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Afferent projections of the subthalamic nucleus in the rat: emphasis on bilateral and interhemispheric connections

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The subthalamic nucleus (STN) is important for normal movement as well as in movement disorders. The STN is a target nuclei in patients with advanced Parkinson's disease (PD). Deep brain stimulation (DBS) is a standard surgical treatment for PD. Although DBS results in a significant reduction in motor disability, several negative side effects have been reported. Thus, to understand the side effects of DBS the connection of the STN should be well known. Therefore, the present study aims to re-examine the STN with an emphasis on poorly- or un-documented connections. Furthermore, the bilateral and interhemispheric connections of the STN are evaluated. Fifteen male albino rats received injections of Fluoro-Gold retrograde and biotinylated dextran amine anterograde tracers into the STN. Following a 7–10 day survival period, the animals were processed according to the relevant protocol for each tracer. The present study demonstrates ipsilateral connections of the STN with cortical regions (i.e., infralimbic, cingulate, frontal, piriform, primary motor, primary sensory, insular and retrosplenial cortices), the endopiriform nucleus, basal ganglia related structures (i.e., caudate putamen, globus pallidus, ventral pallidum, nucleus accumbens, claustrum and substantia innominata) and the deep cerebellar nuclei (i.e., lateral, anterior interposed). Bilateral connections of the STN were observed with limbic (amygdala, bed nucleus of stria terminalis), hypothalamic (ventromedial, posterior, anterior, lateral and mammillary) thalamic (thalamic reticular nucleus), epithalamic (habenular nucleus), and brainstem structures (superior colliculus, substantia nigra, spinal nucleus of the trigeminal nerve, red nucleus, dorsal raphe nucleus, pedunculopontine tegmental nuclei). Interhemispheric connections between left and right STN were also observed. The present study fills important gaps in connectivity of the STN. In particular, we report STN connectivity with cortical areas (i.e., piriform, endopiriform and insular), claustrum, hypothalamic, thalamic reticular, cerebellar, habenular, trigeminal, red, cuneate and gracile nuclei and substantia innominate. These connections, which have not been previously described or poorly described, provide new routes that can alter the conceptual architecture of the basal ganglia circuitry and may modify our view of the functional identity of the STN.

Key words: subthalamic nucleus, ipsilateral, bilateral and interhemispheric connections

INTRODUCTION

The biconvex subthalamic nucleus (STN) is derived from the diencephalon and lies between the zona incerta dorsally, and the cerebral peduncle ventrally. The STN is surrounded by bundles of myelinated fibers, and is thus considered to be a "close nucleus" (Chang et al. 1983, Parent and Hazrati 1995, Yelnik and Percheron 1979), and is mainly composed of glutamatergic cells (Feger et al. 1991, Hammond et al. 1983, Rinvik and Ottersen 1993). The well-known motor role of STN was confirmed by electrophysiological and behavioral studies (Bergman et al. 1994, Hamani et al. 2004, Hammond et al. 1978a). The STN has been regarded as an

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important structure in the modulation of basal ganglia output. Output of the basal ganglia is modulated by "direct" (i.e., substantia nigra pars reticulata and the internal segment of the globus pallidus) and "indirect" (i.e., external segment of the globus pallidus and the STN) pathways (Haegelen et al. 2009). Additionally, a pathway bypasses the striatum and connects the cortex directly to the STN, which subsequently sends excitatory projections to the internal segment of the globus pallidus.

Afferent and efferent connections of the STN were studied widely using various retrograde and anterograde tracers in animals (Benarroch 2008, Hammond et al. 1978a, b, Marani et al. 2008). Recent studies using tractography have revealed connections of the STN in humans (Milardi et al. 2016, Lambert et al. 2012). The results of clinical and experimental studies showed that specific regions of the STN are related to specific functions; whereas the dorsolateral portion is related to sensorimotor functions, the ventromedial portion is involved with oculomotor and associative aspects of motor behavior, and the medial portion represents the limbic area of the STN (DeLong et al. 1985, Romanelli et al. 2004a, Romanelli et al. 2004b).

Data from clinical studies have shown the involvement of STN in movement disorders such as Parkinson's disease (PD) (Benabid 2003, Hamani et al. 2004). Deep brain stimulation (DBS) of the STN is widely used in the treatment of advanced PD (Accolla et al. 2016, Castrioto et al. 2011, Honey et al. 2017). Although DBS results in a significant reduction in motor disability, some patients experience severely impairing conditions, including cognitive, psychiatric and emotional, behavioral, recognition of facial and attention, verbal fluency, memory and weight gain (Dias Abdo Agamme et al. 2015, Drapier et al. 2006, Dujardin et al. 2004, Fraigne and Peever 2013, Le Jeune et al. 2008, Phillips et al. 2012, Schroeder et al. 2002, 2003, Volkmann et al. 2010). The mechanisms underlying these symptoms still remains unclear in PD patients. Thus, to understand the side effects of DBS, an understanding of STN connectivity is essential. To address this, the present study aims to re-examine the afferent projections of the STN using Fluoro-Gold (FG) and biotinylated dextran amine (BDA) tracers in rats. Our focus is on evaluating poorly- or un-documented sources of afferent connections of the STN. Further, bilateral and interhemispheric connections of the STN are evaluated.

MATERIALS AND METHODS

Study animals

Fifteen male Wistar albino rats weighing 250–400 g were fed with a standard laboratory rat chow and tap water ad libitum, and housed in Plexiglass cages with a 12-h light/dark cycle in a temperature-controlled room (20±3°C). The Ethical Committee of Koç University approved all procedures, which followed the New

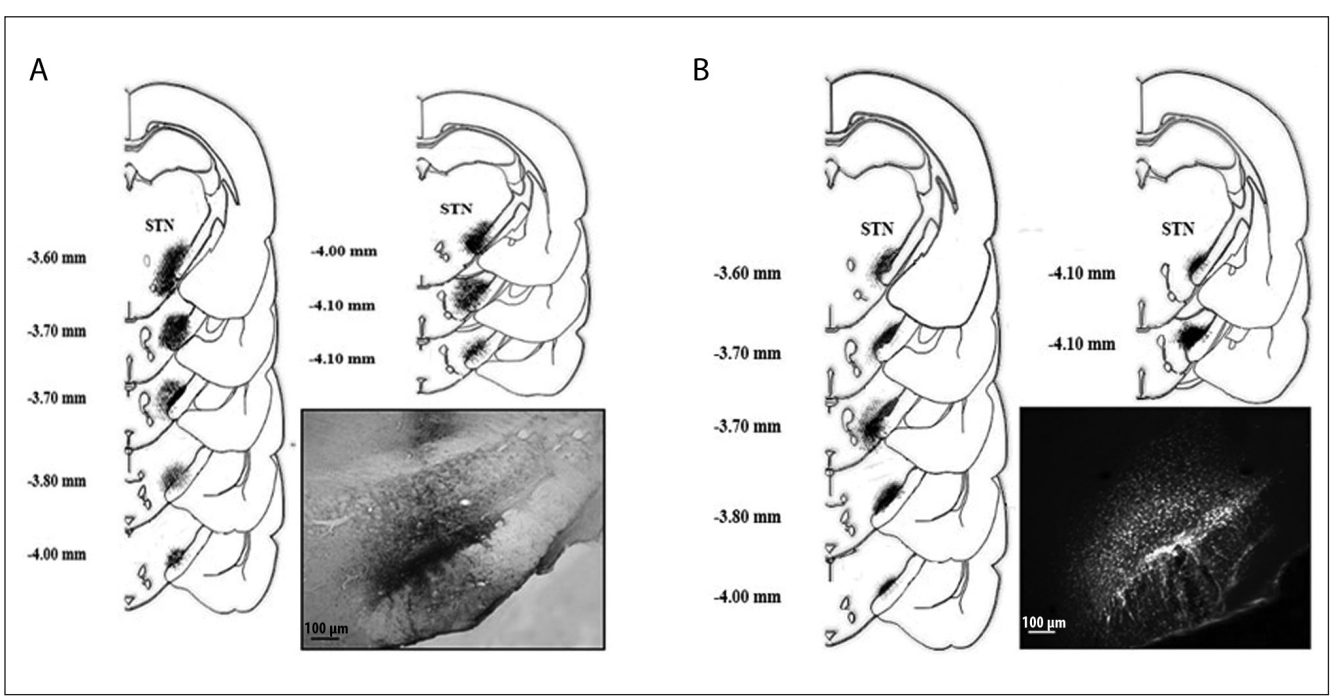

Fig. 1. Schematic illustrations of injection sites to the STN. (A) BDA injection to the STN. (B) FG injection to the STN.

York Academy of Sciences' guidelines for the use of animals in research, testing, and education.

Rats were anesthetized with ketamine (50 mg/kg, intraperitoneally i.p) and chlorpromazine (1 mg/kg, i.p.). The heads of the animals were placed in a stereotaxic frame (Stoelting Model 51600, USA). Next, the scalps were incised longitudinally, and the skulls were exposed between lambda and bregma. A small hole was drilled in the skull at a position appropriate for the unilateral injection of FG (n=7) or BDA (n=8) into the STN. A Hamilton Syringe (Hamilton 80016, 32 ga, 2 in, point style 3) containing either FG or BDA solution was lowered into either the right or left STN unilaterally, according to the rat brain atlas of Paxinos and Watson (1998). The coordinates were selected as follows: 3.6 mm posterior from bregma, 2.4 mm lateral to midline, and 8.2 mm ventral to the surface of the skull (Fig. 1A, B).

FG solution (0.20 µl 2%, FluoroChrome Inc. Englewood, CO, USA) was pressure injected into STN at a rate of 0.02 µl/min. After a 5–7 day survival period, animals were deeply anaesthetized with ketamine (100 mg/kg, intraperitoneally) and chlorpromazine (1 mg/kg, intraperitoneally) and perfused transcardially with 250–300 ml of heparinized saline solution, followed by 4 % paraformaldehyde in 0.1 M phosphate buffer (400–450 ml). Brains were removed and subsequently fixed in the same solution for 24 h at 4°C. Coronal sections (40 µm) were cut on a vibrotome (Leica). Every third section was placed on gelatin subbed glass slides, dehydrated and cleared, covered with DPX, and then examined under a fluorescence microscope.

BDA injection

BDA solution (0.5 µl 10% BDA in 0.1M PBS, Molecular Probes, Eugene, OR) was pressure injected into STN at a rate of 0.02 µl/min. After a 10-day survival period, the animals underwent the same procedure as used under the FG method. Coronal serial sections (40 µm) were cut on a vibrotome (Leica) and free floating sections were collected into a well plates. Immunohistochemistry was applied to every fourth section. BDA was visualized in sections using a standard avidin– biotin complex (Vectastain Elite, ABC kit) horseradish peroxidase staining procedure. Sections were transferred to PBS and washed for 10 min, three times, and then incubated for 3 hours at room temperature in Vectastain working solution. After three additional 10 min PBS washes, tissue was then exposed to diaminobenzidine (DAB, Vector Labs). The reaction was stopped with distilled water, and washed for 5 min. After the dehydration process, the sections were mounted with Entellan.

FG and BDA results that are presented were selected from animals in which the centers of the injection sites were optimal, with minimal contamination of the adjacent structures or along the pipette tract.

RESULTS

Subsequent to the FG and BDA injections into the STN, we observed consistently labeled cells in various

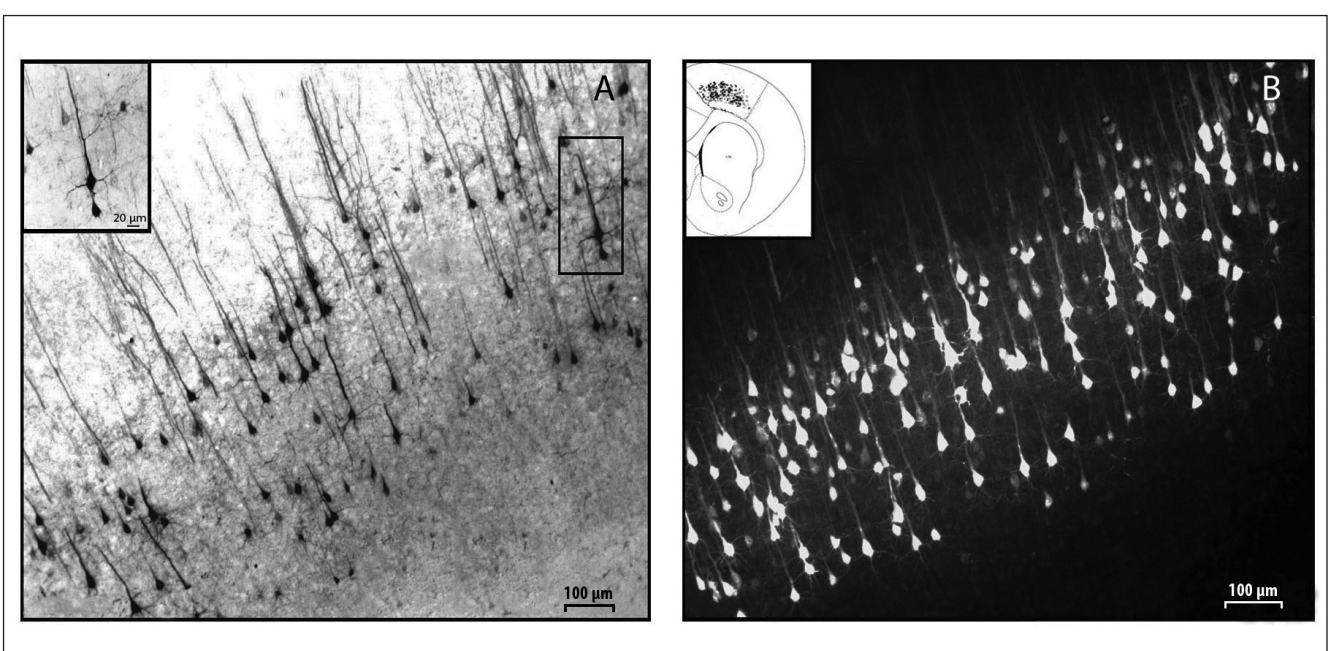

Fig. 2. (A) BDA labelled cells in the primary motor cortex (M1) and magnified motor cell of the M1. (B) FG labelled cells in the M1 and schematic illustration of the labelled cells.

cortical, subcortical and brain stem structures. We also observed bilateral and interhemispheric connections of the STN.

Cortical-STN connections

Dense constant FG and BDA labelled cells were observed in the infralimbic (IL), cingulate (Cg1, Cg2), frontal (Fr3), piriform (Pir1), endopiriform (DEn, IEn), primary motor (M1, M2) (Fig. 2A, B), primary somatosensory (SI), insular (ICx) (Fig. 3A, B) and retrosplenial (RSD, RSG) cortices. All cortical connections were ipsilateral; no labelled cells were observed in any of the aforementioned cortical regions on the contralateral side (Table I). The majority of the labelled cells were located in cortical layers 5 and 6 (Fig. 2A, B). The density of connections of the STN with cortical structures are shown in Table I.

Subcortical-STN connections

FG and BDA injections into the STN showed constant dense labelled cells in the caudate and putamen (CPu), globus pallidus (GP), ventral pallidum (VP), nucleus accumbens (Acb), claustrum (Cl) and substantia innominate (SI). All basal ganglia-related connections were ipsilateral; no labelling was observed on the contralateral side (Fig. 4A, B, C). The density of connections of the STN with basal ganglia related structures are given in Table I.

FG and BDA labelled cells were distributed in large areas of the hypothalamus, including ventromedial (VMH) (Fig. 5), anterior (AHA), posterior (PH), lateral (LH), para and pre mammillary nuclei (MM) (Table I). All hypothalamic connections were bilateral; moderate connections were observed on the ipsilateral side and sparse connections were observed on the contralateral side (Table I).

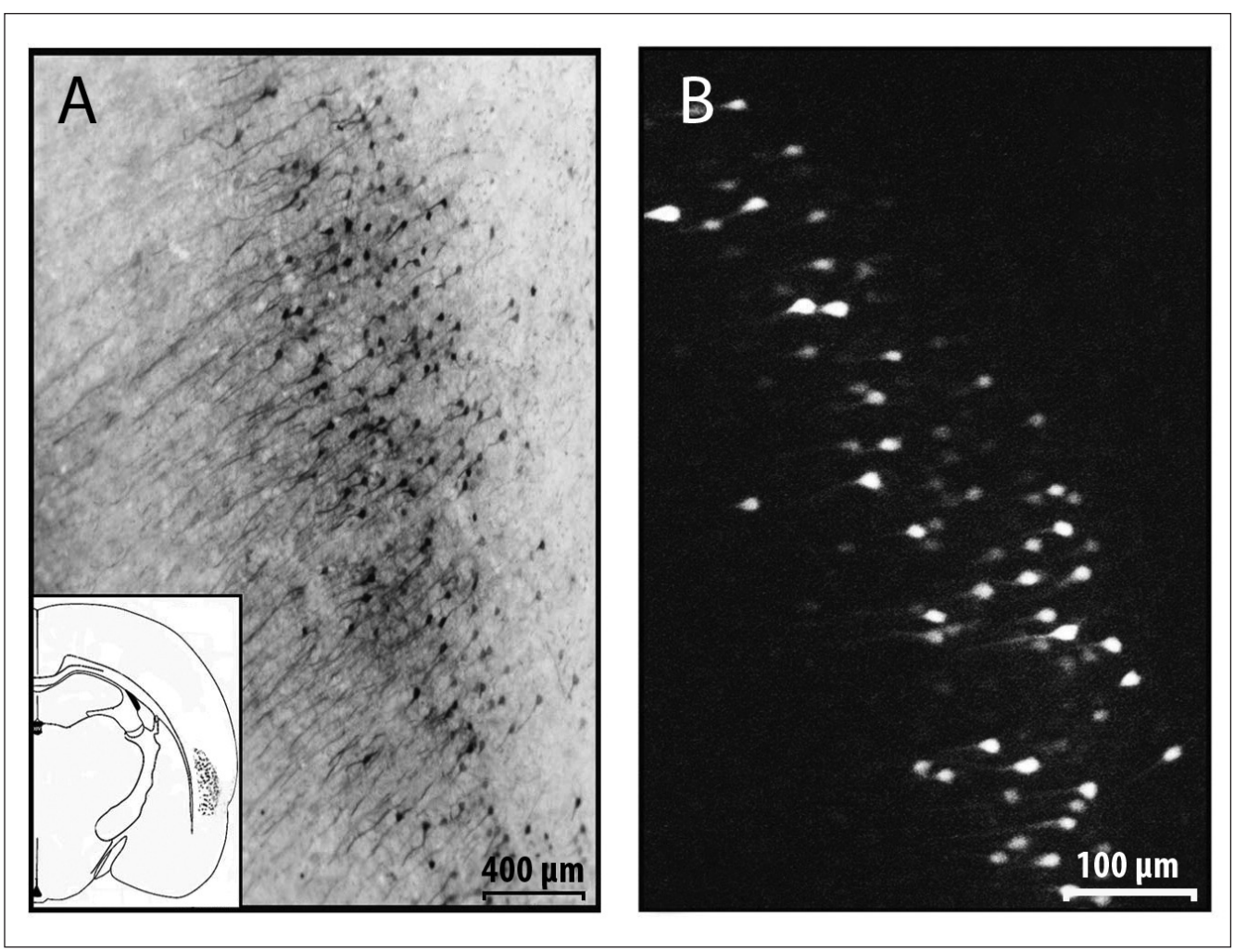

Fig. 3. (A) Labelled cells in the insular cortex (ICx) following BDA injection into the STN, and schematic illustration of the localization of the BDA labelled cells in the ICx. (B) STN labelled cells in the ICx following FG injection into the STN.

Table I. Distribution and relative density of retrogradely labeled neurons sending projections to the subthalamic nucleus.

Source of afferent connections to the STN	FG labeled neurons	BDA labeled neurons	Presence of bilateral connections
Cortex			
Infralimbic (IL)	++	++	–
Cingulate (Cg1, Cg2)	++	++	–
Frontal (Fr3)	++	+	–
Piriform (Pr1)	++	+	–
Endopiriform n. (En, DEn)	++	++	–
Motor (M1, M2)	+++	++	–
Sensory (S1)	++	+	–
Insular (ICx)	+++	++	–
Retrosplenial (RSD, RSG)	++	++	–
Basal Ganglia Structures			
Caudate and putamen (CPu)	+++	++	–
Globus pallidus (GP)	+++	+	–
Ventral pallidum (VP)	++	+	–
Nucleus acumbens (Acb)	++	+	–
Clastrum (Cl)	+	+	–
Substantia innominate (SI)	+	+	–
Limbic Structures			
Amygdaloid n. (CeC, BLA, LaDL)	++	+	+
Bed nucleus of stria terminalis (BST)	+	+	+
Hypothalamus			
Ventromedial n. (VMH)	++	+	+
Posterior n. (PH)	++	+	+
Anterior n. (AH)	++	+	+
Mammillary n. (MM)	+	+	+
Lateral n. (LH)	+	+	+
Thalamus			
Thalamic reticular n. (TRN)	++	+	+
Parafasicular n. (PF)	++	+	–
Central n. (CM, CL, PC)	++	+	–
Ventral n. (VL, VA)	–	+	–
Mediodorsal n. (MD)	–	+	–
Subthalamus			
Subthalamic (STN)	+	+	+
Zona incerta (ZI)	+	+	–
Epithalamus			
Habenular (MHb, LHb)	++	+	+
Brainstem			
Anterior Pretectal (APT)	++	+	–
Parabrachial (PB)	+	+	–
Superior colliculus (SC)	+	+	+
Periaqueductal gray (PAG)	++	+	–
Red nucleus (RMC)	++	+	+
Substantia nigra (SNR, SNC)	++	+	+
Cunate & gracilis (Cu, Gr)	+++	+	–
Trigeminal (Sp5, Pr5, M5)	+++	+	+
Reticular(Gi, PnC, IRt, PMn)	+	+	+
Dorsal raphe (DR)	+	+	+
Locus ceruleus (LC)	+	+	–
Ventral tegmental area (VTA)	++	+	+
Pedinculopontine tegmental (PPTg)	++	+	+
Subthalamic nucleus (STN)	+	+	+
Cerebellum			
Lateral (Lat, LatPC)	++	+	–
Interpositus (IntA)	+	+	–

Note: The density of retrograde labeled cells was estimated as follows: + sparse: for the cell number below 10, ++ moderate: for between 10 and 20, +++ dense: for the cell number above 20 in a specific area.

STN connectivity was observed with various thalamic nuclei (i.e., thalamic reticular [TRN], centromedial [CM], centrolateral [CL], paracentral [PC], parafasicular [PF], ventral anterior [VA], ventral lateral [VL] and mediodorsal [MD]) (Table I). Thalamic connections were primarily ipsilateral; however, TRN connections were bilateral, with dense connectivity ipsilateral to the injection site and sparse connectivity on the contralateral side (Fig. 6A, B) (Table I). The STN is also connected to the zona incerta (ZI) and to the opposite STN. Bilateral connections were observed between the STN and the habenular (MHb, LHb) nuclei of the epithalamus. The density of the hypothalamic, thalamic, subthalamic and epithalamic connections are given in Table I.

Following injections of BDA and FG into the STN, we observed bilateral labelled cells in various limbic structures, including the amygdala (CeC, BLA, LaDL) and bed nucleus of stria terminalis (BST). The density of limbic connections with the STN are given in Table I.

Brainstem-STN connections

Following injections of BDA and FG into the STN, we observed labelled cells in various brainstem structures. Brainstem structures connected to the STN included, the cuneate (Cu) and gracile (Gr) nucleus (Fig. 7A), anterior pretectal nucleus (APT), substantia nigra (SNR, SNC), dorsal raphe (DR) (Fig. 7B), ventral tegmental area (VTA), pedunculopontine tegmental nucleus (PPTg), parabrachial nucleus (PB), superior colliculus (SC), periaqueductal gray (PAG), red nucleus (RMC), locus coeruleus (LC), trigeminal nuclei (Sp5, Pr5, M5) (Fig. 7C), and brainstem reticular nucleus (PnO, Gi, IRt, PMn) (Table I).

Ipsilateral labelled cells were also observed in the lateral (Lat, LatPC) and interpositus (IntPC) nuclei of the cerebellum (Table I).

Bilateral STN connections

Bilateral STN connections were observed with various limbic (amygdala, bed nucleus of stria terminalis, amygdala), hypothalamic (ventromedial, posterior, anterior, lateral and mammillary) thalamic (thalamic reticular nucleus), epithalamic (habenular nucleus), and brainstem structures (superior colliculus, substantia nigra, spinal nucleus of trigeminal, red nucleus dorsal raphe nucleus, pedunculopontine tegmental nuclei) (Table I). None of the aforementioned STN connections with cortical, basal ganglia and cerebellar regions were bilateral.

DISCUSSION

The results of the present study in rats are largely consistent with previously reported afferent projections of the STN that were reported in a human study using diffusion weighted imaging (Lambert et al. 2012). The present study also provides some novel finding; in particular, we found afferent connections from the claustrum, substantia innominate, cerebellum, insular

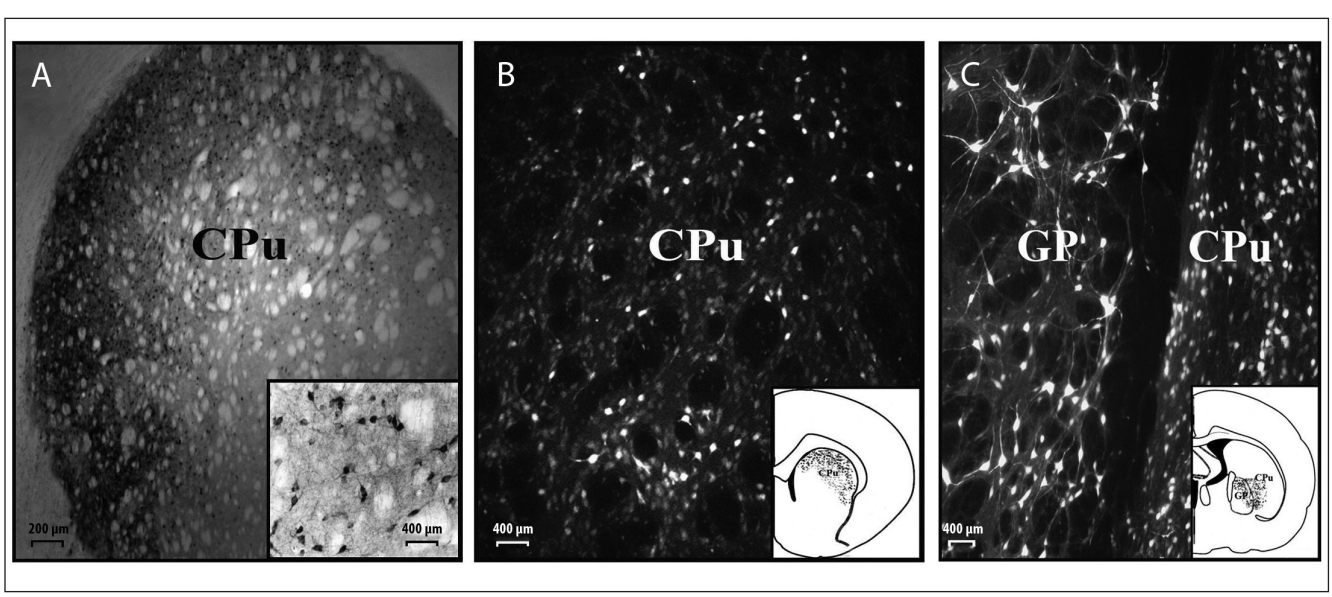

Fig. 4. (A) BDA labelled cells in the caudate and putamen (CPu) and high magnification of the BDA labelled CPu cells. (B) FG labelled cells in the CPu and schematic illustration of the localization of the FG labelled cells in the CPu. (C) FG labelled cells in the CPu and GP and schematic illustration of the localization of the FG labelled cells in the CPu and GP.

cortex, hypothalamic, thalamic reticular, habenular, trigeminal, red, cuneate and gracile nuclei to the STN (Fig. 8). These afferent connections were previously poorly- or un-documented. Further, we documented bilateral connections and interhemispheric connections between the two STN. These connections on various novel routes can change the conceptual architecture of basal ganglia circuitry. Stimulation of the STN is currently the most common target for the surgical treatment of PD. Stimulation of the STN not only affects the STN, but also the anatomical networks which are distributed from the target structure and STN afferents. Therefore, detailed description of STN anatomical connections of the STN will improve our understanding of treatment effects.

Cortical-STN connections

Dense constant FG and BDA labelled cells were observed in large areas of the cortices (IL, Cg, Fr3, Pir1, DEn, M1, S1, ICx, RSG). These labelled cells were specifically localized to cortical layers 5 and 6. Layers 5 and 6 of the cortex send efferent connections to various parts of the brain. The presence of direct cortical-STN connections were confirmed by studies using tracers and electrophysiological studies in animals (Hammond et al. 1978a, b). In primates, a direct route for motor cortical information to the STN has not been defined anatomically (Bergman et al. 1990). However, electrophysiological recordings and high-resolution functional magnetic resonance imaging (fMRI) studies showed the presence of cortico-STN connection in humans (Aravamuthan et al. 2007). Rouzaire-Dubois and Scarnati (1985) reported that cortico-STN projections arise from virtually the entire cortical mantle, and are bilaterally distributed in the rat. The present study also shows large areas of the cortex that were connected to STN; however, the cortical connections were all ipsilateral. The STN is involved in processing cortical information of different types and conveying them to basal ganglia. Studies have shown that cortical projections to the STN are collaterals projecting to the striatum (Feger et al. 1991). In the present study we were not able to define whether these connections were collaterals or direct connections.

Among the cortical-STN connections, the present study shows distinct bilateral connections of the STN with the piriform and endopiriform cortex, which have not been previously reported in the rat. The role of STN connections with the piriform and endopiriform cortex are unknown. The piriform and endopiriform cortex are closely related to limbic structures (e.g., amygdala, hippocampus, and rhinal cortex), and may play a role in transmitting information from the limbic region to neocortex as well as in the physiology of learning and memory. Connections between the STN and the insular cortex have received less attention in the literature. Different functions have been attributed to the insular cortex. Multiple impairments have been reported following injury to the insula in humans, including alterations in autonomic, emotional, sensory functioning (e.g., gustatory, olfactory, auditory, somatosensory, multimodal), body awareness, and language (Ibañez et al. 2010). The disruption of insular cortex-STN connections can be the result of emotional and sensory changes observed in PD patients following DBS.

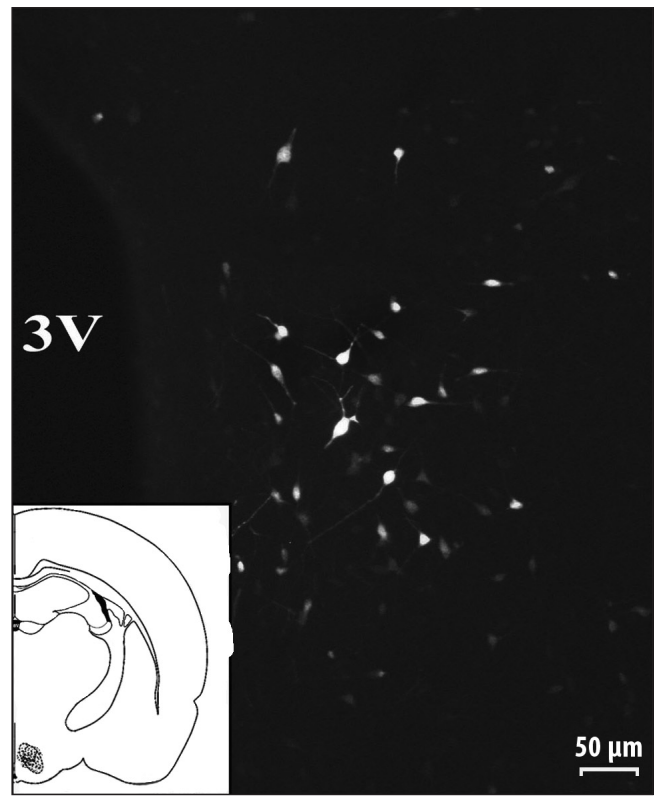

Fig. 5. FG labelled cells in the ventromedial hypothalamic nucleus (VMH) and schematic illustration of the localization of the BDA labelled cells in the VMH.

Subcortical-STN connections

The present study confirms the well-known basal ganglia connections (i.e., caudate putamen, globus pallidus, ventral pallidum and nucleus accumbens). We also demonstrated the presence of ipsilateral claustrum-STN and substantia innominate-STN connections, which have not been previously documented. The claustrum receives input from almost all cortical regions and proj-

ects back to almost all cortical regions (Crick and Koch 2005). Due to the widespread connections of the claustrum, this region may contain specialized mechanisms that permit information to travel widely to synchronize different perceptual, cognitive, and motor modalities.

Although nucleus accumbens-STN connections have been described in various species (Bálint et al. 2011), their functions are still unknown. The nucleus accumbens is responsible for motivation, emotionality, and reward mechanisms (Haegelen et al. 2009). The present study confirms these connections in the rat. Emotional disturbances following DBS surgery in PD patients can be explained by disruption of nucleus accumbens-STN connections.

It has been well demonstrated that the medial lip of the STN in rodents and in primates is connected to limbic forebrain structures (Dong et al. 2000, Haegelen et al. 2009). The results of the present study confirm STN bilateral connections with the bed nucleus of stria terminalis and amygdaloid nuclei. Limbic-STN connections have received less attention in the literature, and their functional role remains unclear. The cognitive loss observed in PD patients with disruption of the medial lip of the STN during DBS may be due to the STN connections with the amygdala and bed nucleus of stria terminalis (Haegelen et al. 2009).

Diencephalic-STN connections

Various nuclei of the thalamus are connected with the STN. Connections with the central and parafasicular thalamic nuclei was revealed in the cat, rat (Royce and Mourey 1985, Sugimoto et al. 1983), and monkey (Carpenter et al. 1981, Sadikot et al. 1990) using autoradiography and tract tracing methods. In accordance with these previous studies, the present study confirms thalamic-STN connections in the rat. The central, parafasicular, ventral and mediodorsal thalamic nuclei may participate in processing of the information that flows through the STN. Additionally, the present study shows that the thalamic reticular nucleus is bilaterally connected to the STN, which has not been documented previously. The thalamic reticular nucleus (TRN) is an important communication hub between the thalamus and cortex. The main afferents of the TRN are collaterals from thalamocortical and corticothalamic fibers. TRN connections with the STN may be involved in transfer-

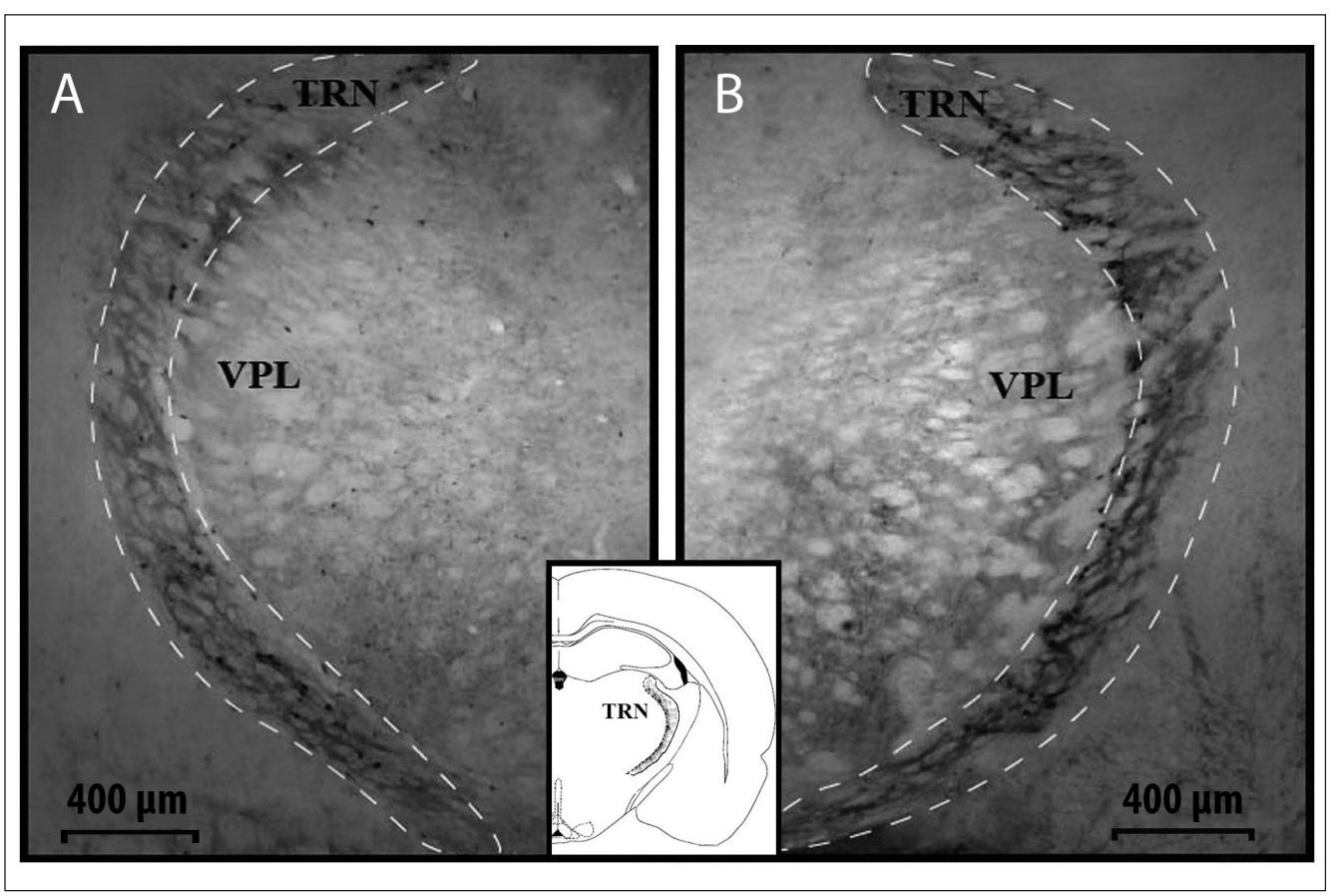

Fig. 6. (A) Numerous BDA labelled cells in the ipsilateral thalamic reticular nucleus (TRN). (B) Sparse BDA labelled cells in the contralateral TRN.

ring information related to thalamus and the cortex, thus, synchronizing this structure with the STN.

Hypothalamo-STN connections have been poorly documented in the literature. Early studies by Morgan (1927) using a fiber degeneration technique showed connections of the STN with the mammillary nuclei in the dog. Similarly, Saper et al. (1976), using a tritium labelled amino acid technique, showed connections of the ventromedial hypothalamic nucleus (VMH) with the STN in the rat. A physiological study showed the involvement of VMH nucleus in inducing running behavior in rats (Iwamoto et al. 1999). Further, kainite injection into the VMH showed a significant increase in the multiunit activity of the STN (Narita et al. 2002). Using a FG retrograde tracer, Chometton et al. (2014) showed that the neurons producing melanin concentrating hormone in the posterior hypothalamic nucleus projected to the STN. Further, Ramirez-Zamora et al. (2016) reported two cases of STN DBS in PD patients who developed reproducible hyperhidrosis with high frequency DBS. The present study clearly demonstrates hypothalamic-STN connections in the rat. Further studies are necessary to reveal the functional significance of these connections.

Brainstem-STN connections

The STN is connected to various nuclei of the brainstem. One of the most well-documented connections of the STN is with the substantia nigra (Canteras et al. 1990). The present study also confirms this connection in the rat. The pedunculopontine tegmental nucleus is being widely studied due to its vast connections with the cerebral cortex, basal ganglia, thalamus, and motor areas of the brainstem and spinal cord. The pedunculopontine tegmental nucleus is thought to be involved in mechanisms of cortical arousal and behavioral state control, and this region participates in control of locomotion and muscle tone (Saper and Loewy 1982). Using diffusion tractography, Aravamuthan et al. (2007) showed pedunculopontine–STN connections in humans. Further, electrical stimulation of the STN modulated the activity of pedunculopontine neurons in the rat (Florio et al. 2007). Hammond et al. (1983) suggested that pedunculopontine tegmental-STN connections mediate the activity of motor structures of the diencephalon and mesencephalon. The present study confirms pedunculopontine tegmental-STN connections in rats. The physiological effects of the pedunculopontine tegmental-STN projections are unknown (Florio et al. 2007, Saper and Loewy 1982).

The presence of bilateral labelled cells in the dorsal raphe nucleus were observe after retrograde tracer injection in the STN of various species (Canteras et al. 1990). We confirm the presence of this connection in the rat. Although serotonin is the most abundant transmitter in the dorsal raphe nucleus, very little is known regarding the physiological action of serotonin–immunoreactive fibers from the raphe nucleus to the STN. The present study also shows connections of the STN with the brainstem reticular nuclei (Gi, IRt, PMn and PnC), which have not been reported in previous studies. However, a clinical study by Pötter et al. (2008) supports our findings. In that study they demonstrated that high-frequency stimulation of the STN alters the excitability of the brainstem startle system in PD, most likely by releasing the reticular motor system. The present study also shows superior colliculus-STN connections. The superior colliculus has a role in re-direction of the

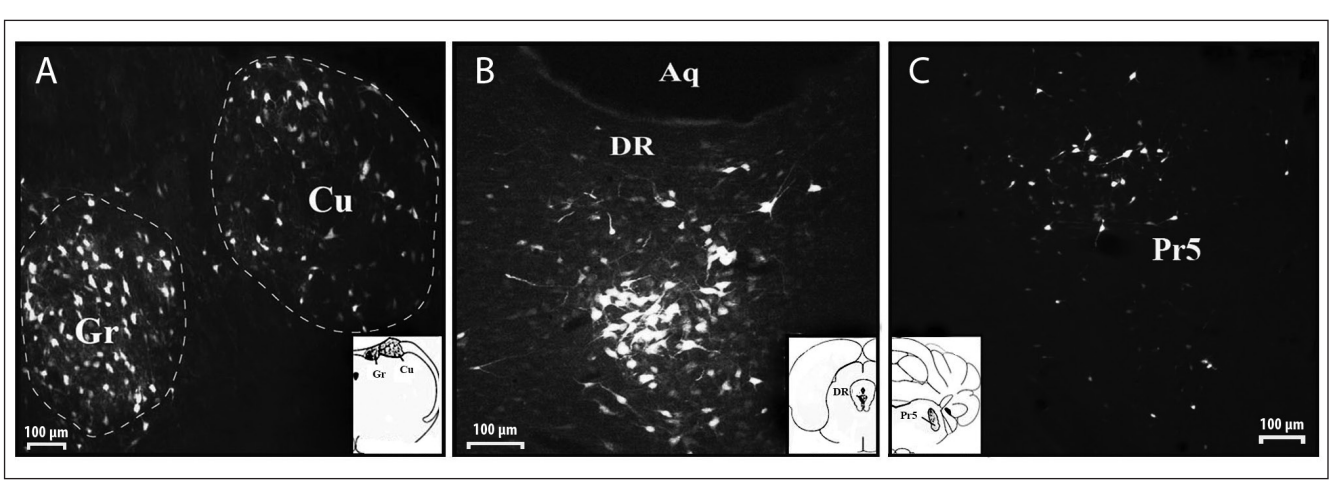

Fig. 7. (A) FG labelled cells in the cuneate (Cu) and gracile (Gr) nuclei and schematic illustration of the localization of labeled neurons in the Cu and Gr. (B) FG labelled cells in the dorsal raphe nucleus (DR) and schematic illustration of the localization of labeled neurons in the DR nucleus. (C) FG labelled cells in the principal nucleus of the trigeminal nucleus (Pr5) and schematic illustration of the localization of labeled neurons in the Pr5.

gaze (Grantyn et al. 2004). Coizet et al. (2009) reported that STN becomes responsive to visual stimuli at short latencies when local disinhibitory injections are made into the deep layer of superior colliculus.

Cerebellar-STN connections have not been documented in previous studies. However, cerebellum-STN connections were described in the human brain by using diffusion magnetic resonance imaging and tractography (Milardi et al. 2016). Further, Moers-Hornikx (2011) performed high-frequency stimulation of the STN in rats, and evaluated neuronal activation in the deep cerebellar nuclei using c-Fos immunohistochemistry. In that they found increased c-Fos expression in the deep cerebellar nuclei. Using a retrograde virus-tracing technique, Bostan et al. (2010) described the STN-ponto-cerebellar pathway. However, the present study shows direct cerebello-STN connections, which may provide an anatomical basis for understanding how the cerebellum influences basal ganglia function.

Sensory information conveyed to motor structures exert an important role in the control of movement (Asanuma 1981). Electrophysiological studies showed that a single-unit recording from the STN in the monkey responded to peripheral somatosensory stimulation. Boivie (1971) observed degeneration in the STN following a lesion of the dorsal column nuclei. Further, Hammond et al. (1978a) reported the existence of STN units that could be activated in the rat by electrical stimuli applied in the vicinity of the vibrissae.

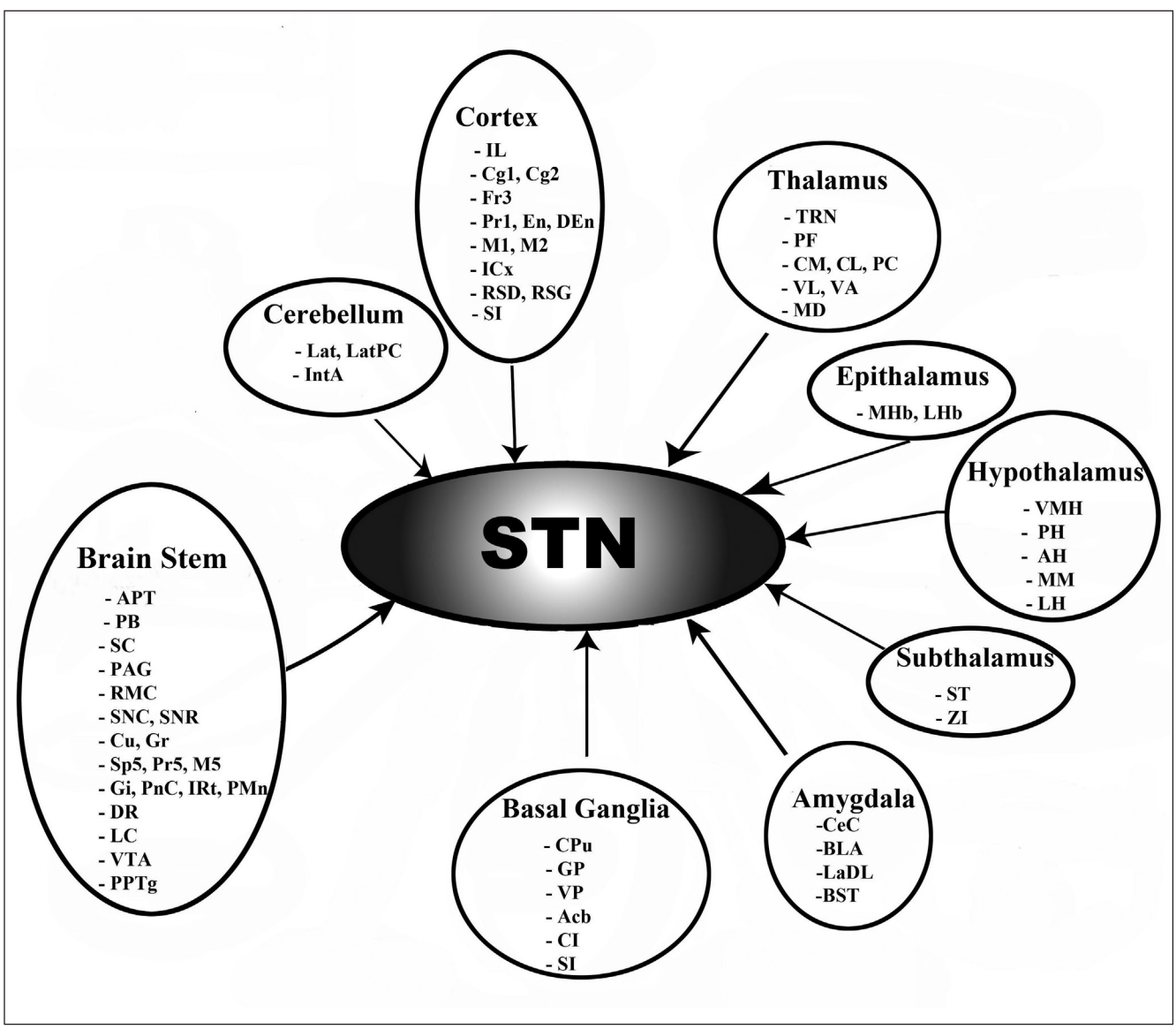

Fig. 8. Schematic illustration showing the afferent projections of the STN.

Moreover, Canteras et al. (1988) showed the existence of very sparse direct projections from the spinal trigeminal and dorsal column nuclei to the contralateral STN, but found the evidence on this point less than conclusive. However, the present study clearly demonstrates the presence of direct pathways between the STN and both the spinal trigeminal and dorsal column nuclei.

Bilateral STN and STN-STN connections

An early study by Carpenter et al. (1981) showed the connections of the STN in monkey using HRP and [^{3}H] amino acid, and found no labelling across the midline, or in the striatum, medial pallidal segment, thalamus, substantia nigra, or dorsal raphe nucleus. The present study confirms a lack of bilateral STN connections with cortical, basal ganglia and cerebellar regions. However, the present study shows bilateral STN connections with various limbic, hypothalamic, thalamic, epithalamic, and brainstem structures (see Table I). Thus, the STN can be influenced by widespread regions of both hemispheres. Lizarraga et al. (2016) compared the effects of bilateral and unilateral STN DBS stimulation on motor scores, gait scores, and kinematics in PD patients. They reported that motor and gait scores improved with bilateral stimulation; however, right-side stimulation improved stride length significantly as compared to left-sided or bilateral stimulation. Results of the aforementioned studies show that STN is modulated by various nuclei of both hemispheres. Thus, the bilateral connections of the STN described in the present study confirm the clinical findings of Lizarraga et al. (2016). Understanding the bilateral connections of the STN will be critical for developing the next generation of DBS systems that minimize negative effects.

Further in the present study we have reported connections between left and right STN, however, no interconnection was documented in literature. The study by Darvas and Hebb (2014) analyzed task specific phase synchronization and causality between left and right STN local field potentials recorded from both hemispheres simultaneously during a cued movement task in four subjects with PD who underwent DBS surgery and found significant phase locking between hemispheres in alpha frequency (8–12Hz) in all subjects concurrent with movement of either hand. They concluded that bilateral network is activated by unilateral motor program. Cross connections of the STN may allow for the interdependent control of activity in one STN and its contralateral part.

The present study shows cross connections of the STN, which has not been previously reported in the literature. The two sides of the STN are connected via the posterior hypothalamic decussation. Darvas and Hebb (2014) analyzed task specific phase synchronization and causality between left and right STN local field potentails, and recorded from both hemispheres simultaneously during a cued movement task in four subjects with PD who underwent DBS surgery. They found significant phase locking between hemispheres in the alpha frequency (8–12 Hz) in all subjects, which coincided with movement of either hand. Based on these results, the authors suggested that this bilateral network is activated by a unilateral motor program. Thus, the cross connection demonstrated in the present study confirms the aforementioned study.

METHODOLOGICAL REMARKS

Although we aimed to inject relatively small volumes into STN, we still observed contamination of the tracer to the neighboring regions. To minimize variation in contamination, we used the same coordinate for all animals, selected animals of similar weight, and injected the same amount of tracer into the STN. However, we observed different density and number of labelled cells. Thus, we tried to overcome the aforementioned limitations by optimizing these parameters, increasing the number of experiments, and by using two tracers. Further, only regions showing labeling with both tracers were reported in the present study.

CONCLUSION

The results of the present study show that the STN has wide ranging connections with different parts of the central nervous system. The novel and the poorly-documented connections demonstrated in the present study may add to the functional identity of the STN. In addition to the well-known motor functions of the STN, this region may also play a key role in non-motor functions in both associative and limbic functions through its neural connections. These new findings may modify the view of the functional identity of the STN.

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