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Hale Soloff Date

GABAergic interneurons in the ventral motor and caudal intralaminar thalamic nuclei in primates: A potential source of GABAergic dysfunction in parkinsonism?

By Hale Soloff

Master of Science

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By

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B.S., Ursinus College, 2016

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Abstract

GABAergic interneurons in the ventral motor and caudal intralaminar thalamic nuclei in primates: A potential source of GABAergic dysfunction in parkinsonism?

By Hale Soloff

Despite extensive research, our understanding of the pathophysiology of the basal ganglia-thalamocortical functional circuitry in Parkinson's Disease (PD) remains incomplete. Key features of parkinsonism are an increased GABAergic inhibition of the ventral motor thalamic nuclei, namely the ventral anterior and ventral lateral (VA/VL) nuclear group, and significant cell loss in the intralaminar centromedian and parafascicular nuclei (CM/Pf). According to current models of the basal ganglia circuitry, this increased thalamic inhibition originates from an abnormally overactive GABAergic tone from the basal ganglia output nuclei. A less studied source of GABA in the VA/VL are the inhibitory GABAergic interneurons. Because these interneurons are absent from the more easily studied rodent motor thalamus, little is known about their anatomy, function, or role in this thalamic region, and in the centromedian/parafascicular (CM/Pf) nuclear group, another main target of basal ganglia outflow in primates.

In order to further characterize the prevalence and potential degeneration of these interneurons in the motor thalamus of normal and parkinsonian monkeys, we performed an unbiased stereological count of interneurons and thalamocortical cells in VA/VL and CM/Pf by immunostaining with anti-GABA and anti-NeuN, respectively, in 3 control and 3 MPTP- (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-) treated parkinsonian monkeys. Data indicate that GABAergic interneurons account 12-14% of neurons in the basal ganglia-receiving motor thalamus (BGMT), 19-20% of neurons in the cerebellar-receiving motor thalamus (CbMT), 9-10% of neurons in the CM, and 7-8% of neurons in the Pf, without any significant difference between control and parkinsonian monkeys. Results of this study set the stage for a deeper understanding of the functional integration of GABAergic interneurons in the physiology and pathophysiology of the basal ganglia-thalamocortical circuitry in normal and parkinsonian state.

GABAergic interneurons in the ventral motor and caudal intralaminar thalamic nuclei in primates: A potential source of GABAergic dysfunction in parkinsonism?

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INTRODUCTION

Despite the significant progress made in our understanding of the anatomical and functional consequences of nigrostriatal dopamine depletion on basal ganglia nuclei in Parkinson's Disease (PD) (Smith et al., 1998; Wichmann & Delong, 2006; Wichmann et al., 2011; Smith et al., 2012), much less is known about the impact of these changes upon the motor thalamus and its connections with motor cortices.

Thalamic activity is relevant to the pathology of PD because abnormal firing patterns and disruptions of neural firing rates have been observed in the thalamus of both animal models of parkinsonism and PD patients (Mitchell et al., 1989; Schneider & Rothblat, 1996; Zirh et al., 1998; Molnar et al., 2005). Studies have shown that neurons in the basal ganglia-receiving region of the motor thalamus (BGMT) in MPTP-treated parkinsonian monkeys display a reduced firing rate, increased incidence of rebound burst activity, pathologic oscillatory activity, and increased synchronization when compared to normal animals (Kammermeier et al., 2014; Devergnas et al., 2015; Galvan et al., 2015).

Although the underlying substrates of these functional changes remains unknown, current explanations have focused primarily on the increased inhibition from the internal globus pallidus (GPi), one of the major GABAergic output of the basal ganglia onto ventral motor thalamic nuclei (Boraud et al., 1996; Wichmann et al., 2011).

The possible contribution to the pathophysiology of parkinsonism of another source of thalamic inhibition, the GABAergic interneurons, remains unknown because the anatomy and function of GABAergic interneurons in the primate motor thalamus have been largely unexplored (Sawyer et al., 1991; Jones, 2007). Knowledge of changes to these interneurons is

crucial for a complete understanding of pathological GABAergic innervation in the thalamus. The reticular thalamic nucleus is one more source of GABAergic innervation throughout the thalamus, but its overall microcircuitry and relationship to PD pathology remains to be examined in future studies, Our project presents the first detailed quantification of GABAergic interneuron and thalamocortical projection neuron prevalence in the primate ventral motor thalamus under normal and parkinsonian conditions. Combined with previous and upcoming results from our research team (Swain et al., 2020), the results of our studies will provide an understanding of how GABAergic interneurons are integrated within the synaptic network which modulates thalamic activity in PD, thus providing a foundation for interpreting future research on the functional importance of these neurons in PD pathophysiology. The absence of GABAergic interneurons in the rodent motor thalamus suggests the potential importance of these cells in high-order information processing unique to the primate brain (Smith et al., 1987; Ilinsky & Kultas-Ilinsky, 1990; Arai et al., 1994; Arcelli et al., 1997; Jones, 2007).

Ventral Motor Nuclei Anatomy: The ventral motor thalamus can be divided into sub-regions based on their main extrinsic inputs. For the purpose of this project, I will refer to two distinct and segregated regions, the cerebellar-receiving motor thalamus (CbMT) and the basal ganglia-receiving motor thalamus (BGMT), which project glutamatergic efferents mainly to the primary and supplementary motor cortices, respectively. Both nuclei receive significant glutamatergic inputs from the cerebral cortex (Kultas-Ilinsky & Ilinsky, 1990; Yamawaki & Shepherd, 2015; Albaugh et al., 2016). Like the glutamatergic input seen in primary sensory thalamic nuclei, the CbMT receives a massive ascending glutamatergic input from deep cerebellar nuclei, recognized as “drivers” of thalamocortical projection neurons in this region. In contrast, the BGMT is devoid of such a powerful extrinsic drive. Its main extrinsic afferents

rather originate from large multisynaptic GABAergic terminals from the GPi or the substantia nigra pars reticulata (SNr), known as the main output nuclei of the basal ganglia (Rovo et al., 2012). Nigral terminals in the BGMT form the majority of symmetric synaptic contacts with thalamocortical projection neurons and also provide significant inputs the distal dendrites of interneurons (Kultas-Ilinsky & Ilinsky, 1990). As such, the pattern of connectivity and overall role of GABAergic interneurons in each region may differ considerably.

Intralaminar Nuclei Degeneration in PD: Like the ventral motor nuclei (BGMT/CbMT), the CM/Pf complex receives projections from the basal ganglia and projects to the cortex; however, unlike those nuclei, the main outputs of the CM/Pf are predominantly glutamatergic projections to the striatum (Galvan & Smith, 2011). According to the published literature, the centromedian and parafascicular nuclei (CM/Pf) undergo significant neuronal degeneration in both PD patients and the chronic low-dose MPTP primate model of PD (Henderson et al., 2000a, b; Brooks & Halliday, 2009; Halliday et al., 2011; Villalba et al., 2014). In chronically MPTP-treated monkeys, this degeneration occurs early, prior to the development of parkinsonian motor symptoms (Henderson et al., 2000a, b; Villalba et al., 2014). These findings are consistent with postmortem human data showing that significant CM/Pf neuronal loss is seen even in early diagnosed patients with mild parkinsonian motor symptoms (Henderson et al., 2000a, b; Henderson et al., 2005; Villalba et al., 2014). To our knowledge, there is no data about the specific loss of GABAergic interneurons in the CM/Pf complex of parkinsonian monkeys and PD patients. This issue will be directly addressed in the present study.

GABAergic Interneurons in the Primate Motor Thalamus: GABAergic interneurons are present in the BGMT and CbMT of primates, which together make up the ventral motor thalamic nuclei. They are also present in the centromedian (CM) and parafascicular (Pf) thalamic nuclei,

two caudal intralaminar thalamic nuclei that receive prominent GABAergic inputs from the basal ganglia and give rise to the bulk of the thalamostriatal projection (Smith et al., 2004; Smith et al., 2014). Non-stereological estimates suggested that these interneurons account for as many as 30% of the total thalamic neurons in the ventral motor thalamic nuclei of squirrel monkeys (Smith et al., 1987; Jones, 2007). Topographical distributions of GABA-immunoreactive neurons in various thalamic groups revealed that different thalamic nuclear groups contain varied density of GABA-immunoreactive interneurons with heterogeneous distribution (Smith et al., 1987), suggesting that the proportions of interneurons in each thalamic nucleus may vary.

Thalamic Interneuron Morphology and Microcircuitry: At the electron microscopic level, dendritic profiles of thalamic GABAergic interneurons can be identified by the presence of flattened pleomorphic synaptic vesicles and the formation of dendro-dendritic symmetric synapses (Ilinsky & Kultas-Ilinsky, 1990; Jones, 2007; Rovo et al., 2012; Cox, 2014). GAD immunostaining at the EM level revealed that interneurons in the CbMT rarely give rise to axonal projections (Kultas-Ilinsky & Ilinsky, 1991). The interneuron axon was identified using electron microscopy based on its dense axolemma undercoat, small bundles of microtubules, and smooth endoplasmic reticulum, and it lacked the ribosomal rosettes observed in projection neurons axons. As it is not known whether interneurons in the BGMT of primates give rise to axonal projections, similar methods of pallidothalamic tracing combined with GAD immunostaining may reveal evidence of interneuron axons. Because GABAergic interneurons are absent in the motor thalamic nuclei of rodents, which are more practical to study in large quantities relative to primates, their potential role as a source of GABA and regulators of thalamic processing of motor-related information in normal and diseased states is largely ignored (Smith et al., 1987; Ilinsky & Kultas-Ilinsky, 1990; Arai et al., 1994; Jones, 2007). Based on data

from the rodent sensory thalamus, which includes GABAergic interneurons, these cells play a critical role in regulating major extrinsic glutamatergic drive of thalamocortical neurons via complex triadic synaptic arrangements made-up of presynaptic axonal and dendritic profiles from interneurons, presynaptic subcortical glutamatergic terminals and post-synaptic dendrites of thalamocortical cells (Jones & Powell, 1969; Sherman, 2004; Casale & McCormick, 2011; Jurgens et al., 2012; Cox, 2014). However, because of significant differences in their sources of innervation, hypotheses about the synaptic microcircuitry of interneurons in the motor thalamic nuclei of primates and humans can only be cautiously informed by what is known about interneurons in sensory nuclei. The precise microcircuitry of interneurons in the ventral motor nuclei of the primate thalamus remains poorly studied. Previous research indicates that small cortical terminals and large GABAergic terminals from the basal ganglia afferents form asymmetric and symmetric synapses, respectively, with distal and proximal dendrites of thalamocortical projection neurons and GABAergic interneurons in the basal ganglia-receiving thalamus (Ilinsky & Kultas-Ilinsky, 1990; Kultas-Ilinsky & Ilinsky, 1991; Jones, 2007; Rovo et al., 2012). However, it remains to be investigated whether these afferents form synaptic triads with interneurons and projection neurons in the ventral motor thalamic nuclei.

Thalamic Pathophysiology in Parkinsonism: In the traditional understanding of the pathophysiology of basal ganglia-thalamocortical circuits in PD, patients have problems initiating movements due to increased inhibition of thalamocortical cells by their basal ganglia inputs (Wichmann, 2019). Electrophysiological data show that stimulation in the GPi changes oscillatory activity in the ventral thalamus, but does not entrain the activity of thalamocortical cells (Kammermeier et al., 2016). Studies of MPTP-treated parkinsonian monkeys demonstrated electrophysiological changes in the BGMT including increased rebound burst firing and changes

in oscillatory activity (Galvan et al., 2015). These complex effects of GPi stimulation on thalamocortical projection neurons cannot be interpreted solely based on increased pallidothalamic GABAergic inhibition onto projection neurons. We suggest that the local influence of interneurons on signaling could account for that complexity. However, without an in-depth knowledge of the anatomy of these neurons in normal and parkinsonian states, such a suggestion remains speculative. The CM/Pf, which undergoes severe degeneration in PD, is thought to primarily regulate cognitive functions such as attention and behavior-switching. Nevertheless, deep-brain stimulation (DBS) in the CM/Pf has yielded anti-parkinsonian effects, likely due to the nucleus' important role as the primary origin of glutamatergic thalamostriatal connections (Smith et al., 2014).

Study Objectives: The present project quantifies changes in the prevalence of GABAergic interneurons and thalamocortical projection neurons in the motor thalamus of MPTP-treated parkinsonian monkeys using unbiased stereological cell counting, which is the current best method for quantitative assessment of neuronal cell number (West et al., 1991). Knowledge of neuronal cell number and identification of which neuron types are lost in neurodegenerative diseases are crucial for creating and evaluating neuroprotective regimens (Cannon & Greenamyre, 2009). This work begins to provide an anatomical foundation for understanding the role of these interneurons in healthy brains and PD pathophysiology. We propose that thalamic interneurons are important cellular components of the thalamocortical loop functioning, and that changes to their structure or synaptic relationships may contribute to pathological changes observed in the thalamus in PD.

MATERIALS AND METHODS

Animals: Six (1 male, 5 female) rhesus macaque monkeys (Macaca mulatta, age range 4-14 years, 5.85-10.80 kg weight range) from the Yerkes National Primate Research Center colony were used in this study (Table 1). All procedures were performed in accordance with guidelines from the National Institutes of Health and approved by Emory's Animal Care and Use Committee. The animals were housed in a temperature-controlled room and exposed to a 12-h light/dark cycle. They were fed twice daily with monkey chow supplemented with fruits or vegetables. The animals had free access to water.

Monkey #	**Age at time of sacrifice (years)**	**Weight at time of sacrifice (kg)**	**Sex**	**Control/MPTP**
MR284R	5.3	6.09	F	Control
MR285R	3.75	10.8	M	Control
MR286R	5.25	9.1	F	Control
MR319R	5.63	8.3	F	MPTP
MR205R	14.33	5.85	F	MPTP
MR265R	7.11	8.7	F	MPTP

Table 1: Summary of age, sex and MPTP treatment of rhesus monkeys used in this study.

MPTP treatment and behavioral observations: Three parkinsonian macaques were generated using a chronic low-dose 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment regimen that is routinely used at the Yerkes National Primate Research Center (Masilamoni et al., 2011; Galvan et al., 2015; Kammermeier et al., 2016). The monkeys received weekly intramuscular injections of MPTP (0.2-0.5 mg/kg; Sigma-Aldrich, St-Louis, MO) for 21 weeks (total dose 2.1-26.79 mg/kg). During the course of the MPTP injections, the monkeys progressively developed moderate-to-severe parkinsonian motor symptoms that were stable for at least 6 weeks leading up to the time of euthanasia (Masilamoni et al., 2011). Prior to MPTP treatment, the monkeys were habituated to a behavioral observation cage and a baseline of motor behavior was established using a modified parkinsonian motor sign rating scale based on one described by (Kurlan, 1991) that scores key parkinsonian motor signs including gross motor activity, balance, posture, bradykinesia, and hypokinesia. Cages equipped with infrared beams to detect and record movement were used to supplement analysis of videotaped recordings. All behavioral analysis tools and methods are routinely used in our laboratory (Raju et al., 2008; Masilamoni et al., 2010; Masilamoni et al., 2011; Villalba & Smith, 2011;Galvan et al., 2015; Mathai et al., 2015; Kammermeier et al., 2016).

Animal perfusion and tissue collection: The animals were euthanized with an overdose of pentobarbital (100 mg/kg, i.v.) and transcardially perfused with cold oxygenated Ringer's solution followed by a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde. The brains of the animals were removed from the skull, cut in blocks, and sectioned with a freezing microtome. At the level of the thalamus, sections were then cut at 50 µm thickness. All sections were serially collected and stored in anti-freeze solution at -20°C until they were processed.

Immunohistochemistry: One out of every ten sections through the whole extent of the ventral motor thalamus was immunostained with specific GABA (for interneurons) or NeuN (for all thalamic cells) antibodies (Table 2, Figure 1). Adjacent sections were immunostained for calbindin D28k (CB) to help delineate nuclear borders between BGMT and CbMT, and to delineate the borders of the CM/Pf complex (Villalba, Wichmann, & Smith, 2014; Villalba et al., 2019). The specificity of these antibodies has been well characterized in our lab and others (Seguela et al., 1984; Mullen et al., 1992; Gonzales et al., 2013). To avoid inter-individual differences in labeling intensity due to inherent variability in reagents used across experiments, tissue from normal and MPTP-treated monkeys was processed together. We used the avidin-biotin complex (ABC) immunoperoxidase method to localize the various markers (ABC-Vectastain Standard kit, PK-4000, Vector Labs Inc, Burlingame, CA, USA). The immunostaining protocol began with sections treated with sodium borohydride at room temperature for 20 minutes, followed by pre-incubation for 1 hour in a solution containing 1% normal horse serum (NHS) for NeuN and CB or normal goat serum (NGS) for GABA, 0.3% Triton-X-100, and 1% bovine serum albumin (BSA) in PBS. Sections were then incubated for 24 hours at room temperature in a nearly identical solution with the addition of primary antibodies. The next day, the sections were thoroughly rinsed in PBS and then incubated for 90 min at room temperature in a PBS solution containing the respective secondary antibodies combined with 1% normal horse or goat serum, 0.3% Triton-X-100, and 1% BSA. Sections were exposed to an avidin-biotin-peroxidase complex (ABC; 1:100; Vector) for 90 min followed by rinses in PBS and Tris buffer (0.05 M; pH 7.6). Sections were then incubated for 10 min at RT within a solution containing 0.025% 3,3′-diamino-benzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO), 0.01 M imidazole, and 0.005% hydrogen peroxide in Tris buffer, followed by rinses with

Antigen	Immunizing Species	Vendor and Catalogue #	Antibody Dilution Used	Secondary	Secondary Vendor and Catalogue #
Neun Mouse	Mouse	Millipore MAB377	1:2000	Horse anti Mouse 1:200	Vector BA-2000
GABA Rabbit	Rabbit	Sigma A2052	1:40000	Goat anti Rabbit 1:200	Vector BA-1000
Calbindin D-28R Mouse	Mouse	Sigma C9848	1:4000	Horse anti Mouse 1:200	Vector BA-2000

Table 2: Antibodies used in immunohistochemistry

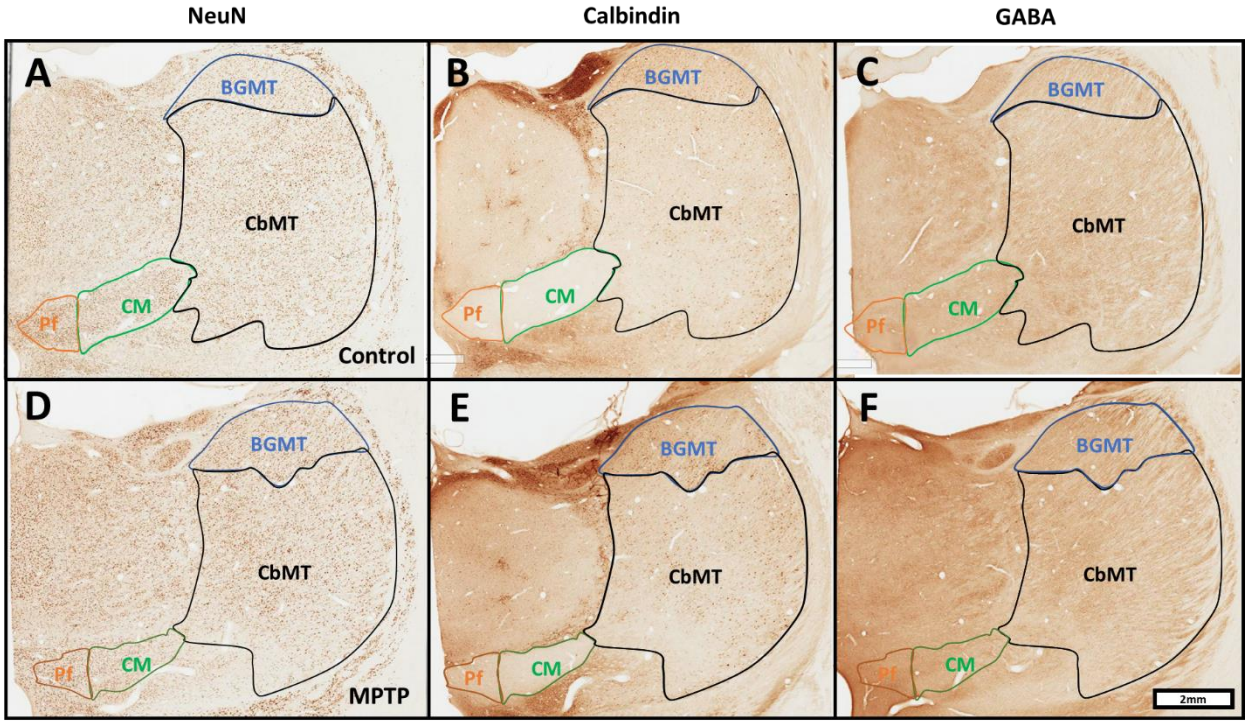

Figure 1: Serial coronal sections (A-F) showing NeuN, Calbindin, and GABA immunoreactivity in the basal ganglia-receiving motor thalamus (BGMT), cerebellar-receiving motor thalamus

(CbMT), centromedian nucleus (CM) and parafascicular nucleus (PF) of control (A-C, N=3) and MPTP-treated (D-F, N=3) monkeys. Scale bar corresponds to 2mm.

PBS, mounted onto gelatin-coated slides, dehydrated in alcohol, immersed in toluene and coverslipped with Permount.

Stereological estimation of GABA-positive and NeuN-positive neurons in BGMT, CbMT, CM, and Pf thalamic nuclei: Unbiased stereological cell counting was performed to compare the total number of interneurons and projection neurons in the BGMT, CbMT, CM, and Pf of MPTP-treated (N = 3) and control (N = 3) parkinsonian monkeys (Figure 2). The immunostained sections were scanned using a Scan Scope light microscope (Aperio Technologies, Vista, CA) at 20x magnification to create low power images. Using these images, the borders of the BGMT, CbMT, CM, and Pf regions of interest were manually delineated based on the presence and absence of CB-positive neurons with assistance from the stereotaxic atlas of the rhesus monkey thalamus (Ilinsky & Kultas-Ilinsky 2002; Lanciego & Vazquez 2012). These tracings were replicated for NeuN- and GABA-labeled slides adjacent to those immunostained for CB. Using the Stereo Investigator cell counting software (MBF Bioscience, Williston, VT) and a Leica DMLB light microscope (Vienna, Austria), the boundaries of the BGMT, CbMT, CM, and Pf thalamic nuclei were outlined under a 4X objective to match the manually traced images. Under a 100X oil-immersion objective, unbiased stereological cell counting was performed using the optical fractionator principle to estimate the total number of GABA-positive interneurons and NeuN-positive thalamic neurons in selected thalamic nuclei in control and parkinsonian monkeys (Gundersen & Osterby 1981; Gundersen 1986; West et al. 1991; West 1999; Schmitz & Hof 2005). Automated software randomly placed optical dissector probes (65 μm × 65 μm) within each ROI (separated by 400 μm for BGMT and CbMT, 250 μm for CM and

150 μm for Pf) to achieve systematic random sampling. Instances of stained cell bodies were manually counted if they were within a probe or touching a probe's inclusion surface, and no cell

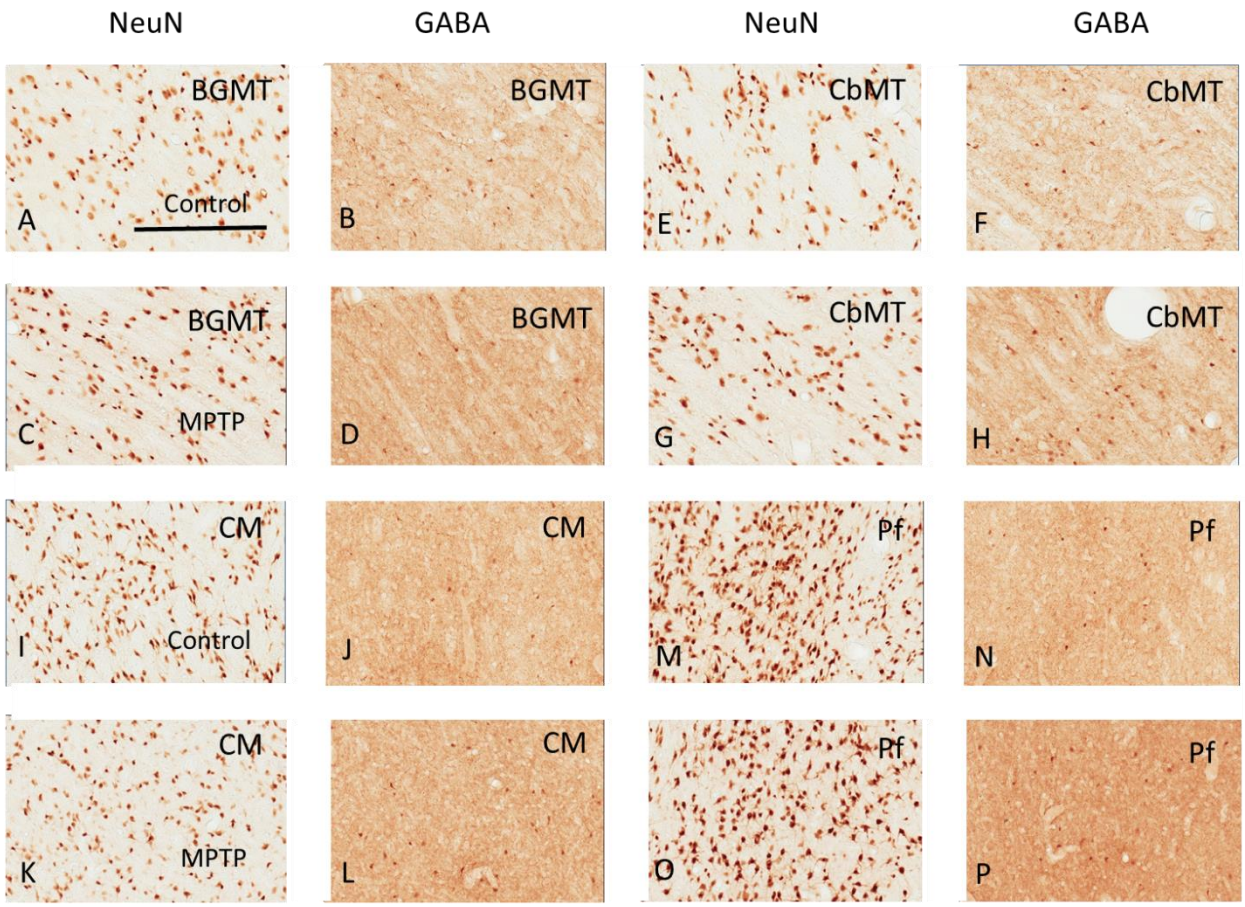

Figure 2: NeuN and GABA immunoreactivity in the basal ganglia-receiving motor thalamus (BGMT) (A-D), cerebellar-receiving motor thalamus (CbMT) (E-H), centromedian nucleus (CM) (I-L) and parafascicular nucleus (PF) (M-P) of control (A-F and I-N, n=3) and MPTP-treated (C-H and K-P, n=3) monkeys. Scale bar corresponds to 0.5mm. The scale bar in panel A applies to all panels.

bodies outside of the probe or touching the probes exclusion surface were counted. On GABA-stained sections, GABA-positive cells were defined as individual, though sometimes overlapping, objects with easily visible borders and obvious staining of at least 8 μm in size. Because NeuN is specific to neurons but not specific to one type of neuron, all obviously stained individual objects were counted, and the number of GABA-positive cells was subtracted from the total NeuN-positive neuron count to find the remaining number of NeuN-positive thalamic projection neurons. The software also controlled the position of the x–y stage of the microscope, so that the entire brain region could be scanned by successively meandering between counting frames. The Stereo Investigator software calculated the estimated volume of the ROIs by multiplying the sum of the ROI areas by the distance between sections, which was then used to calculate an estimate of total cell counts for each region per hemisphere. Cavalieri's principle of volume estimation was used to achieve the most accurate possible estimate of the volume of each ROI in the final analysis (Gundersen & Osterby, 1981; Schmitz & Hof, 2005). This process utilized the same nuclear boundaries created for the cell counting process as explained. The density of cells in each region was calculated by dividing the estimated number of cells by the estimated volume calculated using Cavalieri's principle (neurons/mm^3). Because the four nuclei studied are not always mutually present in the same coronal plane, the average number of sections counted per monkey for each nucleus was 10 sections containing BGMT, 5 sections containing CbMT, and 7 sections containing CM and Pf. The number of sections studied and the distance between probes were adjusted during preliminary data collection to achieve a low coefficient of error (CE) value, which is a useful indicator of within-sample variation over numerous trials (Schmitz & Hof 2005; Slomianka & West 2005; Herculano-Houzel et al., 2015). Our sampling was designed to obtain a CE (Gundersen, m = 1) of <0.15.

***Statistical Analyses*:** The estimated mean density of interneurons and projection neurons between groups was statistically compared using Student t-tests (Table 3). Inter-individual differences between animals of the same group were tested using Levene's test (Table 3). Including more monkeys in our analyses may be necessary to account for large inter-individual variability and potential gender differences.

RESULTS

Count and Density of GABAergic interneurons and projection neurons in thalamic nuclei of control and MPTP-treated parkinsonian monkeys: Our stereological analysis showed that the total number of GABA-stained interneurons in the BGMT, CbMT, CM, and Pf nuclei of MPTP-treated parkinsonian monkeys was not significantly different compared to control monkeys (Figure 3, $p>0.05$). Likewise, no significant difference was observed in the number of projection neurons in the studied nuclei between control and parkinsonian monkeys (Figure 3, $p>0.05$). To account for differences in nuclei volume between individuals, the mean density of neurons in each nucleus was calculated by dividing the estimated number of cells by the estimated volume of the nucleus calculated using Cavalieri's method. No significant difference in density was observed for either interneurons or projection neurons between control and parkinsonian monkeys in any of the four nuclei studied ($p>0.05$).

BGMT					
Control	Int #	Pnu #	Int Den	Pnu Den	Vol
Mean	**113188.7**	**796528.61**	**1897.937**	**13425.83**	**5.92E+10**
CE	0.063333	0.0266667	N/A	N/A	0.014167
SE	22143.1	20210.372	310.3796	216.4695	1.070687
SD	38352.97	35005.391	537.5933	374.9362	2.622637
MPTP					
Mean	**134928.8**	**860346.35**	**2392.883**	**14791.28**	**5.84E+10**
CE	0.056667	0.0266667	N/A	N/A	0.014167
SE	17869.08	167459.68	372.5243	1091.743	7.198847
SD	30950.15	290048.67	645.231	1890.954	17.6335
Levene Significance (1,4)	N/A	N/A	0.625	0.105	0.033*
Student T-test p value	N/A	N/A	0.365081	0.287162	0.918189

CBMT					
Control	Int #	Pnu #	Int Den	Pnu Den	Vol
Mean	**142502.8**	**601288.6**	**2859.86**	**12291.61**	**4.9E+10**
CE	0.063333	0.03	N/A	N/A	0.026833
SE	31202.11	32490.67	510.1787	668.6077	1.167035
SD	54043.63	56275.49	883.6554	1158.063	2.858639
MPTP					
Mean	**130580.8**	**525829.5**	**3248.567**	**14038.67**	**3.92E+10**
CE	0.07	0.033333	N/A	N/A	0.026833
SE	40874.02	113207.8	415.6708	549.2219	6.299549
SD	70795.87	196081.7	719.963	951.2803	15.43068
Levene Significance (1,4)	N/A	N/A	0.749	0.617	0.144
Student T-test p value	N/A	N/A	0.5865	0.113613	0.157561

CM					
Control	Int #	Pnu #	Int Den	Pnu Den	Vol
Mean	**42077.58**	**433925.1**	**2444.691**	**25505.54**	**1.71E+10**
CE	0.063333	0.02	N/A	N/A	0.015333
SE	4856.436	30783.36	151.9392	1526.69	0.601751
SD	8411.593	53318.34	263.1663	2644.305	1.473983
MPTP					
Mean	**41968.01**	**391792.7**	**2793.125**	**25819.37**	**1.52E+10**
CE	0.063333	0.03	N/A	N/A	0.015333
SE	1363.122	50880.5	266.4013	2154.947	0.880142
SD	2360.997	88127.61	461.4206	3732.477	2.155899
Levene Significance (1,4)	N/A	N/A	0.235	0.498	0.6475
Student T-test p value	N/A	N/A	0.319353	0.911135	0.110364

Pf					
Control	Int #	Pnu #	Int Den	Pnu Den	Vol
Mean	**16331.36**	**201144**	**2943.931**	**38089.2**	**5.42E+09**
CE	0.063333	0.026667	N/A	N/A	0.017917
SE	1583.695	13768.79	268.2934	2595.009	0.123083
SD	2743.04	23848.24	464.6978	4494.688	0.301491
MPTP					
Mean	**16528.6**	**221301.8**	**3189.166**	**40677.83**	**5.4E+09**
CE	0.06	0.02	N/A	N/A	0.017917
SE	258.6757	23398.38	387.7835	2166.148	0.397179
SD	448.0395	40527.18	671.6607	3751.879	0.972885
Levene Significance (1,4)	N/A	N/A	0.425	0.817	0.1425
Student T-test p value	N/A	N/A	0.630475	0.486485	0.963519

Table 3: Summary of stereology data and statistics. BGMT: basal-ganglia receiving territory of the motor thalamus, CbMT: cerebellar-receiving territory of the motor thalamus, CM: centromedian nucleus of the thalamus, Pf: parafascicular nucleus of the thalamus, CE: coefficient of error, SE: standard error, SD: standard deviation, Int #: interneuron number, Pnu #: projection neuron number, Int Den: interneuron density, Pnu Den: projection neuron density, Vol: volume (μm^3)

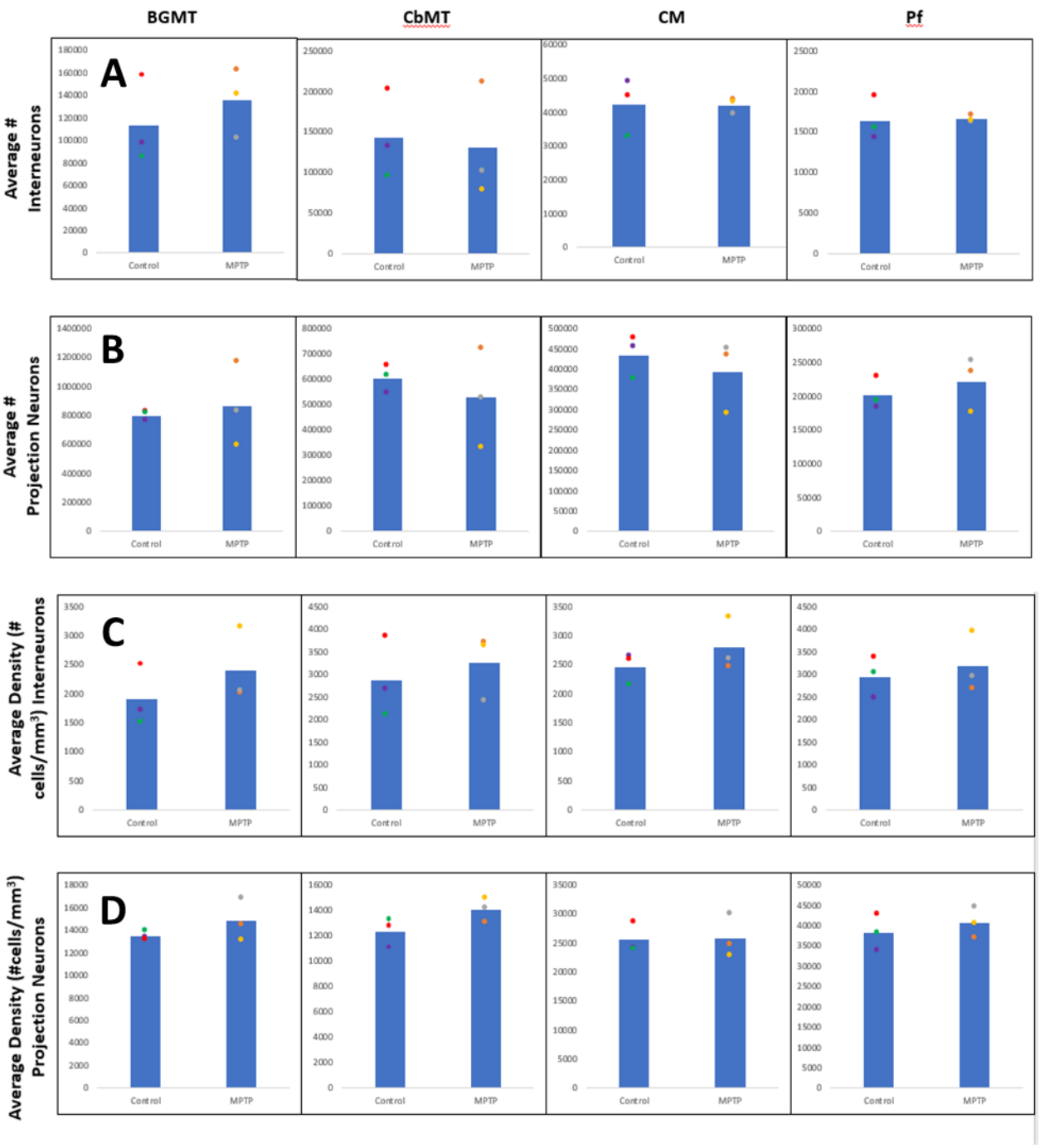

Figure 3: Average estimated number (A,B) and density (C,D) of interneurons (A,C) and projection neurons (B,D) in the BGMT, CbMT, CM, and Pf thalamic nuclei of 3 control and 3 MPTP-treated monkeys. Columns indicate means, dots represent pre-averaged estimated count or density from individual control or MPTP-treated monkeys.

Total volume of the BGMT, CbMT, CM and Pf nuclei in control and MPTP-treated parkinsonian monkeys: The Cavalieri's analysis revealed a no statistically significant change in volume in any of the BGMT, CbMT, CM, and Pf nuclei of the MPTP-treated monkeys compared with controls (Figure 4 $p>0.05$).

Relative Prevalence of Interneurons vs. Projection Neurons in Thalamic Nuclei: The relative prevalence of interneurons and projection neurons was calculated as a proportion of the total NeuN-positive neurons counted in each nucleus. Unique proportions of interneurons to projection neurons were observed in each of the 4 nuclei studied. Of the total neurons counted, interneurons represented 12-14% of neurons in the BGMT, 19-20% of neurons in the CbMT, 9-10% of neurons in the CM, and 7-8% of neurons in the Pf (Figure 5). The remaining neurons counted in each nucleus were identified as projection neurons. No difference was observed in the proportion of interneurons to projection neurons in each nucleus between control and MPTP-treated monkeys.

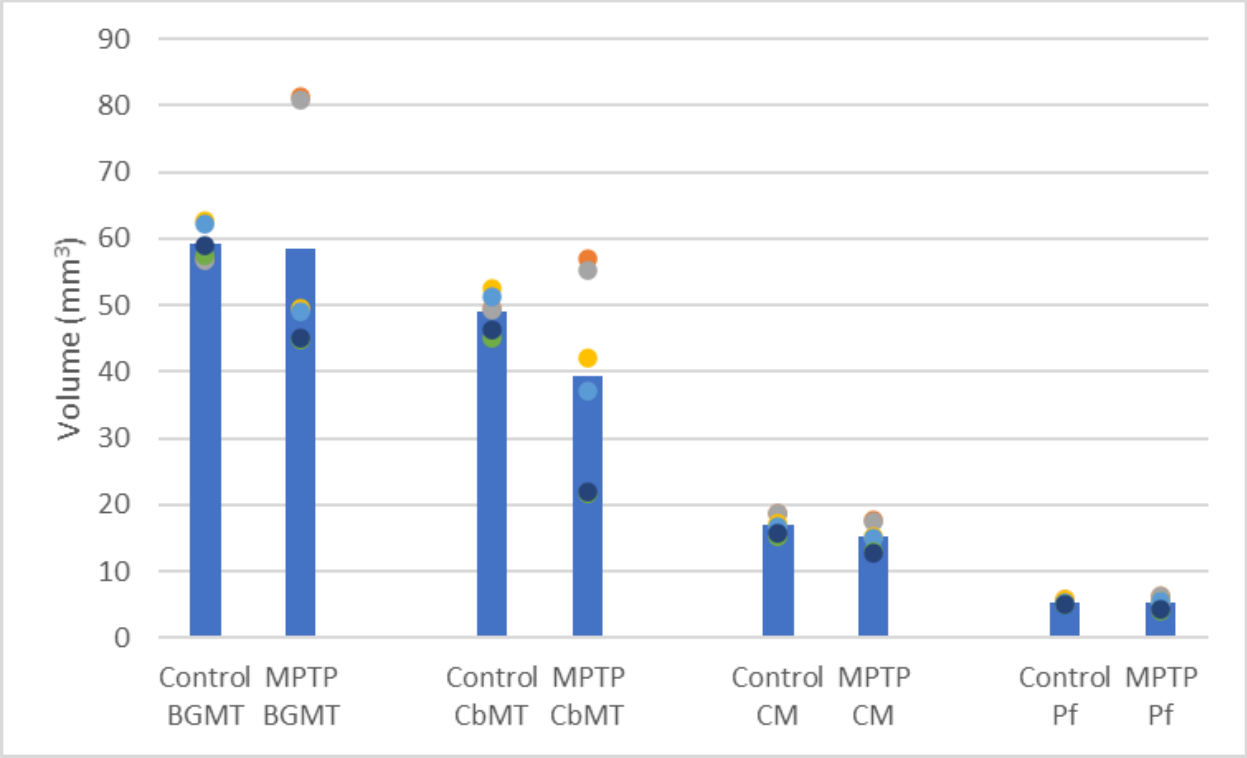

Figure 4: Average estimated volume of BGMT, CbMT, CM, and Pf thalamic nuclei in control (n=3) and MPTP-treated (n=3) monkeys. Volume was estimated using Cavalieri's principle. Columns indicate means, dots represent pre-averaged estimated volume from individual control or MPTP monkeys.

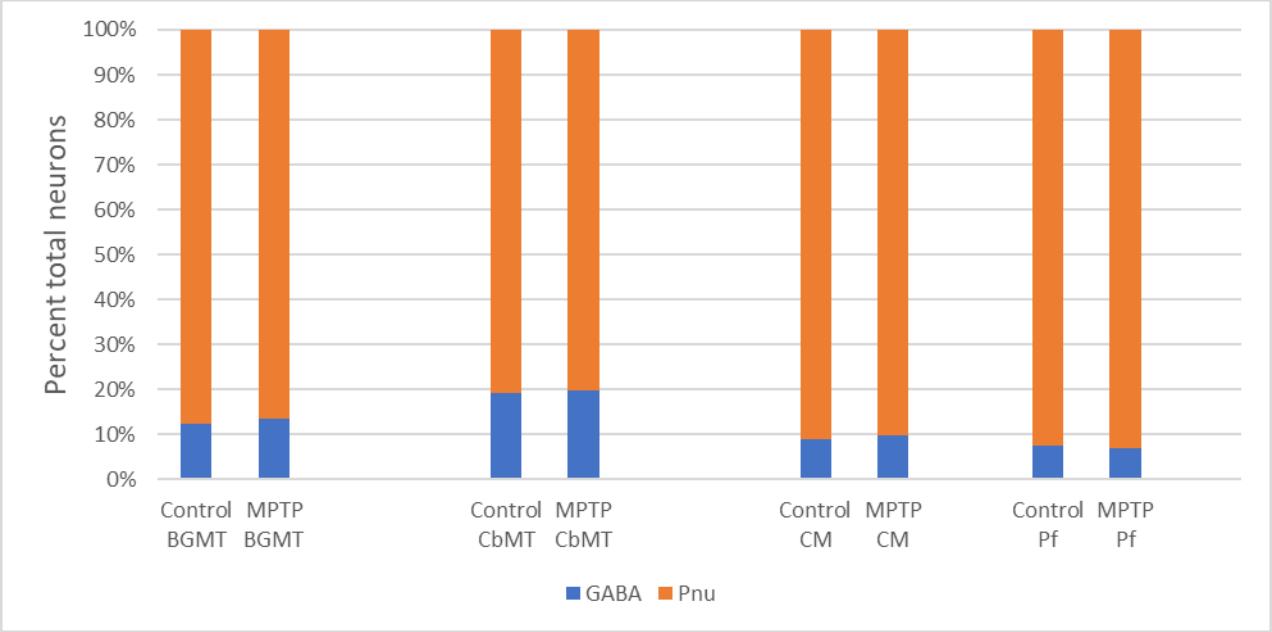

Figure 5: Average estimated percentages of interneurons and projection neurons in the BGMT, CbMT, CM, and Pf thalamic nuclei in individual control (n=3) and MPTP (n=3) monkeys

DISCUSSION

The findings of the present study provide the first stereological assessment of the abundance of GABAergic interneurons in the ventral motor and caudal intralaminar thalamic nuclei in control and parkinsonian monkeys. In line with previous studies, our data demonstrate that GABAergic interneurons account for ~10-20% of the total neuronal population in these nuclear groups, being most prevalent in the cerebellar-receiving region of the ventral motor thalamus. Our comparative analysis did not reveal any significant difference in the relative abundance of these interneurons between control and parkinsonian monkeys. In the following account, I will examine these findings in light of the previous literature on the anatomy and function of GABAergic interneurons in the mammalian thalamus, and relate our observations to the pathophysiology of the GABAergic basal ganglia-thalamic networks in parkinsonism.

Relative Prevalence of GABAergic Interneurons in the Primate Motor Thalamus: Our analysis indicated a heterogeneous distribution of GABAergic interneurons across thalamic nuclei. The lowest density of interneurons was observed in the BGMT (1897 cells/mm^3), with increasing densities observed in the CM (2444 cells/mm^3), the CBMT (2859 cells/mm^3), and the Pf (2943 cells/mm^3) nuclei. We observed a greater proportion of GABAergic interneurons in the CbMT (19-20% of total neurons) compared to the other thalamic nuclei studied (12-14% of BGMT neurons, 9-10% of CM neurons, and 7-8% of Pf neurons). These findings are consistent with previous observations that GABA-immunoreactive cells in the ventral thalamic nuclei of squirrel monkeys are less numerous than other cell types (Smith et al., 1987). However, previous studies indicate that interneurons account for as much as 30% of the BGMT and CbMT in primates (Smith et al., 1987; Arcelli et al., 1997; Jones 2007). The lesser percentage of interneurons composing ventral motor nuclei in primates reported in this study is likely due to

the use of different monkey species (cynomolgus macaque or squirrel monkey) but differences in methodologies such as staining technique or stereological cell counting could also contribute. The variation in relative abundance of interneurons between motor nuclei indicates that the contribution of interneurons to GABAergic regulation of CbMT and BGMT potentially differs in primates.

Due to a limited understanding of thalamic GABAergic interneuron functions in the motor thalamus, we can only speculate on the functional consequences of varied interneuron to projection neuron ratios across motor thalamic nuclei. One idea about the functional role of interneurons in thalamic information processing is that interneurons serve to rapidly modulate synaptic transmission between thalamic inputs and outputs. In the CbMT, which receives excitatory afferents from the cerebellum and cortex, dendritic release of GABA from interneurons could allow for rapid local inhibition of projection neurons in response to excitatory thalamic afferents (Cox, 2014). Previous research on sensory thalamic nuclei informs our hypotheses on the microcircuitry of the CbMT due to their similar driver-like excitatory inputs. Inhibitory interneurons in the sensory thalamus of rodents and primates have been observed in synaptic triads (Sherman, 2004; Jones, 2007; Cox, 2014). These triads are composed of a dendrodendritic synaptic connection between a thalamocortical projection neuron and a thalamic interneuron, both of which are targeted by the same corticothalamic projection neuron. In this synaptic complex, the interneuron may be providing immediate local inhibition of the post-synaptic thalamocortical projection neuron whenever the corticothalamic input fires, thereby reducing the responsiveness of the projection cell to its primary inputs (Sherman, 2004). Evidence suggests the presence of similar triads in the CbMT, but not BGMT, of the primate motor thalamus (Ilinsky & Kultas-ilinsky, 1990; Kultas-Ilinsky & Ilinsky, 1991). Additionally,

interneurons in the CbMT enter complex synaptic arrangements with the axons of projection neurons, as well as with cell bodies and dendrites of both interneurons and projection neurons (Kultas-Ilinsky & Ilinsky, 1991).

In contrast to CBMT, the BGMT lacks large excitatory afferents like those from the cerebellum, instead receiving large multisynaptic inhibitory afferents from the basal ganglia and small cortical glutamatergic modulation from cerebral cortex (Albaugh et al., 2020). The interneurons could still serve as an "inverter" to primary afferent signals, reducing their inhibitory GABA release onto projection neurons in response to shared inhibitory inputs from the basal ganglia. How thalamic cells in this region can be activated despite these prominent tonically active GABAergic inputs from basal ganglia output nuclei is unknown, but evidence suggests that inhibitory basal ganglia inputs can induce excitatory motor signals in the BGMT by increasing incidence of rebound burst firing (Kim et al., 2017). Optogenetic stimulation of cortical interneurons in studies of epilepsy indicate that interneurons can provide inhibitory or excitatory effects depending on context by raising the reversal potential of GABA receptors in postsynaptic cells (Ye & Kaszuba, 2017). However, there is no evidence that such a phenomenon occurs at basal ganglia synapses in the thalamus. At present, the reason more GABAergic interneurons are present in the CbMT than the BGMT of primates is unknown. Speculatively, the BGMT could have less need for intranuclear GABA modulation due to the presence of prominent GABAergic inputs from the basal ganglia, whereas GABAergic modulation by interneurons in the CbMT may be a crucial component of synaptic triads composed of otherwise excitatory neurotransmission.

Species Differences in the Prevalence of Thalamic GABAergic Interneurons: Our study provides the first detailed stereological assessment of the total number of GABAergic

interneurons in the nonhuman primate motor thalamus. A number of previous studies reported the general ratio of cellular composition of the motor thalamus in primates (Smith et al., 1987; Kultas-Ilinsky & Ilinsky, 1991; Jones 2007), but no stereological cell counting has been performed to estimate the total number of interneurons or projection neurons. Because GABAergic interneurons are absent from the motor thalamus of rodents, their prevalence in the BGMT and CbMT can only be examined in primate species (Ilinsky & Kultas-Ilinsky, 1990; Kultas-Ilinksy & Ilinsky, 1991; Jones 2007). We expand upon previous work from Dr. Smith's lab (Smith et al., 1987) by quantifying the relative prevalence of interneurons and projection neurons composing various thalamic nuclei. Quantitative studies of GABAergic interneurons have yet to be performed in the human motor thalamus. A retrospective view on thalamic interneurons in various species can inform our expectations about what may be uncovered in future studies.

Work by Arcelli and coauthors (1997) points out that the visual thalamus of many different species (bat, mouse, rat, guinea pig, rabbit, cat, monkey, and human) contains GABAergic interneurons, but the presence and number of interneurons in other nuclei varied by species. They observe that the presence of GABAergic neurons that enter complex synaptic arrangements in the dorsal thalamus is progressively greater in species with more complex behavior, but that the cellular density of GABAergic neurons in the reticular thalamus decreased with increasing behavior complexity. A clear lack of association was found between the sensorimotor survival needs of specific animals and the presence or absence of GABAergic interneurons in other thalamic nuclei associated with those sensorimotor tasks. Based on these results, they speculate that a greater density of GABAergic interneurons is likely associated with

increased complexity of thalamic local information processing, but not directly related to the ability to perform specific sensorimotor tasks.

Data from our lab (Smith et al., 1987) has demonstrated the presence of GABA-immunoreactive neurons in varying number in all thalamic nuclei of the squirrel monkey. GABAergic neurons in the thalamus of the squirrel monkey appear more numerous and more widely distributed than in any non-primates investigated thus far. For example, GAD-positive or GABA-positive neurons in the thalamus of rats, opossums, and rabbits are mostly concentrated within a few sensory thalamic nuclei, while in the cat these cells are also abundant throughout the dorsal thalamic nuclei and absent from the CM and Pf nuclei (Houser et al., 1980; Penny et al., 1983; Ottersen & Storm-Mathisen, 1984; Penny et al., 1984; Gabbott et al., 1985; Mugnani, 1985). These data further support the hypothesis that there has been an increase in the number of GABAergic neurons in the thalamus during the course of the mammalian phylogenetic evolution, which most likely reflects increasing complexity of local information processing in the thalamus (Penny et al., 1984; Arcelli et al., 1997).

Prevalence of GABAergic Interneurons and Projection Neurons in Control vs Parkinsonian Monkeys: Our analysis indicated no statistically significant difference between control and MPTP-treated primates in the number or density of interneurons and projection neurons within the BGMT, CbMT, CM, and Pf nuclei. These observations are partly different from those previously reported in chronically MPTP-treated monkeys (Villalba et al., 2014) and PD patients (Henderson et al., 2000a, b; Halliday et al., 2005; Brooks & Halliday, 2009; Halliday, 2009) which showed that the CM/Pf complex undergoes severe neuronal degeneration (40-60% cell loss) in parkinsonian monkeys and PD patients. We also did not find a statistically significant difference in the volume of the CM/Pf nuclei between control and parkinsonian

monkeys, which is in contrast to that observed in a previous monkey study which showed a significant decrease of volume (~35%) of the CM and Pf nuclei in chronically MPTP-treated monkeys (Villalba et al., 2014, Villalba et al., 2019). Careful consideration and implementation of stereological counting methods and acceptably low within sample variation (CE < 0.15) lead us to believe that our unexpected findings are not due to methodological error.

An alternative explanation for the difference between our findings and those of previous studies is the consideration of staining methods and their limitations. Whereas our study utilized NeuN to label all neurons in the CM/Pf, the other monkey studies used Nissl staining procedures. The Nissl staining method may have led to a larger estimate of the total neuronal number because Nissl labels nuclei regardless of their origin (García-Cabezas et al., 2016), including those of glial cells, which could have resulted in accidental counting of non-neuronal cell bodies during the sampling of CM/Pf neurons. The potential loss of glial cells induced by MPTP could explain why our findings on the total number of neurons in the CM/Pf of control monkeys were significantly lower than those reported by Villalba et al. (2014) while we estimated a similar number of cells in the CM/Pf of MPTP-treated monkeys. If the Nissl stain is less effective due to MPTP treatment (for example, due to the dissolution of Nissl bodies observed in stressed cells called chromatolysis (Hanz & Fainzilber, 2006)), then it is possible that the results acquired using Nissl could have undercounted CM/Pf neurons only in the MPTP group. Notably, the average number of neurons in the control CM/Pf estimated in our NeuN-based study and the Nissl-based study of Villalba et al (2014) were different, whereas the average number of neurons estimated in the MPTP-treated CM/Pf are similar between studies. Therefore, either A) Nissl is less effective at counting neuronal cell bodies in MPTP-treated monkeys (which brings interpretations of cell loss from Villabla 2014/2019 into question) and NeuN is less effective at

counting neurons in both control and MPTP-treated monkeys (which would explain why our control results were different but MPTP results were the same) or B) the Nissl count included glial cells that were lost after MPTP treatment or C) the Nissl count was accurate and an alternative explanation is needed to explain our NeuN findings.

The NeuN staining method may result in a smaller estimate of the total neuronal number for various reasons. Even when great care is taken with immunostaining methods, the quality of antibody penetration into target cells is likely less than perfect (Melvin & Sutherland, 2010). Some neurons do not express NeuN or express it at undetectably low levels, such as cerebellar Purkinje cells, olfactory bulb mitral cells, gamma motor neurons in the spinal cord, and retinal photoreceptor cells (Mullen, Buck, & Smith, 1992; Gusel'nikova & Korzhevskiy, 2015). Evidence suggests that neurons sometimes downregulate NeuN expression without exhibiting signs of cell death in response to stress caused by physical damage or diseased states, so neurons that were non-immunoreactive to NeuN may have been excluded from the estimate of the MPTP group (Cannon & Greenamyre, 2009; Alvarez et al., 2011; Wootz et al., 2013; Gusel'nikova & Korzhevskiy, 2015). Even if a sub-population of neurons in our regions of interest shared one of the above qualities that excluded them from NeuN staining, it is unlikely that the proportion of neurons not stained with NeuN would be identical within interneuron and projection neuron populations. Because the ratio of projection neurons to interneurons we found was unchanged between control and MPTP groups, these explanations for our findings are unlikely.

Crucially, we would have expected to estimate fewer total neurons in both control and MPTP groups in the CM/Pf relative to the previous study if any of the above explanations were true, but the average number of neurons estimated in the CM of MPTP-treated monkeys in our study is actually slightly higher than the average number of CM neurons reported in the previous

study (Villalba et al., 2014), and the average number of neurons estimated in the MPTP-treated Pf is similar to the average number of Pf neurons previously reported (Villalba et al., 2014). Considering the specific relationship of these different results, we must consider the possibility of whether MPTP-resistant CM/Pf neurons are more effectively stained with NeuN than MPTP-vulnerable neurons. Such an explanation would also clarify why a similar average number of neurons within nuclei was found between control and MPTP groups in this study; both counts of NeuN-stained control and MPTP-treated populations would have labeled MPTP-resistant neurons, but the MPTP-vulnerable neurons would be unnoticed in control sections and unnoticed or missing in MPTP-treated sections. A study by Cannon and Greenamyre (2009) strongly cautions against NeuN as a reliable marker for ventral midbrain neurons, specifically the MPTP-vulnerable substantia nigra compacta (SNc), for this reason. They provide evidence that dopaminergic neurons in the dorsal SNc, which are not as sensitive to degeneration, are well stained with NeuN, while more vulnerable dopaminergic neurons in the ventral SNc are not well labeled with NeuN (Varastet et al., 1994; Cannon & Greenamyre, 2009). Like the ventral SNc, the CM/Pf is sensitive to MPTP treatment, so a similar occurrence in the control and MPTP-treated CM/Pf could lead to NeuN exclusively labeling MPTP-resistant neurons, leading to results like those observed in this study.

Follow-up experiments will be necessary to narrow down the list of alternative explanations. First, an unbiased controlled recount of the sections used here by an independent investigator will confirm the reliability of the results. Additional adjacent tissue sections can be stained with Nissl for a direct within-subject comparison, which will be especially enlightening in the control CM/Pf where the total number of neurons found thus far with NeuN and Nissl were drastically different. If necessary, simultaneous fluorescent microscopy of NeuN and Nissl

can effectively display overlap between the two markers in a single section. Fluorescent microscopy using NeuN in conjunction with positive controls for interneurons (GABA), dopaminergic neurons (tyrosine hydroxylase), glial cells (GFAP), and nuclear DNA (Hoechst stain) will reveal whether NeuN staining is variable within cell types in the CM/Pf region.

Does the lack of change in total number of GABAergic interneurons rule out the possibility of thalamic GABAergic interneurons dysfunction in PD?: Although no difference in the number of thalamic GABAergic interneurons between control and MPTP-treated primates was observed in this study, other mechanisms we did not examine could involve interneurons in the pathology of PD. Plastic changes to the synaptic signaling variables of interneurons such as the efficacy of their GABAergic synapses, the prevalence of various inputs onto their vesicle-containing dendrites, and the density of dendrodendritic synapses that form along individual dendritic branches could all contribute to dysfunctional or compensatory changes to thalamic information processing in PD. Future studies utilizing electron microscopy (EM) and 3-dimensional reconstruction of EM-resolution images of thalamic interneurons will be performed to quantify these characteristics of their synaptic inputs and outputs.

We propose that the increase of thalamic inhibition observed in the MPTP-treated primate model of PD, caused in part by increased GABAergic innervation from the basal ganglia, could also be contributed to by alterations in interneurons signaling or connectivity such as: 1) an increased prevalence and efficacy of dendro-dendritic synapses between GABAergic thalamic interneurons and thalamocortical projection neurons and 2) an increase glutamatergic drive of thalamic interneurons. A recent study from our laboratory (Swain et al., 2020) used electron microscopy to assess structural changes in the microcircuitry of the BGMT and CM nuclei in MPTP-treated monkeys. The findings of this study indicate that the density of vGluT1-

positive corticothalamic terminals is reduced, while the cross-sectional area of the remaining corticothalamic terminals is increased in the BGMT of parkinsonian monkeys (Swain et al., 2020). Notably, similar plastic ultrastructural remodeling occurs in the striatum of MPTP-treated monkeys. Despite a significant reduction of glutamatergic cortical afferents onto striatofugal neurons and a loss of striatal dendritic spines, the overall glutamatergic transmission to the striatum is increased in the parkinsonian state, possibly due in part to a significant increase in the volume, size, and efficacy of synapses of remaining corticostriatal terminals (Calabresi et al., 1993; Day et al., 2006; Raju et al., 2008; Villalba & Smith, 2010; Villalba & Smith, 2011; Villalba et al., 2015). Based on these observations, increased synaptic drive onto thalamic inhibitory interneurons by the larger remaining corticothalamic terminals could potentially contribute to increased thalamic inhibition and altered functional communication between the cerebral cortex and thalamus in animal models of parkinsonism and PD patients (Mitchell et al., 1989; Schneider & Rothblat, 1996; Zirh et al., 1998; Molnar et al., 2005).

A recent study assessed potential changes in the prevalence of cortical and cerebellar glutamatergic inputs to GABAergic interneurons in the BGMT and CbMT of control and MPTP-treated parkinsonian monkeys, finding that interneurons are a major target of both types of glutamatergic inputs and observing no noteworthy difference in the extent of glutamatergic innervation of thalamic GABAergic interneurons between control and parkinsonian monkeys (Albaugh et al., 2020). These results suggest that the decreased density of vGluT1-positive corticothalamic terminals are unlikely to result in proportionally increased targeting of glutamatergic inputs onto interneurons by the remaining terminals.

Further results from our laboratory (Swain et al., 2020) indicated that a larger proportion of GPi terminals tend to contact interneurons in the BGMT and CM of parkinsonian monkeys

relative to controls. We anticipate that such a change would result in the inhibition of GABAergic interneurons and subsequent disinhibition of their thalamocortical or thalamostriatal targets. It is unclear at this stage whether this structural plasticity contributes to dysfunctional thalamic signaling or exists as an adaptive mechanism to compensate for increased inhibitory signaling onto thalamocortical cells. Upcoming experiments will need to determine how to parse the electrophysiological and behavioral consequences of the anatomical plastic changes being investigated by present research.

Future Studies: Future studies of thalamic interneuron morphology, synaptology, microcircuitry, and plastic changes in PD models must be considered to further our understanding of these cells in the primate brain.

Because thalamic GABAergic interneurons in the motor thalamus are only found in primates, our understanding of the physiological properties and function of these cells is limited by of the lack of tools available to selectively interrogate these neurons in the monkey brain. For instance, although GABAergic interneurons and thalamocortical projection neurons can be easily distinguished by their morphology in anatomical studies, there is no approach to distinguish between the two cell types during in vivo electrophysiological studies of the primate motor thalamus (Galvan et al., 2012; Galvan et al., 2016). In vitro slice electrophysiology, an approach which is commonly used to study the synaptic properties of specific neurons in the mouse brain is not as feasible in primates due to the relatively limited number of monkeys one could use. One possible solution to allow for specific recording of GABAergic interneurons using in vivo electrophysiological approaches in the primate motor thalamus is the use of optogenetic activation of GFP-transduced GABAergic interneurons. A viral vector could be injected into the monkey thalamus which contains green fluorescent protein (GFP) to visualize gene expression in

transduced cells, as well as a ChR2, a sequence for the light-sensitive membrane channel channelrhodopsin-2, under the control of a GAD promoter, which will result in selective ChR2 expression in GAD-containing GABAergic interneurons. This way, selective activation of interneurons can be achieved by directly stimulating ChR2-containing interneurons with light. One could also record specifically from the ChR2-positive thalamic interneurons, while stimulating basal ganglia outputs, an approach called Opto Tagging (Lima et al., 2009). Although the efficient use of a GAD promoter to transduce gene expression in GABAergic cells in the primate brain remains to be established, promoters specific for other neuronal populations have been used to preferentially target excitatory neurons, and new methods are being explored to target inhibitory neurons in primates (Dimidschstein et al., 2016; Galvan et al., 2016; Galvan et al., 2017; Galvan et al., 2018). Together, these approaches would offer the possibility of assessing the downstream effects of thalamic GABA interneurons on thalamocortical cells at rest or during the performance of behavioral tasks in control and parkinsonian conditions. The possibility of collecting electrophysiological responses of GABA interneurons to stimulation of extrinsic basal ganglia and cortical afferents in control and parkinsonian states could also be a major step forward in understanding the physiology and pathophysiology of motor thalamic GABA interneurons in normal and diseased states.

We plan to follow this study with a direct investigation of the synaptic microcircuitry of interneurons in the control and parkinsonian primate motor thalamus. Using three-dimensional electron microscopic reconstruction, we can quantify the relative prevalence, density, and efficacy of cortical, nigral, reticular, and interneuron synaptic inputs onto GABAergic interneuron dendrites in the BGMT, CbMT, CM, and Pf in both control and MPTP-treated monkeys. This information will be crucial to understanding the role of interneurons in

modulating and processing signals coming into the thalamus, and how that role could be disrupted by plastic changes that occur to the synaptic network in PD.

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