Ventral Hippocampal Neurons Project Axons Simultaneously to the Medial Prefrontal Cortex and Amygdala in the Rat

Akinori Ishikawa and Shoji Nakamura

Department of Neuroscience, Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan

Submitted 20 January 2006; accepted in final form 6 July 2006

Ishikawa, Akinori and Shoji Nakamura. Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. J Neurophysiol 96: 2134–2138, 2006. First published July 12, 2006; doi:10.1152/jn.00069.2006. The ventral hippocampus (VH) may have an important role in spatial memory processes and emotional behaviors through connections with the medial prefrontal cortex (mPFC) and amygdala. Although the mPFC and amygdala receive afferent projections from the VH, it has not been determined whether the individual VH neurons project to both the mPFC and the amygdala. In this study, antidromic responses to the mPFC and amygdala stimulation were evoked in single VH neurons. In addition, VH neurons were retrogradely double-labeled with fluorescent tracers injected in the mPFC and amygdala. VH neurons projecting to both the mPFC and amygdala were predominantly located in the subiculum and CA1 and bifurcated near or at the soma. Our anatomical and electrophysiological evidence for the presence of VH neurons projecting to both the mPFC and amygdala provides a previously unrecognized pathway from the hippocampus that simultaneously activates the mPFC and amygdala.

INTRODUCTION

It is well established that the hippocampus plays an essential role in memory formation and emotional behaviors (Jarrard 1993; McNaughton and Gray 2000; Squire 1992). Recent studies have proposed that the hippocampus is functionally and anatomically subdivided into dorsal and ventral regions (Bannerman et al. 2004; Trivedi and Coover 2004). The dorsal hippocampus (DH), which receives multimodal information by way of the association and entorhinal cortices, has preferential roles in the acquisition and consolidation of spatial and episodic memories (Bannerman et al. 2004; Jarrard 1993; Squire 1992; Trivedi and Coover 2004). On the other hand, the ventral hippocampus (VH), which has dense anatomical connections with the amygdala, is particularly involved in the regulation of emotional behavior such as anxiety and fear (Bannerman et al. 2004; Hobin et al. 2006; Trivedi and Coover 2004). Furthermore, other series of studies have indicated that the VH neurons directly project their axons to the mPFC and are implicated in spatial short-term and working memory formation (Floresco et al. 1997; Seamans et al. 1998). Although the VH is suggested to contribute both to memory and to emotional functions, it is unclear if the same VH neurons have a role in both of these functions. In these experiments, we examined anatomically and electrophysiologically whether single VH neurons have projections to both the mPFC and amygdala. Some of these data have been presented in abstract form (Ishikawa and Nakamura 2004).

METHODS

Surgery and placement of electrodes

These experimental protocols were reviewed and approved by the Committee of Ethics on Animal Experiments of Yamaguchi University School of Medicine and carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 80–23) revised 1996. Surgical procedures were performed as previously described (Ishikawa and Nakamura 2003). In brief, male rats (n = 5, 290–315 g) were anesthetized with sodium pentobarbital (50 mg/kg, ip). The head was fixed horizontally in a stereotaxic instrument (Narishige) and the body temperature maintained at 36–37°C. Small holes were made in the skull to enable vertical insertions of electrodes. Bipolar stimulating electrodes, which were also used to record field potentials in the mPFC, were positioned in the mPFC [anterior (A) to the bregma, 3.0–3.2 mm; lateral (L) to the midline, 0.75 mm; ventral (V) to the brain surface, 3.5 mm] and in the basolateral nucleus of amygdala (BLA) [posterior (P), 3.0 mm; L, 5.0 mm]. The depths of the electrodes (V, 7.3–8.0 mm) for BLA stimulation were adjusted to maximize the amplitude of the BLA-evoked field potential in the mPFC (Fig. 1). This procedure aided the placement of stimulating electrodes in the BLA, because there are dense reciprocal connections between the mPFC and BLA (McDonald 1987; Sesack et al. 1989).

Electrophysiological recording

Single unit activity in the VH (P, 6.5 mm; L, 5.4 mm; V, 3.0–6.0 mm) was recorded using a glass micropipette (5–10 MΩ). Electrical stimuli were delivered as a single pulse of 0.1 ms at 0.5 Hz (intensity, 0.1–5.0 mA). The following criteria were used to discriminate spikes antidromically driven by mPFC or BLA stimulation: a fixed latency at the threshold for activation; the ability to follow twin pulse stimulation at intervals longer than twice the refractory period; the stability of the latency over 10 trials; and a mean discharge rate of 30 spikes/s. In the case of mPFC stimulation, anodal direct current was used to aid localization. The stimulation sites were marked by applications of horseradish peroxidase (HRP) and diaminobenzidine (DAB) (Flamini et al. 1993). When the recording electrode reached the target site, stimulation was performed at a frequency of 10 Hz. Single unit activity was recorded with a tungsten electrode (200–250 μm). The cells were filled with horseradish peroxidase (HRP) or biocytin (Bion et al. 1993), and the recording electrode was filled with Neutral Red (0.2%) or Lucifer Yellow (0.1%) (Ishikawa and Nakamura 2003). The cells were identified by location, morphology, and responsiveness of spikes to visual stimuli (Ishikawa and Nakamura 2003). The spike activity was amplified by a Neurolog system (Digidata 1200, Axon Instruments, Foster City, CA) and was digitized and stored on a computer using Axon Data Station software for analysis. The duration and amplitude of each spike were measured using the software (Axon Data Station). Each neuron was recorded for at least 10 min.

Verification of recording and stimulating sites

Recording and stimulating sites were marked by applications of pontamine sky blue (10 μA for 5 min) and a DC of 30 μA for 1 min through each electrode. At the end of each experiment animals were perfused with 4% paraformaldehyde (PFA) solution, and the brains were removed. Frozen coronal sections of 50 μm, including the mPFC, VH, or BLA, were stained with hematoxylin-eosin. Recording and stimulating sites were reconstructed according to the rat brain atlas of Paxinos and Watson (1998).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Injection of retrograde fluorescent dyes

True Blue (TB) and Diamidino Yellow (DY) (Sigma, St Louis, MO) were dissolved in distilled water at 2.5 and 2% concentrations, respectively. Three rats were injected with TB and DY using 1-µl Hamilton syringes with 25-gauge needles. After the described surgical operation, TB and DY were injected into the mPFC and BLA, respectively, with a volume of 0.16 µl over a period of 5 min. The stereotaxic coordinates of the mPFC and BLA were determined in the same way as used in the electrophysiological experiments. The inserted needle was withdrawn 10–15 min after injection.

Histological preparation and microscopic examination

After 7 days, the rats were deeply anesthetized with an overdose of sodium pentobarbitone and perfused with 4% PFA solution, and the brains were removed. After post fixation and dehydration with 4% PFA solution containing 10–30% sucrose, the brains were coronally sectioned at 40 µm. The sections including the mPFC, amygdala and VH were mounted onto a gelatin-coated slide glass and examined using a fluorescent microscope (BX-FLA, Olympus) with a UV filter inducing excitation light of ~360-nm wavelength. Labeling with TB and DY was seen as blue fluorescence in the perikarya and yellow fluorescence in the nuclei, respectively.

Data analysis

All data are expressed as means ± SE. We used paired Student’s t-test to compare two sets of data. Differences are considered significant at P < 0.05.

RESULTS

Stimulating and injection sites in the mPFC and amygdala

The tips of stimulating electrodes and centers of injection sites were located in the ventral portion of the prelimbic cortex (v-PrL) and basolateral nucleus of amygdala (BLA). Amygdalar stimulation evoked negative-going field potentials in the mPFC. Increasing the depth of stimulating sites in the amygdala (V 7.0, 7.3, and 7.5 mm) induced shortened latencies of negative peak and augmentation of amplitudes. Arrows show the time of stimulation. The values below each coronal section represent the distance from bregma.

Antidromic responses of VH neurons to mPFC and amygdala stimulations

A total of 25 VH (8 CA1 and 17 subicular) neurons were antidromically activated by mPFC and/or BLA stimulation (Fig. 2). Of these 25 VH neurons, 13 (52%) had antidromical responses to both mPFC and BLA stimulation, in contrast the remaining 12 neurons were antidromically activated by either mPFC (n = 6) or BLA (n = 6) stimulation. Of the 13 neurons projecting to both the mPFC and amygdala, 8 were located in the subiculum and 5 in the CA1 region. The eight subicular neurons had shorter antidromic latencies to BLA stimulation (9.1 ± 0.7 ms) compared with mPFC stimulation (13.1 ± 1.8 ms; t = 2.61, df = 7, P < 0.05). There was no difference in antidromic latencies of the five CA1 neurons to the mPFC stimulation (12.2 ± 1.7 ms) and BLA stimulation (11.9 ± 2.0 ms). A typical example of a VH neuron showing antidromic responses from both the mPFC and the BLA is shown in Fig. 2, A–C. The mean conduction time from the soma to the branch point, which was calculated using the equation described in METHODS, ranged from 0 to 1 ms (mean, 0.3 ± 0.1 ms, n = 8). This shows that the axons of VH neurons projecting to both the mPFC and BLA bifurcated near or at the soma.

VH neurons double labeled with retrograde fluorescent tracers

The VH neurons having blue fluorescence in their perikarya or yellow fluorescence in their nuclei were broadly distributed in the CA1 and subicular regions. A larger number of VH neurons double labeled with both TB and DY were seen mainly in the subiculum and the CA1 (Fig. 3).
**DISCUSSION**

In this study, it was shown electrophysiologically and anatomically that single VH neurons project to both the mPFC and amygdala. VH neurons were antidromically activated by both mPFC and amygdalar stimulation and retrogradely double labeled by TB and DY injected into the mPFC and the amygdala, respectively. VH neurons projecting to both the mPFC and amygdala were located in the CA1 and subiculum.

**FIG. 2.** Antidromic responses (A–C) and distribution (D) of ventral hippocampus (VH) neurons projecting to both the mPFC and BLA. In the neuron located in the subiculum, antidromic responses to stimulation of the mPFC (top in A) and the BLA (bottom in A) had latencies of 10 and 8 ms, respectively. This neuron could follow twin pulse BLA stimulation at an interval of 3 ms (2nd trace in B) with an initial segment-somatodendritic break of an action potential (*), and the refractory time for this neuron was determined to be 2 ms (lowest trace in B). BLA stimulation evoked antidromic responses when given at 21 ms after mPFC stimulation (left in C). Decreasing the interstimulus interval to 20 ms failed to evoke antidromic responses to BLA stimulation because of collision (right in C). From these results, the conduction time between the soma and the branch point was calculated to be 0 ms. In the traces in A, 5 waveforms are superimposed. D: neurons projecting to both the mPFC and BLA (filled circles) were located in the ventral portion of CA1 and subiculum (S). Open triangles and open circles represent the location of neurons antidromically driven by BLA and mPFC stimulation, respectively. Values represent the distance from the bregma.
HIPPOCAMPAL PROJECTIONS TO BOTH THE PREFRONTAL CORTEX AND AMYGDALA

The stimulating and injection sites, which were positioned to maximize amygdala-evoked field potentials in the mPFC, were mainly located in the BLA and v-PrL. Subicular neurons had shorter antidromic latencies to amygdalar stimulation as compared with mPFC stimulation, whereas CA1 neurons showed similar antidromic latencies in response to mPFC and amygdalar stimulations. Furthermore, the shorter conduction time from the soma to the branch point of the axons projecting to both the mPFC and amygdala indicated the bifurcation of the axons near or at the soma.

It was found that VH neurons projecting to both the mPFC and amygdala were predominantly located in the subiculum and CA1. This is consistent with previous reports that the CA1 and subicular neurons in the VH project mainly to the mPFC (Jay and Witter 1991), and the amygdala receives major afferents from the subiculum and the CA1 region (Canteras and Swanson 1992; Pitkanen et al. 2000). Furthermore, these VH neurons are likely to innervate BLA neurons projecting to the v-PrL, which also receives direct inputs from the same VH neurons. This indicates that there are direct and indirect circuits from the same VH neurons to the v-PrL. In view of the modulatory effects of emotional states on the memory formation, it is possible that the indirect pathways through the BLA provide emotional information in the direct signal processing from the VH to the v-PrL, which plays crucial roles in short-term and working memory (Floresco et al. 1997; Seamans et al. 1998).

Because CA1 neurons projecting to both the mPFC and amygdala had approximately the same antidromic latencies to mPFC and amygdala stimulation, it is suggested that activation of those CA1 neurons causes simultaneous activation of both the mPFC and the amygdala. This seems to be in accordance with the hypothesis that synchronized firings of neurons in different brain regions are crucial for integration of multimodal information in cognitive brain mechanisms (Engel et al. 1999; Singer 1999). In contrast subicular neurons, which had shorter antidromic latencies to amygdala stimulation than to mPFC stimulation, may be involved differently in the functions related to the mPFC and amygdala. Both mPFC and amygdalar neurons show firing activity entrained to hippocampal theta rhythm, which is involved in memory formation or emotional regulation (Jensen 2005; McNaughton and Gray 2000; Pape et al. 1998; Seidenbecher et al. 2003). Therefore our findings suggest that simultaneous inputs from the same VH neurons may induce synchronized theta activity in the mPFC and amygdala. Because theta rhythm in the mPFC and amygdala have been reported to have a role in memory and emotion processes, respectively (Jensen 2005; Seidenbecher et al. 2003), theta rhythm synchronization of mPFC and amygdalar neurons may be important for the association of memory and emotion.

Because the VH plays crucial roles in both working memory and emotional behaviors (Hobin et al. 2006; Seamans et al. 1998; Trivedi and Cooper 2004), our study suggests that the same VH neurons may be activated during both of these processes. A recent study has reported that the microinjection of muscimol into the VH induced impairment of fear memory retrieval after extinction (Hobin et al. 2006). Considering that the amygdala and mPFC are implicated in fear memory extinction (Akirav et al. 2006; Milad and Quirk 2002; Morgan et al. 1993; Sotres-Bayon et al. 2006), VH neurons projecting to both the amygdala and mPFC may play a crucial role in fear memory retrieval after extinction. Further experiments are needed to elucidate the functional roles of VH neurons in memory and emotion in relation to the mPFC and amygdala.

ACKNOWLEDGMENTS

We thank Dr. J. Kawano for helpful technical advice and Dr. S. Taha for helpful discussion. We also thank K. Tanaka for secretarial work.

REFERENCES


