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Research report

AMPA and NMDA receptor-mediated currents in developing dentate gyrus granule cells

Gui-lan Ye, Si Yi, Georgi Gamkrelidze, Joseph F. Pasternak, Barbara L. Trommer*

Departments of Pediatrics and Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA
Department of Pediatrics, Division of Neurology, Evanston Northwestern Healthcare, Evanston, IL 60201, USA
Evanston Northwestern Healthcare, 2650 Ridge Avenue, Evanston, IL 60201, USA

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Abstract

Granule cells (GCs) of the hippocampal dentate gyrus (DG) undergo postnatal neurogenesis such that cells at different maturational stages can be studied within an anatomically restricted region and a narrow animal age epoch. Using whole cell patch clamp recordings in hippocampal slices, we have previously found that input resistance (IR) correlates inversely with morphometric indicators of GC maturity. Using IR as an index of maturity we measured developmental changes in synaptic currents mediated by *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in GCs from 5- to 12-day rats. Peak NMDA and AMPA EPSC amplitudes increased, and the NMDA/AMPA ratio reversed with advancing cell age. NMDA EPSCs showed a maturational decrease in rise time but no change in decay time, whereas AMPA EPSCs showed neither rise nor decay time changes with development. Ifenprodil, a high affinity selective inhibitor of NR1/NR2B diheteromeric NMDA receptors, blocked approximately 50% of the peak amplitude of evoked NMDA EPSCs in all tested GCs regardless of their maturity and did not affect the measured kinetic properties. These data suggest that development of glutamatergic synapses follows distinct schedules. AMPA receptors possessed mature kinetics and became the dominant glutamatergic current within the age epoch studied, whereas NMDA receptors showed maturational changes in rise times but not decay kinetics. The reported modifications of EPSC properties are consistent with changes in receptor and synapse number, and relative quantities of AMPA and NMDA receptors. Changes in the subunit composition that determines NMDA decay kinetics may occur beyond the early neonatal period.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Excitatory amino acid receptors: physiology, pharmacology, and modulation

Keywords: Hippocampal slices; Glutamatergic neurotransmission; Ifenprodil

1. Introduction

The hippocampal dentate gyrus (DG) is vital to normal learning and memory [21] and is implicated in neuro-developmental disabilities such as epilepsy, schizophrenia, and autism [20,30] as well as neurodegenerative disorders such as Alzheimer disease [24]. Granule cells (GCs) of dentate gyrus undergo postnatal neurogenesis in many mammalian species including humans [6,10]. Both clinical and experimental data suggest that newly born GCs may contribute to functional recovery from brain injury of diverse etiologies [3,18,25,34] and/or serve as a source of replacement for cells lost in disease [13,32]. Thus, the study of GC integration in the hippocampal network is of potential therapeutic importance.

Because of postnatal neurogenesis and stratification within DG, GCs with staggered birthdates can be studied in situ over a narrow animal age epoch and even within a

* Corresponding author. Fax: +1 847 570 2073.
E-mail address: btrommer@northwestern.edu (B.L. Trommer).

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given hippocampal slice. Using patch clamp recordings of biocytin-filled GCs in rat hippocampal slices in the first postnatal month, we have previously shown that cells with input resistance (IR) ranging from 0.250 to 3.0 GΩ can be found within an anatomically restricted region in the middle third of the suprapyramidal limb, and that the cells with highest IR are the least mature by morphometric criteria such as dendritic length and degree of penetration of the dendritic arbor into the molecular layer [17,19]. In a subsequent qualitative electrophysiologic study of very immature GCs (IR ≥ 1 GΩ) from young rat DG (postnatal days 5–12), we demonstrated a maturational progression of the response to medial perforant path stimulation that paralleled morphometric advances in cell age: glutamatergic currents were absent in the youngest cells, exclusively NMDA-mediated in somewhat older cells, and both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA-mediated in the oldest cells in the population [37]. In the present study, we examined GCs from 5- to 12-day rats (IR range 0.350–2.200 GΩ) that had both AMPA and NMDA-mediated currents. Using IR as an index of maturity, we examined the relative maturation of amplitude and kinetics of these currents, and determined the response of the NMDA currents to the selective NR2B antagonist ifenprodil.

2. Materials and methods

Hippocampal slices (300–350 μm) from Sprague–Dawley rats of both genders (6–12 days) were prepared as previously described [37] and were maintained at 35 °C for at least 30 min, allowed to cool to room temperature and transferred as needed to a small volume perfusion chamber (32 °C). ACSF composition was (in mM): NaCl 124, KCl 3, $CaCl_2$ 2.4, $MgSO_4$ 1.3, NaH_2PO_4 1.25, $NaHCO_3$ 26, and glucose 10 (gassed with 95% O_2/5% CO_2, pH = 7.4). Recording pipettes were pulled from borosilicate glass capillaries (OD 2.00 ± 0.05, ID 1.20 ± 0.05 mm, Garner Class Co.) using a Flaming Brown Model P-97 horizontal puller (Sutter Instrument Co.) and had tip impedance 4.5–6.0 MΩ. Internal solutions contained (in mM): cesium gluconate 107.5, cesium methanesulfonate 17.5, NaCl 8, Mg-ATP 3, GTP 0.2, 5,5′ -dimethyl-bis-(o-aminophenoxy)-N,N,N′ ,N′ -tetracetic acid (BAPTA) 10 (to prevent inadvertent long-term potentiation at depolarized membrane potentials), and *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (HEPES) 10. Liquid junction potentials were determined to be 10 mV for this internal solution [22] and membrane potentials were appropriately corrected.

Visualized patch clamp recordings were made in the suprapyramidal limb of the dentate gyrus granule cell layer approximately midway between the crest and the distal end of the limb using an Axopatch-1D amplifier and pClamp6 software (Axon Instruments). Bipolar platinum stimulating electrodes (tip diameter 25 μm, Rhodes Medical Instruments, Inc.) were placed in the middle third of the molecular layer to activate the medial perforate path. Cell access was obtained in voltage clamp mode and seals were 2–7 GΩ prior to break-in. Resting membrane potential (RMP) was measured immediately after break-in to avoid the cesium-induced depolarization. Post-synaptic currents were evoked at 0.033 Hz with stimuli of 150 μA and 112 μs, the maximal intensity tolerated in this preparation without causing hydrolysis or motion at the stimulating electrode tip [37]. Input resistance (IR) was calculated from the steady state current in response to a 100 ms 5 mV hyperpolarizing pulse. Thereafter, a 21-ms, 5-mV hyperpolarizing pulse (ΔV) was delivered 19 ms before each stimulus. Series resistance (Rs) was uncompensated and was monitored throughout the experiment using the formula Rs = ΔV/amplitude where amplitude = the peak (as extrapolated to time = 0) of the capacitive transient induced by this pulse. Recordings were not used for analysis if DC offset was >5 mV after withdrawal or if Rs in either AMPA or NMDA sweeps was >30 MΩ.

Cells used for these studies were required to have both NMDA and AMPA currents evoked at the same stimulus intensity. AMPA currents were measured at $V_{holding}$ = −70 mV in picrotoxin (PTX, 100 μM) and were completely eliminated by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM). NMDA currents were then measured at Vholding = +40 mV and their identity was confirmed by reversible blockade by AP5 (100 μM). The contribution of the NR2B receptor was assessed by comparing NMDA currents in the absence and presence of the selective antagonist ifenprodil (3 μM). Preliminary recordings during wash-in of ifenprodil in 15 cells of different maturities indicated that steady state effect was reached by 12–14 min of exposure and this duration was used for all measurements.

Stimulus-evoked postsynaptic currents were analyzed using the Clampfit subroutines of pClamp 6.04 software. Peak and 10–90% rise times (average of 4 sweeps) were calculated using standard algorithms. Decay kinetics for the NMDA currents were estimated via a manual determination of the time from peak to 50% [12] or 25% of peak amplitude.

Drugs were applied by bath perfusion. 2-D-aminophosphonopentanoic acid (AP5, Tocris), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Tocris), and ifenprodil tartrate (RBI) were prepared as stock solutions of 50 mM, 20 mM, and 1 mM, respectively in distilled water. Picrotoxin (Sigma) was added directly to the ACSF and mixed at room temperature for ≥1 h until fully dissolved.

All electrophysiologic data were imported into Systat software (SPSS, Chicago) for statistical analysis and graphing. When GCs were subdivided by IR groups for analysis of developmental trends, the cut-off values (in ascending order of maturity) were as follows: Group 1: IR ≥ 1.5 GΩ; Group 2: 1.5 GΩ > IR ≥ 1.0 GΩ; Group 3: 1.0 GΩ > IR ≥ 0.5 GΩ; Group 4: IR < 0.5 GΩ. For some