nature portfolio

Peer Review File

Dopaminergic mechanisms of dynamical social specialization

Corresponding Author: Dr Philippe Faure

Any redactions in this file are there to maintain patient confidentiality, the confidentiality of unpublished data, or to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
In their manuscript, Solie et al. investigates how group socialization of animals contributes to specification of goal-directed behavior using an elegant semi-natural, reinforcement based foraging assay. In this assay mice live in an environment where they must learn to press a lever to obtain food. When single mice live in the environment, they segregate into two groups, “achievers” that rapidly learn to press the lever and almost immediately consume the food pellet earned, and “storers” mice that press the lever but do not necessarily consume the food right away building up food pellets to eat at a later time. Interestingly, when same-sex triplets of mice are placed in the assay, behavior phenotypes are more diverse and sex-dependent. Males segregated into three different groups including “scroungers” that do not press a lever but essentially steal pellets that other mice earned; whereas, females mostly fall into “storer” phenotypes. Combining mathematical modeling, in vivo electrophysiology, fiber photometry and optogenetics the authors implicate ventral tegmental area (VTA) dopaminergic (DAergic) neuron signaling in contributing to specialization of foraging behavior in group settings as well as competition in males underlying emergence of the “scrounging” phenotype which is flexible and may be reduced by decreasing competition. Overall, the concepts introduced in the manuscript are interesting, significant, and thoughtfully described. The idea that behavioral specification is not fixed but is flexible based on learning is a novel concept and the work should open new avenues for investigation of social behavior on learning. Results, particularly in the foraging assay, are robust and the role of dopamine in the assay are intriguing. However, experiments testing the role of exploration/exploitation dynamics and dopamine in shaping behavioral phenotypes need to be better controlled to support the hypotheses proposed. Additionally, the concept that behavioral specification is flexible based on learning could be more directly tested. Specific major points to be addressed include:

1. Data showing that group housed animals exhibit different foraging phenotypes compared to single housed mice is very compelling. In males, however, it seems that “independent” and “worker” mice described in Figure 3 are essentially “achiever” mice described in Figure 1, combining these groups seems warranted to simplify and better focus the manuscript. Ultimately, the most interesting part of the group foraging experiments is the emergence of “scrounger” mice in males and mostly “storer” mice in females (the definition of storer mice needs to be clarified, when do they actually consume the food they earned? Do they really store the food as in leave it in the hopper?). The resulting experiments then should better test the concepts that 1) dopamine contributes to behavioral phenotypes, 2) competition shapes emergence of behavior phenotypes observed, and 3) behavioral specification is flexible based on reinforcement learning.

2. One of the main goals of the manuscript is to understand how individual differences in behavior emerge. Social dominance is a fundamental component of animal social behavior and there is evidence that dominant male animals have higher reward seeking behavior (Davis, J. et al., Neuroscience 2009; Lazano-Montes, M. et al., Front. Behav. Neurosci. 2019) and that dopamine signaling plays a role in social hierarchies (van der Kooij, M.A. et al., Mol. Psy. 2018; Morgan, D. et al., Nat. Neurosci. 2002). In addition, optogenetic stimulation of the mesolimbic pathway can affect dominance (Lazano-Montes, M. et al. Front. Behav. Neurosci. 2019). Authors should include experiments to test if a particular behavioral foraging phenotype such as "independent" animals represents social dominant mice in the group and if they exhibit inherent

differences in dopamine reward responses compared to their group subordinates. This would help clarify whether specifications of goal-directed behavior are intrinsically linked to social hierarchy.

3. All physiology and photometry data presented focus on VTA DAergic neuron activity in general without specifying if activity changes observed occur in selective DAergic circuits or if changes in activity reflect actual DA release in VTA-innervated brain areas. This makes subsequent mechanistic manipulations using optogenetics challenging to interpret. The authors should test if VTA DAergic activity changes in the different foraging phenotypes shown in Figs 1 and 2 results in phasic DA release in the mesolimbic pathway and if so, then specifically manipulate this circuit to test subsequent effects of DA signaling in foraging phenotypes.

4. The specific role of dopamine in shaping foraging phenotypes in the grouped setting is unclear. Some inference is made based on in vivo recording of VTA DAergic activity (Figs. 1h and 2h). However, these experiments are problematic in that recordings are made from anesthetized mice and recordings are made post-foraging assay without baseline measurements (in Fig. 1). In addition, findings differ between single housed and grouped mice with single housed mice showing differences in tonic activity based on foraging phenotype, while grouped animals show differences in bursting activity. A better approach would be to perform longitudinal recordings prior, during, and immediately after the foraging assay in awake behaving mice using neuropixels or other biophysical approaches to measure activity dynamics of VTA DAergic neurons and elucidate how dynamics change over time in different behavioral foraging phenotypes. Alternatively, authors should focus on phasic dopamine signals during the foraging task to test if manipulating these responses during learning alters behavioral specification to more mechanistically link DA to foraging phenotypes.

5. The rationale for the selected VTA DA photostimulation parameters during optogenetic experiments requires clarification. Specifically, the authors deliver light stimulation 15 minutes on the day prior to the experiment and again for 30 minutes just before the start of the task, but it is unclear why these timepoints were chosen. More explanation is needed regarding how this stimulation timing is expected to influence behavior, particularly in relation to aggression or other home cage behaviors that may confound interpretation of social specialization. Given that shifts in behavioral roles may occur independently of VTA DA stimulation, the authors should justify why they did not apply temporally precise stimulation during behaviorally relevant periods. A more targeted approach—for example, optogenetic activation of VTA DAergic neurons during conspecific LP in workers or independents after day 2 when roles have emerged could test whether this induces a switch to scrounger-like behavior, disrupting society roles. This would provide more direct evidence linking VTA DAergic neuron activity to dynamic role changes rather than baseline modulation.

6. Interestingly, from mathematical modeling, the authors find that explorer/exploitation dynamics contribute to specification by modulating competition. To test the model, the authors mixed sexes in the foraging assay which reduced scrounger phenotypes in the male mice and increased worker female mice. However, this approach does not necessarily test competition as mice are likely mating during the assay which could certainly modulate DAergic signaling and foraging behavior independent of competition effects. The authors should design experiments to reduce beta in all male groups or increase beta in all female groups.

7. The concept that behavioral specification is flexible based on goal directed learning is an intriguing concept that is not directly tested in a meaningful way. The observation that scrounger mice do not exhibit an increase in VTA DAergic neuron activity when they lever press for food but when other mice lever press is an exciting result. If scrounger mice are removed from the group and placed in the foraging assay alone to retrain them to lever press for food then reintroduce them into the group setting, will they still be scroungers? If they become independent/workers/achievers, then this would support the flexible specification hypothesis.

8. The manuscript would benefit from clearer rationales and stronger literature support. For instance, in lines 221–222, the authors mention VTA DAergic neuron activity during exploration, but this concept should be introduced and cited earlier. Additionally, the rationale for focusing exclusively on VTA DAergic neurons over other regions like the prefrontal cortex—known for its role in social cognition and behavioral flexibility—needs strengthening. Current citations on dopamine's role as a learning signal and in social behavior are insufficient

Minor Concerns:
1. The claim of a specific phasic increase in VTA DAergic neuron activity after the learning phase is currently only supported by signal traces (Fig. 1d). This should be accompanied by quantitative analysis (e.g., peak amplitude, area under the curve) to allow for a clearer comparison across conditions.

2. In Figure 2h, combining males and females in group analyses is problematic. The "sorters" are all females, and "workers" are all males—who already show differences in post-task firing rates. Analyses should separate groups (e.g., independents, workers, sorters) by sex to avoid confounds.

3. In the Methods section for In vivo electrophysiology (lines 542-564), it indicates that mice received an intravenous catheter and were infused with nicotine or saline during recording but this was not described in the results.

References
Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., Nader, S. H., Buchheimer, N., Ehrenkaufer, R.

L., and Nader, M. A. (2002) Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nature neuroscience 5, 169-174

Davis, J. F., Krause, E. G., Melhorn, S. J., Sakai, R. R., and Benoit, S. C. (2009) Dominant rats are natural risk takers and display increased motivation for food reward. Neuroscience 162, 23-30

van der Kooij, M. A., Hollis, F., Lozano, L., Zalachoras, I., Abad, S., Zanoletti, O., Grosse, J., Guillot de Suduiraut, I., Canto, C., and Sandi, C. (2018) Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. Mol Psychiatry 23, 569-578

Lozano-Montes, L., Astori, S., Abad, S., Guillot de Suduiraut, I., Sandi, C., and Zalachoras, I. (2019) Latency to Reward Predicts Social Dominance in Rats: A Causal Role for the Dopaminergic Mesolimbic System. Front Behav Neurosci 13, 69

(Remarks on code availability)

Referee #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #3

(Remarks to the Author)
Summary of the key results
Behavior

The current paper studied foraging behavior in a semi-natural multi-animal settings. The authors reported divergent strategies mice used during foraging, when they were both alone or in a group context. The authors used a hierarchical approach to cluster mice into behavioral types “based on the percentage of LPs and complete sequences, identified four distinct foraging strategies”. The authors suggest that mice in the isolated context, can be clustered into Achievers and Storers and in the social context into Independents, Workers, Scroungers, and Storers.
The authors found significant differences between foraging strategies of male and female mice. These differences further lead to compositional differences in groups of male and female mice. By taking advantages of the sex related variation, the author proposed a reinforcement model with parameters that can capture this variation, and further predict composition of mixed group, which was verified by experimental data.

Modeling
Using the mice’s behaviors, the authors created a reinforcement learning model to fit behavior data and found inverse temperature parameter (commonly interpreted as balancing exploration and exploitation) being the critical parameter to capture the variability across individual.

DA-recordings
The authors recorded DA activities during behavior and activated DA neurons to test their causal roles.
The authors argue, interestingly, that the different types of behavioral strategies have different responses to food, lever pressing and conspecific lever pressing. The authors observe that for Scrounger mice, conspecific lever pressing leads to DA increases while for the other mice it does not.

DA-manipulations
Using Channelrhodopsin2 delivered to DA-neurons, the authors tested if manipulating DA activity would alter the group composition of mice. The authors found that within a triad of mice, if one or three mice are optogenetically activated on the initial days of the experiment, there were progressively more Storer mice than in the control condition where mice were injected with YFP. It is entirely unclear what this means, and the authors should expand significantly on the justification of their protocol.

Originality and significance: if not novel, please include reference

This study appears to be novel. What is most appealing about it is the behavioral analysis of the different behavioral types. The studies significance is based on its ambitious and extensive bridging of group-level behavior with behavioral mechanisms—algorithmically interpreted—and the DA system that might generate the individual behavioral strategy variability. I was very appreciative of this extensive effort to tackle division-of-labor in a “microsociety”.

Data & methodology: validity of approach, quality of data, quality of presentation

The experimental designs are intriguing. In particular, the triad approach of mixing mice of different types to causally test

their influence on each other was excellent.

However, there is more that could be further explored.
In particular, the authors want to reduce the behavioral differences that they observe to the DA system. However, it seems plausible that the fiber photometry results, which though different, may simply be related to the mice's movement rather than the a social role per se. This doesn't undermine the author's conclusion as the different learned motor parameters could underly the different roles, but it would be useful to look at the trials where the Achievers did not gather the delivered food pellets directly after pressing a lever and contrast them with the trials when the Storers did not gather the food pellets directly after pressing a lever. Are these the same? Similarly, what was the DA response after an Achiever directly gathered food and a Storer directly gathered food?
Does the body mass of the mice predict if they are Achievers or Storers? Does the social rank of the mice predict if they are Achievers or Storers? Does the observed sex difference relate to different variances in the mice's weight of male mice and female mice rather than sex?

Appropriate use of statistics and treatment of uncertainties

The clustering of the mice's behavior could be more thoroughly described and justified. It is unclear if there is a continuum between the mice behavioral types or if they are genuinely discrete classes.
The reports of the DA activity is remarkably brief; it would be useful to see the dynamics of the activity and its relationship to the specific actions of different mice separated by behavior-types under different behavioral trajectories.

Conclusions: robustness, validity, reliability

Problem #1: This could simply be because Scrounger mice learned the relation between the sound of lever pressing and the availability of food.
The claim that mice learn the social cues ("social alertness") is not fully supported. Do they learn the sound of the lever? Is the DA transient related to the vigorous movement of the Scroungers? A yoked condition where a single-housed mouse performs the task but a lever is occasionally automatically depressed and then food is delivered will help disambiguate whether or not the social nature of the lever press is critical to the Scrounger's DA response.

Problem #2: The optogenetic effects are hard to interpret
As the authors note the optogenetic effects are unusual. What do they show? Would a stressed mouse become a Storer? What does the optogenetic pre-task manipulation actually do to the DA ystem?

Problem #3: Sex or other individual difference
The authors don't consider individual differences other than sex to explain their behavior effects, when they could an should.

Suggested improvements: experiments, data for possible revision

The following could improve the paper:
1) Investigation of potential other factors like initial body mass or development of social hierarchy to the behavior.
2) The authors should more thoroughly analys DA transients rather than relying on averages. This could include correlations with specific movements and comparison of complete vs. incomplete sequences.
3) Within the authors's modeling the mices' behavior β plays the key role. The authors should link this directly to the DA measurements on a mouse-by-mouse basis and an completed vs incompleted sequence basis. Does a mouse's β relate to the DA activity and not broken out by behavioral type?
4) The authors should justify their unusual DA manipulation and the subsequent interpretation of optogenetic results, particularly how increased pre-task DA activity leads to a Storer (exploratory/low β) phenotype.

References: appropriate credit to previous work?

Yes. However, these references may assist in justifying some of the DA work:
Chen, Cathy S., et al. "Dopamine and norepinephrine differentially mediate the exploration–exploitation tradeoff." Journal of Neuroscience 44.44 (2024).
Hamid, Arif A., et al. "Mesolimbic dopamine signals the value of work." Nature neuroscience 19.1 (2016): 117-126.
Kakade, Sham, and Peter Dayan. "Dopamine: generalization and bonuses." Neural networks 15.4-6 (2002): 549-559.

Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

The report is clearly written and the effort is greatly appreciated. However, the data appears appears rich and the paper leaves many questions unanswered that make it difficult to understand the reasons for the conclusions.

(Remarks on code availability)

Referee #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)
The authors provided a set of Matlab scripts to simulate the full model and simulate and analyze the reduced model will a concise README file. The code is easy to run followed by instructions.
However, I can not find code for behavioral analysis.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have done an outstanding job of addressing reviewers' concerns regarding their manuscript. They have performed new analysis of their data and several new experiments to 1) better characterize the social behavioral groups they have identified, 2) better elucidate contributions of dopaminergic neuron activity in behavior specification, and 3) directly test the flexibility of social behavior phenotypes in their semi-natural “microsociety” reinforcement paradigm. This includes new optogenetic and chemogenetic experiments to test manipulation of dopaminergic neuron activity directly on social behavior phenotypes and new social behavior flexibility experiments where they demonstrate that phenotypes identified in an initial group setting can switch to different phenotypes in a new group setting. Finally, the authors investigate if social hierarchy can explain behavior phenotypes observed in the group reinforcement paradigm and they find that social hierarchy and behavioral archetypes are independent. Taken together, these new experiments along with revised data analysis and additional electrophysiology recordings significantly strengthen the authors’ findings which should be of broad interest. We have no further concerns.

Susanna Molas and Andrew R. Tapper

(Remarks on code availability)

Referee #2

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referee #3

(Remarks to the Author)
Comments for author during round 2 review
The authors improved the manuscript a lot, pinpointing three core arguments relating to the role emergence: both internal factor (Dopamine) and external factor (competition) are necessary to the role emergence, and role specialization is plastic. For the internal factor, the author constructed a model and find a parameter β controlling balance between exploration and exploitation influencing the foraging behavior and role differentiation most. They further combine in vivo electrophysiology, fiber photometry and optogenetics to show that dopamine activities are correlated to the parameter β. The experiment mixing Scroungers and naïve mice directly test the idea of plasticity in role division. The improvement helps readability.
The inconsistency of DA activities remains the major drawback. The author discussed the paradox about DA activities and proposed a distinction between tonic baseline activities and phasic, behavior event time locked activities. The author proposed that in vivo electrophysiology data after experiment reflect DA responses amplified by competition, so is not related to β. On the other hand, optogenetics and chemogenetics manipulations influence the baseline DA activities, and thus influencing β. The explanation, however, still looks unconvincing to me. If the β is more tightly related to baseline activities at the beginning of the experiment, then we should expect different baseline activities for triads that turn out to be Storers or Worker-Scoungers. Based on the model, higher β corresponds to higher DA activities and higher probability of being Worker-Scoungers triads. However, the in vivo electrophysiology shows comparable DA tonic baseline activities for male and female (though male and female role specification are different). One potential explanation might be different mapping between DA activities and β for male and female. The initial DA activities that correlates more to the β may be the salience (signal-to-noise ratio) of task even aligned phasic activities. If the baseline increased (male optogenetic activation experiment), salience of the phasic DA activities will decrease and thus the β decrease for them, leading to more Storers in the triads. One simple test of this hypothesis would be comparing DA activities on day 1 of the experiment between animals that turn out to be Storers versus Worker-Scoungers. The prediction is higher task event related DA activities on day 1 for Worker-Scoungers.
Minor points
1. Behavior description of Storers archetype is inconsistent. In Fig. 2e, the gains and losses are few in Storers, while in Fig. S3e, storers have many losses and gains (at least as many as Workers and Scroungers).

(Remarks on code availability)

Referee #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)
The authors provide detailed readme files about how to run the code and I can run the code without error following the guide.

Version 2:

Reviewer comments:

Referee #3

(Remarks to the Author)
We grateful to the authors for address our main concerns and for revising the discussion. We have no further recommendations.

(Remarks on code availability)

Referee #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

(Remarks on code availability)

Referees' comments:

Referee #1 (Remarks to the Author): In their manuscript, Solie et al. investigates how group socialization of animals contributes to specification of goal-directed behavior using an elegant semi-natural, reinforcement based foraging assay. In this assay mice live in an environment where they must learn to press a lever to obtain food. When single mice live in the environment, they segregate into two groups, "achievers" that rapidly learn to press the lever and almost immediately consume the food pellet earned, and "storers" mice that press the lever but do not necessarily consume the food right away building up food pellets to eat at a later time. Interestingly, when same-sex triplets of mice are placed in the assay, behavior phenotypes are more diverse and sex-dependent. Males segregated into three different groups including "scroungers" that do not press a lever but essentially steal pellets that other mice earned; whereas, females mostly fall into "storer" phenotypes. Combining mathematical modeling, in vivo electrophysiology, fiber photometry and optogenetics the authors implicate ventral tegmental area (VTA) dopaminergic (DAergic) neuron signaling in contributing to specialization of foraging behavior in group settings as well as competition in males underlying emergence of the "scrounging" phenotype which is flexible and may be reduced by decreasing competition. Overall, the concepts introduced in the manuscript are interesting, significant, and thoughtfully described. The idea that behavioral specification is not fixed but is flexible based on learning is a novel concept and the work should open new avenues for investigation of social behavior on learning. Results, particularly in the foraging assay, are robust and the role of dopamine in the assay are intriguing. However, experiments testing the role of exploration/exploitation dynamics and dopamine in shaping behavioral phenotypes need to be better controlled to support the hypotheses proposed. Additionally, the concept that behavioral specification is flexible based on learning could be more directly tested. Specific major points to be addressed include:

We thank the reviewer for this thorough and constructive assessment. In the revised manuscript, we have undertaken substantial revisions to address the key issues raised. Conceptually, we simplified our behavioral framework by removing the "Independent" category and adopting an archetypal analysis, which emphasizes three robust strategies (Worker, Scrounger, Storer) in a continuous space. Experimentally, we added the results of new experiments that (i) causally manipulate dopaminergic tone in both sexes using optogenetics and chemogenetics, (ii) directly test the flexibility of role adoption through mixing Scroungers with naive mice, (iii) assess social dominance alongside behavioral roles and show their dissociation, and (iv) extend electrophysiological recordings after the microsociety experiment. Together with expanded photometry analyses and clarified rationale for our manipulations, these additions more directly test the three core ideas emphasized by the reviewer: that dopamine contributes to behavioral phenotypes, that competition shapes role emergence, and that specialization is flexible and learning-dependent. Below is our point-by-point response to the reviewer's specific comments.

1. Data showing that group housed animals exhibit different foraging phenotypes compared to single housed mice is very compelling. In males, however, it seems that "independent" and "worker" mice described in Figure 3 are essentially "achiever" mice described in Figure 1, combining these groups seems warranted to simplify and better focus the manuscript. Ultimately, the most interesting part of the group foraging experiments is the emergence of "scrounger" mice in males and mostly "storer" mice in females (the definition of storer mice needs to be clarified, when do they actually consume the food they earned? Do they really store the food as in leave it in the hopper?). The resulting experiments then should better test the concepts that 1) dopamine contributes to behavioral phenotypes, 2) competition shapes emergence of behavior phenotypes observed, and 3) behavioral specification is flexible based on reinforcement learning.

We thank the reviewer for this helpful suggestion. In line with this comment, we have simplified the classification by removing the *Independent* category from the group foraging analysis. As the reviewer rightly points out, this class mostly captured behavioral profiles intermediate between *Worker* and *Scrounger*. In the revised version, we now use archetypal analysis to cluster the behavioral space and

focus on three clear archetypes: *Worker*, *Scrounger*, and *Storer*. Mice previously labeled as *Independents* now fall within this space without being treated as a distinct class. In this archetypal analysis, we also included two new parameters—gained pellets and lost pellets—which further refine our characterization of the animals' behavior.
Regarding the lone context, we have retained the labels *Achiever* and *Storer* to describe two robustly dissociable behavioral strategies. While *Achievers* and *Workers* both perform a high proportion of direct lever press–to–reward sequences, we emphasize that *Worker* specifically refers to a functional role in a social setting — producing food that can benefit other group members. This notion of shared task structure and division of labor does not apply to isolated animals. For this reason, we retained the term *Achiever* to describe efficient task execution in the solitary condition. By contrast, the term *Storer* refers to a consistent pattern of delayed reward retrieval that is expressed both in isolation and in group settings, and can therefore be used across conditions to describe a stable behavioral strategy.
We have also clarified the definition of *Storer* mice. These individuals often perform multiple lever presses before visiting the magazine to collect the reward (hence, effectively leaving the food in the hopper), a pattern observed both when tested alone and in group settings. As shown in the revised Figure 1 and 2, and Supplementary Figure 2b, this behavior results in a low proportion of complete sequences in the social context and could, in principle, create opportunities for conspecifics to steal unretrieved pellets. However, *Storer* mice do not typically rush to the food dispenser when a pellet is delivered, and this lack of urgency appears to limit competitive interactions. In practice, pellets remain in the dispenser until consumed, and Storer mice typically accumulate several pellets before eating them in batches. This delayed consumption, rather than immediate intake, operationally defines the Storer strategy. Thus, the term "storage" is not metaphorical but reflects a reproducible delay in consumption that shapes social interactions around food (see lines 92-94, 104-106 and 109-112)

We also took this opportunity to clarify how the manuscript addresses the three key concepts highlighted by the reviewer, namely, that (1) dopaminergic signaling contributes to behavioral phenotypes, (2) competition shapes the emergence of social roles, and (3) behavioral specification is flexible and learning-dependent. In the revised manuscript, these aspects are now tested more directly through targeted experiments: dopaminergic activity is causally manipulated in both sexes (Figure 6), the role of competition is assessed through mixed-sex and β-asymmetric group configurations (Figures 5a–f), and the flexibility of roles is demonstrated by showing that *Scroungers* can shift their strategy when reintroduced into new social environments (Figures 5g–i). In addition, we now explicitly compare social role and social dominance, and show that the dominance rank does not predict the behavioral profile, suggesting that the division of labor we observe reflects a distinct dimension of social organization (Figure 2k-l). Together, these additions reinforce the central claims and improve the overall coherence of the study. These points will be further detailed in our responses to the specific questions that follow.

2. One of the main goals of the manuscript is to understand how individual differences in behavior emerge. Social dominance is a fundamental component of animal social behavior and there is evidence that dominant male animals have higher reward seeking behavior (Davis, J. et al., Neuroscience 2009; Lazano-Montes, M. et al., Front. Behav. Neurosci. 2019) and that dopamine signaling plays a role in social hierarchies (van der Kooij, M.A. et al., Mol. Psy. 2018; Morgan, D. et al., Nat. Neurosci. 2002). In addition, optogenetic stimulation of the mesolimbic pathway can affect dominance (Lazano-Montes, M. et al. Front. Behav. Neurosci. 2019). Authors should include experiments to test if a particular behavioral foraging phenotype such as "independent" animals represents social dominant mice in the group and if they exhibit inherent differences in dopamine reward responses compared to their group subordinates. This would help clarify whether specifications of goal-directed behavior are intrinsically linked to social hierarchy.

We thank the reviewer for raising this important point regarding the relationship between social dominance and behavioral specialization. In the revised manuscript, we explicitly address this issue.

First, as noted in our response to point 1, we no longer refer to *Independent* animals as a separate category. Instead, we now describe foraging phenotypes within a continuous archetypal space, which allowed us to assess whether proximity to a specific archetype — in particular, the *Scrounger* or *Worker* profiles — is associated with dominance status.
To directly test the question of social hierarchy, we performed tube tests before and after microsociety experiment (Figures 2l–m). These tests revealed that social dominance ranks were not predictive of behavioral phenotype. Specifically, in male triads, dominance did not correlate with distance to either the *Scrounger* or *Worker* archetype, and in female triads, there was no relation between dominance and Storer-like behavior. This suggests that the division of labor we observe — including opportunistic scrounging or sustained food production — reflects dimensions of behavioral specialization that are at least partially independent from classical dominance hierarchies.
Finally, although we did not directly examine the relationship between VTA dopaminergic activity and dominance status, our neural analyses indicate that DA responses are better predicted by the behavioral role than by fixed individual traits such as hierarchy, weight, sex…. For instance, *Workers* show phasic VTA DA activity at their own lever presses, while *Scroungers* respond to conspecific lever presses (Figures 3c–g). These role-dependent dopaminergic signatures support a model in which social roles emerge dynamically through reinforcement and interaction history, rather than being determined by pre-existing social rank.
We believe this dissociation between dominance and functional role within the group is an important result, and we now highlight it more clearly in the revised Results (lines 128-132) and Discussion sections (see lines 301-304). References have been added.

3. All physiology and photometry data presented focus on VTA DAergic neuron activity in general without specifying if activity changes observed occur in selective DAergic circuits or if changes in activity reflect actual DA release in VTA-innervated brain areas. This makes subsequent mechanistic manipulations using optogenetics challenging to interpret. The authors should test if VTA DAergic activity changes in the different foraging phenotypes shown in Figs 1 and 2 results in phasic DA release in the mesolimbic pathway and if so, then specifically manipulate this circuit to test subsequent effects of DA signaling in foraging phenotypes.
All of our electrophysiological, fiber photometry, and opto-/chemogenetic manipulations targeted dopaminergic cell bodies within the VTA using a DAT-Cre approach (cf Methods and figure legend 3a), without distinguishing projection-specific subpopulations. Our objective in this study was to assess how global VTA dopaminergic tone influences role adoption in a social context, rather than to dissect downstream targets.
We agree that projection-specific approaches—for example, using retrograde labeling or dopamine sensors in the nucleus accumbens or prefrontal cortex—would provide additional mechanistic resolution, and we now make this limitation explicit in the Discussion (lines 283). Importantly, our current strategy still allows meaningful causal inference: phasic DA responses differentiated *Workers* from *Scroungers* (e.g., self vs. conspecific lever presses, Fig. 3), and global pre-task manipulations of VTA activity shifted group structure in directions predicted by our computational model (Fig. 6).
Thus, while projection-specific studies will be an important next step, our findings already demonstrate that population-level dopaminergic tone in the VTA is sufficient to bias the emergence of social specialization.

4. The specific role of dopamine in shaping foraging phenotypes in the grouped setting is unclear. Some inference is made based on in vivo recording of VTA DAergic activity (Figs. 1h and 2h). However, these experiments are problematic in that recordings are made from anesthetized mice and recordings are made post-foraging assay without baseline measurements (in Fig. 1). In addition, findings differ between single housed and grouped mice with single housed mice showing differences in tonic activity based on foraging phenotype, while grouped animals show differences in bursting activity. A better approach would be to perform longitudinal recordings prior, during, and immediately after the foraging assay in awake

behaving mice using neuropixels or other biophysical approaches to measure activity dynamics of VTA DAergic neurons and elucidate how dynamics change over time in different behavioral foraging phenotypes. Alternatively, authors should focus on phasic dopamine signals during the foraging task to test if manipulating these responses during learning alters behavioral specification to more mechanistically link DA to foraging phenotypes.

We agree that longitudinal recordings in awake, behaving mice—such as Neuropixels—would provide a finer temporal resolution of dopaminergic dynamics across role acquisition. Our aim here, however, was to establish a functional link between VTA dopaminergic tone and social role differentiation. To this end, we combined: (i) fiber photometry in awake mice during the task, which revealed role-specific phasic DA responses to self- vs. conspecific lever presses (Figs. 1d, 3a–g), and (ii) in vivo electrophysiology post-assay, which allowed us to compare baseline firing properties as a function of emergent phenotype (Figs. 1j, 3h–i). In this revised version of the manuscript, we have implemented significant changes in the analysis of our recordings from anesthetized animals. Specifically, we have: (i) incorporated new recordings obtained after microsociety experiments and (ii) linked electrophysiological experiments with behavioral data through archetypal analysis. These modifications enabled us to use firing rates for all our analyses (Figures 1 and 2) and to correlate the identified profiles with VTA DA activity, as measured by both fiber photometry and extracellular recording.

We also report baseline activity before and after microsociety experiment (Fig. 3h), showing sex-specific plasticity of VTA tone, with increased firing and bursting in males but not females.

Although these recordings do not capture moment-to-moment causality, they indicate that social interaction induces lasting dopaminergic adaptations linked to specialization. Importantly, the convergence of three complementary approaches—fiber photometry for phasic event-locked dynamics, extracellular recordings for baseline tone, and causal manipulations of VTA DA neurons—already provides a coherent mechanistic picture. Together these methods demonstrate both correlation and causation between DA activity and role specialization, making Neuropixels-style longitudinal tracking a valuable but not essential addition for the present study. To test causality more directly, we manipulated VTA DA neuron activity prior to task onset using optogenetics and chemogenetics (Fig. 6). These interventions shifted group composition as predicted by our model, demonstrating that dopaminergic tone biases the emergence of social roles (i.e., along the Storer–Worker/Scrounger axis predicted by the model). Finally, we now explicitly clarify in the Discussion that in vivo recordings capture stable plasticity, while fiber photometry resolves role-specific fast dynamics, together indicating that both phasic and tonic adaptations contribute to social specialization (Discussion, lines 271-289).

Lastly, while closed-loop manipulations of DA transients during behavior represent an exciting next step, our present combination of fiber photometry, electrophysiology, and causal manipulations already provides a mechanistic framework linking dopaminergic activity to the emergence of social specialization.

5. The rationale for the selected VTA DA photostimulation parameters during optogenetic experiments requires clarification. Specifically, the authors deliver light stimulation 15 minutes on the day prior to the experiment and again for 30 minutes just before the start of the task, but it is unclear why these timepoints were chosen. More explanation is needed regarding how this stimulation timing is expected to influence behavior, particularly in relation to aggression or other home cage behaviors that may confound interpretation of social specialization. Given that shifts in behavioral roles may occur independently of VTA DA stimulation, the authors should justify why they did not apply temporally precise stimulation during behaviorally relevant periods. A more targeted approach—for example, optogenetic activation of VTA DAergic neurons during conspecific LP in workers or independents after day 2 when roles have emerged could test whether this induces a switch to scrounger-like behavior, disrupting society roles. This would provide more direct evidence linking VTA DAergic neuron activity to dynamic role changes rather than baseline modulation.

We thank the reviewer for this comment and the opportunity to clarify our rationale. Our primary goal with the optogenetic experiments was to test that the dopaminergic state at the onset of group formation biases subsequent role adoption through its impact on the exploration–exploitation balance (β parameter). For

this reason, we deliberately used a pre-task stimulation protocol, rather than real-time event-locked manipulations. This choice is now explicitly stated in the Results, immediately after the subtitle *Dopaminergic modulation before the microsociety experiment changes role specialization* (lines 229-234), where we clarify that the protocol was designed to bias initial dopaminergic tone instead of directly driving behavior.
The stimulation schedule (15 minutes the day before, and again 15 minutes, 30 minutes immediately prior to group formation) was based on previous studies showing that short ChR2 activation epochs produce long-lasting increases in VTA excitability and network effects (Chaudhury et al., 2013; Tye et al., 2013; Bariselli et al., 2018). We confirmed in our hands that this protocol elevated firing for at least six hours post-stimulation (Fig. S8). Using this design, we observed reproducible shifts in role distribution, both when one animal (1ChR2) or all animals (3ChR2) were stimulated (Fig. 6e–g). Complementary experiments with inhibitory DREADDs in female triads (Fig. 6h–n) produced the opposite shift, further supporting the idea that dopaminergic responsiveness at group formation is a key determinant of emergent social organization.
Lastly, as stated above, while closed-loop manipulations represent an exciting next step, our present combination of fiber photometry, electrophysiology, and causal manipulations already provides a mechanistic framework linking dopaminergic activity to the emergence of social specialization. Moreover, we did not observe overt changes in locomotion or aggression after stimulation, and the behavioral shifts extended to the entire triad, consistent with a collective reorganization of roles rather than an artifact of direct stimulation. This interpretation is also emphasized in the Discussion (line 282-284), where we note that by manipulating tonic DA activity prior to social engagement we demonstrated that baseline neuromodulatory state biases group composition; although DA can also influence arousal, motivation, or mating, the effects were reproducible and consistent across groups, supporting their relevance to social specialization.

6. Interestingly, from mathematical modeling, the authors find that explorer/exploitation dynamics contribute to specification by modulating competition. To test the model, the authors mixed sexes in the foraging assay which reduced scrounger phenotypes in the male mice and increased worker female mice. However, this approach does not necessarily test competition as mice are likely mating during the assay which could certainly modulate DAergic signaling and foraging behavior independent of competition effects. The authors should design experiments to reduce beta in all male groups or increase beta in all female groups.
We thank the reviewer for this insightful comment. We agree that mixed-sex groups could introduce additional variables, such as mating-related behaviors, that influence dopamine signaling independently of competition. Our original goal with these groups was to test a model prediction that heterogeneity in β values increases the likelihood of role differentiation (Results, lines 208–211). The observed reduction in Scrounger phenotypes and the emergence of Worker-like strategies in females were consistent with this prediction, but we acknowledge that mating could act as a confounding factor.
To address this issue, we performed complementary manipulations in single-sex triads. In males, pre-task ChR2 stimulation of VTA DA neurons (predicted to increase β) promoted Storer-like strategies and reduced competitive specialization (Fig. 6e–g). In females, inhibitory DREADDs (hM4Di) reduced VTA DA activity (also predicted to increase β), leading to the emergence of Worker and Scrounger phenotypes and thus a male-like division of labor (Fig. 6l–n). These causal manipulations directly validate the model's prediction that the distribution of β within a group controls the emergence of specialization. Because these effects were observed in single-sex groups, they isolate the contribution of dopaminergic tone from potential mating effects.
We now clarify this rationale more explicitly in the revised manuscript (Results, lines 208–217; Discussion, lines 282-284, 309–312).

7. The concept that behavioral specification is flexible based on goal directed learning is an intriguing concept that is not directly tested in a meaningful way. The observation that scrounger mice do not exhibit

an increase in VTA DAergic neuron activity when they lever press for food but when other mice lever press is an exciting result. If scrounger mice are removed from the group and placed in the foraging assay alone to retrain them to lever press for food then reintroduce them into the group setting, will they still be scroungers? If they become independent/workers/achievers, then this would support the flexible specification hypothesis.

We fully agree with the reviewer that testing the flexibility of behavioral specification is essential to support our claim that social roles are not hardwired but emerge through reinforcement and social interaction.

In response to this helpful suggestion, we performed a new experiment that directly addresses this point (Figure 5g–i). In this protocol, a previously identified *Scrounger* was removed from its triad after 1 week of microsociety experiment. The animal was then reintroduced into a new triad composed of two naïve mice (Figure 5g-i). Strikingly, after group reintegration, this mouse no longer adopted a Scrounger profile but instead behaved consistently with a Worker/Storer-like role, producing food for the group. This result provides direct evidence that behavioral roles are not fixed, and that individual learning history can modulate subsequent role adoption in a new social context. It supports the idea that social specialization arises dynamically from the interaction between internal learning states and external group structure — a central tenet of our model. We thank the reviewer for encouraging this important addition, which we believe significantly strengthens the manuscript. The results are presented in the revised version in Figure 5g–i and in lines 219–226.

8. The manuscript would benefit from clearer rationales and stronger literature support. For instance, in lines 221–222, the authors mention VTA DAergic neuron activity during exploration, but this concept should be introduced and cited earlier. Additionally, the rationale for focusing exclusively on VTA DAergic neurons over other regions like the prefrontal cortex—known for its role in social cognition and behavioral flexibility—needs strengthening. Current citations on dopamine's role as a learning signal and in social behavior are insufficient

In the revised manuscript, we have improved the clarity of our conceptual framework and provided more comprehensive references in support of key claims.

First, we now introduce the notion of VTA dopamine's involvement in exploration/exploitation tradeoffs earlier in the Introduction (lines 49–52), along with relevant citations (e.g., Beeler et al 2010, Cinotti et al., 2019; Dongelmans et al., 2022, Humphries et al 20212, Chen et al 2024, Faure 2025). This allows us to better contextualize the role of the β parameter in our model, which reflects this tradeoff and is influenced by dopaminergic tone. We also clarify the connection between dopaminergic modulation and behavioral variability in social and non-social contexts, by citing recent work on dopamine's role in adaptive exploration.

Second, while we agree that other brain regions — including the prefrontal cortex — are key players in social cognition and behavioral flexibility, our study was specifically designed to test the hypothesis that dopaminergic tone in the VTA can modulate social role adoption through its influence on reinforcement learning and internal uncertainty. This choice was motivated by:

1. The computational model predicting that β, a dopamine-sensitive parameter, governs social differentiation;
2. Previous findings showing that VTA activity reflects learning signals during social interaction (Gunaydin et al., 2014; Solié et al., 2021);
3. The technical feasibility of selectively manipulating VTA dopaminergic neurons in a temporally controlled, cell-type-specific manner using DAT-Cre mice.

We now make this rationale clearer in the revised Introduction (lines 48-51) and Discussion (lines 299–314), and we include additional citations on the role of dopamine in social valuation, goal-directed learning, group-level coordination and hierarchy (e.g., Solié et al., 2021, Morgan et al., 2002; van der Kooij et al., 2018, Bariselli et al., 2018, Davis et al 2009, Lozano-Montes et al 2019 …).

Minor Concerns:

1. The claim of a specific phasic increase in VTA DAergic neuron activity after the learning phase is currently only supported by signal traces (Fig. 1d). This should be accompanied by quantitative analysis (e.g., peak amplitude, area under the curve) to allow for a clearer comparison across conditions.

We thank the reviewer for this suggestion. In the revised manuscript, we have added a quantitative analysis of the photometry signals presented in Figure 1d. Specifically, we now report the peak amplitude and area under the curve (AUC) of the ΔF/F signal aligned to lever presses, both before and after learning (Figure 1e). This analysis confirms a significant increase in phasic VTA DA activity after learning in *Achievers*, consistent with goal-directed reinforcement.

In addition, to further support the link between dopaminergic response and behavioral phenotype, we analyzed peak amplitude as a function of distance to the archetypes defined in our behavioral space. This continuous approach, shown in Figure 3d, reveals that phasic DA responses are modulated by behavioral role: animals closer to the *Worker* archetype show stronger self-LP-locked responses, while those closer to the *Scrounger* archetype respond preferentially to conspecific LPs.

These additions improve the quantification and interpretation of our photometry data. They are described in the revised Results (mainly lines 134-154, see also methods) and incorporated in Figures 1e and 3d.

2. In Figure 2h, combining males and females in group analyses is problematic. The "sorters" are all females, and "workers" are all males—who already show differences in post-task firing rates. Analyses should separate groups (e.g., independents, workers, sorters) by sex to avoid confounds.

In the revised manuscript, we have separated all analyses by sex when examining post-task neural activity and behavioral phenotypes. Specifically:

- In Figure 3f–g, firing rates are now plotted separately for males and females, and the statistical analyses explicitly test for sex × role interactions.
- We removed group-level comparisons that combined males and females into the same categories (e.g., "storers") to avoid misinterpretation due to sex-specific distributions.
- In the archetypal analysis, sex differences are accounted for both in the behavioral space and in their relation to neural measures (Figure 2 and 3).

These modifications ensure that role-specific interpretations are not conflated with sex effects. We thank the reviewer for encouraging this important clarification.

3. In the Methods section for In vivo electrophysiology (lines 542-564), it indicates that mice received an intravenous catheter and were infused with nicotine or saline during recording but this was not described in the results

We apologize for this mistake. The irrelevant sentence has been removed from the Methods section.

References

Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., Nader, S. H., Buchheimer, N., Ehrenkaufer, R. L., and Nader, M. A. (2002) Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nature neuroscience 5, 169-174

Davis, J. F., Krause, E. G., Melhorn, S. J., Sakai, R. R., and Benoit, S. C. (2009) Dominant rats are natural risk takers and display increased motivation for food reward. Neuroscience 162, 23-30

van der Kooij, M. A., Hollis, F., Lozano, L., Zalachoras, I., Abad, S., Zanoletti, O., Grosse, J., Guillot de Suduiraut, I., Canto, C., and Sandi, C. (2018) Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. Mol Psychiatry 23, 569-578

Lozano-Montes, L., Astori, S., Abad, S., Guillot de Suduiraut, I., Sandi, C., and Zalachoras, I. (2019) Latency to Reward Predicts Social Dominance in Rats: A Causal Role for the Dopaminergic Mesolimbic System. Front Behav Neurosci 13, 69

These references have been added

Referee #2 (Remarks to the Author):
I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #3 (Remarks to the Author):
Summary of the key results

Behavior
The current paper studied foraging behavior in a semi-natural multi-animal settings. The authors reported divergent strategies mice used during foraging, when they were both alone or in a group context. The authors used a hierarchical approach to cluster mice into behavioral types "based on the percentage of LPs and complete sequences, identified four distinct foraging strategies". The authors suggest that mice in the isolated context, can be clustered into Achievers and Storers and in the social context into Independents, Workers, Scroungers, and Storers.
The authors found significant differences between foraging strategies of male and female mice. These differences further lead to compositional differences in groups of male and female mice. By taking advantages of the sex related variation, the author proposed a reinforcement model with parameters that can capture this variation, and further predict composition of mixed group, which was verified by experimental data.

Modeling
Using the mice's behaviors, the authors created a reinforcement learning model to fit behavior data and found inverse temperature parameter β (commonly interpreted as balancing exploration and exploitation) being the critical parameter to capture the variability across individual.
DA-recordings
The authors recorded DA activities during behavior and activated DA neurons to test their causal roles.
The authors argue, interestingly, that the different types of behavioral strategies have different responses to food, lever pressing and conspecific lever pressing. The authors observe that for Scrounger mice, conspecific lever pressing leads to DA increases while for the other mice it does not

DA-manipulations
Using Channelrhodopsin2 delivered to DA-neurons, the authors tested if manipulating DA activity would alter the group composition of mice. The authors found that within a triad of mice, if one or three mice are optogenetically activated on the initial days of the experiment, there were progressively more Storer mice than in the control condition where mice were injected with YFP. It is entirely unclear what this means, and the authors should expand significantly on the justification of their protocol.
We thank the reviewer for raising this point. The optogenetic protocol was designed to test a key prediction of our reinforcement learning model: that the initial dopaminergic state, modeled via the inverse temperature parameter β, biases the allocation of social roles within a group by modulating the balance between exploration and exploitation.
To this end, we opted for pre-task stimulation of VTA dopaminergic neurons (15 min the day before, and again 15 mins, 30 min just prior to the microsociety experiment), rather than real-time manipulation during task performance. This strategy allowed us to bias the internal state of the animal at the time of group formation, consistent with the model's assumption that early competition dynamics influence later role emergence. This type of stimulation protocol has been shown to induce long-lasting excitability changes in DA neurons (e.g., Chaudhury et al., *Nature* 2013; Tye et al., *Nature* 2013, Bariselli et al., Nat Comm 2018). We also show that this effect lasts several hours after the stimulation (Figure S8).
Importantly, the observed shift toward increased *Storer*-like behavior in stimulated triads was not restricted to the stimulated animal, but reflected a reorganization of the entire triad, in agreement with the idea that social structure is shaped by dopaminergic biases at the group level.
To strengthen this interpretation, we now include complementary results using inhibitory DREADDs in females (Figure 6h–n). In this experiment, a reduction in VTA dopaminergic tone prior to the task led to the emergence of specialization (Workers and Scroungers) in otherwise uniform female triads. These results mirror the optogenetic findings in males and reinforce the model-derived prediction that β modulates role distribution.

We now clarify this rationale and its relation to the theoretical framework more explicitly in the revised Results (see line 201-207, see section Dopaminergic modulation biases role specialization, lines 229-234) and Discussion (271–289).

Originality and significance: if not novel, please include reference. This study appears to be novel. What is most appealing about it is the behavioral analysis of the different behavioral types. The studies significance is based on its ambitious and extensive bridging of group-level behavior with behavioral mechanisms—algorithmically interpreted—and the DA system that might generate the individual behavioral strategy variability. I was very appreciative of this extensive effort to tackle division-of-labor in a "microsociety".

Data & methodology: validity of approach, quality of data, quality of presentation
The experimental designs are intriguing. In particular, the triad approach of mixing mice of different types to causally test their influence on each other was excellent.
However, there is more that could be further explored.
In particular, the authors want to reduce the behavioral differences that they observe to the DA system. However, it seems plausible that the fiber photometry results, which though different, may simply be related to the mice's movement rather than the a social role per se.
We thank the reviewer for raising this point. We are confident that the role-specific DA signals we report cannot be explained by locomotion alone. First, all photometry analyses were time-locked to discrete behavioral events—self lever presses, conspecific lever presses, and reward retrieval—rather than to general locomotor activity. Second, in Scroungers we now show that the phasic DA response to a conspecific's lever press is contingent on the Scrounger's attention to the event, which would not be predicted by a motor explanation (Figure 3e). Third, DA responses scale with the animal's position in the archetypal behavioral space: proximity to the Worker archetype predicts stronger self-LP responses, while proximity to the Scrounger archetype predicts stronger conspecific-LP responses (Fig. 3d). Finally, in the lone context, DA responses to lever presses depend on whether the animal retrieves the pellet immediately or not (Fig. 1d–e; Supp. Fig. 1b–c), showing that the signal reflects the value of a complete action–outcome sequence rather than movement per se (lines 82–88).
Taken together, these results indicate that the observed DA transients reflect the behavioral relevance and learned value of specific events, and our data provide clear evidence that these responses are not reducible to motor activity.

This doesn't undermine the author's conclusion as the different learned motor parameters could underly the different roles, but it would be useful to look at the trials where the Achievers did not gather the delivered food pellets directly after pressing a lever and contrast them with the trials when the Storers did not gather the food pellets directly after pressing a lever. Are these the same? Similarly, what was the DA response after an Achiever directly gathered food and a Storer directly gathered food?
The fiber photometry analysis presented in Fig. 1d was not separated by Achiever vs Storer categories, but instead by the latency of reward collection—i.e., whether lever presses were followed by direct pellet retrieval (complete sequences) or not (incomplete sequences). This analysis shows that phasic VTA DA responses at lever press are selectively observed during complete sequences, and absent when food retrieval is delayed. We also show a correlation between time to food dispenser and peak amplitude at LP (Supp Figure 1C). Thus, both Achievers and Storers display this dynamic at the level of single trials, but Achievers express it more frequently because they perform a higher proportion of direct sequences.
This pattern is consistent with group data as well: female triads, which are predominantly Storer-like, still show lever-press responses, but these are weaker than in males, who perform more complete sequences.
Together, these results indicate that the DA response reflects the reinforcement value of direct action–outcome sequences, rather than a categorical difference between Achievers and Storers.

Does the body mass of the mice predict if they are Achievers or Storers? Does the social rank of the mice predict if they are Achievers or Storers? Does the observed sex difference relate to different variances in the mice's weight of male mice and female mice rather than sex?

We carefully tested whether somatic or dominance factors could account for the observed profiles. Body weight did not predict whether mice were Achievers or Storers, with substantial overlap between groups (Fig. 1j), and we also analyzed weight in the group context (Fig. 2j). While males and females differ in average weight, variance in body mass was insufficient to explain sex-specific group structure (Results, lines 96, 127). Tube tests performed before and after microsociety experiment also showed no consistent relationship between dominance rank and behavioral profile, confirming that in this task foraging strategy and hierarchy are independent axes of variability (Figs. 2k–l; Results, lines 128–132; Discussion, lines 301–304). Instead, our computational model and causal manipulations point to dopaminergic tone and social competition as the critical drivers of specialization. We now note this explicitly in the revised manuscript.

Appropriate use of statistics and treatment of uncertainties

The clustering of the mice's behavior could be more thoroughly described and justified. It is unclear if there is a continuum between the mice behavioral types or if they are genuinely discrete classes.

We thank the reviewer for this comment. In the revised manuscript we moved from discrete clustering to an archetypal analysis framework, which better captures the continuous nature of behavioral variation. This approach defines a behavioral space bounded by three archetypes—Worker, Scrounger, and Storer—while allowing each individual to be represented as a weighted mixture of these roles. It thereby preserves population structure without imposing artificial categorical boundaries. Importantly, this continuous representation also enabled us to link behavior and physiology more directly, by relating the distance to archetypes with dopaminergic responses measured through fiber photometry and electrophysiology (e.g., Fig. 3d, f–g). We believe this directly addresses the reviewer's concern and provides a more faithful description of naturalistic behavior. Details are provided in the revised Results (lines 88–96, 106–109; Figs. 1e–f, 2d, 3 f,g, i) and Methods (lines 667–720).

The reports of the DA activity is remarkably brief; it would be useful to see the dynamics of the activity and its relationship to the specific actions of different mice separated by behavior-types under different behavioral trajectories.

We thank the reviewer for highlighting this point. In the revised manuscript we expanded our analysis of fiber photometry and electrophysiological data to provide a more detailed account of dopaminergic dynamics. In Fig. 1e, we now quantify both peak amplitude and area under the curve (AUC) of VTA DA transients aligned to lever presses before and after learning. In Fig. 3, we present phasic responses stepwise: first by sex (Fig. 3c), then by behavioral profile (Fig. 3d), and finally as a graded function of distance to behavioral archetypes (Fig. 3e). This analysis demonstrates continuous, role-dependent DA signaling aligned to both self and conspecific lever presses. Supplementary Fig. S4 further illustrates these dynamics by showing individual, per-mouse average photometry traces aligned to specific task events (food delivery, own LPs, and conspecific LPs) for Workers and Scroungers. Together, these additions clarify how dopaminergic activity tracks both learning history and emergent social specialization.

Conclusions: robustness, validity, reliability

Problem #1: This could simply be because Scrounger mice learned the relation between the sound of lever pressing and the availability of food.

The claim that mice learn the social cues ("social alertness") is not fully supported. Do they learn the sound of the lever? Is the DA transient related to the vigorous movement of the Scroungers? A yoked condition where a single-housed mouse performs the task but a lever is occasionally automatically depressed and then food is delivered will help disambiguate whether or not the social nature of the lever press is critical to the Scrounger's DA response.

We thank the reviewer for this comment. In the revised manuscript we removed references to "social alertness," as our dataset does not allow us to conclude whether Scrounger DA transients reflect truly social processing or learned associations with task-related cues (e.g. lever sounds, pellet delivery).
To probe this further, we performed several new analyses. First, spatial tracking revealed that conspecific lever presses are salient events: both Workers and especially Scroungers showed systematic orientation toward the lever zone following conspecific presses, whereas Storers did not (Fig. S3a–b). Second, we found that the phasic DA response of Scroungers to conspecific lever presses depends on their attentional state: a clear transient is observed when the Scrounger is oriented toward the event, but not when it is disengaged (Fig. 3e). This contingency strongly argues against an interpretation in terms of movement vigor or non-specific sensory cues (this is also indirectly confirmed by analysis in lone context were peak amplitude after post lever movement not directed toward dispenser is low, Supp Figure 1b-c). Third, using the archetypal framework, we demonstrate that DA responses scale with proximity to the Worker vs. Scrounger poles (Fig. 3f-g), further indicating that dopaminergic dynamics reflect role-specific valuation strategies rather than generic motor correlates.
Finally, we note that Workers and Scroungers are exposed to the same external events (lever sounds, pellet deliveries), yet only Scroungers show strong phasic responses to conspecific lever presses. Together, these results support the interpretation that VTA DA activity is role-dependent and shaped by learning history and strategy, not reducible to locomotor vigor or external sensory cues alone. We acknowledge that an explicit yoked control (lever sounds without social interaction) would further strengthen this conclusion. While such an experiment was not performed here, our analyses of orientation, attentional state, and archetypal position already provide indirect evidence that the DA signals cannot be explained by lever sound or generic movement alone. We therefore interpret the responses as reflecting role-dependent valuation of conspecific actions, rather than simple motor or sensory correlates.

Problem #2: The optogenetic effects are hard to interpret

As the authors note the optogenetic effects are unusual. What do they show? Would a stressed mouse become a Storer? What does the optogenetic pre-task manipulation actually do to the DA qystem?

Our pre-task stimulation protocol was chosen to test a model-derived prediction that the initial dopaminergic state (β) biases group-level role allocation through its effect on the exploration–exploitation balance. Brief ChR2 stimulation of VTA DA neurons has been shown to increase excitability and alter downstream activity for hours (Chaudhury et al., 2013, Bariselli et al., 2018), and in our hands produced elevated firing for more than 6 hours (Supp. Fig. S8). We therefore interpret this manipulation as biasing the dopaminergic "starting state" of the group rather than driving behavior in real time.
In our experiments, this manipulation consistently shifted triads toward Storer-like strategies. While this outcome may seem counterintuitive, it is consistent with our conceptual framework: social competition elevates DA activity and promotes Worker–Scrounger specialization, whereas non-specific tonic activation through optogenetic stimulation produced Storers. This apparent paradox can be reconciled if β is understood not as a direct readout of firing rate, but as shaped by distinct dopaminergic components. In particular, tonic activation may bias animals toward repetitive food production without immediate consumption, whereas phasic, task-aligned responses amplified by competition favor competitive specialization. Thus, elevated dopaminergic tone constrains the space of possible strategies, which is then resolved by social interactions (see discussion line 271-289).
In the revised version we also include a complementary chemogenetic experiment, showing that reducing VTA DA activity in females before the task produced the inverse effect and promoted specialization. Together, these manipulations demonstrate that dopaminergic state at group formation biases subsequent role adoption, even if the precise mapping between DA firing and behavioral outcome depends on the balance of tonic versus phasic components.
We did not observe signs of acute stress (e.g., freezing, hypoactivity), and the Storer bias was consistent and reproducible across cohorts. Nonetheless, we acknowledge that dopaminergic manipulations can influence arousal and motivation in complex ways, and we now discuss this limitation explicitly in the revised Discussion (lines 283-285, lines 838-839).

Problem #3: Sex or other individual difference
The authors don't consider individual differences other than sex to explain their behavior effects, when they could an should.

We thank the reviewer for emphasizing the importance of considering individual variability beyond sex. In the revised manuscript, we have expanded our analyses to address this point along three complementary axes:

1. Body mass: As discussed in response to Reviewer 1 (Minor Point 2) and Reviewer 3 (Point 4), we tested whether initial body weight predicted behavioral phenotype in solitary or group contexts. We found no significant correlation between weight and role adoption (e.g., Achiever vs. Storer), and weight distributions were comparable across profiles (Figure 1j).
2. Social rank: We also assessed whether dominance status, as measured by tube tests, was predictive of behavioral archetype. As detailed in Figure 2l–m, dominance and role were not systematically associated in either males or females, suggesting that these dimensions of individuality are at least partially independent.
3. Behavioral space representation: To go beyond categorical distinctions (e.g., sex or predefined types), we now describe individual behavior using a continuous archetypal decomposition. This allows us to locate each mouse in a multidimensional space reflecting its strategy, independent of sex or rank. The reinforcement learning model is applied at the individual level, and β is fitted mouse-by-mouse, capturing within-sex variability.

We agree with the reviewer that sex alone cannot explain the full structure of behavioral diversity, and we hope these additional analyses address the broader question of individual-level determinants of specialization.

Suggested improvements: experiments, data for possible revision
The following could improve the paper:
1) Investigation of potential other factors like initial body mass or development of social hierarchy to the behavior.

As detailed in our responses above (Reviewer 3 – Points 4 and 9), we analyzed both initial body mass and dominance rank (assessed via tube tests) in relation to behavioral phenotype.

- We found no significant association between body weight and role adoption in either solitary or group contexts (Figure 1j et 2j).
- Similarly, dominance rank did not predict behavioral archetype in either sex (Figure 2k-l).

These findings support the conclusion that social specialization emerges from processes not reducible to static somatic traits or hierarchy alone, but rather from the interaction between reinforcement dynamics, dopaminergic tone, and group structure.

2) The authors should more thoroughly analys DA transients rather than relying on averages. This could include correlations with specific movements and comparison of complete vs. incomplete sequences.

In the revised manuscript, we expanded our analysis of DA transients in several key ways:

1. We now report peak amplitude and AUC of ΔF/F responses aligned to behavioral events, rather than relying solely on trial-averaged traces (Figures 1d and 3c-e). This allows for a more quantitative assessment of response magnitude across learning stages and roles.
2. In Figure 3f-g, we examine how phasic DA response amplitude varies continuously as a function of distance to behavioral archetypes, capturing fine-grained variation beyond discrete types.
3. In addition, the comparison of complete versus incomplete sequences in the lone context (Fig. 1d–e; Supp. Fig. 1b–c) shows that DA responses depend on whether food is retrieved immediately after a lever press, not simply on the motor act itself.

Together, these results indicate that the phasic DA signals we report reflect the reinforcement value of action–outcome sequences and role-dependent strategies, rather than being explained by motor activity alone.

3) Within the authors's modeling the mices' behavior β plays the key role. The authors should link this directly to the DA measurements on a mouse-by-mouse basis and an completed vs incompleted sequence basis. Does a mouse's β relate to the DA activity and not broken out by behavioral type?

We thank the reviewer for this suggestion. While we agree that a direct mouse-by-mouse estimation of the β parameter followed by correlation with DA activity would be ideal, we found that individual-level model fitting was not sufficiently robust due to variability in behavior and limited sequence length per animal.
To address this, we implemented a complementary data-driven approach using archetypal analysis. This unsupervised method allowed us to:

- Represent each animal's behavior as a weighted combination of three archetypal strategies (Worker, Scrounger, Storer),
- Project individuals into a continuous low-dimensional behavioral space,
- And compute each animal's distance to each archetype.

We then correlated these distances with dopaminergic response features, such as peak amplitude and area under the curve (AUC) of VTA ΔF/F signals aligned to key behavioral events (e.g., lever press, reward retrieval). These analyses, presented in Figure 3f-g, revealed graded, role-specific dopaminergic encoding, thereby linking continuous behavioral structure to DA activity at the individual level — even without explicit reliance on fitted β values.
We believe this approach provides a conceptually and quantitatively meaningful way to test the model's predictions about behavioral differentiation and DA modulation.

4) The authors should justify their unusual DA manipulation and the subsequent interpretation of optogenetic results, particularly how increased pre-task DA activity leads to a Storer (exploratory/low β) phenotype.

The apparent paradox—that increased dopaminergic activity (via optogenetic stimulation) leads to an increase in Storer profiles, which are not obviously "exploratory"—arises from a potential mismatch between intuitive behavioral categories and the formal structure of our reinforcement learning model. In the model, β does not capture exploration in the sense of novelty-seeking, but rather the degree to which recent reinforcement history constrains choice. High β values favor exploitation and, in a competitive social context, drive the symmetry breaking that produces complementary Worker–Scrounger roles. By contrast, low β values promote more stochastic action selection, which suppresses differentiation and favors convergence toward uniform, low-conflict strategies such as the Storer profile, where animals produce food but delay its consumption.
Accordingly, increasing tonic DA activity before group formation biases the system toward lower β-like dynamics, reducing specialization and promoting Storer-like behavior. This interpretation is consistent with our model's predictions and with the idea, developed in the revised Discussion (lines 271-289), that tonic and phasic components of dopaminergic activity have distinct consequences: broad tonic activation biases animals toward storing, while phasic, competition-aligned signals promote Worker–Scrounger specialization. Importantly, the effects of our manipulations were reproducible across groups and extended to all members of a triad, indicating a collective reorganization of roles rather than an artifact of direct stimulation.

References: appropriate credit to previous work?

Yes. However, these references may assist in justifying some of the DA work:
Chen, Cathy S., et al. "Dopamine and norepinephrine differentially mediate the exploration–exploitation tradeoff." Journal of Neuroscience 44.44 (2024).
Hamid, Arif A., et al. "Mesolimbic dopamine signals the value of work." Nature neuroscience 19.1 (2016): 117-126.
Kakade, Sham, and Peter Dayan. "Dopamine: generalization and bonuses." Neural networks 15.4-6 (2002): 549-559.
We thank the reviewer for these excellent suggestions. We have now incorporated these references references into the Introduction and Discussion sections to strengthen our theoretical and empirical framing of dopamine's role in exploration, learning, and valuation. These citations have been integrated into the Introduction and Discussion sections

Clarity and context: lucidity of abstract/summary, appropriateness of abstract, introduction and conclusions

The report is clearly written and the effort is greatly appreciated. However, the data appears appears rich and the paper leaves many questions unanswered that make it difficult to understand the reasons for the conclusions.

In revising the manuscript, we have aimed to address both specific methodological concerns and broader conceptual questions.
In particular, we have clarified and strengthened the evidence for the three core hypotheses highlighted by Reviewer 1:

1. That dopamine contributes to the emergence and differentiation of behavioral phenotypes, through both correlative and causal evidence;
2. That competition shapes the structure of behavioral roles, as demonstrated through sex-based comparisons, group manipulations, and targeted dopaminergic interventions;
3. That behavioral specialization is flexible and shaped by reinforcement learning, as shown through re-training experiments and modeling of role-switching dynamics.

We have also incorporated many valuable suggestions from Reviewer 3, including refined analyses of dopaminergic transients, clarification of the optogenetic logic, consideration of body weight and social rank, and the use of archetypal analysis to represent individual variation without relying on discrete clusters.
We believe these changes have improved the clarity, rigor, and interpretability of the manuscript, and we thank the reviewers again for their constructive input.

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #4 (Remarks on code availability):

The authors provided a set of Matlab scripts to simulate the full model and simulate and analyze the reduced model will a concise README file. The code is easy to run followed by instructions.
However, I can not find code for behavioral analysis.

Code for behavioral analysis have been added and addociated with the revision and will be found with data on zenodo.

Referee #1 (Remarks to the Author):

The authors have done an outstanding job of addressing reviewers' concerns regarding their manuscript. They have performed new analysis of their data and several new experiments to 1) better characterize the social behavioral groups they have identified, 2) better elucidate contributions of dopaminergic neuron activity in behavior specification, and 3) directly test the flexibility of social behavior phenotypes in their semi-natural "microsociety" reinforcement paradigm. This includes new optogenetic and chemogenetic experiments to test manipulation of dopaminergic neuron activity directly on social behavior phenotypes and new social behavior flexibility experiments where they demonstrate that phenotypes identified in an initial group setting can switch to different phenotypes in a new group setting. Finally, the authors investigate if social hierarchy can explain behavior phenotypes observed in the group reinforcement paradigm and they find that social hierarchy and behavioral archetypes are independent. Taken together, these new experiments along with revised data analysis and additional electrophysiology recordings significantly strengthen the authors' findings which should be of broad interest. We have no further concerns.

Susanna Molas and Andrew R. Tapper

We thank Reviewers #1 and #2 for their very positive evaluation of our revised manuscript. We are grateful for their supportive comments, which confirm that the main points are now clear and the conclusions well supported by the data.

Referee #2 (Remarks to the Author): I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #3 (Remarks to the Author):

Comments for author during round 2 review

The authors improved the manuscript a lot, pinpointing three core arguments relating to the role emergence: both internal factor (Dopamine) and external factor (competition) are necessary to the role emergence, and role specialization is plastic. For the internal factor, the author constructed a model and find a parameter β controlling balance between exploration and exploitation influencing the foraging behavior and role differentiation most. They further combine in vivo electrophysiology, fiber photometry and optogenetics to show that dopamine activities are correlated to the parameter β. The experiment mixing Scroungers and naïve mice directly test the idea of plasticity in role division. The improvement helps readability.

The inconsistency of DA activities remains the major drawback. The author discussed the paradox about DA activities and proposed a distinction between tonic baseline activities and phasic, behavior event time locked activities. The author proposed that in vivo electrophysiology data after experiment reflect DA responses amplified by competition, so is not related to β. On the other hand, optogenetics and chemogenetics manipulations influence the baseline DA activities, and thus influencing β. The explanation, however, still looks unconvincing to me. If the β is more tightly related to baseline activities at the beginning of the experiment, then we should expect different baseline activities for triads that turn out to be Storers or Worker-Scoungers. Based on the model, higher β corresponds to higher DA activities and higher probability of being Worker-Scoungers triads. However, the in vivo electrophysiology shows comparable DA tonic baseline activities for male and female (though male and female role specification are different). One potential explanation might be different mapping between DA activities and β for male and female. The initial DA activities that correlates more to the β may be the salience (signal-to-noise ratio) of task even aligned phasic activities. If the baseline increased (male optogenetic activation experiment), salience of the phasic DA activities will decrease and thus the β decrease for them, leading to more Storers in the triads. One simple test of this hypothesis would be comparing DA activities on day 1 of the experiment between animals that turn out to be Storers versus Worker-Scoungers. The prediction is higher task event related DA activities on day 1 for Worker-Scoungers.

We thank the reviewer for the positive and constructive evaluation provided during the first round of revision, which acknowledge the substantial additional analyses and experiments we had incorporated. We also thank them for this thoughtful comment in the second round, which primarily concerns the interpretation of the dopaminergic data

and helped us to refine the discussion and clarify the link between β and dopaminergic activity. We agree that the relationship between β and dopamine cannot be reduced to a simple correspondence with tonic firing rate, and we found the reviewer's alternative interpretation—that β might reflect the salience or signal-to-noise ratio of phasic dopaminergic responses—particularly insightful.

Following this suggestion, we re-examined our fiber-photometry recordings to test whether early dopaminergic activity (day 1) could predict later role specialization (Workers, Scroungers, Storers). We evaluated event-related VTA transients at food delivery and at lever presses on day 1. Food-related transients were already detectable in both sexes, whereas lever-press–evoked responses were clearly present in males but weak or absent in females (Supplementary Fig. 4). These analyses are now included in the Supplementary Results (Supplementary Fig. 4a–b) and described in the revised text.

In light of this, we have substantially rewritten the relevant paragraph in the Discussion (l 271-291), which now explicitly integrates this interpretation. We clarify that β likely depends on the interaction between distinct dopaminergic components—tonic, non-specific activity versus phasic, event-locked responses—and that its effective value may be shaped by the salience (signal-to-noise ratio) of phasic signaling. This modification resolves the previous ambiguity and aligns well with the reviewer's reasoning, while also framing this question as a broader and technically challenging avenue for future investigation.

We hope that these clarifications and the revised discussion will convince the reviewer that our conclusions are now consistent with the data and capture the complexity of the dopaminergic mechanisms underlying social specialization.

Minor points

1. Behavior description of Storers archetype is inconsistent. In Fig. 2e, the gains and losses are few in Storers, while in Fig. S3e, storers have many losses and gains (at least as many as Workers and Scroungers).

The apparent difference between Fig. 2e and Fig. S3e simply reflects the temporal evolution of behavior: Fig. S3e shows data from the first day of group formation, whereas Fig. 2e presents stable behavioral patterns from the final days of the experiment. In addition, Fig. S3e reports raw counts (few events early in the task), whereas Fig. 2e displays normalized percentages, which further explains the visual difference.

Referee #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports.

Referee #4 (Remarks on code availability): The authors provide detailed readme files about how to run the code and I can run the code without error following the guide.

We thank Reviewer #4 for taking the time to test our code and for confirming that it runs correctly with the provided documentation.