Neuroscience 162 (2009) 155–173

TWO DISTINCT POPULATIONS OF PROJECTION NEURONS IN THE RAT LATERAL PARAFASCICULAR THALAMIC NUCLEUS AND THEIR CHOLINERGIC RESPONSIVENESS

J. A. BEATTY,[a,c] E. L. SYLWESTRAK[a,c] AND C. L. COX[a,b,c]*

[a]*Department of Molecular and Integrative Physiology, University of Illinois at Urbana–Champaign, Urbana, IL 61801, USA*

[b]*Department of Pharmacology, University of Illinois at Urbana–Champaign, Urbana, IL 61801, USA*

[c]*2357 Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, 405 North Mathews Avenue, MC-251, Urbana, IL 61801, USA*

Abstract—The lateral parafascicular nucleus (lPf) is a member of the intralaminar thalamic nuclei, a collection of nuclei that characteristically provides widespread projections to the neocortex and basal ganglia and is associated with arousal, sensory, and motor functions. Recently, lPf neurons have been shown to possess different characteristics than other cortical-projecting thalamic relay neurons. We performed whole cell recordings from lPf neurons using an *in vitro* rat slice preparation and found two distinct neuronal subtypes that were differentiated by distinct morphological and physiological characteristics: diffuse and bushy. Diffuse neurons, which had been previously described, were the predominant neuronal subtype (66%). These neurons had few, poorly-branching, extended dendrites, and rarely displayed burst-like action potential discharge, a ubiquitous feature of thalamocortical relay neurons. Interestingly, we discovered a smaller population of bushy neurons (34%) that shared similar morphological and physiological characteristics with thalamocortical relay neurons of primary sensory thalamic nuclei. In contrast to other thalamocortical relay neurons, activation of muscarinic cholinergic receptors produced a membrane hyperpolarization via activation of M_2 receptors in most lPf neurons (60%). In a minority of lPf neurons (33%), muscarinic agonists produced a membrane depolarization via activation of predominantly M_3 receptors. The muscarinic receptor-mediated actions were independent of lPf neuronal subtype (i.e. diffuse or bushy neurons); however the cholinergic actions were correlated with lPf neurons with different efferent targets. Retrogradely-labeled lPf neurons from frontal cortical fluorescent bead injections primarily consisted of bushy type lPf neurons (78%), but more importantly, all of these neurons were depolarized by muscarinic agonists. On the other hand, lPf neurons labeled by striatal injections were predominantly hyperpolarized by muscarinic agonists (63%). Our results indicate two distinct subpopulations of lPf projection neurons, and interestingly lPf neurons respond differentially to muscarinic receptor activation based on their axonal target.

Key words: thalamus, basal ganglia, thalamostriatal, burst, acetylcholine, muscarinic.

*Correspondence to: C. L. Cox, 2357 Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana–Champaign, 405 North Mathews Avenue, MC-251, Urbana, IL 61801, USA. Tel: +1-217-265-0573; fax: +1-217-244-5180.
E-mail address: cox2@illinois.edu (C. L. Cox).

Abbreviations: dLGN, dorsal lateral geniculate nucleus; DMPP, 1,1-dimethyl-4-phenyl-piperazinium iodide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; lPf, lateral parafascicular nucleus; LTS, low threshold calcium spike; MCh, acetyl-β-methylcholine; MGN, medial geniculate nucleus; TTX, tetrodotoxin; 4-DAMP, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide.

Intralaminar thalamic nuclei have long been hypothesized to play an important role in cortical activation and speculated to potentially serve as cellular substrates of consciousness (Morison and Dempsey, 1941; Scheibel and Scheibel, 1967; Llinas and Ribary, 1993; Llinas et al., 1998). The parafascicular nucleus is a bilateral cluster of neurons in the caudal intralaminar thalamic group split into a medial and lateral portion by the fasciculus retroflexus in the rat. The lateral parafascicular nucleus (lPf) is homologous to the centre-médian nucleus in monkeys and humans, and this nucleus has been associated with motor-related structures (Jones, 1985; Groenewegen and Berendse, 1994). The lPf is primarily innervated by the basal ganglia, neocortex, and brainstem structures (Cornwall and Phillipson, 1988; Tsumori et al., 2000; Krout and Loewy, 2000; Krout et al., 2002; Tsumori et al., 2002). The major efferent projections of lPf neurons innervate the basal ganglia (striatum, globus pallidus, and subthalamic nucleus), with fewer cortical efferents relative to other thalamocortical projecting nuclei (Jones and Leavitt, 1974; Berendse and Groenewegen, 1990, 1991; Kincaid et al., 1991; Feger et al., 1994; Deschenes et al., 1996).

While the anatomical relationship between the lPf, neocortex, and basal ganglia is well established, the understanding of the functional relationship is limited. The lPf provides excitatory glutamatergic synaptic innervation to both basal ganglia and neocortex (Wilson et al., 1983; Sugimoto and Hattori, 1983; Dube et al., 1988; Mouroux and Feger, 1993; Mouroux et al., 1995; Marini et al., 1996). The centre-médian-parafascicular complex is of particular interest considering the significant neuronal loss in this region in patients with Parkinson's disease and progressive supranuclear palsy (Henderson et al., 2000b). In light of this pathology, alterations in lPf activity may play a potentially important role in the manifestations of these motor diseases. Anatomical studies indicate rat lPf neurons are morphologically distinct from thalamocortical neurons of primary sensory thalamic nuclei in that they have long poorly branching dendrites (Scheibel and Scheibel, 1967; Deschenes et al., 1996). Until recently only these

0306-4522/09 $ - see front matter.
doi:10.1016/j.neuroscience.2009.04.043

anatomical studies have existed regarding the neurons of the rat lPf. More recently, several laboratories have reported that lPf neurons display electrophysiological properties distinct from thalamocortical relay neurons, most notably being their decrease in burst discharge (Smith et al., 2006; Phelan et al., 2006; Lacey et al., 2007). However, all of these studies indicate a morphologically similar population that is distinct from stereotypical thalamocortical neurons. Ascending cholinergic projections from the pedunculopontine nucleus and the laterodorsal tegmental nucleus to various thalamic neurons are thought to be involved in regulating behavioral states such as arousal, attention, and sleep/wake states (Moruzzi and Magoun, 1949; Hallanger et al., 1987; Pare et al., 1988; Steriade and Llinas, 1988). Autoradiographic studies reveal the localization of both muscarinic and nicotinic receptors throughout the thalamus (Rotter et al., 1979; Clarke et al., 1985). The majority of studies involving cholinergic and thalamic actions are limited to primary sensory thalamic nuclei. In rat thalamocortical relay neurons of primary sensory nuclei, activation of muscarinic receptors depolarizes all relay neurons, presumably via activation of M1 and M_3 (Zhu and Uhlrich, 1998; Varela and Sherman, 2007). In cat and guinea-pig thalamocortical neurons, activation of muscarinic receptors produces biphasic responses consisting of a brief hyperpolarization followed by a membrane depolarization, or only membrane depolarizations (McCormick and Prince, 1987). In the present study, we obtained whole cell recordings from lPf neurons and distinguished two distinct subtypes of neurons, diffuse and bushy, based upon electrophysiological and morphological differences. Furthermore, we surprisingly found that in contrast to primary sensory thalamocortical neurons, muscarinic agonists produced an inhibitory action on the majority of lPf neurons with a minority population responding to the muscarinic agonists with an excitatory, depolarizing action. The inhibitory or excitatory response to the muscarinic agonist is correlated with the different efferent targets of lPf neurons. That is, muscarinic agonists only depolarized cortical projecting lPf neurons, whereas muscarinic agonists predominantly hyperpolarized striatal-projecting neurons. These distinct actions suggest differential function of cholinergic actions in the different neural circuits, thalamocortical versus thalamostriatal.

EXPERIMENTAL PROCEDURES

All experimental procedures were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the University of Illinois Institutional Animal Care and Use Committee. Thalamic slices were prepared from young Sprague–Dawley rats (postnatal age 10–20 days). The rats were deeply anesthetized with sodium pentobarbital (50 mg/kg) and decapitated. The brains were quickly removed and placed into cold (~4 °C), oxygenated (95% O_2/5% CO_2) slicing medium containing (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 10.0 $MgCl_2$, 0.5 $CaCl_2$, 26.0 $NaHCO_3$, 11.0 glucose, and 234.0 sucrose. Tissue slices (300 μm thickness) were cut in the horizontal plane using a vibrating slicer and transferred to a holding chamber containing warmed (~35 °C), oxygenated (95% O_2/5% CO_2) physiological solution containing (in mM): 126.0 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose for at least 1 h before recording. In experiments with lanthanum chloride, we used a physiological solution containing (in mM): 151.0 NaCl, 2.5 KCl, 1.25 $MgCl_2$, 2.0 $CaCl_2$, 10.0 HEPES, and 10.0 glucose, and the pH adjusted to 7.3 with NaOH and oxygenated with 100% O_2. Individual slices were then transferred to a submersion-type recording chamber and superfused (~2 ml/min) with oxygenated physiological solution maintained at ~30 °C.

Recording pipettes were pulled from 1.5 mm outer diameter capillary tubing and had tip resistances of 3–6 MΩ when filled with solution containing (in mM): 117.0 K-gluconate, 13.0 KCl, 1.0 $MgCl_2$, 0.07 $CaCl_2$, 0.1 EGTA, 10.0 HEPES, 2.0 Na_2–ATP, 0.4 Na-GTP, and 0.2% biocytin. The pH of this solution was adjusted to 7.3 and osmolarity was adjusted to 290 mOsm. The use of this intracellular solution results in ~10 mV junction potential that has been corrected for in all voltage measures.

Whole-cell recordings were obtained with the visual aid of a modified microscope equipped with differential interference contrast optics (Axioskop 2FS, Carl Zeiss Instruments, Thornwood, NY, USA). A low-power objective was used to identify specific thalamic nuclei and anatomical landmarks, and a high-power water immersion objective was used to visualize individual neurons. Recordings were limited to 500 μm lateral to the fasciculus retroflexus. An Axoclamp 2B or Multiclamp 700A amplifier (Molecular Devices Corporation, Sunnyvale, CA, USA) was used in bridge mode for voltage recordings. An active bridge circuit was continuously adjusted to balance the drop in potential produced by passing current through the recording electrode. Voltage clamp recordings were limited to neurons that had a stable access resistance of <18 MΩ. Current and voltage protocols were generated using pCLAMP software (Molecular Devices Corporation), and data were digitized and stored on computer for off-line analyses.

Initial input resistances were calculated from the linear slope of the voltage–current relationship obtained by applying constant current pulses ranging from −40 to +40 pA (1 s duration). To evoke the transient inward current, we used an inactivation protocol in which command voltage steps were given to different hyperpolarized levels (−110 to −50, 5 mV increments, 5 s duration) followed by a return to the holding potential (−50 mV). Peak amplitudes were measured and averaged over three consecutive trials for each neuron tested. Action potential characteristics were measured in a subset of neurons where the membrane potential was manually adjusted to −50 mV, and a series of depolarizing current pulses were injected in each neuron (range: 5–225 pA, 5–10 pA increments, 1 s duration, 0.2 Hz). The slopes of the frequency–intensity relationships were calculated from the linear plots of current versus maximum discharge rate, typically the first interspike interval. The calculated correlation coefficient of the resulting linear regression was >0.9 in all neurons tested. Spike adaptation was calculated by the ratio of the last instantaneous frequency and initial instantaneous frequency in response to the maximum current intensity tested in each neuron. Only neurons that responded with greater than six action potentials to the current step were included in this analysis. All data are presented as mean±standard deviation. Differences between means were considered significant when $P<0.05$.

Concentrated stock solutions of pharmacological agents were prepared in distilled water and diluted in physiological solution to final concentration before use. Agonists were applied by injecting a bolus into the input line of the chamber for 60–80 s using a motorized syringe pump. Based on the rate of agonist injection and chamber perfusion rate, the final bath concentrations of agonists were estimated to be approximately one-fourth of the concentration introduced in the flow line (Cox et al., 1995). Concentrations listed in the text are the final bath concentrations after the fourfold dilution. During drug application, changes in input resistance were determined by membrane responses to single-intensity constant hyperpolarizing current pulses (5–20 pA, 250–500