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Experience-dependent modulation of collective behavior in larval zebrafish

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Abstract:

Complex group behavior can emerge from simple inter-individual interactions, yet how prior social experience shapes these interactions remains unclear. Using naturalistic and virtual-reality experiments, we varied prior social experience by exposing zebrafish larvae to high or low group densities, then measured its effects on future social interactions and group structure. We find that inter-individual distances decrease after exposure to high population densities and increase after exposure to low densities. These adaptations develop gradually over tens of minutes and remain stable for hours. Mechanistically, larvae estimate group density from the frequency of neighbor-evoked retinal looming events and couple interaction strength to that estimate. A time-varying computational model incorporating previous visual-social experiences accurately describes our observations. Our findings show that inter-individual interactions are not static, but continuously evolve with social experience and environmental demands, establishing larval zebrafish as a model for studying the neurobiological mechanisms of experience-dependent modulation of collective behavior.

Introduction:

Collective behavior is crucial for animal survival, and can emerge from simple local interactions among individuals[1–8]. Understanding the exact nature of these local interactions, or the behavioral 'rules' that animals use to respond to their group mates has been the focus of extensive experimental and theoretical studies[1–14].

Importantly, moving animal groups, such as schooling fish or flocking birds, can exhibit a diversity of stable collective states such as schooling (directed group motion), swarming (disordered motion) and milling (circular motion around a center point) and the ability to dynamically switch between states. Theoretical and experimental studies indicate that such diversity of collective states can emerge from static local interactions among individuals. Specifically, simulated groups of moving agents exhibit different emergent collective states depending on the initial and boundary conditions of the system[2,15–18]. Experimental evidence in schooling fish support these theoretical findings, indicating that groups can transition between multiple stable behavioral regimes, or states, while implementing static interactions among individuals [19–24].

However, assuming inter-individual interaction rules to be static and hardwired ignores the fact that all living systems require plasticity and feedback regulation to maintain homeostasis. In particular, social interactions need to be flexible to allow individuals in groups to respond to evolving social and environmental demands. Indeed, experimental studies indicate that shoaling and schooling behaviors in fish, as well as collective motion in locusts, depend on previous experience with conspecifics and early-life social exposure, suggesting that these behaviors have a learned component[25–29]. Yet, little is known about the mechanisms that allow animals to adaptively modulate their inter-individual interactions and the resulting collective behaviors, based on previous experiences[29], internal states[30,31] and behavioral goals[22,32].

One key macroscopic feature of groups that animals may need to adaptively control is population density[33]. Group density is a prominent factor that affects key animal behaviors, such as foraging[34–36], mating[37] and predator avoidance[35,36,38], and affects parasite and disease transmission[39]. The need for density sensing and control seems to be a general feature for all taxa, from bacteria that use quorum sensing to assess local population density[40,41] to humans responding to pedestrian flow[42]. To achieve such control, animals must be able to continuously sense or estimate the density of neighbors in their current environment, overcome local fluctuations and noise in their assessment and to adaptively modulate their behaviors in response to these estimates[1,43–46]. The mechanisms that allow fish to sense, internalize, and change behaviors in response to different population densities are not known.

Larval zebrafish is an emerging animal model in behavioral neuroscience, and recently, the inter-individual interactions underlying collective behavior in larval, juvenile and adult zebrafish were described [10,12,13,27,46,47]. It was shown that at younger ages, up to 7dpf (days post fertilization), these fish exhibit primarily repulsive inter-individual forces which lead to overdispersed group structures. Around 14 dpf, attractive forces and aggregation tendencies start to emerge which become robustly expressed at 21 dpf[12,13,48]. Yet, little is known about the ability of larval zebrafish to adaptively modify their inter-individual interactions based on recent social experiences. In contrast, long term modulation of low level sensorimotor programs and algorithms was shown to affect individual behaviors such as hunting[49] and defensive strategies[50,51] already at the larval stage. Therefore, the simple social interactions exhibited at early developmental stages together with the unique ability to study the nervous system at the whole-brain single-cell resolution at these times, offers a unique opportunity to tap into the behavioral and neural mechanisms of experience dependent modulation of collective behavior.

Here, we use naturalistic and virtual-reality (VR) experiments and explore how young larval zebrafish - at 7 dpf - modulate their repulsive interactions in response to persistent changes in population density. We infer the specific visual cues that fish use to estimate their current group density and we precisely track experience-dependent modulation of inter-individual avoidance behavior in response to these estimates. We show that modulation is a slow and stable process that develops within tens of minutes and lasts for several hours. Finally, we present a time-varying computational model that implements experience dependent gain changes in the sensorimotor transformations that connect the visual inputs generated by conspecifics into concrete swim and turn behaviors. This model accurately accounts for the experience-dependent modulation of collective behavior in groups of larval zebrafish and reproduces our behavioral findings. Our results demonstrate how even at a very early age, when collective social behavior is just beginning to emerge, larval zebrafish are already able to adjust their social interactions and the resulting collective behavior to adaptively cope with changing social conditions.

Results:

Previous social experience of larvae modulates their collective behavior

To study how recent social experiences of young larvae modulate their collective behavior, we divided 7 dpf (days post fertilization) larvae into groups of either 5 (low density - 0.22 fish/cm^3) or 20 (high density - 0.88 fish/cm^3) animals, allowed each group to swim together for 4 hours in opaque circular arenas (diameter = 6cm, water depth = 0.8cm) and measured their collective swimming behavior (Fig. 1a-b, Supplementary Movie 1, Methods). We then redistributed fish into groups of the opposite density and

measured their collective swimming behavior again (Fig.1a-b). This procedure allowed us to compare fish swimming in similar group densities that differ only in their history of social experiences (prior to experiments, all fish were raised in the same density of 0.55 fish/cm^3) (Fig. 1a, compare dotted blue to solid blue and dotted orange to solid orange).

As previously shown[12,48], 7 dpf larvae tend to swim in overdispersed groups in both high and low densities, with average nearest neighbor distances that are higher than chance levels ($\widehat{NN}_5$=1.42±0.11cm [mean±SD], $\widehat{NN}_5^{\text{Shuffled trajectories}}$=1.32cm, $\widehat{NN}_{20}$=0.65±0.03cm, $\widehat{NN}_{20}^{\text{Shuffled trajectories}}$=0.6cm, $p_{5,\text{bootstrap}}$<1/100,000, Cohen's d =0.7, 95% bootstrap interval (BI_{NN5}): [1.38, 1.46]; $p_{20,\text{bootstrap}}$<1/100,000, Cohen's d = 0.87, 95% BI_{NN20}: [0.65, 0.67])(Fig. 1c-d, Methods). When comparing the effects of previously experienced social density on the current behavior of fish, we found that group structure was modulated by past social experience: fish swimming in low density groups that were previously exposed to high density swam closer to their neighbors ($\widehat{NN}_5$=1.42±0.11cm, $\widehat{NN}_{20\rightarrow5}$=1.34±0.1cm [mean±SD], p_{Fisher}=0.001; Cohen's d=-0.69, 95% confidence interval of the mean difference $CI_{NN20\rightarrow5\text{ - }NN5}$: [-0.11, -0.03]), and conversely, fish swimming in high density groups that were pre-exposed to low density swam farther away from one another ($\widehat{NN}_{20}$=0.65±0.03cm, $\widehat{NN}_{5\rightarrow20}$=0.67±0.02cm, p_{Fisher}=0.049, Cohen's d=0.6, 95% $CI_{NN5\rightarrow20\text{ - }NN20}$: [0, 0.032]) (Fig. 1c-d, dashed blue vs blue, and dashed orange vs orange, Fig. S1a). Importantly, while we observed changes in other individual and group properties such as distance to the walls, average swimming speed, and average distance traveled in a bout, these changes were independent of the specific previous social experiences of the fish (Fig. S1b-f).

The change in inter-individual distances due to previous social experience is likely the result of the modulation of inter-individual interactions. Larval zebrafish are known to use retinal occupancy of neighbors to guide their inter-individual interactions[12]. Specifically, 7 dpf larvae were found to turn away from the more occupied eye, and this rule was shown to be sufficient to reproduce the over-dispersion phenotype in these young animals[12,48].

Here, we confirmed these results for larvae swimming in groups of either 5 or 20 animals, and show that fish in our experiments also tend to turn away from the more occupied eye (Fig. 1e). Critically, we found a history dependent modulation of these inter-individual interactions that are aligned with the changes in collective swim behavior described in Figure 1c,d: larvae swimming in groups of 5 animals showed a reduced tendency to turn away from the more occupied eye, after experiencing a higher density (experimental condition 20→5 vs. 5)(p_{Fisher}=0.013, F(1,89)=6.49, partial eta-squared η_p^2=0.07, 95% $CI_{p20\rightarrow5\text{ - }p5}$: [-0.039 -0.005], main effect for previous social density, two-way mixed-design ANOVA, Methods), while larvae swimming in groups of 20 animals showed an increased tendency to turn away after experiencing a lower density, but only at intermediate occupancy values (experimental condition 5→20 vs. 20) (Holm-adjusted Fisherian p-values: $p^{[10^o-20^o]}$ = 0.026, Cohen's d=0.78, 95% CI: [0.002 0.026], $p^{[20^o-30^o]}$ = 0.029, Cohen's d=0.75, 95% CI: [0.003, 0.419], two-way mixed-design ANOVA followed by post hoc comparisons at the different Δoccupancy bins, Methods)(Fig. 1e).

We note that pre-exposure to large groups of 20 fish in a large arena (diameter=12cm), such that global density is kept low (0.22 fish/cm^3), did not elicit a detectable behavioral modulation when fish were subsequently tested in groups of 5 fish in small arenas (6cm, 0.22 fish/cm^3), emphasizing the importance of density in eliciting behavioral modulation (Fig. S1g-h).

Taken together, these results show that collective behavior depends on the previous social experiences of the larvae, and that fish can either increase or decrease the strength of their social interactions, depending on the specific experienced history.

Interactions with virtual neighbors elicit similar modulations of collective behavior

To precisely characterize the history-dependent modulation of larval collective behavior and to infer the mechanisms at the basis of this modulation we utilized a high throughput virtual-reality (VR) assay that allowed us to study how larvae modulate their behavior when exposed to different spatial and temporal retinal occupancy patterns (Fig. 2a, Supplementary Movie 2). To this end we projected dots that mimicked the size and motion of real groups of fish and measured the behavior of a single fish interacting with these virtual neighbors (Fig. 2a-b, S2a, Methods). This virtual-reality assay allowed us to easily transition between neighbor densities (Fig. 2a, right) without the need to physically disturb the fish or move them between groups, and also to fully control the physical and dynamical properties of the simulated virtual neighbors (see Fig. 3 and below).

To test the effects of previous social experiences in virtual reality on inter-individual distances, we first exposed fish to either a high or low density of virtual neighbors (4 or 19 virtual neighbors) (Fig. S2a). We then switched the virtual neighbor densities (from low to high or vice versa) and recorded the responses of the fish before and after density switches (Fig. 2b).

We found that similar to experiments with real groups of larvae, fish swimming with 4 virtual neighbors that were previously exposed to 19 virtual neighbors swam closer to their neighbors compared to fish that did not experience a change in density ($\widehat{NN}_5$=1.59±0.17cm, $\widehat{NN}_{20\rightarrow5}$=1.45±0.09cm [mean±SD], p_{Fisher}=0.003; Cohen's d=-1.03, 95% $CI_{NN20\rightarrow5\ -\ NN5}$: [-0.23, -0.05]) (Fig. 2c, S2b). In contrast, fish that swam with 19 virtual neighbors and were previously exposed to 4 virtual neighbors tended to swim farther away from their neighbors ($\widehat{NN}_{20}$=0.6±0.04cm, $\widehat{NN}_{5\rightarrow20}$=0.63±0.04cm [mean±SD], p_{Fisher}=0.041, Cohen's d = 0.64, 95% $CI_{NN5\rightarrow20\ -\ NN20}$: [0.001, 0.054]) (Fig. 2c, S2b). Similar to the group swimming experiments, while we observed changes in other individual and group properties such as distance to the walls, the bout rate of the fish, and average distance traveled in a bout, these changes were independent of the specific previous social experiences of the fish (Fig. S2c-e).

Here again, the change in inter-individual distances was consistent with the observed strength of responses to difference in retinal occupancy between the eyes: fish that were previously exposed to high density of virtual neighbors, exhibited a weaker tendency to turn away from the more occupied eye (p_{Fisher} = 0.031, F(1,30) = 4.88, partial eta-squared $\eta_p{}^2$=0.14, 95% $CI_{p20\rightarrow5\ -\ p5}$: [-0.104, -0.004] main effect for previous social density, two-way mixed-design ANOVA, Methods), while fish that previously experienced a lower density of virtual neighbors showed a slightly higher tendency to turn away from the more occupied eye, but the effect did not cross a significance threshold (p_{Fisher} =0.059, *F*(1,30) = 3.85, $\eta_p{}^2$=0.11, 95% $CI_{p5\rightarrow20\ -\ p20}$: [-0.002, 0.089] main effect for previous social density, two-way mixed-design ANOVA)(Fig. 2d).

These results confirm that visual social interactions shape collective behavior of 7 dpf larvae and also that history-dependent modulation of social interaction strength, especially the effects of previous exposure to high group densities, can be reliably induced in a VR setting.

History dependent modulation of collective behavior is slow, stable and reversible

Next, we analyzed the quantitative dynamics of experience dependent modulation and the stability of behavioral changes. We focused on the modulation caused by previous exposure to high densities, as it resulted in a more robust change in group behavior (Fig. 1c-d and Fig. 2c). To measure the temporal dynamics of collective behavior modulation, we compared group behavior at low neighbor density (four virtual neighbors) before and after exposing the fish to varying times (5 to 40 minutes) of high neighbor density (19 virtual neighbors)(Fig. 2e, Methods). We found that 20 minutes or longer of high-density exposure times are needed to cause a decrease in the distances that fish kept from their virtual neighbors (p_{Fisher} = 0.0002, F(1,57) = 12.13, partial eta-squared $\eta_p{}^2$=0.1, main effect for time (pre vs post NNd), p_{Fisher}=0.11, F(3,57) = 2.13, interaction of time and duration of high density; two-way mixed-design ANOVA followed by planned simple-effect tests of the ΔNNd in each high neighbor density duration: ΔNN_{5m}=0.002±0.09cm [mean±SD], p_{5m}=0.47; ΔNN_{10m}=-0.04±0.12cm, p_{10m}=0.28; ΔNN_{20m}=-0.07±0.09cm, p_{20m}=0.009, Cohen's d = -0.81, 95% CI: [-0.11, -0.01], ΔNN_{40m}=-0.1±0.12cm, p_{40m}=0.007, Cohen's d = -0.96, 95% CI: [-0.14, -0.05]; Holm-adjusted Fisherian p-values, Methods)(Fig. 2e). Surprisingly, the decrease in inter-individual distances due to exposure to high neighbor density (19 virtual neighbors for 20 min), remained stable for more than 2 hours after switching back to a low neighbor density (mixed-effects models with categorical timepoint as a fixed effect and fish identity as a random intercept; no evidence for drift across post-training timepoints (20-120 min): p_{Fisher} = 0.95, F(5,133) = 0.215, $\eta_p{}^2$ =0.008; followed by two planned contrasts: baseline vs 120min ΔNNd = -0.14±0.12cm, p = 0.027, Cohen's d = -0.87, 95% CI: [-0.2, -0.07]; 20 min vs 120 min ΔNNd = -0.02±0.15 cm, p = 0.27, Cohen's d =-0.16, 95% CI: [-0.09, 0.05]; Holm-adjusted Fisherian p-values for contrasts) (Fig. 2f).

To explore ways to reverse these behavioral modulations, we tested whether a subsequent exposure to the lowest possible density (0 neighbors) can negate the effects of exposure to high neighbor density. Indeed, we found that increasing times of exposure to 0 neighbors can reverse this effect and bring inter-individual distances among fish back to baseline in approximately 50 minutes (p_{Fisher} = 0.0002, F(3,65) = 8.08, $\eta_p{}^2$=0.27, interaction between duration of 0 neighbors and time (pre vs post NNd); two-way mixed-design ANOVA followed by planned simple-effect tests of the ΔNNd at each 0 neighbor durations: ΔNN_0=-0.25±0.14cm [mean±SD], p_0=0.0001, Cohen's d = -2.34, 95% CI: [-0.33, -0.18]; ΔNN_{10}=-0.17±0.12cm, p_{10}=0.0001, Cohen's d = -1.23, 95% CI: [-0.25, -0.1]; ΔNN_{20}=-0.07±0.17cm, p_{20}=0.095, Cohen's d = -0.41, 95% CI: [-0.16, 0.007], ΔNN_{50}=-0.02±0.18cm, p_{50}=0.26, Cohen's d = -0.19, 95% CI: [-0.04, 0.09]; Holm-adjusted Fisherian p-values) (Fig. 2g, Fig. S2f-g).

These results indicate that exposure to high neighbor density elicits a slow cumulative change in the collective behavior of the fish, characterized by closer proximity to neighbors, a phenomenon that remains stable for at least two hours. This process can be reversed by removing all virtual social stimuli, which causes the inter-individual interactions and distances between fish to slowly decay back to their baseline measured values.

We note that the behavioral adaptations observed in VR must rely exclusively on visual experiences. In order to isolate the precise visual features of neighbors that trigger this modulation we independently tested the ability of specific visual features, such as the object's density, their retinal occupancy and their motion statistics, to elicit behavioral modulation.

Persistent increase in retinal occupancy drives modulation of neighbor proximity

To test which statistics of the visual scene are used by larvae to modulate their social responses, we varied the size and motion statistics of virtual neighbors independently from their density and measured their ability to elicit modulation of nearest neighbor distances (Fig. S3a-b). Thus, instead of increasing the number of virtual neighbors (as in the high-density VR exposure), we held the number of neighbors fixed and altered their visual properties. We found that increasing either the size or the speed of virtual neighbors, also led fish to swim closer to their virtual neighbors (although increasing the size of the neighbors had a weaker effect on nearest neighbor distances) (Fig. S3a-b). We did not observe changes in group structure or inter-individual distances when virtual neighbors were replaced with a whole-field black background for twenty minutes, before testing again their nearest neighbors distances in a social context. Thus, changes in global luminance alone are not sufficient to reproduce the experience-dependent modulation observed with virtual neighbors (Fig. S3c)[52].

We hypothesized that changes in the perceived motion energy of neighbors on the retina (translational, looming, or both) are at the basis of the observed behavioral modulation. We therefore sought to determine the specific contributions of each of those components. To that end, we presented fish with two sets of stimuli, each isolating either looming or translational motion: a high density of virtual neighbors (19 neighbors) that are stationary but randomly increase and decrease in size (looming, not moving), or a high density of virtual neighbors moving on concentric circles around the focal fish (moving, not looming). As a control, we also presented fish with a similar high density of stationary neighbors that lacked neighbor-evoked looming on the retina (Fig. 3a left, Supplementary Movie 3, Methods).

We found that only changes in looming energy caused a reduction in inter-individual distances, whereas for stationary neighbors and translational motion energy we did not detect a significant modulation of inter-individual distances ($\Delta NN_{stationary}$=0.03$\pm$0.13cm [mean$\pm$SD], $p_{Stationary}$=0.58; $\Delta NN_{Translational}$=-0.025$\pm$0.17cm, $p_{Translational}$=0.58; $\Delta NN_{Looming}$=-0.09$\pm$0.15cm, $p_{Looming}$=0.025, Cohen's d = -0.61, 95% CI: [-0.16, -0.027]; Holm-adjusted Fisherian p-values) (Fig. 3a right). Our data did not indicate that the reduction in inter-individual distances was associated with consistent changes in other individual swim properties such as the path traveled in a bout or the bout rates of these fish (Fig. S3e-f).

To corroborate these findings, we directly manipulated translational and looming motion using a single dot stimulus, mimicking a neighboring fish swimming in the vicinity of the focal fish, and tested its ability to elicit modulation of inter-individual interactions[12] (Fig. 3b left, Supplementary Movie 4, Methods). The single dot stimulus moves in bouts 'rotationally' around the fish from $\pm$75^o to $\pm$30^o for 5 seconds (with fish heading taken as 0^o). We either kept the size and distance of the dot constant to elicit only translational motion on the retina (corresponding to 'Translational motion' condition in figure 3a) or slowly changed the size of the dot or its distance to the fish, to add a looming component (corresponding to 'Looming motion' in figure 3a). We specifically hypothesized that the two stimuli that include a looming component will also elicit a behavioral modulation.

We find that fish robustly responded to the presentation of all three single dot stimuli (Fig. 3b, bottom), as shown previously[12] and similar to the case of multiple virtual neighbors (Fig. 2d). However, the addition of looming, reduced future response probabilities to the single dot stimulus, whereas fish kept responding robustly to a moving dot stimulus with no looming component ('Moving' vs. 'Moving closer': p_{Fisher} = 0.022, Cohen's d = -0.59, $CI_{Moving\ closer\ -\ Moving}$: [-0.080, -0.004]; 'Moving' vs. 'Moving and growing': p_{Fisher} = 0.004, Cohen's d = -0.84 , $CI_{Moving\ and\ growing\ -\ Moving}$: [-0.09, -0.02]; Holm-adjusted Fisherian p-values) (Fig. 3b, bottom).

We next tested whether changes in response probability to the single looming dot stimuli ('Moving closer' and 'Moving and growing') also translate into a reduction in nearest neighbor distances in a group context. As predicted by our results in Fig. 3a, we found that after training with single dot looming stimuli,

fish swam closer to their virtual neighbors compared to their baseline neighbor distances (ΔNN_{moving}=-0.01±0.14cm, p_{moving}=1, $\Delta NN_{moving\ closer}$=-0.05±0.12cm, $p_{moving\ closer}$=0.021, Cohen's d = -0.4, 95% CI: [-0.09, -0.01]; $\Delta NN_{moving\ and\ growing}$=-0.06±0.14cm, $p_{moving\ and\ growing}$=0.037, Cohen's d = -0.67, 95% CI: [-0.11, -0.01]; Holm adjusted Fisherian p-values) (Fig. 3c).
Taken together, these results demonstrate that the modulation of collective behavior, characterized by a reduction in interindividual distances, is driven by repeated looming events, such as those triggered by the movement of neighbors in the animal's vicinity. Further, they show that it is not the number of neighbors per se that elicits behavioral modulation, but rather the increase in looming frequency that is associated with changes in the social environment of the fish.

Modeling experience-dependent modulation of collective behavior

We devised a modeling framework that links visually based inter-individual interactions to the history of experienced visual scenes (Fig. 4a-c). The model has two time-varying components - an interaction function $f(\mathbf{v})$ that defines the way a larval zebrafish will respond to the retinal occupancy $\mathbf{v}$ generated by its neighbors, and an unobserved internal state variable $s(t)$ that represents a cumulated value of previously experienced retinal occupancy changes and modulates the response to visual occupancy $f[\mathbf{v}, s(t)]$.
Previously, we have shown that larval zebrafish base their turning responses on precise retinal occupancy computations: fish spatially integrate retinal occupancy in the vertical dimension at different visual angles, and these integrated values elicit directional turning biases. Turn biases are then horizontally averaged within each eye and the resulting values are compared between the two eyes to elicit a turning response[12] (Fig. 4b, S4a, Methods). We estimated $f(\mathbf{v})$ based on these algorithms, in the low-density case (4 virtual neighbors) before and after exposure to high density (19 virtual neighbors) (Methods). As expected, the estimated probability to turn away from the more occupied eye is weaker after exposure to high density (Fig. 4b, right).
Next, we linked the transition between stronger and weaker responses, to the history of experienced changes in retinal occupancy by the fish. To that end, we allow agents to update an internal state variable $s(t)$, by introducing a leaky integrator module that temporally integrates looming events, which we hypothesize to be extracted at the level of each retina and then combined in a downstream retinofugal region[50,53,54] (Fig. 4c-d). This module is represented by the linear differential equation:

$$\tau \frac{ds}{dt} = s_{rest} - s(t) + r \cdot loom_{max}(t) \qquad (1)$$

where τ represents the time constant of the process, $loom_{max}(t)$ is the experienced maximal loom at time t, r is a scaling variable, and s_{rest} is the value the process will relax to in the absence of any input. Based on the temporal dynamics observed in our experiments (Fig. 2e-g) we equipped this integrator module with a very slow time constant (τ) of ~6 hours (see Fig. S4b-c, for integrator dynamics at different choices of τ, Methods). We then linearly link the strength of the evoked turning response to this internal state variable, where higher integrated values result in a weaker turning response, and small values of $s(t)$ in a stronger bias to turn away (Fig. 4c-d, Methods).
We note that based on the slow time constant, the linear differential equation governing our model (Eq. 1), has a steady state (equilibrium) solution that is only reached after many hours, which implies that the model operates largely in the initial domain, where sensitivity to changes in the input is high (Fig. S4b).

The model accurately predicts experience dependent modulation of collective behavior

We tested the ability of the model to explain and predict our behavioral observations (Fig. 1-3). To that end we first quantified the behavior of a single simulated fish (agent) exposed to virtual neighbors of different group sizes, as described for real fish in Fig. 2 (Fig. 5a, Methods). We find that the nearest neighbor distances of an agent interacting with neighbors in low density conditions (4 virtual neighbors, swimming according to the pre-recorded trajectories used in VR experiments) were similar to those exhibited by real fish in VR experiments (Fig. S5a). The transition to high density (19 virtual neighbors) was accompanied by a linear increase in values of the internal state variable (due to the related increase in looming events, Fig. S4b, S5a) and a corresponding decrease in the strength of fish turning responses (Fig. 4d), which resulted in a decrease in nearest neighbor distances (Fig. 5b). This new group structure then remained stable for more than 2 hours of simulation time, in line with the long time constant of the leaky integrator module (Fig. S4b), which captured the stability of the modulation observed in VR experiments (Fig. 2f).

Increasing the exposure time to high density of virtual neighbors caused a corresponding decrease in the average nearest neighbor distance (Fig. 5c), accurately capturing similar trends observed in VR experiments (Fig. 2e). Furthermore, removing all visual stimulation after exposure to high density, caused the strongest decrease in internal state variable values (Fig. S4b) and an exponential return to baseline nearest neighbor distances in about ~50 minutes of simulated times ($R_{exponential}{}^2$ =0.99)(Fig. 5d), which again matched the experimental observations in VR (Fig. 2g).

The model also accounts for more subtle observations in our experiments. Simulated agents exposed to a high density of stationary objects (non-moving neighbors), did not show a decrease in their response to neighbors, or their inter-individual distances, capturing the finding that occupancy changes due to own motion are insufficient to elicit behavioral modulation (Fig. S5b, Fig. 3a). Exposure to even higher neighbor densities (39 virtual neighbors compared to 19 virtual neighbors) caused a stronger decrease in nearest neighbor distances in both experiments and simulation (Fig. S5b, Fig. S3d). Similar to experimental findings, exposure to low density of faster moving neighbors (4 virtual neighbors moving at 4 times the original speed) caused a subsequent decrease in nearest neighbor distances (Fig. S5c, Fig. S3b). In addition, simulated agents exhibited an increase in the distance kept from the walls of the arena after exposure to high neighbor density mimicking the effect observed in real fish (Fig. S5d, Fig. S2g), even though no explicit change to wall interactions was introduced into the model (Methods). The model also predicts that priming fish with 0 neighbors before introducing a low neighbor density (4 virtual neighbors) should cause an increase in inter-individual distances. Experiments priming fish with 0 neighbors confirmed this prediction (Fig. S5e).

Finally, we replaced the non-interacting virtual neighbors with groups of fully responsive, interacting agents (Fig. 5e-f, Methods) and compared their behavior to those of real animals swimming together in groups of varying densities (Fig. 1a-d). Here again, agents that were previously exposed to high-density groups (20 interacting agents) and were randomly picked out of the cohort and virtually ‘transferred’ to a low-density group (Methods), exhibited a decrease in nearest neighbor distances and swam closer to their neighbors (Fig. 5f, S5f). Conversely, simulated agents that were exposed to a low-density scenario (5 interacting agents) and were virtually ‘combined’ to create a high-density group, swam at higher distances from their neighbors (Fig. 5f, S5f).

Taken together, these results show that simulated agents that are equipped with an internal state variable that integrates and 'remembers' the history of experienced retinal occupancy changes, can comprehensively account for most if not all observations of collective behavior modulation in larvae.

Discussion

The ability to respond to changes in social density is a crucial behavioral component across taxa, from bacteria to humans[42,55–57], offering flexibility in coping with changing social environments. In zebrafish, young larvae swim in overdispersed groups, presumably to optimize their personal space and to avoid potentially harmful collisions with each other[12,27]. Nonetheless, the tendency to disperse and maximize nearest neighbor distance ought to be regulated to match different contexts. For example, tight aggregation might be unavoidable in the presence of local attractors such as food sources, or restricted areas of preferred temperature, luminance, flow or salinity. It is critical in such cases to attenuate and balance the drive to disperse with the tendency to aggregate around a spatially attractive location. Since the spatial profile of such sensory attractants usually changes slowly over time, the corresponding time constants with which desired population density setpoints are adjusted should change with comparable dynamics. Interestingly, we find that the temporal modulations of inter-individual interactions of young larvae indeed have relatively long time-constants, with notable modulations in behavior appearing after tens of minutes and lasting for several hours. We hypothesize that these long time constants are related to the natural occurrences of density modulation in the larvae's environment, allowing fish to filter out and ignore fast, abrupt and stochastic changes in local population densities[58].

The mechanisms that allow zebrafish to estimate their population density are largely unknown. A few studies have looked into the role of olfactory[51] and somatosensory[27,46] cues that larval zebrafish can use to estimate the presence of neighbors. In the current study, we show that fish also strongly rely on visual information to estimate population density by integrating looming events on their retina over time. In contrast, translational motion and static retinal occupancy estimations, that were shown to guide the acute turning responses of fish to their neighbors[12,48], seemed to contribute less to adjusting the strength of repulsive interactions (Fig. 3). Notably, visual looming events are particularly informative in identifying conspecifics invading one's 'personal space' since the invader's perceived expansion velocity is a highly non-linear function of its distance[53]. Thus, looming is more strongly influenced by the distances to nearest neighbors and less affected by the overall group size, making it a more accurate estimate of local density, which is presumably more relevant to the animals.

We found that modulations of avoidance behavior induced by such persistent looming events cause stable changes in collective behaviors. We predict that our findings will also generalize to older ages of larvae, when fish begin to aggregate and to exhibit both repulsive and attractive interactions with their neighbors, as early as 14 dpf[12,13,48]. At these later developmental stages, exposure to high densities might cause fish to similarly attenuate only their repulsive interactions, resulting in tighter aggregations,

or alternatively to attenuate both attractive and repulsive interactions, consequently forming more loose aggregations. Future studies can analyze history-dependent modulation in older zebrafish larvae and to test these different hypotheses.

The modulation of social interactions we report here bears some relation to a previously well characterized behavior in larval zebrafish - habituation to visually evoked predator looming[50,54,59,60]. Indeed, young larvae turn away from their neighbors at 7 dpf, and attenuate these responses due to repeated neighbor evoked looming events. However, key differences distinguish these two phenomena. 1) Repulsive social interactions exhibit a dichotomy between the nature of the stimuli that elicit the turning responses (static visual occupancy on the retina), and the stimuli that elicit modulation of these responses, (neighbor evoked looming events; Fig. 3b and [12]). This dichotomy does not exist for predator looming responses[50]. 2) The statistics of the looming stimuli are different, with neighbor evoked looms reaching much smaller terminal sizes on the retina at slower expansion rates. 3) The statistics of the responses themselves are different, with neighbor evoked responses being mostly routine turns, unlike the large abrupt escape responses to predator looms[12,59]. We therefore hypothesize that these two behaviors are controlled by different neural circuits (see below).

To quantify the history dependent modulation of collective behavior we introduced a time varying state-space model[61,62] that accurately describes both the static and dynamic features of collective behavior in varying social densities. This model deviates from the common approaches to modeling collective motion which rely only on static interaction rules[3,5]. Here, we propose a transition to a more general framework for describing collective motion, that can naturally incorporate additional modulating factors such hunger or stress, and varying environmental contexts such as presence of prey, water currents and changes in temperature.

This algorithmic modeling framework facilitates the generation of realistic circuit models that implement the underlying computations[63–65]. An explicit example of such a model, that implements directed turning in response to retinal occupancy, has been described previously[12]. Here, we provide the experimental and behavioral framework that allows us to extend this circuit model to include how such turning behavior is modulated by exposure to persistent looming events. A core element of the extended model would be a population of looming sensitive neurons that exerts modulatory control on the critical, turning inducing nodes.

Explicit circuit implementations underlying the detection of looming objects have been described previously in various organisms, including 7dpf larval zebrafish[50,53,54,60]. We propose that the dedicated retino-tectal circuitry in the zebrafish is well poised to extract looming specific information[50,54,59,60]. Our results suggest that looming events can then be integrated over longer time scales and lead to the updating of modulatory circuits possibly located in the dorsal raphe, the habenula or the hypothalamus. Such modulatory networks are well suited to change internal state-dependent output patterns as described, for example, for stress and hunger[66,67].

The validation of such anatomically inspired circuit models requires functional interrogation at a brain-wide level and at cellular resolution in a behaving animal[63,65,68,69]. Our ability to detect experience dependent modulation of collective behaviors as early as 7 dpf, facilitates such investigation of the underlying biological and neural mechanisms, since larval zebrafish at this early stage are especially amenable to various brain wide neuroimaging and optogenetic perturbation approaches[70].

Our description of experience-dependent modulation of social interactions in larval zebrafish shows that even young animals, which are sometimes considered to have only a few simple and reflexive behaviors, can achieve increased behavioral diversity and cope with dynamic conditions. We hypothesize that other behavioral paradigms such as position holding in a moving stream and hunting moving prey might also be influenced by internal state variables, similar to the one described here for population density[49,71–73]. The behavioral assays and modeling framework developed here lend themselves readily to future studies testing these hypotheses.

Methods:

1. Fish husbandry

All larvae used in the experiments were obtained by crossing adult AB zebrafish. Larvae were raised in densities of approximately 50 fish in large petri dishes (D = 12 cm) filled with 0.8 cm of water (Density = 0.55 fish per cm^3). Dishes were filled with filtered fish facility water and were kept at 28°C, on a 14–10h light dark cycle. From age 5 dpf, fish were fed paramecia once a day. All experiments followed institutional IACUC protocols as determined by the Harvard University Faculty of Arts and Sciences standing committee on the use of animals in research and teaching.

2. Group swimming experiments with density switches

At age 7dpf, fish were transferred from their holding dishes to round custom-designed experimental arenas (d = 6cm, depth 1cm), filled with filtered fish facility water up to a height of ~0.8 cm[12]. Briefly, these plastic experimental arenas had a flat bottom and curved walls (a quarter of a sphere of radius 1 cm) and were sandblasted to prevent reflections and allow for stimulus projection directly on the arena's surface[12]. Every experimental arena was filmed using an overhead camera (Grasshopper3-NIR, FLIR System, Zoom 7000, 18–108 mm lens, Navitar) and a long pass filter (R72, Hoya). Arenas were lit from below using 4 infrared LED panels (940 nm panels, Cop Security) and from above by indirect light coming from 4 32W fluorescent lights. Measured light levels at the water surface were ~650 lux. Every 4 cameras were connected to a single recording computer that recorded 4MP images at 30 fps per camera. To prevent overload of the RAM we performed online segmentation of the recorded images and saved only a binary image from the camera stream. The segmented images were then analyzed offline to extract the continuous tracks of the fish. All acquisition, online segmentation and offline tracking were performed using costume designed software written in Matlab[12,31].

Fish were pre-acclimated in either low density (5 fish) or high density (20 fish) for 4 hours prior to behavioral imaging. After the pre-acclimation period, fish were imaged for 20 minutes to obtain a baseline measure of their behavior. We then combined 5 fish groups into 20 fish groups or separated 20 fish groups into 5 fish groups (Fig 1a). Fish were then imaged again for 20 minutes to measure how pre-exposure to one density affects behavior in the current density. Groups were eliminated from subsequent analysis in the case that one or more of the fish were immobile for more than 50% of the experiment. All and all 7.2% and 9.3% of groups of 5 and 20 fish were eliminated from the analysis due to immobility of the fish. Choosing a more stringent, or a less stringent criteria for elimination did not change the qualitative nature of the results.

2.1. Fish kinematics and group level properties

The position of each fish *i* at time *t* is defined as the center of mass of the fish extracted from offline tracking and is denoted as $\mathbf{x}_i(t)$. The velocity of each fish *i* is given by $\mathbf{v}_i(t) = [\mathbf{x}_i(t+dt) - \mathbf{x}_i(t-dt)]/2dt$, where dt is 1 frame or 0.033s. The speed of the fish is then $s_i(t) = |\mathbf{v}_i(t)|$, and the direction of motion is $\mathbf{d}_i(t) = \mathbf{v}_i(t)/|\mathbf{v}_i(t)|$. Distance between fish *i* and *j* is the Euclidean distance $d_{ij}(t) = |\mathbf{x}_i(t) - \mathbf{x}_j(t)|$. Chance levels for interindividual distances (Fig. 1c-d) were calculated from randomized groups created by mixing fish from separately recorded groups and randomly shuffling their time signatures. For 20 fish groups, we allocated fish from different groups to the extent possible by the number of tested groups, filling in with fish that did swim together but randomly shuffling their time signatures.

2.2. Estimating retinal occupancy

We defined the effective retinal field of each eye as the visual angle extending from the fish's heading direction (0º) towards its tail (±165º), assuming a 30º blind angle directly behind the fish[74]. To estimate retinal occupancy, we used the physical length of a neighboring fish (a straight line from snout to tail), the distance to the focal fish's head, and the relative angle with respect to the heading direction of the focal fish to calculate the specific visual angles out of the retinal field occupied on each eye. When estimating total occupancy within each eye, we corrected for instances of neighboring fish occluding one another (Fig. 1e).

2.3.Turning in response to the difference in retinal occupancy between the eyes

To estimate turning direction (left vs. right) in response to difference in retinal occupancy between the eyes p(turn right|Δretinal occupancy) (Fig. 1e), we measured the change in heading angle $\Delta\mathbf{d}_i(t) = \mathbf{d}_i(t) - \mathbf{d}_i(t-\tau)$ around a detected bout event and used the visual occupancy measured at the beginning of that bout. Specifically, p(turn right|Δretinal occupancy) is the fraction of right turns out of all left/right turns recorded for 5º (groups of 5 fish) or 10º (groups of 20 fish) bins of Δretinal occupancy. We discarded all turning events at distance < 1.25 body length from the wall, as not to confound wall avoidance with neighbor responses (Fig. S1c).

3. Virtual reality experiments

Every VR experimental arena was filmed using an overhead camera (Grasshopper3-NIR, FLIR System, Di II VC, 18–200 mm lens, Tamron) and a long pass filter (IR720, Zuma). All experimental arenas were lit from below using an infrared LED panel (850 nm panels, Univivi) and from above by indirect light coming from four 32W fluorescent lights. Stimuli were projected from below, directly onto the sandblasted arenas using P7 mini-LED projectors (AAXA)(contrast ratio 2000:1)(Fig. 2a). Measured light levels at the water surface with bottom projection were ~1250 lux. Every 8 experimental arenas were connected to a single computer controlling stimulus projection, video recording and online behavioral tracking. Stimuli were updated at 60hz and behavioral data was recorded at 90hz. All and all, we used 24 experimental arenas allowing us to test different experimental conditions on the same experimental day within the same clutch of fish. All fish tracking and posture analysis were done using custom software written in Python 3.7 and OpenCV 4.1[12,63]. Briefly, movie images were background subtracted online to obtain an image of the swimming fish, and body orientation was estimated using second-order image moments. In all experiments, fish were first pre-acclimated in their designated groups (5 or 20 real fish) for at least 4 hours. Single fish were then transferred to individual arenas

and interacted with the projected virtual neighbors. Fish with bout rates < 0.5Hz were removed from the analysis, as 7 dpf larvae are expected to bout at around 1Hz. All and all ~10% of the fish were discarded from further analysis. To filter out instances where fish were temporarily immobile, we limit our data to instances when fish actively performed bouts. While this filtering removes some noise in our data it does not change any of our findings.

3.1. Trajectory replay stimuli

Virtual neighbors were presented as dark dots (d=0.15cm) on a light background using the maximal contrast possible by our projectors. We used example trajectories of 5 and 20 fish, randomly chosen from real groups recorded in the free-swimming experiments. In cases where stimuli presentation time in the experiments exceeded the length of the recorded trajectories (20min), we restarted the stimulus causing a momentary discontinuity in the presented stimuli. Similarly, when switching between low and high densities, a momentary discontinuity exists in the dots' trajectories. To confirm that our findings are not unique to the chosen stimuli trajectory sets, we created surrogate fish trajectories by randomly sampling from the distribution of bout sizes, interbout intervals and turning angles of the recorded fish. Experiments using these surrogate datasets, closely matched our findings from experiments using the replayed trajectories. Below we describe the various stimuli sets we used in all VR experiments:

- *Increasing time of high density* (Fig. 2e). We repeatedly presented a 5 min segment of the 20 min trajectories to better compare between shorter and longer exposures. For example, fish that were exposed to 20 min of high density, were presented with the same 5 min stimuli as fish that were exposed to 5 min of high density, but the stimuli looped 4 times for the 20 min condition.

- *Stationary dots* (Fig. 3a, Supplementary Movie 3). We used a single, randomly chosen frame from the recorded fish trajectories. Dots were stationary for the entire stimulus presentation time.

- *Low density with increased speed* (Fig. S3b). To speed up fish swimming by a factor of 2 or 4, we down sampled the recorded trajectories. This allowed fish to traverse the same distance by either 1/2 or 1/4 of the time. We then replayed the trajectories to compensate for the shorter total presentation time.

- *Rotational trajectories* (Fig. 3a, Supplementary Movie 3). To independently test the effects of rotational energy we presented a high density of dots (19) moving on 1d rings centered around the focal fish. Radial distances from the focal fish were chosen based on the most likely distance to find the 1st, 2nd…19th nearest neighbor in the recorded trajectories of real groups. Angular position with respect to the heading direction of the focal fish was changed in a bout-like fashion, with clockwise/counterclockwise direction of motion chosen randomly for each bout.

- *Stationary Looming dots* (Fig. 3a, Supplementary Movie 3). To independently test the effects of looming energy, we arranged 19 dots equally spaced from one another within a circle (forming a hexagonal formation). Dot sizes increased and decreased with temporal dynamics taken from the recorded changes in angular size experienced by a focal fish swimming with virtual neighbors. Minimum and maximum dot radii were chosen at $r_{min} = 0.075$cm and $r_{max} = 0.9$cm.
- *Trajectories of 39 neighbors* (Fig. S3d). We combined the real recorded trajectories from two 20 fish groups to replay as a 39-neighbor group.

3.2. Single dot stimulus

We used a similar protocol for single dot presentations as described in[12]. Briefly, in each trial, a single dot appeared at one side of the fish at a given radial distance and moved with fish-like bouts tangentially around the fish from ±75º to ±30º for 5s, with 0º taken as the fish heading direction. The presentation side was chosen at random and once a dot reached the end of its trajectory a new dot immediately appeared (0s inter-stimulus interval) (Fig. 3b, Supplementary Movie 4). We manipulated the dot's radial distance or the dot's radius according to the following stimulus conditions:

- *'Moving' dot stimulus.* We set dot radius = 0.15cm and distance to dot center = 0.42cm. Hence, dots had a constant angular size of ~40º throughout the trial.
- *'Moving closer' dot stimulus.* Dots had a constant radius = 0.075cm, and the distance to the dot center gradually decreased over the 5s presentation time from 0.92cm to 0.22cm, i.e. angular size changed from ~9º to ~40º throughout the trial.
- *'Moving and growing' dot stimulus.* Dots were presented at a constant distance of 0.35 cm, and dot radius was increased from 0.025cm to 0.13, i.e. angular size changed from ~9º to ~40º throughout the trial.

3.3. Estimating fish response to a single dot stimulus

To analyze fish responses to the single dot stimuli, we calculated the change in body orientation of the fish for each bout during stimulus presentation. We then estimated the overall probability to turn left or right in each trial. These estimated probabilities were then averaged over blocks of trials and over fish (Fig. 3b).

3.4. Estimating fish responses to global retinal occupancy based on monocular averaging and binocular comparisons.

Previously, we showed that 7 dpf larvae turn away from the eye that experiences a higher retinal occupancy[12]. Specifically, responses to the global retinal occupancy of neighbors occupying the set of visual angles $\theta^{left}, \theta^{right}$ (on the left and right eyes) follow the form:

$$p(turn\ right|\theta^{left}, \theta^{right}) = 0.5 + \sum_{i}^{\theta^{left}} {w_i}^{left} \cdot bias({v_i}^{left}) + \sum_{i}^{\theta^{right}} {w_i}^{right} \cdot bias({v_i}^{right}) \quad (2)$$

Where $bias(v_i) = p(turn\ right|v_i) - 0.5$ is the turning direction bias in response to vertical retinal occupancy v_i, w_i is the relative weight assigned to each response bias and represents a weighted average $w_i = v_i/\Sigma v_i$ of the occupied vertical visual angles in each eye. The intercept 0.5, centers the summed responses around that value and $p(turn\ right|\theta^{left}, \theta^{right})$ is bounded between 0 and 1. If we assume that $p(turn\ right|v_i) = av_i + 0.5$ and $p(turn\ left|v_i) = -av_i + 0.5$, i.e. response can be approximated as a linear function of v_i , we can rewrite the above equation as:

$$p(turn\ right|\theta^{left}, \theta^{right}) = 0.5 + \sum_{i}^{\theta^{left}} {w_i}^{left} \cdot {av_i}^{left} - \sum_{i}^{\theta^{right}} {w_i}^{right} \cdot {av_i}^{right} \quad (3)$$

And since $w_i = v_i/\Sigma v_i$, we get:

$$p(turn\ right|\theta^{\ left},\theta^{\ right}) = 0.5 + a[\sum_{i}^{\theta^{left}}(v_i^{\ left})^2/\Sigma v_i - \sum_{i}^{\theta^{right}}(v_i^{\ right})^2/\Sigma v_i] \quad (4)$$

Hence, the turning direction of the fish is dependent linearly on the difference in the weighted average of the squared vertical visual angles in each eye. We estimate this function from data in Fig. 4b and use it in simulating fish responses to virtual neighbors (see below) (Fig. 5a-d).

4. Time varying models of fish social interactions

Basic individual agent swim properties, responses to arena walls and static inter-individual interactions based on retinal occupancy used in the models here, were previously described in[12]. We briefly describe the details again here and expand on the additional aspects of experience dependent modulation of inter-individual interactions.

4.1. *Basic swim properties*. Each stationary fish, at every time step, probabilistically decides to perform a bout according to the average bout rates of real fish. Similarly, bout duration and distance traveled in a bout followed that of the average bouts of real fish.

4.2. *Wall interactions*. Similar to real fish, simulated agents' first priority is to avoid crushing into the walls. Therefore, agents ignore their neighbors when in close proximity to the arena walls and turn away from the direction of the closest wall. In every bout, the probability to respond to the walls and to turn in the opposite direction is drawn from the empirical wall response functions observed in real fish (Fig. S1c). If the executed bout was expected to end outside of the arena, it was truncated to ensure the fish stayed inside the simulated arena.

4.3. *Turning behavior in response to global retinal occupancy of neighbors*. We used the algorithms described in[12] and in Eq. 2 (see above) to simulate the response of agents to the global retinal occupancy of their neighbors. When modeling agents responding to stimuli from VR experiments (Fig. 5a-d), we used the estimated response to vertical retinal occupancy extracted from VR experiments (Fig. 4b) and the linear approximation presented in Eq. 4. In models simulating a group of interacting agents (Fig. 5e-f), we used the estimated responses to vertical retinal occupancy described in[12] (these estimated response functions are included in the simulation codes). Vertical retinal occupancy of neighbor j at visual sub-angle i (Vc_{ji}) was calculated using the simulated height (H_j) and distance (d_j) of neighbor j: $Vc_{ji} = 2 \cdot arctan(H_j/d_j)$. For simplicity, we did not account for occlusions among neighbors in estimating retinal occupancy as initial simulations showed that it did not affect our simulation results. In addition, we also assume that the height of the fish along its body axis is constant, allowing us to treat the vertical occupancy at all visual sub-angles occupied by neighbor j as a single value.

4.4. *Leaky Integrator module for temporal integration of neighbor-evoked looming events*. The experimental findings from VR experiments (Fig. 2e-g, Fig. 3a-c) indicate that agents slowly integrate (and remember) neighbor-evoked looming events on their retina. This process develops slowly, remains stable for many hours, and can be reverted back to baseline via the removal of all retinal inputs (Fig. 2e-g). We therefore introduce a leaky integrator module, that temporally updates an internal state variable $s(t)$ according to the the input to the integrator $Input(t)$, which is the maximal increase in retinal occupancy of any neighbor experienced at time t (Eq. 1). In the

absence of visual input, the internal state variable will exponentially decay back to a resting state value s_{rest} with a time constant τ.

4.5. *Linking internal state $s(t)$ to agents' occupancy based turning behavior*. We assume that higher internal state values $s(t)$ indicate weaker responses to retinal occupancy of neighbors, as seen in behavioral experiments. Therefore, we linked $s(t)$ to the strength of response to retinal occupancy $p(turn\ right|v_i) = av_i + 0.5$. For simplicity, we assumes a linear relationship between $s(t)$ and the slope of the response a, and define it as $a' = a(1 - \frac{s(t)-s_{start}}{D})$, where s_{start} is the starting value of the integrator, D is a scaling factor and α is the value estimated from groups of 5 fish that were not previously exposed to changes in social density[12] (Fig. 4b). Since 7 dpf larvae do not show attraction to neighbors at this age, we constrain a' to be between [0 3a].

4.6. *Switching simulated agents between high and low density*. When switching a group of simulated agents between densities mid simulation (Fig. 5e-f), we either removed (high to low) or increased the number of simulated agents. When removing agents, we simply chose $N_{to\ remove}$ agents randomly, and eliminated them from the rest of the simulations. When adding agents, we assigned the $N_{to\ add}$ agents starting positions and heading orientations at random. The internal state values $s(t)$ of the added agents, were chosen such that they represent the distribution of values of internal states of the agents already swimming together. Therefore, we randomly drawn $s(t)$ for the newly added agents from a normal distribution with mean and sd calculated from the N agents already swimming together.

4.7. *Model parameters used in simulations*

Parameter name	**Description**	**Values (Single agent in VR/ Group of agents)**
Arena diameter	Similar to the arena sizes in group swimming experiments	6 / 6
Time interval (Δt)	Time between simulated steps	1/50 s/ 1/50 s
Simulation time	Total simulated time per group	Matching real fish experiments
Number of repetitions	Random repetitions of a given model - matching experimental repetitions	24 per condition / 24 per condition
Fish starting positions	Random positions within 0.9*arena diameter	
Fish length	Estimated from fish	0.4cm/ 0.4cm
Fish height	Estimated from fish	0.4 cm/ 0.2cm
Bout Rate	Estimated from group	1.64 Hz/1.64 Hz

	swimming experiments	
Bout size	Estimated from group swimming experiments	0.1cm / 0.1cm
Bout duration	Estimated from group swimming experiments	320 ms/ 320ms
a	Slope of response to retinal occupancy $p(turn\ right\|v_i) = av_i + 0.5$	0.0104 / 0.00372 (Estimated from data / estimated from VR data in[12])
s_{rest}	Resting state potential of leaky integrator	0 / 0
s_{start}	Starting value of integrator - steady state value for 5 fish groups.	0.6 / 0.6
τ	Leak time constant. Estimated from data (Fig. 3e-g, S4b-c)	6 hrs / 6 hrs
r	Scale parameter for input. Fitted to data.	13 /13
D	Scale parameter for linearly relating $s(t)$ to a. Fitted to data.	0.19 for all simulations. 0.1 for figure 5d to account for stronger response in this experimental fish batch.

5. Statistical methods.

Sample sizes and power consideration. Sample sizes for group-swimming experiments were chosen primarily to support stable estimation of group-level metrics (e.g., mean nearest-neighbor distance, NNd) and turning response probabilities, guided by prior larval zebrafish studies[12,48]. We anticipated that N>15 groups in each condition would provide reasonable sensitivity to detect between-condition differences of moderate magnitude (Cohen's d ≈ 0.5), based on previously reported variability and effect sizes in comparable assays[12,48]. Because our design combines sets of 5-fish groups into 20-fish groups (and also splits 20 fish groups into 5 fish groups), the dataset contains more observations from 5-fish groups than from 20-fish groups. In virtual-reality experiments, we used 16–24 fish per experiment, consistent with prior work[12] and pilot measurements of response variability, which indicated that these sample sizes yield sufficiently precise estimates of behavioral responses and allow comparisons across experimental conditions.

Statistical reporting. Estimated effect sizes are reported as Cohen's d = $\frac{\bar{x}_1-\bar{x}_2}{SD_{pooled}}$ for difference of means, and partial eta-squared ${\eta_p}^2 = \frac{SSeffect}{SSeffect+SSerror}$ for omnibus ANOVA-type effects. Confidence intervals (CIs) for mean estimates and for mean differences were obtained through bootstrap resampling (100,000 resamples), and are reported as 95% CIs unless stated otherwise[75]. For parametric analyses, model assumptions were evaluated using standard diagnostic checks (e.g., assessment of variance homogeneity, inspection of residual distributions). Unless stated otherwise, tests were two-sided. In line with current statistical reporting recommendations (Wasserstein et al.[76]), we emphasize the report of estimated effect sizes and CIs alongside Fisherian (permutation) p-values rather than dichotomous significance thresholds. All analyses were performed in Matlab (MathWorks).

Hypothesis testing

5.1. Bootstrap for a single sample (Fig. 1d). To test whether groups' mean nearest-neighbor distance (NNd) differed from a baseline expected under random grouping, we generated a null ("baseline") distribution by shuffling group composition (mixing the trajectories of fish from different real groups) and computing the corresponding NNd estimate. We then approximated the sampling distribution of the experimental estimator by nonparametric bootstrap resampling at the group level[75]: for each condition, we sampled with replacement N 5-fish groups (where N is the number of observed 5-fish groups in that condition) and recomputed the NNd estimate, repeating this procedure 100,000 times. We report 95% bootstrap intervals (95% BI) for the estimand. A two-sided p-value ($p_{bootstrap}$) associated with the null hypothesis (i.e., estimand equal to the baseline value) was obtained by inversion of the 95% BI.

5.2. Fisherian (permutation) tests.

We conduct Fisher tests in all experiments comparing two or more conditions[77]. Briefly, we assess a sharp null hypothesis using permutations consistent with the experimental design. For between-subject factors, condition/group labels were permuted across fish (or groups of fish) while preserving all repeated measurements within each fish/group. For within-subject pre–post comparisons, pre/post labels were independently swapped within each fish. Assuming the sharp null hypothesis, a Fisherian p-value corresponds to the proportion of permutations yielding a test statistic as or more extreme than the observed test statistic. We approximated the Fisher-exact p-value using 100,000 permutations. Our analysis follows the procedure initially described in[77] and more recently by various statisticians[78,79].

Test statistics. Two sample comparisons are tested using Welch's t-statistic. In all ANOVA models, omnibus main effects and interactions are tested using the omnibus F statistic and post-hoc main effects using Welch's t-statistic (see specific models below).

5.2.1. Comparing two independent groups (Fig. 1d, 2c). We compared nearest-neighbor distances (NNd) between groups that differed in their *previous* social-density exposure, analyzing 5-fish groups and 20-fish groups separately. For each group j, let Y_j denote the group mean nearest neighbor distance averaged over the full experiment. Let A_j denote the previous social density condition of group j, where A_j = 0 for high previous social

density (20 fish) and A_j = 1 for low previous density (5 fish). Within each group-size (5-fish groups; 20-fish groups), we tested whether Y_j depended on $A_j$using a permutation test with the difference in sample means as the test statistic.

Sharp null hypothesis. Using potential outcomes $Y_j(A_j = 0)$ and $Y_j(A_j = 1)$, we tested the sharp null hypothesis $Y_j(A_j = 0) = Y_j(A_j = 1)$ for all groups j for the current group size.

5.2.2. Comparing turning behavior as a function of visual-occupancy differences using a two-way mixed-design ANOVA (Fig. 1e, 2d). We analyzed turning behavior using a two-way mixed-design ANOVA with previous social-density condition (previous exposure to high vs low social density; between-group factor), binned occupancy difference (categorical; within-group factor), and a condition x bin interaction term capturing effect modification by occupancy levels.

Sharp null hypothesis. Let $Y_{j,b}(a)$ denote the potential outcome of group j, in visual occupancy difference bin b under previous-density condition a. For the omnibus main effect of condition and for effect modification (implemented as the condition x bin interaction term), we tested the Fisher sharp null that condition has no effect in any bin: $Y_{j,b}(0) = Y_{j,b}(1)$ for all groups j and all bins b. For post-hoc simple effects within a given bin b, we tested the bin-specific sharp null: $Y_{j,b}(0) = Y_{j,b}(1)$ for all groups j in that bin.

5.2.3. Testing pre-post changes in nearest-neighbor distance (NNd) across different high/low density exposure times (Fig. 2e, g). We analyzed pre-post changes in NNd using two-way mixed-design ANOVA with time (pre vs post; within-subject factor), condition (between-subject factor; exposure regimen), and a time-by-condition term capturing whether the pre-post change differed across exposure condition. Primary inference was based on the relevant omnibus effect (e.g., the main effect of time, where changes were expected across multiple conditions (Fig. 2e)), followed by *a priori* contrasts (simple effects) testing pre-post changes within specific exposure conditions and reporting the Holm adjusted p-values across these comparisons.

Null hypothesis. Let $Y_{i,t}(a)$ denote the outcome of fish i, measured at time t (pre or post manipulation) in condition a. Define the individual pre-post change as $\Delta_i(a) = Y_{i,post}(a) - Y_{i,pre}(a)$. For the omnibus main effect of time, we tested the null of no pre-post difference for any fish under its assigned condition, i.e., $\Delta_i(a) = 0$ for all fish i. For the condition x time term, we tested the null of no effect modification of the pre-post change by condition, i.e. $\Delta_i(a) = \Delta_i(a')$ for all fish i and all pairs of conditions a, a'. For post-hoc simple effects within condition a, we tested the condition specific null: $\Delta_i(a) = 0$ for all fish i assigned to condition a.

5.2.4. Testing pre-post changes in nearest-neighbor distance (NNd) across VR manipulations (Fig. 3a, c). To test whether each VR manipulation (stationary high-density, translational-motion cues, and looming-motion cues) induced changes in group structure, we compared the NNd before and after the manipulation using a permutation test for paired data. For each manipulation, we defined the pre–post change and computed a two-sided permutation p-value via within-group label swapping. Because

multiple manipulations were tested, we report Holm-adjusted p-values across these comparisons.

Null hypothesis. For each manipulation a, let $Y_{i,pre}(a)$ and $Y_{i,post}(a)$ denote the outcomes of fish i measured before and after manipulation a, and let $\Delta_i(a) = Y_{i,post}(a) - Y_{i,pre}(a)$. we tested the manipulation specific null: $\Delta_i(a) = 0$ for all fish i in condition a.

5.2.5. Testing stability of NNd changes over time (Fig. 2f). To test whether NNd changes persisted over time after exposure to high density, we fit linear mixed-effects models with categorical timepoint as a fixed effect and fish identity as a random intercept (within-fish repeated-measures design). Stability across post-manipulation timepoints was assessed with an omnibus constraint test (i.e., equality of post manipulation timepoint means 20-120 min) followed by targeted contrasts (i.e., baseline vs 120 min; 20 min vs 120 min). Permutation p-values were used for the omnibus stability test and for all planned timepoint contrasts; for each permutation, we refit the mixed-effects model and recomputed the relevant test statistic. P-values for the planned contrasts were adjusted using the Holm procedure.

Null Hypothesis. Let $Y_i(t)$ denote the NNd of fish i at time point t. For the omnibus post manipulation stability test (20-120 min), we tested the null hypothesis that each fish's post manipulation outcomes are invariant across post timepoints, i.e., $Y_i(t) = Y_i(t')$ for all fish i and all post manipulation timepoints t, t'. For the planned pairwise contrasts, we tested the corresponding pairwise sharp nulls $Y_i(baseline) = Y_i(120)$ and $Y_i(20) = Y_i(120)$ for all fish i.

5.3. Multiple comparisons. When multiple follow-up (post-hoc or planned) comparisons were conducted within a given analysis, we controlled the family-wise error rate by adjusting p-values using the Holm–Bonferroni procedure across the set of comparisons defining that family.

Data availability.

All data are publicly available at: https://doi.org/10.7910/DVN/KWA7DK. Source data are provided with this paper.

Code availability.

All codes used for model simulations are publicly available at:
https://github.com/harpazone/Experience-dependent-modulation-of-collective-behavior

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Author Contributions.
RH, MP, MCF and FE designed research, RH, MP and RG performed experiments, RH, FE and MAB analyzed the data, RH, FE and MAB interpreted the data, RH, MCF, MAB and FE wrote the paper.

Competing interests.
The authors declare no competing interests.

Figure Legends.

Figure 1 - Previous social experience modulates collective swimming behavior. A. High throughput experimental design to study experience dependent modulation in groups. Fish are pre-exposed to their designated groups for 4 hrs and behavior is monitored in high and low densities (Test 1). Groups are then combined or split to study the opposite density (Test 2). **b.** Example of the joint trajectories of groups of 5 or 20 fish over 20 seconds. Colors represent different fish. **c.** Time dynamics of the average nearest neighbor distance of groups swimming in either low (dashed blue) or high (dashed red) neighbor density, and for groups that were previously exposed to the opposite density (solid lines). Individual group data are averaged in 60s blocks. Shaded areas are SEM. **d.** Average nearest neighbor distance of groups (N_5=44, $N_{20\rightarrow5}$=45, N_{20}=27, $N_{5\rightarrow20}$=14). Dots represent individual groups; error bars are mean$\pm$SEM. Black dashed lines (in c. and d.) represent the expected average nearest neighbor distances of non-interacting fish from shuffled trajectories (Methods). A zoomed-in inset is shown to better contrast the NNd in large and small groups, which span different scales. **e.** Probability to turn away from the more occupied eye as a function of difference in retinal occupancy (higher occupied eye - lower occupied eye). Lines represent mean turning probability calculated as the fraction of turns away from the more occupied side out of all turns in 5^o (groups of 5) or 10^o (groups of 20) bins. Shaded areas are SEM. Previous social experience modulates fish responses to retinal occupancy. Source data are provided as a Source Data file.

Figure 2 - Dynamics of experience dependent modulation. a. High throughput VR assay to study experience dependent modulation of collective behavior. Fish are pre-exposed to their designated group sizes for 4 hrs. Single fish are then moved to VR assays and exposed to virtual neighbors moving according to real, pre-recorded trajectories. The density of virtual neighbors is switched mid experiment. **b.** Example trajectories (over 20s) of single real fish (red/blue) and their virtual neighbors (gray) while fish are exposed to either low (blue borders) or high (red borders) virtual neighbor densities. **c.** Average nearest neighbor distance of real fish from their virtual neighbors. Dots represent individual fish; error

bars are mean±SEM (N=16 for all conditions). A zoomed-in inset is shown to better contrast the NNd in large and small groups, which span different scales. **d.** Probability to turn away from the more occupied eye as a function of difference in retinal occupancy (higher occupied eye - lower occupied eye). Lines represent mean turning probability calculated as the fraction of turns away from the more occupied eye out of all turns in 5^o (4 virtual neighbors) or 20^o (19 virtual neighbors) bins. Shaded areas are SEM. **e.** Average change (after-before) in nearest neighbor distances of fish swimming in a low neighbor density after exposure to increasing times of high neighbor density ($N_{t=5m}$=11, $N_{t=10m}$=15, $N_{t=20m}$=17, $N_{t=40m}$=18). **f.** Average change (after-before) in nearest neighbor distances of fish swimming in a low neighbor density after exposure to a high density of virtual neighbors (20 min). Bars represent averages over fish in blocks of consecutive 20 minutes after exposure to high density. Lines connect individual fish (N=20). **g.** Average change (after-before) in nearest neighbor distance of fish swimming in a low neighbor density after exposure to 20 min of high virtual neighbor density followed by increasing times of 0 neighbors ($N_{t=0m}$=19, $N_{t=10m}$=16, $N_{t=20m}$=13, $N_{t=50m}$=21). In panels (**e-g**) top panels represent experiment conditions, single dots are individual fish, and error bars represent mean±SEM. Source data are provided as a Source Data file.

Figure 3 - Persistent increase in retinal occupancy drives experience-dependent modulation of collective behavior. a. Average change (after-before) in nearest neighbor distances of fish swimming in low density (4 virtual neighbors) after exposure to 20 min of high density (19 virtual neighbors) with different motion statistics ($N_{Stationary}$=21, $N_{Translational}$=24, $N_{Looming}$=22, Methods). **b. Top:** Experiments testing the ability of a single dot stimulus with different motion statistics to elicit changes in nearest neighbor distance (Methods). **Bottom:** Probability per bout to turn away from a moving dot in the 3 conditions**.** Bold lines are the mean probability over fish, and shaded areas are SEM. Probability is calculated as the fraction of turns away from the presented side out of all turns, and each block is the average of 60 stimulus presentations. Dotted lines represent no preference in turning direction. **c.** Average change (after-before) in nearest neighbor distance of fish swimming in a low-density group after exposure to 30 min of single dot stimuli as shown in **b**. (N_{moving}=24, $N_{moving\ closer}$=24, $N_{moving\ and\ growing}$=23). Single dot stimuli with a looming component to their motion cause a subsequent reduction in nearest neighbor distances of fish in a group. In panels (a, c) single dots are individual fish, and error bars represent mean±SEM. Source data are provided as a Source Data file.

Figure 4 - Time varying models of experience-dependent modulation of collective behavior a. A sketch of a time varying model with two components - a hidden state variable $s(t)$ and an observed response to retinal occupancy of neighbors $f[\mathbf{v}, s(t)]$ that depends on the hidden state variable. **b. Left:** The response to retinal occupancy $f(\mathbf{v})$ depends on the difference in average retinal occupancy within each eye[12] (Fig. S4a, Methods). **Right:** Estimating $f[\mathbf{v}, s(t)]$ from fish responses to virtual neighbors reveals that fish turn away from the more occupied eye and that responses are modulated by previous exposure to a different social density. Lines represent mean per bout turn probabilities; shaded areas represent SEM (Methods) **c.** A leaky integrator module tracks the values of the hidden state variable $s(t)$. The input to the integrator is the experienced maximal increases in retinal occupancy (i.e. looming motion on the retina). **d. Top:** Example of experienced looming events when fish are exposed to a low-

high-low density experimental design. **Bottom:** The values of the internal state variable $s(t)$ and the corresponding change in p(turn away) in response to a constant average difference in retinal occupancy. Increasing values of $s(t)$ cause a reduction in directed turning responses and vice versa.

Figure 5 - Models accurately predict experience dependent modulation of collective behavior. a. Example trajectories (20s) of simulated agents (blue), interacting with 'virtual neighbors' (red) based on the model presented in Fig 4. (Methods). **b.** Average change (after-before) in nearest neighbor distances of simulated agents swimming in low neighbor density after exposure to a high density of virtual neighbors (20 min)**.** Bars represent averages over blocks of consecutive 20 minutes after exposure to high density. Lines connect individual agents. **c.** Average change (after-before) in nearest neighbor distance of simulated agents in a low neighbor density after exposure to increasing times of high 'virtual neighbor' density. **d.** Average change (after-before) in nearest neighbor distance of simulated agents in a low 'virtual neighbor' density after exposure to 20 min of high 'virtual neighbor' density followed by increasing times of 0 neighbors. In panels (b-d) top panels represent simulation conditions, single dots are individual agents (N=24), error bars (blue) represent mean$\pm$SEM of simulated data. Dark red circles and error bars represent mean$\pm$SEM of the real fish data presented in (Fig. 2e-g). **e.** Example trajectories (20s) of groups of simulated agents interacting according to the model presented in Fig. 4 (Methods). Simulated agents are virtually 'split' and 'combined' to mimic the experimental design used for real groups of fish and presented in Fig. 1a. **f.** Average nearest neighbor distances of groups of simulated agents that swim in low (left - blue) and high (right - orange) group densities. Solid lines show simulated groups that were pre-exposed to the opposite density; dots represent individual groups of agents and error bars are mean$\pm$SEM in simulations. Dark red circles and error bars represent mean$\pm$SEM of the real fish data presented in (Fig. 1d). Zoomed-in insets are shown to better contrast the NNd in large and small groups, which span different scales. N=24 for all simulations.