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A rat brain slice preparation for characterizing both thalamostriatal and corticostriatal afferents

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Abstract

The striatum, the primary input nucleus of the basal ganglia, is crucially involved in motor and cognitive function and receives significant glutamate input from the cortex and thalamus. Increasing evidence suggests fundamental differences between these afferents, yet direct comparisons have been lacking. We describe a slice preparation that allows for direct comparison of the pharmacology and biophysics of these two pathways. Visualization of slices from animals previously injected with BDA into the parafascicular nucleus revealed the presence of axons of thalamic origin in the slice. These axons were especially well-preserved after traversing the reticular nucleus, the location chosen for stimulation of thalamostriatal afferents. Initial characterization of the two pathways revealed both non-NMDA and NMDA receptor-mediated currents at synapses from both afferents and convergence of the afferents in 51% of striatal efferent neurons. Annihilation of action potentials was not observed in collision experiments, nor was current spread from the site of stimulation to striatum found. Differences in short-term plasticity suggest that the probability of release differs for the two inputs. The present work thus provides a novel rat brain slice preparation in which the effects of selective stimulation of cortical versus thalamic afferents to striatum can be studied in the same preparation.

Keywords: Basal ganglia; Striatum; Brain slice; EPSCs; Thalamostriatal; Corticostriatal

1. Introduction

The striatum is the primary input nucleus of the basal ganglia, a group of subcortical nuclei critically involved in motor and cognitive function (Graybiel et al., 1994; Packard and Knowlton, 2002). Crucial to striatal function are the large glutamate afferents from the cerebral cortex and thalamus (Kemp and Powell, 1971; Kitai et al., 1976; Herrling, 1985; Smith et al., 1994). The corticostriatal (CS) afferent has been studied in some depth and much is known about the postsynaptic receptor composition and plasticity that occurs at this synapse (Herrling, 1985; Jiang and North, 1991; Calabresi et al., 1992, 1999; Centonze et al., 2003). Likewise, it has been known for some time that significant thalamostriatal (TS) input arises from midline and intralaminar nuclei, such as the parafascicular (Pf) and centromedian (CM) nuclei (Powell, 1952; Berendse and Groenewegen, 1990). Recent anatomical studies have discovered that the TS input is more significant in size than once thought and includes ventral group nuclei (McFarland and Haber, 2001, 2002; Van der Werf et al., 2002; Smith et al., 2004). Additionally, studies of the Pf nucleus in monkeys have recently demonstrated a role of TS input from the Pf nucleus in strategy switching during decision-making (Matsumoto et al., 2001; Minamimoto and Kimura, 2002; Kimura et al., 2004), thus mediating a fundamentally different function than the CS afferent, which is thought to provide the striatum with salient cortical somatosensory information on which action selection occurs (Mink, 1996; Bar-Gad et al., 2003). To date, however, relatively little is known about the synaptic physiology of the thalamic input to striatal efferent neurons.

One of the primary obstacles to our comprehensive understanding of the thalamostriatal pathway has been the lack of a rat brain slice preparation that allows for the electrophysiological characterization of the thalamostriatal pathway and direct comparison of the characteristics of that excitatory input with the characteristics of the CS input to striatal efferent neurons.

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To address this issue, we have developed a novel rat brain slice preparation that preserves both the CS and TS afferents to the spiny efferent neurons of the striatum. Due to the diffuse and convoluted nature of the thalamostriatal projection, we chose an off-horizontal slice preparation and verified the existence of functional TS projections in this slice. We found that there is convergence of both CS and TS afferents onto single spiny efferent neurons in the slice and that following stimulation, both pathways activate both AMPA and NMDA receptors. Of particular interest, we demonstrated differences in short-term plasticity in these two afferents, suggesting that the probability of release for these two afferents differs. This preparation should offer a useful tool for the further characterization of the TS afferent and the direct comparison of the TS and CS pathways.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (75–190 g) were used in all experiments. Rats were housed in groups in a room controlled for temperature and lighting and had free access to food and water. All animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee of the University of Utah and were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. BDA injections

Male, Sprague–Dawley rats (~190 g) were housed for 2 days prior to surgery for monitoring of their health and to reduce stress from transportation. At the time of surgery, the animals were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg). Infusion cannulae (29 gauge, 10 mm length, Small Parts, FL) were placed at −4.2 mm AP, 1.2 ML and −6.4 DV from Bregma and left in place for 1 min before infusion of biotinylated dextran amine (BDA, 10,000 MW, NeuroTrace® kit, Invitrogen, CA). A syringe pump (Havard Apparatus, Model 22) and a 25-μl Hamilton syringe were used to infuse 3% BDA in artificial cerebral spinal fluid (144 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 0.4 mM KH_2PO_4 in ddH_2O, pH 7.4) at a rate of 0.05 μl/min for a total of 0.2 μl in 4 min. After the infusion was complete, the infusion cannula was left in place for 1 min, then removed. Wound clips (9 mm, Stoelting Co., IL) were used to close the wound and a post-operative injection of Banamine (flunixin HCl, 1.2 mg/kg, s.c.) was given for prophylactic pain management. After recovery, animals were returned to their home cages for 7–10 days before being sacrificed.

Slices (400 μm) were prepared from BDA-injected rats as for an electrophysiology experiment, as described below. After 2 h of rest in oxygenated Ringer's saline, the slices were fixed in 4% paraformaldehyde at 4 °C overnight. Slices were then rinsed in phosphate buffered saline (PBS) and subsequently washed in 1% H_2O_2 (in 10% methanol and 90% PBS) for 15 min. Following this step, the slices were rinsed in PBS again and then permeabilized in 2% Triton X-100 (Triton X) in PBS for 2 h. An ABC Elite Kit (Vector Laboratories, CA) was used to visualize the BDA signal. The slices were left in this solution also containing 1% Triton X in PBS overnight. After a series of washes in PBS, the slices were incubated in diaminobenzidine (2.5 mg/ml) for 10 min. Slices were rinsed again and mounted in a glycerol solution containing MOWIOL 488 (Calbiochem, CA), a polyvinyl alcohol that helps to clear thick tissue slices.

2.3. Striatal slice preparation

Rats were anesthetized with Nembutal (25 mg/kg) and decapitated. The brains were rapidly removed and placed in ice-cold sucrose Ringer's solution bubbled with 95% O_2/5% CO_2 and containing (in mM): sucrose (124), KCl (3), $NaPO_4$ (1.2), $MgSO_4$ (2), $NaHCO_3$ (6), glucose (10) and $CaCl_2$ (2). The solution pH was between 7.35 and 7.38, and the osmolality was 295–305 mOsm. Descriptions of the trajectory of the thalamostriatal afferents from a number of tracing studies led us to select an oblique horizontal preparation of approximately 30° off-horizontal (Berendse and Groenewegen, 1990; Deschenes et al., 1996; Yasukawa et al., 2004). The brain was laid on a chilled cutting surface ventral side down and blocked along the midline. The cerebellum was removed, and the left hemisphere laid medial side down. The angle of the slice preparation is shown in Fig. 1A, which also shows the perspective when blocking the brain. The olfactory bulb was used as a reference to make the oblique 30° cut. The brain was bluntly blocked again rostrally and dorsally and then glued, ventral side down, on the Vibratome chuck. Slices were cut at a thickness of 400 μm while bubbling the slice in chilled sucrose Ringer's solution.

2.4. Electrophysiological measurements

Immediately after each slice was cut, it was placed in oxygenated normal Ringer's solution (NaCl substituted for sucrose) and left for a minimum of 1 h for recuperation at room temperature. For recording, slices were placed in a submersion chamber and constantly superfused at room temperature (2.5–3.0 ml/min) with oxygenated Ringer's solution. The whole-cell patch-clamp technique was used to record from spiny efferent neurons within striatum, as previously reported (Chapman et al., 2003) using the blind technique (Blanton et al., 1989). A microscope equipped with Nomarski optics and infrared illumination (Axioskop FS microscope, Zeiss) was used to visualize the slice, thus facilitating patching onto cells. Borosilicate patch electrodes (World Precision Instruments, FL) were pulled to 3–6 MΩ resistances using a micropipette electrode puller (Sutter Instruments, CA). The internal solution was comprised of (in mM): K gluconate (130), KCl (10), HEPES (10), EGTA-KOH (1), $CaCl_2$ (0.1) and glucose (10).

Whole-cell recordings were obtained from spiny efferent neurons using a MultiClamp 700 A amplifier (Molecular Devices, CA). Signals in current-clamp mode were acquired at 20 kHz and filtered at 10 kHz for off-line analysis using Clampfit 9 (Molecular Devices). Input and series resistance values of 50–100 and <15 MΩ, respectively, were used as selection criteria for accepting recordings. Only recordings that did not exhibit sub-