Route Finding in a Complex Maze in Wild-Type and CA1 NR-1 KO Mice: Hippocampal Local Field Potentials, Single Units and Relationship with Behavior

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ABSTRACT

Spatial navigation relies on the brain ability to combine information from multiple streams in order to generate a ***cognitive map***, which is then used, for example, to find routes to a goal. This takes place in a network of brain structures centered on the hippocampus. Hippocampal place cells, cells that activate only when the animal is in a particular location in the environment combine sensory information with the estimate of animal's position updated by self-motion signals. Synaptic plasticity is important for these processes: Transgenic mice with a disfunctional NR-1 receptor in the CA1 hippocampal subfield are impaired in a route finding task on a star-shaped maze. We use a combination of neural ensemble electrophysiology and behavioral video tracking to analyze place cells in these mice and wild-type controls, showing how they adapt to the strategy adopted by the animal for route finding.

Author Keywords

Hippocampus, spatial behavior, place cells, NMDA, transgenic models, mouse.

INTRODUCTION

Navigation in a complex environment can rely on several different strategies: for example, subjects may decide on the route to take based on external landmarks (***allocentric*** strategies), or memorize a well-known route as a sequence of body movements (***egocentric strategy).*** The hippocampus is at the center of the network of brain structures supporting spatial memory and route computations, embodying the ***Cogntive Map*** function that have been proposed in the '40s by Tolman (1). In rodents, interference with hippocampal function with lesions studies, see e.g. (2), pharmacological see e.g. (3) and genomic means (4), impairs the animals' ability to navigate to a goal. On the other hand, when a route can be computed in terms of a simple set of body movements (e.g. right/left turns), the role of hippocampus seems less important (5). Interestingly, different hippocampal subregions appear to have different involvement in the acquisition of spatial memories (4), with CA1 disruption being more effective than intervention on CA3. For these reasons, the availability of specific genomic manipulation that affect only one of the hippocampal subfield have been particularly fruitful in order to dissect the neural circuitry of spatial navigation. In particular, a series of knockout models have targeted the NR-1 subunit of the NMDA receptor specifically in CA1 (6), CA3 (7) an the dentate gyrus (8), offering an unprecedented chance to explore how this receptor activity affects synaptic and system plasticity in each of these structures.

In addition to these behavioral effects, the implication of the hippocampus in spatial processing is critically supported by the correlation between space and the activity of hippocampal cells: in all hippocampal substructures ***place cells*** have been found**,** cells that activate each one as the animal traverses a different place in the environment. Taken together, place cells activities form a veritable map of the environment, which can be used to localize the animal. Place cells activity is the result of a computation involving multiple inputs. Most importantly, the hippocampus has to combine inputs related self motion, or ***path integration*** (9) and external cues. In order to dissociate these different contributions, and different navigational strategies, Rondi-Reig and co-workers. Rondi-Reig and co-workers (10) tested wild-type and CA1 NR-1 KO mice in a complex, star-shaped maze, where animals had to find the one (out of five) rewarded arm. In standard trials, mice always started from the same departure arm. NR-1 KO mice were found to be impaired in acquiring this task. In probe trials, mice started from a different arm, so that, if they followed a body-turn based strategy, they should end up in an arm different from the goal arm. If however, they based their route on external cues, they should still be able to reach the goal arm. Thus, NMDA receptors in CA1 are important for this type of route learning. However, nothing is known about how hippocampal cells encode routes computed according to different strategies.

We recorded neural activity in the CA1 hippocampal subfield, extracted instantaneous mouse position by video tracking and found that a large proportion of neurons there were place cells. Interestingly, during probe trials, place cells shifted their place cells according to the strategy used by the animal: in "egocentric" trials place fields rotated as if the new departure arm corresponded to the standard one. In "allocentric" trials, the map was instead preserved. These results suggest that the hippocampus is fully involved in the system responsible for path finding. We are currently analyzing the differences between KO and wild-type mice, also with respect to Local Field Potential (LFP) activities and behavioral learning curves.

MATERIALS AND METHODS

Animals

NR-1 KO specific for CA1 mice (C57Bl6 background) were bred in the lab of Dr. Rondi-Reig at the Université de Paris 6. They were transported in the Amsterdam lab upon weaning, in a litter contaning both homozygous (i.e. with an active mutation) and heterozygous subjects acting as controls. Behavioral training on the star-maze was performed for 10 sessions in 5 days. After that, a pair of subject composed by one control and one KO mouse was selected for surgical implant. Because the KO mutation remains specifically expressed in CA1 only until 2-1/2 months of age (11), all experimental procedures were performed up to that age limit. All experiments were carried our in accordance with the Dutch law, and upon approval of the institutional Committee for Animal Usage in Research (DEC).

Surgical Procedures

A microdrive containing 6 independently movable tetrodes, weighing less than 2 grams (12) was implanted over the right dorsal hippocampus (bregma AP: -2.0 mm ML: 2.2 mm) and anchored to the skull with micro-screws, under isoflurane-buprenorphine anesthesia. Tetrodes are bundles of 4 13 µm nichrome wires, which allow, by triangulation, discrimination of up to ~12 cells per tetrode. In general, our setup allows yields of 10-20 simultaneously recorded hippocampal cells.

After surgery, tetrodes were gradually lowered in order to reach the pyramidal layer of the CA1 hippocampal subfield. Electrode location was first assessed by distinctive signs in the LFP (theta rhythm, sharp wave/ripples compleces) and confirmed ***ex-post*** by histological examination.

Electrophysiological Techniques, Data Pre-Processing

Tetrode signals were amplified 2000x, bandpass filtered (600-6000 Hz) and digitized at 30 kHz sampling rate by the Neuralynx Cheetah data acquisition system (Neuralynx Inc., Bozeman MT U.S.A.). Spikes from the surrounding cells were detected by a threshold mechanism and a 1 ms snippet was recorded on disk for each spike. Additionally, the same tetrode signals were low pass filtered (475 Hz cutoff) and acquired as LFP.

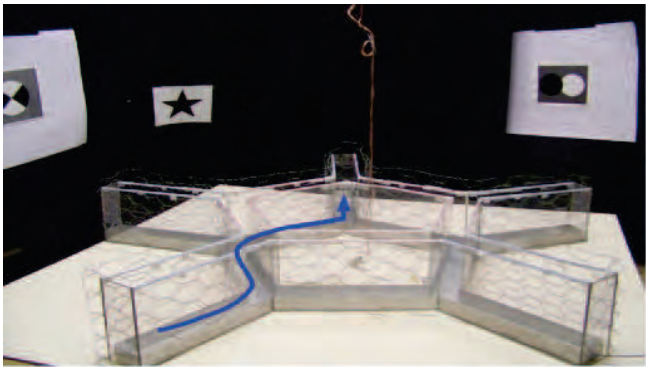

Figure 1. The star-maze, the behavioral apparatus used for this experiment.

After recording, spike signals were sorted and assigned to putative emitting neurons by means of a semi-automated clustering algorithm, KlustaKwik (13), further refined manually in the Klusters user interface (14).

Behavioral Monitoring

Correlating neural signals with animals position requires precise tracking of the mouse position. We accomplished this with the Ethovision XT software package, which was analyzing signal form a color video camera placed directly over the center of the maze. Synchronization of video and electrophysiological signals was obtained by recording timestamps signals from Ethovision (TCAP signals) in one of the Cheetah system's digital inputs. Custom-written MATLAB routines decoded the TCAP signals, providing a lookup table between the Ethovision and Cheetah timelines.

Behavioral Task.

The behavioral task took place in the starmaze (10), a maze formed by 5 alleys arranged in a pentagon, and 5 further arms connecting radially to the vertex of the pentagon, on its exterior (Figure 1). The maze was located in a room lined with black curtains, with a number of large geometrical cues hanging from the curtains. We placed mildly food-deprived mice in a departure (D) arm, and had to find a sugar pellet reward in another arm, the target (T) arm. In order to avoid that mice could find the reward by an odor trace, all arms are baited with sugar pellet, but the pellets are covered by a Plexiglas grill that makes them inaccessible in all arms but the T arm.

In probe trials, which occur every 3-5 normal trials, the mouse is placed in a different arm (P arm) at the beginning of the trial. In general probe trials are classified as allocentric if the mouse enters the T arm, egocentric if it ends up in the arm that bears the same spatial relationship with the P arm as the T arm does with the D arm. Trials with different outcomes are classified as error trials.

Mice are trained for 5 days (two 15 trials sessions per day). At the end of that period, animals animals that did not learned the task are discarded, and two of the remaining ones (1 mutant, 1 control) are implanted. The pair of implanted mice undergoes recording sessions during the

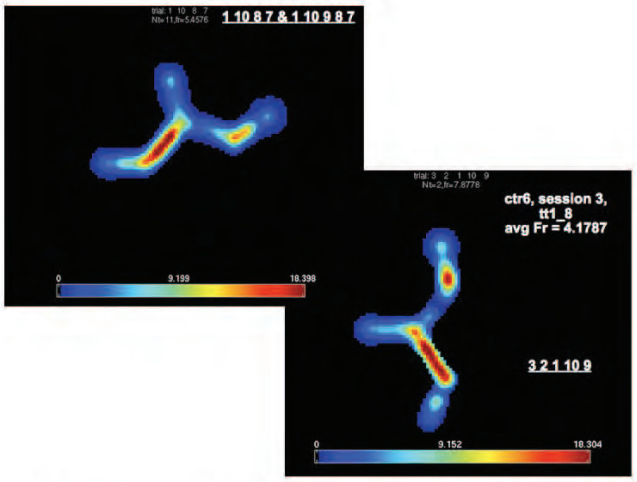

Figure 2. Place field map for a cells rotating its spatial correlates during an egocentric probe trial.

same days, with a counterbalanced schedule. Recording sessions entail 2 20-30 minutes sleep episodes before and after task performance.

RESULTS

After pre-training, both control and mutant mice were able to consistently find the T arm. However, consistently with Rondi-Reig et al. (2006), mutant performance was significantly lower ($p < 0.05$ 2-way ANOVA), using two specifically designed behavioral measures (15). During probe trials, both mutant and control mice were about equally likely to use a allocentric or egocentric strategy.We recorded from 5 control and 5 mutants mice (1 control mouse had to be excluded from analysis due to poor signal quality). From these mice, a total of 369 cells were recorded and so far analyzed. Data analysis is now under way: in our initial findings, there is a slight impairment in the spatial selectivity of CA1 place cells in mutants. One aspect of interest in the data is whether strategy selection affects

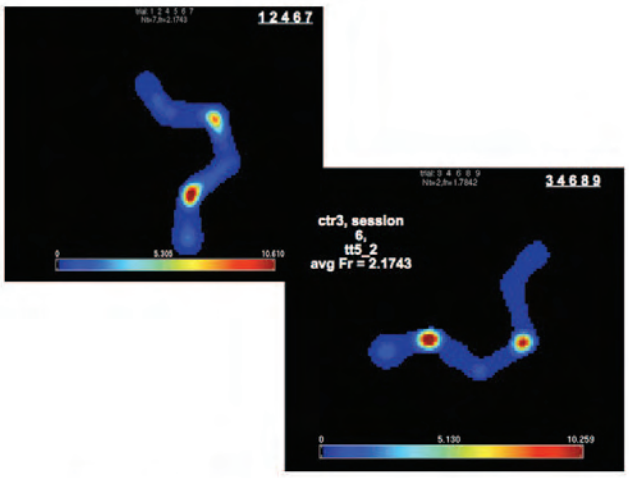

Figure 3. A second example analogous to Figure 2.

place cells firing. In fact, in egocentric probe trials, place cells tended to rotate their place field, maintaning a consistent relationship with the departure point. In contrast, during allocentric trial, different populations of place cells encode the visited arms (which differ from the arms traversed during regular trials; Figures 2-3). We are at the moment quantifying possible differences in this behavior between the mutants and the controls.

CONCLUSIONS

Our combination of neural ensemble electrophysiology in the mouse, genomic manipulations, and behavioral tracking offers novel possibilities for dissecting the neural basis of behavior. In addition to the mentioned results, we are now in the process of analyzing the detailed temporal structure of neural ensemble activity and its relationship with LFP oscillations.

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