Journal of Chemical Neuroanatomy 37 (2009) 33–45

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Cholinergic innervation and thalamic input in rat nucleus accumbens

Michael Ligorio [a,b], Laurent Descarries [c,d], Richard A. Warren [a,b,*]

[a] Centre de recherche Fernand-Séguin, Université de Montréal, Montreal, Que., Canada
[b] Department of Psychiatry, Université de Montréal, Montreal, Que., Canada
[c] Departments of Pathology & Cell Biology and of Physiology, Université de Montréal, Montreal, Que., Canada
[d] Groupe de recherche sur le système nerveux central, Université de Montréal, Montreal, Que., Canada

ARTICLE INFO

Article history:
Received 29 May 2008
Received in revised form 8 July 2008
Accepted 9 August 2008
Available online 19 August 2008

Keywords:
Acetylcholine
Choline acetyltransferase
Phaseolus vulgaris-leucoagglutinin
Thalamus
Cholinergic interneurons
Midline thalamic nuclei
Paraventricular nucleus

ABSTRACT

Cholinergic interneurons are the only known source of acetylcholine in the rat nucleus accumbens (nAcb); yet there is little anatomical data about their mode of innervation and the origin of their excitatory drive. We characterized the cholinergic and thalamic innervations of nAcb with choline acetyltransferase (ChAT) immunocytochemistry and anterograde transport of Phaseolus vulgaris-leucoagglutinin (PHA-L) from the midline/intralaminar/paraventricular thalamic nuclei. The use of a monoclonal ChAT antiserum against whole rat ChAT protein allowed for an optimal visualization of the small dendritic branches and fine varicose axons of cholinergic interneurons. PHA-L-labeled thalamic afferents were heterogeneously distributed throughout the core and shell regions of nAcb, overlapping regionally with cholinergic somata and dendrites. At the ultrastructural level, several hundred single-section profiles of PHA-L and ChAT-labeled axon terminals were analyzed for morphology, synaptic frequency, and the nature of their synaptic targets. The cholinergic profiles were small and apposed to various neuronal elements, but rarely exhibited a synaptic membrane specialization (5% in single ultrathin sections). Stereological extrapolation indicated that less than 15% of these cholinergic varicosities were synaptic. The PHA-L-labeled profiles were comparatively large and often synaptic (37% in single ultrathin sections), making asymmetrical contacts primarily with dendritic spines (>90%). Stereological extrapolation indicated that all PHA-L-labeled terminals were synaptic. In double-labeled material, some PHA-L-labeled terminals were directly apposed to ChAT-labeled somata or dendrites, but synapses were never seen between the two types of elements. These observations demonstrate that the cholinergic innervation of rat nAcb is largely asynaptic. They confirm that the afferents from midline/intralaminar/paraventricular thalamic nuclei to rat nAcb synapse mostly on dendritic spines, presumably of medium spiny neurons, and suggest that the excitatory drive of nAcb cholinergic interneurons from thalamus is indirect, either via substance P release from recurrent collaterals of medium spiny neurons and/or by extrasynaptic diffusion of glutamate.

1. Introduction

The nucleus accumbens (nAcb) is the largest part of the ventral striatum. It is a point of convergence for projections from several limbic regions: the medial prefrontal cortex, the hippocampus, the amygdala, and several thalamic nuclei (Groenewegen et al., 1980, 1982, 1987; Newman and Winans, 1980; Krayniak et al., 1981; Kelley and Domesick, 1982; Kelley et al., 1982; Kelley and Stinus, 1984; Jayaraman, 1985; Jones, 2007). nAcb also receives a dense dopamine innervation from the ventral tegmental area (Fallon and Moore, 1978; Sesack and Pickel, 1990) and therefore may play a role in behavioral reinforcement, including addiction (Nestler, 2001; Hyman et al., 2006). The primary output of nAcb is to the ventral pallidum (Yang and Mogenson, 1985; Zahm and Heimer, 1990; Hakan et al., 1992), which is involved in the activation of voluntary movements (Swerdlow and Koob, 1987; Heimer et al., 1994). Thus, nAcb may be viewed as an interface between motivation and motor systems of the brain. It is implicated in various psychophysical states related to goal-directed behavior, arousal, attention and cognition, as well as in a number of psychiatric and neurological disorders, such as Alzheimer's disease, Tourette's syndrome, schizophrenia and depression (Snyder, 1973; Matthysse, 1983; Comings, 1987; Csernansky et al., 1991; Grace, 1992; Braun et al., 1993; Gray et al., 1994; Nestler and Carlezon, 2006).

* Corresponding author at: Centre de recherche F-Séguin, 7331 Hochelaga, Montréal, Que. H1N 3V2, Canada. Tel.: +1 514 251 4015x3415; fax: +1 514 251 2617.
E-mail address: richard.warren@umontreal.ca (R.A. Warren).

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doi:10.1016/j.jchemneu.2008.08.003

The cellular composition of nAcb is similar to that of the dorsal striatum, in that medium-sized densely spiny GABAergic projection neurons (MS neurons) constitute more than 90% of its neuronal population. The remaining neurons consists of local interneurons, including a variety of moderately spiny GABAergic neurons, and large sparsely spiny or aspiny neurons (Kawaguchi, 1993; Kawaguchi et al., 1995; Meredith, 1999) that are choline acetyltransferase (ChAT) immunopositive (Bolam et al., 1984a,b; Wainer et al., 1984; Phelps et al., 1985; Phelps and Vaughn, 1986; DiFiglia, 1987; Izzo and Bolam, 1988; Pickel and Chan, 1990). Although few in number, cholinergic interneurons have large dendritic fields and very dense axonal arborizations (Wilson et al., 1990; Contant et al., 1996), which accounts for the high concentrations of acetylcholine (ACh), ChAT, and acetylcholinesterase (AChE) in the dorsal and ventral striatum (Hoover et al., 1978). Their role in striatal function is still being investigated.

ACh increases the excitability of MS neurons by acting on postsynaptic muscarinic receptors (Uchimura and North, 1990; Sugita et al., 1991; Zhang and Warren, 2002). Accordingly, anatomical observations suggest that cholinergic terminals synapse on the cell bodies, dendritic shafts, and dendritic spines of MS neurons in the striatum (Izzo and Bolam, 1988). ACh also acts presynaptically to affect the release and/or the effect of various neurotransmitters, including glutamate, GABA and dopamine (Sugita et al., 1991; Pennartz and Lopes da Silva, 1994; Zhou et al., 2001; de Rover et al., 2002; Zhang and Warren, 2002; Yang and Warren, 2003). Notably, ACh exerts a powerful and rapid inhibition of glutamatergic input to MS neurons (Pakhotin and Bracci, 2007; Zhang and Warren, 2002; see also Narushima et al., 2007). Appositions between cholinerigic axon terminals and glutamatergic terminals have been observed (Pickel and Chan, 1990), which may account for some of the presynaptic effects of ACh.

Striatal cholinergic interneurons have been shown to be tonically active (Wilson et al., 1990; Wilson, 1993). In primates, they exhibit a pause in activity in response to various stimuli associated with rewarding and/or aversive events (Zhou et al., 2002; Kimura et al., 2003). What maintains their tonic drive, however, is still unclear. Either tonic activity is intrinsic to cholinergic interneurons (Bennett and Wilson, 1999), or it is evoked by extrinsic excitatory inputs. In the rat dorsal striatum, electrical stimulation of the thalamus or cerebral cortex evokes excitatory postsynaptic potentials in cholinergic interneurons (Wilson et al., 1990; Kawaguchi et al., 1995; Bennett and Wilson, 1998, 1999), and causes a glutamate receptor dependent increase in ACh levels (Baldi et al., 1995; Consolo et al., 1996a,b). Likewise, in nAcb, electrical stimulation of the medial prefrontal cortex evokes excitatory postsynaptic potentials in presumptive cholinergic interneurons, which are blocked by ionotropic and/or metabotropic glutamate receptor antagonists (Yang and Warren, 2003). Moreover, the release of ACh induced in nAcb by stimulation of the hippocampus/fornix can be suppressed by an NMDA receptor antagonist (Kraus and Prast, 2001). These physiological data suggest that cholinergic interneurons in the dorsal and/or ventral striatum might be directly contacted by glutamatergic afferents from the thalamus, cerebral cortex, and hippocampus. In nAcb, however, there is limited anatomical data to support this contention.

There has been only one study in which a neuroanatomical tracing technique was combined with ChAT immunocytochemistry at the electron microscopic level to investigate the relationships between thalamic afferents and cholinergic interneurons in nAcb. This study combined ChAT immunocytochemistry with neuronal degeneration after relatively large electrolytic lesions including all midline, intralaminar and paraventricular nuclei. Its authors reported that 15% of degenerating axon terminals made synaptic contact with the cholinergic interneurons in the medial part of rat nAcb (Meredith and Wouterlood, 1990). Based on the work of Berendse and Groenewegen (1990), who had demonstrated a significant projection of the paraventricular thalamic nucleus (PV) to the medial shell of nAcb, they assumed that the PV was the source of these contacts on nAcb cholinergic interneurons. They also argued that they might have seen more synapses on these neurons if they could have identified smaller and more distal dendritic branches with the anti-ChAT serum available to them at the time. Indeed, in a subsequent study of rat dorsal striatum after Phaseolus vulgaris-leucoagglutinin (PHA-L) transport from the parafascicular nucleus, Lapper and Bolam reported that there were many thalamostriatal terminals in synaptic contact with the perikarya and dendrites of ChAT immunopositive neurons.

In this context, we decided to combine PHA-L tracing and immunocytochemistry with a monoclonal antibody against whole rat ChAT, to reassess the relationship between midline/intralaminar/paraventricular thalamic afferents and cholinergic interneurons in rat nAcb. This particular anti-ChAT antibody allows to visualize the fine dendritic branches and the whole axonal arborization of cholinergic interneurons throughout rat brain, including the striatum (e.g., Contant et al., 1996; Aznavour et al., 2003). It was therefore expected that it would also allow for the first detailed description of the morphological and relational ultrastructural features of the cholinergic innervation in rat nAcb.

2. Materials and methods

Fifteen male and female Wistar rats (Charles River, St. Constant, QC), ranging from 235 to 350 g in weight, were used in this study. All animal procedures were conducted in strict accordance with the Guide to the Care and Use of Experimental Animals (Second edition) of the Canadian Council on Animal Care. Protocols were approved by the Comité de Déontologie pour l'Expérimentation sur des Animaux at the Centre de recherche Fernand-Séguin and the Université de Montréal.

2.1. PHA-L injections

PHA-L injections were performed as follows in eight rats. After anesthesia with pentobarbital (65 mg/kg) and ketamine (10 mg/kg), the rat was placed on a thermostatically controlled heating pad (37 ± 0.5 °C) and its head secured in a stereotaxic frame. Under aseptic conditions, the skull was exposed, and a burr hole (about 1.0 mm in diameter) was drilled over the midline, 2.0–3.0 mm posterior to bregma. A small incision was made in the dura mater, bleeding was controlled, and a 27-gauge needle was lowered 2–3 mm deep into the brain, withdrawn, and replaced by a borosilicate glass micropipette (20–25 μm tip in outer diameter) half-filled with PHA-L solution (Vector L110; 2.5% in 0.1 M sodium phosphate buffer, pH 8.0). In seven rats, three separate PHA-L injections were made at stereotaxic coordinates 5.0, 5.5, and 6.2 mm ventral to bregma, aiming for the paraventricular, intermediolateral, and central medial thalamic nuclei, respectively (Paxinos and Watson, 1986). In one animal, a single PHA-L injection was made 5.3 mm ventral to bregma. PHA-L was injected iontophoretically using 5 μA positive current and a 7-s on/off cycle for 15 min at each site, after which the wound was staple-closed and covered with betadine and cicatrin. The rat was then subcutaneously administered 1.0–2.0 ml of sterile saline, returned to the animal facilities, and given normal care until perfused 2–7 days later.

2.2. Fixation and sectioning

All rats were anesthetized with a lethal dose of pentobarbital and perfused transcardially for 1 min with 50 ml of 0.1 M sodium phosphate buffer (PB, pH 7.4) followed by 450 ml of fresh fixative containing a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in PB over a period of 20 min. The brain was removed, immersed whole in the same fixative for 3 h, rinsed in PB, and marked with two razor blade slashes on the right cerebral cortex. A Vibratome was used to cut serial 60-μm-thick transverse sections spanning the entire nAcb, as well as 100-μm-thick sections of the thalamus.

2.3. ChAT and PHA-L immunocytochemistry for light microscopy

Eleven rats were used in all, seven for ChAT labeling, two for PHA-L-labeling, and two for labeling both antigens, including double-labeled sections. In all cases, some sections were processed immediately after sectioning; the remainders were