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Feline subthalamic nucleus neurons contain glutamate-like but not GABA-like or glycine-like immunoreactivity

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The identity of the neurotransmitter of subthalamic nucleus neurons has not been definitively established. GABA, glycine, and glutamate have all been hypothesized to be the neurotransmitter of these neurons. Immunohistochemistry with 3 well characterized antisera against glutamate, GABA, and glycine were used to study feline subthalamic nucleus neurons. These neurons were found to contain intense glutamate-like but not GABA- or glycine-like immunoreactivity. The surrounding neuropil contained glutamate-like and GABA-like but not glycine-like immunoreactivity. These results support the hypothesis that subthalamic nucleus neurons are glutamatergic.

The subthalamic nucleus (STN) plays an important role in the control of motor behavior. Lesions of the STN in both human and non-human primates and pharmacological inhibition of its activity in non-human primates give rise to a profoundly disabling hyperkinetic movement disorder termed hemiballism[2]. Virtually all STN neurons are projection neurons[30]. STN neurons are uniquely positioned to modulate the output of the basal ganglia because they receive afferents from lateral globus pallidus, sensorimotor cortex, and centre-median/parafascicular complex and project to entopeduncular nucleus (or its primate homologue, medial globus pallidus) and substantia nigra pars reticulata (SNr)[17,27]. While the importance of the STN in motor function has been recognized for decades[10], the identity of the neurotransmitter of STN neurons has not been defined. Physiological studies with extracellular recording techniques showed that STN stimulation produced excitation of neurons in the SNr[6,7], and inhibition of neurons in the entopeduncular nucleus and globus pallidus[12,18,24,32]. These results appear to be paradoxical as physiological and anatomical studies have established that STN projection neurons send branched axons to both the pallidum and the substantia nigra[3,30]. The observation of inhibition of pallidal neurons by STN stimulation led to suggestions that γ-aminobutyric acid (GABA)[24] or glycine (Gly)[31] is the neurotransmitter of STN neurons. This inhibitory response could be abolished by the GABA antagonists picrotoxin and bicuculline[24], indicating that GABA might be responsible for the inhibition of pallidal neurons. In addition, the finding that [^{3}H]GABA was transported into STN neurons when injected into the pallidal complex of cats[15] and the globus pallidus[28] of rats suggested that GABA was indeed the neurotransmitter of STN neurons.

The conclusion that GABA is the neurotransmitter of STN neurons has been undermined by a series of recent studies. Immunohistochemical experiments with antisera directed against glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, and GABA itself, have failed to reveal evidence of GABA[25,26]- or GAD[13]-immunoreactive STN neurons. An in situ hybridization study with a ribonucleotide probe for GAD failed to demonstrate that STN neurons contain GAD mRNA[1]. A recent study of STN efferent projections in rat showed that subthalamopallidal terminals contain round vesicles

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and form asymmetrical synapses, whereas previous studies had shown that GABAergic boutons within the pallidum contain pleomorphic vesicles and form symmetric synapses[11]. No previous study has used immunocytochemical techniques to test the hypothesis that Gly is the neurotransmitter of the STN.

Nakanishi et al. have recently used intracellular recording techniques to demonstrate that STN stimulation gives rise to monosynaptic excitatory postsynaptic potentials in SNr neurons, suggesting that the neurotransmitter of STN is excitatory[14]. Extracellular recordings in the medial globus pallidus (MGP) of non-human primates after STN lesions have revealed a decrease in unit activity, supporting the hypothesis that the STN exerts an excitatory influence on MGP neurons[5]. A hyperkinetic movement disorder identical to that produced by inhibition of STN function in rhesus monkeys[2] has been induced in rhesus monkeys by injection of the excitatory amino acid antagonist kynurenic acid into the MGP[21]. This latter observation suggests that the neurotransmitter of STN neurons may be an excitatory amino acid. The hypothesis that an excitatory amino acid is the neurotransmitter of STN neurons is buttressed by reports that STN neurons in rodents and squirrel monkeys contain glutamate-like immunoreactivity[16,25].

To explore further the identity of the STN neuron neurotransmitter we undertook an immunohistochemical study of feline STN neurons using antisera directed against the putative excitatory amino acid neurotransmitter glutamate (Glu) and the inhibitory amino acid neurotransmitters GABA and Gly.

Two adult cats were deeply anesthetized with pentobarbital and perfused transcardially with 1.0 liter of 0.9% NaCl solution followed by 3.5 liters of 4% paraformaldehyde-0.2% glutaraldehyde-0.1 M sodium phosphate buffer (pH 7.4). After extraction from the cranial vault, the brains were postfixed overnight in the same fixative at 4 °C. The brains were then serially immersed in 10, 20 and 30% sucrose in 0.1 M phosphate buffer. One of these animals had received an ibotenic acid lesion of the right medial geniculate nucleus while the other had received a lesion of the right STN. These lesions were placed as part of a study on the effects of STN ablation on single unit activity in the pallidum and did not affect the present observations. Forty-μm thick frozen sections were cut on a sliding microtome and stored in 0.1 M phosphate buffer plus 0.01% NaAzide until the time of assay. Three well charac-

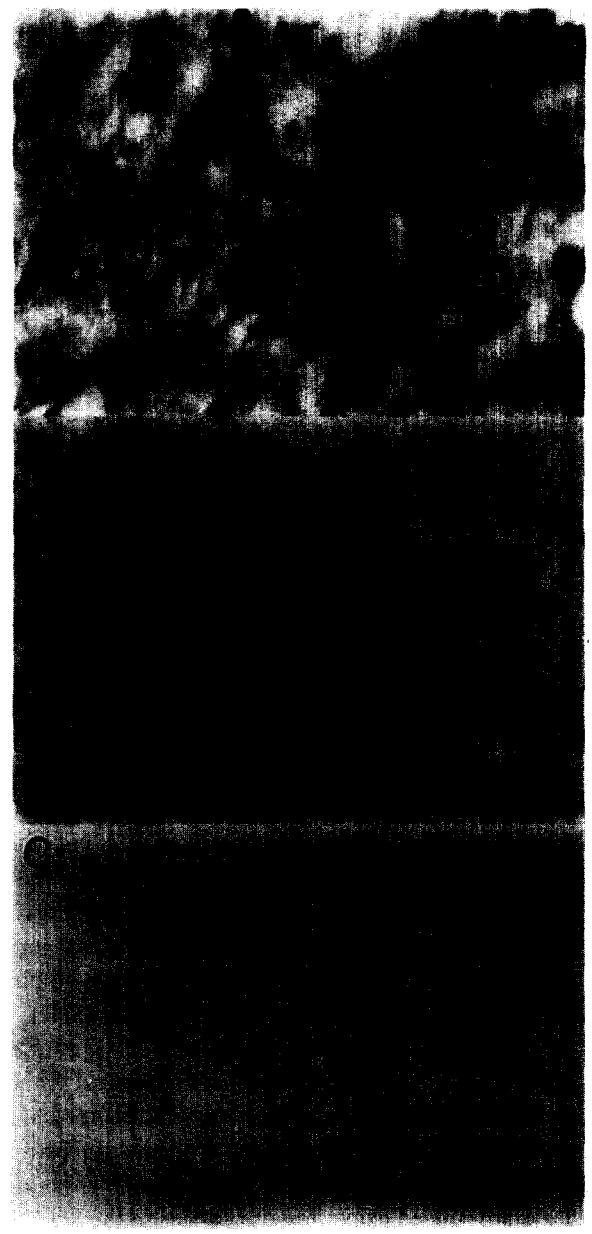

Fig. 1. Feline subthalamic nucleus neurons stained with anti-glutamate (A), anti-GABA (B), and anti-glycine (C) antisera. With the anti-glutamate antisera, neurons are robustly stained and the surrounding neuropil is stained as well. The anti-GABA antiserum produces weaker cell staining that outlines some STN neuronal perikarya (arrow in B). Neuropil staining is also evident with the anti-GABA staining. No glycine-like immunoreactivity is present in either neurons or neuropil. Bar = 80 μm.

terized polyclonal antisera directed against glutamate[9], GABA[8], and Gly[19] were utilized. The concentrations of primary antisera were 1/1000 for the glutamate antiserum, 1/1000 for the GABA antiserum, and 10.4 μg/ml for the Gly antiserum. Free floating sections were processed with the peroxidase–antiperoxidase technique as previously described[20].

Examination of sections exposed to the Glu antiserum revealed an abundance of intensely immunoreactive STN neurons (Fig. 1A). While quantitation was not performed, it appeared that virtually all STN neurons contained Glu-like immunoreactivity. The Glu-like immunoreactivity appeared to fill the cytoplasm and proximal neuronal processes. In sections exposed to the GABA or Gly antiserum no STN neurons contained GABA-like or Gly-like immunoreactivity (Fig. 1B and C). As previously reported by Smith and Parent in monkeys[25], the STN neuropil was also positively stained by the Glu and GABA antisera. The STN contained large numbers of Glu-like immunoreactive fibers and GABA-like immunoreactive puncta that seemed to surround STN neurons. No Gly-like immunoreactivity of any kind was present in the STN. Methodological control sections were run by omitting the primary antisera or substituting non-immune rabbit serum for primary antiserum. No staining was seen under these control conditions. The Gly antiserum positively stained neurons and neuropil in sections of cervical spinal cord. The GABA antiserum stained neurons and neuropil in the molecular layer of the cerebellum.

Our finding of Glu-like and GABA-like immunoreactive STN neuropil is consistent with evidence that afferents to the STN use these substances as neurotransmitters. While the Glu-like immunoreactive fibers could be recurrent collaterals, recent physiological data suggest that the neurotransmitter of the cortico-STN projection is an excitatory amino acid[23]. The presence of GABA-like immunoreactive puncta is consistent with biochemical and neurochemical evidence that GABA is the neurotransmitter of the pallidal-STN projection[4,22]. Recently, Takada and Hattori described retrograde transport of [^{3}H]-Gly by rat pallido-STN projection neurons and suggested that Gly is the neurotransmitter of the pallidal-STN projection[28]. Their conclusion is not supported by our results and a previous study in rats that demonstrated a paucity of Gly-like immunoreactive neurons and fibers in the forebrain[29].

As with previous immunohistochemical studies in rodents and non-human primates, our findings support the hypothesis that the neurotransmitter of STN neurons is an excitatory amino acid.

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