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Evaluation of the thesis
„Social learning about rewards – how information from others
helps to adapt to changing environment"
by Maciej Winiarski
prepared under the supervision of dr hab. Ewelina Knapska and dr Alicja Puścian
in the Laboratory of Neurobiology of Emotions
of the Nencki Institute of Experimental Biology of the Polish Academy of Sciences

Maciej Winiarski's thesis focuses on the exploration of changes in the interactions among a group of animals involved in social transmission of information about a novel stimulus introduced in the environment. The thesis explores a potential mechanism of neuronal plasticity involved in social learning and places an emphasis on the evolutionarily conserved development of adaptive social behaviors, and thus investigates a problem with significant relevance for our understanding of the causes of maladaptive changes in social behaviors associated with psychopathologies in humans. The topic of the thesis is of broad interest in the field of neuroscience and has potential translational relevance.

The thesis has 99 numbered pages and was written in English. The organization of the thesis follows the usual structure, with main chapters of Introduction, Materials and Methods, Results, and Discussion, and also including required summaries in Polish and English. The literature section cites over 350 references. The thesis also includes lists of publications co-authored by the student and research projects he participated in. I have no major criticisms with regard to the language of the thesis, only noticed minor editing mistakes (e.g., missing bracket on page 10, double dot on page 15, '2/12h' on page 32, a dot in the middle of a sentence on page 68, etc…). I am afraid my command of English might not be sufficient to provide a complete evaluation of the thesis language, nevertheless, it was my impression that there were scattered errors in the use of articles. This probably includes the title, where an 'a' appears to be missing before 'changing'.

The Introduction has 20 pages and extensively describes the state of research on social interactions in animals, including empathy, the ability to learn by observing other individuals, and mechanisms involved in the transfer of information among animals. The references cited included observations from the study of laboratory animals as well as a very broad overview of behaviors in lower vertebrates, arthropods, and even single-cell organisms. The final part of the Introduction describes the role of the prefrontal cortex in controlling social behaviors and also the involvement of extracellular matrix metalloproteinases in neuronal plasticity involved in social learning. There are two major issues I would like to rise with regard to the Introduction. References cover the last 5 decades of research, which clearly shows a good command of the scientific literature related to the subject of the thesis. However, I think that in some cases the references are very loosely related to the statement they are attached to (for instance: page 23 Noworyta-Sokołowska et al. 2019; page 27 Bellone and Luscher 2006, Mameli et al., 2011, Marquez et al., 2013; page 29 Cieślak et al., 2018). Moreover, in my humble opinion in some cases references to the work of the groups of Kay Tye or Gül Dölen and Roberta Malenka are missing.

My second issue is related to the structure of the Introduction. While the part focused on social interactions in different animal species is extensive, the section focused on the role of the prefrontal cortex is rather brief, and the introduction to the mechanisms of action of the extracellular protein matrix metalloproteinases and the tissue inhibitor of metalloproteinases 1 (TIMP1) is very short. The latter two receive much more emphasis in the Results and Discussion, and thus the Introduction should have been more comprehensive with regard to their potential roles and the rationale for their study in the context of neuronal plasticity involved in social learning. Finally, the Introduction lacks a summary at the very end and probably also a final paragraph defining specific problems to be tackled.

The aims of the thesis are stated as the development of novel models for the study of socially-mediated learning, analysis of the transfer of information about potential rewards, the effect of environment familiarity on social learning, and the role extracellular matrix metalloproteinase 9 in neuronal plasticity involved in social learning. The aims are presented in a way that resembles a research plan rather than a set of hypotheses or a description of the area of exploration.

Materials and methods are presented on 13 pages and include 5 figures, among them helpful diagrams of the Eco-HAB instrument. The research methodology includes several protocols for the assessment of animal behavior in Eco-HAB instruments, a U-tube social dominance test, and intracerebral injections of nanoparticles carrying the human TIMP1 protein. The Python language scripts used for the analysis of behavioral data are provided through a GitHub repository and are openly available. I have no significant issues to rise with regard to the description of the Methods, only a few minor comments. The description of behavioral procedures lacks references to specific local ethics committee permits for the behavioral experiments and I could not find the Kolmogorov-Smirnov test under statistical methods (5.12). Additionally, it was not clear to me during which day phase the 12-h experimental stages were conducted. I also have a few more pedantic remarks: Figure 1 showing an experimental subject is probably not necessary, Figure 2 with the transponders is definitely not necessary (they are a different type from the ones used), and also there is no actual ‘materials’ section under ‘Materials and Methods’.

The Results section is 21 pages long and divided into 4 chapters describing the main experiments: analysis of changes in the behavior of a group of animals in the Eco-HAB upon exposure to a novel stimulus, the effect of intra-cerebral TIMP1 application on the persistence of behavioral changes, social learning in a novel environment and finally identification of group hierarchies and their effect on the transfer of social information. I found the second part of the results to be particularly interesting, in particular, the evidence for stable and persistent social hierarchies and reported changes induced by a novel stimulus and treatment with TIMP1. The experimental framework presented in the thesis may have potential further use in analyses of the mechanisms involved in the social transfer of information, and associated changes in neuronal plasticity occurring in the prefrontal cortex. Nevertheless, I have some major criticisms with regard to this part of the thesis. First concerns the analysis of the data and its presentation in figures. The results of statistical analyses are listed in the text, but missing from figures, and in some instances, it was not clear to me how the comparisons were performed (e.g., Figures 20 and 23, panels C&D). Furthermore, in some cases analysis of variance followed by a *post hoc* test was probably required, instead of simple pairwise comparisons (e.g., data presented in Figures 14, 15A, 16, 20 C & D and 23 C & D). More rigorous analysis may have affected the statistical significance of some of the results reported (for instance the effect of TIMP1 administration on bottle preference presented in Figure 14A), nevertheless in my opinion the majority of the conclusions is sufficiently cautious to remain intact after a revision of statistics. Additionally, some of the

figures have very low resolution (Figure 20 A&B and Figure 23 A&B) in particular, to the extent that it is difficult to decipher the results presented.

A second major criticism of the Results section regards the parts which are missing I could not find the results of some of the experiments mentioned, in particular, the localization of nanoparticle injections (described in section 5.5 of the Methods), and also the very limited presentation of the results from U-tube social dominance test (only as a correlation in Figure 22). Moreover, the description of the analysis of the strengths of social links is rather brief. The results presented in Figure 23 are awkwardly placed at the very end of the text and summarized in just a few sentences. As noted, these results could be of particular interest, and in my opinion, deserved a more comprehensive description. Finally, I have one additional minor issue regarding Results: the D'Agostino-Pearson test does not confirm the normality of a sample distribution but rather may detect potential significant departures from normality.

The Discussion is presented on 10 pages and focuses on the interpretation of experimental results, emphasizing methodological aspects and the role of extracellular matrix metalloproteinase 9 in neuronal plasticity. The focus on conclusions in the discussion confirms the candidate's capacity for data analysis and inference based on results. Extensive references to relevant previous studies are made, including citations of reports focused on different animal species, and also comprehensive references to previous studies from the candidate's research group. A separate section with Conclusions completes the Discussion, highlighting the new methodology for the study of socially transferred information and the role of MMP9 in neuronal plasticity underlying social learning.

In the response to the evaluation I would like to ask for responses to the following issues related to the experiments and their interpretation:

1. Why were male mice selected as the object of study? Disregarding the current recommendations for including both sexes in research, *Mus musculus* males and females appear to have significant differences in their social behaviors. Briefly, while female mice were reported to display prosocial and cooperative behaviors, interactions between adult male mice appear to be mostly antagonistic.

2. Is the scent left by an animal who had access to saccharose a reward? If I understand correctly, in the first two sets of experiments described under Results, the animals remaining in the Eco-HAB had no access to saccharose, and thus could not associate the taste or metabolic effects of saccharose with scent.

3. Why choose the human TIMP1 for intracerebral injections? Is it identical to murine?

4. What was the rationale for choosing the prelimbic cortex as the site of TIMP1 injection to modulate the social transfer of information? The prelimbic cortex in mice is a functional, but not an anatomical equivalent of some subareas of the human prefrontal cortex.

My evaluation lists extensive criticisms and even challenges some of the conclusions presented in the thesis. Nevertheless, I am convinced that the thesis confirms that the candidate has general knowledge in the area of research and is capable of conducting research independently. The methodology for the analysis of social interactions presented in the thesis is a valuable contribution to the field and together with data on the

mechanisms of plasticity in the prefrontal cortex represents an original solution to a research problem. Therefore, the thesis of Maciej Winiarski fulfills the requirements of Polish law (specifically: art. 187 Ustawy z dnia 20 lipca 2018 roku Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2021 r. poz. 478, 619, 1630)). I am recommending that Maciej Winiarski is allowed to proceed with further steps toward the defense of the thesis.

Sincerely,

dr hab. Jan Rodriguez Parkitna
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10/04/2023

Scientific Council,

Nencki Institute for Experimental Biology,

Evaluation of Ph.D. dissertation of Maciej Winiarski

This dissertation details a very nice and original study which cleverly uses the cutting-edge Eco-HAB experimental system to explore important questions related to social learning and group behavior in a semi-naturalistic environment, and utilizes a examinenanoparticles delivery system to explore how TIMP1 release in the prelimbic prefrontal cortex (PL) influences these behavioral parameters. The thesis is very well written and organized in a logical and clear manner. The student deonstrates excellent knowledge of the professional literature, a very clever design of the experimental procedures and very good methodological skills. In my eyes, this dissertation should certainly be confirmed. I do have several reservations, though, and I recommend that the student and supervisors will take them in account and consider correcting the dissertation accordingly:

Major issues

1. The main issue with the dissertation is the use of the term “reward” to describe the information transferred by the demonstrators in the various experiments. This reservation emerges from the fact that the study doesn’t really show that the information is related to reward and not to diet change. It is true that sucrose is well known to be rewarding to mice, but it is also significantly changing the diet of the mice and most probably influences the gut microbiome and leaves traces in the bedding which may attract a lot of attention by the group members in the Eco-HAB system. Such enhanced attention to the bedding may explain the preference of this bedding and the higher consumption of water

from bottles located near it. This is indeed social transmission of information but not necessary regarding reward, in the hedonic sense of the word, but regarding diet changes. In order to show that reward is indeed involved here, the student should show similar results regarding other rewarding stimuli, amphetamines, for example, which do not involve diet change. Since this was not shown, I would suggest to attenuate the use of the term "reward" in the dissertation in general, especially in the discussion and specifically in the title.

2. I am intrigued by the lack of histological analysis of the injection sites in the TIMP1 experiments. Especially this is important since the student claims that it specifically targets the PL, a very small target in mice. Thus, I am not sure that the students can really claim that it reached this area and no other prefrontal areas without a proper histological analysis.
3. The student claims that the effect of the TIMP1 nanoparticles is mainly on synaptic plasticity, but there is no validation of this claim. While MMP9 is known to be important for synaptic plasticity it most probably participate in many neuronal processes and the effect of TIMP1 may be on other process as well. Hence, I would refrain from using the term synaptic plasticity and just claim that proper function of the PL (if the histology justifies that) is necessary for the social learning in the Eco-HAB system.

Minor issues:

1. Page 14, last paragraph: I think that you can't talk about group behavior in semi-natural environment without referring to Shemesh et al. 2013 (VPMID: 24015357) and Weissbrod et al. 2013 (PMID: 23771126).
2. Page 15, 2nd paragraph, line 5: 1991).. – correct to 1991).
3. Page 15, 3rd paragraph, line 7: helping behavior in rats should also refer to Ben0Ami Bartal et al., 2011 (PMID: 22158823).
4. Page 16, 1st paragraph, line 7: we use mice, instead of "we show mice".
5. Page 16, 2nd paragraph, line 8: inter-species, instead of "between species".

6. Page 17, 2nd paragraph, line 8: encoded by urine or by skin instead of "encoded in urine or in skin"
7. Page 17, 3rd paragraph, line 2: use the term chemosensory instead of "olfaction", as the vomeronasal system is not "olfaction".
8. Page 18, 1st line: parasite-free instead of "parasite free".
9. Page 18, 4th paragraph, 1st line: "The most robust types of social cues that inform about the potential danger are fresh carcasses or wounded individuals" – I don't think that these may be called "social cues"!
10. Page 21, tile of 3.9: Relationship between animals istead of "Relationship between the animals".
11. Page 32, 1st paragraph, line 10: 12h/12h instead of "2h/12h".
12. Page 33, last two line: phosphate-buffered saline (PBS).
13. Page 37, last paragraph, 7 lines from bottom: "It was defined as a proportion of visits to the compartment containing social olfactory stimulus, to the visits to the compartment containing non-social (control) olfactory stimulus during the testing phase" – definition is not clear.
14. Page 38, 3rd paragraph, 1st line: environment,
15. Page 39, 1st paragraph, last line: Why not normalizing for individual motor activity within cohort?
16. Page 45, last paragraph: use locomotion activity instead of "activity", which is too general.
17. Page 47, 1st paragraph: p = 0.094). Approach
18. Fig. 14A: ANOVA test is needed.
19. Fig. 15A: How come the Y-axis title is "Followings" if the values are fractions? Also, how do you know that TIMP1 by itself does not reduce following regardless of the reward? The comparison with the CTRL is borderline significant.
20. Page 68, 1st paragraph, line 5: just ate and intensified.
21. Page 71, 2nd paragraph, line 8: "its significant boost" is wrong in English. Try using "significant augmentation".
22. Page 73, last sentence of the 1st paragraph: "Nevertheless, my results show that the proper level of MMP9 activity, even if not exclusively involved, is crucial in

social learning about rewards." As discussed above, I don't think that this conclusion is justified, neither for the use of the "reward" term, nor for the use of the "social learning" term.

23. Page 74, 2nd paragraph, line 2: Dang et al., 2019;
24. Page 74, 3rd paragraph, line 4: transferred social information instead of "socially transferred information".

Summary: This is a very good, scholarly-written dissertation that should be confirmed and justifies the degree of Ph.D.

Sincerely,

Professor Shlomo Wagner, Ph.D.

Head, Sagol Department of Neurobiology,

The University of Haifa, Haifa, Israel.

Evaluation by Dr. Cristina Marquez

EVALUATION OF THE PhD THESIS BY Maciej Winiarski

Social learning about rewards – how information from others helps to adapt to changing environment

Conclusion of the evaluation: Positive.

Please find below the reasoning of my evaluation, of what I think is an exceptional PhD thesis, not only for its structure and formal presentation, but for the rigor and clarity of the results obtained.

The present thesis addresses a very important and unsolved question in neuroscience, how animals learn from others, using a very interesting semi-naturalistic paradigm that allows for the quantitative study of groups of animals (mice). Specifically, this doctoral thesis focuses on how animals use information of other to locate rewards and how social structure of the group affects this transmission of socially relevant information.

Very well-articulated introduction, very comprehensive and up to date review of the literature. Nicely organized to introduce and justify the experiments proposed in this thesis, in a global and multispecies approach. The introduction reflects a high mature view of the field of the author and perfectly guides the reader into the presentation of the research goals. The purpose of the present doctoral thesis is aimed at describing and testing how appetitive learning is modulated by social experience, meaning, how animals learn from others in foraging contexts, how it is affected by familiarity and social structure, and elucidate the role of synaptic plasticity at the level of prefrontal cortex in these processes. All these are very relevant questions, important and unsolved in the field, to my evaluation of the questions addressed is also excellent.

Regarding the design and methods of the experiments, they are very innovative, complex and excellent, allowing to address these important questions in a highly controlled manner.

The results section is structured in the 4 main questions performed in 3 experiments. The section is very well written with adequate statistics analysis and visualization of the results, and stating in every step the rationale for each experimental question.

Scent of a rewarded mouse attracts other mice and changes the pattern of social interactions

In the first set of experiments, the author performed a new protocol for the study of reward social learning using the Eco-HAB system. The authors demonstrate that the introduction of reward information through smell of conspecifics bedding influences reward seeking in the colony and interestingly alters the sociability levels of the group (info about reward in the experimental arena increased the number of followings of the animals). These results are extremely interesting and novel.

Disrupting synaptic plasticity in the prelimbic cortex impairs response to the scent of a rewarded mouse

Once the behavioral effect is clearly described, the author went one step further and questioned how disrupting synaptic plasticity in PrL would affect this social learning of the scent of a rewarded mouse. The main finding here is that synaptic plasticity in PrL mostly abolishes the persistence in odor seeking. Although there is a tendency in reducing the proportion of visits of the reward area, it is not significant. The main effect seen is in how persistent this exploration is, meaning that animals with altered PrL synaptic plasticity can detect the social information, but they do not seek for it. Interestingly this

manipulation did not affect the sociability levels (both control and TIMP1 sociability distributions are shifted to the right) but did reduce the number of followings in the setup, with no changes in locomotor activity.

Social olfactory information helps to find the reward in a novel environment, which requires an intact prelimbic cortex

This section represents a very interesting expansion of the above described results. In the previous experiments, animals would explore familiar environments. In this section, the author explores how social learning is expressed when animas move in a novel environment and they need more information in order to find actual reward. This is, again a very pertinent, interesting and unexplored question.

In these experiments, animals that do not have intact synaptic plasticity in PrL learn worse about the location of the reward (drink less the rewarding bottle) and interestingly, persist much less in seeking the reward. It seems that PrL has a specific importance in how persistent and maybe motivated the animals are in seeking for socially informed reward. This effect is not explained by a hedonic effect of the PrL manipulation.

Disruption of PrL in this experiment again reduces the followings of the animals as before, but here it does affect in cohort sociability. Moreover, in this novel context, manipulation in PrL does affect general locomotor activity, maybe explained by novelty induced exploration.

Study of social networks in this task

In the last chapter of the results section, the author performs a very interesting study on how social structure and social networks might be affected and affecting the results described above.

Manipulation in the PrL alters the progressive stabilization of the social hierarchy. This results are very interesting, however, what is really impressive is the demonstration that some animals specifically change more their behavior when socially relevant information about rewards is present. The author finds that not the whole social network changes, but specifically some of the animals increase their following behavior, and furthermore demonstrates that this correlates with social hierarchy as measured in another test (u test).

This type of analysis is really rich and has a lot of potential to extract more information about what is happening in the social group in the process of studying social learning of rewards. I personally enjoyed very much this section, which I find of utmost interest and novelty compared with the type of analysis and approaches performed in the field regarding these subjects.

The thesis ends with an extended discussion that recapitulates and puts in the same frame all the results obtained in this thesis. In general, the field has tended to study the effects of social learning (and social behavior in general) focusing on processes that are based in threatful or fearful information. I agree with the author of this thesis that information obout social processes based on rewards are very important, too, and have been largely neglected in the literature. This thesis reproduces in a semi-naturalistic environment a very interesting process, how information contained in the urine of an animal carries information about rewards, that is used by other animals of the group. This behavioral protocol that the authors establish for the first time in this thesis is very important, as it allows for the manipulation and

study of its neurobiological processes. This thesis also provides work in this sense, by manipulating synaptic plasticity in prefrontal cortex.

In conclusion, this thesis is exceptional and supposes an advancement in the knowledge that we have on how animals use social cues to guide behavior. The results obtained here are of basic research nature, and do no aim to provide a direct practical application. However, the implementation of this behavioral protocols into the Eco-HAB system is providing proof on how potent this system is and the extensive possibilities that it has, once the experimental controls are well into place and time is devoted to analyze the powerful datasets that this system provides.

Cristina Marquez, PhD

Evaluation by Dr. Cristina Marquez

To whom it might concern,

I was asked to provide my opinion on whether this doctoral thesis should be considered as outstanding. In my opinion, there is no doubt that it deserves this recognition.

As explained in my report, the questions that this doctoral thesis addresses are complety new and they are addressed with such a rigor and clarity that the author manages to obtain very interesting and important results. I am sure that these results will form a very important publication (a part of the other publications where the author has already participated as a co-author). In my opinion, it is exceptional that a PhD student finishes his doctoral training with such a good record of publication being the main subject still to out in the public.

In conclusion, yes, I recommend the recognition of this thesis as outstanding.

Kind regards

Cristina Marquez, PhD