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Assessment of the Doctoral Thesis of Nikhil Ahuja

Title: Neuronal representation of a moving object in a novel spatial avoidance task

Supervisor: Prof. Ales Stuchlik, DSc.

The subject of this review is the Ph.D. thesis of Nikhil Ahuja *Neuronal representation of a moving object in a novel spatial avoidance task*. The central goal of the thesis was to develop a new behavioral paradigm in rats to 1) evaluate the spatial behavior with respect to the moving object, and 2) characterize the neuronal correlates of this behavior. The knowledge about the neuronal correlates associated with the perception of the moving object is still lacking when compared to the knowledge about the neuronal activity which accompanies spatial navigation in the space. Therefore, the topic of the thesis is timely.

In the introduction, the author reviews the existing literature and knowledge about the mechanisms of the neuronal correlates of various sorts of behaviors with a primary focus on spatial navigations. The author starts with a brief description of the hippocampal anatomical organization and each of its subfields. It is followed by a detailed description of the hippocampal electrophysiological features. The priority is given to CA1 neurons known as place cells and local field potentials generated within the hippocampus like theta activity, gamma rhythms, or sharp-wave ripples. The substantial part of the introduction is dedicated to the neuronal substrates of navigation and orientation in the space. The author cites relevant studies that explored the properties of place cells, their place-field or modification under various spatial conditions. The author also describes other functional cell types like head direction cells, grid cells, boundary cells, etc. The introduction then slowly shifts to the description of the

theoretical concept of the hippocampal cognitive map introduced by O'Keefe and on the role of the extra-hippocampal structures in the various forms of spatial navigation. The introductory section finishes with the latest knowledge about the neuronal substrates of socio-cognitive behavior and mainly how the brain encodes moving objects. The introduction is written well and summarizes the latest observations and data from this research field. The text is accompanied by appropriate figures and schematics that help the reader quickly understand complex processes like neural coding of various spatial features.

The goals of the thesis and Ph.D. research are clear. Firstly, the author aims to set up a new paradigm to to test animal behavior in response to the moving object. The author then focuses on the elucidation of the neural codes underlying the detection of the animals' position in respect to the moving object in various moving object representations. Relevant hypotheses accompany the aims.

The methods section describes surgical, behavioral, and electrophysiological techniques used in the research. The designed behavioral protocol is well described accompanied but comprehensive schematics which help the reader to understand the complexity of the behavioral testing. The techniques of tetrode fabrication, microdrive assembling, and implantation are also well described. The author presents procedures of spike sorting and single unit separation from the recorded data. It is followed by a section that describes the properties of the robot, its movement and the structure of implemented behavioral paradigms. Each method has its figure that helps to visualize the experimental procedures.

The result section's initial part is focused on the detailed analysis of the animal's behavior and robot avoidance learning in various stages of the training session. The author analyzed a myriad of parameters of rat's movement and avoidance in comparison to slow and fast-moving robots. The data shows that the designed protocol can be used to evaluate changes in behavior in relation to moving objects. Also, the animals can be trained to avoid either front, left, or right side of the robot. The results also demonstrated that the animals can detect and avoid slow as well as fast-moving robots. To obtain these results, the author used sophisticated techniques to analyze, quantify and visualize various features from the position tracking. Each result is accompanied by a brief summary that helps to interpret the results from a mechanistic viewpoint and explains well what type of information each parameter provides about the avoidance strategy. The behavioral section is followed by neuronal correlates of the observed behavior. The author was able to identify activity from 46 cells in three rats in no-shock conditions and 46 cells from one rat trained in robot avoidance task. The author identified a population of cells that seems to respond to the position of the robot and typical place cells. The cells, however, displayed heterogeneous responses characterized by a change of firing in respect to the position to the room, the position of the robot relative to the rat and vice versa. The results are accompanied by appropriate statistical tests.

Summary: The central goal of the thesis was to develop a new behavioral paradigm in rats to 1) evaluate the spatial behavior with respect to the moving object, and 2) characterize the neuronal correlates of this behavior. The knowledge about the neuronal correlates associated with the perception of the moving object is still lacking when compared to the knowledge about the neuronal activity which accompanies spatial navigation in the space. Therefore, the topic of the thesis is timely. From a methodological point

of view, it was an extremely demanding project because it required deep knowledge in behavioral neuroscience and good skills in *in vivo* electrophysiology and advanced techniques of signal and statistical processing. During the project new tools, methodological approaches and paradigms were successfully developed to achieve the proposed goals and test the hypotheses. From a scientific perspective, the thesis is of very good quality and the author brings novel observations and data about the behavioral and neuronal responses to moving objects that were not published yet. The scientific significance of obtained results is supported by several articles published in impacted journals where Nikhil Ahuja is the first author or co-author. The results of Nikhil Ahuja's work bring new and important insights into the mechanism of mammalian brain function.

Overall, I believe that Nikhil Ahuja has demonstrated considerable achievements as a researcher and fully warrants the most serious consideration of the Ph.D. viva committee to award Nikhil Ahuja a Ph.D. degree.

Here, I propose questions that could be asked during the candidate's viva.

1. Neural correlates of behavior represent an exciting research field of modern neuroscience? Place cells or similar cell types were identified in humans. Could the author comment about the existence of moving object cells in humans?
2. How are the studied moving-object cells related to mirror cells?
3. The animals responded to a different color of robots. Could the shape of the robot also impact the learning of the avoidance task, especially in the case of a white robot?
4. Did the author analyze local field potentials during the behavioral tasks? Is there any study that explored changes in the hippocampal oscillations with the moving object?
5. What is the role of the interneurons in the formation of cognitive maps or spatial representation? Does the author expect that interneurons could be involved in the neural mechanisms underlying the moving-object recognition?

Yours sincerely,

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