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SUBCORTICAL EXCITATORY INPUTS TO NIGRAL DOPAMINERGIC AND NON-DOPAMINERGIC NEURONS: A LIGHT AND ELECTRON MICROSCOPIC STUDY

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Midbrain dopaminergic neurons are involved in the symptomotology of motor disorders such as Parkinsonism as well as psychiatric disorders such as schizophrenia. The normal functioning of substantia nigra (SN) dopaminergic neurons is greatly influenced by their afferent inputs as evidenced by significant differences in the physiological characteristics of *in vivo* vs. *in vitro* preparations. Although the sources and neurotransmitters of many afferents to the SN are known, because dopaminergic and non-dopaminergic dendrites co-mingle in pars reticulata, the precise postsynaptic targets, particularly those of the presumed excitatory inputs, remain to be determined. In the present study inputs from the subthalamic (STN) and the pedunculopontine (PPN) nuclei (both presumed excitatory) to the SN were investigated by combined anterograde tracing and tyrosine hydroxylase (TH) immunocytochemistry at the light and electron microscopic levels.

The anterograde tracers biocytin or PHA-L were iontophoretically injected into either the STN or the PPN. Following appropriate survival times the animals were perfused transcardially with a mixture of 4% paraformaldehyde and 0.2% glutaraldehyde. Cryoprotected, free-floating brain sections were freeze-thawed and immediately processed for the visualization of the tracer with standard Vector ABC reagents and 3,3'-diaminobenzidine (DAB) or nickel-enhanced DAB. Sections containing the SN were incubated in TH antibody (Eugene Tech.), followed by sequential incubations in a bridging antibody and peroxidase anti-peroxidase solutions. The reaction product was visualized with DAB or benzidine dihydrochloride. Alternate sections were examined at the light microscopic level and the remainder were further processed for electron microscopic evaluation. Sections were post-fixed in 1% osmium tetroxide, dehydrated in an ascending series of ethanols (with 1% uranyl acetate in 70% ethanol), and propylene oxide, and embedded in resin (Durcupan ACM) between liquid release-coated glass slides and cover slips. After curing at 60° C, slides were examined and photographed in the light microscope, the slides and cover slips removed, and areas of interest trimmed out. Ultrathin sections were collected on formvar coated grids, counterstained with 0.3% lead citrate, and examined on a Phillips CM-10 electron microscope. All measurements were done with the aid of MacMeasure (NIH) and a MacTablet (Summagraphics) digitizing pad .

In the light microscope, both the PPN and STN were seen to give rise to numerous fine varicose axons in the SN. The PPN input appeared densest in the pars compacta while the STN input was densest in the pars reticulata. Many of the axonal varicosities seemed to be associated with TH positive (TH^+) dendrites and perikarya (Fig. 1, 4). At the electron microscopic level, the terminal boutons of PPN and STN origin had an average area of 0.53 ± 0.07 μm^2 (SEM) and 0.43 ± 0.06 μm^2, respectively. Both terminals contained small round vesicles, and 1-3 mitochondria, and formed asymmetric synapses mostly onto small dendrites approximately 1 μm in diameter. Most of the terminals contained one active zone, but occasionally PPN terminals synapsed with more than one element. Although terminals of PPN and STN origin were quantitatively similar, PPN terminals were less tightly packed with vesicles than STN terminals, and unlike the latter, they contained some large dense core vesicles in addition to the more numerous small round vesicles. PPN terminals were observed to form synapses with both TH^+ (Fig. 2) and TH- dendrites (Fig. 3), consistent with the light microscopy. In contrast, even though at the level of the light microscope some STN inputs seem to be in close apposition to TH^+ dendrites, electron microscopy revealed that the overwhelming majority of these same terminals formed synaptic contacts with TH^- dendrites (Fig. 5) immediately adjacent to the TH^+ dendrites (Fig. 6). These data suggest that the PPN exerts a monosynaptic excitatory influence on dopaminergic neurons, whereas the STN influences on dopaminergic neurons are largely polysynaptic.