Differential neural activity patterns mediate learning across contexts in a social cichlid fish

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Author contributions:
MRS, AJ, and HAH designed experiments, MRS performed experiments and statistical analysis, MRS and HAH wrote the manuscript, MRS, AJ, and HAH revised manuscript.

Abstract

Learning and decision-making are greatly influenced by the social context surrounding individuals. 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 behavioral processes require neural mechanisms that are flexible across contexts. Here we examined how the social context influences the learning response during a visual 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 quantified the expression of Fos, an immediate-early gene, across candidate brain regions known to play a role in social behavior and learning, such as the putative teleost homologues of the mammalian hippocampus, basolateral amygdala, and medial amygdala/BNST complex. We found that neural activity patterns differ between social and non-social contexts. Our results suggest that while the same brain regions may be involved in the learning of a discrimination task independent of social context, activity in each region encodes specific aspects of the task based on context.

1. Introduction

For group-living species, social interactions provide a key source of information that can greatly impact the fitness and well-being of individual group members. 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* (Kendal et al., 2005). The benefits of social learning allow individuals to gain information from conspecifics as to, for instance, which foods to eat, which routes to take to feeding locations, and how to escape from predators (Ward et al., 2016). These wide-ranging behaviors have been studied across species, such as in investigations of socially-transmitted food preferences (Posadas-Andrews and Roper, 1983; Galef and Wigmore, 1983), social learning of certain skills (Visalberghi and Fragaszy, 1990; Bugnyar and Kotrschal, 2002; Claidiere et al., 2013), mate preference learning (reviewed in White, 2004), predator avoidance (Griffin, 2004), and fear transmission (Bruchey et al., 2010; reviewed in Olsson and Phelps, 2007). The behavioral mechanisms that underlie these behaviors are diverse, ranging from stimulus enhancement (when another individual draws the observer's attention to a particular stimulus or object) to observational learning (Heyes, 1994; Byrne, 1994; Zajonc, 1965), allowing animals to acquire new information important for their survival and which can incidentally be transmitted to conspecifics (Whiten et al., 2016; Laland and Galef, 2009; Laland and Hoppitt et al., 2003; Rendell and Whitehead, 2001). While a lot is known about the neural basis of learning in non-social contexts (reviewed in Cox & Whitten, 2019), few studies have examined whether and how these mechanisms might operate in the context of social learning.

Studies in traditional model systems such as rodents and songbirds have expanded our understanding of the neurobiological mechanisms that mediate social learning. In particular, studies across decades have explored which brain regions are important for the acquisition and

maintenance of socially-transmitted food preferences in rats (reviewed in Hoppitt and Laland, 2013). Subregions of the hippocampus (specifically, the subiculum and dentate gyrus) have been shown to be critical for the retention of socially acquired food preferences (Bunsey and Eichenbaum, 1995; Alvarez et al., 2001). In addition, the basal forebrain and the frontal, piriform, and orbitofrontal cortices play important roles in mediating the acquisition and retention of socially-transmitted information in rodents (reviewed in Choleris et al., 2009). Social learning of fear has been found to be also modulated in part by the lateral nucleus of the amygdala in both rhesus monkeys and humans (Amaral, 2003; Olsson et al., 2007).

At the molecular level, social learning requires neural activity-dependent changes in gene expression, much like long-lasting alterations in the strength of synaptic connectivity important for associative learning (Cole et al., 1989; Sheng & Greenberg, 1990). Activation of immediate early genes (IEGs) is a critical mediator in this process (Bozon et al., 2003; Loebrich and Nedivi, 2009). Previous studies in rodents have shown that IEGs such as *cfos* are expressed following acquisition and consolidation of associative learning (Countryman et al., 2005; Smith et al., 2007; Bertaina and Destrade, 1995; Tischmeyer and Grimm, 1999). In addition, rats trained on a test of social transmission of food preference show greater *cfos* expression in subregions of the hippocampus in a time-dependent manner (Countryman et al., 2005; Smith et al., 2007). The medial amygdala plays a key role in mouse social cognition, as oxytocin receptors in this region are essential for recognizing familiar conspecifics (Ferguson et al., 2001). In songbirds, differential Fos expression has been shown to underlie different aspects of song learning and production (Bolhuis et al., 2000; Bolhuis et al., 2001). There is also evidence in songbirds 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 (Bischof &

Rollenhagen, 1999). Taken together, these findings suggest that associative learning in social contexts are driven by differential neural activity patterns across multiple brain regions in various species.

Here, we investigate the neural activity patterns that differentiate social and non-social learning in a model system that readily forms naturalistic social groups in the laboratory. The African cichlid fish, *Astatotilapia burtoni*, is a model system in social neuroscience because of its remarkable phenotypic plasticity and sophisticated social cognition (Hofmann, 2003; Maruska and Fernald, 2018). Dominant males of this species are territorial and aggressive, while subordinates typically do not hold territories and are overall less aggressive (Hofmann and Fernald, 2001; Maruska and Fernald, 2018). In a recent study, we found that although dominant males of this species had strong influence over the movement of their social groups under normal conditions, they were less influential in a more complex learning task (Rodriguez-Santiago, Nührenberg et al., 2020). This effect was primarily driven by the socially aversive behavior of dominant males, which, although central in interaction networks, occupied peripheral positions in spatial networks. IEG expression in response to different types of social information has also been shown in this species (Fernald and Maruska, 2012; Maruska, Zhang, et al., 2013; O'Connell et al., 2013; Weitekamp and Hofmann, 2017), suggesting that differences in learning in social or non-social contexts 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 or without a conspecific informant. We first compared the learning response rates in a social and non-social context 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 how the brain acquires a cued

association across social contexts, we then 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; O'Connell and Hofmann, 2011, 2012). We predicted that neural activity during learning in a social context would be highest in brain regions important for mediating social behavior in this species, 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, such as specific sub-regions of the lateral part of the dorsal telencephalon (Dl, the putative homologue of the hippocampus). In addition, 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 behavior would be relatively low in the non-social context. Our results reflect differences in how new information is acquired in different social contexts.

2. Methods

Animals

Astatotilapia burtoni descended from a wild caught stock population were maintained in stable naturalistic communities of eight males and eight females, as described previously (in O'Connell & Hofmann, 2012), until being transferred to experimental aquaria. Any 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.

Visual cue discrimination task

In a protocol broadly following that of Rodriguez-Santiago, Nührenberg et al. (2020), all animals were housed and tested in 200 L aquaria (108 x 54 x 46 cm) with digital video cameras mounted above (see Supplemental Figure 1 for schematic). Cameras were scheduled to record behavior for 20 minutes prior to each trial and for 10 minutes after. Each aquarium had two automatic feeders (Eheim) attached at opposing ends. Each pair of feeders was controlled by an Arduino Uno microcontroller board to present either an RGB 255,60,0 (orange) or RGB 0,255,255 (cyan) for three seconds every three hours. Following the offset of the LED cues, the feeder programmed to shine the orange light immediately dispensed food (cue+feeding sides were randomized across trials to ensure the animals did not develop a side preference and that there was no local enhancement). The first cue was at 8:00h every day, for a total of four cue trials per day spaced out every three hours. Food reward was dispensed after every cue regardless of behavioral response. This was the learning task used for training informants, as well as the social groups with informants and the individuals in the non-social context (detailed below).

Training informants on the discrimination task

Twenty males and twenty females 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 tagged males and four tagged and non-gravid females (n = 5 groups) were initially placed together in the experimental aquaria 16 hours prior to the first learning trial. Following twenty-two trials, individuals were placed in holding aquaria and remained in the same

social groups in which they were trained. These individuals are now considered *informants* for observers in the subsequent learning experiments.

Visual cue discrimination task training in a social context

Following informant training, one male (n=11 dominant male informants, n=11 subordinate male informants) was placed in a new group of all naïve fish (deemed *observers*). Groups of seven observers (three males, four females) and one informant male were trained on the visual discrimination task described above (n=11 total groups with dominant informant, n=11 total groups with subordinate informant). Every group (including the informant) consisted of four males and four females (females measured on average 41.5±0.55 mm and were within 5 mm length of other females in the tank). Groups were assembled and placed in the experimental aquaria 15 hours prior to the first trial at 8:00h the following day. Groups were trained for either 6, 14, or 22 trials (depending on their pre-determined experimental group). Three naïve study subjects (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. The last trial (either 6, 14, or 22) for all groups was at 11:00h.

We examined the aggressive behavior of informants during the ten minutes prior to every trial by quantifying approach behavior, defined as the focal informant swimming directly towards any part of another fish's body, within 3 body lengths. If the approached fish responded by moving away in any direction, the behavior was recorded as a displacement for the focal initiator.

Visual cue discrimination task training in a non-social context

In the non-social condition, naïve 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 socially stimulated but receives no social facilitation while responding to the visual cue. All males tested were dominant as all males transitioned into the dominant phenotype immediately after being put in isolation. 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.

Establishing a response criterion for successful task completion

For each behavioral trial, we assessed the response of each individual fish 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. For all fish, a correct response required the fish to move directly under the correct feeder in order to be scored. 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 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 responded correctly, or *learned* the task. For the social context, this criterion was met when at least five of the seven naïve group members (71%) move to the correct cue. Note that this variable is right censored, because we stopped the experiment after either six, fourteen, or twenty-two trials.

We tested the sensitivity of this response criterion by examining how changes to the response parameters impacted the cumulative response rate (Supplemental Figure 2). We used survival analysis to compare the response rates of 50-100% of the group in two, three, and four

consecutive trials. We found no significant difference in the cumulative response probability (Supplemental Table 1) of the group when we compared each required group percent response after two, three, or four consecutive responses. When we examined how the cumulative response probability differs depending on the percent of group members that responded across trials, we found a significant difference (log-rank test: $X^2 = 42.7$, $P < 0.001$; Supplemental Figure 2). For the non-social context, we tested the response criterion by examining how the results are affected by changing the number of consecutive trials required to reach criterion. We found that there is a significant difference in response probability between two, three, and four consecutive trials as the response criterion (log-rank test: $X^2 = 6.1$, $P = 0.05$; Supplemental Figure 2d). Based on these validation experiments, we concluded that using a group response criterion of 70% after two or more consecutive trials was most biologically meaningful since it does not show a response floor or ceiling effect.

Accounting for social hierarchy dynamics and group behavior in the social context

To better understand the social and individual-level factors that might contribute to variation in informant influence, we examined both the aggressive behavior of informants as well as the total behavioral activity of the entire community. To determine whether informants retained their social status over time, we examined the number of displacements as a measure of their aggressive behavior). A repeated-measures analysis of variance (ANOVA) indicated that the number of displacements did not change over time ($F_{21,239} = 0.91$, $p = 0.579$), demonstrating that informants maintained their social status throughout the experiment (Supplemental Figure 3a). As expected, there was a significant effect of informant status ($F_{1,279} = 22.229$, $p < 0.001$), confirming that DOM and SUB informants differed in their overall aggression. Importantly, however, the

number of displacements by an informant (as a proxy of aggression) did not affect the number of trials it took the group to reach criterion (r =0.65, $F_{21,281}$ = 0.646, p = 0.882; Supplemental Figure 3b).

We also examined the relationship between behavioral activity and learning response in both social and non-social treatments (Supplemental Figure 4). We found that overall behavioral activity (as measured by total number of line crosses in the 10 minutes prior to the trial, normalized to the number of group members) increased in both social and non-social treatments over time ($F_{1,42}$ = 17.645, p = <0.001), although there was no interaction between treatment (social v. non-social) and behavioral activity over trials ($F_{3,42}$ = 2.041, p = 0.123; Supplemental Figure 4a). Although social groups had higher overall behavioral activity than the non-social context, this was not driven by the number of displacements by informants ($F_{1,301}$ = 0.084, p = 0.77; Supplemental Figure 4b). In addition, although there was a significant increase in mean behavioral activity over trials across all social groups, there was no main effect of learning rate or interaction between learning rate and trial on behavioral activity (ME of learning rate: $F_{2,16}$ = 0.497, p=0.618, learning rate x trial: $F_{2,16}$ = 0.289, p = 0.753; Supplemental Figure 4c). In the non-social context treatment, there was a main effect of trial and learning rate on mean behavioral activity (trial: $F_{1,23}$ = 15.156, p = 0.001, learning rate: $F_{2,23}$ = 11.638, p < 0.001), but no significant interaction between learning rate and trial ($F_{1,23}$ = 1.044, p = 0.317; Supplemental Figure 4d).

Sample processing and immunohistochemistry for examining neural activity

To examine neural activity patterns across learning trials, three individual samples were collected from each community. In groups with a dominant male informant, the second largest male, subordinate male, and a female were collected. In groups with a subordinate male informant,

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 (see Table 2 for sample sizes).

Brains were rapidly dissected, fixed overnight in 4% paraformaldehyde at 4°C, then washed in 1X phosphate-buffered saline (PBS) and cryo-protected in 30% sucrose at 4°C, then finally embedded in O.C.T. compound (Tissue-Tek; Fisher Scientific Co., Pittsburgh, PA, USA), and stored at -80°C until further processing. Brains were sectioned on a cryostat at 30 μm and thaw-mounted onto Super-Frost Plus slides (Fisher Scientific) in four series of alternating sections that were stored at -80°C until further processing. For Brightfield detection of the IEG Fos, one series of sections was removed from -80°C, dried on a slide warmer and processed for immunohistochemistry, as described previously (Weitekamp and Hofmann, 2017), using a rabbit anti-c-Fos primary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA; catalog # sc-253) at a dilution of 1:500.

Quantification of Fos-positive cells

Slides were coded such that the experimenter was blind to treatment. Cell nuclei labeled by Fos immunohistochemistry were clearly marked by dark brown staining and counted using the Optical Fractionator workflow of the Stereo Investigator software package (Microbrighfield, Williston, VT, USA). We quantified Fos induction in two subregions, Dm-1 and Dm-3, of the medial part of the dorsal telencephalon (Dm, the putative teleost homolog of the mammalian basolateral amygdala), the supracommissural nucleus of the ventral pallium (Vs, the teleost homolog of the medial amygdala/bed nucleus of the stria terminalis), and the lateral subdivision (Dlv) and the granular zone (Dlg) of the lateral part of the dorsal telencephalon (Dl, the putative

teleost homolog of the hippocampus) (O'Connell and Hofmann, 2011). Three sections of each brain region were quantified and averaged per individual. A region of interest was defined using a 2x objective, then positive cells were counted using a 20x objective. The counting frame and sampling grid parameters varied for each brain region to account for differences in cell density and area of each region (Vs: 30x30 counting frame, 100x100 sampling grid; Dm, Dlv, Dlg: 50x50 counting frame, 150x150 sampling grid). For each brain region, data are presented as the estimated population of Fos immunoreactive nuclei using number weighted section thickness divided by the area of the region.

To examine how neural activity patterns change in the social context during the acquisition of a cue association, we counted Fos expression in key brain regions of interest, namely the ventral (Dl-v) and granular (Dl-g) subregions of the lateral part of the dorsal telencephalon (Dl), the Dm-1 and Dm-3 subregions of medial part of the dorsal telencephalon (Dm), and the ventral pallium (Vs) (O'Connell & Hofmann 2011). We first confirmed that there was not a correlation between fish standard length (in mm) and IEG positive cell numbers in any brain region across both contexts (data not shown). Next, we compared how activity in these regions changed across trials in different social conditions (non-social / individuals trained by themselves, and social / observers trained with an informant in groups). For the non-social context, we further examined how this activity across trials was influenced by the animal's sex. For the social context, we examined the impact of informant social status (dominant or subordinate males) as well the sex and size of the observers.

Statistical analysis

All statistical analyses were conducted using R Studio (version 1.0.143) and the 'survival' package (Therneau, 2020). We analyzed the learning response using a survival analysis. We used the nonparametric log-rank test because the proportional hazard assumption was not met, given that it does not support multiple response variables, such as social context, informant status, and individual sex. Thus, we used a series of log-rank tests to examine the overall effect of social context and pairwise differences between informant status in the social context and sex in the non-social context. In a separate analysis, we examined differences between informant status effects in a social context, as well as sex differences in response rate in the non-social context using repeated measures analysis of variance (ANOVA).

We used Principal Components Analysis (PCA) to identify how neural activity patterns across brain regions clustered based on the social context conditions and individual-level traits. Independent ANOVA tests were used to compare principal component scores between social condition (social v non-social), trial, and learning response. To account for repeated measures of the same fish across treatments, generalized linear mixed models (GLMM) were used for Fos expression analyses, which are a proxy for neural activity. To examine how learning context, trial, and individual-level traits influence learning and neural activity patterns, we used the R package glmmTMB which ranks models based on Akaike Information Criterion scores, corrected for sample size (AICc) (Calcagno and Mazancourt 2010), and allows for usage of the beta family, which is appropriate for modeling proportional data. We first performed an overall GLMM that included both social and non-social learning conditions, and also did a separate model on the social and non-social conditions. In the overall model, we included learning condition, trial, sex, whether the learning criterion was met, and group as dependent variables and brain region (Dl-g, Dl-v, Dm-1, Dm-3, and Vs) as the independent variables. In the social condition model, the dependent

variables were trial, sex, observer status, informant status, whether the learning criterion was met, and group. In the non-social condition model, the dependent variables were trial, sex, and learning as the dependent variables.

3. Results

Social context facilitates faster response rates compared to a non-social context

We first asked whether the cumulative response rate differed between the social and non-social contexts and found that the cumulative probability of consecutive group responses during the cue discrimination task is significantly greater than the response rate of individuals in an non-social context (log-rank test: $X^2 = 8.1$, $P = 0.004$; Figure 1a). However, the number of trials it took to reach the response criterion did not differ between the social and non-social contexts (Wilcox test: W = 41, $p = 0.426$; Figure 1b). To our surprise, the social status of the informant – dominant vs. subordinate – did not have any effect on learning rate (log-rank test: $X^2 = 0.005$, $P = 0.94$), contrary to our previous study (Rodriguez-Santiago, Nührenberg et al., 2020).

Neural activity patterns depend on social context

We used PCA to determine which aspects of the social context and individual-level traits influence neural activity patterns during a learning task, and how these contextual aspects contribute to a learning response. We first conducted a PCA that included variables in both social conditions: namely the trial at which individuals were taken (trial), the context condition (social v non-social), and whether the response criterion was met (yes or no). We found that principal component 1 (PC1) accounted for 59.6% of the total variance and differed significantly between

social conditions across trials (Figure 2). There was a main effect of both social context ($F_{1,91}$ = 385.4, $p < .001$) and trial ($F_{2,91}$ = 7.47, p = 0.0009), though no significant interaction effect ($F_{2,91}$ = 1.911, $p = 0.154$; Figure 2d). However, there was a significant interaction between trial and learning response (learning response: $F_{1,91}$ = 22.37, $p < .001$; trial: $F_{2,91}$ = 3.396, p = .04; response x trial: $F_{2,91}$ = 8.3, $p < .001$; Figure 2e), and strong main effects of learning response and context (learning response: $F_{1,91}$ = 71.038, $p < .001$; context: $F_{1,91}$ = 269.57, $p < .001$; Figure 2f). There was a strong main effect of trial and learning response on PC2, as well as interactions between trial and context, and learning response and context (Figure 2g: context: $F_{1,91}$ = 1.086, p = .3; trial: $F_{2,91}$ = 76.24, $p < .001$; interaction: $F_{2,91}$ = 19.45, $p < .001$; Figure 2h: learning response: $F_{1,91}$ = 59.61, $p < .001$; trial: $F_{2,91}$ = 26.16, $p < .001$; trial x learning response: $F_{2,91}$ = .064, p = .938; Figure 2i: learning response: $F_{1,91}$ = 47.93, $p < .001$; context: $F_{1,91}$ = 13.493, p = .004).

Given the striking differences in neural activity patterns between the social and non-social contexts in both the comparisons of estimated Fos+ cells across brain regions and the PCA, we conducted separate PCAs on the social (Supplemental Figure 5) and non-social contexts (Supplemental Figure 6). In this analysis, we found that PC1 accounts for 43.98% of the total variance in the social context, and while there was not a clear separation by informant status (Supplemental Figure 5a), Dm-1 and Vs regions loaded most strongly on PC1. There was a main effect of trial and learning response (trial: $F_{2,63}$ = 19.43, $p < .001$; learning response: $F_{1,63}$ = 77.28, $p < .001$; Supplemental Figure 5e) but no significant main effect of informant status on PC1 ($F_{1,63}$ = 1.525, p = 0.221) (Supplemental Figure 5d). There was not a significant interaction between learning response and informant status on PC1 in the social context ($F_{2,63}$ = 1.889, p = 0.160) (Supplemental Figure 5f). In addition, there was a main effect of trial on PC2 ($F_{2,63}$ = 6.168, p = .003), but no effect of informant status or learning response (Supplemental Figure 5g-i). In the

non-social context, we found similar striking differences in the brain regions that loaded on PC1 (53.97% of the total variance) and PC2 (31% of the total variance) (Supplemental Figure 6b). There was a main effect of learning on PC1 ($F_{1,22} = 7.732$, $p = .011$; Supplemental Figure 6e-f) but no main effect of sex or trial. For PC2 there was a main effect of trial ($F_{2,22} = 10.22$, $p < .001$; Supplemental Figure 6g-h) but not main effect of sex or learning.

Neural activity patterns during acquisition of learning differ across social contexts

To disentangle the factors that contribute to the stark PCA differences we see with the learning context, we examined neural activity in key nodes of the SDMN across trials and contexts. We used relative Fos expression as a marker of neural activity across Dl-g, Dm-1, and Vs brain regions involved in social behavior and association learning. We compared neural activity across social conditions (social, non-social) and learning task trial (6, 14, 22) using two-way ANOVAs (Figure 3a,c,e; Supplemental Table 3 for statistics). We found that the trial and context had significant main effects on Fos expression in the Dl-g, but there was no significant interaction. In the Dm-1, there was both a significant main effect of trial and context as well as an interaction. In the Vs, there was a main effect of trial and context. In addition, we also examined neural activity in the Dl-v and the Dm-3 subregions and found no significant effect of trial, although there was a significant effect of context (data not shown, statistics in Supplemental Table 3).

When we examined whether Fos expression changed with learning, we found a significant difference between context treatments (Figure 3b,d,f; see Supplemental Table 4 for all statistics). Across all brain regions, there was a main effect of context and learning response. There was an interaction between learning and context in the Dm-1 only. There was no difference in Fos expression in the non-social context based on whether individuals learned the task, while in the

social context Fos expression was highest in observers that learned the task in the Dl-g ($p < 0.001$; Figure 3b) as well as in the Vs ($p = 0.001$; Figure 3f). Despite the large differences between Fos expression across social contexts in the five 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 the social rank of observers or based on the informant status (data not shown).

Experimental variables drive neural activity in a brain region-specific and social context-dependent manner

Next we employed generalized linear mixed models and AIC_c scores to understand which variables influenced activity in each brain region given that the PCA results show that differential brain region activity underlies learning across contexts (Figure 2). We compared the AIC_c scores to pick the model that best represents the variables that influence activity in each brain region within each social context. The first models for each social condition included all variables accounted for in each context (social context included: informant status, observer status, trial, learning response; non-social context included: sex, trial, learning response). For the Dl-g, we found that the learning response in the model of best fit for both the social and non-social context had the greatest effect on activity (social: AIC = -532.3, $p < .001$; non-social: AIC = 259.1, $p = .049$). Meanwhile in the Dm-1, activity in both contexts was mostly driven by trial (social: AIC = -504.5, $p < .001$ for both trials 14 and 22; non-social: AIC =278.2, $p = .018$ for trial 14, $p < .001$ for trial 22). For the Vs, activity in the model of best fit was driven by trial 14 in the social context (AIC = 468.3, $p = .004$), while there was no effect of any predictor variable in the non-social context (for full statistical models, see Supplemental Table 5 for model comparison in just the

social context, and see Table 6 for model comparison in just the non-social context). Together, these data suggest that neural activity across social conditions was predominantly driven by factors that were not part of the social context, such as whether individuals learned the task and the task trial.

4. Discussion

In the present study, we found large differences between a social and non-social context in behavioral and neural activity during an associative learning task. Specifically, we discovered a significant difference in learning rate between contexts, such that social groups had a higher cumulative probability of reaching the response criterion sooner than individuals in a non-social context. This striking behavioral difference is reflected in the neural activity pattern differences between contexts, with specific brain regions encoding different aspects of our learning paradigm, suggesting that the acquisition of a learning response to a cue association is mediated by different brain regions depending on the social context.

Observational learning and stimulus enhancement accelerate associative learning of a visual cue discrimination task

By examining the cumulative learning rate probability of acquiring a cue association response across two experimental contexts, we found that social groups had a significantly higher cumulative probability of learning 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 more adaptively beneficial as it confers fewer costs and allows individuals to

gain new information more quickly (Kendal et al., 2005; Galef and Laland 2005). In addition, information diffusion is typically accelerated in social groups (Hoppitt and Laland, 2013).

There are at least two mechanisms by which learning might have occurred in our social paradigm, social facilitation (when the presence of an informant affects the observer's behavior) and stimulus enhancement (where the observer's behavior changes after watching an informant interact with a stimulus). To demonstrate that the group response is due solely to the presence of an informant (i.e., social facilitation), it would be necessary to test individual group members by themselves following acquisition. While we did not examine this retention by observers in the present study, it should be noted that the informants themselves were trained in naïve groups and then transplanted to new groups, where they were the only informed individual. Importantly, all informants displayed a correct response to the cue within one or two trials in their new communities, suggesting that the observers in our study in fact acquired the association and were not just copying other group members' behavior. It seems thus likely that individuals in social groups learned by means of observation or stimulus enhancement, which ultimately led them to respond faster than lone individuals. However, it cannot be ignored that *A. burtoni* is a highly social species, and although individuals in the non-social context had blind cave fish as a social buffer, their slow learning rate could be due to stress factors from being apart from conspecifics.

Hippocampal sub-regions differentially mediate learning in social and non-social contexts

When we examined the neural activity across brain regions in different trials of the learning task, we found significant differences in neural activity (measured as number Fos-immunoreactive cells) between the social and non-social contexts as well as depending on whether the task had been learned or not. In Dl (the lateral part of the dorsal telencephalon and putative homologue of

the mammalian hippocampus), we found a significant increase in Fos expression (or ‘activity’) from trial 6 to trial 14 in the social context in the Dl-g sub-region, which was also significantly higher in groups that learned the task. In the Dl-v sub-region, there was a significant main effect of social context across trials, and a significant decrease in activity between trials 14 and 22. Activity in the Dl-v was not correlated with learning. The Dl-g and Dl-v are subdivisions of the dorsal pallium, a region implicated in the learning of spatial and temporal relationships in teleosts (Portavella et al., 2002; Broglio et al., 2005). Previous work has also shown that the major pathways within the dorsal pallium are highly recursive and have complex reciprocal connections with subpallial regions (Elliott et al., 2017). Based on tract-tracing neuroanatomical data, as well as lesions studies that implicate the Dl and other dorsal pallial regions in learning and memory tasks, Elliott et al. (2017) suggested that the dorsal 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. Taken together, these results suggest a differential role for these Dl subregions in the acquisition of this association learning task.

The basolateral amygdala likely encodes social group formation, not learning of the association task

We found a significant difference in neural activity across social contexts in subregions of Dm (medial part of the dorsal telencephalon and putative homologue of the mammalian basolateral amygdala). More specifically, we found a significant decrease in activity across trials in the social context in Dm-1, and a significant decrease from trial 14 to 22. Activity in Dm-1 was not associated with group learning, and there was no difference across learning response in the Dm-3 (not shown). These findings are consistent with previous studies in goldfish that have shown that Dm lesions

disrupt trace and delay avoidance conditioning (Portavella et al., 2004; Broglio et al., 2005), as well as fear and heart-rate classical conditioning (Alvarez et al., 2003), while such lesions have no effect on spatial memory and cue learning (Rodriguez et al., 2002; Salas et al., 2003). The effects of these lesions in fish are similar to lesions of the amygdala in mammals (Davis et al., 1992; Gentile et al., 1986; LeDoux, 2000, Lee and Kim, 2004), 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 (Broglio et al., 2005). In *A. burtoni* males, Dm activity is correlated with the level of engagement in joint territory defense, although the sign of the correlation depends on an individual's role in this cooperative behavior (Weitekamp and Hofmann, 2017). In the present study, we found that activity in the Dm complex was significantly higher in trial 6 compared to 14 and 22. Given that few groups had learned the task prior or by trial 6, it is not surprising that Dm activity was also higher in groups that had not yet successfully learned the task. Interestingly, individuals from groups that did reach the learning criterion by trial 6 or sooner showed lower Dm activity, which further indicates that Dm is not involved in learning the cue association task. Instead, this result suggests that the Dm regions, and the Dm-1 in particular, may play a role in some aspect of social group formation rather than being involved in the acquisition of the cue association task, providing further support for a role of this brain region in affective processing.

The extended medial amygdala encodes the social context

In Vs (the supracommissural part of the ventral pallium and putative homologue of the mammalian medial amygdala/BNST) we found a significant main effect of social context. Also, Vs activity 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 (Wullimann and Mueller, 2004; Yamamoto et al., 2007; Braford, 2009; Nieuwenhuys, 2011). However, developmental studies have found similar genetic markers, namely *Dlx2, Lhx7, Nkx2.1b,* between the Vs and the extended amygdala (Alunni et al., 2004). Stimulation of the Vs has been shown to increase aggression in male bluegill fish (Demski and Knigge, 1971) spawning in salmon (Satou et al., 1984). In our species, *A. burtoni*, this region is under social and reproductive modulation (Maruska et al., 2013) and shows varying levels of sex steroid receptor expression in males when given the opportunity to ascend or descend in status. Taken together, 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.

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

While we see evidence for differential neural activity across multiple brain regions during the acquisition of an association in both social and non-social contexts, we are unable to fully separate the effects of group formation 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 group formation impacts learning. In other words, 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 behavioral activity levels. Disentangling the effects of social stability

formation from the increased probability of learning after repeated trials in both social and non-social contexts will require subsequent rigorous behavioral examination with automated tracking.

What Fos expression tells us about the observed neural activity patterns and its limitations

An important aspect of examining IEG induction as a measure of neural activity is that we examined this expression 1 hour 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 (Clayton, 2000; Clayton et al., 2019) as most IEGs encode transcription factors or DNA-binding proteins that coordinate the cellular response to a stimulus (Loebrich and Nedivi, 2009). By examining Fos protein expression within 60-90 minutes following the last stimulus exposure, we aimed to capture the brain regions that were active, and presumably important, for the animal's behavioral 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 DOM male). However, 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. In addition, we found no correlation between neural activity and informant aggression (data not shown), although the aggressive behaviors of other observer males could have had an effect on the neural activity patterns seen in the social context.

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 with females, while the majority of males are socially subordinate and reproductively suppressed (Fernald and Hirata, 1977; Hofmann, 2003). We have previously shown for this species that the social status of an informant can have a strong effect on how fast a group learns the visual cue discrimination task we used in the present study. Specifically, even though socially dominant males strongly influence their social groups through aggressive displays and space use, they are significantly less effective in generating group consensus during the association task than subordinate males (Rodriguez-Santiago, Nührenberg et al., 2020). In contrast, we did not find a significant effect of informant status on group learning in the present study. 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, Nührenberg et al. (2020) 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 (Maguire et al., 2021). Although we did not quantify group stability here, the behavioral traits that determine whether an individual is an effective informant – aggression and space use – are highly context-specific and might explain the absence of a social status effect. These factors may also explain why we did not find differences in neural activity patterns between dominant and subordinate observers when learning the task. One interesting observation of relevance here comes from social fear learning in rats, where subordinate animals display increased fear responses after interacting with a dominant informant, which is also reflected in distinct neural activity patterns (Jones and Monfils, 2016).

Possible neural pathways that mediate social learning

We found that neural activity patterns differ by measuring the number of Fos+ cells across brain regions. While this immediate-early gene expression implicates various brain regions in different aspects of learning in a social compared to a non-social context, it remains unknown in what type of cells this Fos activity was highest. Possible neural pathways that may mediate this social learning effect involve the dopaminergic reward pathway, as well as nonapeptides important for social behavior.

The dopaminergic system plays an important role in encoding the salience and rewarding properties of social stimuli, and is also highly active during reinforcement learning. Dopamine is a neurotransmitter involved in reward and risk assessment, behavior reinforcement, and anticipatory responses to reward-associated stimuli (Berridge and Robinson, 1998; Heimovics et al., 2009). In mammals, dopaminergic neurons of the ventral tegmental area (VTA) and substantia nigra have long been identified with the processing of rewarding stimuli (Schultz et al., 1997). These neurons send projections to brain structures involved in motivation and goal-directed behavior, and have been shown to code the prediction error of a reward in association tasks (Schultz et al., 1993). In association learning tasks, dopamine signals the delivery of an unexpected reward (or punishment), which is usually preceded or paired with a stimulus (Schultz, 2006). Through repeated exposures, individuals learn to associate the reward with the paired stimulus, and the dopaminergic response progressively shifts to the event-predicting stimuli rather than responding to the reward itself. In addition, disruption of dopaminergic signaling has been shown to impair various facets of social interactions across species (Messias et al., 2016a; Weitekamp et al., 2017; Messias et al., 2016b). Due to its role both in association learning and social behavior,

activity in the dopaminergic pathway could mediate the behavioral difference in learning between social and non-social contexts. In a companion study, we used a second immunohistological series of the animals examined here to quantify activity in dopaminergic neurons in *A. burtoni* and found that Fos-positive dopaminergic neurons in both the posterior tuberculum of the ventral diencephalon (a putative partial homologue of the mammalian ventral tegmental area; O'Connell and Hofmann, 2011; O'Connell et al., 2013) and Vc (central part of the ventral telencephalon and putative homologue of the mammalian striatum; O'Connell & Hofmann, 2011) were significantly more active in the social context and became activated following learning in the social (Vc) or non-social context (TPp), respectively (Rodriguez-Santiago and Hofmann, in prep.). These results open up a promising avenue of future research in the study of the mechanisms underlying social learning.

Nonapeptides play a fundamental role in the regulation of social behavior. In particular, arginine vasopressin (AVP), have been implicated in regulating affiliative, reproductive, and aggressive behavior in many vertebrate species (Goodson, 2013; Goodson and Bass, 2001). In the brain, AVP exerts its effects in particular regions by binding to distinct receptors that differ in expression across tissues and function. The AVP receptor subtype, V1a, has been shown to regulate sex and species differences in many social behaviors in mammals, birds, amphibians, and fish (Semsar et al., 2001; Insel et al., 1994; Goodson and Wang, 2006; Baeyens and Cornett, 2006). In addition, the nonapeptide oxytocin (OT) in particular improves performance on a preference task based on social transmission and has facilitatory effects on social learning when administered after the acquisition of the preference (Popik and Van Ree, 1993; Choleris and Kavaliers, 1999). Both OT and AVP have been found to improve performance in a social transmission of food preference task, such that animals pharmacologically treated with the nonapeptides are more likely

to acquire the demonstrated preference (Popik and Van Ree, 1993). Given the fundamental role of nonapeptides in social behavior across species, examining activity levels in OT and AVP-expressing cells during association learning in a social context is a potentially fruitful avenue for future research into the mechanisms mediating social learning in large groups.

5. Conclusion

We used the highly social African cichlid fish *A. burtoni* to demonstrate that social learning is associated with increased neural activity (as measured by the expression of Fos, an IEG) when compared to non-social learning across key brain regions important for learning and social behavior. These brain regions are important for modulating learning (hippocampus), emotional learning and fear avoidance (basolateral amygdala), and social behavior (medial amygdala/BNST), and are part of a greater Social Decision-Making Network that is important for mediating various aspects of social behavior (O'Connell & Hofmann, 2011, 2012). 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 informants in social groups. Thus, while these regions are important for different aspects of social learning (O'Connell and Hofmann, 2011), they do not appear to be modulated by group dynamics or individual-level traits in a social learning context. While future studies are needed to fully understand the mechanisms that drive social learning contexts (e.g. neuroendocrine or dopaminergic pathways), our results in *A. burtoni* highlight that there are neural activity pattern differences in how individuals acquire information in different social contexts.

6. Acknowledgements

We thank members of the Hofmann lab for many fruitful discussions. In particular, we thank Caitlin Friesen and Isaac Miller-Crews for comments on earlier drafts of this manuscript, and Nupur Shambharkar for performing the Fos cell counts. In addition, we thank Julie Butler and Morgan Gustison for detailed comments on earlier drafts of this manuscript. This work was supported by a UT Austin Graduate School Bruton and Summer Fellowships, and a Department of Integrative Biology Doctoral Dissertation Improvement Grant (MRS); the National Science Foundation Bio/computational Evolution in Action Consortium (BEACON) Center for the Study of Evolution in Action (H.A.H. and A.J.), Dr. Dan Bolnick and the Howard Hughes Medical Institute (A.J.); and NSF grant IOS1354942 (HAH).

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Figures

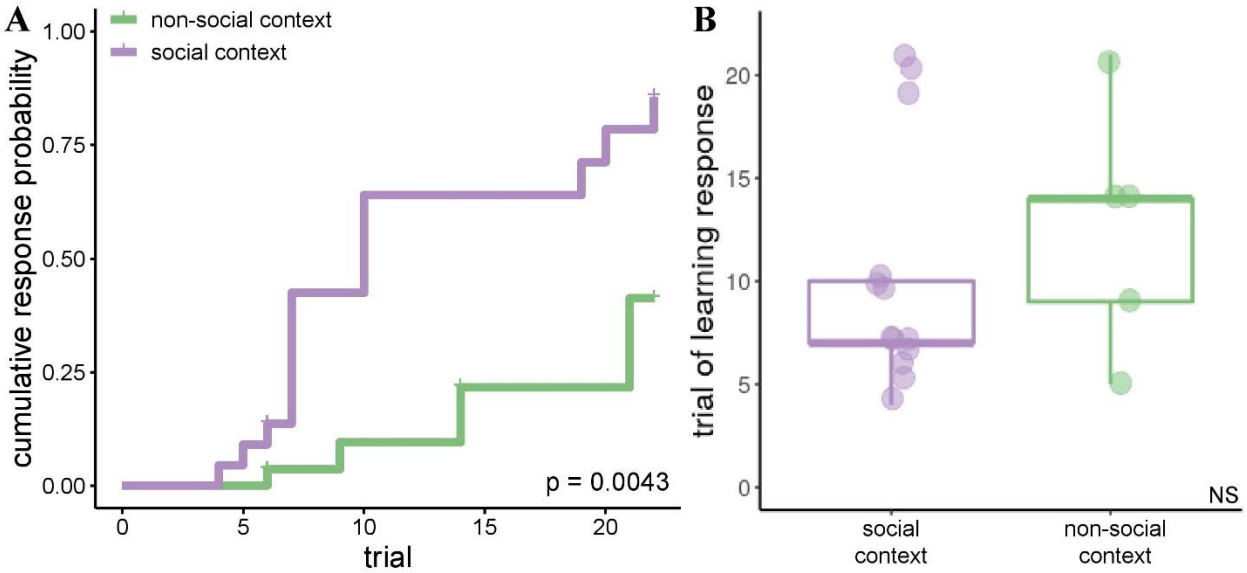

Figure 1. Learning rate is faster in a social context. A) Comparison of the cumulative response probability to a visual cue discrimination task between a social and a non-social context shows that groups have a higher response probability than individuals in a non-social context (p = 0.004). B) Although the rate of response probability is significantly different between social contexts, the total number of trials required to achieve this response criterion is not statistically different between contexts (p = 0.426).

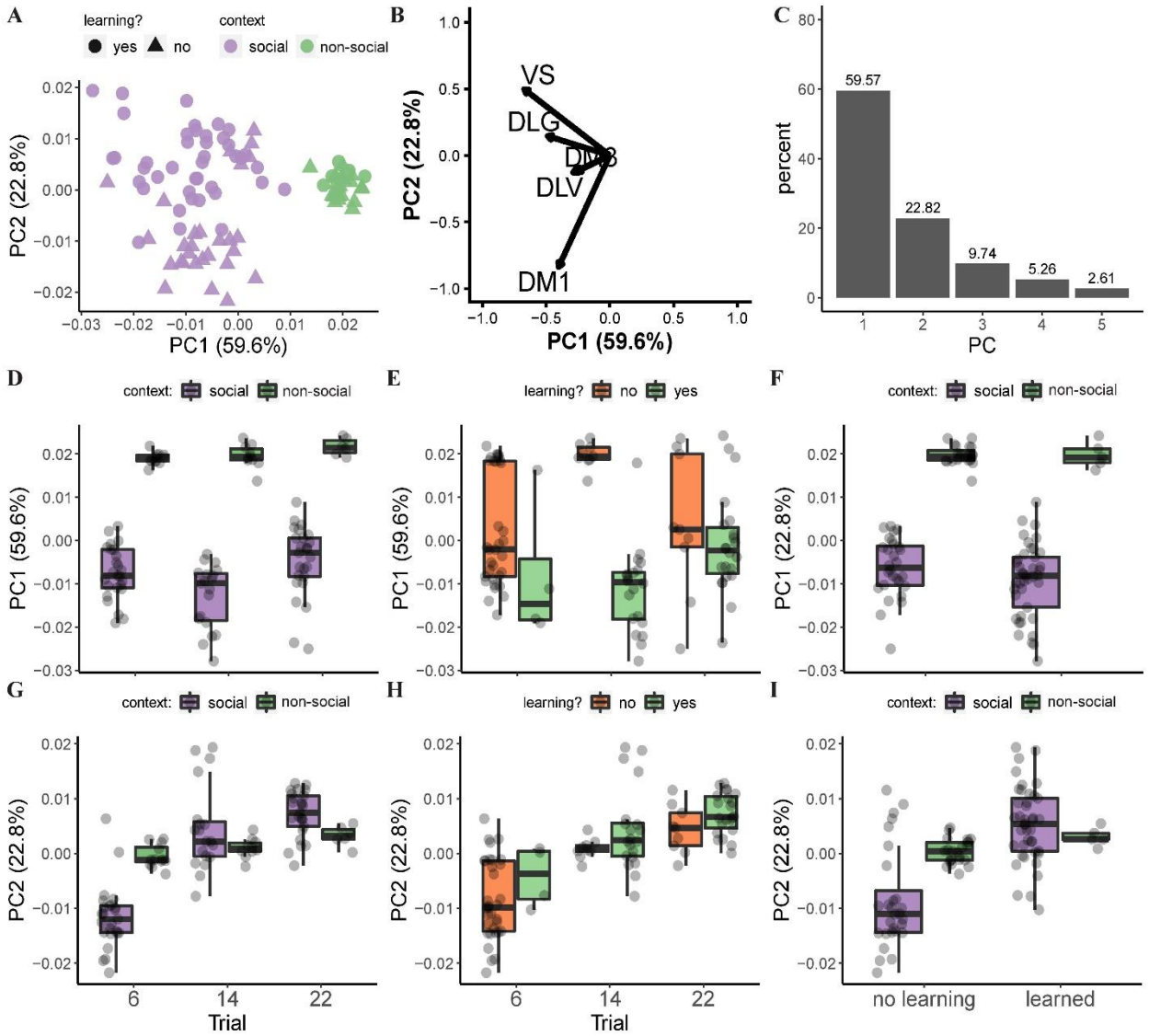

Figure 2. Principal component analysis (PCA) of neural activity shows differential expression pattern with learning context. A) Scatter plot of all Fos expression data separates out by social condition (social, non-social) across PC1. B) Vector plot showing the PCA variables that load on PC1 and PC2. C) Plot showing the percent of the variance explained by each PC. PC1 (D-F) and PC2 (G-I) loadings plotted across trials based on social condition (D and G) and whether learning response was reached (E and H). Boxplot showing that PC1 loadings (F) differentiates data by social condition but not by learning response while PC2 loadings (I) differentiate do not differentiate context across learning response.

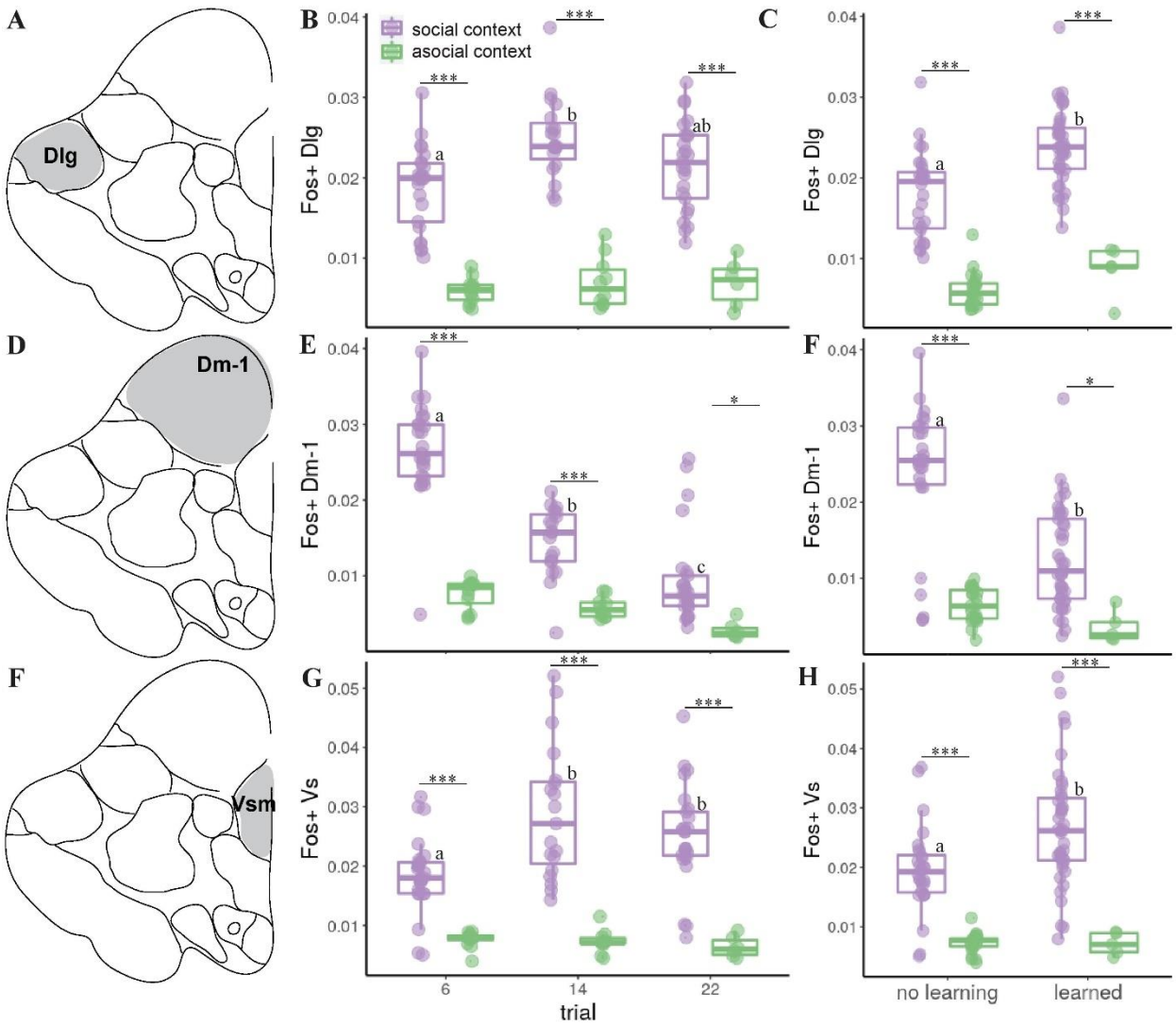

Figure 3. Neural activity across brain regions varies over trials and with learning. Fos expression was quantified as a marker of neural activity in the Dlg, Dm-1, and Vs regions of the forebrain (A, D, F). In the Dlg, there was a significant increase in activity from trial 6 to trial 14 in the social 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 (G), and was significantly higher in the social context with learning (H).