Functional segregation and integration within the human subthalamic nucleus from a micro- and meso-level perspective

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The subthalamic nucleus (STN) is a core basal ganglia structure involved in the control of motor, cognitive, motivational and affective functions. The (challenged) tripartite subdivision hypothesis places these functions into distinct sensorimotor, cognitive/associative, and limbic subregions based on the topography of cortical projections. To a large extent, this hypothesis is used to motivate the choice of target coordinates for implantation of deep brain stimulation electrodes for treatment of neurological and psychiatric disorders. Yet, the parallel organization of basal ganglia circuits has been known to allow considerable cross-talk, which might contribute to the occurrence of neuropsychiatric side effects when stimulating the dorsolateral, putative sensorimotor, part of the STN for treatment of Parkinson’s disease. Any functional segregation within the STN is expected to be reflected both at micro-level microscopy and meso-level neural population activity. As such, we review the current empirical evidence from anterograde tracing and immunocytochemistry studies and from local field potential recordings for delineating the STN into distinct subregions. The spatial distribution of immunoreactivity presents as a combination of gradients, and although neural activity in distinct frequency bands appears spatially clustered, there is substantial overlap in peak locations. We argue that regional specialization without sharply defined borders is likely most representative of the STN’s functional organization.

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(Nambu, Tokuno, & Takada, 2002), the STN is considered to play a pivotal role in the control of motor, cognitive, motivational and affective functions. This is exemplified by its altered neural activity patterns observed in relation to Parkinson’s disease (PD) severity (Sharott et al., 2014), and its recognized role in resolving conflict during response selection (Frank, 2006). The use of the STN as target region for deep brain stimulation (DBS) treatment has proven successful in alleviating motor symptoms in PD (e.g., Kleiner-Fisman et al., 2006), and is also clinically effective for other neurological and psychiatric disorders such as dystonia (Ostrem et al., 2011), and obsessive-compulsive disorder (Mallet et al., 2008). Although STN-DBS has become an established treatment for advanced PD, it is associated with a cost. Next to a reduction in bradykinesia, rigidity, and tremor, a substantial number of patients experience neuropsychiatric side effects due to stimulation including depressive episodes, apathy, impulse control problems, impaired word fluency and cognitive decline (Benabid, Chabardes, Mitrofanis, & Pollak, 2009; Voon, Kubu, Krack, Houeto, & Tröster, 2006). This is often explained in light of the prominent, but challenged hypothesis that the STN is comprised of three distinct subdivisions: a sensorimotor, cognitive (or associative), and limbic part (Alkemade, Schnitzler, & Forstmann, 2015; Haynes & Haber, 2013; Lambert et al., 2012; Parent & Hazrati, 1995; Temel, Blokland, Steinbusch, & Visser-Vandewalle, 2005), see Fig. 2. Stimulating the STN outside the putative sensorimotor zone, e.g., due to the choice of stimulation parameters or the anatomical location of the active contact(s), may contribute to the side effects observed. The labelling of the STN’s subregions into sensorimotor, cognitive, and limbic parts suggests clearly anatomically separable functions. However, all three are closely intertwined from a neuropsychological point of view, therefore raising questions to what extent they can be regarded as independent functions controlled by distinct subregions and parallel pathways.

Here, we aim to reassess the prominent tripartite hypothesis of the human STN by zooming into the micro- and meso-level. Concretely, in the first part we review evidence based on human and non-human primate microscopy approaches. We argue that the anatomical microstructure with sub-millimeter precision can serve to understand the intrinsic make-up of the STN. Next, we aim to highlight the meso-level by means of local field potential (LFP) recordings from DBS electrodes in patients with PD. Together with simultaneous electroencephalography (EEG) or magnetoencephalography (MEG) these recordings give exciting insights into the neural dynamics and networks associated with the STN, eventually helping to better understand its role in cognition and behaviour. Finally, we discuss how findings from micro- and meso-level studies together provide converging evidence for both segregated and integrated functional representations within the STN. We focus on aspects such as local and global information processing, the separation of sensorimotor, cognitive, and limbic task-aspects into distinct functions, and the implication of spatially overlapping subregions for DBS treatment.

2. Micro-level perspective

In 1995 Parent and Hazrati published a highly influential review paper, in which they brought together results from various neuronal tracing studies in rodents and non-human primates on the STN, uniting them in the tripartite hypothesis (Parent & Hazrati, 1995). The studies on which the tripartite hypothesis is based are of high quality, and at a later stage the results were reproduced in non-human primates (Haynes & Haber, 2013). However, the studies that formed the basis to formulate the tripartite subdivision hypothesis, leave room for an alternative interpretation. It is therefore not surprising that other studies report different numbers of subdivisions, ranging between zero and four (for review see Keuken et al., 2012).

The tripartite subdivision hinges on the (relative) absence of neuronal tracer from specific parts of the STN. An inherent bias of tracing techniques is a systematic underestimation of the projection fields of cortical injection regions (Alkemade, 2013; Haynes & Haber, 2013). This means that the (relative) absence of cognitive/associative connections in the limbic medial tip of the STN could, at least in part, be the result of technical limitations. The underestimation of the projection field allows the alternative hypothesis that neurons connected to cortical sensorimotor, cognitive, and limbic areas are largely intermingled in the STN, and that no subdivisions can be distinguished. One could speculate that the tripartite

![Schematic representation of the major anatomical connections between cortex, basal ganglia, and thalamus.](image)

Excitatory (glutamatergic) connections are indicated in green, inhibitory (GABAergic) connections in red. Typically, three distinct pathways are distinguished: the direct pathway (1) with a net excitatory influence on thalamocortical projections, the indirect pathway (2) and the hyperdirect pathway (3) with a net inhibitory influence on thalamocortical projections. The labels of the involved structures are placed approximately near the anatomical locations as visible on a coronal view of the ICBM/MNI2009b MRI template image shown in the background. GPe = external pallidum, GPi = internal pallidum, STN = subthalamic nucleus.
The tripartite hypothesis is based on long-range anatomical projections to the STN from brain structures with associated sensorimotor, cognitive, and limbic functions. A) Topographic organization of cortical projections with terminals in the STN as determined by anterograde tracing in non-human primates (Haynes & Haber, 2013). Terminal fields of dense projections (left panel) were topographically aligned with their corresponding diffuse projections (right panel), although the diffuse projections showed a higher overlap. Colours denote the tracer injection sites. Adapted from Haynes and Haber (2013). B) Winner-takes-all parcellation of the human STN by Ewert et al. (2016) based on diffusion-weighted images from 32 healthy adults and 90 people with Parkinson’s disease. The directionality of connections cannot be inferred from this technique. This figure was generated with the Lead-DBS software package (Horn & Klühn, 2015). vmPFC = ventromedial prefrontal cortex, OFC = orbitofrontal cortex, ACC = anterior cingulate cortex, dPFC = dorsal prefrontal cortex, SMA = supplementary motor area, M1 = primary motor cortex, Hipp = hippocampus, Amyg = amygdala, s/m/iPFC = superior, middle, inferior frontal gyri.

The tripartite subdivision hypothesis received more traction, since it provides an attractive theoretical framework that could potentially explain differences in clinical outcomes observed in patients who receive DBS. The alternative hypothesis of spatially intermingled functional areas in the STN in combination with the technical limitations of neuronal tracing techniques stress the importance of complementary research approaches to resolve the internal structure of the STN.

Testing of the tripartite hypothesis is challenging in view of the required anatomical detail together with the functional information that needs to be incorporated in order to provide a definitive vote on the validity of the hypothesis. Immunocytochemistry provides a complementary research approach providing a high level of anatomical detail, and through the principle of functional segregation can provide a handle on potential functional specialization within the STN. Functional segregation leads to the development of different neuronal cell types that move apart during development, determined by the acquired specialization (Arendt, 2008). Neuronal wiring of the nervous system is dependent on guidance events, which provide a framework that allows the formation of functional circuits (Kolodkin & Tessier-Lavigne, 2011). The development of the formation of the STN’s functional connections is guided by attraction and repulsion molecules expressed in neurons. To form the putative subdivisions of the STN, the expression of such guidance molecules is required to differ between distinct parts of the STN, and thus would be reflected by differences in the molecular fingerprint of the connected neurons. By extension this would mean that different neuronal populations as identified based on their molecular fingerprint are indicative of a potential functional specialization within the STN.

Older studies in humans and non-human primates using antibodies raised against markers for serotonergic signalling, and expression of calcium binding proteins showed an inhomogeneous distribution throughout the STN (Augood, Waldvogel, Münkle, Faull, & Emson, 1999; Mori, Takino, Yamada, & Sano, 1985; (Parent et al., 1996); Parent, Wallman, Gagnon, & Parent, 2011). We recently confirmed and extended these descriptive findings with a quantitative approach using statistical modelling of the immunohistochemical characteristics of the human STN using detailed 3-dimensional reconstructions of the human STN. We investigated the spatial distribution of twelve individual protein markers (serotonin transporter (SERT), calretinin (CALR), parvalbumin (PARV), tyrosine hydroxylase (TH), synaptophysin (SYN), transferrin (TF), glutamic acid decarboxylase (GAD65/67), neurofilament H (SMI32), ferritin (FERR), GABA receptor subunit A3 (GABRA3), vesicular glutamate transporter 1 (VGLUT1), myelin basic protein (MBP)) to determine whether we could find evidence for subdivisions or functionally enriched zones in the STN (Fig. 3). Statistical modelling of the data revealed that the distribution patterns of the majority of tested markers was best described by gradual differences in expression patterns in the STN along varying axes of the STN, which we could not reconcile with a tripartite subdivision (Alkemade et al., 2019). Our findings were in concordance with tracing studies, as well as the gradual distribution of iron throughout the STN (Alkemade et al., 2019; de Hollander et al., 2014; Haynes & Haber, 2013). Taken together, the neuroanatomical results obtained from various microscopy techniques can be reconciled across studies, and variations in the description of the internal structure of the STN appear to be the result of differences in interpretation of the data, rather than differences in the data themselves.
3. Meso-level perspective

The macroelectrodes implanted for DBS treatment allow for LFP recordings that reflect changes in the extracellular electric field generated by synchronous post-synaptic currents, and to a lesser extent action potentials, from many neurons around the electrode (Buzsáki, Anastassiou, & Koch, 2012). Additionally, intra-operative microelectrode recordings provide insights into the firing patterns of single neurons. The focus of most research studies has been on spectral features of these recordings including time-varying amplitude modulations of oscillations and their role in the pathophysiology of PD. In particular beta oscillations (13–30 Hz) and their burst-like activity pattern are linked to bradykinesia and rigidity symptoms (e.g., Kühn, Kupsch, Schneider, & Brown, 2006; Kühn et al., 2009; Neumann et al., 2016; Tinkhauser et al., 2007; van Wijk et al., 2016), which is in the putative sensorimotor subregion.

Neural activity in separate frequency bands is suggestive of distinct functional roles, and could therefore indicate regional specialization within the STN. Compared to the beta band, theta (4–8 Hz) and alpha power (8–12 Hz) seem to be more uniformly distributed (Geng et al., 2018; Kühn et al., 2005; Trotttenberg et al., 2007) but with peak amplitudes for the alpha band at more ventromedial locations, within the putative cognitive subregion and around its border with the sensorimotor subregion (Horn, Neumann, Degen, Schneider, & Kühn, 2017), see Fig. 4. Activity at higher frequencies such as gamma band activity (60–90 Hz) and high-frequency oscillations (200–400 Hz) appears to be located slightly superior to the beta band, around the STN’s dorsal border (Geng et al., 2018; Trotttenberg et al., 2006; van Wijk et al., 2017), but have also been reported less close to the dorsal border (Telkes et al., 2018; Wang et al., 2014; Zaidel et al., 2010). In general, these observations from rest recordings are mimicked by power changes during task performance. Movement induces spatially diffuse beta and gamma modulations mostly in the dorsolateral part of the STN (Geng et al., 2018; Lofredi et al., 2018; Tinkhauser et al., 2019), whereas theta/alpha modulations induced by emotional stimuli occur more ventromedially (Rappel et al., 2020). Notably, in all these studies there is substantial spatial variability in identified peak locations, with maximum power values in individual hemispheres often detected outside the STN, and large spatial overlap across frequency bands. A complicating factor is the inherent unequal sampling of the STN through surgical targeting of the dorsolateral part, resulting in very few recordings from the medial tip. This renders it difficult to divide the STN into subregions based on these recordings alone.

Several factors compromise the spatial resolution of LFP localization studies. Firstly, electrophysiological recordings require a reference. DBS electrodes implanted for treatment typically contain at least four contacts that are spaced 2 mm apart. It is conventional to use a bipolar derivation between adjacent contacts, hence resulting in three time series per hemisphere. The Euclidian midpoint between the contacts is then defined as the location of the recording. However, activity measured by either contact contributes to the time series, and cannot be distinguished. Secondly, each contact...
is sensitive to neural activity in a certain volume around it. The spatial extent from which DBS electrodes pick up LFP signal can comprise several millimetre (Lempka & McIntyre, 2013), which renders it likely that activity detected just outside the borders originates from neurons within the STN. Although bipolar recordings are more representative of local neural activity patterns compared to monopolar recordings with a distant reference (Marmor et al., 2017), detailed computer simulations show that the source origin of oscillations within the STN may not necessarily be at the location of the bipolar LFP recording with largest signal amplitude due to polarity reversal around the source origin (Maling, Lempka, Blumenfeld, Bronte-Stewart, & McIntyre, 2018). This is further complicated by the inhomogeneity of neuronal density across the STN (Lévesque & Parent, 2005) that may influence LFP signal amplitude. Furthermore, inaccuracies in electrode localization on post-operative CT or MRI scans and the subsequent warping to MNI space may introduce additional variability when grouping results across hemispheres. For these reasons, it is difficult to pinpoint the exact origin of meso-level neural activity within the STN.

4. From micro- and meso-level to functional networks

Electrophysiological recordings obtained from micro- or macroelectrodes may serve as a bridge between biochemical processes and overt behaviour. Linking the spatial distribution of recorded neural activity to variations in protein markers, for example as investigated by Alkemade et al. (2019), is however not straightforward. Per definition, these markers represent only a subset of biological markers that contribute to the LFP and their relation may not be directly evident. It is known that the amount of synchronous postsynaptic currents is a major determinant of the STN’s LFP signal amplitude (Lempka & McIntyre, 2013). The distribution of SYN as a marker for the density of local synapses is therefore of particular interest. Unfortunately, LFP recordings from the rostral part of the STN, which shows highest expression of SYN, are scarce. SMI32 is a marker for Neurofilament-H, a major cytoskeletal component that could reflect the presence of more and/or thicker axons, and hence possibly synaptic input. The Neurofilament-H gradient along the dorsal-ventral direction indeed roughly follows the alpha and beta power peak locations reported by Horn et al. (2017) but does not dissociate between these frequency bands, for which it might be necessary to additionally consider the synaptic interactions with other regions in the cortico-basal ganglia network.

Computational modelling studies provide valuable insights into the emergence of neural oscillations within brain networks. The circuit with recurrent excitatory/inhibitory projections between the STN and the external pallidum (GPe) has been a popular focus of investigation in the context of Parkinson’s disease as it intrinsically supports oscillations. Striatal and cortical projections to the STN-GPe circuit have been found critical for triggering and modulating STN oscillations (e.g., Gillies, Willshaw, & Li, 2002; Kumar, Cardanobile, Rotter, & Aertsen, 2011; Nevado Holgado, Terry, & Bogacz, 2010; Terman, Rubin, Yew, & Wilson, 2002). The balance between excitation and inhibition in the network influences the frequency and amplitude of the oscillation. Although speculative, neurons showing immunoreactivity for protein markers with an increased expression along the
ventromedial-dorsolateral axis (GAD6567, GABRA3, and VGLUT1) may be linked to the distinct peak locations of alpha and beta oscillations. Alternatively, long feedback loops involving cerebral cortex are capable of generating oscillatory activity (Leblois, 2006; Pavlides, Hogan, & Bogacz, 2015), and oscillations might arise elsewhere in the cortex or thalamo-cortical network and propagate to the STN (Hahn & McIntyre, 2010; Pavlides et al., 2015; van Albada, Gray, Drysdale, & Robinson, 2009). Hence, spatial variability in the spectral features of LFP signals may not be fully reflected by differences in micro-architecture of the STN, since these features may be influenced by signals originating outside the STN.

Neural synchronization between STN and cortex can be studied by combining LFP recordings from DBS electrodes with simultaneous electroencephalography (EEG) or magnetoencephalography (MEG). This has revealed separate networks, in the alpha band with temporal cortex and in the beta band with motor cortex, that have proved highly replicable across patients from different medical centres (Hirschmann et al., 2011; Litvak et al., 2011). Beta band connectivity can be further subdivided into low-beta (13–21 Hz) coherence with lateral cortical motor areas and high-beta (21–30 Hz) coherence with mesial areas (Oswal et al., 2016; Toledo et al., 2019). The shorter phase delays for high-beta compared to low-beta coherence suggest differential involvement of the hyperdirect and indirect pathways in generating the functional coupling (Oswal et al., 2016). At lower frequencies, theta band coherence between STN and midfrontal cortical regions emerges during conflict in decision-making tasks (B. A. Zavala et al., 2014; B. Zavala et al., 2016) and has been observed during response inhibition in the most ventral DBS electrodes (Alegre et al., 2013). These studies, however, do not address the level of overlap between functional networks within the STN and whether a clear division into subregions is supported.

Even though tracing studies do not provide an unequivocal mapping of long-range anatomical projections, findings can be further supplemented by those obtained from in vivo diffusion-weighted imaging. Overall, this creates a picture of considerable integration between parallel circuits throughout the cortico-basal ganglia-thalamic network (Huber & Knutson, 2010). The topography of projections from (pre-)frontal cortical regions associated with limbic, cognitive and motor functions is largely preserved across dorsolateral, central, and ventromedial parts of the striatum and pallidum but with a high degree of overlap (Draganski et al., 2008). The lack of clear subdivisions within STN does not seem to be an exception.

## 5. Behavioural and clinical implications

The position of the STN in the inhibitory indirect and hyperdirect cortico-basal ganglia pathways has led researchers to ascribe a primary role to the STN in inhibiting undesired movements (Albin, Young, & Penney, 1989; DeLong, 1990; Mink, 1996). The relation between altered activation in basal ganglia pathways and the occurrence of movement disorders has inspired this view. Enhanced activation of the indirect relative to direct pathway is associated with hypokinetic symptoms such as bradykinesia and rigidity in Parkinson’s disease. Vice versa, diminished STN output, e.g., due to white or grey matter lesions or induced by levodopa or DBS treatment, may underlie hyperkinetic symptoms as observed in hemiballismus and other forms of dyskinesia. Several lines of evidence suggest that the function of the STN as a “brake” extends into the cognitive domain of action selection and is one of global motor suppression (Aron, Herz, Brown, Forstmann, & Zaghrou, 2016). Corticospinal excitability of the hand is reduced during task instructions of stopping vocal responses, in correlation with an increase in beta band activity in the STN (Wessel, Ghahremani, et al., 2016). Similarly, the presentation of surprising stimuli leads to a general reduction of corticospinal excitability and activates the STN (Wessel & Aron, 2013; Wessel, Jenkinson, et al., 2016). The withholding of primed actions during surprise or conflict might allow for more time to evaluate the sensory information and select the most appropriate action.

The monosynaptic hyperdirect pathway connections from the right inferior frontal cortex and pre-supplemental motor area, both considered key for inhibitory control, to the STN are particularly suited to ensure a rapid initiation of motor suppression. Interference with the braking function of this pathway could explain the occurrence of more impulsive choices during conflict in decision-making tasks when DBS is switched on (Cavanagh et al., 2011; Frank, Samanta, Moustafa, & Sherman, 2007). More generally, DBS is associated with both impaired and improved performance on tests of executive function that rely on inhibitory control or require cognitive switching (Jahanshahi et al., 2000). It is unclear whether these can all be ascribed to a diminished ability to inhibit automatic responses. Moreover, multiple brain regions and networks might be influenced by the stimulation and therefore alter behaviour. Neumann et al. (2018) used detailed computer simulations of neuronal firing within the cortico-basal ganglia-thalamic circuit in combination with precise DBS electrode localizations and fiber tracking in order to distinguish the contribution of hyperdirect and indirect pathways during a visuomotor task under changing conditions. Their findings indicate that reaction time adaptations could be attributed to modulation of the hyperdirect pathway while kinematic aspects such as movement time were best explained by a suppression of the indirect pathway. This type of multimodal and computational approaches may become increasingly valuable in linking anatomy and function and for revealing potentially common neural mechanisms across domains.

A growing number of LFP studies demonstrate neural activity within the STN to be associated with diverse behavioural tasks. Amplitude modulations in different frequency bands can, by and large, be attributed to sensorimotor, cognitive, and limbic task aspects but do not strictly adhere to it. For example, a suppression of beta band activity is typically associated with classical movement tasks but also occurs during motor imagery (Kuhn, Doyle, et al., 2006), action observation (Alegre et al., 2010), (in preparation to) counting of salient stimulus occurrences (Oswal, Litvak, Sauleau, & Brown, 2012), and is modulated by the cognitive load of the action (Oswal, Litvak, et al., 2013). A suppression of alpha band activity during presentation of affective pictures has been found to correlate with the perceived valence (Brücke et al., 2018).
variability and overlap in peak locations. Technical restrictions for both micro-level and meso-level studies prevent a definite conclusion on the existence of distinct subregions. Even so, drawing parallels between these levels provides complementary information on the local and global organisation of neural representations. With DBS technology and imaging techniques becoming more sophisticated, we will be able to map neural involvement during sensorimotor, cognitive, and limbic aspects of these tasks and their shared representations at finer spatial resolutions. Unravelling to what extent these representations are truly segregated or integrated is essential for understanding the functional role of the basal ganglia, and could have important consequences for the optimization of DBS treatment.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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