Comments to the Author

Review of the preregistration "Are the more flexible great-tailed grackles also better at inhibition?"

This is an interesting proposal which has the potential to answer very important questions about a topic critical to behavioral ecology – the role that inhibition may (or may not) play in the evolution of behavioral flexibility.

The authors provide a brief introduction to the project, focused hypotheses/ predictions, and great detail about their project timeline and methods. My comments for each section are below:

- Abstract.
    - The background makes it seem like the goal of this project will be to test if our test-based measures of behavioral flexibility can reliably predict realized behavioral flexibility (i.e. answering the question – can we use tests of behavioral flexibility in order to predict a species' ability to move into a new environment)? However, the goal of this project is to test whether or not behavioral flexibility predicts inhibition and the consistency of relevant tests. I suggest restructuring the abstract to reflect the goals of this project – they need to explicitly address why linking behavioral flexibility to inhibition is interesting and important to their overall question.
- Predictions.
    - P1 is well structured, as are the alternatives. However, if the hypothesis is that "flexibility requires inhibition", then they should test whether or not inhibition predicts flexibility (rather than the other way around).
    - P2 does not follow from the hypothesis – I suggest switching P2 with its alternative to maintain a consistent structure.
    - The authors should explain why the go-no go test is being validated against the delayed gratification test (rather than against the detour test or vice versa).
    - P3 needs to be restructured to follow that of P1 – include P3 and alternatives separately.
- Methods.
    - As written, the methods and analysis plan are difficult to follow. I suggest organizing the methods per prediction (i.e. listing the dependent/independent variables under each section).

General comments:

This preregistration describes a series of experiments (detour, go no-go, delayed gratification) in order to investigate inhibition in the great-tailed grackle. The goal is then to combine these results with other experiments on flexibility on the same individuals, and ask whether behavioural flexibility requires a certain level of inhibition. While the role of inhibition in cognition has been broadly studied, few or no studies have attempted to understand the relationship between inhibition and flexibility. The experiments described are well designed, the predictions clearly laid out and the analysis appears to be statistically sound. Therefore, I believe that this project would constitute a welcome addition to the literature once it is completed.

Nevertheless, I have a few reservations with regards to the preregistration itself. My main comment is that it is hard for the reader to fully understand the rationale and the execution of the experiments. This project is part of a larger scale endeavour, and little effort was made to make all the relevant information easily accessible in this preregistration, sometimes making it difficult to assess. Additionally, the structure is confusing at times. For instance, all the details of the methodology are given in the very last section, while many of the predictions and other aspects of the experimentation are described elsewhere. It would be easier to understand if the rationale, the methodology, the predictions and the analysis for each experiment was given as a clear sequence.
My second main comment is that it is unclear why great-tailed grackles are a good system for answering the questions of interest. The main reason given to study flexibility & inhibition in this system is that "they have rapidly expanded their range into North America over the past 140 years". However, little information is given on the ecology of this species. Furthermore, I feel that ecological relevance in the cognitive tasks has been neglected. Species-specific traits can promote or impair individuals' performance in laboratory tasks, and hence I believe it is important to integrate the ecological challenges encountered by these birds when predicting the outcome of experiments. For instance, under what circumstances is inhibition beneficial for these birds? Do they require to be behaviourally flexible in their expanded range? Do they often need to choose between several options? Is there a lot of competition between individuals for accessing food? What do they typically feed on? How do they access this food? Such attributes might affect the outcome of experiments. It would thus be wise to predict, in the light of the grackles' ecology, which experiments might represent an additional challenge, and which ones might be more straightforward for them.

Specific comments:

Please note that my comments refer to the pages of the PDF I was given for review.

Page 3

Section C

P1: Why test for higher quantity of food rewards if this is not linked to inhibition? Does it bring additional information? Or is it to confirm the results from Hillemann et al. 2014?

P1 alternatives: It seems that it will be virtually impossible to disentangle between these two alternatives. How will this be interpreted?

P2 alternative: what about the detour task?

Figure 2.
"…where food items are transferred from the serving lid to the storing lid with delays ranging from 2-1280 seconds"
Shouldn't it be "from the storing lid to the serving lid"?

Page 4

Go no-go task: why introduce the negative stimulus? For each coloured dot there is a correct & an incorrect behaviour, one could thus simply reward all correct behaviour, equally for both situations. I am wondering whether the introduction of a negative stimulus will actually help understand the results. In both cases, the bird must choose to peck or to refrain to peck. When it pecks, it can get a reward. When it doesn't peck, it can only avoid getting the negative stimulus, but never gets a reward. A simple strategy could be to just peck all the time, and have exactly the same amount of rewards than if it did the task correctly, although with additional delays. I fear that it will remain unclear how the negative stimulus affected the bird's ability to solve this task. If the negative stimulus has a huge impact, birds might learn fast, but if this is a minor disturbance, they might accept many more errors. Similarly, is it mostly the stimulus itself, or the added delay that is influencing learning? I believe it would be more straightforward to give rewards for correct choices, and no rewards for incorrect choices, all the time.

Page 5

P3: why only two and not ½? Testing half of the individuals would allow for more power in the comparisons, and this would provide a fully counterbalanced design.
Furthermore, what will happen to these two birds doing the detour task before the flexibility manipulations, will they also experience the other inhibition manipulations (delayed gratification, go no-go)? If so in which order?

Page 6

P1.2.a 85% correct over how many trials? I now see this is described at the end of the

manuscript. Would be good to include this here, or at least to refer to the section where the details are explained.

Page 7

Flexibility 1 & 2: What is "the last reversal an individual experienced"? Similarly, it is not clear what is "the first 40 trials in their final reversal after the individual has seen the newly rewarded option once". I understand this must be described in another preregistration, but the reader is left with the task of reading a whole other preregistration to figure out what is going on. Maybe expand this section a little to give more details? I think just a couple sentences might be enough. Or at least point to the relevant section of the other preregistration?

It is quite unclear what is the measure described in "flexibility 4".

Page 8

I would consider leaving the random effect "experimenter" in the analysis whether or not including it creates statistically significant differences across models. There is always some variance explained by such random effects, even when not significant.

Page 14

Have you decided to change the analyses after reading McElreath (2016)?

Page 15

I entirely understand the need for a day off in the experiments. However, does this mean that some individuals will experience a 1-day break during their experiments? Or between training and experiments? If so, this should be included in the analysis as a random factor, whenever possible.

Page 21

Here the training criterion is >1 item in at least 3 trials. In the detour task (page 22), the criterion is to correctly retrieve the food reward on the first attempt in 4 of 5 consecutive trials before receiving the test. In the go no-go task (page 24), the criterion is to retrieve food immediately in 8 out of the most recent 10 opportunities. Why use different criteria for the different experiments?

Page 22

Test: "subjects were allowed to retrieve the item on all trials regardless of the accuracy of their first attempt".
With this method, the bird gets a reward both when it makes the correct choice and the incorrect choice. This has the potential to reduce learning drastically, since making a "wrong" choice only slightly delays access to the reward, but is still a rewarding choice. Is there a specific reason for letting the bird access the reward regardless of its performance in the test?

Page 24

If some of the birds experience other manipulations (or a significant time interval between the initial training and the test phase) whilst other birds are directly trained and tested, this has the potential to affect their learning abilities. If this is the case, I'd recommend giving a few training trials again to the birds which don't go straight from training to testing.

Page 25

It is not clear when the bird receives the reward. Does it get rewarded immediately after pecking on the rewarded stimulus? Or at the end of the 10s presentation?
Rewarding the bird at the end of the 10s presentation will also introduce a delay in getting the reward which can affect their abilities to associate the stimulus with the reward.

This is an interesting study that examines the relationship between behavioural flexibility and inhibition using great-tailed grackles as a study model. This investigation for the understanding about whether flexibility as a trait would co-evolve with other traits in facilitating a species establishment and expansion. Overall, I find the study design is appropriate; tasks are set to measure behavioural flexibility as well as inhibition. However, as the submitted document is a preprint (as opposed to a written manuscript with completed data collection and analyses), my comments here are solely down for the submitted version. I hope the authors could address each comment during the revision.

1) Rationale of the study: The title itself highlights the investigation interest lies in behavioural flexibility and inhibitory control. Yet, the abstract and the introduction have not even explained why inhibitory control would be selected as the main investigation of the study. Accordingly, some information about the relationship between behavioural flexibility and how it is likely related to other traits, as well as the reasons for selecting inhibition in particular, would have strengthen the rationale for the study. This additional information will greatly help to work out the logics in hypotheses testing.

2) Rationale of the tasks: Authors should highlight the reason(s) for using the three inhibition tasks when measuring inhibition, in particular there are some critics about the use of the cylinder task (van Horik et al., 2018).

3) Clarity in concepts: Authors defined behavioural flexibility is the behavioural changes with increased experience or the outcome of learning. While flexibility in discrimination-reversal learning task is clearly learning based, flexibility in novel food-extraction task (or some forms of variants as in multi-access box in this project) has been discussed is a different form of flexibility from those that seen in discrimination-reversal learning task (Audet & Lefebvre, 2017). In this case, it is not entirely sure what forms of flexibility authors are measuring and if these forms of flexibility are correlated with inhibition at all. Inhibition in novel food-extraction tasks could be seen in a design that requires individuals to absolute a previously learned technique in the face of a similar task. At any rate, could authors clarify which form of flexibility they would like to measure?

4) Procedures of tasks: a) P.3 Figure 2. Add ‘but far from the bird’ in ‘(near the experimenter)’; this will make a contrast statement for ‘(near the bird)’.

b) P.3 Figure 2. Three trials, consecutive trials?

c) P.3 Figure 2. In the sentence ‘Once they pass training (by waiting for more than one food item in three trials), they move on to the test where food items are transferred from the serving to the storing lid with delays ranging from 2-1280 seconds.’, ‘food items are transferred from the serving to the storing lid’ should be 'from the storing to the severing lids', right?

d) Colour discrimination test - I understand authors have counterbalanced the colour presentation for birds in the colour discrimination test. However, are the chosen green and purple colours neutral to the birds (i.e. birds should not show a colour preference in either colour)? This is because if a bird shows any preference to one colour and that colour is rewarded in the discrimination phase, it could mask the true learning rate. If a bird is rewarded with the non-preferred colour in the discrimination phase, this would be measuring inhibition.

e) To speed up birds’ learning process in using touch screen, authors could also consider shaping. The stimulus (0.5cm diameter) in the ‘moving dot’ phase might be relatively small for birds, which would reduce the probability for birds to hit the target and likely prolong the training process. Therefore, shaping process in which a much larger stimulus (say 2.5cm diameter) at this stage would increase the probability of pecking the touch screen for a bird, followed by slowly reducing the diameter of the stimulus to 0.5cm may facilitate birds’ learning to use touch screen.

f) Authors might also want to consider a habituation phase to the touch screen for birds to explore the testing environment/operant box or, to minimise any neophobic responses. Also, make sure birds are always tested in the same compartment/box.

References Audet and Lefebvre (2017). What's flexible in behavioral flexibility? Behav. Ecol., 28, 943–947,https://doi.org/10.1093/beheco/arx007

van Horik, Whiteside, Laker, Beardsworth, Madden (2018). Do detour tasks provide accurate assays of inhibitory control? Proc Biol Sci. 285,1875). pii: 20180150. doi: 10.1098/rspb.2018.0150.
Review as PDF
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