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Affective Cognitive Neuroscience Laboratory

Evaluation of Ph.D. thesis of Mateusz Kostecki, MSc, entitled: "Social transmission of information about the localization of food in rodents and its influence on the hippocampal representation of space" and conducted in the Laboratory of Emotions Neurobiology of the Nencki Institute of Experimental Biology Polish Academy of Sciences, under the supervision of professor Ewelina Knapska.

Kraków, 23 August 2024

In human societies, we often take for granted the accessibility of food in nearby shops, relying on well-established supply and distribution systems. However, for many animal species, transmitting information about food locations between individuals is critical for survival. For these species, obtaining reliable information about where food resources are available is not merely a convenience but a necessity that can determine their chances of survival. This knowledge exchange among peers allows individuals to exploit food sources more efficiently, reducing the time and energy spent searching for sustenance. Moreover, in environments where food is scarce or unpredictably distributed, the ability to share and receive information about food locations provides significant adaptive advantages, enhancing the survival and reproductive success of individuals within the group.

Understanding the mechanisms and conditions under which such social information is shared and utilized is essential for comprehending the broader dynamics of animal behavior and ecology. In this spirit, Mr. Mateusz Kostecki's Ph.D. thesis is devoted to developing and validating a behavioral paradigm based on the place preference test to study rodents' ability to detect food sources using social cues obtained during interactions with conspecifics. By combining this behavioral paradigm with studies of social interaction and brain imaging techniques, the Ph.D. candidate aimed to demonstrate that mice and rats can exchange information about food locations in their environment and that this information guides their exploratory behavior. Mr. Kostecki also sought to identify the neural mechanisms and likely channel for this information transfer. Based on the experimental results, the doctoral student proposed that both mice and rats can transfer information about the location of food to their conspecifics. He also suggested that cells encoding the food location in the hippocampus are

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reactivated during the purported information transfer and that this process is associated with increased neuronal activity in brain structures related to metabolism and homeostasis. In turn, the prolonged anogenital contact time was interpreted as a potential channel for information transfer. The thesis, supervised by Professor Ewelina Knapska, was prepared in the Laboratory of Emotions Neurobiology at the Nencki Institute of Experimental Biology Polish Academy of Sciences.

The dissertation counts 85 pages, and consists of the following sections: Summary in English and Polish, Contents, Acknowledgements, a general Introduction including aims, Materials and methods, Results, Discussion, Conclusion, Bibliography, and the Publication record of the PhD candidate.

A brief introduction, written with knowledge of the subject and flair, contains references to numerous studies by predecessors and vivid descriptions of the experiments they conducted. I appreciate that this section is framed within the interaction between social neuroscience and spatial cognition. The subsection on spatial navigation is engaging and contains a wealth of relevant information. The role of the hippocampus in olfaction is well described, and the suggestion regarding the involvement of the olfactory tubercle in the processing of social information offers a solid basis and justification for fiber photometry imaging in this brain region. Although I am a great proponent of brevity and a concise description of the topic, it seems that the subject could be treated a bit more broadly by adding, for example, a paragraph on social behaviors in rodents and the transmission of social information among them. The introduction would also benefit from better text formatting, as there are currently many one-sentence paragraphs without any justification, such as on page 16. The objectives of the work are described clearly and concisely. However, the mix of tenses can be somewhat confusing—sometimes the present perfect tense is used, sometimes the simple past, and other times the present tense. In my opinion, it also seems more appropriate to write a personal doctoral dissertation in the first-person singular, reserving the use of 'we' for writing publications. This observation leads to another point regarding the role and independence of the doctoral candidate in conducting the described experiments. Nowhere in the dissertation (except for a brief and very casual mention in the acknowledgments) did I find information about who else, and to which extent, was involved in carrying out the experiments. This information is important because, without it, it is difficult to assess the doctoral student's leading role in the presented work.

The methodology is described somewhat modestly. There is no comprehensive outline of the experiments conducted or their timeline. The description of the conduct and analysis of social interactions is brief (two sentences), and there is no description of the analysis of ultrasonic vocalizations. Additionally, details such as the total number of animals used or phase of the cycle, during which the animals were tested, are missing. Such a general description of the procedures might be more acceptable if the main results had already been published. However, in the context of a doctoral

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dissertation that is not based on publications, I would expect a detailed account of all the procedures used.

My greatest methodological concern is the unequal time that the donors spent in the different compartments of the apparatus—8 minutes in the target compartment and 3 minutes in the second compartment. This uneven exposure to the odor associated with each compartment seems to be the simplest explanation for the recipients' behavior. In my view, the combination of the longer exposure to the smell of the target compartment and the potential smell of food from the donors' mouths may have caused the recipients to spend more time in the target compartment. If that were the case, the only conclusion that can be drawn from the obtained results is that the animals associate the smell of the compartment with the smell of food and that the donor might be the vector of this information. In a sense, this is a social transfer of information about food, but it seems to have little to do with the intentional transmission.

There are also concerns about the statistical analysis of data from the behavioral experiments. It appears that the "F" and "no int" groups were compared using t-tests against a common "F+L" group. This approach is questionable because these three groups should be subjected to a common analysis, such as ANOVA. The description of the statistical analysis also lacks details regarding the analysis of social behavior and USV data, calcium imaging and c-Fos activity mapping.

The Results section contains well-described and clearly presented experimental data. However, it is unfortunate that the figure descriptions do not include information about the number of animals per group, clarification of whether the bars represent the mean or median or something else, and what the error bars represent. Additionally, there is no justification provided for the choice of data presentation—whether a bar graph or a box-and-whisker plot was used.

Like the introduction, the discussion is eloquently written and demonstrates a deep understanding of the subject. The doctoral student masterfully navigates the intricacies of his work with expertise and finesse, earning genuine admiration. However, also here, I have a few critical remarks. First, I believe the discussion should begin with a concise summary of the obtained results. Instead, it opens with a definitive statement that mice can transmit information about the location of food. As noted by the doctoral student himself, this assertion raises many questions, yet these questions are not listed in the end. On the other hand, I really appreciate placing the discussion in an evolutionary context and considering the ecological relevance of the obtained results. Indeed, in the case of mice, which are territorial animals, it isn't easy to imagine the ecological relevance and evolutionary sense of the social transfer of information about food. The doctoral candidate undertook the discussion of this issue and did so interestingly and appropriately.

The discussion somewhat lacks considerations on how the information about the location of food would be generated, transmitted, and interpreted without the use of some form of language. The

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assumption that the channel of information transfer is scents excreted by the Donor in the anogenital region during the interaction, or even by mouth is somewhat exaggerated, as it assumes that this method can describe reality. It would be extremely helpful if, during the defense, the candidate could attempt to explain how scents could be used to abstractly convey information about a location.

Concluding, despite a few critical remarks that came to my mind while reading the thesis, I evaluate this dissertation positively. I have not found serious flaws or errors in the experiments forming the core of the thesis. The overall quality of the thesis is high and it has been a pleasure to read and evaluate it. The candidate demonstrated an excellent command of an impressive array of scientific tools and techniques, and an ability to combine these skills to address an important and novel research question.

To sum up, the evaluated dissertation reports the results of original research that addresses a well-defined scientific problem, demonstrates the candidate's general theoretical knowledge and his capability of conducting independent research. Therefore, I conclude that the presented doctoral dissertation meets the conditions specified in Article 187 of the Act of 20 July 2018, the Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended) and I recommend the Scientific Council of the Nencki Institute of Experimental Biology Polish Academy of Sciences to admit Mr. Mateusz Kostecki to the further stages of the doctoral procedure.

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Review of doctoral thesis of PhD candidate Mateusz Kostecki

Below is a review of the dissertation titled: “Social transmission of information about the localization of food in rodents and its influence on the hippocampal representation of space” by PhD candidate Mateusz Kostecki supervised by Prof. Ewalina Knapska.

Summary of aims

In social species, the social environment is a rich source of information, that influences motivated behavior such as exploration. Social cues can indicate to an animal that a certain spatial location should be approached or avoided. In the beautifully written introduction, the rationale and background for this research are presented with a comprehensive reference to previous literature. The introduction lays out the challenge of understanding how mapping of spatial information is related to complex behavior and emphasized the gap in the field stemming from the use of highly artificial behavioral paradigms. The introduction reveals a deep neurobiological perspective and raises some tantalizing ideas about the function of the hippocampus in donating meaning to environmental stimuli. This thesis aims to study different aspects of this behavior, ranging from behavioral to molecular, with the overarching aim of uncovering neural circuits involved in motivated behavior. Specifically, the aim of these studies is to develop an ethological behavioral paradigm that will test neurobiological mechanisms underlying the representation of contextual information transmitted by social cues. The focus of the study is on hippocampal and olfactory interactions, especially regarding remapping of spatial information based on social information about food availability. In contrast with previous studies that used artificial settings, the ethological setup of this set of studies renders it more translationally relevant.

Employment of research methodology

To test these fascinating questions, the experimenters used a combination of cutting-edge neuroscience methods in both rats and mice. In vivo calcium imaging was performed with fiber photometery to record activity of cell populations, in vivo single cell activity was performed via miniscope GRIN lens recordings of the hippocampus. Moreover, whole-brain imaging of the immediate early gene c-Fos was performed via brain clearing with the iDISCO protocol and lightsheet microscopy. These methods were used to determine the neural representation within an adapted version of the place preference test with odor discrimination for different contexts. Setting up each of these methods is a complicated task, and require extensive training from the surgeries to data collection, and the successful culmination of these efforts in the presented

results evidence independent work, stamina and trouble-shooting abilities. Furthermore, the data analysis of each of these is complex (signal processing for the calcium imaging and big data network analysis for the brain-wide imaging), and demand the experimenter to develop a vast range of expertise.

Presented results

The experiments presented in the dissertation show some evidence that mice as well as rats develop a place preference for an arena containing food after interacting with a demonstrator or donor who ate the food in this arena. However, in terms of the neurobiological mechanisms, most of the hypothesized effects were not found. There was no difference in stability of cells between the F and F+L groups. This finding is interpreted as showing remapping in both conditions in the discussion, but, perhaps outside of my expertise, I am not sure what led to this conclusion, in the lack of a stable point of comparison (i.e. maybe this is as stable as the system gets? As mentioned in the discussion, this could be a technical issue). Next, cells 10X more active in the target arena compared to non-target were classified as context cells. I assume non-target cells were 10X more active in the non-target arena although this is not specified, nor the percentage of cells that fit these criteria. Some evidence for hippocampal modulation by the task was identified, with indication of reduced activity in the non-target cells during onset of nose-to-nose interactions in the F+L condition but not the F condition. This is interpreted as increased activity in the F+L condition but not the L condition in the discussion, but it would be interesting to see other interpretations, such as the non-target cells are inhibited as being irrelevant. Or that some cells are temporarily inhibited following social interaction to allow a focus on social cognition. Notably, It does appear that there is a significant difference in between the target and non-target cells 2 frames after initial nose-to-nose interaction, apparently in the F condition, but no difference in this time period in the F+L condition but an opposite trend, maybe due to high variability. For target cells in the F+L group, It seems like most cells are briefly inhibited one second prior to the initiation of interaction. Perhaps the coding of the initiation is slightly inaccurate or animals plan the interaction before it occurs. Finally, no effect was observed in the olfactory tubercle, however increased c-Fos levels were observed in the tuberomammillary and ventral premammillary nuclei, areas associated with metabolism and homeostasis.

Discussion

The thoughtful discussion covers a range of topics and offers additional future experiments to address the questions raised by the results. Before delving into these, in the beginning of the discussion the possible purpose for using social information of food location in mice. The need to conduct more naturalistic

experiments is discussed. I agree, and would say that despite the framing of this paradigm as an ethologically valid test, I would say that the place preference task is probably missing several important aspects of the behavior being studied. But it is very difficult to perform miniscope and photometry experiments in a complex setting, so this is an understandable compromise.

Choice of literature cited

the introduction and discussion comprehensively cover a range of areas relating to the transfer of food preferences in rodents as well as relating to hippocampal functions in memory and learning and meaning attribution. The researcher can find interest in the work of Prof. David Eilam, who has published several studies and reviews on the social modulation of foraging behavior in rats, some of these are added below. In addition, the possible role of the dopaminergic system is mentioned in the discussion, with VTA as a target of interest. In that line, previous research has shown that the nucleus accumbens plays a role in STFP, indicating this as an interesting region to investigate. Finally, the idea of a systemic view of predictive homeostatic coding raised in the discussion is wonderful, and a recent publication may also be of interest to the author.

Specific comments:

- If hierarchy was quantified, I would check if this could be integrated into the analysis. This parameter may cause increased variability in the results as animals treat information differently depending on the dominance hierarchy position of the individual transmitting information. As mice were housed in groups of 4, a dominance test would provide this information, this assuming donors and recipients were from the same homecage (this is not specified).
- I am curious to hear why the “test” with food information was given to the donor on the same day as the information transfer to the recipient. Wouldn’t it have been stronger to condition the donor to receive food in one compartment but not the other one, and only then test the transfer of knowledge without the presence of actual food?
- For the miniscope recording, the donor was placed in a mesh tube, was the affective state of the donor altered by this?
- The authors suggest that mice learn the target arena based on olfactory cues from anogenital region that combine information about the scent and food. Yet, they found that even in the absence of odor discrimination mice still developed a preference for the target arena. This was not mediated by time near the door. Moreover, rats had the interaction with the donor in a separate location, so they could not point to the correct arena. I am curious

to hear what is the alternative hypothesis. the interesting finding about different frequencies of the vocalisations of rats in the F vs. F+L groups, suggest that vocal transmission of information may be playing a role.

- I would split the animals into learners and non-learners and look at the data that way, since in the behavioral figures it appears that there is a categorical difference, where some animals develop the preference for the target area while others do not.
- It does appear that there is a significant difference in between the target and non-target cells 2 frames after initial nose-to-nose interaction, apparently in the F condition, but no difference in this time period in the F+L condition but an opposite trend, maybe due to high variability as there were only 24 cells in one of these groups. For target cells in the F+L group, It seems like most cells are briefly inhibited one second prior to the initiation of interaction. Perhaps the coding of the initiation is slightly inaccurate or animals plan the interaction before it occurs.
- What is the translation of frames to time? It would have been easier to interpret.
- Some of the figures were called out of order, a brief interpretation of each result along the text would have been helpful. Fig 21 & 22 have the same title accidentally.

Suggested References

Dorfman A, Weiss O, Hagbi Z, Levi A, Eilam D. Social spatial cognition. Neurosci Biobehav Rev. 2021 Feb;121:277-290. doi: 10.1016/j.neubiorev.2020.12.023. Epub 2020 Dec 26. PMID: 33373664.

Dorfman A, Eilam D. Social spatial cognition: social distance dynamics as an identifier of social interactions. Anim Cogn. 2021 May;24(3):407-418. doi: 10.1007/s10071-020-01441-9. Epub 2020 Oct 13. PMID: 33048261.

Dorfman A, Eilam D. The hierarchy of food, sociality, and experience in spatial decision-making by food-deprived rats. Behav Processes. 2018 Dec;157:1-6. doi: 10.1016/j.beproc.2018.08.006. Epub 2018 Aug 22. PMID: 30142379.

Weiss O, Dorfman A, Ram T, Zadicario P, Eilam D. Rats do not eat alone in public: Food-deprived rats socialize rather than competing for baits. PLoS One. 2017 Mar 9;12(3):e0173302. doi: 10.1371/journal.pone.0173302. PMID: 28278246; PMCID: PMC5344391.

Noguer-Calabús I, Schäble S, Kalenscher T. Lesions of nucleus accumbens shell abolish socially transmitted food preferences. Eur J Neurosci. 2022 Nov;56(10):5795-5809. doi: 10.1111/ejn.15827. Epub 2022 Sep 30. PMID: 36151057.

Ciaunica A, Shmeleva EV, Levin M. The brain is not mental! coupling neuronal and immune cellular processing in human organisms. Front Integr Neurosci. 2023 May 17;17:1057622. doi: 10.3389/fnint.2023.1057622. PMID: 37265513; PMCID: PMC10230067.

Conclusion

Thank you for sharing this work with me, I enjoyed reading it and can appreciate the technical challenges that it posed. I would like to encourage further exploration of this data, including more sophisticated behavioral analysis of movement and interactions, and matching the neural data with behavioral types. This work shows maturity and understanding of the field and the problems of interest in the field, wishing you the best of luck going forward!

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Evaluation for the PhD thesis " *Social transmission of information about the localization of food in rodents and its influence on the hippocampal representation of space*" by Mateusz Kostecki

I am very happy to evaluate this thesis, which, in my eyes, is outstanding. If you have any questions, feel free to contact me. The thesis does not only demonstrate that rodents can use social information to find food, but also describes the mechanisms behind this in the brain using an array of state-of-the-art methods and shows planning by the animals based on their energetic state. I have thoroughly enjoyed reading it and the student can be admitted for the defence without reservation. I do find the thesis exceptional, but have minor problems here and there. For example, I think it could have profited by being read by a native speaker. There are no profound mistakes, but just the typical mistakes I am familiar from other Polish colleagues (too many "the" here and none "there...) small typos and formatting errors. I feel like the presentation of figures is very inconsistent. Some are very pretty, others seem of low resolution....find most of these really very small comments below.

The introduction is well structured and gives a good overview over the existing literature, clearly identifying the knowledge gaps that can be addressed. I really like how he challenges some existing ideas and then leads the reader up to a hypothesis that logically follows from this, for example in the chapter about spatial navigation and how place cells might not be completely understood yet. I happen to know that Mateusz is very interested in the effects captivity has on the behaviour of animals, an understudied but hugely important topic and an interest that I share and I was interested to see how he discussed it in the context of planning his own work. He also ties the subchapters together well, for example when establishing a potential paradigm for testing remapping of place cells without a change in the physical environment by using social cues, discussed in the previous subchapter and then putting it into the greater context again. He makes a compelling case at the end that the use of

social cues may allow bridging knowledge between areas of research more naturally than previous studies have done. He even formulates hypotheses, which should be standard, but I find it appallingly rare in most theses I read.

The methods are generally well described. Sometimes it would have been nice to repeat at the beginning of each section what it was used for as in this format (one document instead of divided up into papers as is the norm here) the sections referring to each method are far apart and for example at first, I did not understand why viral vectors were injected for the endoscopic imaging, which probably would be clear for an expert on this method. I did understand after reading the caption of Figure 1. Overall, the methods are clear, detailed enough but not too much with citations where appropriate.

One thing that was not clear to me is why the sections in the behaviour setup were analysed by 3cmx3cm bins as really it only matters in which of the three sections they were.

The behaviour results are fascinating for me (I was not familiar with place preference setups). I like how we are led through the results (there is a preference -> it is mitigated by social interaction -> it is transferred via the anogenital region -> rats can do it, too/broaden the scope....) and how alternative explanations are carefully tested and excluded.

In the results of the MINISCOPE recordings I find it a bit hard to compare the many figures. Maybe instead of such large ones, fewer figures with a and b panels allowing to compare visually (for example figure 16 and 17 or figs 23 and 24, which do not need to be so big anyway and are actually examples of ones that seem low resolution). And as one has to navigate a lot between the text and the corresponding figures the captions could have been a bit more informative (one example: Fig. 16. The closest text above refers to figure 24 and so I really have to think about what these target cell activities refer to). I think there must be a mistake in figures 21 and 22, the titles (which are unusual for figures anyway both say F group, but the captions say F and F+L, which makes more sense. In general I wonder why there are so many plots for the behaviourally non-significant nose-to-nose interactions and none for the nose-to-anogenital, which actually were the relevant ones. At least some examples would have been nice. What do I learn from these results, given that they were not relevant for the behaviour? Or maybe I misunderstand something here. Some figures also have gigantic axis labels (e.g., figs 29 and 30). So the result section, even though so interesting per se, is probably my least favourite of the thesis.

The discussion then is more up to the general standard of the rest of the thesis. I would argue about the placement of the ecological and

evolutionary relevant paragraph (which I really like), which for me would be long more at the end of the discussion after discussing the actual results first.

In the following section (on the channels of info transfer) the student discusses the result that anogenital sniffing was more explanatory than nose-to-nose and concludes saying that "breath smell is probably still the most important". I am not sure I found an explanation anywhere how information about place might be transferred in ANY odour be it smell or anogenital. At the same time I myself can detect, for example, alcohol on the breath of someone in the same room, I don't have to put my nose close to their mouth for that, so the relevant bit of information might be something novel? That discussion section is a tiny bit weak. I do like the discussion of some sections further down where he has to explain why the hypotheses were not met (which I always find the most interesting results). Throughout he offers scenarios for further testing and also points out potential weaknesses in his own designs, which is really all that can be expected. I particularly like how he links his results to homeostasis and energetics at the end, he really thinks beyond the limitations of his immediate study system and I am interested to follow what he does in the future.

Overall, again, I think this thesis is more than sufficient for obtaining a PhD and I look forward to (hopefully) hearing his defense.

Sincerely,