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Neural activity patterns differ between learning contexts in a social fish

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Learning and decision-making are greatly influenced by context. When navigating a complex social world, individuals must quickly ascertain where to gain important resources and which group members are useful sources of such information. Such dynamic behavioural processes require neural mechanisms that are flexible across contexts. Here we examine how the social context influences the learning response during a cue discrimination task and the neural activity patterns that underlie acquisition of this novel information. Using the cichlid fish, *Astatotilapia burtoni*, we show that learning of the task is faster in social groups than in a non-social context. We quantify the neural activity patterns by examining the expression of Fos, an immediate-early gene, across brain regions known to play a role in social behaviour and learning (such as the putative teleost homologues of the mammalian hippocampus, basolateral amygdala and medial amygdala/BNST complex). We find that neural activity patterns differ between social and non-social contexts. Taken together, our results suggest that while the same brain regions may be involved in the learning of a cue association, the activity in each region reflects an individual's social context.

Keywords:
animal behaviour, cichlid fish, neural activity, social learning

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1. Introduction

Social interactions provide a key source of information that can greatly impact the fitness of individuals within groups. It is commonly assumed that learning from others, or *social learning*, is inherently adaptive as it allows individuals to avoid costs associated with learning by themselves, or *non-socially* [1]. The benefits of social learning allow individuals to gain information from conspecifics that is important for their survival [2–5]. These wide-ranging behaviours have been studied across species, such as in instances of socially transmitted food preferences [6,7], social learning of certain skills [8–10], mate preference learning [reviewed in 11], predator avoidance [12] and fear transmission [13, reviewed in 14]. The behavioural mechanisms that underlie these behaviours are diverse, ranging from stimulus enhancement (when another individual draws the observer's attention to a particular stimulus or object) to observational learning [15–17]. To disentangle the processes of social learning, we need to understand its underlying mechanisms.

Previous studies in rodents and songbirds have expanded our understanding of the neurobiological mechanisms that mediate social learning by examining changes in neural activity across brain regions [14,18–21]. At the molecular level, learning requires activity-dependent changes in gene expression [22,23], and activation of immediate early genes (IEGs) is a critical mediator in this process [24,25]. Studies in rodents have shown that IEGs such as *cfos* are expressed following acquisition and consolidation of associative learning [26–29]. In addition, IEG expression in the hippocampus specifically has been shown to reflect neural activity during a social learning task [26,27]. The medial amygdala also plays a key role in mouse social cognition, as oxytocin receptors in this region are essential for recognizing familiar conspecifics [30]. In songbirds, differential Fos expression has been shown to underlie different aspects of song learning and production [31,32], and

there is evidence that differential neural activity underlies different phases of sexual imprinting, a type of social learning by which a juvenile learns specific characteristics of a parent or other familiar individual [33]. While these findings demonstrate that neural activity patterns can inform our understanding of the neural substrates underlying social behaviours, not many studies have examined how this activity changes over the course of the learning process in different social contexts (but see [34]).

Here, we investigate the neural activity patterns that differentiate social and non-social learning in *Astatotilapia burtoni*, an African cichlid fish used as a model system in social neuroscience because of its remarkable phenotypic plasticity and sophisticated social cognition [35,36]. Dominant males of this species are territorial and aggressive, while subordinates typically do not hold territories and are overall less aggressive [36,37]. In a recent study, we found that although dominant males had strong influence over the movement of their social groups under normal conditions, they were less influential in a more complex learning task [38]. IEG expression in response to different types of social information, such as territory intruder, has also been shown in this species [39–42], suggesting that differences in the learning context may induce differential patterns of neural activity.

We examined IEG expression in different brain regions of *A. burtoni* males and females during learning in social groups with a demonstrator and in a non-social context. We first compared learning rates between contexts as measured by the latency to acquire a cue association. We hypothesized that social facilitation mechanisms would allow groups to learn the task faster than individuals in the non-social context. To understand the neural activity patterns that distinguish learning in different social contexts, we quantified expression of Fos, an IEG, across the putative teleost homologues of the mammalian hippocampus, basolateral amygdala, and medial amygdala/bed nucleus of the stria terminalis (BNST) complex, which are key nodes of the social decision-making network (SDMN) [43,44]. We predicted that neural activity during learning in a social context would be highest in brain regions important for mediating social behaviour in this species (see, e.g. [45] and 36]), such as the supracommissural part of the ventral pallium (Vs, the putative homologue of the mammalian medial amygdala/BNST complex) and the medial part of the dorsal telencephalon (Dm, the putative homologue of the basolateral amygdala); as well as those important for associative learning [46,47], such as the lateral part of the dorsal telencephalon (Dl, the putative homologue of the hippocampus). In addition, given the well-known role of the hippocampus in associative learning, we expected neural activity in Dl to increase in both contexts once learning occurs. Finally, we predicted that neural activity in regions important for social behaviour would be relatively low in the non-social context. Our results reflect differences in activity patterns depending on the social context.

2. Methods

(a) Animals

Astatotilapia burtoni descended from a wild caught stock population were kept in stable naturalistic communities of eight males and eight females, as described previously [44] until being transferred to experimental aquaria. Brooding females were stripped of fry immediately prior to being placed in experimental aquaria. All work was done in compliance with the Institutional Animal care and Use Committee at The University of Texas at Austin. All relevant code and analyses are available online at https://github.com/neuromari/neuro_social_learning.

(b) Visual cue discrimination task

In a protocol broadly following that of Rodriguez-Santiago *et al*. [38], all animals were housed and tested in 200 L aquaria with digital video cameras mounted above (see electronic supplementary material, figure S1 for schematic). Behaviour was recorded for 20 min prior to each trial and for 10 min after. Each aquarium had two automatic feeders (Eheim) attached at opposing ends of the tank that were controlled by an Arduino Uno microcontroller board attached to an LED. This LED presented either an orange or a cyan light for 3 s every 3 h, and when the lights went off a food reward was dispensed from the feeder that shone the orange light cue. Cue sides were randomized across trials to ensure the animals did not develop a side preference. There were four trials per day spaced every 3 h, and on the last day we collected brains after the second trial. The food reward was dispensed after every cue regardless of behavioural response.

(c) Cue discrimination task training in a social context

All animals were tagged with visible implant elastomer tags (Northwest Marine Technology) for identification one week prior to the start of the experiment. Social groups of four males and four non-gravid females ($n = 5$ groups) were initially placed together in the experimental aquaria 16 h prior to the first learning trial. Following 22 trials, individuals were placed in holding aquaria and remained in the same social groups in which they were trained. These individuals are now considered *demonstrators* for observers in the subsequent learning experiments.

One male demonstrator ($n = 22$ total) was placed in a new group of all naive fish (deemed *observers*). Groups of seven observers (three males, four females) and one demonstrator male were trained on the task described above ($n = 11$ total groups with dominant demonstrator, $n = 11$ total groups with subordinate demonstrator). Females measured on average 41.5 ± 0.55 mm and were within 5 mm length of other females in the tank. Males were on average 5–10 mm larger than the females in their groups. Groups were assembled and placed in the experimental aquaria 15 h prior to the first trial at 8.00 h. Groups were trained for either six, 14 or 22 trials. The last trial (either six, 14 or 22) for all groups was at 11.00 h.

(d) Visual cue discrimination task training in a non-social context

In the non-social condition, naive individual males ($n = 14$) and individual females ($n = 14$) were trained on the same visual discrimination task while housed with seven blind cave fish (Mexican tetra, *Astyanax mexicanus*) such that the focal fish is not socially isolated but receives no social facilitation while responding to the visual cue. Individuals were placed in the experimental aquaria along with blind cave tetras the night before the first trial. All individuals were taken after either trial 6, 14 or 22 for subsequent brain collection and histology.

(e) Criterion for the learning response

For each trial, we assessed the behavioural response to the onset of the LED and quantified the number of fish that moved toward the correct feeder prior to the food reward being dispensed. A correct response required the fish to move directly under the correct feeder prior to food presentation. In the social context, the percent group response was calculated by dividing the number of fish that moved by the total number in the group. In the non-social context, individual response was binary—either the fish

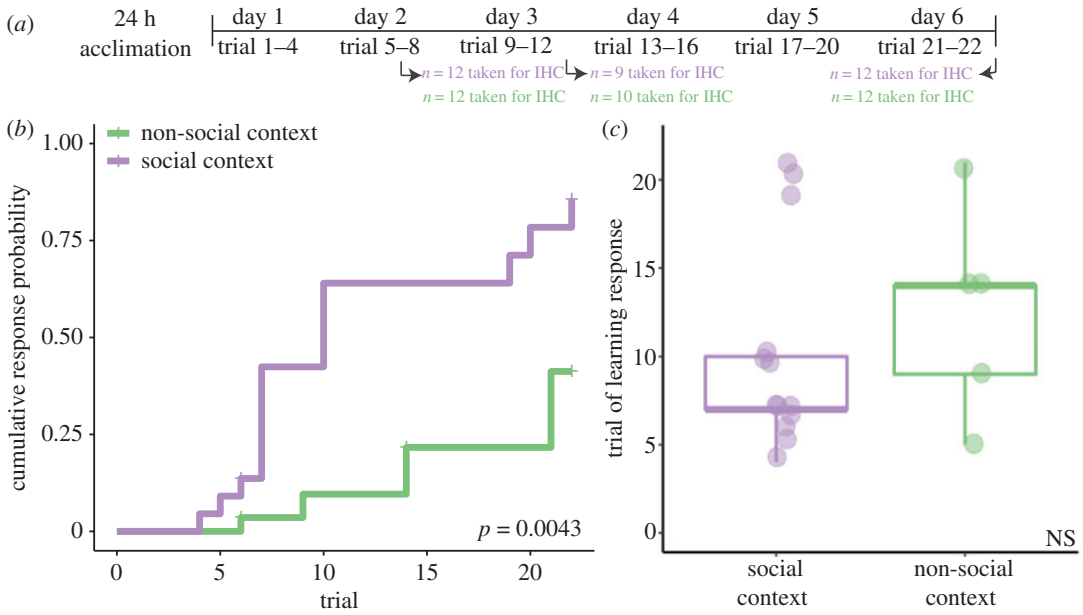

Figure 1. Learning rate is faster in a social context. (*a*) Timeline of experimental design. All individuals were placed in tanks 24 h prior to first trial. Ns represent number of observers and individuals from non-social context taken to examine neural activity patterns. (*b*) Comparison of the cumulative response probability, or learning rate, between social and non-social context shows that groups have a higher response probability than individuals in a non-social context ($p = 0.004$). This includes groups and individuals that never reached the response criterion. (*c*) Although the rate of learning is significantly different (shown in *b*), the total number of trials it took groups and individuals that did learn to reach the response criterion is not statistically different between contexts ($p = 0.426$). (Online version in colour.)

moved or did not. We established a response criterion where groups and individuals had to correctly respond to the task in two or more consecutive trials for them to be considered to have *learned* the task. For the social context, this criterion was met when at least five of the seven naive group members (71%) move to the correct cue (see electronic supplementary material, Materials for how we arrived at this criterion). Note that this variable is right censored because we stopped the experiment after either six, 14 or 22 trials. Learning thus was a dependent variable and we did not include a non-learning control group.

(f) Sample processing and immunohistochemistry for examining neural activity

To examine neural activity patterns across learning trials, three naive observers (the largest (51 ± 1.3 mm) and smallest (48.5 ± 1.2 mm) male observers, and one female) were taken from each group after either trial 6, 14 or 22 for subsequent brain collection and histological processing (design outlined in figure 1*a*). In groups with a dominant male demonstrator, the second-largest male, subordinate male, and a female were collected. In groups with a subordinate male demonstrator, the dominant male, and third largest male, and a female were collected. For all non-socially trained individuals, males and females were euthanized after trials 6, 14 or 22. A detailed description of the immunohistochemical procedures and the quantification of Fos-positive cells is provided in the electronic supplemental material.

(g) Statistical analysis

All statistical analyses were conducted using R Studio (v. 1.0.143). We analysed the learning response using a survival analysis and the 'survival' package [48] in R language. We used a series of log-rank tests to examine the effect of social context on the learning rate, measured as the cumulative successful response rate based on groups and individuals meeting the learning criterion. For the individuals and groups that *did* learn the task, we compared the total number of trials it took reach the learning criterion in a social versus non-social context using a Mann–Whitney–Wilcoxon test since the data did not meet parametric assumptions. All boxplots shown represent the median and the first and third quartiles. Boxplot whiskers extend to the largest and smallest observations within or equal to 1.5 times the interquartile range. Results were considered significant at the $p < 0.05$ level. We calculated effect sizes as Cohen's *D* using the R function 'cohen.d()' in the R package 'effsize'. We conducted principal component analysis (PCA) with the R function 'prcomp' to identify how neural activity patterns across brain regions clustered based on social context conditions and individual-level traits. We used two-way ANOVAs with the group animals came from as a random effect to identify significant effects of context (social, non-social), time point (trial 6, 14, 22) and learning (yes met learning criterion, did not met criterion) on brain region Fos expression. We compared pairwise differences between groups using *post-doc* TukeyHSD tests.

3. Results

(a) Social facilitation results in faster learning compared to a non-social context

We first asked whether learning of the cue discrimination task differed between the social and non-social contexts, which was measured as the cumulative response probability. We found that the learning probability is significantly greater in social groups than individuals in a non-social context (log-rank test: $X^2 = 8.1$, $p = 0.004$, Cohen's $D = 0.4$; figure 1*b*). However, the total number of trials it took to reach the response criterion did not differ between contexts (Wilcoxon test with FDR correction: $W = 41$, $p = 0.426$; figure 1*c*).

(b) Neural activity patterns depend on the social context

We used PCA to determine what factors of the context contribute to differential neural activity patterns during learning. We first conducted a PCA that included variables in both social conditions: the trial at which individuals were taken (trial), the context condition (social versus non-social), and whether the response criterion was met (yes or no). We found that principal

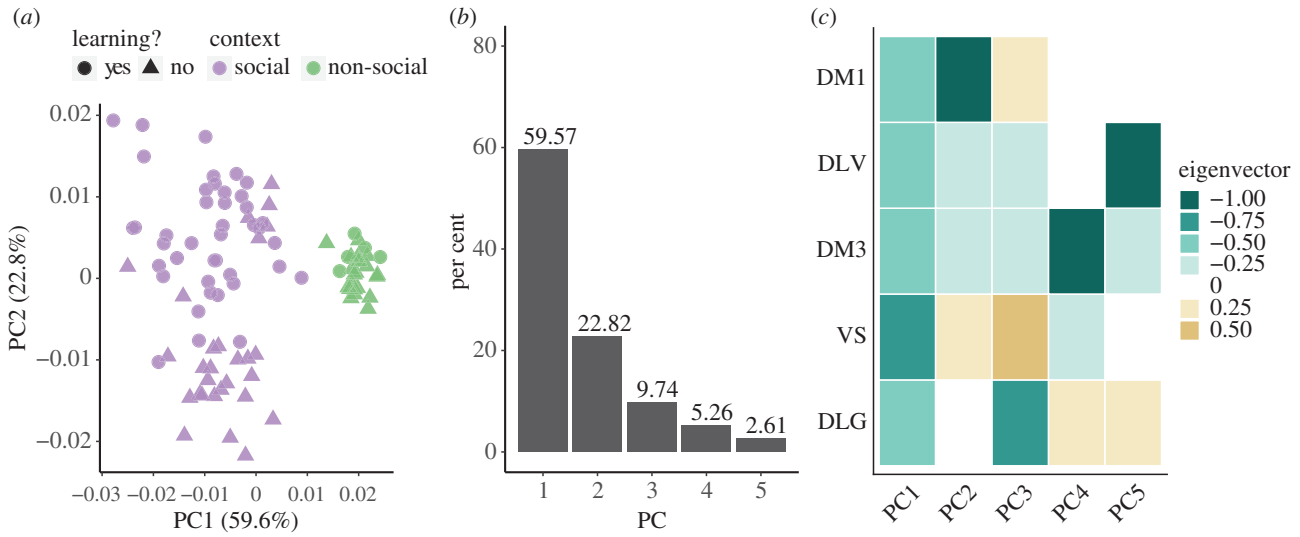

Figure 2. PCA of neural activity shows differential expression pattern with learning context. (*a*) Scatter plot of all Fos expression data separates out by social context across PC1. (*b*) Bar graph showing the percent of the variance explained by each PC. (*c*) Heat map illustrating the eigenvector values for each PC. Eigenvector values closer to −1 and 1 represent stronger loadings on that PC. (Online version in colour.)

component 1 (PC1) accounted for 59.6% of the total variance and differed significantly between contexts across trials (figure 2). When we examined the eigenvector values for each PC to determine the loadings each brain region contributes, we found that PC1 was most strongly loaded by the Vs, while Dm-1 loaded strongly on PC2 (figure 2*c*). We also conducted ANOVAs to examine if there was a main effect of context and trial on these PCs, and found that these direct comparisons reflect our raw data results (figure 3). Given the striking differences in neural activity patterns between contexts, we conducted separate PCAs on the social and non-social contexts. The results of these analyses, along with the ANOVA results, are in the electronic supplemental material.

(c) Neural activity patterns during acquisition of learning differ across social contexts

To disentangle how neural activity patterns change over learning trials across contexts, we examined neural activity across Dl-g, Dm-1 and Vs brain regions. We compared neural activity across context (social, non-social) and learning task trial (6, 14, 22) using two-way ANOVAs (figure 3; electronic supplementary material, table S2 and S3 for statistics). In the Dl-g, we found significant main effects of trial and context on Fos expression but no significant interaction (ME trial $F_{2,91} = 8.878$, $p < 0.001$, Cohen's $D = 0.44$, ME context $F_{1,91} = 203.754$, $p < 0.001$, Cohen's $D = 1.5$, trial×context $F_{2,91} = 2.22$; $p = 0.114$, Cohen's $D = 0.22$). In the Dm-1 there was both a significant main effect of trial and context as well as an interaction (ME trial $F_{2,91} = 54.63$, $p < 0.001$, Cohen's $D = 1.10$, ME context $F_{1,91} = 120.92$, $p < 0.001$, Cohen's $D = 0.93$; trial×context $F_{2,91} = 11.97$, $p < 0.001$, Cohen's $D = 0.51$). In the Vs there was also a significant main effect and interaction between trial and context (ME trial $F_{2,91} = 8.776$, $p < 0.001$, Cohen's $D = 0.44$, ME context $F_{1,91} = 95.324$, $p < 0.001$, Cohen's $D = 1.02$, trial × context $F_{2,91} = 4.103$, $p = 0.02$, Cohen's $D = 0.3$; all trial × context in figure 3*b,e,g*).

When we examined whether Fos expression changed with learning, we found a significant difference between context (figure 3*c,f,h*; see electronic supplementary material, table S3 for statistics). Across all brain regions, there was a main effect of context (Dl-g: $F_{1,93} = 146.3$, $p < 0.001$, Cohen's $D = 1.25$; Dm-1: $F_{1,93} = 87.84$, $p < 0.001$, Cohen's $D = 0.97$; Vs: $F_{1,93} = 58.552$, $p < 0.001$, Cohen's $D = 0.79$) as well as learning (Dl-g: $F_{1,93} = 115.6$, $p < 0.001$, Cohen's $D = 1.12$; Dm-1: $F_{1,93} = 10.21$, $p = 0.002$, Cohen's $D = 0.33$; Vs: $F_{1,93} = 46.167$, $p < 0.001$, Cohen's $D = 0.7$), and an interaction between learning and context in the Dm-1 ($F_{1,93} = 5.137$, $p = 0.03$). There was no difference in Fos expression in the non-social context based on learning, while in the social context Fos expression was highest in observers that learned the task in the Dl-g ($p < 0.001$) as well as in the Vs ($p = 0.001$). Despite the large differences between Fos expression across social contexts in all brain regions measured over trials, when we looked closer at factors that impact this difference within the social context, we found no significant differences in expression based on either observer or demonstrator social rank (electronic supplementary material, tables S4 and S5).

4. Discussion

We found that learning under different contexts (social versus non-social) affects neural activity patterns at various times during the learning process. Specifically, we discovered a significant difference in the learning rate between contexts, such that social groups reached the learning criterion earlier than individuals in a non-social context. This behavioural difference was reflected in the neural activity pattern differences between contexts, with activity in brain regions being higher across different learning trials depending on the context. Overall, although animals performed the same learning task, our results demonstrate that neural activity patterns during the learning process reflect the context.

(a) Groups learn a cue association task faster than individuals

We found that groups with a demonstrator had a significantly faster learning response than individuals in a non-social context. This is not necessarily surprising given the prevalence of social learning strategies across species and the notion that social learning is adaptively beneficial as it confers fewer costs and allows individuals to gain new

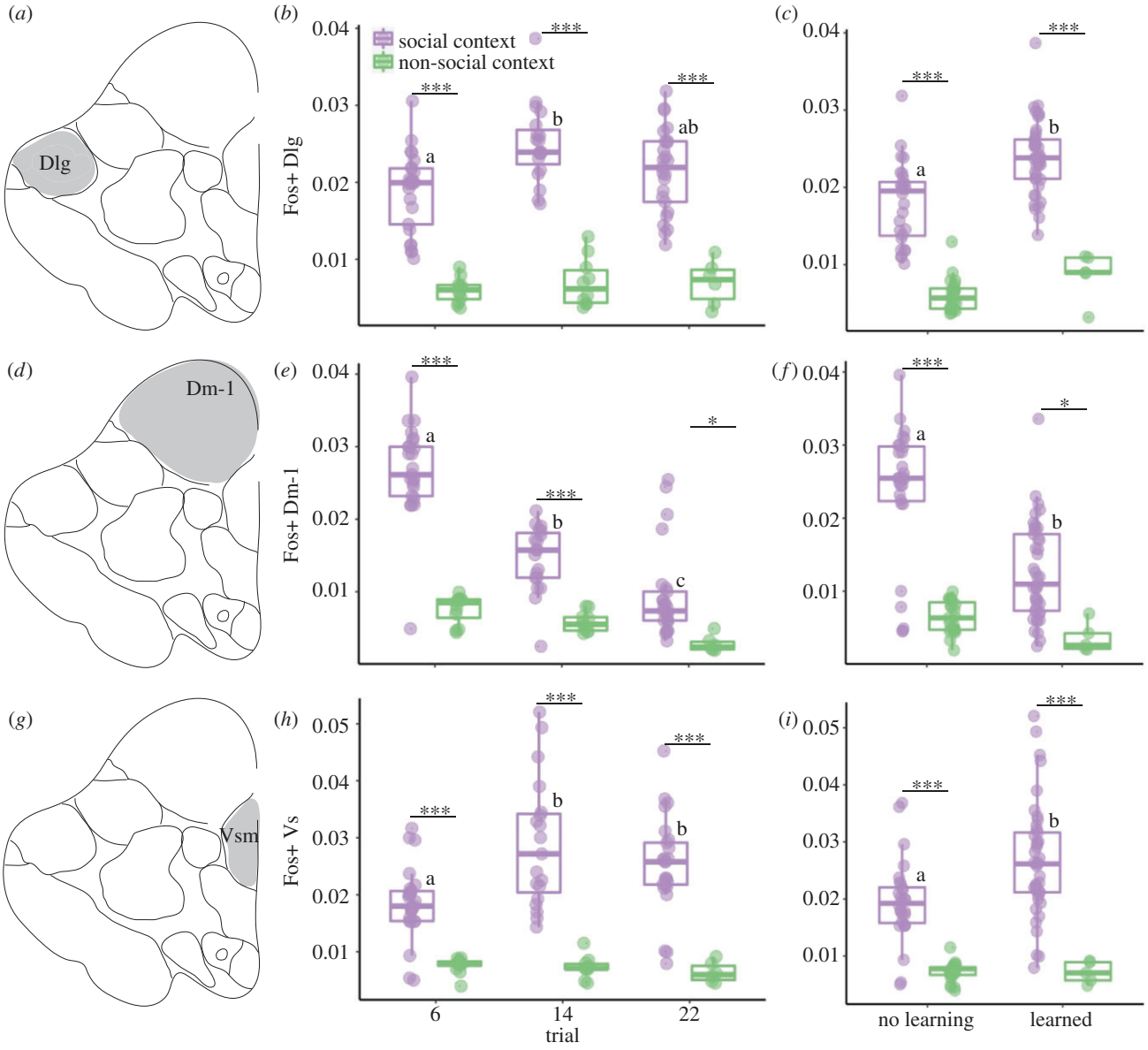

Figure 3. Neural activity across brain regions varies over trials and with learning. Fos expression was quantified as a marker of neural activity in the Dlg, Dm-1 and Vs regions of the forebrain (*a*,*d*,*g*). In the Dlg, there was a significant increase in activity from trial 6 to 14 in the social context, while there was no difference in activity across trials in the non-social context (*b*). Neural activity was significantly highest in learners in the social context (*c*). In the Dm-1, activity significantly decreased over trials (*e*). Activity was significantly highest in the Dm-1 in the social context when learning had not occurred (*f*). In the Vs, activity significantly increased after trial 6 in the social context (*h*) and was significantly higher in the social context with learning (*i*). (Online version in colour.)

information more quickly [49]. In addition, information diffusion is typically accelerated in social groups [50].

There are at least two mechanisms by which learning might have occurred in our social paradigm—social facilitation (when the presence of a demonstrator affects the observer's behaviour) and stimulus enhancement (where the observer's behaviour changes after watching a demonstrator interact with a stimulus). To demonstrate that the group response is due solely to the presence of a demonstrator (i.e. social facilitation), it would be necessary to test individual group members by themselves following acquisition. While we did not test for this retention in observers, it should be noted that the demonstrators themselves were trained in naive groups and then transplanted to new groups where they were the only informed individual and correctly displayed the learning response. This suggests that the individuals in groups can in fact acquire the association since they display the correct learning behaviour in subsequent trials and in new groups. However, this learning response may not be entirely attributable to social learning mechanisms. Conspecific cueing and local enhancement may also have contributed to individual-level learning within a social context. Cue locations were randomized during every trial to buffer local enhancement effects. In addition, it cannot be ignored that *A. burtoni* is a social species, and although individuals in the non-social context had blind cave fish as a buffer, their slow learning rate could be due to social isolation stress factors, although we saw no behavioural evidence to suggest this was the case.

(b) Differential hippocampal sub-region activity between a social and non-social context

When we examined neural activity across brain regions in different trials of the learning task, we found significant differences associated with learning in the social context in the Dl-g, a subdivision of the lateral pallium of teleosts, a region implicated in the learning of spatial and temporal relationships in teleosts [46,51]. We also found an overall increase in activity across trials

and learning in the social context, a pattern that remains true across all the brain regions we measured. Previous work has shown that the major pathways within the lateral and dorsal pallium are highly recursive and have complex reciprocal connections with subpallial regions [52]. Based on tract-tracing neuroanatomical data, as well as lesions studies that implicate the Dl and other pallial regions in learning and memory tasks, Elliott *et al*. [52] suggested that this pallial circuitry (which includes the Dl subregions) can implement the same pattern separation and completion computations ascribed to the mammalian hippocampal dentate gyrus and CA3 fields. Given previous work implicating Dl subregions as hippocampal-like in function, we hypothesize that higher neural activity in the Dl-g reflects learning of the task and that the overall higher activity in the social context compared to the non-social reflects background activity in response to stimuli within the social environment.

(c) The basolateral amygdala likely encodes group acclimation, not learning of the association task

We found a significant difference in neural activity across social contexts in subregions of Dm—specifically, a significant decrease in activity across trials in the social context and a significant decrease from trial 14 to 22. Activity in Dm-1 was associated with no learning, and there was no difference with learning in the Dm-3 (not shown). Previous studies in goldfish that have shown that Dm lesions disrupt trace and delay avoidance conditioning [47,51], as well as fear and heart-rate classical conditioning [19], while such lesions have no effect on spatial memory and cue learning [53,54], consistent with our findings that the Dm showed no significant activity change with learning. The effects of these lesions in fish are similar to lesions of the amygdala in mammals [55–58] and in part based on this evidence the teleost medial pallium (which includes the Dm) has been proposed as homologous to the pallial amygdala of mammals [51]. In *A. burtoni* males, Dm activity is correlated with the level of engagement in joint territory defense, although the direction of the correlation depends on an individual's role in said behaviour [42]. Here, we found that activity in the Dm-1 was significantly higher in trial 6 compared to 14 and 22. Given that few groups had learned the task by trial 6, it is not surprising that Dm activity was also higher in groups that had not yet successfully learned the task. Interestingly, observers from groups that did reach the learning criterion by trial 6 had lower Dm activity, indicating that Dm is not involved in learning the cue association task. We hypothesize that this overall decrease in Dm-1 neural activity reflects an aspect of tank or group acclimation, although testing this hypothesis was outside the scope of the present study.

(d) The extended medial amygdala encodes social context

In Vs, we found a significant main effect of social context. Vs activity also increased in social groups in trials 14 and 22, possibly as a consequence of more groups successfully learning the task at these later trials. Homology of this brain region has historically been difficult to characterize due to the eversion, rather than invagination, of the neural tube during teleost development [59–62]. However, developmental studies have found similar genetic markers, namely *Dlx2*, *Lhx7*, *Nkx2.1b*, between the Vs and the extended amygdala [63]. Stimulation of the Vs has been shown to increase aggression in male bluegill fish [64]. In *A. burtoni*, this region is under social and reproductive modulation [40] and shows varying levels of sex steroid receptor expression in males when given the opportunity to ascend or descend in status. This suggests that Vs plays a predominant role in mediating social information, which is why we see large differences in neural activity here between the social and non-social learning contexts.

(e) Disentangling the effects of group formation and learning on neural activity patterns

While we see evidence for differential neural activity across brain regions during learning of an association in both social and non-social contexts, we are unable to fully separate the effects of group acclimation time from the effects of learning. Even though there are significant differences in neural activity in specific brain regions (Dl, Dm) based on whether groups demonstrated learning, it remains unclear how acclimating to a new group while receiving new information impacts learning. There could be a dampening of response in early trials due to social instability simply because the groups did not have time to acclimate prior to the start of the trials. In the non-social context, we observed a general dampening of neural activity specifically in early trials that coincided with lower behavioural activity levels. Disentangling the effects of social group formation and acclimation from the increased probability of learning after repeated trials in all contexts will require examining individual behaviour within groups by using automated tracking. This would allow us to relate an individual's neural activity patterns to its attentional structure (inferred from, e.g. visual field measurements) as well as to its position within a proximity or social network, independent of learning the cue [38].

(f) What Fos expression tells US about the observed neural activity patterns

An important aspect of our experimental design is that we examined Fos expression 1 h after the last learning trial the animals underwent—whether it was trial 6, 14 or 22. Expression of IEGs such as Fos is widely used as a measure of neural activity [65,66] as most IEGs encode transcription factors or DNA-binding proteins that coordinate the cellular response to a stimulus [25]. By examining Fos protein expression within 60–90 min following the last stimulus exposure, we aimed to capture the brain regions that are active, and presumably important, for the individual's behavioural response. Animals did not perform these behaviors in isolation, and it is possible that both in the social and non-social contexts their neural activity reflects a response to the environment rather than the stimulus cue itself. For example, there could have been a salient social signal occurring in the aquarium at the same time as the cue (such as high territorial aggression by a dominant male). We posit that the differential activity patterns reflect these differences in environmental cue saliency. For example, based on previous work examining the role of the Dm in behaviour and given the high Fos expression in the Dm-1 in trial 6 compared to later trials in both the social and non-social contexts, the observed IEG pattern in this region is likely reflective of the animal's response to other salient cues in the (social) environment besides the stimulus cue.

Since we only sampled brain activity at specific trials, it is possible that an individual learned the task during an intervening trial. In such a case, the neural activity measured at a subsequent trial could reflect a memory instead of the learning process itself. This would be particularly relevant for activity in the Dl, which has been shown to be important for memory processes in goldfish [46,53] and weakly electric fish [52]. However, if activity in this region did reflect a memory of the already learned cue, we would expect such activity to be highest at trial 22 across all groups and contexts, since most of the behavioural responses have occurred by then. Instead, we found that activity in the Dl-g is highest at trial 14, suggesting that this region was not activated with continued cue task recall.

(g) Group learning and neural activity patterns are independent of social status

Communities of *A. burtoni* naturally form rank hierarchies with some males establishing social dominance by aggressively defending territories for mating, while most males are socially subordinate and reproductively suppressed [35,67]. We have previously shown for this species that the social status of a demonstrator can have a strong effect on how fast a group learns the visual cue discrimination task. Specifically, even though socially dominant males strongly influence their groups through aggressive displays and space use, they are significantly less effective in generating group consensus during the task than subordinate males [38]. By contrast, we did not find a significant effect of demonstrator status on group learning in the present study (data not shown). This may not be surprising given that the present study was not designed to examine the effects of social status on group learning, and thus lacks the statistical power to robustly detect such an effect. It should also be noted that in the Rodriguez-Santiago *et al*. [38] study, dominant males were considerably larger than subordinate males, while in the present study the size difference was much smaller. Previous work has shown that small size differences result in lower stability of the social hierarchy in this species [68]. Although we did not quantify group stability here, the behavioural traits that determine whether an individual is an effective demonstrator—aggression and space use—are highly context-specific and might explain the absence of a social status effect.

5. Conclusion

We used the social cichlid fish *A. burtoni* to demonstrate that learning in a social context is associated with increased neural activity (as measured by the expression of Fos) when compared to learning in a non-social context across key brain regions important for learning and social behaviour. These brain regions are important for modulating learning (hippocampus), emotional learning and fear avoidance (basolateral amygdala), and social behaviour (medial amygdala/BNST), and are part of a greater SDMN that is important for mediating various aspects of social behaviour [43,44]. In addition, we found that activity in these regions was not modulated by the sex or social status of individuals, nor was it impacted by the status of demonstrators in social groups. Thus, while these regions are important for different aspects of learning [43], they do not appear to be modulated by group dynamics or individual-level traits in two different learning contexts. Taken together, our results highlight that the context individuals are in strongly affects neural activity patterns throughout the learning process.

Authors' contributions. M.R.-S.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft, writing—review and editing; A.J.: conceptualization, methodology, resources, software, writing—review and editing; H.A.H.: conceptualization, funding acquisition, resources, software, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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