Social-like responses are inducible in the asocial and blind Mexican cavefish despite the continued exhibition of strong repetitive behavior

Motoko Iwashita and Masato Yoshizawa

Department of Biology, the University of Hawai‘i at Mānoa, Honolulu, Hawai‘i, USA

Corresponding:
Masato Yoshizawa
2538 McCarthy Mall, EDM 216, Honolulu, HI 96822
yoshizaw@hawaii.edu

Abstract

Collective behaviors, such as schooling of fishes and mass migrations of ungulates, are hallmark features of a wide variety of animal species. Such phenotypic characters can be lost, either through evolutionary process across generations or by certain environmental stressors within a generation. Such stressors simultaneously promote stereotypic repetitive behaviors in many mammals, such as those exhibited in certain *ex-situ* captive settings. However, in asocial species, it is unclear whether social ability is permanently lost or only suppressed. It is also unclear if this antagonistic regulation between repetitive and social behaviors is deeply conserved among vertebrates. An evolutionary model, the Mexican tetra, *Astyanax mexicanus*, has a blind cave-dwelling morph lacking collective behaviors but exhibiting repetitive circling. In contrast, the sighted surface riverine morph of *A. mexicanus* shows more normative behaviors. We here report that social-like interactions are recoverable in blind asocial cavefish under familiar environments but are suppressed under the stress-associated unfamiliar environments. In contrast, surface fish revealed robust social-like behaviors in the dark, regardless of familiarity to the environment. Treatment of a human antipsychotic drug also induced social-like interactions in cavefish even in unfamiliar environments. In addition, the level of repetitive behavior is positively correlated with that of asociality in cavefish, suggesting that the antagonistic regulation between repetitive and social-like behaviors is present in teleost fish. Asocial cavefish therefore, with a deeply conserved framework of behavioral regulation, are still capable of expressing social-like behavior following *ca*. 200,000 years of adaptation to caves.

Introduction

Many animals across a wide range of taxa express collective behaviors (e.g. shoaling and herding) for multiple benefits including foraging and/or anti-predator effects[1]. Despite the benefits of collective behavior, some species have reduced their collective behavior over evolutionary time to become to exhibit marked asociality when compared to their social closest relatives[1]. Also, even among social species, this collective behavior can disappear when the animal experiences a stress-associated environment[2,3]. Instead, these animals may express asocial behavior and/or stereotypic repetitive behaviors, which are particularly well reported in mammals (eg. "zoochosis")[2,4]. Stereotypic repetitive behaviors, in contrast to collective behaviors, are generally thought of as non-functional[2]. Psychiatric conditions in humans, such as autism spectrum disorder share similarities to atypical repetitive behavioral responses in other animals in that social activity is decreased and there is an overall increase in stereotypic repetitive behaviors, especially under stress-inducing environment[5–7]. The neural mechanisms underlying the contrasting expression of social and repetitive behaviors and how the 'choice' between stereotypic repetitive and social modes of behavior are made, also remain largely unknown[2,3,8–10]. However, researches in mammals have hinted at such neural mechanisms by indicating that the dopaminergic striatal system is key for behavioral choices. For example, the treatment of anti-agitation drugs (drug to reduce repetitive behavior, eg. aripiprazole and risperidone) attenuated but not eliminated the activity of the D2-dopamine receptor-based indirect pathway in mammals[3,11]. Importantly, this core dopaminergic system exists in all vertebrate models from lamprey, teleost (zebrafish), amphibian (Xenopus), bird (chicken) to mammalian (mouse and human)[12,13]. However, among simpler vertebrate, it is largely unknown whether an evolved asocial population lost the ability to cooperate each other or just suppressed it, and whether repetitive behaviors are enhanced under stress in an antagonistic manner with the collective behavior.

To solve these two questions, we used an evolutionary model, the Mexican tetra, *Astyanax mexicanus*, and developed a novel experimental workflow to assay collective and stereotyped behavior[14]. *A. mexicanus* is an emerging as a key model system in diverse aspects of evolution and development, including those with relevance to human medicine e.g. cataract formation, obesity, diabetes, albinism-related syndrome, and insomnia[15–23]. *A. mexicanus* is composed of surface riverine epigean (surface fish) and cave-dwelling hypogean (cavefish) forms. Cavefish diverged from their surface-dwelling relatives 20,000~200,000 years ago[24,25] and since then have evolved many distinct morphological and behavioral phenotypes including eye regression/loss, pigment reduction, increased lateral line mechanosensors, adherence to vibration stimulus, sleeplessness, hyperactivity and repetitive circling[22,26]. Compared to cavefish, surface fish show normative phenotypes including, eyed and pigmented morphologies, no-strong adherence to vibration stimulus, and nocturnal sleep patterns. Cavefish have also been shown to have reduced social-like interactions such as no detectable schooling and shoaling behavior[27–29] or hierarchal dominance[30]. In contrast, surface fish school/shoal with cohorts and model fish[27] and show group hierarchical dominance[30]. Importantly, the surface fish schooling behavior was concluded to be driven by visual stimulus because it diminished in the dark[27]. In contrast, surface fish in the dark still showed similar levels of aggression, thought to establish hierarchical dominance, to those kept in the light[30]. This motivates us to test any other social-like interaction in the dark, with which we can compare non-visual based social interactions between surface fish and cavefish.

We then analyzed social-like nearby interactions and repetitive behavior in the dark and, surprisingly, revealed numerous social-like interactions in surface fish in the dark. Surface fish, however, did not show repetitive turning. Furthermore, we also uncovered weak but existing social-like interactions in cavefish while exhibiting significantly higher level of repetitive behavior than that of surface fish. During these nearby interactions, cavefish decelerated their swimming velocity, similar to surface fish. The comparison of these behaviors under familiar and unfamiliar environments, indicated that cavefish significantly increased nearby interactions in the familiar environment. Atypical dopamine psychiatric drug, aripiprazole, which is known to increase social behaviors and reduce repetitive motions in human[31–33], slowed swimming velocity and increased nearby interaction in both surface and cavefish. Cavefish also reduced repetitive turning after aripiprazole treatment. In summary, cavefish showed a robust repetitive circling by largely ceding social-like nearby interactions, where this social-like interaction still kept being plastic in response to the environmental cues. Together with the reported parallels in pharmacological response, a set of behavioral symptoms, and gene expression profiles between cavefish and human, the presented result suggests a deeply shared pathway that controls social-like and repetitive behaviors between teleost and mammals.

Results

Shift in spatial preference in an unfamiliar environment

Since there are few reports for social-like interaction of teleost species in the dark (e.g., [34]), we began by systematically assessing close-distance interactions using social and non-social morphs of *Astyanax mexicanus*—surface fish and cavefish—in the dark. Fish were introduced into an unfamiliar environment (the recording arena), where we could observe nearby interactions at close range (see below). The behaviors of four fish were recorded for 10 min (Fig. 2**a,b**). The separate trajectory of each of the four individual fish was tracked by idTracker[35]. After their introduction to the recording arena, surface fish showed bursts of fast swimming and moved aimlessly with regards to direction, likely associated with their high anxiety state. Within one minute after the release, four surface fish gathered at the center of the arena then gradually started exploring the periphery (Fig. 2**b**). Cavefish also showed their increased swimming velocity and swam in random directions following transfer, and unlike surface fish, they did not gather at the center of the recording arena, and did not change their location preference. Instead, cavefish were scattered randomly with respect to tank location and frequently occurred at the periphery of the arena, from the beginning to the end of the analysis (9 min), and they swam longer distance overall than surface fish (Fig. 2**c,d**).

Shorter nearest neighbor distance and inter-individual distance in surface fish in the dark

Surface fish are known to school/shoal in lighted environments, exhibited the same strong shoaling and schooling in our setup under light (Supplemental Movie 1)[27], and did not respond to any visual stimuli in the dark (Supplemental Movie 2). In the dark, two standard shoaling measurements for fish—the averages of Nearest Neighbor Distance (NND: the distance between a focal individual and its nearest neighbor) and averages of Inter-Individual Distance (IID: the mean distance between each individual) of surface fish were significantly shorter than those of cavefish ($P < 0.001$; Fig. 2**e** and **h**; NND and IID, respectively: one to 10 minutes after the introduction to the recording arena; See statistics scores in Supplemental Table 1). To validate whether fish were closer to each other than under random expectations, we employed a permutation test by randomly choosing 4 fish among 12 groups (each group consists of 4 fish). The 1,000 permutation were independently performed between surface fish and cavefish (See Materials and Methods). By using these permutated X-Y coordinates of fish, NND and IID were re-calculated and plotted as simulated no-interaction conditions. Surface fish then showed significantly shorter NND and IID comparing with the surface fish permutation result (Fig. 2**f** and 2**i**, respectively: $P < 0.001$. See statistics scores in Supplemental Table 1) whereas the NND and IID of cavefish were undistinguishable from the those of the permutation result (Fig. 2**g** and 2**j**, respectively: $P > 0.1$, Supplemental Table 1). This result indicates that surface fish swam significantly closer to each other compared to random expectations during the recording. In contrast, cavefish swam randomly as if there was no cohort in the same arena.

A measurement of nearby interaction in the dark and the involvement of the lateral line mechanosensors.

Due to the relatively loose shoaling tendency of surface fish in the dark and the difficulty in detecting in detail the behaviors of any two arbitrary fish, a new measurement of this social-like interaction was needed (e.g., Fig. 2**e** and 2**h**). Here, we define "nearby interaction" as when two fish come within a short distance from each other after changing their swimming direction toward one another, suggesting intent in the movement rather than random locomotion with

respect to their conspecifics. We set this distance as 6 cm for adult *A. mexicanus* according to (1) observed distances of when two fish were first attracted to one another through 8 surface fish videos in the dark (32 individuals in total, 9 min in total after back-ground subtraction, also (2) histogram plots of the 'nearby bouts' across inter-pair fish distances (example of a video of each surface fish and cavefish, Supplemental Fig. 1, Supplemental information). We also set a minimum duration of nearby interaction as 4 sec according to the direct observation of videos and histogram plots (Supplemental Fig. 2) (Materials and Methods; Supplemental information). We further checked if the swimming tracks of two focal fish were nearly congruent by visualizing them in each bout of the detected nearby interaction (Materials and Methods; Supplemental information; Supplemental Fig. 3,4).

Using this nearby interaction criteria, cavefish showed low but detectable levels of nearby interaction (eg. nearby interaction duration: 9.9 ± 1.7 s, N = 32 at Fig. 4**h**; 12.2 ± 2.2 s, N = 22 at Fig. 6**c** for mean ± standard error of means; Both in the unfamiliar environment during 9 min assays; see below), which contradicts previous reports of 'no detectable shoaling/schooling behavior in cavefish[27,36,37]. As the predominant turning direction was towards cohorts during the bout of nearby interaction in cavefish (Supplemental Fig. 4) and no sign of fin or scale damage was observed after each of the nearby interaction-assays (cf. foraging or aggression against intruders which has previously been reported in *A. mexicanus*[30]), we counted nearby interactions in cavefish as the same social-like behavior as those seen in surface fish.

To investigate the sensory systems mediating nearby interactions in the dark, we first tested the involvement of the lateral line mechanosensors. Previous research has strongly suggested that the lateral line is the major sensor controlling shoaling/schooling among the non-visual sensory systems[38]. We employed a method of the pharmacological ablation for the lateral line by applying gentamicin aminoglycoside antibiotics—an established method for ablating the lateral line sensors of teleosts including *A. mexicanus*[39]. Gentamicin treatment inhibited function of the neuromasts—the sensory units of the lateral line—in both surface and cavefish (Supplemental Fig. 5**e-h**)[39,40], and also reduced the duration of nearby interactions in surface fish ($P = 0.0112$, Supplemental Fig 5**a,b.** See Supplemental Table 1). In contrast, there was no detectable change of nearby interactions in cavefish ($P > 0.05$, Supplemental Fig 5**a,b**, Supplemental Table 1), although the neuromasts were similarly ablated to surface fish (Supplemental Fig 5**e-h**). Accordingly, this result suggests that the lateral line sensors contribute to social-like interaction in surface fish.

<u>Turning bias index as a measure of repetitive circling</u>

In human patients with psychiatric disorders, social tendencies seem to be suppressed in those who show stereotypic repetitive motions[3,41,42], that is, the repetitive behavior seems to exclude social behavior. This antagonistic control between social and repetitive behaviors has been extensively reported in mammals[2,3,43] but there are few reports from other vertebrates. In cavefish we observed a highly biased one-way turning, from the ratio of the numbers of left and right turnings occurring per fish every 0.33 second interval, during the 9 min of recording (Fig. 3**a,c,e**). There was; however, no detectable left-right preference in the direction of turning among individual cavefish (Fig. 3**c**). In contrast, surface fish showed symmetrical and balanced turning tendencies (Fig. 3**b,d**). As with cavefish, there was no significant left or right preference in the turning direction in surface fish (Fig. 3**b**). The biased levels and direction preference were consistent for each cavefish individual over at least 10 days, indicating that biased turning is a

stable phenotype (interclass correlation: kappa = 0.92, P = 0.0016, N = 6) (Supplementary Fig. 6)[44]. Using this biased turning index, we analyzed its relationship with nearby interaction.

Effects of familiar and unfamiliar environments on nearby interaction and biased turnings

In humans, stereotypic repetitive motion is enhanced within novel unfamiliar environments[45,46]. In contrast, social tendency is suppressed in these environments[45,46]. We therefore tested if similar behavioral patterns could be observed in the two *Astyanax* morphs by measuring the respective levels of repetitive turning and social-like interaction in response to familiar and unfamiliar environments (Fig. 4**a**).

In the unfamiliar environment, cavefish swam longer distances (Fig. 4**d**, Supplemental Table 1), and both surface fish and cavefish preferred to avoid the periphery of the arena, compared to the familiar environment (Fig. 4**b,c**, Supplemental Table 1). Note that, as described in the above, cavefish did not change their location preference whereas surface fish shifted from the center to the periphery during the 9 min assay in the unfamiliar environment (Fig. 2**b,c**). Cavefish kept showing the high-levels of the repetitive turning behavior in both familiar and unfamiliar environments, where the shift of the level was not detectable (Fig. 4**e**, Supplemental Table 1). Also, surface fish showed detectable repetitive turning in neither the familiar nor unfamiliar environment (Fig. 4**e**, Supplemental Table 1). Assaying social-like interaction, the IID and NND indexes for shoaling could not detect any change in distance among the four focal individual fish between the unfamiliar and familiar environments for either surface or cavefish (Fig. 4**f,g**, Supplemental Table 1). In contrast, implementing the novel metric for nearby interactions on the same videos as used for IID and NND measures, revealed that surface fish and cavefish did indeed respond differently in the unfamiliar environmental (Fig. 4**h,i**; Pop × Env, $P < 0.05$; Supplemental Table 1). In surface fish, the unfamiliar stimulus significantly reduced the variation of the nearby interaction duration although there was no detectable shift in its average (Fig. 4**h**: Levene's test; $F(1,29) = 4.8$, $P = 0.0361$). In contrast, the unfamiliar stimulus strongly reduced the social-like nearby interaction in cavefish ($P < 0.001$; Fig. 4**h,i**: both the duration and bout number; Supplemental Table 1). Of note, in both the familiar and unfamiliar environments, surface fish showed overall significantly more nearby-interaction than cavefish (two-sample Wilcoxon tests (surface fish vs cavefish), $W = 415$, $P = 0.0006$ and $W = 503$, $P = 1.70 \times 10^{-10}$ in the duration of interaction, in the familiar and unfamiliar environments, respectively; $W = 412$, $P = 0.0008$ and $W = 501$, $P = 2.21 \times 10^{-10}$ in the interaction bout number, in the familiar and unfamiliar environments, respectively. See Supplemental Table 1). Thus, surface fish were consistently swimming closer together than cavefish.

It is likely that interacting fish reduce their swimming velocity to swim close to one another. To test this prediction, we measured average swimming velocities: (i) during a 4 sec period directly before the bout of nearby interaction, (ii) during the bout, (iii) a 4 sec period after the bout, and (iv) the rest of the recording time (Supplemental Fig. 7). In the familiar environment, surface fish significantly reduced their swimming velocity during the bout compared to other time periods (Supplemental Fig. 7**a**, Supplemental Table 1). In contrast, in the unfamiliar environment, surface fish didn't change swimming velocity across the recording period, which significantly reduced the variation of swimming velocity ($F(1,156) = 35.6$, $P = 1.50 \times 10^{-8}$ in surface fish). Cavefish did not change in their velocity profile between the familiar and unfamiliar environments ($F(1,294) = 1.1$, $P = 0.2988$ in cavefish), however it is noteworthy that cavefish significantly reduced their swimming velocity during the interaction bout, suggesting that they can respond to their cohorts, regardless of their familiarity to the

233 environment (Supplemental Fig. 7**a**, Supplemental Table 1). Whilst, cavefish, unlike surface fish,
234 did not change their velocity profile under the unfamiliar environment; also, cavefish showed
235 much less nearby interactions in the unfamiliar environment (Supplemental Fig. 7**a** and Fig. 4**h**,**i**;
236 Supplemental Table 1).
237 We next tested whether repetitive turning is associated with the reduced social-like
238 interaction in cavefish. We did not include surface fish in this correlation analysis because
239 surface fish showed almost no variation in the repetitive turning (Fig. 4**e**). By fitting a
240 logarithmic curve, significant correlations between the turning bias vs. the duration, and the
241 turning bias vs. the bout number of the nearby interactions were detected in cavefish in the
242 familiar environment ($R^2 = 0.118$, $P = 0.0308$ and $R^2 = 0.146$, $P = 0.0176$, respectively; Fig.
243 5**a,b**). However, in the unfamiliar environment, where the nearby interactions were reduced in
244 cavefish, no significant correlation between the nearby interactions and the turning bias was
245 detected, possibly due to the significantly reduced variation in the nearby interaction ($P > 0.05$;
246 Fig. 5**c,d**).
247 In summary, an overall negative correlation between social-like nearby interaction and restricted
248 repetitive behavior was discovered in cavefish under non-stressed conditions. This relationship
249 is shared between mammals and cavefish ([45,46] and Personal communication with Dr. Ryan Lee).
250 Particularly, the reduction of nearby interaction under the unfamiliar environment in cavefish is
251 parallel to mammals[45,46], suggesting a possibility of shared neural processing between fish and
252 mammals in behavioral choices.
253
254 <u>Involvement of dopaminergic system in the balance of the levels between nearby interaction and</u>
255 <u>turning bias</u>
256 In mammals, dopaminergic inputs from the ventral tegmental area (VTA) and substantia nigra
257 pars compacta (SNpc) play an important role for behavioral choice in the cortico-striatum
258 system[3,12,13]. Within the striatum, the balance between the D1 direct- and D2 indirect-pathways is
259 crucial, from lamprey to mammals, in balancing behaviors[12,13]. An antipsychiatric drug,
260 aripiprazole, is one of two approved drugs for autism spectrum disorder (ASD) treatment by the
261 U.S. Food and Drug Administration (FDA). Aripiprazole suppresses the phasic stimulation-
262 dependent activity of the indirect D2 pathway but preserves the basal tonic level of the D2
263 indirect pathway due to its partial antagonistic action on the D2 receptor in mammals[33].
264 Aripiprazole is prescribed for the reduction of irritability, perhaps because it maintains the basal
265 inhibitory activities of the D2 pathway and reduces repetitive behaivors[33]. To address whether
266 there is a parallel response to this drug between *A. mexicanus* and humans, we treated surface
267 and cavefish with aripiprazole through a bath application and tested whether the treatment affects
268 social-like nearby interaction and repetitive turning in both surface fish and cavefish.
269 Consistent with our previous findings[26], both surface fish and cavefish reduced the overall
270 swimming distance travelled under the aripiprazole treatment compared to the control or
271 pretreatment levels ($P < 0.01$, Fig. 6**a**, Supplemental Table 1). Under this treatment, cavefish
272 reduced repetitive biased turning ($P < 0.01$, Fig. 6**b**, Supplemental Table 1), and both surface and
273 cavefish increased the social-like nearby interactions ($P < 0.01$, Fig 6**c,d**, Supplemental Table 1;
274 also see the NND and IID results Fig. 6**e,f**).
275 In this aripiprazole treatment, we used the unfamiliar condition where the surface fish reduced
276 the variation of velocity between during and out of the bout (Supplemental Fig. 7**b,c**). The
277 aripiprazole treatment did not change the profile of velocity shifts during the bout (Supplemental
278 Table 1). Still, it significantly reduced the velocity in the aripiprazole treated group compared to

the control group at the post treatment (Wilcoxon test: W = 2,135, P = 1.51×10^{-15} and W = 1,561, P = 9.36×10^{-12} for surface fish and cavefish, respectively. Corrected by Bonferroni's method).

Discussion

In this study, we document that the sighted surface-dwelling form of *Astyanax mexicanus* are able to follow and interact with conspecifics in the dark. We also reveal that so-called 'asocial' cavefish retain a low but detectable level of ability to engage in social-like interactions. The current definition of nearby interaction is close to the one of 'attraction' in zebrafish larvae under the lighted condition[47], and we believe these two indexes indicate the same interactions. However, since the measuring methods are distinct, we used the term 'nearby interaction' in the current study. The location preference within the recording arena and social-like interaction depended on the familiarity to the environment: when fish are in the familiar environment, both surface and cavefish tended to explore the peripheral and cavefish showed more social-like nearby interactions. In contrast, in the unfamiliar environment, surface fish tended to gather at the center of the recording arena and showed less variation in the duration of nearby interaction, while cavefish simply reduced nearby interaction. Cavefish also showed a significant negative correlation between the levels of repetitive turning behavior and social-like interactions under the familiar environment. This quantitative antagonistic control between repetitive behavior and social-like interaction has also been reported in studies involving humans and rodents[43,45,46] but few in teleosts. The treatment of the partial D2 receptor antagonist aripiprazole enhanced social-like interaction and suppressed repetitive turning in cavefish, which is consistent with aripiprazole treatment results in human patients[32,33,48].

The majority of studies on the shoaling and schooling behaviors of teleost fish have been conducted under environmental conditions where visual cues predominate, potentially exaggerating the importance of the visual system in these behaviors[49]. Observations of the maintenance of shoaling behavior in blinded fishes supports the involvement of lateral line mechanosensing in shoaling in addition to the visual sensing in some teleost species, yet, few studies used intact fish to test the involvement of the lateral line[50–52]. Furthermore, many shoaling tests were conducted in somewhat physically constrained conditions (e.g. using a transparent glass divider between stimulus fish and test fish[53] or using a small mesh-cage constraining the swimming area of stimulus fish[34]), where the true extent of mechanosensing in controlling shoaling behavior may be underplayed. In this study, we conducted the first systemic analysis of social-like nearby-interactions in intact fish under free-swimming conditions in the dark.

Our method, measuring nearby interactions via idTracker-based tracking of each individual, a 'nearby interaction' is defined by both a threshold maximum proximity distance between two fish (≦ 6 cm) and a minimum threshold interaction duration (≧ 4 s). This method is sensitive enough to detect changes in nearby interaction under different environments (familiar and unfamiliar; Fig. 4) and drug treatments (Fig. 6 and Supplemental Fig. 5). This method is also applicable to measure a unit of collective behavior among larger groups (i.e., two fish attraction or nearby interaction among ≧ 5 individuals) in other animal species.

As for non-visual collective behavior in *A. mexicanus*, a quantitative trait locus (QTL) for non-visual schooling has been reported where F_2 hybrid fish generated from a pair of a surface fish and a Tinaja cavefish (an independently evolved cave population from that of Pachón cave; Pachón cavefish were used in this study) were used[27]. The QTL for non-visual schooling was detected by using only F_2 which showed negative phototaxis (i.e., having visual capacity) but exhibited a wide variation from non-schooling to schooling. Interestingly, F_2 individuals carrying homozygous cave genotypes at a marker underlying this QTL increased in schooling behavior,

while F_2 with homozygous surface or heterozygous genotypes less schooled[27]. This genetic study suggests that (1) Tinaja cavefish evolved a 'schooling gene' independently from Pachón cavefish and surface fish, or (2) the cavefish collective behavior is regulated by different molecular mechanism from that of surface fish if Tinaja and Pachón cavefish share the 'schooling gene' cave allele. According to our environmental and pharmacological treatments that indicated the reduced but existing social-like response in Pachón cavefish, we expect that the former scenario (1: Tinaja has a distinct allele for schooling) is likely the case. Crossing experiment between Tinaja and Pachón cavefish is necessary to answer this question.

From our results of pharmacological ablation of the lateral line, the nearby interaction of surface fish in the dark is likely regulated by the lateral line mechanosensors (Supplemental Fig. 5). However, this result does not mean that the involvement of other sensory systems, such as tactile and olfactory, can be excluded[54]. Further tests are needed to address which of these sensory systems plays the more crucial role in regulating nearby interaction in fish in the dark.

In the dark, surface fish changed their swimming direction according to the other individuals, as if they sensed and were attracted to others, similar to what has been observed in zebrafish larvae in lighted conditions (Supplemental Fig. 3)[47]. However, surface fish failed to form a clear cluster like 'shoal' in the dark. Instead, surface fish responded to one another, individually. We believe that the failure to form a shoaling cluster is based on the lack of the visual information (see also Supplemental Movie 1 under the light), yet, surface fish keep their collective tendency in the dark by using lateral line sensing.

The nearby interactions detected in this study do not seem to be related to foraging activities or aggression (i.e., two fish come close during expressing aggression or cannibalistic foraging[30]). We carefully assessed damage of the fins and scales, and despite these tissues being good indicators of aggressive encounters, noticeable damage was not detected. Consistent with this observation, both surface fish and cavefish rarely showed startle responses after touching one another. We therefore conclude that *A. mexicanus* rarely attacked one another in our assay system, and that the majority of detected nearby interaction can be characterized as shoaling-like activities.

The degree and type of nearby interaction in the dark recorded in this study appears relevant within the context of each morph's natural habitat. Surface fish have a known nocturnal predator, prawn fish[55], and the adult surface fish form a tighter shoaling cluster at night (personal observation in the lab). These facts imply that the shoaling can be advantageous by simply lowering individual risk among cohorts, so called 'attack dilution effect', and/or increased sensitivity in detecting potential predators by collective-effect of more sensors of multiple individuals[1]. In cavefish, the higher level of nearby interactions and lower repetitive behaviors observed in the familiar lab setting were also observed in their natural habitat, Cueva de El Pachón, Tamaulipas, Mexico (2019, personal observation). Whilst they did not form a recognizable shoal, cavefish responded to conspecifics and tended to follow each other. This social-like tendency could be merely a residual of regressive evolution to a troglobitic niche following their divergence from surface riverine ancestors[56,57]. Alternatively, this weak social-like tendency could remain advantageous in mate-finding, food location or evasion responses to potential dangers within their cave environment.

The young adult surface fish used in this study had reached an appropriate developmental stage to express the shoaling behavior and all of their behaviors were assayed only in the day. Since the shoaling motivation was seen in the night with very limited visual inputs (see above), it is important to investigate the shoaling capacity in the night in future studies.

In the unfamiliar setting, the cavefish did not enhance the social-like interaction and rather increased repetitive turning, suggesting that cavefish lost a strategy to use the social-like interaction to confront the unfamiliar environmental inputs.

Restricted repetitive behaviors are observed in stereotypic movement disorder, autism spectrum disorder in humans, and also observed in mammals kept in a restricted/isolated environments[2,4]. This motor disorder has been thought of as non-functional. However, since these stereotypic behaviors are seen in many vertebrate species as well as normally developing human infants, there may be an advantage to repetitive behaviors. Karl Friston proposed the Free-Energy Principle that explains how the brain processes a given stimulus[58]. In this principle, the brain or the central nervous system is continuously attempting to minimize prediction errors (differences between the environmental signals sampled by an animal's sensory systems, and animal's internal model of the world within the brain). The brain has two strategies to minimize differences between sensory input and the internal model when novel stimuli are encountered: (i) to update the internal model, or (ii) to locomote back to a 'no-novel stimulus environment', to sample the sensory inputs that are already implemented in the current internal model[58]. Stereotypic repetitive circling seems a result of choosing the latter strategy by sampling predictable sensory inputs (strong lateral-line inputs on the one side of the body, and much less inputs from the other side of the body). This method can reduce the prediction errors between the sensory inputs and those predictions from the internal model (e.g., left turning provides the strong right lateral line signals in cavefish). In contrast, surface fish responded to the unfamiliar stimuli by avoiding the peripheral area and homogenizing the nearby interaction duration, yet, did not increase repetitive turning. Therefore, surface fish and cavefish have apparently different strategies to minimize the errors between the sensory inputs and the predicted inputs by the internal model. Given that surface fish appear to acclimatize to novel environments much quicker than cavefish, this suggests that surface fish can update their internal model more frequently than cavefish. This possibility should be tested in *A. mexicanus* in the near future.

Previous studies have indicated that there is an antagonistic relationship between repetitive and social behavior[2,3]. For example, social impairment and repetitive behavior are thought to be linked together, as revealed by the results of oxytocin infusion (reviewed in [3]). In contrast, in an autism study of the ketogenic diet treatment, some of treated patients recovered their social behavior to the normative level, while repetitive behavior was not significantly attenuated[59]. This result suggests that the repetitive behavior can be independent from social impairment. Therefore, the mechanism which controls switching between social and repetitive behaviors is still largely unknown. Many studies of repetitive behavior and social behavior have focused on the cortex-striatal system, where many patients who show repetitive behavior (including Gilles de la Tourette syndrome, obsessive–compulsive disorder, autism spectrum disorders, Parkinson's disease, and Huntington's disease) have abnormal functional and/or histological features diagnosed by PET and fMRI[9]. Importantly, this striatal system, particularly its dopaminergic system, is also suggested to drive social behavior, where the dopamine signal from the ventral tegmental area (VTA) stimulates the D1-dopamine receptor positive-striatal neurons in the nucleus accumbens, driving social behavior in mouse[3,60]. Among vertebrates, the striatum was proposed as the conserved bridging center. Located at the basal ganglia, the striatum integrates (1) higher-level of sensory inputs from the cerebral cortex/dorsal pallium and (2) the information-evaluating dopaminergic input from substantia nigra par compacta (SNc).

The dopaminergic striatal pathway is largely conserved among vertebrate species. This includes the direct and indirect pathways, each associated with a specific type of dopamine receptor— D1 and D2 receptors, respectively. The major function of this striatal dopaminergic system is thought to be to select behavioral modules (eg. mating, foraging module, and aggression modules) according to given sensory inputs and internal states[12]. For example, a fish may ignore or follow to the mechanical signals from cohorts according to the fish's internal state. The treatment of a partial D2-dopamine receptor antagonist, aripiprazole, showed a parallel response between cavefish and human, suggesting a potential shared pathway that controls social-like and repetitive behaviors between teleost and mammals[3,11]. It will be exciting to address the evolutionary and neurophysiological changes in the striatal dopaminergic system between cavefish and surface fish, and how it is associated to the integration of internal model, which Friston proposed, in the future.

Conclusion

In the current study, surface fish still expressed strong social-like interaction in the darkness. We also report that cavefish retain a lower level of the ability for the social-like interactions through 200,000 years of evolution in the dark. Both surface fish and cavefish were capable of responding to familiar and unfamiliar environmental stimuli, and changed social-like interaction accordingly. This was surprising given: (1) surface fish were thought to be unable to express social-like collective behavior in the dark[14,61], and (2) cavefish were thought to be asocial in both light and dark environments[14,61]. Cavefish also showed a quantitatively negative correlation between repetitive turning and nearby interaction, paralleling results documented in humans for the negative interactions between stereotyped behavior and sociality[45,46], which is, as far as we know, the first time that this quantitative pattern has been shown in teleost species. The behavioral shifts caused by the treatment of a partial D2 receptor antagonist also overlapped between cavefish and mammals[32,33,48]. The relevance of these findings reveals cavefish as an evolutionary model for the choice of social and repetitive behavioral modules, as well as a new translational biomedical system for the balance between simple repetitive and complex behaviors.

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Data availability

The video datasets generated during and/or analyzed during the current study are available at university's shared server and will be deposited to Zenodo. All program scripts used in this study is attached in this submission (DEMO_code.zip) and will be deposited in GitHub, shortly.

Materials and Methods

Fish maintenance and rearing in the lab

Astyanax mexicanus surface fish used in this study were laboratory-raised descendants of original collections made in Balmorhea Springs State Park, Texas by Dr. William R. Jeffery. Cavefish were laboratory-raised descendants originally collected from Cueva de El Pachón in Tamaulipas, Mexico by Drs. William R. Jeffery and Richard Borowsky.

Fish (surface fish and Pachón cave populations) were housed in the University of Hawai‘i at Mānoa aquatic facility with temperatures set at 21°C ± 0.5°C for rearing, 24°C ± 0.5°C for behavior experiments, and 25°C ± 0.5°C for breeding [62,63]. Lights were maintained on a 12:12 light:dark cycles [62,63]. For rearing and behavior experiments, light intensity was maintained between 30-100 Lux. Fish were raised to adults and maintained in standard 42 L tanks in a custom-made water-flow tank system. Adult fish were fed a mixed diet to satiation two times daily starting 3 hours after the lights came on (Zeitgeber time 3 or ZT3), and ZT9 (TetraColor Tropical Fish Food Granules and TetraMin Tropical Fish Food Crisps, Tetra, Blacksburg, VA; Jumbo Mysis Shrimp, Hikari Sales U.S.A., Inc., Hayward, CA). All fish in the behavioral experiments were between 4 - 5 cm in standard length and between 10 - 24 months old. All fish care and experimental protocols are approved under IACUC (17-2560) at University of Hawai‘i at Mānoa. All populations of *A. mexicanus* used in this study are readily available from us upon request.

Recording group fish behavior

Prior to experimentation, four fish comprising group-raised siblings were housed in a “home” container (15.6 × 15.6 × 5.7 cm: Ziplock® containers, S. C. Johnson & Son, Inc., Racine, WI, USA) filled with conditioned water (pH 7.0; conductivity 600-800 µS) for 5 days or more. For the unfamiliar environment experiment, all of four fish were gently transferred to a 37.85 L tank (50.8 × 25.4 × 30.5 cm) with a 2 cm depth of water on the stage of a custom-made infrared side- or back-light system in the darkroom or within a custom-built black box (75 × 50 × 155 cm). The Infrared back-light system is composed of a side- or bounce lighting of infrared LED strip (SMD3528 850nm strip: LightingWill, Guang Dong, China) (Fig. 1**a**). Videos were recorded using a USB webcam (LifeCam Studio 1080p HD Webcam, Microsoft, Redmond, WA, USA) fitted with a zoom lens (Zoom 7000, Navitar, Rochester, NY, USA) installed on the top of the custom-built black box. An IR high-pass filter (Optical cast plastic IR long-pass filter, Edmund Optics Worldwide, Barrington, NJ, USA) was placed between the camera and the lens to block visible light. The video was captured at 16-20 frames per second using VirtualDub software (version 1.10.4, http://www.virtualdub.org/) with a vfw codec (https://sourceforge.net/projects/x264vfw/). After 10- or 6-min recording, fish were returned to housing container. For the familiar environment experiment, fish were transferred to 37.85 L tank (50.8 × 25.4 × 30.5 cm) with a 3 cm depth of water and kept for four days to let them acclimate. On the fourth day, the movements of acclimated fish were recorded using the same recording tank setup. After all recording sessions, study fish were visually checked for damage to the fins and for lost scales as an indicator of overt aggression.

Drug treatment

The bath treatment of Aripiprazole was performed as described in Yoshizawa et al [26]. Briefly, the Aripiprazole working solution was prepared by mixing 1.0 mM Aripiprazole stock solution into fish conditioned water to make a 1,000x dilution (Final concentration: 1.0 µM Aripiprazole, 0.1% DMSO in the conditioned water). For the stock solution, Aripiprazole (Selleck, Houston,

511 TX) was dissolved in 100 % of Dimethyl sulfoxide (DMSO, MilliporeSigma). For the control,
512 0.1 % of DMSO in the conditioned water was used. The four fish in each home container were
513 treated with either the Aripiprazole working solution or the 0.1 % DMSO control solution
514 overnight (~16 hrs). In the next morning, group fish behaviors in the 37.85L tank was recorded
515 as described in the "Recording group fish behavior" section.
516
517 Tracking
518 The first minute of each original video was discarded since this time interval contains the
519 moment that four fish were released from their home container and agitating the water surface,
520 disrupting observation. Before tracking each fish's position by idTracker software[35], the
521 background visual artefacts were subtracted by a custom made ImageJ macro script. Briefly, if a
522 video was recorded with white background, each frame image was adjusted by the "Add"
523 operation of the 'Image Calculator' function with an inverted median z-stacked image of 4,000
524 frames calculated by 'Z Project...' and 'Invert' functions of ImageJ [64]. If a video has a black
525 background, each frame image was adjusted by "Subtract" operation of the 'Image Calculater'
526 with a median z-stacked image of 4,000 frames calculated with 'Z Project…' function of ImageJ
527 (no 'Invert'). Then, the frame images' contrasts were adjusted according to each video. Each
528 processed video was saved as the AVI format by using the jpeg compression in ImageJ. Using
529 these background-subtracted videos, the X-Y coordinate of each fish was extracted by tracking
530 each fish's ID under the ID-detection algorithm of idTracker [35]. Parameters of idTracker were
531 usually set as Number of individuals as 4, Intensity threshold as 0.75-0.89, Minimum size as 30-
532 50, and "Select region by Polygon" function, and check the "Remove background" option. We
533 aimed for a successful tracking frame ratio of above 92 % by changing these parameters. Even
534 with a high ratio of successful tracking frames (e.g., 99%), the outputs of idTracker can have
535 errors in IDs by switching the IDs between different fish. These errors were manually surveyed
536 frame-by-frame by using idPlayer, an accompanied software of idTracker [35]. The corrected IDs
537 were re-assigned by a custom python script. If a fish had periods without ID during the analyzing
538 videos, the estimated X-Y coordinates were calculated in those durations by averaging velocity
539 and swimming direction between the X-Y coordinates before and after the missing ID period.
540 Tracking results were used for the repetitive turning and nearby interaction analyses (see below).
541
542 Biased repetitive turning analyses
543 Changes of the angles in swimming directions in every 5 frame window (i.e., every 0.33 s) were
544 calculated as radian. When the X-Y coordinates of the three-time points (t0, t1, and t2) were as
545 (x0, y0), (x1, y1) and (x2, y2), the swimming angle ɵ between two vectors (x1-x0, y1-y0) and
546 (x2-x1, y2-y1) is: ɵ=arctan(((x1-x2)*(y0-y1)-(y1-y2)*(x0-x1)) / ((x1-x2)* (x0-x1) + (y1-y2)*
547 (y0-y1)) (the cross product of two vectors). The numbers of frames that have positive radian
548 values (N1), representing anticlockwise turns, and negative radian values (N2), representing
549 clockwise turns, were totaled. The repetitive turning index was then calculated as Log 10
550 (N1/N2). As fish did not show any preference in the direction of turning (Fig. 3**b,c**, and
551 Supplemental Fig. 6), the repetitive turning index was expressed as the absolute values of Log 10
552 (N1/N2).
553
554 Recording of single fish repetitive behavior
555 Repetitive behavior assays were carried out on single fish, each individual fish being, in turn,
556 transferred from the home container to a 25.5 x 38.0 cm Pyrex Grass Baking Dish filled with ~4

557 cm depth of water by using a fine brine shrimp net. Two of these dish arenas were lit by a
558 custom-made infrared light box from underneath (a 58.4 × 41.2 × 15.2 cm opaque storage box
559 equipped with infrared LED strip SMD3528 850nm strip: LightingWill). Videos for this
560 behavioral assay were recorded with the same camera setup as the familiarity experiment, except
561 that a c-mount zoom lens (macro 1.8/12.5-75mm C-mount zoom lens, Toyo Lens) was fitted.
562 A repeat behavioral assay using the same fish was recorded 10 days after the first. To identify
563 individuals, each fish was labeled with a physical implanted dye (Visible Implant Elastomer
564 (VIE) tags, Northwest Marine Technology, Inc. Anacortes, WA, USA) whilst anesthetized in a
565 bath treatment of the ice-cold 0.2 mg/mL of MS-222 (MilliporeSigma, St. Louis, MO), directly
566 after the first recording. After 10 days, the fish behavior was recorded using the same procedure.
567
568 Social-like nearby interaction analysis
569 The inter-individual distances (IID) and nearest-neighbor distances (NND) were calculated
570 according to Partridge et al [65]. For IID, the average of the distances between the four fish being
571 assayed (6 distances) was calculated per video frame. Then, the mean and standard error of
572 means (s.e.m.) were calculated by using one 4-fish group as N = 1 (for example, N = 12 in each
573 of the surface fish and cavefish experimental groups in Fig. 2**e**). For NND, the average of 4
574 nearest distances between a focal fish and the nearest individual per video frame, then the mean
575 and s.e.m. were calculated as in IID.
576 The permutation test of NND and IID was performed by random selection of one individual from
577 each group (one of four fish) and forming a fake 4-fish group, where none of each individual
578 physically swam in the same arena. We then calculated nearest neighbor distances and inter-
579 individual distances among these 4 fish by using their 9 min X-Y coordinates (> 8,600 frames).
580 The fake 4-fish group were randomly and exclusively formed 1,000 times in each surface fish
581 and cavefish.
582
583 A 'nearby interaction bout' was defined by a minimum interaction duration threshold of four
584 seconds and a maximum physical distance threshold of 6 cm distance, between the two fish. As a
585 note, the definition of nearby interaction is close to the one of 'attraction' by Hinz et al., where
586 attraction was expressed as the probability that the focal turns to the side where the other fish is
587 located[47]. The current nearby interaction is to measure the preference to stay or follow one
588 another without considering the turning direction. We use a term 'nearby interaction' in this
589 study to avoid confusion.
590 Nearby interactions were extracted from each fish's X-Y coordinates by a custom-made Matlab
591 script. The bout duration was adjusted by subtracting the passing-by duration (PbDur) (see
592 following and Supplemental Information, Supplemental Fig. 2). The PbDur is defined as the
593 average duration of a fish passing-by another fish where these fish do not express the nearby
594 interaction. The purpose of subtracting the PbDur from the bout durations is to remove the period
595 from the nearby interaction events that resulted from just physical proximity rather than the
596 actual social-like interaction. PbDur was calculated as: PbDur = 6 cm /V, where V is an average
597 velocity of four fish in the same group during 9 min or 5 min periods (Supplemental Information;
598 Supplemental Fig. 2). If V is low, PbDur becomes longer; therefore, it excludes 'by-chance'
599 events where two fish may remain within 6 cm of one another while not expressing attraction
600 toward one another. The swimming velocities of 'during the bout,' '4 seconds before bout
601 commencement,' and '4sec directly following the bout' were also extracted and calculated using
602 a custom made Matlab script.

603
604 Statistical analysis
605 For statistical comparisons, we performed tests including student's t-tests and Wilcoxon's signed
606 rank tests, and two-way or three-way generalized linear model analyses to compare between
607 surface vs cavefish, treated and non-treated, and pre vs post treatment. We calculated Akaike's
608 Information Criterion for each linear and generalized linear models and chose the model with the
609 lowest AIC. The post-hoc Holm correction was used for understanding which contrasts were
610 significant [66]. Above calculations were conducted using R version 3.6.3 software (packages of
611 car, lme4, and lmerTest) [67–69] and all statistical scores are available in Supplemental Table 1,
612 figure legends or the body text.
613

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Figures

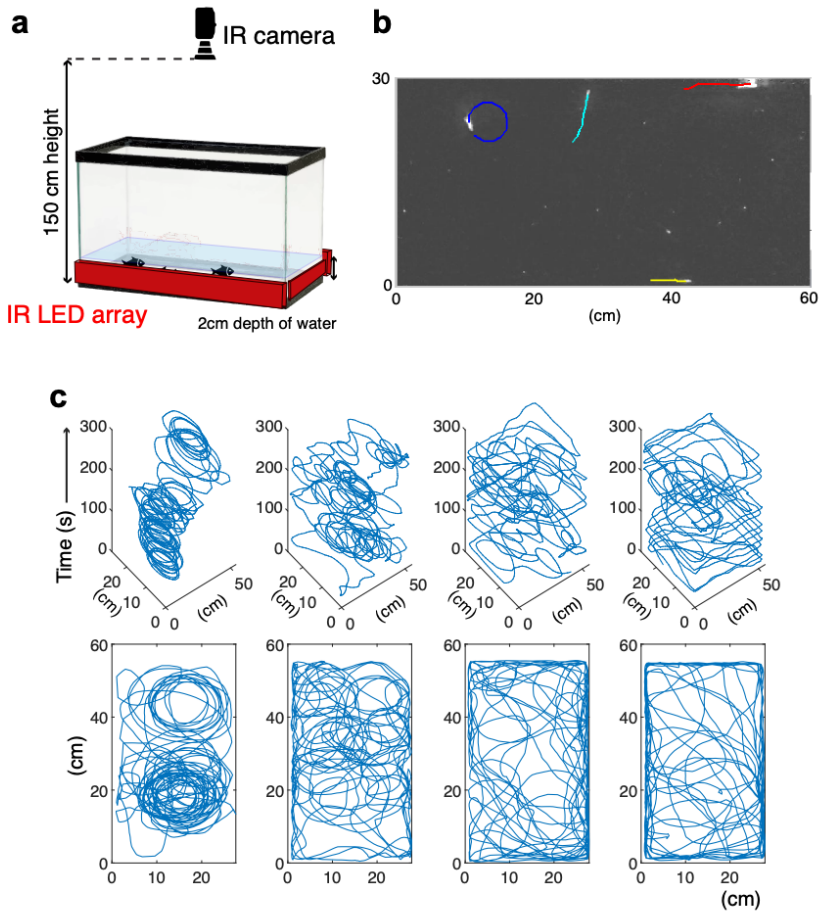

Figure 1. Schematics of the behavior assay.
a Schematics of the assay system. The recording tank was side-lighted by IR LED array. The IR camera was set at the top of the tank and recorded fish movement. **b** One example frame of recorded video. Colored lines represent trajectories of individual fish detected by idTracker software. **c** Example trajectories of each fish from a 5 min recording. Each fish shape's ID was detected by idTracker, and traced its X-Y coordinates across the entire recording time. The top row (4 panels) shows the X-Y-Time traces of each of four fish. The bottom row represents the X-Y traces by stacking the time series of the each representing top row panel.

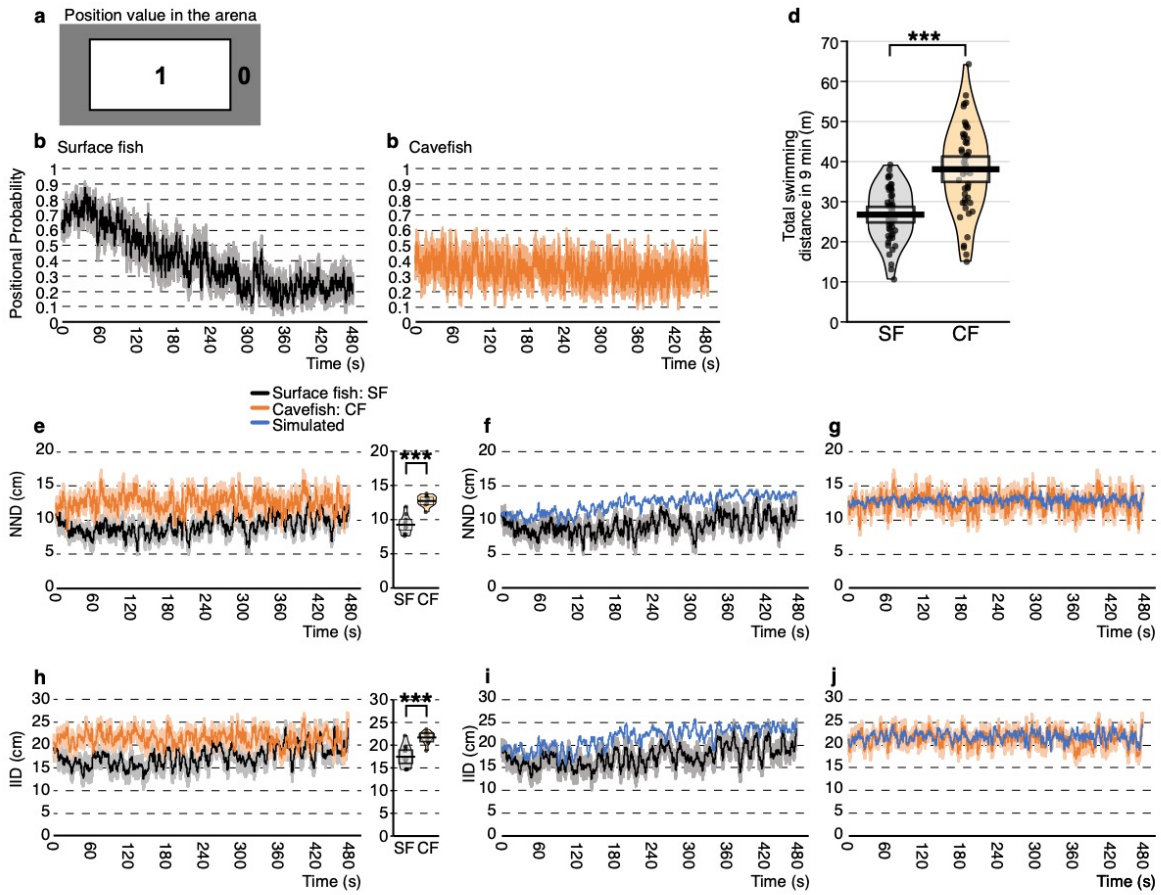

Figure 2. Distinct responses to the unfamiliar environment between surface fish and cavefish.

a The position index used in this study to characterize location preference (top view of the recording arena). Region 1 represents the center and region 0 represents the peripheral area of the recording. **b**, **c** The positional probability of four fish for the region 1 in every frame (each 0.33 sec). Surface fish are shown in **b** and cavefish are in **c** (N = 48; 12 groups of four). The means ± standard error of the mean (s.e.m.) at each frame were shown). **d** Pirate plots depicting total swimming distance of 48 surface fish and 48 cavefish during 9 minute behavioral assays. The top and bottom of the white bands in each of the surface fish (SF) and cavefish (CF) plots represent 75 and 25 percentiles, respectively. The thick bars at the middle of data points represent median of data, and thickness of bean pod represent data density. Measurements of nearest neighbor distance (NND) (**e**, **f**, **g**) and inter individual distance (IID) (**h**, **i**, **j**) in each recording frame (each 0.0625 sec). Black lines represent surface fish (SF) and orange lines are for cavefish (CF). Blue lines represent the mean of the simulated NND or IID calculated by a thousand of the permutated X-Y coordinates of individuals. The means ± s.e.m. were shown (N = 48 from 12 groups of four surface fish or cavefish). Right panels of **e**, **h** are the summary pirates plots of the left panels. ***: $P < 0.001$. All statistics scores are available in Supplemental Table 1.

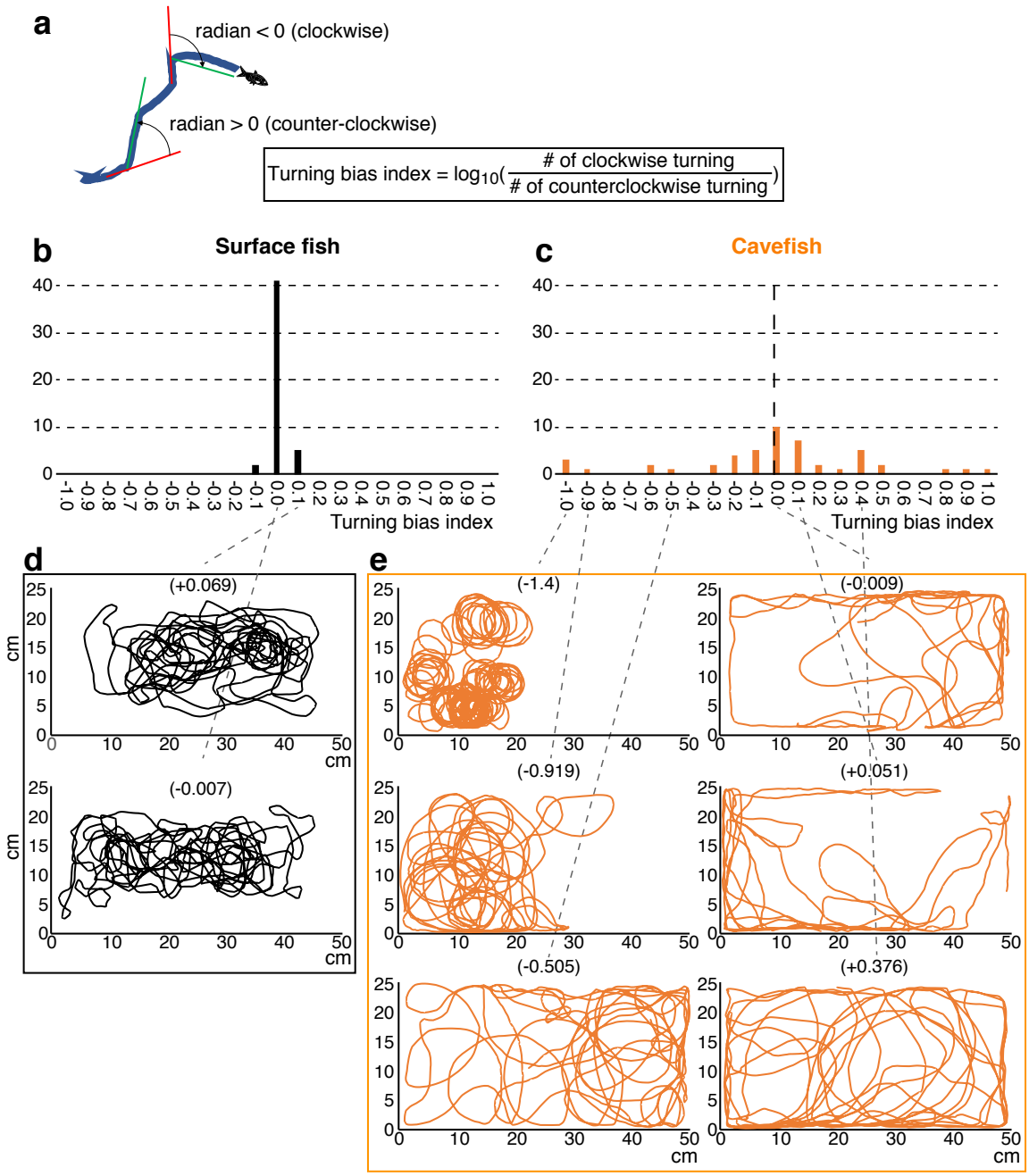

Figure 3. Turning bias index showed strong biased turning in cavefish
a Diagram showing calculation formula for turning bias index. The changes traveling directions were calculated every 5 frames (every 0.33 sec) across all trajectory and were expressed as radian. The positive radian value represents left (counterclockwise) turning, and *vice versa*. Then the $\log_{10}$ of the ratio between the numbers of clockwise and counterclockwise turnings was used as the turning bias index. **b, c** Histograms of the turning bias index for 48 surface fish (**b**) and 48 cavefish (**c**) in the unfamiliar environment (see the main text). **d, e** Swimming trajectories representing a range of turning bias indexes in surface fish (**d**) and cavefish (**e**)

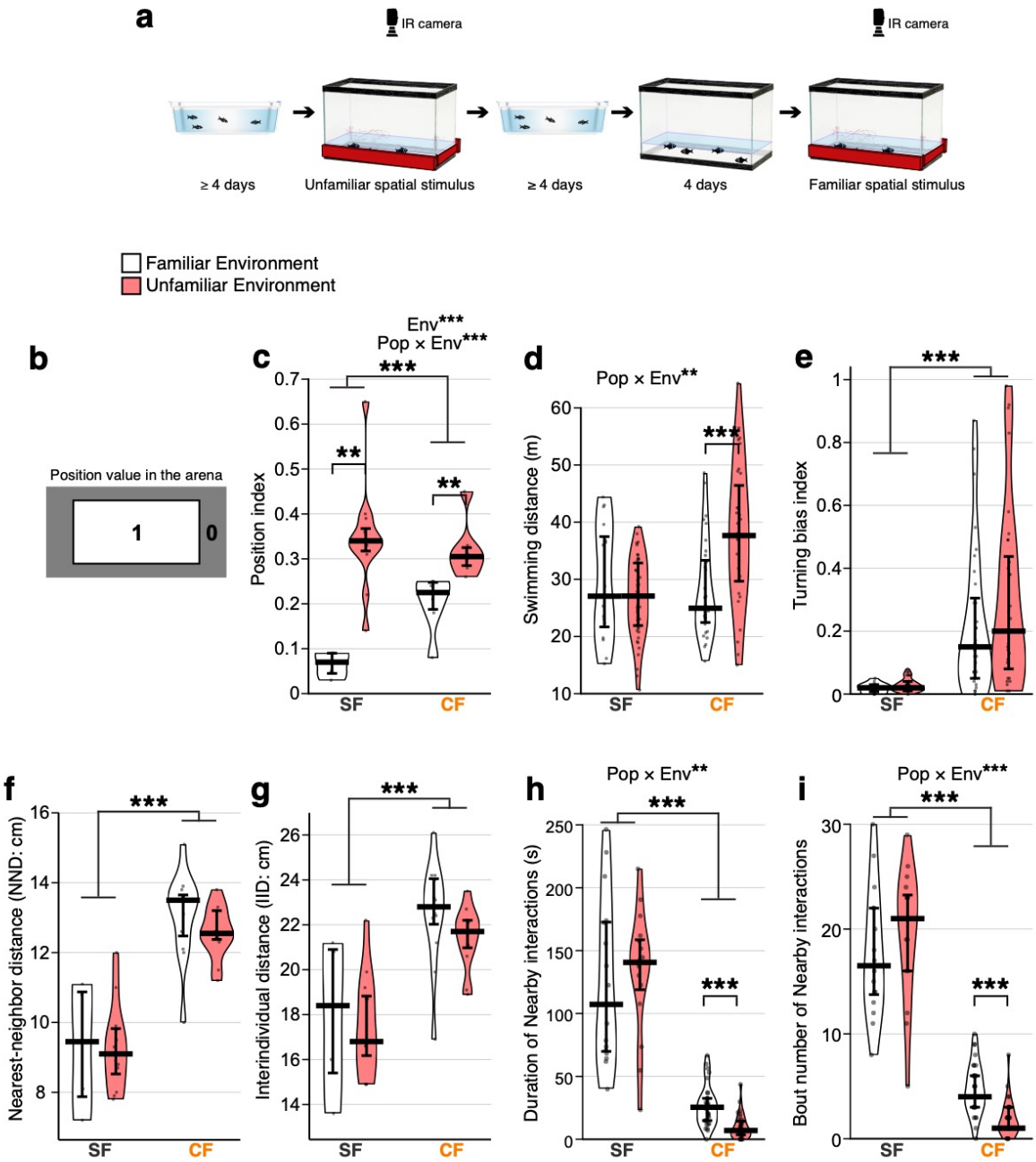

Figure 4. Cavefish reduced social-like nearby interactions under the unfamiliar environment comparing those under the familiar environment.

a Schematics of experimental procedure. Fish were acclimated in the home tray for more than 4 days and were transferred to the recording tank in this unfamiliar environment. After the recording, fish were return to the home tray and kept for more than 4 days. Then, fish were returned to the recording tank and were acclimated to the recording tank for 4 days. After the acclimation, the IR LED array was set to illuminate fish from the side, and fish movements were recorded under this familiar(ized) environment. **b** a diagram explains the Position index. **c** Plots for Positional preference of the fish for region 1. Three bars in the bean pods represent 25 (bottom bar), median (middle) and 75 (top) percentiles of data. The generalized linear mixed-effects model indicated that environment (Env: familiar vs unfamiliar) and the interaction of Env

× population (Pop: SF vs CF) were significant. See Supplemental Table 1. **d** Swimming distance was significantly increased in the unfamiliar environment in cavefish. **e, f, g** Turning bias index (**e**), Nearest neighbor distance (NND) (**f**), and Interindividual distance (IID) (**g**) showed no significant change between the familiar and unfamiliar environments but differed between SF and CF. **h, i** The duration (**h**) and the bout number of nearby interactions (**i**) showed the significant difference in Pop, and the significant interaction in Env × Pop via generalized linear mixed-effects model (Supplemental Table 1). Post-hoc test indicated the significant reduction of nearby interaction in the unfamiliar environment comparing with the familiar environment. SF: surface fish, CF: cavefish. **: $P < 0.01$, ***: $P < 0.001$. All statistics scores are available in Supplemental Table 1.

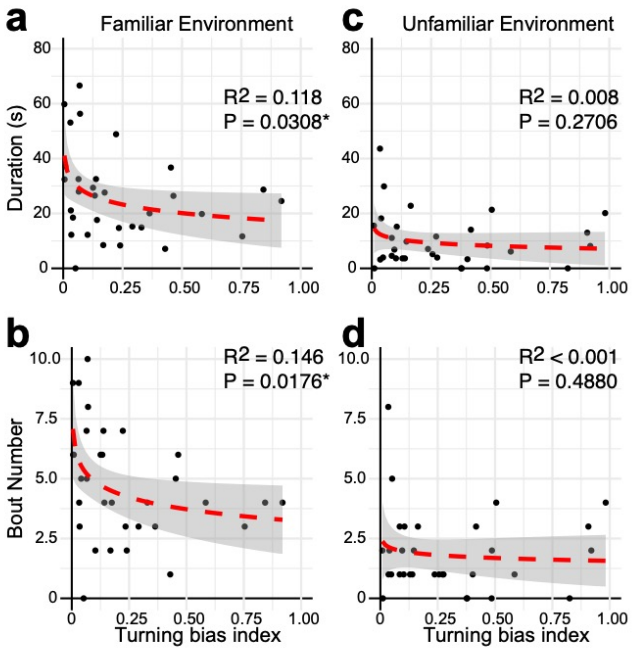

Figure 5. The relationship between the repetitive turning behavior and nearby social behavior in cavefish.

a, b In the familiar environment, the logarithmic transformed turning bias index and nearby interaction are significantly negatively correlated. $F(1,30) = 5.1$, adjusted $R^2 = 0.1178$, $P = 0.0308$ for the turning bias index against the duration of nearby interactions (**a**); $F(1,30) = 6.3$, adjusted $R^2 = 0.1464$, $P = 0.0176$ for the turning bias index against the bout number of nearby interactions (**b**). **c, d** In contrast, in the unfamiliar environment, the nearby interactions were reduced and correlation was not detected. $F(1,30) = 1.3$, adjusted $R^2 = 0.0083$, $P = 0.2706$ for the turning bias index against the duration of nearby interactions (**c**); $F(1,30) = 0.5$, adjusted $R^2 = 0.0162$, $P = 0.4880$ for the turning bias index against the bout number of nearby interactions (**d**). *: $P < 0.05$. Red dashed lines: regression lines with the logarithmic transformation on x-axis. Gray shades represent 95 % confidence intervals of the regression lines. (N = 32 from 8 groups).

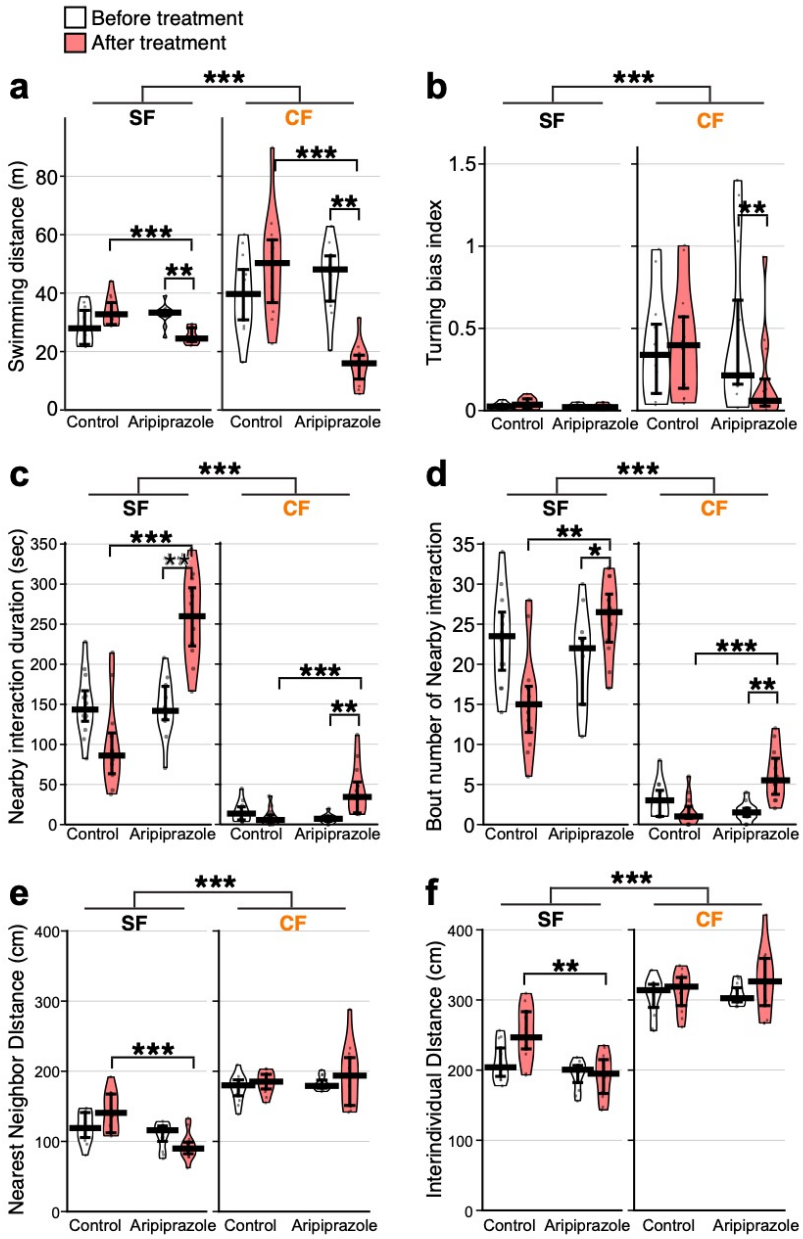

Figure 6. The partial agonist for the D2 receptor, aripiprazole, enhanced nearby interactions and reduced turning bias in cavefish.

The pretreatment behaviors were recorded more than four days before the aripiprazole treatment. Aripiprazole and the control DMSO were treated for ~16 hours before the posttreatment recording under the unfamiliar environment. **a** Total swimming distances before (blank) and after (red shaded) the treatment, between the aripiprazole and control treatments, and between surface fish and cavefish. Both surface fish and cavefish significantly reduced swimming distance after the aripiprazole treatment (N = 12 for each of surface fish and cavefish). **b** Turning bias index indicated that cavefish significantly reduced biased turning after the aripiprazole treatment (N = 12 for each of surface fish and cavefish). **c, d** The duration (**c**) and the bout number of nearby interactions (**d**) showed the significant difference in Pop (surface fish and cavefish), and the significant interaction in PrePost (pre and post treatments) × Exp (control

875 and aripiprazole treatments) × Pop via generalized linear mixed-effects model (Supplemental
876 Table 1). Post-hoc test indicated the significant increase of nearby interaction in the aripiprazole
877 treated groups of surface fish and cavefish. N =12 for each of surface fish and cavefish. **e, f**
878 Nearest neighbor distance (NND) (**e**), and Interindividual distance (IID) (**f**) showed no detectable
879 change in cavefish but were significantly shortened in surface fish under the aripiprazole
880 treatment. N =12 for each of surface fish and cavefish.
881 All statistics scores are available in Supplemental Table 1
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