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AMAZING PAPERS IN NEUROSCIENCE
Teaching Principles of Place Cells

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Animals navigate within their surrounding environment to find food, shelter, and mates; this behavior forms one of the most basic means of survival. The vertebrate hippocampus acts as an integration hub for varied dynamic processes such as attention, memory, perception, and decision-making. This ultimately allows an animal to move efficiently in its surroundings in search of food or to escape from predators. Place cells are neurons located within the hippocampus which are triggered in response to an animal entering specific places in its local environment. John O' Keefe first described the firing patterns of these cells in 1976 in a paper published in Experimental Neurology. This was a pioneering effort in combining the efficacy of electrophysiological recordings with the value of behavioral approaches in freely moving animals. The author also presented testable hypotheses of plausible mechanisms governing place cell activation which in turn provided a conceptual scaffold for a diverse range of subsequent work in the field. This is an excellent paper for undergraduate education because it provides the historical context to an important research avenue while simultaneously showing how clear and concise hypotheses can emerge from studying how neural activity correlates with animal behaviour.

Key words: hippocampus; place cell; displace cell; misplace cell; spatial receptive field; behavior

The hippocampus is a functional region in the vertebrate brain that has been implicated in memory consolidation for encoding the sequences of events. Lesions in hippocampal regions in rats have been observed to cause impairments in memory formation (Fortin et al., 2002). Long-term potentiation is observed in the hippocampus through AMPAR mediated long lasting increases in postsynaptic responses in hippocampal slices implicating the hippocampus in learning and memory (Whitlock et al., 2006). Hippocampal functionality interestingly also extends to navigation and acts as positioning system for rats (O'Keefe and Dostrovsky, 1971; Taube et al., 1990; Hafting et al., 2005). The complexity and diversity of hippocampal functions have led to divergent views and vigorous debate within the neuroscience community (reviewed in Brasier, 2017).

John O'Keefe and his colleagues pioneered the techniques of extracellular recordings from neurons in the medulla in freely moving rats, which were then extended to study the hippocampus (Ainsworth et al., 1969; reviewed in Moser et al., 2015). Work in the O'Keefe laboratory revealed the existence of neurons in the CA1 (*Cornu Ammonis*) region of the hippocampus that fire when an organism is positioned in particular locations in space. The discovery of these 'place cells' and related cell types in the rodent brain set into motion a series of discoveries which have helped uncover the principles governing the neural mechanisms of navigation and spatial learning and memory.

Here, I review one of the pioneering research papers in the field, "Place units in the hippocampus of the freely moving rat," published in the *Journal of Experimental Neurology* by John O' Keefe (1976). This paper outlines and familiarizes students with traditional, yet relevant methods for recording neural activity and observing corresponding behaviors in freely moving animals. It also provides a springboard to explore subsequent literature. In the early 1970s, O'Keefe and his colleagues hypothesized that place cells in the CA1 region of the hippocampus could have a conserved mechanism for signalling an animal's position in the environment. Since then, place cells have been found in rats, bats, birds, and even humans (Ulanovsky and Moss, 2011). Hippocampal lesions impair spatial navigation in rats (Morris et al., 1982). Research on place cells and grid cells (space-selective cells firing at multiple regularly spaced positions) resulted in a Nobel prize being awarded to John O'Keefe jointly with Edvard Moser and May Brit Moser in 2014. O'Keefe (1976) discusses traditional methodologies employed in recording from the hippocampal region in freely behaving rodents. Critically, O'Keefe presents four testable hypotheses of the possible role of these cells.

Students are often not exposed to the foundational papers underlying the results mentioned in textbooks. O'Keefe (1976) provides such a foundation and opens avenues for critical analysis of subsequent literature.

DESCRIPTION

O'Keefe performed electrophysiological recordings from rat hippocampus using implanted extracellular electrodes. Distinct spikes from individual cells (i.e., 'units') were selected for further analysis. Once a unit was selected, individual rats were forced to traverse a maze in search of rewards which were placed in distinct regions of the maze, all while the animal's behavior was recorded using a videotape recorder. The responses of each unit were observed as the rat performed a variety of behaviors (e.g., moving into different arms of the maze, eating, drinking, grooming, and sniffing). Extracellular recordings were taken from hippocampal units under visual and olfactory cues and unit responses were studied in the presence of varied orientations of the surroundings - for example, by changing the position of lights in the room, switching off lights, and providing patterned environmental stimuli. The experiments aimed to explore the environmental and intrinsic factors underpinning the specialized functionality of hippocampal units.

O'Keefe characterized the responses of CA1 region hippocampal cells relative to the environment and classified them into three types:

1. 'Place units' (firing depended on the rat's location in space);
2. 'Displace units' (firing depended on the rat's motor behavior but not on its position in space); and
3. 'Others' (firing patterns did not correlate with either position in space or behavior of animal).

Place units/cells fired preferentially in response to specific locations in the maze. These cells had a low basal spontaneous firing frequency which increased when the rat approached specific locales. Various behaviors like eating, drinking, and grooming also increased the activity of place units but much less so than the firing frequency of place units in their appropriate receptive fields. Neighboring units often had their receptive fields adjacent to each other- this suggested the possibility of cognitive spatial mapping of the surroundings in the brain of the animal. Interestingly, a sub-category of place cells termed 'misplace' cells were also documented. They were named misplace units because they fired when there was an apparent "misplacement" of what was actually perceived experienced and what was expected, i.e., when the rat encountered a novel object (O'Keefe, 1976). These cells fired in specific places in the maze, but the firing frequency was maximal when the rat encountered a novel object in its receptive field (the area in space where a particular unit fired preferentially with increased frequency).

Displace units, unlike the place units, responded to changes in a rat's motor behavior irrespective of its position in the maze – a pattern of activity similar to theta cells found in research from others (e.g., Feder and Ranck, 1973; Ranck, 1973). Activity in displace units was also positively correlated with the speed at which the animal ran. Responses were characterized by rhythmic sinusoidal activity (6 - 12 Hz) observed during voluntary behaviors such as running, exploring, sniffing, and jumping and an irregular high amplitude low frequency activity observed during automatic species specific behaviors like resting, eating, drinking, and grooming (Vanderwolf, 1971).

O'Keefe presented four hypotheses which aimed to provide a mechanistic understanding of place units. The first hypothesis suggested that place units might be responding to simple sensory cues like hearing, olfaction, and vision. Interestingly, earlier works (O'Keefe and Dostrovsky, 1971; Ranck, 1973) had never shown dependence on visual or tactile cues for the responses of hippocampal cells explored previously. In addition to preferential firing of place units when the rat was in the place field, the neuronal responses changed when there were changes in the environment, such as moving lights or their orientation in a particular direction. Subsequent studies showed that place cells fired in the blind rats as well as normal rats kept in the dark suggesting the role of combination of multimodal cues in addition to vision in modulating place cell activity (Quirk et al., 1990; Save et al., 1998). The experiments done in O'Keefe, 1976 could not pinpoint which specific sensory cues were responsible for place cell firing but could state that place cell responses were modulated by a combination of cues of different modalities.

The second hypothesis suggested that place cells responded to a sensory cue while performing a specific behavior. This hypothesis hoped to explain the working of misplace cells which usually fired in the presence of novel stimuli accompanied by sniffing and exploratory behavior. O'Keefe's suggestion that place cells fired as a response to a sensory cue while executing a particular behavior had its drawbacks because some place cells fired when the rat engaged in stationary sniffing in a particular location while some others fired when the rat ran past a part of a maze. It was difficult to decrypt the conditions of firing owing to varied responses of the rat while executing similar behaviors. This suggested that place cells had varied responses while rats performed the same behaviors and multimodal stimuli might have a confounding effect on cellular responses.

The third hypothesis proposed by O'Keefe postulated that stimuli had to be encountered in a specific temporal sequence for a place cell to be triggered. He suggested that the occurrence of a certain combination of stimuli in a particular temporal sequence could elicit activity in place cells. However, this raised the question of how the nervous system 'decided' to respond to a combination of stimuli. One possible explanation for the apparent response selectivity was reward and reinforcement (food or water) associated with the specific combinations of stimuli. A weakness of this hypothesis was that it could not explain observed responses of place cells in locations in the maze where there was no reward. Often, place cells fired only when the rat performed a specific behavior in one part of the maze but not in the other. This, yet again, implied that there was involvement of environmental factors that shaped place unit firing.

The fourth hypothesis was an attempt to incorporate all his observations and reconcile them with the effects of environmental factors. O'Keefe proposed the idea of a cognitive map which represented the position of the rat in its environment. It suggested that place cells fired when there were specific environmental factors that impinged upon the animal. Each place cell would convey two information streams – one conveying information of wide field environmental stimuli in the surroundings, and the other keeping track of movement of the animal based on its speed and the direction of motion. An accurate measure of the distance travelled, and the direction of motion could be carried out by displace cells, as these were observed to fire in response to changes in the movement and speed of the animal. Similarly, misplace units would fire when there was a mismatch between stimuli actually present in an environment and stimuli expected. The rat would have an expectation of which stimuli to expect while exploring its surroundings. Therefore, the cognitive map would be formed by the animal exploring its surroundings or correcting for any variances in the existing cognitive maps.

The cognitive map theory predicted that hippocampal lesions would prevent rats from performing well in spatial learning tasks and this was confirmed by O'Keefe et al. (1975). Ensuing studies enriched this idea and led to discoveries of more specialized cells like head direction cells (Taube et al., 1990) and grid cells (Hafting et al., 2005) that function in tandem with place cells to enable navigation.

VALUE

This paper shows how rodents can be used to study the neural basis of navigation. Moreover, this research pioneered the technique of neural recordings in freely behaving rats. Today, genetic manipulation capabilities and techniques like deep brain stimulation, electrophysiology, and optogenetics can be employed efficiently in genetically tractable rodents (i.e., mice) to explain to students how to better probe brain regions and get a holistic view of the role of place cells (Rickgauer et al., 2014; Pfeiffer and Foster, 2013).

In contrast to hypotheses driven research – which involves experimenting based on a scaffold built from hypotheses about the plausible guiding principles behind a phenomenon - this paper is an example of observation driven research. It highlights how empirical observations can generate testable hypotheses. Moreover, it gives students a *modus operandi* for when they encounter a wholly new phenomenon – it shows how descriptive studies of animal behaviour can give valuable information.

This paper is one of the pioneering research papers in the field, employing novel techniques to generate hypotheses for understanding how animals track their own location in space, and laid the groundwork for subsequent work. Significant strides have been made in recent times with researchers exploring place cell contributions in processing tactile (Gener et al., 2013), gustatory (Herzog et al., 2019) and auditory information (Aronov et al., 2017). Place cells have also been demonstrated to respond to passage of time (Kraus et al., 2013, Colgin, 2020). Interestingly, place cell firing can also undergo modulations depending on past experiences (Gereke et al., 2018) and goal / reward locations (Hollup et al., 2001; Gauthier and Tank, 2018) suggesting that place cells might be integration centers of attention, memory, and reward related behaviors. Overall, this research paper provided the initial foundation for a wide variety of work that ultimately resulted in O'Keefe, Moser and Moser winning the Nobel prize for Physiology and medicine.

AUDIENCE

This paper would be a good fit for 3rd and 4th year neuroscience and psychology undergraduate classes. Students reading this paper would need a basic background in neurophysiology and vertebrate brain anatomy. This paper could be used in behavioural neuroscience seminar courses to highlight foundational research into neural mechanisms of spatial navigation, how work progressed in this field in ensuing years, and how this work shaped other fields. The paper could also be deployed in courses focused on learning and memory and / or the neural basis of animal navigation. Importantly, this requires students to evaluate the validity of a set of proposed hypotheses, making it ideal for promoting critical thinking. This paper along with other papers of this time may help students assimilate different trains of thought prevalent during the 1970s and their condensation into current consensus views about place cell function.

GUIDELINES

In addition to research papers and reviews on this topic, this paper would fit well with the content of Chapter 30 (Memory) in the textbook *Neuroscience* by Dale Purves (Purves et al., 2019). Interactive sessions with students can be followed by a detailed discussion of scientific literature beginning with O'Keefe (1976), followed by subsequent research articles and reviews to clearly establish the train of thought of researchers over time. Instructors (and students) can refer to online lectures as primers (Center for Brains, Minds and Machines (CBMM), 2018; Grieves R 2019). Videos showing the progress of research and the life of Dr. John O'Keefe can be viewed to put the work in a personal context (Nobel Prize, 2014; Downstate TV, 2016). One interesting activity can be a discussion in proposing experiments and behavioral studies to better the experiments as mentioned in the paper. This will stimulate scientific thinking and critical analysis of the general process of experimentation.

A timeline can be constructed showing the growth and progression of ideas and research leading to a Nobel Prize. Works of Ranck (Ranck, 1973; Muller and Kubie, 1987) and O'Keefe (O'Keefe and Dostrovsky, 1971; O'Keefe and Conway, 1978; O'Keefe, 1978, O'Keefe and Nadel, 1978; O'Keefe and Speakman, 1987; O'Keefe et al., 1998; O'Keefe, 1979) led to the basic understanding of place cells and the idea of a cognitive map of surroundings (Nadel,1991; Ekstrom et al., 2003, Ulanovsky and Moss, 2011, reviewed in Moser et al., 2015). These articles can guide students to develop a sense for how ideas evolved and were distilled down to the general principles of spatial navigation and place cells now presented in textbooks (e.g., Chapter 30 in Purves et al., 2019).

CONCLUSIONS

Overall, O'Keefe (1976) is a pioneering study that serves as an ideal way to introduce the value of combining behavioural and electrophysiological analyses and to provide theoretical knowledge about specialized neurons in the hippocampus. Follow up articles and new literature give students a sense for how research in this field burgeoned and led to important discoveries of the underlying mechanisms governing learning and memory and spatial navigation in animals.

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