Collective Decision-making in Response to Visual Looming Stimuli in Medaka Fish

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Abstract

Various social animals synchronously make behavioral choices among alternatives (i.e., collective decision-making) to efficiently migrate, forage, or escape from predators. Here, we demonstrated that medaka fish, *Oryzias latipes,* make collective decisions in response to visual looming stimuli (LS), a simulated predator attack. The group-level response to the LS could be classified into 2 patterns: freezing synchronously or not freezing, indicating that medaka selected their response to the LS from 2 alternative choices. Furthermore, the behavioral response to the LS persisted over multiple behavioral trials for 3 days, indicating that each group made a consistent decision to freeze or not to freeze. Finally, we showed that the group response could be predicted by the maximum swimming speed among individuals during the LS presentation. If a group included at least 1 individual that responded to the LS with high-speed movement (>7.4 cm/s), the group tended to freeze synchronously in response to the LS. If no member of the group was moving at high-speed during the LS presentation, the group tended to continue moving after the LS presentation. Our findings suggest that the presence of 1 or more individual medaka with a strong fear response in a group determines the group-level decision of how to respond to a threatening stimulus.

Introduction

Group-living animals, including humans, make choices based on the decisions of others to behave synchronously during predator escape/migration (i.e., collective decision-making). In collective decision-making during escape from a predator, animals are believed to exhibit a certain behavior depending on the number of other members of the group exhibiting that behavior, which is known as a quorum response (Sumpter & Pratt, 2009). For example, three-spine stickleback individuals in a small fish group tend to follow a certain course to escape from a predator depending on a threshold number of individuals already taking that course in the presence of the predator (Ward et al., 2008). In human groups, a simulated predator detection task showed that individuals tend to detect and decide to flee when they see a threshold number of escapees. When at least a half the members of an entire group detect a predator, the entire group decides to escape (Clément et al., 2015).

Here we used medaka fish, *Oryzias latipes*, to investigate how groups detect the approach of a predator and make decisions. Medaka fish are small teleost fish that exhibit coordinated and cohesive movement (Imada et al., 2010; Ochiai et al., 2013) and have the ability to recognize familiar individuals (Okuyama et al., 2014; Wang & Takeuchi, 2017). In the present study, small fish groups (6 fish each) were exposed to a predator-informed looming stimuli (LS) to trigger defensive behaviors. In most cases, we observed that medaka groups made 1 of 2 alternative collective decisions: to freeze

or not to freeze after presentation of the LS. Their collective choice tended to persist over time (15 times/3 days). Based on the findings of the present study, we propose that at least 1 fish with high responsiveness to presentation of the LS determines the group-level decision to freeze or not to freeze.

Methods

Animal

Medaka fish *(Oryzias latipes*; fading strain) were obtained from the National BioResource Project Medaka (Takeuchi & Manabe, 1984). All fish were hatched and bred in our laboratory. Medaka fish were maintained in groups in plastic aquariums (22.6 cm length × 14.6 cm width × 14.5 cm height, Sanko) under controlled temperature (26 ± 1°C) and light (14 h: 10 h light: dark) conditions. Every day, fish were fed brine shrimp between 12:00 to 13:00 and solid bait Otohime β-2 (Marubeni Nissin Feed, Tokyo, Japan) at least once around 10:00 and 17:00 on weekdays. A month after hatching, 6 individuals were randomly allocated to each group irrespective of sex, and 10 groups were formed. The 6-fish groups were maintained for a month (body length ~2 cm, 2-months-old) before beginning the experiment.

LS presentation

The LS, a simulated predator attack, was created using the "Zoom" animation effect in Microsoft PowerPoint, that covered a tank up to 14 cm wide in 6 s and gradually became darker and larger in 10 s (see Supplementary Fig. S1a). The LS was presented on a liquid crystal display (EXLDH271DB, I-ODATA) placed on the side of the tank (see Supplementary Fig. S1a). At 1 h after transferring the tank from the breeding system to the experimental setup (see Supplementary Fig. S1b), the LS was presented 5 times at 30-min intervals, for 3 consecutive days (see Supplementary Fig. S1c). Videos (4k, 30 frames per second [fps]) were recorded from above using a camera (M80 Air action camera, Apexcam). The videos were recorded a total of 30 s: before LS (10 s), during LS (10 s), and after LS (10 s), and used for behavioral analysis.

Software for data preprocessing and analysis

The video file (mov file) was replaced by a jpeg file with 1 fps using FFMPEG v4.2.2, and the coordinates were obtained by labeling the medaka heads using the UMA tracker algorithm (pochi-pochi) (Yamanaka & Takeuchi, 2018). These data (converted coordinates and speed) were pre-processed in a Matlab2020b environment. Data analysis was performed in a python v3.7.7 environment, numpy v1.19.5 for matrix computation, pandas v1.2.3 for spreadsheet computation, seaborn v0.19.1 and matplotlib v3.3.4 for chart creation. Scipy v1.6.0 was used for hierarchical clustering and sklearn v0.24.1. was used to calculate the silhouette coefficient. To clarify whether or not freezing individuals were consistent by group, we used PypeR v1.1.2 and the R v3.6.0 package

(lme4 v1.1.27.1 and performance v0.7.3).

Searching parameters during the LS presentation that could predict group responses after the LS presentation ended

We searched parameters by logistic regression models using the speed (min, max, mean, and median value in each trial) with pystan v2.19.1.1 and selected the best fit model by the Watanabe-Akaike Information Criterion (WAIC)(Watanabe, 2010) and area under the curve (AUC).

$$\mathrm{x}[i] = \mathrm{alpha} + \mathrm{beta} \cdot \mathrm{V}_i \qquad i = 1 \ldots \mathrm{I} \qquad (1)$$

$$\mathrm{theta}[i] = \mathrm{inv_logit}(\mathrm{x}_i) \qquad i = 1 \ldots \mathrm{I} \qquad (2)$$

$$\mathrm{Y}[i] \sim \mathrm{Bernoulli}(\mathrm{theta}_i) \qquad i = 1 \ldots \mathrm{I} \qquad (3)$$

$$\text{Inflection point} = \text{-alpha / beta} \qquad (4)$$

I is the number of data (I = 97) excluding the data of 2 to 5 freezing individuals, and i is the index of the number of data. V in equation (1) represents the index of speed, where the maximum, minimum, mean, and median values of speed before and during the LS presentation per trial in each group were substituted. Also, alpha is the intercept, and beta is the variable of V. Equation (2) was converted into theta for the number of freezing individuals 0 and 6 as 0 and 1, respectively. Equation (3) shows that the reaction pattern Y at the group level follows a Bernoulli distribution. The estimation was then carried out by a Markov chain Monte Carlo (number of iterations = 2000, number of chains = 4, number of warm-ups = 1000), which generates random numbers. Equation (4) shows the inflection point of the sigmoid curve.

Results

Classification of individual responses to the LS

We carried out a total of 15 behavioral trials for 3 days (5 times/day) using 10 groups comprising 6 fish each. First, we extracted coordinate data of individual fish in each trial using 30-s movies (1 frame/s): before LS (10 s), during LS (10 s), and after LS (10 s). To visualize the response patterns in each trial after the LS presentation, we logarithmically transformed the average speed and standard deviation of individual responses in each trial, which suggested 2 clusters (see Fig. 1a); a cluster of fish that continued moving and a cluster of fish that suspended moving (see Supplementary Fig. S2a and S2b). Therefore, we used the Ward hierarchical clustering method to estimate how many clusters could exist in the scatter plot. Next, the optimal number of clusters, which is the highest intra-cluster cohesion, and the highest inter-cluster divergence was selected by the silhouette coefficient. The closer the silhouette coefficient is to 1, the higher the intra-cluster cohesion and the inter-cluster divergence. The result revealed that the silhouette coefficient closest to 1 was 0.6976, when the number of clusters was 2 (see Table 1). Therefore, the number of clusters could be classified in hierarchical clustering as

2 (see Fig. 1b). From the logistically transformed values after the LS presentation ended, the mean and standard deviation speed values of cluster 0 and cluster 1 tended to be low and high, respectively (see Fig. 1b). We confirmed this finding by drawing line graphs of the speed of each individual after the LS labeled by the cluster number (0 or 1), showing that there were 2 response patterns (see Supplementary Fig. S2c). Accordingly, we defined individual response patterns belonging to cluster 0 and 1 as freezing and not freezing, respectively.

Group responses after LS

To classify group response patterns after the LS, we counted the number of freezing individuals in each group and classified them by hierarchical clustering (see Fig. 2), then reviewed the optimal number of clusters by the silhouette coefficient. The result showed that the silhouette coefficient closest to 1 was 0.6335, when the number of clusters was 2 (see Table. 2). To confirm whether or not the response patterns by group were consistent, we estimated the intraclass correlation coefficient (ICC) using generalized liner-mixed models (GLMMs), in which freezing individuals are the objective variable following a binomial distribution and groups are the random factor, and trials, days, and interaction term are the fixed factors (see Table 3). The adjusted ICC of the GLMM showed that group response patterns were consistent for 3 days (adjusted ICC = 0.703).

Possible prediction of group responses after the LS presentation ended based on the maximum speed among individuals during the LS presentation

We focused on 97 of 150 cases in which all individuals did nor did not exhibit freezing after the LS. Also, we searched some behavioral parameters that could predict a synchronous response after the LS presentation ended, using logistic regression models from the speed (min, max, mean, and median value) by the group “before LS” and “during LS” as the explanatory variable. Therefore, we selected the best fit model by the WAIC. The result showed that the Vmax of at least 1 individual in the group during the LS was the smallest WAIC (WAIC = 41.45, see Table. 4). Eventually, the threshold of the sigmoid curve was calculated from result of estimation (inflection point = 7.412 cm/s) (see Fig. 3). The result suggested that the group chooses to freeze or not to freeze based on the highest swimming speed of 1 or more individuals in the group in response to the LS presentation; i.e., when at least 1 individual in the tank moves at a speed greater than ~7.4 cm/s during the LS presentation, the entire group decides to freeze after the LS presentation.

Discussion

The present findings demonstrated that the behavior of each individual per trial after the LS presentation ended could be classified into 2 patterns: freezing or not freezing, and group-level response patterns were consistently polarized into 1 of the 2 patterns. Our logistic regression models

suggested that at least 1 fish with high responsiveness to the LS presentation could determine group-level decision-making to freeze or not to freeze. Previous studies of the three-spine stickleback (Ward et al., 2008) and humans (Clément et al., 2015) indicated that quorum responses are important for collective decisions on how to direct an escape from a predator. Here, however, we showed that the behavioral mechanism underlying the collective response of medaka fish is quite different from that of so-called "quorum responses". This mechanism can reduce the latency to the collective choice, which may be useful in case of an emergency, like a rapidly approaching predator. In addition, we demonstrated that the collective choice tended to persist over time (15 times/ 3 days), suggesting that the presence of an individual with high responsiveness to the LS presentation could determine the collective decision-making. Recent attention has been paid to individual heterogeneity (animal personality) as an essential intermediary mechanism with collective decision-making regarding migration and foraging behavior (Jolles et al., 2020). Animal personality can be defined as behavioral differences among individuals of the same species that are consistent across time and different contexts. For example, bolder (active) individuals tend to belong to the top of the hierarchy in flocks of pigeons and are more influential in the collective choice of a direction for migration than shy (non-active) individuals (Sasaki et al., 2018). Our findings imply that the presence of 1 and more individuals with a certain animal personality (strong fear response) in a group could determine group-level decision-making.

What is a possible neural mechanism underlying the collective choice in medaka fish? Visual input from a fish moving at a high speed (>7.4 cm/s) might cause synchronous freezing of a 6-fish group. Thus, it raises a possibility that subpopulations of neurons, integrating visual inputs from the LS and high-speed movement during the LS presentation, could induce freezing after LS presentation ends in the medaka brain. Like zebrafish, medaka fish are an established model in the field of molecular genetics and state-of-the-art molecular-genetic methods are available. Several efficient methods for targeted gene knock-in using the CRISPR/Cas9 system have been established in medaka fish (Ansai & Kinoshita, 2014; Seleit et al., 2021; Watakabe et al., 2018), where a reporter gene of interest can mimic the expression of endogenous target genes in transgenic strains. Such advanced molecular genetic methods will allow us to identify subpopulations of neurons that mediate animal collective behaviors. The present study is an important first step toward unveiling the neural mechanism, for which our current understanding is very poor.

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Ethics

The work in this paper was conducted using protocols specifically approved by the Animal Care and Use Committee of Tohoku University.

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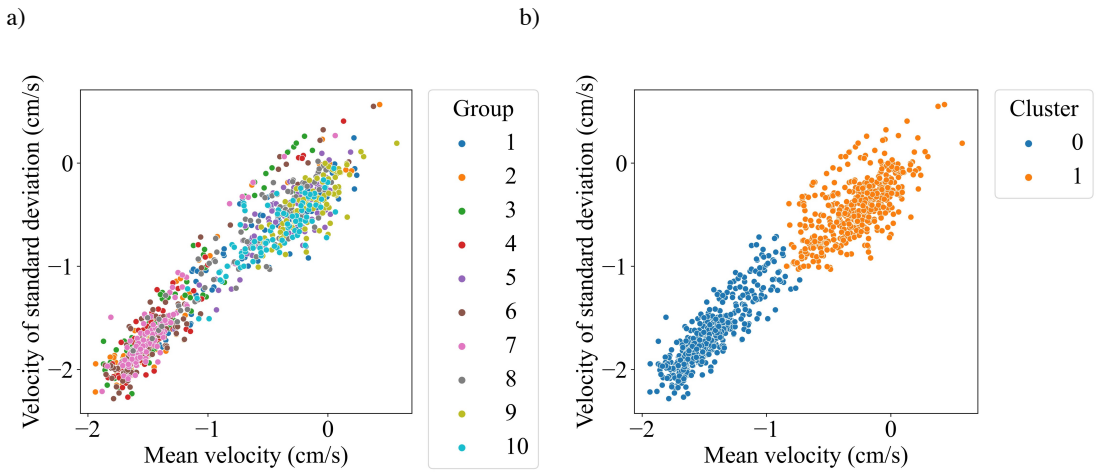

Fig. 1 Scatter plot of log-transformed speed (base 10) of the mean and standard deviation of each individual per trial post-LS. Horizontal axis and vertical axis indicate the mean speed and standard deviation, respectively. Each point indicates each individual per trial. a) Group ID is indicated by color and number. b) Cluster ID by the Ward hierarchical clustering method is indicated by color and number.

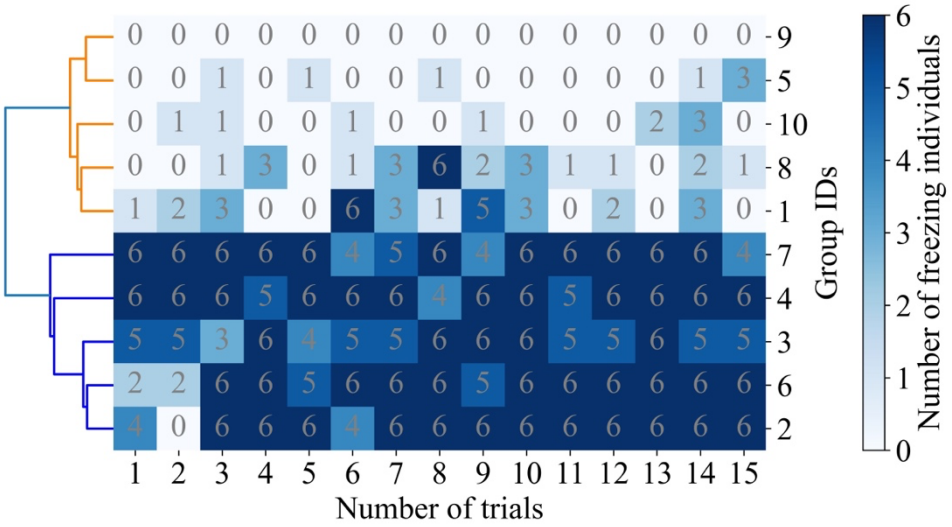

Fig. 2 Heatmap of freezing individuals in each group per trial post-LS. Horizontal axis and vertical axis indicate trial and group, respectively. Colors in the heatmap correspond to the number of freezing individuals. Groups were classified by the Ward hierarchical clustering method.

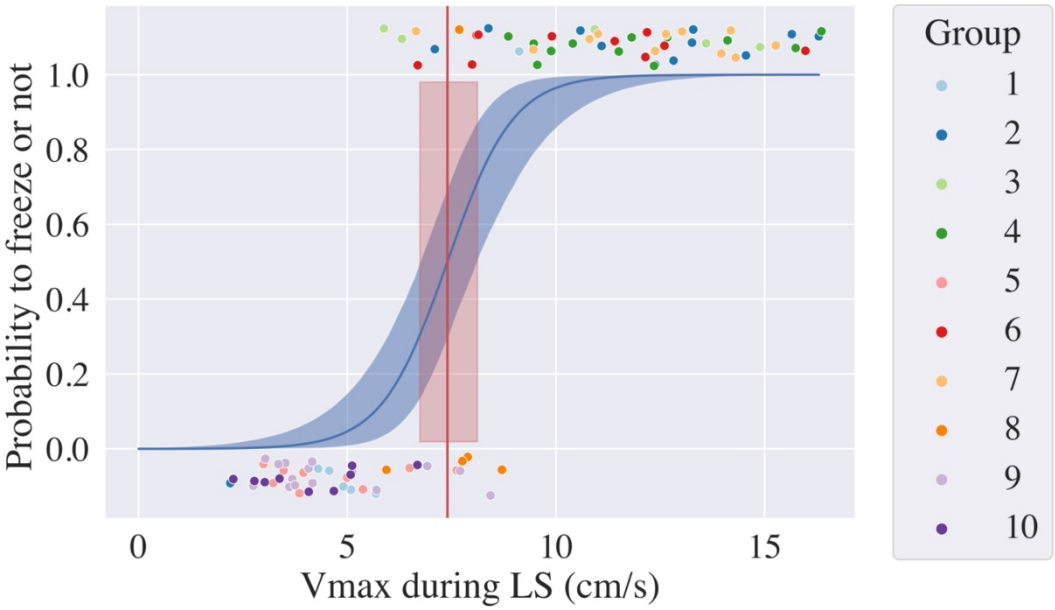

Fig. 3 Sigmoid curve and inflection point from Bayesian estimations of speed for each group per trial during LS. Horizontal and vertical axes indicate Vmax for each group per trial during the LS presentation and the probability to freeze or not to freeze synchronously, respectively. Each point shows each group per trial and point color indicates the group ID. For easier visualization, each point was placed randomly on the graph. Blue curve and red vertical line indicate sigmoid curve and inflection point, the widths of which indicate 95% credible interval, respectively.

Table. 1 Number of clusters and silhouette coefficient for each individual per trial

The number of clusters	2	3	4	5	6	7	8	9	10
Silhouette coefficient	0.697561378	0.554739526	0.483075046	0.403119192	0.365639723	0.34468743	0.329727915	0.347102994	0.355335503

Table. 2 Number of clusters and silhouette coefficient for each group per trial

The number of clusters	2	3	4	5	6	7	8	9	10
Silhouette coeffcient	0.633459934	0.484882542	0.470671624	0.322349117	0.257469957	0.140505406	0.116659037	0.116659037	0.116659037

Table. 3 Generalized linear-mixed model results (Akaike information criterion) by model

model	AIC	Δ AIC
$Y \sim T + D + T{:}D + (1 \mid G)$	400	0
$Y \sim T + D + (1 \mid G)$	409.9	9.9
$Y \sim 1 + (1 \mid G)$	421.2	21.2

Y, T, D, and G indicate the number of freezing individuals in each group per trial, the number of trials, the number of experiment days, and the group identity, respectively. The first, second, and third row indicate the model: model including an interaction of fixed effects, a model not containing an interaction of fixed effects, and a model not without an interaction and fixed effects, respectively.

Table. 4 Explanatory variables and Watanabe-Akaike Information Criterion (WAIC)

Explanatory variable	WAIC	Δ WAIC
LS_Vmax	41.451	0
LS_Vmean	78.507	37.056
pre-LS_Vmedian	100.358	58.907
pre-LS_Vmean	103.722	62.271
LS_Vmin	109.197	67.746
LS_Vmedian	115.689	74.238
pre-LS_Vmin	122.002	80.551
pre-LS_Vmax	124.557	83.106