhibitory WTA network has been criticized based on the weakness of synapses between MSN (Czubayko and Plenz, 2002; Jaeger et al., 1994; Tepper et al., 2004; Tunstall et al., 2002). Still, a complex WTA network may be formed by MSN together with FSI (Plenz, 2003a). FSI provide a strong feedforward inhibi-
tion to MSN (Kita, 1993; Koós and Tepper, 1999; Plenz and Kitai, 1998; Tepper et al., 2004) and are a key factor in controlling MSN firing (Plenz, 2003a). Following cortical activation, FSI suppress prior striatal network states. Through GABAergic lateral connections, the network can then establish a new focus of activity that corresponds to the dominant cortical input (Plenz, 2003a). To establish the new focus MSN and FSI need to receive strong phasic bursts of cortical activity that switch an assembly of striatal neurons to an up-state (Calabresi et al., 2000).

Fukai and Tanaka (1997) point out that WTA is an extreme on a continuum of network signal selection computations. In networks with weaker lateral inhibition unambiguous selection between competing signals can still occur. Signals that fall under a lower threshold lose while the signal that rises above an upper threshold is the winner. The threshold changes dependent on overall input. This concept was implemented in a three-dimensional network model of the striatal microcircuit (Tomkins et al., 2014). Modelling weak, sparse feedback connectivity between MSN neurons and dense, strong feedforward inputs from FSI demonstrated a form of transient selection. This transient selection in the striatum was sufficient to enhance selection in a BG-thalamo-cortical loop model. Increasing the feedback connectivity between MSN within the bounds of computational anatomical estimates (Humphries et al., 2010, p. 2010) gave rise to a steady state selection or WTA. This is in line with findings that many weak synapses from other MSN might be sufficient to collectively modulate activity in a MSN (Chuhma et al., 2011; Guzmán et al., 2003; Humphries et al., 2010; Yim et al., 2011). Both transient and steady state selection could exist in different parts of the striatum depending on the local density of projection neuron connections (Beste et al., 2014a; Tomkins et al., 2014).

1.4 An Overview of Past Research

1.4.1 The Striatum in Action Control

Computational models show that the striatal network plays an important part in action selection and control (Bar-Gad et al., 2003; Humphries and Prescott, 2010; Plenz, 2003b; Redgrave et al., 1999). In line with this, the striatum has been shown to be involved in response inhibition (Aron, 2011; Bari and Robbins, 2013; Beste et al., 2010b; Boehler et al., 2010), conflict monitoring (Beste et al., 2012a, 2008d; Fielding et al., 2005; Willemssen et al., 2011; Wylie et al., 2012, 2010), general task switching (Aarts et al., 2010; Cools, 1980; Cools et al., 2004) and specifically action cascading (Beste and Saft, 2015; Ness and Beste, 2013). GABAergic neurotransmission in the striatum is assumed to constitute a WTA net-
work that is implemented via MSN (e.g. Gurney et al., 2004; Plenz, 2003a; Redgrave et al., 1999). If the integrity of this network is impaired, this should be reflected in altered action control processes. Hence, a lot of insight has come from studies that examine action selection mechanisms in diseases characterized by neuropathology of striatal neurons.

PD is a hypokinetic disorder caused by the degeneration of dopaminergic neurons in the SNc and their projections to the striatum (Mark F. Bear et al., 2007; Nieoullon, 2002). MSN are affected in PD (Chase and Oh, 2000) as their effective processing is dependent on the functioning of the nigro-striatal DA-system (Gurney et al., 2004; Surmeier et al., 2007). Consequently, numerous studies found impaired action selection processes in PD (conflict monitoring; Praamstra and Plat, 2001; Schmiedt-Fehr et al., 2007; Willemssen et al., 2011; Wylie et al., 2009; inhibition: Beste et al., 2010b, 2009a; task switching: Cools et al., 2001). Similar deficits have been demonstrated in elderly subjects (Beste et al., 2010b; Willemssen et al., 2011) in accordance with the finding that the nigro-striatal system and MSN are also marginally impaired in healthy aging (Cass et al., 2007; Collier et al., 2007). However, MSN dysfunction alone cannot account for the changes in PD because much of the impairment may be caused by the damaged nigro-striatal pathways (Beste et al., 2010b).

HD is an autosomal dominant neurological disorder that leads to neurodegenerative changes in the BG as well as thalamic and cortical structures (Kassubek et al., 2005; Rosas et al., 2008; Tabrizi et al., 2009). MSN are affected prominently (Cepeda et al., 2007; Thomas et al., 2011) and early in the disease (Tabrizi et al., 2009) while FSI are more resistant (Cepeda et al., 2007; Mitchell et al., 1999). In addition, the inhibitory GABAergic neurotransmission is drastically changed in HD (Cepeda et al., 2007; Melone et al., 2005). Several studies showed impaired action selection processes in HD patients (Beste et al., 2013a, 2012a, 2011, 2010b, 2009b, 2008a, 2008b, 2008d; Georgiou et al., 1995; Georgiou-Karistianis et al., 2007; Holl et al., 2013; Rosas et al., 2006; Thiruvady et al., 2007). The changes in MSN are evident even before clinical symptoms are developed (Tabrizi et al., 2009). Accordingly, impaired action selection has also been found in presymptomatic HD (Beste et al., 2013a, 2012a, 2011, 2010b, 2009b, 2008b, 2008d).

In both HD and PD structural changes in MSN occur together with alterations in MSN modulation by presynaptic DA signalling in the nigro-striatal system (Cepeda et al., 2007; Chase and Oh, 2000). Therefore, studies on HD and PD cannot give direct insight into the functional neuroanatomy of MSN. Benign hereditary chorea (BHC) may serve as a ‘neuroanatomical model’ to research MSN dysfunction and its effect on action control without other potentially biasing modulations. BHC is a rare autosomal dominant disease that presents with
choreatic movements. It is caused by a mutation of the TTF1 gene that encodes the thyroid transcription factor and leads to a dysgenesis of striatal MSN (Inzelberg et al., 2011). In a recent study, patients with BHC showed deficits in action selection and cascading (Beste and Saft, 2015).

FSI, and to a lesser degree TAN, are reduced in Tourette’s syndrome (TS; Kalanithi et al., 2005; Kataoka et al., 2010), a neuropsychiatric disorder characterized by motor tics that are thought to result from dysfunction in fronto-striatal pathways (Buse et al., 2015; Worbe et al., 2015). Whereas response inhibition is generally impaired in children with TS (Cavanna et al., 2009; Eichele et al., 2010; Müller et al., 2003; Watkins et al., 2005), they show normal performance in cognitive flexibility, without ADHD as a comorbid disorder (Cavanna et al., 2009; Channon et al., 2006). However, this might be due to compensatory prefrontal activity and cognitive flexibility has been found to be impaired in children with more severe symptoms (Baym et al., 2008).

1.4.2 GABA in Action Control

Given the importance of GABA for MSN functioning, it seems evident that striatal GABA plays a crucial role in action control. Still, so far there are no studies that directly link striatal GABA to individual differences in action control. However, converging evidence from the study of cortical regions suggest that GABAergic inhibition in general plays an important role in EFs.

According to the GABA-hypothesis of schizophrenia the impairment of EFs results from reduced GABA concentration and neurotransmission. Reduced GABA activity in schizophrenia has been shown several times, mainly in cortical and limbic areas (for a review see: Guidotti et al., 2005; Marsman et al., 2014). Reduced GABA concentrations have also been found in several other disorders that present with impaired EFs (ADHD, S1 and M1: Edden et al., 2012; panic disorder, occipital cortex: Goddard et al., 2001; MDD, PFC: Hasler G et al., 2007; TS, S1: Puts et al., 2015; MDD, occipital cortex: Sanacora G et al., 2004).

In healthy subjects a more responsive GABA system in M1 and S1 is related to faster motor learning (Floyer-Lea et al., 2006; Stagg et al., 2011a). Furthermore, GABAergic inhibition plays an important role in working memory (Bañuelos et al., 2014; Durstewitz et al., 2000, 1999; Rao et al., 2000) and working memory performance is diminished by blocking GABA_ARs in the PFC (Durstewitz et al., 2000, 1999; Rao et al., 2000). Higher GABA levels in the supplementary motor area (SMA; Boy et al., 2010) and visual cortex (Yoon et al., 2010) are correlated with more efficient response inhibition processes.
In the frontal cortex higher GABA activity has been shown repeatedly to be associated with more effective inhibition of task-irrelevant information and superior selection processes (lateral PFC: de la Vega et al., 2014; Durstewitz et al., 2000, PFC: 1999; dIPFC: Rao et al., 2000; vIPFC: Snyder et al., 2010; FEF: Sumner et al., 2010; M1: Swann et al., 2009). Accordingly, injection of a GABA agonist improved selection in a language task (Snyder et al., 2010). An explanation may be that decreased GABA function leads to reduced competitive dynamics in neural networks. In line with this, decreasing inhibition in a PFC network model allowed non-winning response options to become more active which in turn slowed and impaired the selection process (Snyder et al., 2010).

1.4.3 GABA and Neural Synchronization in Action Control

Another way that GABA may influence network efficiency is by determining network oscillations and their synchronization. Synchronization of neural firing plays a crucial role in neuronal functions and is thought to be a mechanism that allows the brain to execute tasks that require the coordinated function of disparate neural networks (Buzsáki and Draguhn, 2004; Engel and Singer, 2001; Singer, 1993). In accordance, a variety of cognitive and perceptual functions are associated with synchronization (attention: (Buzsáki, 2005; Fries et al., 2001; Klimesch et al., 2007; Uhlhaas et al., 2009).

For cognitive control processes especially oscillations in the theta frequency seem to be of relevance (Cavanagh et al., 2012; Cavanagh and Frank, 2014; Cohen, 2014; De Blasio and Barry, 2013; Harper et al., 2014). They are believed to play a role in establishing cognitive control by organizing related neural processes (Anguera et al., 2013; Buzsáki and Draguhn, 2004; Cohen et al., 2009; Hanslmayr et al., 2007; Nigbur et al., 2011; Uhlhaas et al., 2010; Womelsdorf et al., 2010). In line with this, studies examining response inhibition and conflict monitoring frequently report modulations in theta frequencies when demand on cognitive control is high (Beste et al., 2011; Cohen and Donner, 2013; Lavalle et al., 2014; Ocklenburg et al., 2011; Tang et al., 2013; Wang et al., 2014).

In cortical structures the generation of oscillations and the organization of neuronal ensemble synchronization relies on inhibitory GABAergic networks (Andersen et al., 1966; Buzsáki et al., 1992). GABAergic interneurons form strong connections with multiple pyramidal cells and can synchronize their firing through IPSPs (Cobb et al., 1995). When several interneurons coordinate their activity through reciprocal inhibition and gap junctions their synchronized inhibitory output entrains the activity of numerous target cells in the network (Gonzalez-Burgos and Lewis, 2008; Kopell and Ermentrout, 2004). Accordingly, higher
GABA concentrations in the cortex increase oscillatory activity (Feshchenko et al., 1997; Greicius et al., 2008; Kiviniemi et al., 2005; Licata et al., 2013; Muthukumaraswamy et al., 2009). For example, the cognitive deficits in schizophrenia are linked to an impaired regulation of synchronized oscillatory activity in cortical neurons in association with decreased GABA (Cho et al., 2006; Lewis et al., 2008; Spencer et al., 2004). Treatment with a GABA agonist restored synchronous activity and improved cognitive control (Lewis et al., 2008).

Coherent network oscillations have been found in fronto-striatal circuits and are one of the mechanisms underlying their functional organization (Magill et al., 2004; Marsden et al., 2001; Sharott et al., 2005; Williams, 2002). Nevertheless, the relevance of striatal GABA for these oscillations is not straightforward and cannot be simply generalized from cortical structures. Even though, oscillatory activity in the striatum has been found and is modulated by task performance (Berke et al., 2004; Brown et al., 2004; Courtemanche et al., 2003; DeCoteau et al., 2007a, 2007b; Gervasoni et al., 2004; Masimore et al., 2004) the exact function of these oscillations is still unclear.

Nevertheless, there is evidence that specifically links striatal oscillations to cognitive control processes. Gage et al. (Gage et al., 2010) found that FSI show a pulse of coordinated activity just before choice execution when one action must be enabled and another suppressed. This fits well with the increased oscillatory activity observed during high-demand cognitive control processes described above (e.g. Beste et al., 2011; Ocklenburg et al., 2011). Courtemanche (2003) recorded oscillations in the striatum of alert macaque monkeys. Small sites in the striatum with neurons that exhibited task-related firing increase disengaged from the synchrony during response execution. The authors hypothesized that the striatal oscillations act as a spatiotemporal filter to sharpen action selection and only cortical inputs that overcome the generalized level of synchronous activation could achieve differential activation of striatal outputs. Furthermore, neural synchronization in MSN has been hypothesized to encode task relevant information (Adler et al., 2013). All this speaks for improved action control due to increased synchronizational processes in the striatum.

However, dynamics of inter- and projection neurons in the striatal network differ from those in laminar structures and it is uncertain if, and how, these oscillations are generated in the striatum. Oscillations in the striatum may instead be the result of oscillations in striatal afferents that are mediated by FSI (reviewed by Berke, 2005). Thus, the effects of GABA in the cortex cannot simply be extrapolated albeit there is evidence that striatal GABA modulates synchronizational processes in fronto-striatal networks. Injection of a GABA antagonist in the striatum of rhesus monkeys increased the correlation between striatal local field poten-
tials and EEG. Furthermore, HD patients are known to show a substantial loss of GABA in the striatum (Bonilla et al., 1988; Glass et al., 2000; Reynolds and Pearson, 1990) and also disturbed synchronization processes related to action control (Beste et al., 2011). Finally, GABA\textsubscript{A}R binding is decreased in the striatum of TS patients and TS symptoms have been suggestes to arise from aberrant oscillations (Buse et al., 2015; Hong et al., 2013; Leckman et al., 2006).

GABA concentrations seem to influence cognitive control not only by modulating synchronized activity locally but also at long-range network levels (Duncan et al., 2014; Stagg et al., 2014). Recent results suggest that local GABA levels modulate functional connectivity within the default mode network (Arrubla et al., 2014; Duncan et al., 2014; Kapogiannis et al., 2013; Northoff et al., 2007; Shin et al., 2013; Stagg et al., 2014). Resting state networks have been reported for the BG (Damoiseaux et al., 2008; Martino et al., 2008; Robinson et al., 2009) but the relationship to local GABA concentrations has not yet been explored.

Finally, MSN have been shown to form groups that exhibit recurrent and synchronized bursting and display spatiotemporal pattern generation at behaviourally relevant time scales (Carrillo-Reid et al., 2008; Humphries et al., 2009; Ponzi and Wickens, 2010). These assemblies are likely formed by cortical and/or thalamic excitatory input during learning and later activated by a trigger (Adler et al., 2012). Blockage of glutamatergic transmission abolished the correlated activity whereas blockage of GABA\textsubscript{A}Rs (synaptic and extrasynaptic) locked the network into a single dominant state (Carrillo-Reid et al., 2008). Hence, higher GABA may ensure efficient switching between functional network states defined by cell assemblies (Carrillo-Reid et al., 2008).

1.5 On the Approach Taken in this Thesis

1.5.1 Thesis Aim

The present thesis aims to examine the importance of the striatal GABAergic system for interindividual differences in action control processes and thereby provide direct empirical evidence for the role of striatal GABA in neurocomputational BG models. GABAergic neurotransmission in the striatum is assumed to constitute a selection network that is implemented via MSN (e.g. Gurney et al., 2004; Plenz, 2003a; Redgrave et al., 1999). The efficiency of this network is dependent on neurotransmitter signalling and unimpaired cell functioning (e.g. Beste et al., 2010b; Humphries et al., 2010; see section [→] 1.3.3 and 1.4.1).

Typically, insight into the neuronal mechanisms underlying action control is attained via the examination of neurological disorders. As outlined above (→1.4.1) these results can-
not be used to directly infer GABAergic functioning in the striatal microcircuit because in most cases other interacting transmitter systems and brain regions are also affected by the disorder. Conversely, neuronal mechanisms that mediate superior performance have barely been studied. The second and third study therefore include airplane pilot trainees (APTs) as a potential model of superior performance in action control. This allows to investigate how striatal GABA levels relate to individual differences in action control and to examine neuro-biological processes that are related to superior cognitive control mechanisms.

The investigation of regional neuro-biochemical relationships and their association with psychological functions calls for a multimodal approach (Duncan et al., 2014). Thus, a novel combination of biochemical measures (GABA and glutamate, MRS), behavioural assessment (performance in action control tasks), and neural activity measures (EEG and fMRI) is applied. The influence of striatal GABA is tested on tasks that cover different subprocesses of action control. Interindividual differences in task performance and their association with striatal GABA levels are explored in relation to the processing of sensory striatal input, synchronization processes, and fronto-striatal networks.

1.5.2 Measuring Neurotransmitter Concentrations

GABA concentration in the brain is measured in vivo with magnetic resonance spectroscopy (MRS). MRS is a non-invasive method to quantify neurotransmitter concentrations in discrete regions of the human brain. Like magnetic resonance imaging (MRI) MRS typically uses the signal from hydrogen protons (\(^1\)H; although other nuclei may also be used to obtain MR spectra). MRS uses this signal to provide physiological and chemical information as opposed to the spatial or anatomical information provided by MRI. The concentration of neurochemicals is assessed within a voxel placed over a region of interest and often referenced to total creatine (tCr).

Because the GABA spectrum overlaps with the spectrum of other molecules with higher concentrations, GABA is difficult to measure with standard single voxel techniques. The current state-of-the-art technique for measuring metabolites whose peaks are overshadowed by other spectra is the MEGA-PRESS MRS sequence (Mescher et al., 1998; Mullins et al., 2014). MEGA-PRESS is a difference-edited technique that collects two interleaved datasets. In the ‘ON’ dataset an RF pulse is applied to GABA spins at 1.9 ppm to selectively refocus the evolution of J-coupling to the GABA spins at 3 ppm. In the ‘OFF’ dataset the RF pulse is applied elsewhere and GABA J-coupling is unaffected. A difference spectrum is obtained by subtracting the refocused ON spectrum from the OFF spectrum and retains only the peaks.
that are affected by the RF pulse. Achieving high-quality MRS data for the striatum is particularly challenging and this is the first time that GABA-MRS data is acquired from this region.

It is not possible to determine the relative contribution of the vesicular, extracellular, and cytoplasmic GABA from the MRS signal, or relate it to specific aspects of GABAergic function (Stagg, 2014). However, considering the demonstrated relationship with performance measures (e.g. Edden et al., 2009), it seems likely that MRS GABA levels are at least correlated with neurotransmitter and neuromodulatory pools of GABA (Stagg et al., 2011b). Accumulating evidence points to the fact that MRS may be less sensitive to GABA in presynaptic vesicles, bound to macromolecules (Floyer-Lea et al., 2006) and might primarily reflect extrasynaptic GABAergic tone, rather than either GABA_A or GABA_B synaptic activity (Mason et al., 2001; Stagg, 2014; Stagg et al., 2011c). Nevertheless, extrasynaptic GABAergic tone may still be related to synaptic processes since the primary source for extracellular GABA is synaptic spillover from action potential-dependent vesicular release (Ade et al., 2008; Bright et al., 2007). Thus, extrasynaptic GABA concentration raises with sustained activity of GABAergic neurons (Semyanov et al., 2004).

MRS gives a composite measure of glutamate and glutamine (Glx). The glutamate concentration in the brain is 10 times higher than glutamine. Thus, Glx is primarily driven by glutamate and will be referred to as glutamate from here on (Stagg, 2014). Even though MRS glutamate levels are closely related to local excitability, the majority of glutamate in the brain is involved in non-synaptic, metabolic roles (Stagg et al., 2011c). Therefore, local glutamate levels and local excitability are not as strongly linked as local GABA levels and local inhibition (Rae, 2013; Stagg, 2014; Taylor et al., 2012).

1.5.3 Measuring Action Control

To investigate if striatal GABA is relevant for general action control, each study implements a task of a different action control core functions. In the following these core functions will be defined and ways to measure them will be described.

Response inhibition: Response inhibition is a form of behavioural inhibition that involves action postponing, action withholding, and action cancelation (for a review see Bari and Robbins, 2013). It takes places when a reaction to environmental stimuli needs to be suppressed due to situational requirements (Aron, 2011; Logan et al., 2014).

Typical tasks that assess response inhibition processes establish a specific response that must be inhibited when a similar but altered stimulus is presented or when the stimulus is
followed by a stop signal. Two classical response inhibition paradigms that provide a good basis for the assessment of EEG are the GoNogo task (e.g. Aron and Poldrack, 2005; Beste et al., 2008a; Falkenstein et al., 1999; Ocklenburg et al., 2013, 2011) and the stop signal task (e.g. Hofmann et al., 2012; Robbins, 2007; Verbruggen and Logan, 2008; White et al., 2014). In the stop signal task a prepotent response or an already initiated response to a stimulus has to be withheld or cancelled if the stimulus is followed by a stop signal. GoNogo tasks typically feature a Go- and a Nogo stimulus. In Go trials subjects are asked to respond while in Nogo trials the planned response has to be withheld.

**Conflict monitoring:** Botvinick et al. (2004; Matthew M. Botvinick et al., 2004; 2001) describes conflict monitoring as a function that translates the occurrence of conflict into a complementary adjustment in control. The conflict monitoring system evaluates the level of conflict and passes this information on to control centres. This prompts them to adjust the strength of influence on processing in order to prevent conflict in subsequent performance. Botvinick et al. (2004, 2004; 2001) propose that conflict monitoring is part of a general monitoring function. This function detects internal states that signal a need to enhance or redirect attentional control. If the general monitoring function detects contradictory information, this conflict information activates the conflict monitoring function.

Tasks that are used to assess conflict monitoring typically present a stimulus that requires a certain reaction, but also involve task-irrelevant information that can be incompatible with the original stimulus. In incompatible trials the level of interference is increased and induces an increased involvement of the conflict monitoring system. Several well-established paradigms make use of this mechanism such as the Stroop task (Stroop, 1935; e.g. Beste et al., 2012a; Coderre et al., 2011; Gajewski et al., 2012), the Eriksen flanker task (Eriksen & Eriksen, 1974; e.g. Beste et al., 2014b, e.g. 2008d; Stins et al., 2008) and the Simon task (Simon, 1990; e.g. Fielding et al., 2005; Stock and Beste, 2014; Wylie et al., 2012). In the Simon task subjects are asked to react to one stimulus with a button press with the right hand and to react to another stimuli by pressing a button with the left hand. The target on the screen is presented in two locations. Conflict is established whenever the location on the screen and the response finger are on opposite sides. Responses are faster and less error-prone if the task-irrelevant stimulus location matches the location of the correct response. This finding is referred to as the Simon effect (Keye, Wilhelm, Oberauer, & Stürmer, 2013; Simon, 1990).

**Task switching:** Task switching describes the control processes that configure the mental resources required to switch between subsets of a task. This is accompanied by
switching costs; subjects are usually more error-prone and react slower following a switch. These switching costs likely result from carry-over of task-set activation or inhibition as well as a task-set reconfiguration process (Monsell, 2003).

In task switching paradigms two different rules are established that should be applied depending on certain criteria. Cues or instructions signalling task switching can be presented randomly or after a fixed number of trials and require rule changing. Dual-task paradigms are similar in that the first task set needs to be actively suppressed to proceed to the next task set. Trials typically involve two stimuli that are presented in rapid succession so that processing of the first stimulus is not finished before presentation of the second stimulus. This causes the response to the second stimulus to be slowed. An example is the psychological refractory paradigm (PRP; e.g. Chmielewski et al., 2014; Pashler, 1994; Sigman and Dehaene, 2008).

Stop-change tasks (SCT) are closely related to task switching paradigms as they also require the interruption of a single task and a shift to another (Beste et al., 2013b; e.g. Beste and Saft, 2015; Chmielewski et al., 2014; Ness and Beste, 2013; Stock et al., 2014a; Verbruggen et al., 2008; Yildiz et al., 2013; Yildiz and Beste, 2014). This task allows to investigate the temporal organization and overlap of task-goals and response-related processes (Verbruggen et al., 2008). In this sense, stop-change tasks can be used to test if subjects cascade actions in a rather serial or a rather parallel way. The rational is that a rather serial processing mode shows smaller response time (RT) differences (a flatter slope) between trials with serial and synchronous stimuli presentation of stop stimulus and change stimulus (Miller et al., 2009; Mückschel et al., 2013). Although this serial/parallel interpretation can be problematic (for a detailed account of this issue please refer to: Verbruggen et al., 2008), a flatter slope unequivocally reflects more efficient action cascading. The analysis of EEG components associated with the processing of the auditory and visual change signals as well as processes related to mapping the stimulus onto the appropriate response is well-established for the SCT (Mückschel et al., 2013; Stock et al., 2014b).

1.5.4 Measuring Neural Activity

EEG Recording electroencephalography (EEG) during task performance enables the differentiation between subprocesses of neural events that underlie sensory, perceptual, and cognitive processes. EEG measures are usually considered to be sensitive to cortical but not subcortical processes. Nevertheless, striatal processes and striatal processes are likely to modulate EEG activity because they affect the functioning of fronto-striatal circuits as a whole (Beste and Saft, 2015). Furthermore, intrastriatal circuit properties may influence the spread, extent, and occurrence
of oscillations both locally and in other brain structures (Berke et al., 2004). Indeed, DA loss in the rat striatum leads to changes in the nature and incidence of oscillations in the cortex and other parts of the BG (Belluscio et al., 2003; Buonamici et al., 1986; Goldberg et al., 2002; Magill et al., 2004). In line with this, electrophysiological correlates of cognitive control and inhibition are modulated by pathophysiological processes in the BG of PD, HD, and BHC patients (Beste et al., 2012a, 2011, 2009a; Beste and Saft, 2014).

**Nogo-N2 and Nogo-P3:** During response inhibition in stop- or Nogo trials, a pronounced fronto-central, negative event-related potential (ERP) is observed at around 200-300 ms after stimulus onset (Nogo-N2) and followed by a positive, fronto-central-parietal ERP (Nogo-P3; Huster et al., 2013). The Nogo-N2 reflects mechanisms related to pre-motor processes of inhibition such as conflict monitoring or updating of the response program whereas evaluative processing of the successful outcome of inhibition is associated with the Nogo-P3 (Beste et al., 2011, 2010b; Falkenstein et al., 1999; Huster et al., 2013). Both ERPs have been shown to be affected in BG disease and are therefore likely to reflect processing in fronto-striatal circuits (Beste et al., 2012a, 2011, 2009a, 2008b).

In addition, GoNogo EEG components are well suited to study synchronization processes associated with action control. Even though EEG is always evidence of synchronized neural activity it is not possible to determine if changes in EEG power reflect changes in the synchronization or magnitude of the rhythmic field potentials (Roach and Mathalon, 2008). However, neural synchronization mechanisms can be analysed with time frequency decomposition of EEG. One example is the phase locking factor (PLF), a measure of the reliability of neuronal synchronization processes in time and frequency across trials (Beste et al., 2011, 2010a; Roach and Mathalon, 2008). The PLF is independent of the signals amplitude and varies between 0 and 1, with high values indicating precise phase-synchrony across trials (Kolev and Yordanova, 1997). The Nogo-N2 and Nogo-P3 best correspond to oscillations in the theta and delta frequency band, respectively (Huster et al., 2013). Studies on BG disease and genetic associations suggest that the Nogo-N2 is associated with the striato-nigral system and D1 receptor function, whereas the Nogo-P3 is modulated rather by the striato-pallidal (mesocorticolimic) system and the D2 receptor function (Beste et al., 2016, 2011, 2010b). Together with the importance of theta oscillations for cognitive control, this suggests that striatal processes may especially affect synchronizational processes related to pre-motor inhibition (Nogo-N2). In line with this, Beste et al. (2011) showed that the PLF in the Nogo-N2 time window is attenuated in HD.
P3: During action selection the P3 reflects processes between stimulus evaluation and responding, i.e. mapping of a stimulus onto the appropriate response (Falkenstein et al., 1994; Polich, 2007; Sigman and Dehaene, 2008; Verleger et al., 2005). The P3 has been shown to be sensitive to the temporal spacing of stimuli in dual-tasks (Arnell, 2006; Brisson and Jolicoeur, 2007; Sigman and Dehaene, 2008). In line with this, earlier findings suggest that the P3 in the SCT is predictive of the processing mode (Mückschel et al., 2013; Stock et al., 2014b). A smaller P3 was associated with less overlap between the stop and change process and thus, a rather serial processing mode and more efficient action cascading. In a study by Ness and Beste (2013) blood-oxygen-level dependent (BOLD) activity in the caudate was correlated with the efficiency of action cascading. Hence, the striatum seems to modulate action cascading efficiency and possibly P3 related processes.

P1 and N1: P1 and N1 are ERP components that reflect early attentional processing of stimuli (Herrmann and Knight, 2001). They are modality specific and heavily depend on glutamatergic and acetylcholinergic signalling (e.g. Javitt et al., 2000; Logemann et al., 2014; Sarter et al., 2006; Turchi and Sarter, 2001). Therefore, N1 and P1 may be influenced by glutamatergic transmission in the striatum that receives information from different modalities via glutamatergic cortio-striatal synapses (Kropotov et al., 2000; Nagy et al., 2006; Redgrave and Gurney, 2006; Znamenskiy and Zador, 2013). Indeed, increases in glutamatergic neural transmission and the sensitivity of cortico-striatal NMDA receptors have been shown to promote attentional gating, reflected in P1 and N1 (Beste et al., 2012b, 2008c; Beste and Saft, 2015; Schulz et al., 2012; Turchi and Sarter, 2001). Furthermore, animal studies demonstrated a direct link between attentional processing and glutamatergic transmission at cortico-striatal synapses (Agnoli et al., 2013; Agnoli and Carli, 2011; Sippy et al., 2015).

fMRI

Resting state activity is measured with an functional magnetic resonance imaging (fMRI) scan during which subjects rest quietly with their eyes closed (usually 3 – 11 minutes; Birn et al., 2013). Independent component analysis of the BOLD signal can then identify brain resting state networks. The power of these networks typically lies in the low frequency range (0.01–0.1 Hz) that is characteristic of the grey matter signal (Cordes et al., 2001). The sum of low frequency power in the network is a measure of network activity. Temporally correlating the BOLD signal of two networks gives a measure of functional connectivity between those networks.
1.5.5 Working Hypotheses

The first study investigates the importance of the striatal GABA concentration for response inhibition processes and related synchronized oscillations. Based on computational models and studies on cortical GABA concentration, it seems likely that higher striatal GABA levels lead to a more efficient selection network and thus superior action control processes. Therefore, higher striatal GABA levels should be related to better task performance, i.e. less responses to Nogo stimuli. Given the importance of GABA for the synchronization of network oscillations (→1.4.3) higher striatal GABA levels should correlate with more synchronized oscillations in the theta or delta frequency band that reflect pre-motor and evaluative subprocesses of response inhibition, respectively (Beste et al., 2011, 2010b; Huster et al., 2013).

The second study examines the relation of striatal GABA levels and response selection with a focus on the processing of sensory inputs in the striatum. The convergence of sensory information from different modalities may allow the striatum to integrate information in order to select appropriate actions (e.g. Nagy et al., 2006). This is especially relevant in tasks where several actions have to be coordinated and cascaded because attentional selection processes have been shown to influence performance in these tasks (Brisson and Jolicœur, 2007). In an action cascading task with auditory cues APTs should show enhanced attentional selection processes (reflected in a stronger P1 and N1; (Herrmann and Knight, 2001)), more efficient processes mediating between stimuli and response (reflected in a smaller P3; Mückschel et al., 2013; Stock et al., 2014b), and superior action cascading and response selection. Striatal GABA levels should predict interindividual differences in all three measures. Because the sensory afferent information is fed into the striatum via glutamatergic cortico-striatal synapses (e.g. Bolam et al., 2000), performance measures should also correlate with the glutamate concentration in the striatum.

The third study investigates the relevance of striatal GABA for conflict monitoring processes in the broader context of fronto-striatal networks. To investigate the effect of the BG on motor output, the functional connectivity between the basal ganglia network (BGN) and the somatomotor network (SMN) is examined. Resting state activity in cortical regions has been shown to correlate with performance measures (Motor functions: Fox et al., 2007; Sensory functions: Haag et al., 2015; EFs: Hao et al., 2013; Xu et al., 2014). Thus, in addition to a correlation with higher striatal GABA levels, conflict monitoring performance is assumed to correlate with network activity in the BGN.
Chapter 2

Study 1: Striatal GABA-MRS predicts response inhibition performance and its cortical electrophysiological correlates
Striatal GABA-MRS predicts response inhibition performance and its cortical electrophysiological correlates

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Abstract Response inhibition processes are important for performance monitoring and are mediated via a network constituted by different cortical areas and basal ganglia nuclei. At the basal ganglia level, striatal GABAergic medium spiny neurons are known to be important for response selection, but the importance of the striatal GABAergic system for response inhibition processes remains elusive. Using a novel combination of behavioral, EEG and magnetic resonance spectroscopy (MRS) data, we examine the relevance of the striatal GABAergic system for response inhibition processes. The study shows that striatal GABA levels modulate the efficacy of response inhibition processes. Higher striatal GABA levels were related to better response inhibition performance. We show that striatal GABA modulate specific subprocesses of response inhibition related to pre-motor inhibitory processes through the modulation of neuronal synchronization processes. To our knowledge, this is the first study providing direct evidence for the relevance of the striatal GABAergic system for response inhibition functions and their cortical electrophysiological correlates in humans.

Keywords Striatum · GABA · Response inhibition · EEG · Magnetic resonance spectroscopy

Introduction

Response inhibition processes are important for cognitive control (for review: Bari and Robbins 2013). Several lines of research using functional imaging in humans, clinical data as well as animal studies have shown that prefrontal cortical areas, such as the dorsolateral prefrontal cortex (e.g., Hester et al. 2004), pre- and supplementary motor areas (pre-SMA, SMA) (e.g., Isoda and Hikosaka 2007; Mostofsky et al. 2003), the inferior frontal cortex (e.g., Aron et al. 2004) and the anterior cingulate cortex (e.g., Rubia et al. 2001) are part of the response inhibition network. The response inhibition network also includes subcortical nuclei, which are hypothesized to receive stop commands from cortical structures used to intercept or withhold a response (Duann et al. 2009). It has been shown that besides the subthalamic nucleus (e.g., Forstmann et al. 2012; van den Wildenberg et al. 2006), the striatum is important in response inhibition (e.g., Boehler et al. 2010; Beste et al. 2010). The striatum contains GABAergic medium spiny neurons (MSNs) (Bolam et al. 2000) that are regarded as the major computational element for action control processes in the basal ganglia (Humphries et al. 2010;
The GABAergic MSNs are involved in the selection of different actions (Bar-Gad et al. 2003) and possibly also in the suppression of responses (Bari and Robbins 2013). Yet, the striatum seems to be particularly important for proactive as opposed to reactive inhibition (Aron 2011). Given this putative MSN-mediated suppression of response options, it is possible that striatal GABA levels largely determine the efficacy of response inhibition processes. Yet, the role of striatal GABAergic neural transmission in response inhibition remains elusive.

We investigate the role of the striatal GABA system for response inhibition combining data obtained from magnetic resonance spectroscopy (MRS) with EEG correlates of response inhibition. Using EEG data, different response inhibition subprocesses can be distinguished: (1) a frontal-midline N2 component closely related to theta oscillations reflecting pre-motor processes like conflict monitoring or updating of the response program; and (2) a P3 component related to delta oscillations reflecting evaluative processing of the successful outcome of the inhibition (e.g., Huster et al. 2011; Beste et al. 2013; Beste et al. 2011, 2010). Beste et al. (2010) compared different basal ganglia diseases that differentially affect distinguishable basal ganglia subsystems. Using this comparative approach, Beste et al. (2010) was able to show that especially the Nogo-N2 is affected by the nigro-striatal dopaminergic pathway, hence pointing to a role of striatal structures for cortical electrophysiological correlates of response inhibition. However, action selection in the striatum is largely determined by GABA (Humphries et al. 2010). Recent results suggest that especially neural synchronization processes in striatal GABAergic medium spiny neurons are important for information encoding and behavioral control (Adler et al. 2013). It is therefore likely that striatal GABA levels strongly modulate response inhibition processes occurring in the fronto-striatal networks. We hypothesize that higher striatal GABA levels come along with better performance in response inhibition; i.e., a lower rate of false alarms. False alarms are responses on Nogo stimulus, i.e., an error to withhold a response on Nogo stimuli. Striatal GABA concentrations largely determine striatal MSNs’ connectivity (Humphries et al. 2010) and it is the degree of connectivity in a neural assembly that is important for network synchronization (Kitano and Fukai 2007). For the electrophysiological processes, we therefore hypothesize that higher striatal GABA levels are correlated with the more synchronized electrophysiological oscillations in the delta or theta frequency bands and related response inhibition subprocesses.

Materials and methods

Participants

The study sample consisted of 40 right-handed healthy subjects (age 24.5 ± 5.9 years, range 20–30 years, 10 females and 30 males) with no history of neurological or psychiatry disease and normal or corrected-to-normal vision. The study was approved by the Ethics Committee of the Medical Faculty of the Ruhr-University Bochum, Germany.

Task

A standard Go/Nogo paradigm was used, similar to previous studies by our group (e.g., Ocklenburg et al. 2011; Beste et al. 2010). During the paradigm the words ‘PRESS’ and ‘STOPP’ (translating to DRUCK and STopp in German) were presented on a computer screen. Upon presentation of the ‘PRESS’ stimulus, subjects were required to respond via a custom-made button as fast as possible (Go stimulus). Upon presentation of the ‘STOPP’ stimulus, the subjects were required to withhold a response (Nogo stimulus). Subjects were asked to respond within 400 ms on the ‘DRUCK’ stimulus and refrain from responding on the ‘STopp’ stimulus. Each trial lasted 1,200 ms. To increase time pressure to strengthen response tendencies in trials exceeding this time, a feedback stimulus (1,000 Hz, 60 dB sound pressure level SPL) was given 1,200 ms after the response, which was to be avoided by the subjects. The inter-trial interval (ITI) (time between two consecutive trials measured from the end of one trial to the beginning of the next trial) was jittered between 1,200 and 1,400 ms. The short period for reaction together with short ITI induced a strong response tendency and hence a high rate of false alarms.

EEG recording and analysis

The EEG was recorded from 65 Ag/AgCl electrodes using the extended 10/20 system (Pivik et al. 1993) against a reference electrode located on electrode position Cz. The sampling rate of all recordings was 1 kHz. Electrode impedances were kept below 5 kΩ. The EEG was digitally filtered off-line using an IIR filter with filter bandwidth between 0.5 and 20 Hz. Horizontal and vertical eye movements were corrected in the EEG using independent component analysis (ICA) (infomax algorithm). Artifact rejection procedures were applied twice: automatically, with an amplitude threshold of ±80 µV, and visually by rejecting all trials contaminated by technical artifacts. Before quantifying event-related potentials (ERPs), the
current source density (CSD) of the signals was calculated to achieve a reference-free evaluation (Nunez et al. 1997; Perrin et al. 1989) using the following parameters: order of splines \((m = 4)\), and the maximum degree of the Legendre polynomials \((n = 10)\), with a precision of \(2.72^{-7}\). The data were segmented into 4,096 ms-long epochs. These long epochs were segmented to allow a reliable estimation of slow-oscillating frequencies in subsequent calculations of the phase-locking factor (PLF) (e.g., Beste et al. 2012, 2011). Time point zero denoting the time point of Go and Nogo stimulus delivery was placed in the middle. With this epoch length, a reliable quantification of slow oscillations (delta and theta frequencies) is possible. For the time domain analysis, baseline correction was applied in the interval ranging from \(-200\) ms to stimulus presentation. For the time–frequency analysis, the baseline was set between \(-600\) and \(-400\) ms before stimulus presentation.

On trials denoting response inhibition, the Nogo-N2 was defined as the most negative deflection within the range of 150–300 ms after stimulus onset. The Nogo-P3 was defined as the most positive deflection from 320 till 500 ms. Amplitudes of the Go-N2 and Go-P3 were measured at the corresponding time point, where the Nogo component reached its maximum (Beste et al. 2010). The potentials were quantified at electrode FCz. As can be seen in the scalp topography plots on the Nogo-N2 and Nogo-P3 in Fig. 3, the negativity for the Nogo-N2 is centered around electrode FCz and also the positivity of the Nogo-P3 is seen at this electrode site. The topography map on the phase-locking factor (PLF), which gives an estimate of neural synchronization processes also shows that electrode FCz is the important electrode to analyze.

The PLF gives an estimate of the reliability of neural synchronization processes in time and frequency across trials (PLF; Roach and Mathalon 2008; Tallon-Baudry et al. 2001). The PLF is independent of the signal’s amplitude (Kolev and Yordanova 1997) and varies between 0 and 1, with a value of 1 indicating perfect phase-locking across trials (i.e., high reliability of neural synchronization processes in time and frequency across trials). To obtain the PLF we first ran a time–frequency analysis using complex Morlet wavelets. These wavelets \(w\) can be generated in the time domain for different frequencies, \(f\), according to the equation:

\[
w(t, f) = A \exp(-t^2/2\sigma_t^2) \exp(2i\pi ft),
\]

where \(t\) is time, \(A = (\sigma_t \sqrt{\pi})^{-1/2}\), \(\sigma_t\) is the wavelet duration and \(i = \sqrt{-1}\). For analysis and TF-plots, a ratio of \(f_0/\sigma_f = 5.5\) was used, where \(f_0\) is the central frequency and \(\sigma_f\) is the width of the Gaussian shape in the frequency domain. The analysis was performed in the frequency range 0.1–20 Hz with a central frequency at 0.5 Hz intervals. For different \(f_0\), time and frequency resolutions can be calculated as \(2 \sigma_t\) and \(2 \sigma_f\), respectively. \(\sigma_t\) and \(\sigma_f\) are related by the equation \(\sigma_t = 1/(2\sigma_f)\). For each trial, the time-varying power in a given frequency band was calculated, which was obtained by squaring the absolute value of the convolution of the signal with the complex wavelet. The EEG data were collected outside the scanner in a different session. This was done to avoid the scanner artifacts that could impose problems in the analysis of the EEG data, especially when it comes to the time–frequency decomposition and the quantification of the phase-locking factor.

**MRS data acquisition and analysis**

MRS data were acquired on a 3 T Philips Achieva whole-body scanner using a 32-channel head coil. Fast T2-weighted images were obtained in axial, coronal and sagittal planes to enable placement of a \(30 \times 30 \times 25\) mm\(^3\) MRS volume of interest (VOI) centered on the striatum. MRS spectra were acquired from VOIs placed in the left as well as the right striatum to rule out any laterality effects. MEGA-PRESS edited GABA spectra (Edden and Barker 2007; Mescher et al. 1998) were acquired from each VOI using the following parameters: repetition time (TR) = 2 s, echo time (TE) = 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 and 16 OFF acquisitions of 2,048 data points each and a spectral bandwidth of 2 kHz were interleaved 16 times, resulting in a total of \(16 \times 16 = 256\) averages per MEGA-PRESS scan; total acquisition time = 8.5 min. 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. LCModel (Provencher 1993) (version 6.2-0R), which fits in vivo MR spectra as a linear combination of single metabolite “basis spectra”, was used for quantification (for details see suppl. material of Dydak et al. 2011). In particular, GABA was quantified from the MEGA-PRESS difference spectra with basis spectra created using density matrix simulations. Instead of using the in-built simplified GABA-fitting routine, we optimized the LCModel fitting parameters to allow the confounding macromolecule peak at 3.0 ppm to be largely fit by the flexible baseline function of the LCModel (Dydak et al. 2011). While this additional degree of freedom results in slightly larger %SDs, it provides a more accurate estimation for pure GABA (Murdoch and Dydak 2011; Long et al. 2011; Dydak et al. 2011). All spectra had a linewidth of \(<10\) Hz as determined by the LCModel. All metabolite concentrations were computed as ratios to total creatine (tCr). The value of tCr for computing GABA/tCr was obtained from fitting the edit OFF spectrum of the MEGA-PRESS acquisition.
The fraction of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) were calculated by superimposing the coordinates of the spectroscopy VOI on the high-resolution T1-weighted images using the partial volume correction tool provided by N. Goulden and P. Mullins (http://biu.bangor.ac.uk/projects.php.en). The corresponding volume in the T1-weighted data set was then segmented into GM, WM and CSF fractions using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) as part of SPM8.

In addition to MEGA-PRESS spectra, regular short echo time Point REsolved Spectroscopy (PRESS) spectra (TR = 2 s, TE = 30 ms, 32 averages) were also obtained from the same VOIs and used for quantification of major metabolites including N-acetyl aspartate (NAA), total choline (tCho), tCr, myo-inositol (mI) and glutamate + glutamine (Glx). PRESS metabolite fits with a percentage standard deviation (%SD) value from LCModel over 20 % were excluded from further analysis. For MEGA-PRESS, the corresponding %SD threshold was chosen to be 25 % due to the flexible baseline approach. This threshold lies well within accepted standards used in GABA-MRS studies (e.g., Marjanska et al. 2013; Chowdhury et al. 2014; Silveri et al. 2013). However, 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. A representative striatal GABA spectrum is displayed in Fig. 1. Since no statistically significant differences were found within metabolite ratios between sides, prior to the regression analyses, the concentrations of different metabolites in the left and the right striatum were averaged across the left and right VOI for each subject (refer Fig. 1).

The MRS data were collected prior to the conduction of the response inhibition experiment using EEG. Hence, MRS data do not reflect possible differential GABA levels for experimental trials in which response inhibition was successful and where response inhibition was not successful. The GABA levels taken for the analysis reflect the general GABA level in the striatum; i.e., GABA levels were not determined in an event-related fashion for correct and incorrect Nogo trials separately. This is because a reliable estimation of GABA concentrations requires several acquisitions of the same volume. These several

Fig. 1 Representative MRS VOI placement in the right striatum (top) and the LCModel fits of MEGA-PRESS GABA spectrum (bottom left) and short TE PRESS spectrum (bottom right) acquired from the VOI.
acquisitions cannot be performed in the time a single trial lasts. MRS data were integrated with the behavioral data and EEG data using linear regression analyses; i.e., we used the striatal GABA/tCr ratio as an independent variable to predict the degree of phase-locking and the amplitude values of the Nogo-N2 and Nogo-P3 signals. All significances for the correlations calculated were corrected using Bonferroni–Holm correction. The results presented are therefore controlled for possible type I errors.

**Results**

For descriptive statistics the mean and standard error of the mean (SEM) are given. For the left striatal VOI the GABA/tCr ratio was 0.224 ± 0.01 (SD = 0.05) and for the right striatal VOI 0.220 ± 0.01 (SD = 0.04) GABA/tCr averaged over left and right striatal VOI was 0.221 ± 0.01 (SD = 0.03). Assuming a gray matter creatine concentration of 6 mM (Pouwels and Frahm 1998) allows for an estimate of the striatal GABA concentration of 1.34 ± 0.03 mM, which is well in line with values reported for gray matter in general (Boer et al. 2011; Choi et al. 2006) and a thalamic volume of interest (Dydak et al. 2011).

For the behavioral data the rate of false alarms (i.e., percentage of Nogo trials on which a response occurred) in the Nogo condition was the main dependent variable of interest. The rate of false alarms was 13.87 ± 0.4. The regression analysis revealed a substantial inverse correlation ($r = -0.541; R^2 = 0.292; \ p < 0.001$) between average striatal GABA/tCr level and the rate of false alarms (refer Fig. 2). There were no such correlations when using reaction times on Go and Nogo trials as well as reaction time slowing after the inhibition of responses (all $r < 0.2; \ p > 0.4$).

![Fig. 2 Scatter plot denoting the correlation between striatal GABA levels and the rate of false alarms in the Nogo condition](image)

The event-related potentials on Go and Nogo trials are shown in Fig. 3 at electrode FCz. The N2 amplitudes differed between Go ($-13.15 \pm 1.35$) and Nogo trials ($-21.16 \pm 2.08$) ($t_{39} = 6.37; \ p < 0.001$). The same was evident for the P3, where the P3 was smaller on Go (10.92 ± 1.68), than on Nogo trials (30.19 ± 2.69) ($t_{39} = -8.49; \ p < 0.001$). The regression analyses revealed that neither the Nogo-N2 amplitude nor the Nogo-P3 amplitude was correlated with average striatal GABA/tCr levels (all $r < 0.15; \ p > 0.4$) (refer Fig. 2d). Similarly, there was also no correlation for the Go amplitudes (all $r < 0.2; \ p > 0.4$; not scatter plots shown). However, since especially neuronal synchronization processes in striatal GABAergic MSNs are important for information encoding and behavioral control (Adler et al. 2013), we repeated the regression analyses after the calculation of the phase-locking factor (PLF). The PLF is independent of the amplitude of the EEG signal (e.g., Kolev and Yordanova 1997) and therefore provides information about the neuronal synchronization processes that are unrelated to the above analysis of ERP amplitudes. Figure 4 shows the mean PLF on Nogo trials.

As can be seen in Fig. 4a, the PLF was highest in the theta frequency band around a frequency of 5 Hz, which agrees with literature (for review: Huster et al. 2013). The mean PLF was 0.57 ± 0.02. The regression analysis using the average striatal GABA/tCr level revealed a strong positive correlation with the PLF ($r = 0.612; R^2 = 0.374; \ p < 0.001$) as shown in Fig. 4 in the Nogo-N2 time range. No correlation between average striatal GABA/tCr was evident in the Nogo-P3 time range at the 5 Hz frequency ($r = 0.142; \ p > 0.5$) as well as other frequencies (all $r < 0.12; \ p > 0.6$). To test whether the gray matter fraction (GM %) of the MRS VOIs confounds the correlation between GABA/tCr and the response inhibition measures, we included the fraction of gray matter (GM %) (0.34 ± 0.006), white matter (WM %) (0.58 ± 0.005) and cerebrospinal fluid (CSF %) (0.07 ± 0.004) as additional predictors to GABA/tCr in the regression analyses. This regression model also revealed a predictive effect of average GABA/tCr for the rate of false alarms ($\beta = 0.541; t = 3.87; \ p < 0.001$), while neither GM, WM nor CSF % explained the variance (all $\beta < 0.042; t < -0.39; \ p > 0.9$) (overall model: $F(1,38) = 14.95; \ p < 0.001$). The same was the case for PLF in the Nogo-N2 range. There was a predictive effect of GABA/tCr ($\beta = 0.612; t = 5.85; \ p < 0.001$), but no effect of GM, WM and CSF % (all $\beta < 0.141; t < 1.21; \ p > 0.2$) (overall model: $F(1,38) = 34.27; \ p < 0.001$).

Using the levels of metabolites obtained from the PRESS spectra (i.e., NAA, tCho, tCr, mI and Glx), there were generally no significant correlations with behavioral and neurophysiological parameters of response inhibition.
The total (induced) wavelet power on Go and Nogo trials at electrode FCz are shown in Fig. 4b and c. Using the total wavelet power, there were no correlations with the striatal GABA/tCr levels or any other metabolite obtained from the PRESS spectra (all $r < 0.15; p > 0.3$). This again underlines the specificity of effects regarding the obtained associations with the synchronization parameter.

Discussion

In the current study we examined the relevance of striatal GABA levels for response inhibition processes. Striatal GABA levels were measured using MRS. While the data quality and variability of our MRS data lie well within the normal reported ranges for other brain regions, it should be noted that this is the first study reporting GABA-MRS data acquired from the striatum, which is a particularly challenging brain area from which to achieve high-quality MRS data. Thus a direct comparison to other literature values, validating the interpretation of our results, is not available. Response inhibition processes were examined using a standard Go/Nogo paradigm in combination with EEG data that was integrated with the MRS data. The results show that striatal GABA levels were predictive for response inhibition performance; i.e., higher striatal GABA levels were related to a lower rate of false alarms in the Nogo condition and hence better response inhibition performance. The results are unbiased with respect to the fraction of GM, WM and CSF in the voxel. Other metabolites using the PRESS spectra (i.e., NAA, tCho, tCr, mI and Glx) did not predict parameters of response inhibition performance underlining the specificity of the results obtained for the GABA levels.

The results provide evidence for the assumption that the striatal GABAergic system, known to be involved in the selection of different actions through GABAergic neural transmission (Bar-Gad et al. 2003), are also involved in the suppression of responses. Previous studies on neurodegenerative disorders (e.g., Beste et al. 2010) were not able to show a role of the GABAergic system unequivocally, since GABAergic alterations were confounded with alterations in the dopaminergic system, which is of known importance for response inhibition processes (for review: Bari and Robbins 2013).

While Silveri et al. (2013) have previously shown that higher GABA levels in the anterior cingulate cortex are
related to better response inhibition, our results show that this is also true for striatal structures. In this regard, the results of our study are also in line with recent findings by Caprioli et al. (2014) who demonstrated that reductions in glutamate decarboxylase in rats are related to impulsive behavior. However, our results reveal three important aspects.

First, EEG measures are usually considered to be sensitive only to cortical processes, but not sensitive to subcortical basal ganglia processes. The current results show that a large amount of variance in EEG data is explained by neurobiochemical parameters of the basal ganglia, suggesting that EEG measures are sensitive to ‘remote’ basal ganglia processes. This is underlined by studies in basal ganglia diseases showing that pathophysiological processes affecting the basal ganglia modulate cortical electrophysiological correlates of cognitive control and inhibition processes recorded using the EEG (e.g., Beste and Saft 2014; Beste et al. 2012, 2011, 2009). Possibly, this effect arises as a consequence of the close functional and structural neuroanatomical connection between the prefrontal cortex and the basal ganglia. Regarding the EEG data, striatal GABA levels were predictive for the PLF in the Nogo-N2 time window, but not in the Nogo-P3 time window. For the amplitudes of the Nogo-N2 and Nogo-P3, striatal GABA levels were not predictive.

Second, the striatal GABA system modulates the reliability of neural synchronization processes as reflected by the PLF and not the intensity of an electrophysiological process. As the strength of striatal MSN interconnectivity is largely determined by GABA (Humphries et al. 2010) and the degree of connectivity is known to determine network synchronization (e.g., Kitano and Fukai 2007) as a major requirement for information encoding and behavioral control in the basal ganglia (Adler et al. 2013), it

**Fig. 4** a Grand average plot of the phase-locking factor (PLF) on Nogo trials at electrode FCz. *Warm colors* indicate high phase locking; *cold colors* denote low phase locking. The scatter plots show the correlation between striatal GABA levels and the PLF in the Nogo-N2 time window and the Nogo-P3 time window. *Black lines* linking the scatter plot with the relative time point (region in the PL-plot) indicate the time point from which the PL for the N2 and the P3 component was extracted. *Warm colors* in the maps denote electrode sites where the phase-locking factor was high; *cold colors* denote electrode sites where the phase-locking factor was low. b Time frequency plot denoting the total (induced) wavelet power at electrode FCz for Go trials. c Time frequency plot denoting the total (induced) wavelet power at electrode FCz for Nogo trials.
seems plausible that striatal GABA levels strongly modulate an electrophysiological measure of such neural synchronization processes. The importance of neural synchronization processes for response inhibition has also been shown by other electrophysiological studies (e.g., Beste et al. 2012; Swann et al. 2011). However, since the data structure obtained is necessarily correlative, it is also possible that altered neural synchronization processes at the cortical level may impact striatal GABA levels. Yet, in both cases the results stress the importance of the striatal GABAergic system for response inhibition. It is possible that GABA levels fluctuate between correct and incorrect Nogo trials; however, with the current MRS methods it is not possible to measure GABA levels in an event-related fashion.

Third, the results show that the striatal GABA system is only predictive of circumscribed electrophysiological subprocesses of response inhibition. It seems that striatal GABA levels modulate mechanisms reflecting pre-motor processes such as conflict monitoring or updating of the response program (N2-related processes), but not mechanisms reflecting evaluative processing of the successful response program (P3-related processes) (e.g., Huster et al. 2013; Beste et al. 2011; 2010). The Nogo-N2 is more related to the motor aspects of response inhibition than is the case of the Nogo-P3 (e.g., Huster et al. 2013). Since the striatum plays a particularly important role in motor control and in the canceling and restraining of responses (Bari and Robbins 2013; Bar-Gad et al. 2003), processes closely related to motor aspects of inhibition are more likely to be affected by GABA as a major basal ganglia neurotransmitter than those related to subsequent evaluative processes.

The current study underlines the relevance of the GABAergic system for response inhibition processes. Besides these processes, other executive control functions such as task switching, error monitoring and conflict processing have been shown to be modulated by striatal processes (Beste et al. 2014; van Schouwenburg et al. 2013; Willemssen et al. 2009, 2011). It is therefore conceivable that psychophysiological correlates of these functions may also show modulations by the striatal GABAergic system.

A limitation of the study is that the menstrual cycle of the female participants and hence levels of steroid hormones which have a known impact on GABA levels (Harada et al. 2011; Epperson et al. 2006) was not controlled for. Due to the low number of females in the present study, it was also not possible to run reliable regression analyses using the factor ‘sex’ as predictor in the regression models. A further limitation of the study may be the lack of a control region used for GABA measurements that may be useful to determine the specificity of effects obtained in this study. It should also be kept in mind that the estimated average tissue GABA concentration is the sum of metabolic GABA located in the cell body, within the synaptic cleft, coupled to GABA receptors and at extrasynaptic sites (Rae 2014). It is therefore unclear whether this metric is better considered to be a determinant of the strength of GABAergic inhibition or a reflection of net inhibitory activity. In the striatum, GABAergic interneurons represent only a small fraction of the cell population (and therefore a small fraction of tissue volume), but appear to contain much higher concentrations of GABA than projection neurons, so it is not clear which striatal GABAergic neurons (overview: Tepper and Bolam 2004) mainly drive the effects.

In summary, the study shows, using a novel combination of MRS data and time–frequency decomposed EEG data, that striatal GABA levels predict the efficacy of response inhibition processes. To our knowledge, this is the first study providing direct evidence for the relevance of the striatal GABAergic system for response inhibition functions and their cortical electrophysiological correlates in humans. Using EEG methods, we show that striatal GABA seems to affect only specific subprocesses of response inhibition that are related to pre-motor inhibitory processes through the modulation of the reliability of neural synchronization processes.

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Chapter 3

Study 2: Feeling Safe in the Plane: Neural Mechanisms Underlying Superior Action Control in Airplane Pilot Trainees—A Combined EEG/MRS Study
Feeling Safe in the Plane: Neural Mechanisms Underlying Superior Action Control in Airplane Pilot Trainees—A Combined EEG/MRS Study

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Abstract: In day-to-day life, we need to apply strategies to cascade different actions for efficient unfolding of behavior. While deficits in action cascading are examined extensively, almost nothing is known about the neuronal mechanisms mediating superior performance above the normal level. To examine this question, we investigate action control in airplane pilot trainees. We use a stop-change paradigm that is able to estimate the efficiency of action cascading on the basis of mathematical constraints. Behavioral and EEG data is analyzed along these constraints and integrated with neurochemical data obtained using Magnetic Resonance Spectroscopy (MRS) from the striatal gamma-aminobutyric acid (GABA)ergic system. We show that high performance in action cascading, as exemplified in airplane pilot trainees, can be driven by intensified attentional processes, circumventing response selection processes. The results indicate that the efficiency of action cascading and hence the speed of responding as well as attentional gating functions are modulated by striatal GABA and Glutamate + Glutamine concentrations. In superior performance in action cascading similar increases in the concentrations of GABA and Glutamate + Glutamine lead to stronger neurophysiological and behavioral effects as compared to subjects with normal performance in action cascading. Hum Brain Mapp 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

Key words: magnetic resonance spectroscopy (MRS); EEG; action control; attention; action cascading
INTRODUCTION

Many little boys dream of becoming an airplane pilot, however, only a few of them have the chance to fulfill their dream. One reason is that prospective airplane pilot trainees (APTs) undergo an extensive assessment of cognitive performance to ensure that they are able to cope with the multiple demands in a cockpit, especially in case of emergency [for review: Borghini et al., 2012]. Currently, knowledge about the neuronal mechanisms underlying action control is acquired through the examination of neurological disorders. However, almost nothing is known about the neuronal mechanisms mediating superior performance above the normal level. APTs may reflect a possible model to examine which neurophysiological and neurobiochemical processes underlie superior performance in action control.

When faced with multiple response options it is necessary to prioritize and cascade different actions [Mückschel et al., 2013]. Previous results from cognitive psychology suggest that in situations requiring a prioritization and chaining of actions, responses can be selected along a continuum from a more serial to a more parallel mode [e.g., Miller et al., 2009; Mückschel et al., 2013; Oberauer and Kliegel, 2004; Verbruggen et al., 2008] and therefore in a strategic nature that differ in efficiency [Meyer and Kieras, 1997]. In the serial mode action selection is performed in a step-by-step fashion, while in the parallel mode actions are activated in an overlapping manner, i.e., one action is activated before a previous one is terminated [Verbruggen et al., 2008]. A more parallel processing mode is accompanied with slow response times in the different actions to be cascaded, and hence an inefficient unfolding of behavior [Mückschel et al., 2013; Miller et al., 2009]. Previous results using electrophysiological techniques suggest that interindividual differences in these processing modes and action cascading efficiency are reflected in a network encompassing the anterior cingulate cortex (ACC) and the temporoparietal junction (TPJ) [Mückschel et al., 2013]. The ACC and the TPJ are areas constituting the multiple demand system (MD-system), known to be important for the execution of multi-component behavior [Duncan, 2010, 2013]. Mückschel et al. [2013] have shown that these areas are involved in processes mapping stimuli on appropriate responses (reflected by the P3 event-related potential) and that the P3 predicts the degree of overlap between two actions to-be-cascaded: a smaller P3 being related to a more efficient mode of action cascading [see also: Stock et al., 2014]. Functional imaging studies further show that striatal activity predicts the degree of overlap between two actions to be cascaded [Ness and Beste, 2013], suggesting a role for the striatum. The importance of the striatum is also corroborated by clinical data [Beste and Saft, 2013] and is well in line with several accounts suggesting that the basal ganglia and GABAergic striatal medium spiny neurons (MSNs) play an important role in the selection and coordination between different actions [e.g., Bar-Gad et al., 2003; Redgrave et al., 1999].

However, the basal ganglia can be seen as a structure that is not only involved in processes related to motor aspects of actions, but also as a structure that receives sensory information from different modalities including the visual and the auditory system [Kropotov et al., 2000; Nagy et al., 2006; Redgrave and Gurney, 2006; Znamenskiy and Zador, 2013]. This sensory afferent information is fed into the basal ganglia via glutamatergic cortico-striatal synapses [e.g., Bolam et al., 2000]. This convergence of sensory information in the striatum is interesting, as it also has been noted that besides response-related processes, attentional selection processes are important in situations where two or more actions have to be coordinated and cascaded to fulfill a task [Brisson and Jolicoeur, 2007]. The basal ganglia and especially the striatum may therefore be seen as a structure that integrates converging information in order to select and coordinate the selection of the appropriate action [Redgrave and Gurney, 2006]. The striatum may thus be central to the understanding of possible neurophysiological and neurobiochemical mechanisms underlying performance differences in action cascading processes.

As information processing within the striatum is highly dependent upon GABAergic and glutamatergic neural transmission we apply MR-spectroscopy (MRS) and integrate this data with event-related potential (ERP) data reflecting attentional selection processes [i.e., P1 and N1 ERPs; Herrmann and Knight, 2001], as well as processes mediating between stimuli and response (i.e., P3) [Falkenstein et al., 1994a;b; Polich, 2007; Verleger et al., 2005]. We hypothesize that APTs show enhanced attentional selection processes (stronger P1 and N1) and more efficient processes mediating between stimuli and responses [i.e., a smaller P3; see Mückschel et al., 2013]. This should come along with a more efficient behavioral performance in response selection in APTs. We further expect that GABA levels and metabolites of glutamatergic neural transmission (i.e., Glu and Glx) as revealed by MRS predicts individual variations in P1, N1, and P3 amplitude as well as behavioral data, because striatal structures may serve as a central element in the integration of the different streams of information and processes necessary for action cascading.

MATERIALS AND METHODS

Participants

A sample of 20 airplane pilot trainees (age 23.18 ± 1.97 years, range 21–29 years, two females) after passing the entrance examination and in process of obtaining their Multi-crew Pilot License (MPL) was recruited for the experimental group. The control group consisted of 20 subjects (age 24.94 ± 2.82 years, range 20–31 years, two females); two control subjects were discarded from analyses because the MRS data was of poor quality. All subjects
had normal or corrected-to-normal vision. All subjects received financial compensation or course credit for their participation. Experiments were carried out in accordance with the Declaration of Helsinki and the study was approved by the ethics committee of the medical faculty of the University of Bochum.

**Task**

The task used to examine action cascading processes and allowing for a classification of the mode of action selection along a continuum ranging from a more serial to a more parallel mode and hence the efficiency of action cascading is a stop-change (SC) paradigm. This paradigm has been used previously [e.g., Muckschel et al., 2013; Stock et al., 2014] and is adapted from a paradigm by Verbruggen et al. [2008]. The paradigm is shown in Figure 1.

Throughout every trial, a rectangle (20 × 96 mm²) containing four vertically aligned circles (8 mm diameter) and three horizontal reference lines (line thickness: 1 mm, width: 8 mm) separating the circles were presented on the screen. At the start of each trial, all lines were white and the four circles were filled black. After 250 ms, one of the circles was filled white thus becoming the GO1 target stimulus. In the GO1 condition (67% of all trials), the participants’ response was expected to indicate whether this filled white circle (target) was located above or below the middle reference line. Responses were given by pressing the outer right key with the right middle finger (“above” judgment) or by pressing the inner right key with the right index finger (“below” judgment). All stimuli remained visible until the participant either responded or 2,500 ms had elapsed. In case of reaction times (RTs) longer than 1,000 ms the German word “Schneller!” (translating to “Faster!”) was presented above the box until the participant responded thereby ending the trial.

The remaining 33% of trials were SC trials. The SC condition started with the presentation of a white GO1 stimulus. After a variable ‘stop-signal delay’ (SSD), which was adjusted using a staircase procedure [Logan and Cowan, 1984], a STOP signal (a red rectangle replacing the usual white frame; depicted grey in Fig. 1) was presented, putting an end to the GO1 trial. This STOP signal remained on the screen until the end of the trial and requested the participant to try to inhibit the right hand response to the GO1 stimulus. The SSD was initially set to 450 ms and adapted to the participants’ performance by means of a “staircase procedure” yielding a 50% probability of successfully inhibited GO1 responses. In case of a completely correct SC trial (no response to GO1 stimulus, no response before the GO2 stimulus (explained below) in SCD300 conditions and a correct left hand response to the GO2 stimulus), the SSD was adjusted by adding 50 ms to the SSD of the evaluated trial. In case of an erroneous SC trial (if any of the above criteria were not met), the SSD was adjusted by subtracting 50 ms from the SSD of the evaluated trial. Limiting this procedure, SSD values were set not to go below a value of 50 ms and not to exceed a value of 1,000 ms.
Irrespective of the inhibition performance, every stop signal was combined with one of the three possible CHANGE stimuli. The CHANGE stimulus was a 100 ms sine tone presented via headphones at 75-dB SPL and could be either high (1,300 Hz), medium (900 Hz) or low (500 Hz). It assigned a new reference line in relation to which the GO2 stimulus (the previous GO1 white target circle that had remained on the screen during the whole trial) had to be judged. While the high tone represented the highest of the three lines as the new reference, the medium tone represented the middle line and the low tone represented the lowest line (see Fig. 1). All three reference lines were in effect equally often. The required GO2 response had to be performed with the left hand. If the target circle was located above the newly assigned reference line, an outer left key press (left middle finger) was required and if the target circle was located below the newly assigned reference line, a left inner key press (left index finger) was required. In half of the SC trials, there was a stop-change delay (SCD) with a stimulus onset asynchrony (SOA) of 300 ms between the STOP and the CHANGE signal (“SCD300” condition) while in the other half of SC trials, the two stimuli were presented simultaneously (SOA of 0 ms, “SCD0” condition). In case of RTs longer than 2,000 ms the German word “Schneller!” (translating to “Faster!”) was presented above the box until the participant responded to end the trial. During the intertrial interval (ITI; fixed duration of 900 ms), a fixation cross was presented at the center of the screen. Participants were instructed to respond as fast and accurately as possible. All conditions were presented in a randomized order. Therefore, preparatory effects in the motor system biasing the results are very unlikely. The experiment participants completed an exercise until they understood the task.

**Estimation of the Mode and Efficiency of Action Cascading**

The mode of action cascading and hence the efficiency of this process was estimated using a mathematical model: As described above, the paradigm introduces two different SCD intervals. On the basis of these SCDs (i.e., 0 and 300 ms) we calculated a slope value for the GO2 RTs using the equation:

$$\text{slope} = \frac{(\text{GO2 RT}_{\text{SCD0}} - \text{GO2 RT}_{\text{SCD300}})}{(\text{SCD0} - \text{SCD300})}$$

The slope value was individually calculated for each participant. This value becomes steeper the more SCD0-RT and SCD300-RT differ. The interpretation of the slope value as a measure of response selection processes on a serial–parallel continuum is based on the following rationale [see Verbruggen et al., 2008]: The SOA in the SCD300 condition enforces a serial processing of the STOP- and CHANGE-related processes because the STOP process has usually been finished when the CHANGE stimulus is presented 300-ms later. In contrast to this, the SCD0 condition provides the participants with a choice of how to cascade STOP- and CHANGE-associated processes. Bottleneck models suggest that response selection can be done rather serially (one step is executed after another) or rather parallel [steps are processed in parallel so that there is a temporal overlap of processes; Miller et al., 2009; Verbruggen et al., 2008; Wiu and Liu, 2008]. Because response selection depends on a restricted resource, the processing strategy differentially affects the GO2 RT in the SCD0 condition: When the STOP process has not finished at the time the CHANGE process is initiated (parallel processing), the slope value becomes larger and action cascading less efficient. If it has finished (serial processing), the slope is closer to 0 and action cascading more efficient [Verbruggen et al., 2008].

Obtaining a mean slope value in between 0 and −1 hence suggests that the initiation of some (but not all) of the GO2 response processes were initiated before the termination of the inhibitory process of stopping the GO1 response. Therefore, the slope of the SOA-RT function is flatter in case of more efficient processing than in the case of less efficient processing mode [quoted from Stock et al., 2014].

**EEG Recording and Analysis**

The EEG recording and analysis was performed similar to our previous study [Mückschel et al., 2013; Stock et al., 2014]: The EEG was recorded from 65 Ag/AgCl electrodes against a reference electrode located at FCz. Electrode impedances were kept below 5 kΩ. After data recording, a band-pass filter (0.5–20 Hz) at a slope of 48 db/oct was applied and a raw data inspection was conducted to remove technical artifacts. Periodically recurring artifacts (pulse artifacts, horizontal, and vertical eye movements) were corrected using an independent component analysis (ICA; Infomax algorithm) which was applied to the unpocched data. Stimulus-locked segments based on the stop signal were formed. Automated artifact rejection procedures were applied. Rejection criteria included a maximum voltage step of more than 60 µV ms⁻¹, a maximal value difference of 150 µV in a 250 ms interval or activity below 1 µV. Artifact rejection was followed by a current-source-density (CSD) transformation yielding a reference-free evaluation of the electrophysiological data and helping to identify the electrodes showing the strongest effects. Baseline correction was conducted using the interval from 900 ms to −700 ms as prestimulus baseline (i.e., a baseline set before the occurrence of the GO1 stimulus). The electrodes used for the quantification of the P1, N1, and

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1One limitation to this interpretation remains: According to Verbruggen et al. [2008], it is impossible to distinguish between the behavioral effects (RT slope values) of nondeterministic serial processing and parallel processing based on the RT slope value [c.f., Verbruggen et al., 2008 for a detailed discussion on this issue].
P3 ERPs, were selected in a data-driven manner. Based on the scalp topography maps, the visual P1 and N1 were quantified at electrodes P7 and P8 (P1: 0 ms till 140 ms; N1: 150 till 250), the auditory P1 at electrodes TP9 and TP10, the auditory N1 at C5 and C6 (0 ms till 500 ms), and the P3 at Cz (200 ms till 600 ms). All ERP components (peak amplitudes) were quantified relative to the prestimulus baseline on the single subject level. For each subject peak amplitudes and latencies were obtained.

MRS Acquisition and Analysis

MRS data was acquired on a 3 T Philips Achieva whole-body scanner using a 32-channel head coil. Fast T2-weighted images were obtained in axial, coronal and sagittal planes to enable placement of a 30 × 30 × 25 mm³ MRS volume of interest (VOI) centered on the striatum. MRS data was acquired from VOIs placed in the left as well as the right striatum to rule out any laterality effects. Automatic shimming resulted in linewidths of 5 Hz for all spectra. Both, short echo time point resolved spectroscopy (PRESS) spectra (echo time (TE) = 30 ms, repetition time (TR) = 2,000 ms, 32 averages) and MEGA-PRESS edited GABA spectra (TE/TR = 68/2,000 ms, edit ON acquisitions = 16, edit OFF acquisitions = 16) [Edden et al., 2007; Mescher et al., 1998] were acquired from each VOI. In addition, a reference scan without water suppression was also acquired for frequency and phase correction. LCMR [Provencher et al., 1993] (v6.2-0R), which fits the in vivo MR spectra as a linear combination of single metabolite “basis spectra,” was used to quantify the spectra. PRESS spectra were used for the quantification of major metabolites including N-acetyl aspartate (NAA), total choline (tCho), total creatine (tCr), myo-inositol (mi), and glutamate+glutamine (Glx). GABA was quantified from the MEGA-PRESS difference spectra as GABA plus macromolecules (GABA+) using basis spectra obtained by density matrix simulations. The value of tCr for computing GABA+/tCr was obtained from fitting the edit OFF spectrum of the MEGA-PRESS acquisition. Metabolite concentrations are reported in institutional units (uM) as well as ratios to tCr. PRESS metabolites with LCMR percentage standard deviation (SD) over 20 were excluded from further analysis. Prior to the regression analyses the concentrations of the different metabolites were averaged across the VOI placed in the left and the right striatum.

The fractions of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) were calculated by superimposing the coordinates of the spectroscopy VOI on the high resolution T1-weighted images using the partial volume correction tool provided by N. Goulden and P. Mulkins (http://biu.bangor.ac.uk/projects.php.en). The corresponding volume in the T1-weighted data set was then segmented into GM, WM and CSF fractions using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) as part of SPM8. To control if the fraction of GM, WM and CSF change the predictive values of the spectroscopy parameters on neurophysiological and behavioral data these fractions were also used as predictors in the regression analyses.

Statistical Analyses

The data was analyzed using mixed effects ANOVAs with the factors “SCD interval (SCD0 vs. 300)” and “electrode” (whenever necessary) and the between-subject factor “group” (APT vs. control). Post hoc tests were Bonferroni-corrected whenever necessary. All included variables were normally distributed as tested with Kolmogorov-Smirnov tests (all z < 0.9; P > 0.3). Additionally, regression analyses were calculated using the “enter” method. For these regression analyses, the individual striatal GABA+/tCr and Glx/tCr concentration ratios measured using MRS were used to predict the amplitudes of the ERP components differing between APTs and controls. The group effect (i.e., APTs vs. controls), was also modeled in the regression analyses.

RESULTS

Behavioral Data

The reaction time data was analyzed in a mixed effects ANOVA using “condition” (GO1, SCD0 and SCD 300) as within-subject factor and “group” (APT vs. controls) as between-subject factor. There was a main effect “condition” (F(2,72) = 136.02; P < 0.001; η² = 0.781) showing that RTs were fastest in the GO1 condition (517 ± 16 ms) followed by the SCD300 (778 ± 28 ms) and SCD0 condition (942 ± 27 ms). All conditions differed from each other (P < 0.001). There was an interaction “condition × group” (F(2,72) = 3.99; P = 0.023; η² = 0.095), but no main effect group (F(1,36) = 2.90; P = 0.12). Bonferroni-corrected two samples t tests revealed that APTs did not differ from controls in the GO1 condition (APTs: 514 ± 22 ms; controls: 519 ± 24 ms; t36 = −0.14; P = 0.442) and in the SCD0 condition (APTs: 744 ± 55 ms; controls: 799 ± 44 ms; t36 = −1.18; P = 0.122). However, in the SCD0 condition APTs revealed faster RTs (870 ± 35 ms) than controls (1,013 ± 46 ms) (t36 = −2.84; P < 0.01). The slope of the SCD-RT2 function was hence flatter in APTs than in controls (t36 = −2.32; P = 0.011). The stop signal reaction time (SSRT) did not differ between APTs and controls (P > 0.5). Similarly, the mean stop-change delay (SSD) was not different between APTs and controls (P > 0.6). With respect to the accuracy, APTs and controls did not differ in their error rates on SCD trials, neither in the SCD0 nor in the SCD300 condition (P > 0.6).

Electrophysiological Data

Auditory P1 and N1 effects

The first step was analysis of auditory and visual attentional selection processes and quantification of the
auditory P1 and N1 and the visual P1 and N1. The corresponding ERP plots are shown in Figure 2.

For the auditory P1 (electrodes TP9 and TP10) the ERPs are shown in Figure 2A. There was a main effect “SCD interval” \( (F(1,36) = 15.64; P < 0.001; \eta^2 = 0.292) \) showing that the P1 was larger in the SCD0 (25.37 ± 1.85 \( \mu \)V m\(^{-2} \)) compared to the SCD300 (17.13 ± 1.43 \( \mu \)V m\(^{-2} \)). There was also a main effect group \( (F(1,36) = 4.65; P = 0.037; \eta^2 = 0.109) \) showing that the P1 was larger in APTs (25.28 ± 1.65 \( \mu \)V m\(^{-2} \)) than in controls (15.39 ± 1.54 \( \mu \)V m\(^{-2} \)). Importantly, there was an interaction “SCD interval × group” \( (F(1,36) = 16.11; P < 0.001; \eta^2 = 0.298) \).

Bonferroni-corrected independent samples \( t \) tests revealed that APTs showed a larger P1 (30.11 ± 2.33 \( \mu \)V m\(^{-2} \)) than controls (19.23 ± 2.64 \( \mu \)V m\(^{-2} \)) in the SCD0 condition \( (t_{36} = 3.87; P < 0.001, \eta^2 = 0.173) \) showing that the N1 was less negative in the SCD0 (-11.08 ± 3.1 \( \mu \)V m\(^{-2} \)) compared to the SCD300 condition (-20.28 ± 2.16 \( \mu \)V m\(^{-2} \)). There was also a main effect “group” showing that the N1 was larger in controls (20.81 ± 2.89 \( \mu \)V m\(^{-2} \)) than in APTs (-10.55 ± 2.75 \( \mu \)V m\(^{-2} \)) \( (F(1,36) = 6.28; P = 0.017; \eta^2 = 0.142) \). In addition there was an interaction “SCD interval × group” \( (F(1,36) = 20.01; P < 0.001; \eta^2 = 0.345) \) and the N1 differed between APTs and controls in the SCD0 condition \( (P = 0.01) \), but not in the SCD300 condition \( (P > 0.3) \). As with the P1, there were also no latency effects for the N1 (all \( F < 0.3; P > 0.6 \)).

**Visual P1 and N1 effects**

For the visual P1 amplitude, there were no main effects of “electrode,” “group,” and “SCD interval” or any interaction between these factors as well as no latency effects (all \( F < 0.4; P > 0.4 \)). For the visual N1 there was only a
main effect “electrode” ($F(1,36) = 31.94; \ P < 0.001; \ \eta^2 = 0.457$) showing that the N1 was smaller at electrode P7 ($-24.02\pm 2.35 \ \mu V \ m^{-2}$), compared to P8 ($-33.95\pm 2.77 \ \mu V \ m^{-2}$). All other main or interaction effects were not significant (all $F < 0.6; \ P > 0.3$). There were also no effects for the visual N1 latency (all $F < 0.7; \ P > 0.3$). The visual P1 and N1 are shown in Figure 3.

**P3 effects**

The P3 at electrode Cz is shown in Figure 4.

The mixed effects ANOVA revealed a main effect “SCD interval” ($F(1,36) = 13.99; \ P < 0.001; \ \eta^2 = 0.233$), with the P3 being larger in the SCD0 condition, compared to the SCD300 condition. No other main or interaction effects were significant (all $F < 0.4; \ P > 0.5$).

**MR spectroscopy and regression analyses**

A representative MRS short-TE PRESS spectrum and MEGA-PRESS GABA spectrum acquired from a striatal VOI (Fig. 5A) are shown in Fig. 5B,C, respectively. The concentrations of the different metabolites are given in Table I. tCr was found to be stable across the test groups and could thus be used as reference concentration. As can be seen in Table I, striatal GABA+/tCr concentrations did not differ between APTs and controls. Similar results were obtained for the other MRS metabolites like NAA/tCr, Glx/tCr, mI/tCr and tCho/tCr.

However, the behavioral data revealed a flatter slope of the SCD-RT2 function suggesting for a more serial mode action cascading in APTs, compared to controls. Similarly, the auditory P1 was larger in APTs. To integrate the model-based behavioral and electrophysiological data with the neurochemical (MRS) data regression analyses were used. In the regression analyses the slope of the SCD-RT2 function or the amplitude of the auditory P1 in the SCD0 conditions were the dependent variables. The striatal GABA+/tCr ratio and the factor “group” (APTs vs. controls) and the Glx/tCr ratio were used as independent variables in separate regression analyses. The results of the regression analyses are shown in Figure 6.

**GABA+/tCr**

For the slope of the SCD-RT2 function the regression model was significant ($F(2,35) = 12.38; \ P < 0.001$). Within the model, both, the GABA+/tCr ($\beta = 0.471; \ t = 3.62; \ P = 0.001$)
and the factor group were significant ($\beta = 0.489$; $t = 3.76$; $P = 0.001$). For the APTs there was a correlation between SCT-RT2 and GABA/tCr ($r = 0.736$; $R^2 = 0.53$; $P < 0.001$). The same was evident for the controls ($r = 0.438$; $R^2 = 0.17$; $P = 0.021$). However, as can be seen in Figure 6A the slope of the regression line relating SCD-RT2 with GABA+/tCr was steeper in APTs than in controls.

For the amplitude of the auditory P1 in the SCD0 condition the regression model was also significant ($F(2,35) = 25.46$; $P < 0.001$). Within the model, both, the GABA+/tCr ($\beta = 0.578$; $t = 3.48$; $P = 0.001$) and the factor group were significant ($\beta = 0.710$; $t = 6.54$; $P < 0.001$). There was a correlation between SCT-RT2 and GABA+/tCr for the APTs ($r = 0.588$; $R^2 = 0.33$; $P = 0.003$) as well as for the controls ($r = 0.485$; $R^2 = 0.23$; $P = 0.021$). However, to control whether the correlations obtained were driven by 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 $\beta < 0.123$; $t < 0.94$; $P > 0.4$). To control further for a possible influence of GM, WM and CSF fraction we also used these fractions in other separate regression models. All these models revealed that neither, GM, nor WM, nor CSF explained further variance in the models (all $\beta < 0.139$; $t < 0.99$; $P > 0.4$). Independent samples $t$ tests showed that APTs and control did not differ in their GM ratio (APT: $0.34 \pm 0.02$; controls:

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Airplane pilot trainees (APTs)</th>
<th>Controls</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA/tCr</td>
<td>0.22 (0.08)</td>
<td>0.39 (0.15)</td>
<td>ns</td>
</tr>
<tr>
<td>NAA/tCr</td>
<td>0.92 (0.09)</td>
<td>0.90 (0.11)</td>
<td>ns</td>
</tr>
<tr>
<td>Glu/tCr</td>
<td>1.02 (0.11)</td>
<td>1.01 (0.08)</td>
<td>ns</td>
</tr>
<tr>
<td>Glx/tCr</td>
<td>1.59 (0.11)</td>
<td>1.57 (0.09)</td>
<td>ns</td>
</tr>
<tr>
<td>ml/tCr</td>
<td>0.57 (0.08)</td>
<td>0.57 (0.10)</td>
<td>ns</td>
</tr>
<tr>
<td>GPC+PCH/tCr</td>
<td>0.27 (0.01)</td>
<td>0.27 (0.08)</td>
<td>ns</td>
</tr>
<tr>
<td>tCr (i.u)</td>
<td>6.12 (0.42)</td>
<td>6.11 (0.21)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Figure 5.

(A) Illustration of the placement of the volume of interest for the left striatum. In the right striatum the volume was placed similar. (B) Representative example of a PRESS spectrum showing the concentrations of ml, tCho, tCr, Glx and NAA. (C) Representative example of the MEGA-PRESS edited GABA spectrum showing the peaks of GABA+, NAA, and lipids. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
0.34 ± 0.01; t_{38} = 0.22; P > 0.8), WM ratio (APT: 0.58 ± 0.04; controls: 0.57 ± 0.03; t_{38} = 0.62; P > 0.5) and CSF ratio (APT: 0.06 ± 0.02; controls: 0.07 ± 0.01; t_{38} = −1.01; P > 0.3). Also, when using raw GABA (i.u.) and Cr (i.u.) values corrected for tissue content (i.e., raw GABA and Cr values multiplied by 1-CSF) in other regression analyses using the “enter” method there was still a correlation for GABA in APTs (r = 0.504; R² = 0.25; P = 0.003) and controls (r = 0.444; R² = 0.19; P = 0.029), but none for Cr (all r < 0.102; P > 0.5) with the P1 amplitude. The same was evident for SCT-RT2 parameter, where correlations with GABA were (r = 0.703; R² = 0.49; P < 0.001) for APTs and (r = 0.422; R² = 0.17; P = 0.026) for the controls. There were no correlations with Cr (all r < 0.09; P > 0.6).

**Glx/tCr**

The scatterplots correlating the slope of the SCD-RT2 function and the amplitude of the auditory P1 with the Glx/tCr ratio are shown in Figure 6B. Using the Glx/tCr ratio, the above analyses were repeated. Using the slope of the SCD-RT2 function there was a significant model (F(2,35) = 10.22; P < 0.001) in which both, the Glx/tCr (β = 0.455; t = 3.44; P = 0.001) and the factor group were significant (β = 0.432; t = 3.33; P = 0.001). For the APTs there was a correlation between SCT-RT2 and Glx/tCr (r = 0.673; R² = 0.44; P < 0.001). The same was evident for the controls (r = 0.401; R² = 0.16; P = 0.021).

The regression model was significant for the amplitude of the auditory P1 in the SCD0 condition (F(2,35) = 20.22; P < 0.001). Within the model, both, the Glx/tCr (β = 0.388; t = 3.55; P = 0.001) and the factor group were significant (β = 0.670; t = 6.34; P < 0.001). Again there was a correlation between SCT-RT2 and Glx/tCr for the APTs (r = 0.621; R² = 0.38; P = 0.001) and for the controls (r = 0.431; R² = 0.18; P = 0.025). To control whether the correlations obtained were driven by creatine (tCr) and not by Glx, we used the tCr concentration (i.u.) as obtained from the PRESS spectra as additional regressor in the analyses. These analyses revealed no effect of tCr on the neurophysiological and the behavioral parameter (all β < 0.088; t < 0.77; P > 0.5). To control further for a possible influence of GM, WM, and CSF fraction we also used these fractions in other separate regression models. All these models revealed that neither, GM, nor WM, nor CSF explained further variance in the models (all β < 0.095; t < 0.84; P > 0.5). As with the GABA parameter, we used raw Glx (i.u.) and Cr (i.u.) values corrected for tissue content.

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**Figure 6.** Scatterplots denoting the results of the regression analyses. Plots in column (A) show the correlation between the slope of the SCD-RT2 function and GABA+/tCr (top) as well as the correlation between the auditory P1 amplitude and GABA+/tCr (bottom). Plots in column (B) show the correlations of the SCD-RT2 function and Glx/tCr (top) as well as the correlation between the auditory P1 amplitude and Glx/tCr (bottom). Open circles denote APTs and black dots denote controls.
content in regression analyses using the “enter” method. There was no correlation with Cr in APTs and controls for the P1 and the SCD-RT2 parameter (all \( r < 0.133; P > 0.5 \)). Yet, there were still correlations with the P1 parameter in APTs (\( r = 0.599; R^2 = 0.34; P = 0.001 \)) and controls (\( r = 0.401; R^2 = 0.16; P = 0.029 \)), as well as for the SCD-RT2 parameter in APTs (\( r = 0.623; R^2 = 0.38; P < 0.001 \)) and controls (\( r = 0.391; R^2 = 0.15; P = 0.031 \)).

**DISCUSSION**

We studied APTs as a possible model to examine which electrophysiological and neurobiochemical processes may underlie superior performance in action selection. Currently neuroscientific research heavily investigates declines in performance, but little is known about the mechanisms mediating superior performance in action control.

To this end, we integrated MRS data with electrophysiological data (ERP data) recorded in a stop-change paradigm that allows an estimation of the efficiency of action cascading using mathematical constraints. The behavioral data suggest that APTs use a more efficient mode during action cascading, compared to controls. The slope of the SCD-RT2 function in controls is comparable to other studies in healthy young subjects [see, Muckschel et al., 2013; Stock et al., 2014]. Changes in the slope of the SCD-RT2 functions were driven by the SCD0 condition, where APTs showed faster response times than controls. In the SCD300 condition, there was no difference between APTs and controls. Because SCD0 is the only condition in which participants can cascade their actions according to their “preferred” processing strategy (see Methods section), this allows for the interpretation that the effects were confined to specific variations in response selection/action cascading processes with respect to efficiency of action cascading. However, the P3 data was not different between controls and APTs, suggesting that processes related to the mapping of a stimulus onto the appropriate response, as reflected by P3 [Falkenstein et al., 1994a,b; Polich, 2007; Verleger et al., 2005], do not contribute to the more efficient unfolding of behavioral control observed in APTs. With respect to other studies using the same paradigm [e.g., Muckschel et al., 2013; Stock et al., 2014] the results suggest that the efficiency of action cascading is not necessarily dependent on processes mapping the stimuli onto the response. The effects observed at the behavioral level can therefore not unequivocally be attributed to serial-parallel differences in the response selection mode. It cannot be excluded that APTs exhibit faster parallel processing capabilities than controls. It is possible that APTs use other mechanisms to achieve efficient unfolding of action cascading. In this regard it seems to be an altered perceptual/attentional processing that may be of relevance. In line with this, the electrophysiological data showed that the P1 on the auditory change signal in the SCD0 condition was stronger in APTs, compared to controls. The auditory N1 was also larger in APTs, however, this may reflect a simple aftereffect of the enlarged auditory P1 and is therefore hard to interpret. In the SCD300 condition, no differences between APTs and controls were evident in the auditory P1 and N1. The visual P1 and N1, as well as the P3 were not different between APTs and controls. Further evidence corroborating the assumption that efficient action control in APTs is related to attentional processing comes from the regression analysis showing that the amplitude of the auditory P1 was predictive for the steepness of the slope of the SCD-RT2 function. These results support a role for attentional gating in response selection, with higher response to the auditory stimulus predicting faster processing in the task where STOP and CHANGE signals simultaneously demand processing resources. Perhaps this implies that better cross-modal interaction between stimulus response systems is the driver for faster processing.

Integrating the ERP and behavioral data with the MRS-data revealed that the GABA+/Cr level in the striatum was predictive for the modulation of these attentional gating functions probably underlying efficient action cascading processes in APTs. These results are unbiased with respect to the concentration of creatine and the GM, WM and CSF fractions in the voxel. Because this allows for the conclusion that it is indeed the GABA+ concentration and not the tCr that drives any associations, we will further speak about GABA+ concentrations in the discussion. The same is true for results on Glx/tCr. A stronger auditory P1 and a smaller (less negative) SCD-RT2 parameter were related to higher striatal GABA+ concentrations. Similar results were found for Glx (i.e., glutamate + glutamine). These results suggest that striatal structures are involved in processing of the change stimulus, a result that is plausible against other findings reporting auditory sensory neurons in the striatum [Kropotov et al., 2000; Nagy et al., 2006; Saft et al., 2008; Znamensky and Zador, 2013].

The results further suggest that superior performance in action cascading depends at least on two neurobiochemical factors related to the glutamatergic and GABA-ergic system. While correlations between GABA+ concentrations, Glx concentrations and the auditory P1 amplitude as well as the slope of the SCD-RT2 function in controls and APTs where evident in APTs and controls, the regression analyses showed that the slope of the regression lines were steeper in APTs, compared to controls. Hence, similar increases in GABA+ and Glx -levels led to stronger increases in the auditory P1 and a more efficient processing in action cascading in APTs, compared to controls. This suggests that the neurobiochemical-electrophysiological and neurobiochemical-behavioral coupling is stronger in APTs than in controls. It is likely that this stronger neurobiochemical-electrophysiological and behavioral coupling partly underlies the more efficient unfolding of action cascading processes, besides the more
intense attentional gating in APTs as evidenced by the stronger auditory P1.

In striatal structures GABA is abundant and constitutes the key element in the functioning of medium spiny neurons (MSNs) [e.g., Bolam et al., 2000]. These neuron types have recently been shown to affect action cascading processes and it has been found that dysgenesis of striatal MSNs is associated with a more parallel and hence inefficient mode of action cascading [Beste and Saft, 2013]. The current MRS results showing that higher GABA+ concentrations are related to a more efficient processing mode nicely complement these findings. Striatal MSNs have been suggested to form a winner-takes-all network [WTA; Gurney et al., 2004; Plenz, 2003; Redgrave et al., 1999], i.e., meaning that the network of inhibitory connections between MSNs is assumed to inhibit neighboring neurons. As a consequence competing actions are suppressed and the network converges to a single winner [Bar-Gad et al., 2003]. It is possible that higher striatal GABA concentrations lead to a sharper WTA and hence a stronger suppression of alternative actions in striatal structures. As alternative actions may hence not be evident in these structures, a strong WTA mechanism entails a more efficient processing mode in action cascading processes and leads to faster reaction times during changing to another task. In addition to the putatively sharper WTA mechanism the input to the striatal network, mediated by glutamatergic system, is also more efficient in APTs, as suggested by the slope of the regression line interrelating striatal Glx concentrations with behavioral performance and P1 amplitude. The fact that this was the case despite GABA+ and Glx concentrations did not differ between the groups suggests that there may be a third factor influencing the relation between GABA+/Glx with behavioral performance and neurophysiological processes. This third factor may be related to a possible altered strength of structural white matter connectivity between cortical and basal ganglia structures as well as factors related to the NMDA and GABA receptor sensitivities. This may be subject to future studies.

In summary, the current study shows that high performance in action cascading and multicomponent behavior, as exemplified in airplane pilot trainees, cannot only be driven by altered processes mapping stimuli onto the response [Mückschel et al., 2013; Yildiz et al., 2014], but also by intensified attentional processes. The results show that the efficiency of action cascading and hence the speed of responding as well as attentional gating functions seem to depend on striatal GABA+ and Glx concentrations with higher striatal concentrations being related to a more efficient mode of action cascading and stronger attentional gating. The results suggest that the strength of neurobiochemical-behavioral and -electrophysiological coupling is important for superior action control in situations requiring a cascading of actions and hence differentiates between “high performer” and “normal performer.”

REFERENCES


Chapter 4

Study 3: Interrelation of Resting State Functional Connectivity, Striatal GABA Levels, and Cognitive Control Processes
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. *Human Brain Mapp 36:4383–4393, 2015. © 2015 Wiley Periodicals, Inc.*
**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-relevant 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; Willemsen 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.

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**Key words:** GABA; resting state functional connectivity; executive control; Simon task

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**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.

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 [Tkáč 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 [Marjanska 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 ($tCr_{normal}$ = 7.01 ± 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.

**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 principal 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

**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.]
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 R².

RESULTS

Behavioral Data

For the RT data, the mixed-effects ANOVA revealed a main effect “hand position” (F(1,38) = 52.17; P < 0.001; η² = 0.58) 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; η² = 0.84) 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; η² = 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; η² = 0.819). There was also a main effect “group” (F(1,38) = 32.81; P < 0.001; η² = 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; η² = 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; t38 = 1.46; P > 0.15), but in the incongruent condition (Pilot trainees: 182 ± 1.2; controls: 167 ± 1.5; t38 = 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 congruent trials (IL – IC) from the effect following correct congruent trials (IL – IC). 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 PBS, 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; t38 = −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
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, substantia 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 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.]

**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 (r2 = −0.43, P < 0.01), LFP (r2 = 0.34, P < 0.02), gender (−0.30, P < 0.03), and GABA+/tCr concentration (r2 = 0.30, P < 0.04), with the P-values representing the partial correlations. The full model explained 39% of the variance (adjusted R2) 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 R2 = 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.

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 susceptible 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 relation 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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Chapter 5

General Discussion
5.6 Discussion of the Main Findings

5.6.1 Summary of the Main Findings

The first study investigated the relevance of striatal GABA levels for response inhibition processes and related synchronized oscillations. Response inhibition processes were examined using a standard GoNogo paradigm in combination with EEG Behavioural and neurophysiological data were then integrated with MRS measures. Here, and in the following two studies, correlations with striatal GABA+/tCr (and Glx/tCr) levels were unbiased with respect to the concentration of creatine and the grey matter, white matter, and cerebrospinal fluid fractions in the voxel. This suggests that it is indeed the GABA concentration that drove the associations and therefore GABA+/tCr will be referred to as GABA from here on. Higher striatal GABA levels were predictive for a lower rate of false alarms in the Nogo condition and thus better response inhibition performance. Furthermore, striatal GABA levels were predictive for the PLF in the Nogo-N2 time window, but not the Nogo-P3 time window. The amplitudes of Nogo-N2 and Nogo-P3 were unrelated to striatal GABA levels, i.e. striatal GABA modulated the reliability of the neural synchronization but not the intensity of this electrophysiological process. The striatal GABA system was only predictive for mechanisms that reflect pre-motor processes like conflict monitoring or updating of the response program (Nogo-N2) but not mechanisms that reflect evaluative processes of the successful outcome of inhibition (Nogo-P3). None of the other metabolites measured (NAA, tCho, tCr, mI, and Glx) were predictive for response inhibition performance.

The second study introduced airplane pilot trainees (APTs) as a potential model for superior action control performance to examine neurobiological processes that are related to superior cognitive control mechanisms. APTs and aged-matched controls performed a stop-change task while EEG was recorded. As in study 1, behavioural and electrophysiological data were then integrated with MRS measures. Group differences were only found when stop and change stimuli were present simultaneously (SCD0 condition); i.e., only when interindividual variation in action cascading processing strategy was possible. APTs showed more efficient action cascading than controls as evident by a flatter slope of the SCD-RT2 function. This was driven by the faster response times of APTs in the SCD0 condition. There was a group difference in attentional stimulus processing as characterized by a stronger P1 on the auditory change signal in APTs compared to controls. The auditory N1 was also larger in APTs. However, this may reflect a simple aftereffect of the enlarged auditory P1. Further, a stronger P1 predicted faster processing when two response options simultaneously demand
processing resources (SCD0) and needed to be coordinated and cascaded. There were no group differences for the P3 which suggests that processes associated with the mapping of a stimulus onto the appropriate response did not contribute to the more efficient unfolding of behavioural control in APTs. Neither GABA nor other metabolite concentrations in the striatum differed between APTs and controls. However, striatal GABA and glutamate levels were predictive for the modulation of the attentional gating functions (auditory P1) and the SCD-RT2 parameter. The strength of this correlation differed between the groups; similar increases in GABA and glutamate concentration led to stronger increases in the auditory P1 and more efficient action cascading in APTs than it did in controls.

The third study investigated the relevance of striatal GABA for conflict monitoring processes in association with the resting state activity and functional connectivity of fronto-striatal networks. The identified SMN consisted of M1, somatosensory cortices, PMA and SMA. The identified BG network included the striatum, GP, SNr, subthalamic nucleus, and the thalamus. This is consistent with previous reports (Neta et al., 2015; Robinson et al., 2009). In a Simon task APTs were more accurate than controls, which was pronounced in the most difficult condition with spatially incongruent stimulus presentation and crossed hands. In addition, APTs showed a higher resting state activity in the BGN. For both groups higher striatal GABA levels, a higher resting state activity in the BGN, and a lower functional connectivity between the BGN and SMN predicted better conflict monitoring and response selection. In APTs striatal GABA levels were positively correlated with the resting state activity in the BGN whereas this was only a trend for controls. Striatal GABA levels showed no relation with the functional connectivity between BGN and SMN.

5.6.2 Mechanisms Underlying Superior Action Control

In general better action control was associated with higher striatal glutamate levels, higher BGN resting state activity, lower functional connectivity between BGN and SMN, a higher auditory P1 amplitude, and higher PLF in the Nogo-N2 time window. In addition, higher striatal GABA levels were predictive for better action control performance in all three tasks. The role of striatal GABA in action control is discussed in detail in section 5.6.3. below.

Higher reliability of neural synchronization in the Nogo-N2 time window being a sensitive indicator for successful response inhibition is in line with previous studies and fits well with the pre-motor inhibition hypothesis, stating that the Nogo-N2 reflects the inhibition of a mistakenly selected motor program (Beste et al., 2009a, 2008a; Falkenstein et al., 1999). Fur-
thermore, this supports the relevance of theta oscillations for cognitive control and suggests that superior response inhibition relies on the organisation of relevant neural processes related to pre-motor inhibition (Beste et al., 2011; e.g Cavanagh and Frank, 2014; De Blasio and Barry, 2013; Harper et al., 2014; Ocklenburg et al., 2011).

More efficient action cascading in APTs was driven by P1, but not P3, related processes. This suggest, that the efficiency of action cascading is not necessarily dependent on processes related to mapping the stimulus onto the appropriate response as previously assumed (Mückschel et al., 2013; Stock et al., 2014b) and cannot unequivocally be attributed to serial/parallel differences in the response selection mode of APTs. Instead, APTs may exhibit faster parallel processing capabilities than controls due to enhanced attentional gating in situations where two response options demand processing resources simultaneously.

Striatal glutamate levels were predictive for the attentional gating underlying superior action cascading, restating the importance of glutamatergic transmission at cortico-striatal synapses for early attentional processing and selection (Agnoli and Carli, 2011; Beste et al., 2014a; e.g. Schulz et al., 2012; Turchi and Sarter, 2001). Glutamate levels were not associated with P3 related processes. Integration of cortical information by D1 MSN seems to be a key mechanism of the input selection process of attention (Agnoli et al., 2013; Agnoli and Carli, 2011; Granon et al., 2000; Sippy et al., 2015) whereas switching from one response to the next in a complex motor sequence relies on the integration of cortical signals by D2 MSN (Agnoli et al., 2013). It is plausible that striatal glutamate levels were predictive for D1 but not D2 related processes as glutamatergic signalling in MSN is enhanced by D1 receptor activity whereas the opposite is true for D2 receptors (Surmeier et al., 2007). A more parsimonious explanation is that striatal neurotransmitter levels are unrelated to P3 processes because they are mainly mediated by a fronto-parietal network, including the ACC (Mückschel et al., 2013). The previously found association of striatal activity with action cascading efficiency (Ness and Beste, 2013) may therefore reflect enhanced attentional processing. Considering all the above, attentional gating seems to plays a role in response selection, and faster processing may be driven by better cross-modal interaction between stimulus response systems.

Lower functional connectivity between BGN and SMN was associated with higher accuracy in the Simon task and hence better conflict monitoring performance. This is well in line with several previous studies showing that decreased resting state functional connectivity between striatal and cortical regions enhances response inhibition (Baudrexel et al., 2011; Davis et al., 2013; Fernández-Seara et al., 2015; Kang et al., 2013; Li et al., 2013). At first glance it seems counterintuitive that lower functional connectivity between the BG and sen-
sensorimotor areas would lead to better action control given the importance of fronto-striatal
networks for these processes. However, the BG show a differential response to cognitive con-
lict in tasks. Whereas other regions, including motor and pre-motor areas, are consistently
activated (Liu et al., 2004; Wittfoth et al., 2008), the BG show a delayed temporal response
(Neta et al., 2015) and contradictory decreased BOLD response following errors (Wittfoth et
al., 2009). A disconnect of BG and cortical sensorimotor areas may thus allow the network to
be less influenced by top-down mechanisms during a task and to retain more network stabili-
ty. Then again, resting state connectivity may be unrepresentative for task activity. Connect-
vitiy between the striatum and sensorimotor as well as attention areas has been shown to
increase with higher task demands (Gopinath et al., 2011).

Higher resting state activity in the BGN network was predictive for better conflict mon-
itoring and response selection. Similar results have previously been reported for higher rest-
ing state activity in motor areas (Fox et al., 2007). A possible explanation may be that higher
BGN activity leads to a higher “readiness” in the BGN in the sense that processing resources
may be permanently pre-activated at a higher level which could result in enhanced response
selection. This explanation is congruent with the finding that preparatory effects can augment
performance in a Simon task (Strack et al., 2013). However, the relationship of task perfo-
mance with BGN resting state may be partly mediated by the relationship between striatal
GABA and BGN resting activity (Greicius et al., 2008; Kiviniemi et al., 2005; Licata
et al., 2013).

APTs proved to be a viable model of superior action control. Apart from behavioural
measures, APTs only differed from controls by intensified attentional processes (P1) and
higher BGN resting state activity. Both these measures were positively correlated with task
performance and striatal GABA levels. Although striatal GABA levels were also predictive
for superior task performance, they (or any other MRS metabolites concentration) did not
differ between the groups. Therefore, higher striatal GABA levels themselves cannot account
for superior action control processes in APTs. This suggests that there may be a third factor
shaping the relation between GABA/glutamate, behavioural performance and neurophysi-
ological processes. In APTs, compared to controls, similar increases in GABA and glutamate
levels led to stronger increases in the auditory P1 and to more efficient action cascading.
Likewise, striatal GABA levels correlated with BGN resting state activity in APTs whereas
this was only a trend in the control group. Therefore, stronger neurobiochemical-
electrophysiological and neurobiochemical-behavioral coupling could be responsible for the
superior action control processes in APTs. The stronger coupling may be caused by factors
related to GABA and NMDA receptor sensitivities or altered strength of structural white matter connectivity between cortical and BG structures and may be subject of future studies.

In summary, superior action control was associated with reliable synchronization and organisation of pre-motor inhibition processes, attentional gating related to striatal glutamate levels, higher BGN resting state activity, and lower resting state functional connectivity between BG and cortical motor areas. Superior performance is likely to rely on stronger neurobiochemical-electrophysiological and neurobiochemical-behavioral coupling.

5.6.3 The Relevance of Striatal GABA in Action Control

Striatal GABA levels were predictive for better task performance in all action control core functions. This mirrors positive effects on cognitive function by higher GABA activity in cortical regions (e.g. Bañuelos et al., 2014; Boy et al., 2010; Floyer-Lea et al., 2006; Marsman et al., 2014; Rao et al., 2000; Yoon et al., 2010) and is consistent with earlier findings in disorders with decreased and disordered GABAergic functioning in the striatum (Beste et al., 2012a, 2010b, 2009c, e.g. 2008d; Beste and Saft, 2015). As opposed to previous reports, the present results are not confounded by alterations in other transmitter systems or brain regions in BG disorders and therefore unequivocally demonstrate the importance of striatal GABA in action control for the first time. Furthermore, this strongly supports the importance of the striatum in response inhibition, conflict monitoring, and task switching/action cascading (e.g. Beste and Saft, 2015; Boehler et al., 2010; Cools et al., 2004; Ness and Beste, 2013; Willemsssen et al., 2011). Striatal GABA levels explained a large amount of variance in the EEG data which confirms that EEG parameters are sensitive to remote BG processes (Beste and Saft, 2015). The correlation of GABA with specific behavioural and electrophysiological task measures makes it possible to zero in on the involvement of the striatal GABA in specific subprocesses of action control.

Higher striatal GABA levels were related to more reliable neural synchronization processes in the Nogo-N2 time window that were predictive for better response inhibition (Study 1). A GABAergic modulation of the Nogo-N2 is plausible against earlier findings (Beste et al., 2012a, 2011, 2008b). There was no effect from GABA on the intensity of this electrophysiological process (i.e. Nogo-N2 amplitude). This is consistent with findings from various brain regions showing that GABA concentrations modulate the frequency but not the power of oscillatory activity (Atallah and Scanziani, 2009; Duncan et al., 2014). Furthermore, mechanisms reflecting the evaluative processing of the successful outcome of inhibition (Nogo-P3) were not related to striatal GABA levels. This could be due to the fact that the
striatum is especially relevant for motor control and the canceling and restraining of responses (Bar-Gad et al., 2003, p.; Bari and Robbins, 2013). So, processes closely related to motor aspects of inhibition are more likely to be affected by GABA than later evaluative processes. In addition, the Nogo-P3 is primarily associated with ACC activity and parietal areas (Beste et al., 2012a, 2008a; Fallgatter et al., 2004; Schmajuk et al., 2006). However, even though striatal dysfunction has a more universal effect on the Nogo-N2, the Nogo-P3 has also been shown to be sensitive to changes in the striatum (Beste et al., 2012a, 2009a). Considering that MRS GABA levels seem to primarily reflect extrasynaptic GABAergic tone (e.g. Stagg, 2014), which has a stronger impact on D1 MSN (e.g. Santhakumar et al., 2010), leads to another possible explanation. Outcome evaluation processes (Nogo-P3) and pre-motor processes (Nogo-N2) of response inhibition are modulated by the D2 receptor/striato-pallidal system and the D1 receptor/striato-nigral system, respectively (→1.5.4; Beste et al., 2016, 2011, 2010b). The opposing findings on Nogo-N2 and Nogo-P3 in study 1 could thus reflect the stronger influence of extrasynaptic GABAergic inhibition in D1 MSN.

Both higher striatal glutamate and GABA levels were predictive for the enhanced attentional gating (P1) that seems to underlie more efficient action cascading (Study 2). This corroborates the importance of glutamatergic transmission at cortico-striatal synapses for early attentional processing and selection (Agnoli and Carli, 2011; Beste et al., 2014a; e.g. Schulz et al., 2012; Turchi and Sarter, 2001). Furthermore, this confirms the involvement of the striatum in processing of the change stimulus suggested by reports of auditory sensory neurons in the striatum (Kropotov et al., 2000; Nagy et al., 2006; Saft et al., 2008; Znamenskiy and Zador, 2013). There were no associations of striatal neurotransmitter levels with P3 related processes that are thought to reflect mapping the stimuli onto the appropriate response and, possibly, processing mode in the SCT (Falkenstein et al., 1994; Mückschel et al., 2013; Stock et al., 2014b). Integration of cortical information by D1 MSN seems to be a key mechanism in the input selection process of attention (Agnoli et al., 2013; Agnoli and Carli, 2011; Granon et al., 2000; Sippy et al., 2015) whereas switching from one response to the next in a complex motor sequence relies rather on the integration of cortical signals by D2 MSN (Agnoli et al., 2013). It is possible that striatal glutamate levels were predictive for D1, but not D2, related processes given that glutamatergic signalling in MSN is enhanced by D1 receptor activity whereas the opposite is true for D2 receptors (Surmeier et al., 2007) and the fact that striatal DA synthesis and glutamate levels are positively coupled (Gleich et al., 2015). The association of P1 amplitude with striatal GABA is interesting because early attentional processing is only indirectly influenced by GABA and instead heavily depend on glutamatergic
and acetylcholinergic signalling (e.g. Javitt et al., 2000; Logemann et al., 2014; Sarter et al., 2006; Turchi and Sarter, 2001). However, the correlation could be due to the mutual association of attentional processing and striatal GABA MRS signal with D1 MSN or simply arise from the coupling of striatal GABA and glutamate (e.g. Agnoli et al., 2013). A more parsimonious explanation for the finding that striatal neurotransmitter levels are unrelated to processes mapping the stimuli onto the response is that P3 related processes are mainly mediated by a fronto-parietal network, including the ACC (Mückschel et al., 2013). Thus, the previously found association of striatal activity with action cascading efficiency (Ness and Beste, 2013) may reflect primarily enhanced attentional processing.

Higher striatal GABA levels in APTs were predictive for the higher resting state activity in the BGN that correlated with better task performance. This is in line with studies that showed increased BOLD synchrony in resting state networks after sedation with a GABAAR agonist (Greicius et al., 2008; Kiviniemi et al., 2005; Licata et al., 2013) and reduced resting state activity after stimulant administration (Rack-Gomer et al., 2009). In contrast, a negative correlations between cortical local GABA concentration and BOLD response was repeatedly demonstrated (review: Donahue et al., 2010; Duncan et al., 2014; Muthukumaraswamy et al., 2012, 2009; Stagg et al., 2011a). However, considering the differences in GABAergic network dynamics a generalization of cortical results is not necessarily viable. Still, the assumption that striatal GABA levels lower activity in the striatum could be consistent with higher BGN network activity given the inhibitory effect of the striatum on its target structures. A negative correlation of local GABA levels with local activity is also consistent with the general down regulation of network excitability by extracellular GABA (Semyanov et al., 2004, 2003).

Finally, not all aspects of striatal GABAergic function are represented in the MRS signal. Therefore, the role of striatal GABA levels in action control presented here is not necessarily comprehensive and striatal GABA may very well also modulate additional aspects of action control.

5.6.4 Striatal GABA – Potential Mechanisms of Action

5.6.5 Or: How do Higher Striatal GABA Levels lead to Better Action Control?

The exact functioning of GABA in the striatum, the striatal microcircuit, and also the BG as a whole is still not fully understood. Thus, one can only speculate through which mechanisms higher striatal GABA levels may lead to better action control processes. The simplest explanation would be that higher striatal GABA levels directly relate to improved
action control via the differential shunting inhibition of D1 and D2 MSN (Ade et al., 2008; Santhakumar et al., 2010). That is, increased extracellular GABA would selectively decrease the excitability in direct pathway MSN, thereby allowing for selective facilitation of goal-directed behaviour. Conversely, the lower level of tonic inhibition in indirect pathway MSN would more readily activate inhibition in the indirect pathway MSN and enable efficient response inhibition. However, this relies on an oversimplification of BG functioning (→1.2.2) and in the following other possibilities with respect to the complex architecture of the striatal microcircuit will be explored.

The results of this thesis support neurocomputational BG models that implement response selection as a function of GABAergic inhibition in the striatal microcircuit (e.g. Beste and Saft, 2015; Humphries et al., 2006; Redgrave et al., 1999). Higher GABA concentrations could sharpen the striatal signal selection network and thereby increase competitive dynamics. In turn, conflicting response options and tendencies are inhibited more readily which leads to a more efficient processing mode and faster reaction times. This is in accordance with the selection enhancing effect of increased inhibition in a PFC network model (Snyder et al., 2010) and with impaired action control due to defective down-regulation of MSN activity (Burguière et al., 2013). In neurocomputational models of the striatal microcircuit, inhibitory strength is not a freely variable parameter and extracellular GABA or extrasynaptic GABA\(_A\)Rs are not implemented. Therefore, these models cannot directly confirm the ameliorative effect of higher striatal GABA levels on action selection (also see →5.7.1). However, extracellular GABA acting on extrasynaptic receptors in other brain regions has been shown to play an important role for the regulation of network excitability and information processing by modulating neuronal gain and firing threshold (Scimemi et al., 2005; Semyanov et al., 2004, 2003). When network excitation increases, elevated extracellular GABA could ensure that only a small fraction of cells are active, thus maintaining sparse coding even during high levels of network excitation. By a similar mechanism synaptic spillover could theoretically contribute to lateral inhibition as well as act as a homeostatic regulator of synaptic inhibition (Semyanov et al., 2004).

In the first study higher striatal GABA concentration was predictive for the reliability of neural synchronization processes in the theta frequency (Nogo-N2 PLF). In addition, the association of higher P1 amplitude and BGN activity with higher striatal GABA levels could also indicate increased synchronization since both measures rise with either higher, or more synchronized, underlying neural activity (Laufs et al., 2003; Roach and Mathalon, 2008; Yu-Feng et al., 2007). Thus, GABA may improve action control processes by modulating neural
synchronization. This is plausible in the light of earlier research. Oscillations are thought to arise from inhibitory networks and higher GABA concentrations are known to increase their oscillatory activity (Feshchenko et al., 1997; Greicius et al., 2008; Kiviniemi et al., 2005; Licata et al., 2013; Muthukumaraswamy et al., 2009). Furthermore, striatal oscillations have been linked to cognitive processes (Adler et al., 2013; Courtemanche et al., 2003; Gage et al., 2010). Finally, tonic inhibition acting on extrasynaptic GABA$_A$Rs modulates the generation of rhythmic activity and network oscillations (Huntsman et al., 1999; Lagier et al., 2007; Macdonald et al., 2010; Mann and Mody, 2010; Nusser et al., 2001; Towers et al., 2004).

However, the effect of striatal GABA on local synchronizational processes cannot be inferred from either EEG or BGN activity. Still, the findings are in line with earlier research suggesting that striatal GABA regulates synchronizational processes in fronto-striatal networks (Beste et al., 2011; Bonilla et al., 1988; Darbin and Wichmann, 2008; Glass et al., 2000; Reynolds and Pearson, 1990).

Related to modulating of neural synchronization, GABAergic striatal transmission seems to enable multistable network dynamics via switching between functional network states defined by synchronized cell assemblies (→1.4.3; Carrillo-Reid et al., 2008). In a computational model, these cell assembly population dynamics were locked to stimulus onset times and their reproducibility across repeated stimulus presentations hinged on the strength of inhibition in the network (IPSP size; Ponzi and Wickens, 2012, 2013). This fits well with the association of striatal GABA levels with electrophysiological correlates of action control found in study 1 and 2. Furthermore, the synchronized firing of MSN assemblies could allow for a more reliably information transfer to their target cells (Bruno, 2011; Bruno and Sakmann, 2006) and, indeed, a large number of synchronized striatal outputs may be required to induce large IPSPs in GPi neurons (Kita, 2001).

In summary, striatal GABA most likely enhances action selection and control by regulating network dynamics in the striatal microcircuit. More specifically, and not mutually exclusive, this could be achieved by increasing competitive dynamics in signal selection, modulating synchronizational processes, and enabling to switch between functional network states.

5.7 Outlook

5.7.1 Neurocomputational Models of the Striatum

Computational models are a vital tool towards understanding the operations of the striatal microcircuit and its contribution in information processing in the BG. In an effort to constrain assumptions, it is advisable to build models based closely on actual anatomy and phys-
iology. In the following suggestions for future modelling against the background of the findings in study 1-3 will be proposed. For comprehensive considerations on modelling the striatal microcircuit please see Wickens et al. (2007) as well as Humphries et al. (2010).

Reports of extrasynaptic inhibition in MSN are relatively recent and its properties need to be further investigated (Santhakumar et al., 2010). Thus, neurocomputational BG models have not yet implemented extrasynaptic inhibition. In current models inhibitory strength is dependent on the properties of ion channels, synapses, and cell membrane (modelled and tuned to reflect available data as closely as possible), the firing rate of connected FSI and MSN, and most importantly, the degree of connectivity in the network. The connectivity of FSI may control their spike rate and timing (Berke, 2008; Gage et al., 2010; Lau et al., 2010; Wiltsho et al., 2010). More significantly, the degree of connectivity between MSN influences their firing patterns and rate, cell assembly formation, and ultimately decides the operation carried out by the network (e.g. transient-, steady state-, or absence of selection (e.g. transient-, steady state-, or absence of selection; Beste and Saft, 2014; Ponzi and Wickens, 2012, 2010, 2013; Tomkins et al., 2014). Generally the connection probability between any two MSN depends on the size of overlap between their dendritic and axonal arborization (Kong et al., 2004; Lau et al., 2010; Plenz, 2003a; Tepper et al., 2004; Wickens, 2002; Wickens et al., 2007). However, there are several new findings regarding connectivity in the striatum that have yet to be implemented into computational models. D1 MSN form less connections between themselves than D2 MSN; D1 MSN primarily synapse with other D1 MSN whereas D2 MSN form connections with both types of MSN (Taverna et al., 2008). FSI preferentially connect to D1 MSN (Gittis et al., 2010). Accordingly, currents through GABA$_A$Rs always depress the response to cortico-striatal stimulation in D2 MSN whereas they help to depolarize the response in D1 MSN (Flores-Barrera et al., 2010). Furthermore, MSN collaterals form synapses on the somas of other MSN that are likely to shunt all dendritic input to the soma, providing powerful feedback inhibition (Oorschot et al., 2013). Finally, extracellular GABA acting on extrasynaptic receptors is an important factor for the regulation of network excitability and information processing may enhance competitive network dynamics (→5.6.4; Scimemi et al., 2005; Semyanov et al., 2004, 2003).

Thus future models should implement extrasynaptic GABA$_A$Rs, mechanisms of volume transmission, and extracellular GABA concentration, similar to the simulation of different DA concentration in Humphries et al. (2009). Moreover, it would be interesting to examine the effect of differential distribution of extrasynaptic GABA$_A$Rs on D1 and D2 MSN (Ade et al., 2008; Santhakumar et al., 2010).
5.7.2 The Multimodal Approach - Methodological Considerations

5.7.3 Or: How Should We Study Biochemical-Functional Relationships?

The investigation of regional neurobiochemical relationships and their association with psychological functions calls for a multimodal approach with a combination of neural activity measures (fMRI, EEG), biochemical measures (PET, MRS), and task measures. Besides opening up intriguing research possibilities, the multimodal approach raises several methodological issues.

First, researchers need to consider the physiological mechanisms underlying functional measures and choose the method that best serves to test their hypothesis. EEG (or MEG) is most appropriate to target links between biochemistry and temporal dynamics and mainly reflects cortical postsynaptic potentials. Furthermore, it allows differentiating between subprocesses of neural events that underlie sensory, perceptual, and cognitive processes. With the introduction of time-frequency analysis EEG became a powerful tool to study neural oscillations and their synchronization, a core principle of neural functions in health and disease (Roach and Mathalon, 2008). In addition, source localization allow for estimation of location and distribution of EEG current sources (Jatoi et al., 2014; Pascual-Marqui et al., 2002). Still, fMRI provides more regional specificity and is especially useful for studying neural networks besides stimulus- and task-related activity. The BOLD signal mainly reflects input signals and internal processing of neurons rather than spiking or output (Logothetis, 2008). It is also possible to combine the temporal and spatial advantages of EEG and fMRI by applying both simultaneously (e.g. Gonçalves et al., 2006; Purdon et al., 2009).

Second, the choice of biochemical measure must consider the targeted transmitter and functional context. Whereas PET gives a measure of the density and affinity of particular receptors, including benzodiazepine binding GABA_ARs (Odano et al., 2009), the MRS signal reflects transmitter concentration (→ 1.5.2, for a more detailed description). MRS is particularly suited to measure glutamate and GABA whereas PET should be chosen to investigate serotonin and DA related processes. It is possible to combine functional measures with both GABA_AR density measured in PET and MRS GABA levels. This could shed light onto the stronger neurobiochemical-electrophysiological and behavioural coupling that may underlie the superior performance in APTs (→5.6.2).

Third, multimodal studies should aim to demonstrate neural, biochemical, regional, and psychological/functional specificity, i.e. results should be specific for resting- or stimulus-induced activity, the targeted transmitter, the targeted region, and task or trial-type, respectively. However, inclusion of the necessary controls is often constrained by technical availa-
bilities, e.g. generally only one transmitter system can be measured with PET. Regional specificity in MRS requires an appropriate control region. Furthermore, it would be desirable to improve the sensitivity of GABA-edited MRS to enable the use of smaller volumes. The association of GABA and action control could differ between striatal subregions since they vary in many potentially relevant aspects, e.g. afferent projections (Brodal, 2010), information encoding and task related firing (Adler et al., 2013; Burton et al., 2015), oscillatory activity (Berke, 2005; Berke et al., 2004), and FSI distribution (Kita et al., 1990; Kubota et al., 1993). Thus, future studies may investigate the role of GABA in striatal subregions where especially the caudate may be relevant for goal-directed behaviour and specifically action control (Brasted et al., 1999; Eagle and Robbins, 2003; Miyachi et al., 1997; Ness and Beste, 2013; Ragozzino, 2003; Ragozzino and Choi, 2004; Yin et al., 2005a, 2005a). It is difficult to achieve specificity of neural activity as MRS and PET neurotransmitter measures are usually acquired in resting state and may ignore critical changes in concentrations that occur during task performance. Part of this problem may be overcome through the recent integration of PET with MRI and EEG (Estorch and Carrio, 2013; Shah et al., 2013). Alternatively, including an fMRI or EEG resting state measure may serve as a bridge between biochemical resting state and induced activity measures (Duncan et al., 2013; Enzi et al., 2012).

Finally, given the correlational nature of MRS studies, modulation is necessary to establish causal relationships. MRS measurements before, during, and after a task could be used to investigate a causal link between biochemistry and function (Kim et al., 2013; Schaller et al., 2013). Moreover, there is progress towards event-related MRS designs (e.g. Lally et al., 2014). Another way towards causal relationships is to modulate neurotransmission.

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<tr>
<th>MSN</th>
<th>FSI</th>
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<tr>
<td>synaptic: $\alpha_2\beta_2/3\gamma_2$</td>
<td>$\alpha_1\beta_2/3\gamma_2$</td>
<td>$\alpha_3\beta_2/3\gamma_2$</td>
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<tr>
<td>extrasynaptic: D1</td>
<td>D2</td>
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<tr>
<td>(adult) $\alpha_4\beta_3\delta$</td>
<td>$\alpha_4\beta_3\delta$</td>
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<tr>
<td>(juvenile) $\alpha_5\beta_3\gamma_2$</td>
<td>$\alpha_5\beta_3\gamma_2$</td>
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<td>GABA$_{B1/2}$</td>
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Figure 4. Most likely GABA receptor distribution in the Striatum. Based on autoradiography, in situ hybridization, immunocytochemistry, antibody light and electron microscopic, and subunit assembly rules (e.g. Goetz et al., 2007). Relatively higher expression is in bold.
5.7.4 Modulators of GABAergic Phasic and Tonic Inhibition

To gain a better understanding of interindividual differences and the role of fronto-striatal GABA in action control processes, it is vital to identify and investigate as many modulating factors as possible. Unfortunately, it is not possible to selectively target GABAergic transmission in the BG or striatum outside of operative settings (cf. Bjarkam et al., 2010). Even so, some approaches are particularly relevant for striatal and fronto-striatal processes and action control and will be the focus of the following section. For a review of GABA modulators in the BG see Goetz et al., (2007). Against the background of the results of this thesis especially modulation of tonic inhibition may be of relevance and is more likely to be reflected by MRS measurements (e.g. Stagg, 2014; →1.5.2). As of now there is no established method to target extrasynaptic inhibition (Brickley and Mody, 2012). Nevertheless, many modulators differ in their relative effect on synaptic and extrasynaptic transmission. In particular ethanol and neurosteroids exhibit dose-dependent paradoxical effects that seem to arise from different sensitivity of synaptic and extrasynaptic GABA\textsubscript{A}Rs (Bäckström et al., 2014). In addition to the modulators discussed below, nicotine, wakefulness, circadian rhythm, and stress can affect GABA concentration and need to be taken into account in future studies (Fuxe et al., 1989; Harada et al., 2011; Lang et al., 2011; Morgan et al., 2012; Skilbeck et al., 2010). Furthermore, it is important to consider the balance and interactions between GABA and other transmitter systems. For further information please refer to de la Vega et al. (2014) for an example of excitatory/inhibitory network balance in an MRS study, Eichler and Meier (2008) for excitatory/inhibitory balance in cognitive function, Do et al. (2013), and Bergman et al. (2006) for modulation of striatal circuits by DA and acetylcholine, and Agnoli et al. (2013) for interactions of striatal glutamate, GABA and DA.

**Exogenous Modulators, GABA\textsubscript{A}Rs:** Benzodiazepines enhance synaptic inhibition at GABA\textsubscript{A}\textbf{βγ}$\alpha$2 receptors (all striatal synaptic GABA\textsubscript{A}Rs; see figure 4) but do not affect receptors with the α4/6 or δ subunit (striatal extrasynaptic GABA\textsubscript{A}Rs; Goetz et al., 2007). The BZ1 receptor type, α1β2γ2/3, is the most abundant in the brain and expressed in all parts of the BG (Benke et al., 2004). Although the main effects of benzodiazepines are sedation, anxiolysis, seizures suppression and muscle relaxation, they reduce PFC activity and have diminishing long- and short term effects on EFs and action control (Acheson et al., 2006; Coull et al., 1999; Deakin et al., 2004; Stewart, 2005). There is progress in developing α1, α2, and α3 subtype specific compounds which would allow to target synaptic transmission of FSI, MSN, and TAN, respectively (Atack, 2011; Divljaković et al., 2013). L-655,708 is an orally bioavailable, negative allosteric agonist at the benzodiazepine binding site and selective for the α5
subunit (Santhakumar et al., 2007). In the striatum L-655,708 slightly decreased tonic current of D1 MSN in adult mice and significantly of D2 MSN in young mice (Santhakumar et al., 2010; → this section: Age).

Ethanol affects several neurotransmitter systems but primarily acts as a positive allosteric modulator at GABA<sub>A</sub>Rs, with a higher affinity for α1/4/6β3δ receptors (Santhakumar et al., 2007). Ultra low (1-3 mM) concentrations of alcohol have been shown to selectively increase Cl<sup>-</sup> currents mediated by the α4/6β2/3δ GABA<sub>A</sub>R (Hanchar et al., 2005, 2004; Sundstrom-Poromaa et al., 2002; Wallner et al., 2003). The expression of this receptor type is constrained to the striatum, hippocampus and cerebellum (Lee and Maguire, 2014). Thus, between 0.004 and 0.013% BAC should selectively increase tonic inhibition in these regions. Indeed, although low to high amounts of ethanol (0.2 g/kg, ~0.03% BAC - 1.2 g/kg, ~0.12% BAC) have dose dependent, but generally diminishing effects on action control (e.g. de Wit et al., 2000; Dougherty et al., 2008; Field et al., 2010; Stock et al., 2014b), very low doses can have ameliorative effects in tasks associated with striatal, hippocampal and cerebellar functions (Bruce and Pihl, 1997; Kalev-Zylinska and During, 2007; Lloyd and Rogers, 1997). Still, the effects of blood alcohol concentrations between 1mM and 3mM (~ 0.03g/kg - 0.09 g/kg; Holford, 1987; Kalant et al., 1975) on GABA concentration and action control have not been researched and may be subject to future studies.

**GABA Concentration:** GABA concentration can be increased with gabapentin, a synthetic GABA-like agent that seems to act by enhancing GABA synthesis (Cai et al., 2012; Errante et al., 2002; Maneuf et al., 2003). Gabapentin has been shown to improve action cascading performance making it a promising candidate to study fronto-striatal GABA levels in association with action control (Steenbergen et al., 2015). However, the exact mechanisms of action is unclear and the effects of gabapentin differ between brain regions (Löscher et al., 1991). Therefore future studies using this compound should foremost verify increased striatal GABA levels. Transcranial stimulation is a non-invasive method that can modulate GABA concentration and plasticity. Cathodal transcranial direct current stimulation (tDCS) decreases GABA levels within stimulated areas whereas anodal tDCS decreases both GABA and glutamate levels (Stagg et al., 2011a, 2009a). GABA concentration and cortical excitability can be increased with continuous theta burst stimulation (cTBS; Stagg et al., 2009b). Although only cortical regions can be targeted with transcranial stimulation, subcortical areas are also affected (Bolzoni et al., 2013; Nonnekes et al., 2014). Combined MRS and tDCS or repetitive transcranial magnetic stimulation can be used to specifically modulate and study neurotransmitters, activity, and connectivity in fronto-striatal circuits (Schouwenburg et al., 2015).
Finally, transcutaneous vagus nerve stimulation (tVNS) unselectively increases GABA levels in the brain (Ben-Menachem et al., 1995; Marrosu et al., 2003; Van Leusden et al., 2015). Finally, medication used in the treatment for partial seizures and anxiety disorders can raise ambient GABA levels; Tiagabine by blocking GABA transporters (primarily GAT-1) on nerve terminals (Pollack et al., 2005) and Pregabalin by enhancing the activity of GAD (Tassone et al., 2007).

**Endogenous Modulators, Genetic Variation:** There is complex genetic variability associated with the GABA system (review: Mulligan et al., 2012). The α2 subunit is highly expressed in the cortex and striatum and variants in the encoding gene (GABRA2) are associated with beta oscillations in EEG and suggested to play a role in inhibitory control (Kareken et al., 2010; Villafuerte et al., 2013). Mutations in genes encoding extrasynaptic GABA_ARs are generally associated with epilepsies (for a review see: Macdonald et al., 2010). Missense point mutations in gene encoding for the δ subunit (GABRD) lead to diminished currents and shorter duration of open time (Dibbens et al., 2004; Feng et al., 2006). Of special interest is the R220H variation that has also been identified in the general population with so far unknown associations (Dibbens et al., 2004).

**Neurological and Psychiatric Disorders:** GABAergic function in fronto-striatal circuits is affected in several neurological and psychiatric disorders, among the most well researched are PD and HD (e.g. Glass et al., 2000; Tepper et al., 2007; also see introduction). Furthermore, GABAergic function is affected in several brain regions including the striatum in dystonias (Levy and Hallett, 2002), epilepsies (Mathew et al., 2010; Sperk et al., 2004), TS (Leckman et al., 2010; Lerner et al., 2012), and ADHD (Bollmann et al., 2015; Castelli et al., 2011). Especially the latter two are relevant for fronto-striatal circuits and action control processes. Symptoms in TS are thought to result from dysfunction and aberrant oscillations in fronto-striatal circuits (Buse et al., 2015; Cavanna et al., 2009; Hong et al., 2013; Leckman et al., 2006). Action control, especially response inhibition, is impaired in TS (Baym et al., 2008; Cavanna et al., 2009; Eichele et al., 2010; Müller et al., 2003; Watkins et al., 2005). In line with the putative relevance of FSI for striatal oscillations and downregulation of MSN (Berke, 2005; Burguière et al., 2013), the number and density of striatal FSI is reduced in TS (Kalanithi et al., 2005; Kataoka et al., 2010). Furthermore, there is evidence for widespread abnormalities in the GABA system (Draper et al., 2014; Leckman et al., 2010; Lerner et al., 2012; Puts et al., 2015). Henceforth, TS symptoms are likely to be associated with a GABA/glutamate imbalance (Leckman et al., 2006; Singer et al., 2010) and improve with positive GABA modulation (Singer et al., 2001; Wang et al., 2012).
ADHD is characterized by impaired action control and EFs (Coghill et al., 2014; Seidman, 2006) as well as associated with structural and functional abnormalities in fronto-striatal circuits (Nakao et al., 2011; Seidman, 2006). Abnormalities in the GABA system, including striatal GABA levels and imbalance of excitatory and inhibitory transmitters, plays an important role in ADHD pathophysiology (Bollmann et al., 2015; Buitelaar and Medori, 2009; Edden et al., 2012; Ferreira et al., 2009; Freese et al., 2012; Miyazaki et al., 2006). Of special relevance for MSN function are attenuated D2 related and enhanced D1 related processes in ADHD (Badgaiyan et al., 2015; Gerfen and Bolam, 2010) in accordance with diminished Nogo-P3 (but not Nogo-N2) compared to age-matched controls (Albrecht et al., 2013; Bluschke et al., 2016; Fallgatter et al., 2004).

Age: The GABAergic system undergoes marked maturation during childhood and adolescence (Owens and Kriegstein, 2002)(Chugani et al., 2001; Owens and Kriegstein, 2002; Schmidt and Mirnics, 2015). GABA concentrations, GABergic interneurons, and GAD as well as GABA<sub>A</sub>R density increase during childhood and adolescent and reach adult levels around age 14 and 19 in the BG and PFC, respectively (Chugani et al., 2001; Coyle and Enna, 1976; Fung et al., 2010). Improvements in action control during adolescence (Bedard et al., 2002; Levin et al., 1991; Williams et al., 1999) can be partially ascribed to this development (Rubia et al., 2007, 2006; Silveri et al., 2013). In mice tonic inhibition of striatal MSN increases during development (Kirmse et al., 2008) and switches in adolescence from stronger impact on D2 MSN, mediated by higher expression of the α5 subunit, to stronger impact on D1 MSN, mediated by higher expression of the α4 and δ subunit (see figure 4; Ade et al., 2008; Laurie et al., 1992; Santhakumar et al., 2010). It is unclear if an analogues switch takes places in the human striatum. However, converging evidence points to an upregulation of α4βδ GAB<sub>A</sub>Rs during puberty (Smith, 2013). Considering the respective relation of Nogo-N2 and Nogo-P3 with the D1 and D2 receptor system (e.g. Beste et al., 2016) a future study, analogues to study 1, should compare striatal GABA levels and their relation to Nogo-ERPs in healthy children and adults.

Neurosteroids: Endogenous neurosteroids, synthesized in the brain from ovarian and adrenal cortical steroid hormones, are positive allosteric modulators of GABA<sub>A</sub>Rs, have a high affinity for the δ subunit, and generally act by enhancing tonic inhibition (Belelli and Lambert, 2005; Farrant and Nusser, 2005; Goetz et al., 2007; Stell et al., 2003). Neurosteroid concentration changes during puberty, menstrual cycle, pregnancy, and stress and is associated with related mood and anxiety disorders (Bäckström et al., 2014; Skilbeck et al., 2010). Converging evidence suggest that the lower neurosteroid concentration during the follicular
phase primarily increases tonic inhibition whereas both synaptic and extrasynaptic GABA$_A$Rs are modulated in the luteal phase when neurosteroid levels increase (Bäckström et al., 2014; Matthew and Samba, 2013; Schumacher et al., 2014). In line with the putative performance enhancing effect of striatal tonic inhibition, EFs and action control are generally enhanced during the follicular phase (for reviews see: Souza et al., 2012; Sundström-Poromaa and Gingnell, 2014). However, differences between cycle phases are usually small and difficult to replicate. The interpretation of neurosteroid modulation is further complicated by the direct effects of progesterone and estrogen in the brain, their interaction with the glutamate, DA, GABA and serotonin system, and region specific differences in GABA levels as well as δ subunit expression and phosphorylation between cycle phases (Barth et al., 2015; Epperson et al., 2005; Harada et al., 2011; Matthew and Samba, 2013; Silveri et al., 2013). Thus, future studies should investigate the direct relationship of striatal GABA levels, action control, and the fall in neurosteroid concentration during the menstrual cycle in an between subject design. Furthermore, exogenous modulation of tonic inhibition is possible via a neurosteroid analogue (ganaxolone; Biagini et al., 2010).

Figure 5. Modulators of phasic inhibition, tonic inhibition, and GABA concentration. Axon terminal of a GABAergic neuron (top) releases GABA into the synaptic cleft. There GABA is bound by low-affinity synaptic receptors (αβγ2: left) and raises ambient GABA which is bound by high-affinity extrasynaptic receptors (α4/6β3: right). GABA concentration can be increased by transcutaneous vagus nerve stimulation (tVNS), continuous theta burst stimulation (cTBS), enhancing GABA synthesis, and by blocking GABA transporters (GAT). Transcranial direct current stimulation (tDCS) decreases GABA concentration. Modulators vary in their relative selectivity for Synaptic and extrasynaptic GABA$_A$ receptors and in turn influence phasic inhibition or tonic inhibition/conductance. White trace: brief postsynaptic conductance change (left) and tonic inhibition (right). GAT-1: GABA transporters in axon terminals.
5.8 Summary and Conclusion

This thesis investigated the relevance of striatal GABA levels for action control processes. Neurocomputational models suggest that the striatal microcircuit constitutes an inhibitory network that performs response selection. The efficiency of this network increases with inhibitory strength and is dependent on unimpaired cell functioning. A novel combination of MRS, EEG, fMRI and behavioural data was applied to examine the relation of striatal GABA levels with action control and its electrophysiological correlates as well as activity in fronto-striatal networks. To investigate mechanism underlying superior performance, APTs were compared to age-matched controls. Higher striatal GABA levels were predictive for better task performance in all three action control core functions. This is the first direct demonstration of the importance of striatal GABA and MSN functioning for action control processes and corroborates neurocomputational models of the striatal microcircuit. The MRS signal primarily reflects extrasynaptic GABAergic tonic inhibition that plays an important role for the regulation of network excitability and information processing. Thus, it is plausible that higher GABA levels enhance action control by optimizing the regulation of network dynamics resulting in sharper signal selection, more reliable synchronisational processes, and flexibility between functional network states. The effect of GABA is mediated by enhanced attentional gating, increased reliability of neuronal synchronization processes, and higher BGN network activity. Especially mechanisms related to D1 functioning likely contributed to these results which points to a stronger tonic inhibition of D1 MSN. Superior performance in APTs stems from an increased effect of striatal GABA, likely mediated by potentiated receptor function or altered strength of connectivity in fronto-striatal circuits.

Modulation of GABAergic transmission seems to relate to cognitive performance through an inverted U-shaped function. This may be the result of positive effects mediated by extrasynaptic GABA$_{A}$Rs which have a higher sensitivity to many modulators, whereas negative effects due to overstimulation of synaptic GABA$_{A}$Rs only emerge at high doses (see figure 5). To gain insight into these processes future studies should selectively target synaptic and extrasynaptic GABAergic inhibition in the striatum and fronto-striatal circuits. Furthermore, implementing tonic inhibition, mechanisms of volume transmission and differential extrasynaptic receptor density on MSN into computational models may further elucidate the exact mechanism of striatal GABA and move modelling closer towards actual anatomy and physiology. Finally, understanding the role of GABA in fronto-striatal circuits together with
its dysfunction in related diseases will pave the way for emerging treatment targeted at this transmitter system.

References


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Addendum
Addendum A: Curriculum Vitae

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Schulische Ausbildung

10/2012-Heute Promotionsstudium, International Graduate School of Neuroscience/Fakultät für Psychologie, Arbeitseinheit Biopsychologie, Ruhr-Universität Bochum
Thema: Basalganglienfunktion bei Parkinsonpatienten und manganexponierten Schweizern

10/2010-02/2013 Psychologie M.Sc., Ruhr-Universität Bochum
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10/2007-07/2010 Psychologie B.Sc., Ruhr-Universität Bochum
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Note: 1,4

Arbeitserfahrung

05/2013-Heute Wissenschaftliche Mitarbeiterin im Rahmen der Doktorarbeit am Institut für Prävention und Arbeitsmedizin der deutschen gesetzlichen Unfallversicherung, Bochum
- Durchführung von medizinischen und kognitiven Tests, EEG und MRT
- Datenverarbeitung und -analyse
- Aufbereitung und Präsentation von Ergebnissen

03/2012-10/2012 Praktikum im LWL-Universitätsklinikum Bochum im Rahmen einer Studie mit depressiven Patienten
- Rekrutierung von Probanden
- Neuropsychiatrisches Interview und EEG

- Supervision von Kleingruppen beim Erlernen von Grundtechniken
der therapeutischen Gesprächsführung

04/2012-07/2012
  • Seminarleitung
  • Benotung und Hilfe beim Erstellen von Referaten und Hausarbeiten

01/2011-02/2011 Praktikum im ‚Neuroimaging Center‘ in Groningen, Niederlande
  • Auswertung einer fMRT Studie zu kognitiver Empathie bei Menschen mit ‚at risk mental state‘

04/2010-08/2010 Studentische Hilfskraft in der Arbeitseinheit Entwicklungspsychologie der Ruhr-Universität Bochum
  • Durchführung und Auswertung von Intelligenz- und Aufmerksamkeitstests in einer Studie mit Grundschulkindern

02/2010 Praktikum in der Klinik für Psychotherapie der evangelischen Kliniken Gelsenkirchen

08/2009-09/2009 Praktikum in der psychiatrischen Klinik Vitos Eichberg
  In beiden Klinikpraktika:
  • Assistent bei Gruppen- und Einzeltherapie
  • Teilnahme an Visiten und Teambesprechungen
  • Durchführung diagnostischer Tests

Publikationen und Vorträge

11/2014 Posterpräsentation, Society for Neuroscience 2015, Washington, D.C., USA
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Fähigkeiten und Kenntnisse

Sprachen: Englisch Niveau C2, Niederländisch und Französisch Niveau B1
Software: Microsoft Office, SPSS, SAS, MATLAB, SPM, Presentation, Vision Analyzer, E-Prime
Addendum B: List of Publications
(Dissertationsrelevante Teilpublikationen)


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Clara Quetscher