Neuroscience 252 (2013) 154–168

ELECTROPHYSIOLOGICAL IDENTIFICATION OF MEDIAL AND LATERAL PERFORANT PATH INPUTS TO THE DENTATE GYRUS

R. P. PETERSEN,[a,b] F. MORADPOUR,[b] B. D. EADIE,[a,d,e] J. D. SHIN,[a,b] T. S. KANNANGARA,[a,d,e] K. R. DELANEY[b,c,d] AND B. R. CHRISTIE[a,b,c,d,e]*

[a] *Division of Medical Sciences, University of Victoria, BC, Canada*

[b] *Department of Biology, University of Victoria, BC, Canada*

[c] *Graduate Program in Neuroscience, University of Victoria, BC, Canada*

[d] *Graduate Program in Neuroscience and The Brain Research Centre, University of British Columbia, BC, Canada*

[e] *Department of Cellular and Physiological Sciences, University of British Columbia, BC, Canada*

Abstract—The medial perforant path (MPP) and lateral perforant path (LPP) inputs to the hippocampal dentate gyrus form two distinct laminar inputs onto the middle and distal aspects of granule cell dendrites. Previous evidence indicated that paired stimuli reliably produced paired-pulse depression (PPD) in the MPP and paired-pulse facilitation (PPF) in the LPP. Despite this, several years of practical experience in our laboratory questioned the utility of using paired-pulse administration to reliably differentiate the MPP and LPP *in vitro*. Using visualized field and whole-cell recordings in male Sprague–Dawley rats, we demonstrate that both pathways show net PPF of the excitatory postsynaptic potential (fEPSP) at 50-ms interpulse intervals. LPP afferents did reliably exhibit greater PPF than MPP afferents. Thus, the LPP reliably exhibits a greater paired-pulse ratio than the MPP. The magnitude of the paired-pulse ratio was reduced in both afferents by raising calcium levels or lowering the temperature of the recording chamber. PPD of MPP-evoked fEPSPs was only reliably detected at moderate to high stimulus intensities when population spike activity was evident. PPD was more evident in whole cell voltage clamp recordings but nonetheless was not completely diagnostic as PPD was occasionally observed with LPP stimulation as well. We found the MPP and LPP could be reliably identified using conventional microscopy with hippocampal slices, and that they could be distinguished through the analysis of evoked waveform kinetics. This work refines our knowledge of electrophysiological differences between MPP and LPP projections and will help to facilitate the selective activation of these pathways.

Key words: short-term plasticity, paired-pulse depression, paired-pulse facilitation, population-spike activity, vesicle release probability.

INTRODUCTION

The hippocampus is critical for certain types of memory formation (Squire, 1992; Eichenbaum, 2004; Kesner, 2013) and experimental evidence continues to reveal the nuances of how the hippocampal formation processes information. Recently, different roles for the medial and lateral perforant path inputs to the dentate gyrus (DG) have begun to emerge (Hunsaker et al., 2007; Vivar and van Praag, 2013). The DG is the first component of the hippocampal formation that receives information from the cortex. Its primary input comes from layer 2 of the entorhinal cortex via a bundle of axons that is collectively known as the ''perforant path'' because of the way the axons perforate the subiculum to enter the DG. Information received by the DG is then largely relayed to the CA3 and CA1 subfields of the *cornu ammonis* in a unidirectional circuit, before being transmitted to regions of the cortex where the memories are presumably ''stored.''

In the past few years, evidence has accumulated to indicate that the types of information that the perforant path carries into the DG can be physically separated (Hunsaker et al., 2007). Novel object information appears to be carried by fibers of the lateral perforant path (LPP) that terminate on dendrites in the outer third of the molecular layer (Hunsaker et al., 2007). Spatial information appears to be conveyed by fibers of the medial perforant path (MPP) that originate in the medial aspects of entorhinal cortex layer 2 (Hjorth-Simonsen and Jeune, 1972; Steward and Scoville, 1976; Hunsaker et al., 2007). It has been known for some time that although both pathways originate from pyramidal and stellate cells in layer 2 of entorhinal cortex, they can be distinguished from one another both pharmacologically (Macek et al., 1996) and electrophysiologically (McNaughton, 1980; Wang and Lambert, 2003). Distinguishing MPP and LPP inputs electrophysiologically has primarily relied on the use of paired-pulse protocols where stimuli are administered at 50 ms apart. In the DG, the administration of paired-pulse

*Correspondence to: B. R. Christie, Division of Medical Sciences, University of Victoria, 3800 Finnerty Road, Victoria, BC, Canada V8P 5C2. Tel: +1-250-472-4244; fax: +1-250-472-5505.
E-mail address: brain64@uvic.ca (B. R. Christie).

Abbreviations: ACSF, artificial cerebrospinal fluid; BMI, bicuculline methiodide; DG, dentate gyrus; EGTA, ethylene glycol tetraacetic acid; fEPSP, excitatory postsynaptic potential; Hepes, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; IPI, interpulse interval; LPP, lateral perforant path; MPP, medial perforant path; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline-2,3-dione; NMDA, *N*-methyl-D-aspartic acid; PPD, paired-pulse depression; PPF, paired-pulse facilitation; PPR, paired-pulse ratio.

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.07.063

stimuli usually results in the second pulse being facilitated (PPF) when the LPP is stimulated, and paired-pulse depression (PPD) when the MPP is stimulated (McNaughton, 1980; Christie and Abraham, 1992a,b; Colino and Malenka, 1993; Froc et al., 2003). These findings are taken to indicate MPP presynaptic terminals have a higher vesicle release probability than those of the LPP (McNaughton, 1980).

Despite the common use of paired-pulse stimuli to distinguish the MPP and LPP inputs, several years of practical experience in our laboratory has led us to question the reliability of using paired-pulse stimuli to differentiate the MPP and LPP inputs to the DG *in vitro*. In addition, other researchers have also shown that vesicle release probability can vary significantly (1) within a homogeneous population of hippocampal synapses (Dobrunz and Stevens, 1997), (2) as a function of animal age (Speed and Dobrunz, 2008), (3) across the dorso-ventral/rostral-caudal hippocampal axis (Maruki et al., 2001) and (4) between newly formed and mature neurons in the DG (Wang et al., 2000). In the present experiments, we re-assessed the reliability of the paired-pulse ratio (PPR) to differentiate the MPP from the LPP input in the DG. We also performed a detailed analysis of the kinetics of synaptic waveforms evoked by stimulation of the medial and lateral DG molecular layer as a potential alternative means to distinguish these pathways *in vitro* (McNaughton, 1980; Abraham and McNaughton, 1984). Our results indicate that PPRs are almost always higher for LPP than for MPP stimulation but that PPD of excitatory postsynaptic field potentials (fEPSPs) alone is not a reliable way to identify the MPP. Furthermore, we demonstrate that factors including differences in extracellular calcium concentration, temperature and stimulation intensity significantly alter the balance between depression and facilitation at these synapses and have likely contributed to variability observed in previous studies.

EXPERIMENTAL PROCEDURES

Subjects

Experiments were conducted using hippocampal slices from male Sprague–Dawley rats obtained from the Charles River Laboratories (QC, Canada) and acclimatized to our vivarium for a minimum of 7 days. All animals were housed in polyethylene cages on a 12-h light/dark cycle at constant ambient temperature (21 ± 1 °C) and humidity (50% ± 7%) with *ad libitum* access to standard rat chow and water. Animal procedures were conducted in accordance with the University of Victoria and the Canadian Council on Animal Care principles of laboratory animal care.

Slice preparation

Subjects were anesthetized with isofluorane (Sigma–Aldrich, Ontario, Canada) and decapitated using standardized procedures to minimize stress. After decapitation, the brain was removed while being submerged in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) the following: 125 NaCl, 2.5 KCl, 1.25 $NaHPO_4$, 25 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgCl_2$, and 10 dextrose (pH = 7.2, 280–290 mOsm), bubbled with 95% O_2:5% CO_2. Transverse sections (400 μm) were cut while the brain was submerged in ACSF at 2 °C. Slices were then transferred to a custom 12-well holding chamber with ACSF at room temperature (23 ± 1 °C). All slices were incubated in the holding chamber for > 1.0 h prior to electrophysiological recordings to allow recovery from the dissection.

Field recordings

For most experiments, brain slices were transferred to a recording chamber and perfused with ACSF at 30 ± 1 °C at 1.5–2.0 ml/min. To examine potential dorso-ventral variation in paired-pulse plasticity, hippocampal slices were taken from the dorsal or ventral one-third extent of the DG. The DG was visualized using an upright, fixed stage Olympus BX51WI microscope with a 10× water immersion objective lens. Two sharp-tip concentric bipolar electrodes (25 μM tip, FHC, Bowdain ME) were carefully positioned in the outer and middle one-third DG molecular layer to activate the LPP and MPP (Fig. 1A). Square wave (0.12 μs) electrical stimuli were applied at 15-s intervals to evoke dendritic granule cell field fEPSPs. To determine the maximum response, current injection was increased by 5-μA increments delivered every 3 s until the initial linear going slope of the fEPSP reached a plateau. Once a stimulus current for 15% response was established the duration of the stimulus was adjusted to explore the effects of varying stimulus intensity on the PPR. Recordings were obtained by positioning a pair of glass electrodes (1–2 MΩ) filled with standard ACSF near the crest of the suprapyramidal blade and parallel to the orientation of the stimulation electrodes. Stimulation and recording electrodes were spaced > 300 μm apart to prevent direct depolarization of recorded postsynaptic granule cell dendrites and to minimize inhibitory postsynaptic potential activity (Davies et al., 1990). For some recordings, a single stimulation electrode was used, first positioned in one perforant pathway and then repositioned in the adjacent pathway to replicate the dual recording conditions. The results obtained for single and dual recording electrode configurations were indistinguishable and were therefore pooled. Only hippocampal slices producing signals with response amplitude greater than 1 mV and with a favorable fEPSP to fiber volley ratio (2:1 or greater), qualitative factors indicative of a healthy tissue response (Bortolotto et al., 2001), were used for experimentation.

To achieve reliable and segregated activation of MPP and LPP projections to the DG, several anatomical and electrophysiological criteria were applied. First, sharp-tip stimulation electrodes (25 μM tip) were positioned precisely in the middle and outer one-third molecular layers, corresponding to the strict termination zones of the MPP and LPP (Nafstad, 1967; Steward and Scoville, 1976). Second, stimuli were delivered at a low-stimulus intensity (15% max fEPSP) to reduce current