Neuroscience 166 (2010) 994–1007

HIPPOCAMPAL CELLS ENCODE PLACES BY FORMING SMALL ANATOMICAL CLUSTERS

N. H. NAKAMURA,[a,b]* M. FUKUNAGA,[c] K. T. AKAMA,[a] T. SOGA,[a,d] S. OGAWA[a,e] AND C. PAVLIDES[a]

[a]*Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10065, USA*

[b]*Okinawa Institute of Science and Technology, Okinawa 904-2234, Japan*

[c]*Advanced MRI, LFMI, NINDS, National Institutes of Health, Bethesda, MD 20892, USA*

[d]*Monash University, Bandar Sunway 46150, Malaysia*

[e]*University of Tsukuba, Tsukuba 305-8577, Japan*

Abstract—The hippocampus has been hypothesized to function as a "spatial" or "cognitive" map, however, the functional cellular organization of the spatial map remains a mystery. The majority of electrophysiological studies, thus far, have supported the view of a random-type organization in the hippocampus. However, using immediate early genes (IEGs) as an indicator of neuronal activity, we recently observed a cluster-type organization of hippocampal principal cells, whereby a small number (~4) of nearby cells were activated in rats exposed to a restricted part of an environment. To determine the fine structure of these clusters and to provide a 3D image of active hippocampal cells that encode for different parts of an environment, we established a functional mapping of IEGs *zif268* and *Homer1a*, using *in situ* hybridization and 3D-reconstruction imaging methods. We found that, in rats exposed to the same location twice, there were significantly more double IEG-expressing cells, and the clusters of nearby cells were more "tightly" formed, in comparison to rats exposed to two different locations. We propose that spatial encoding recruits specific cell ensembles in the hippocampus and that with repeated exposure to the same place the ensembles become better organized to more accurately represent the "spatial map." Published by Elsevier Ltd on behalf of IBRO.

Key words: place cells, functional organization, cell assemblies, nearest neighbor distance, *zif268*, *Homer 1a*.

*Correspondence to: N. H. Nakamura, Molecular Neuroscience Unit, Okinawa Institute of Science and Technology OITC 3FL, 12-2, Suzaki, Uruma, Okinawa 904-2234, Japan. Tel: +81-98-929-1358; fax: +81-98-929-0592.
E-mail address: nakamun@oist.jp (N. H. Nakamura).

Abbreviations: CON, home-cage control; D_{1-2}, the difference between the first and second nearest-neighbor distances; D_{1-2}', the normalized difference between the first and second nearest-neighbor distances; D_{2-3}, the difference between the second and third nearest-neighbor distances; D_{3-4}, the difference between the third and fourth nearest-neighbor distances; DAB, 3,3′-diaminobenzidine; DL, two different locations exposure; IEGs, immediate-early genes; ISH, *in situ* hybridization; Ku_{DG}, kurtosis; N_1, the nearest-neighbor distance; N_1', the normalized nearest-neighbor distance; N_2, the second nearest-neighbor distance; N_3, the third nearest-neighbor distance; Sk_G, skewness; SL, the same location exposure twice; 3D, three-dimensional; λ, the evenly scattered nearest-neighbor distance; ρ, the average cell density.

0306-4522/10 $ - see front matter. Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2009.12.069

Based on the discovery of "place cells" (O'Keefe and Dostrovsky, 1971), one of the functions of the hippocampus is proposed to be that of a "spatial" or "cognitive" map (O'Keefe and Nadel, 1978). The functional cellular organization of place cells, within the hippocampus, however, remains a mystery. Part of the problem in discerning a functional organization could be that single unit recordings are limited to a few neurons at a time which could account for the lack of a correlation between topographical and spatial organization. Perhaps due to this limitation, the majority of studies have reported little or no correlation between neighboring neurons and adjacent place fields. If anything, adjacent place cells could have very disparate place fields, and further, there is very little predictability between place fields in different environments (Muller and Kubie, 1987, 1989; Thompson and Best, 1989; Wilson and McNaughton, 1993; Tanila et al., 1997; Knierim et al., 1998). Thus, the consensus view, to date, is that the "spatial map" is randomly arranged, i.e., there is no topographic organization of place cells.

A handful of electrophysiological studies, however, have hinted at a cluster-type functional organization in the hippocampus. For example, Eichenbaum et al. (1989) reported that place cells in the CA1 field formed clusters, whereby, neighboring cells had adjacent and overlapping place fields. Furthermore, by recording from multiple cells with an array of electrodes in the CA1 and CA3, Hampson, Deadwyler and associates found that place cells encoding for space were concentrated in clusters, spaced approximately 200–400 μm apart (Deadwyler et al., 1996; Hampson et al., 1996). Interestingly, a discrete cluster-type organization was also observed in animals performing a spatial task (Hampson et al., 1999, 2002). All of the above mentioned studies, however, are limited in the ability to determine possible cluster-type organization in the hippocampus due to the small number of simultaneously recorded cells. A more representative organization, therefore, could only be achieved through the use of different methods which allow the sampling of the entire neuronal population.

Immediate-early genes (IEGs) have been identified as critical indicators of neuronal activity (Worley et al., 1993; Clayton, 2000). In hippocampal pyramidal neurons, spatial environmental stimuli rapidly and transiently induce expression of IEGs, such as *zif268* (also known as *Egr1* and *NGFI-A*; Cole et al., 1989; for reviews, see Davis et al., 2003; Knapska and Kaczmarek, 2004), *Arc* (Link et al., 1995; Lyford et al., 1995; for a review, see Guzowski, 2002), and *Homer1a* (Brakeman et al., 1997; Kato et al., 1998; for a review, see de Bartolomeis and Iasevoli, 2003).

In situ hybridization (ISH) and immunohistochemical techniques have detected context-specific cell ensemble activity in the hippocampus and neocortex at the single cell level (Chaudhuri et al., 1997; Guzowski et al., 1999; Vazdarjanova et al., 2002). We have recently found that in animals exposed to a restricted part of an environment, zif268-immunoreactive cells formed clusters of a few (3–5) active cells adjacent to clusters of non-active cells in the CA1 and CA3 (Pavlides et al., unpublished observation). A critical question that arises then is how this "spatial map" is formed in the hippocampus—does a cluster of neighboring neurons encode for each spatial component, or does the same cluster of cells become engaged in many different components of an environment, or a combination of both? The present study was aimed at investigating the precise configuration of IEG-expressing cell clusters, and determining how different clusters may participate in different parts of an environment. We have investigated patterns of active nearby CA1 neurons in animals exploring spatial environments, using ISH with *zif268* and *Homer1a* mRNAs and three dimensional (3D)-reconstruction imaging methods.

EXPERIMENTAL PROCEDURES

Subjects and behavioral manipulations

All procedures performed on animals were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996) and were approved by The Rockefeller University Animal Care and Use Committee. Adult male Sprague–Dawley rats (275–300 g on arrival; Charles River Laboratories, Wilmington, MA, USA) were housed with food and water available *ad libitum*, under a 12/12 h light/dark cycle in a temperature-controlled (22 °C) facility. Each animal was handled for 10 min daily for 3–4 weeks before the experiment. A radial eight-arm maze with only two arms attached was placed in a black painted room approximately 2.5 m^2 in diameter (Fig. 1A). Visual cues were placed distally to each of the arms. Proximal to one arm there was a single cue while proximal to the other arm there were several cues. The size of the animal's location on both arms was set to 10×30 cm^2 because place fields have been estimated to be on the average 20–25 cm region of space (Maurer et al., 2006; estimated from Wilson and McNaughton, 1993). Since place fields form rather quickly but are stabilized with repeated exposure (Kubie and Muller, 1991; Best and White, 1999), on two consecutive days before the experiments, each animal was exposed to

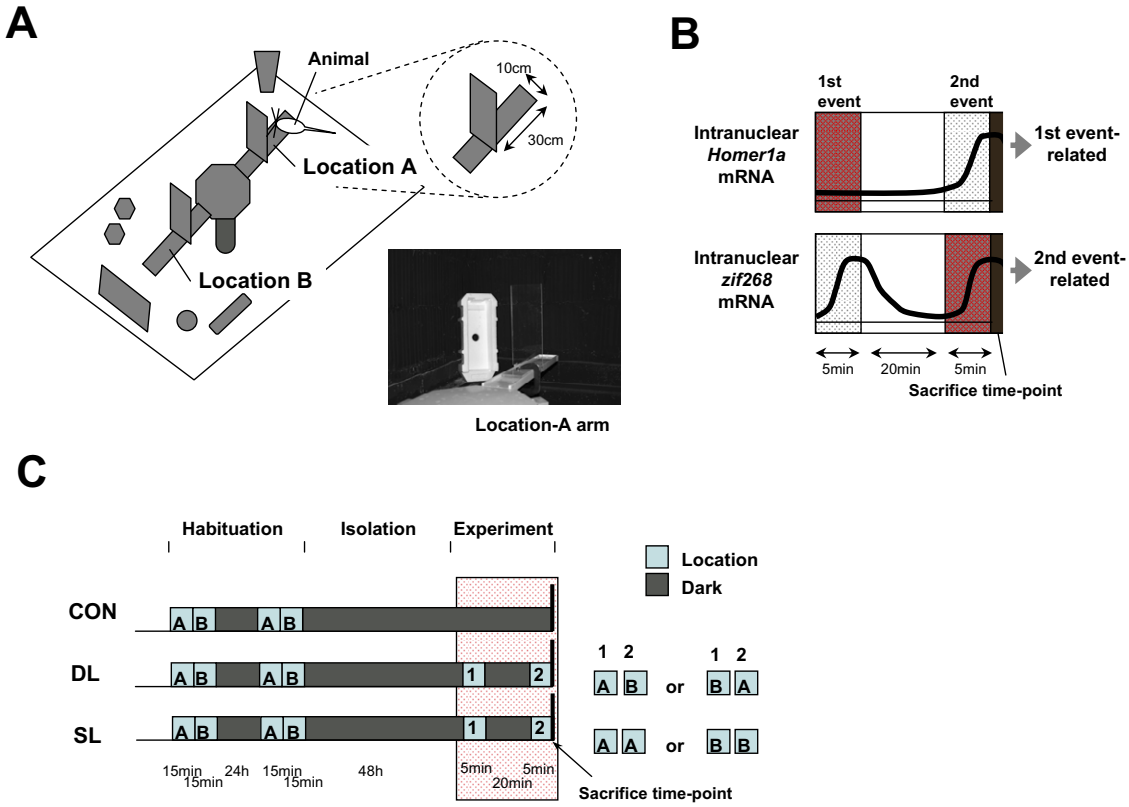

Fig. 1. Behavioral setup and experimental paradigm. (A) Environment. Behavioral testing was performed in a black painted room approximately 2.5 m^2 in diameter. An elevated eight-arm maze, with only two arms attached, was placed in the center of the room. The arms, which were 60 cm long, were sectioned off with a barrier in the middle of the arm and the animals were placed on Locations A or B (size: 10×30 cm^2 each) on the end of the arms. Distal to each arm were a number of cues. The room was always illuminated with red light. During place exposure of an animal, a white light was turned on. Included in the room was the isolation chamber in which the animals were kept. (B) Qualitative time course of peak expression of *Homer1a* and *zif268* mRNAs. The differential time course of these two genes allows for identification of neuronal activity of the two exposures. (C) Experimental Paradigm. Three experimental groups of animals were used—non-exposed controls (CON), exposed to two different locations once (DL), or exposed to the same location twice (SL). All animals were habituated to the arms for 15 min on two consecutive days. They were then returned to their home cage and placed in an isolation chamber for 48 h. On the day of the experiment, they were exposed to the arms as indicated and 30 min after the initial exposure were anesthetized and sacrificed. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.