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HOW STIMULUS DIRECTION DETERMINES THE TRAJECTORY OF THE MAUTHNER-INITIATED ESCAPE RESPONSE IN A TELEOST FISH

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Summary

Fishes use the Mauthner-initiated C-start for short-latency evasion of predators. C-starts consist of a sudden turn (stage 1) and a rapid acceleration (stage 2). We analyzed high-speed ciné films of goldfish C-starts elicited by dropping a ball into the water. It was previously thought that stage 1 angle does not vary concomitantly with the angle of the threatening stimulus relative to the position of the fish. We found, however, a significant inverse relationship between the direction of the impact of the ball and the angle turned by the end of stage 1. When starting near a wall, or when its usual trajectory was blocked by a wall, the fish used an escape route that was not predictable from the stimulus angle. The fish did not appear to correct its trajectory if it began to turn towards the ball. This behavioral evidence supports the previous notion that the underlying neural command is ballistic and does not use sensory information from the stimulus once the movement begins. If this is so, the fish probably utilizes information on obstacle location in the interval leading up to the trigger stimulus.

Introduction

The C-start is the familiar escape response of many fishes and of amphibian larvae when given a sudden aversive stimulus, such as when the side of an aquarium is tapped (Bullock, 1984; Eaton *et al.* 1977; Harper and Blake, 1990; Webb, 1976; Will, 1991). Under natural conditions, fishes use the C-start for short-latency evasion of predatory attacks (Blaxter and Fuiman, 1990; Katzir and Intrator, 1987; Webb and Skadsen, 1980; Webb, 1986). A typical C-start is shown in Fig. 1A,B. Stage 1, the initial component, involves a major contraction of the musculature on one side of the body. When a goldfish is viewed from above at the end of stage 1, its body forms a C-like shape with head and tail bent to one side (Fig. 1A). Thus, during stage 1 the fish changes its orientation to one away from the side of the attack and the tail becomes bent at an angle relative to the starting axis of the body. This allows the animal during stage 2 to propel itself in an escape

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trajectory (ET, Fig. 1B). As well as a forward propulsion, stage 2 may also include a turn. The end of stage 2 is marked by the onset of swimming, or the animal may simply glide (Weihs, 1973).

The long-term goal of our studies on the C-start is to understand how sensory signals are converted into the neural commands leading to stages 1 and 2. Neurophysiological studies show that prior to the C-start one of the Mauthner neurons fires an impulse and thereby triggers a major component of the stage 1 contraction (Eaton *et al.* 1981, 1982, 1988; Nissanov *et al.* 1990; Zottoli, 1977). The Mauthner neurons reside as a bilateral pair in the reticulospinal formation of the brainstem. The cranial and spinal networks of the Mauthner cell system have been well studied (Faber *et al.* 1989, 1991; Fetcho, 1991). The Mauthner dendrites and soma receive a diverse array of sensory inputs; the Mauthner axons cross the midline, descend in the spinal cord, and synapse on primary motoneurons that activate the trunk musculature. To relate Mauthner cell firing and associated activity in other neurons to the C-start, we need to know the fundamental relationship between the angle of attack and the magnitude of the stage 1 and 2 turns.

It was previously believed that stage 1 angle does not vary concomitantly with the direction of the stimulus (Eaton *et al.* 1977, 1981). Escape trajectories are known to be oriented away from the direction of the stimulus (Blaxter *et al.* 1981;

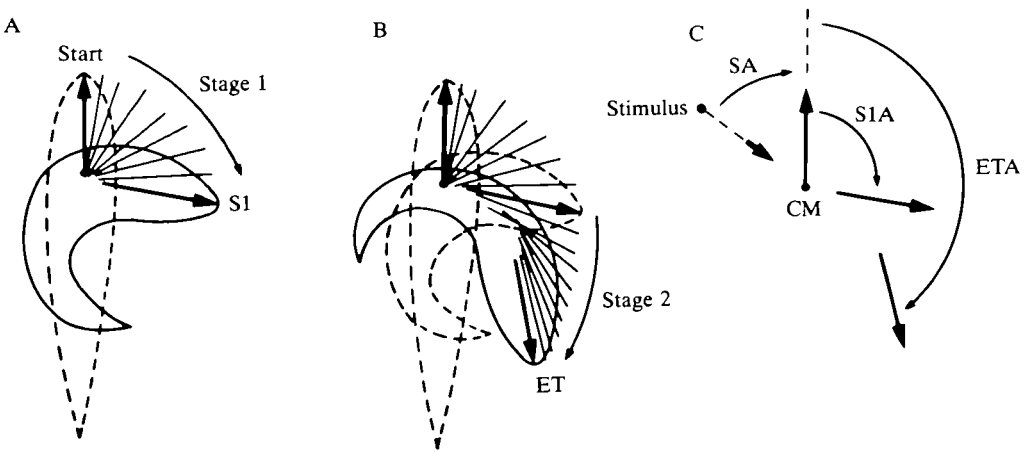

Fig. 1. The goldfish C-start and its analysis. (A,B) Stages 1 and 2. Heavy arrows are midlines at the frame immediately preceding movement onset (Start), at the end of stage 1 (S1) and at the time the escape trajectory angle was measured, 70 ms after movement onset (ET). The midline arrows should not be confused with movement vectors, but are intended only to indicate the position and orientation at specific points in the escape sequence. Images were gathered at 500 frames s^{-1} but we show only every alternate midline for purposes of illustration. (C) Definitions of angular measurements in the analysis. The stimulus was a ball dropped into the water above the fish. The dot labelled Stimulus is the point of impact of the ball relative to the position of the fish. SA, stimulus angle; S1A, stage 1 angle; ETA, escape trajectory angle; CM, stretched body center of mass. See text for details and rationale.

Eaton *et al.* 1981). Until recently, however, it was incorrectly thought that stage 1 was a stereotypic movement in which the fish flexed its body by a relatively constant angle of about 30–50° before beginning stage 2 (Fig. 2). This seemed consistent with the notion that stage 1 might be due to the firing of only one Mauthner cell and its pool of postsynaptic motoneurons in the spinal cord (Eaton *et al.* 1981). According to this *Mauthner reflex hypothesis*, stage 1 begins the movement in the correct *general direction*, either to the left or right, but the stage 1 and stage 2 turns are largely independent of each other. Thus, by implication, the steering for fine trajectory control should occur only during stage 2. Any variability that was seen during stage 1 could have been due to a fixed output from the Mauthner cell processed by spinal networks having different initial biases.

However, because of technical advances in high-speed digital motion analysis, we have recently been able to analyze a very large number of C-starts (Eaton *et al.* 1988; DiDomenico *et al.* 1988; Nissanov *et al.* 1990). As a result, we now know that the Mauthner reflex hypothesis is inadequate. Stage 1 angle varies from 20° to 110° and stage 1 and stage 2 turns are not independent. That is, small stage 1 angles tend to be followed by stage 2 turns that are small. Large stage 1 turns are followed by large turns during stage 2. Thus, except for escape trajectories of 30–50°, the Mauthner reflex hypothesis would either overestimate or underestimate the size of

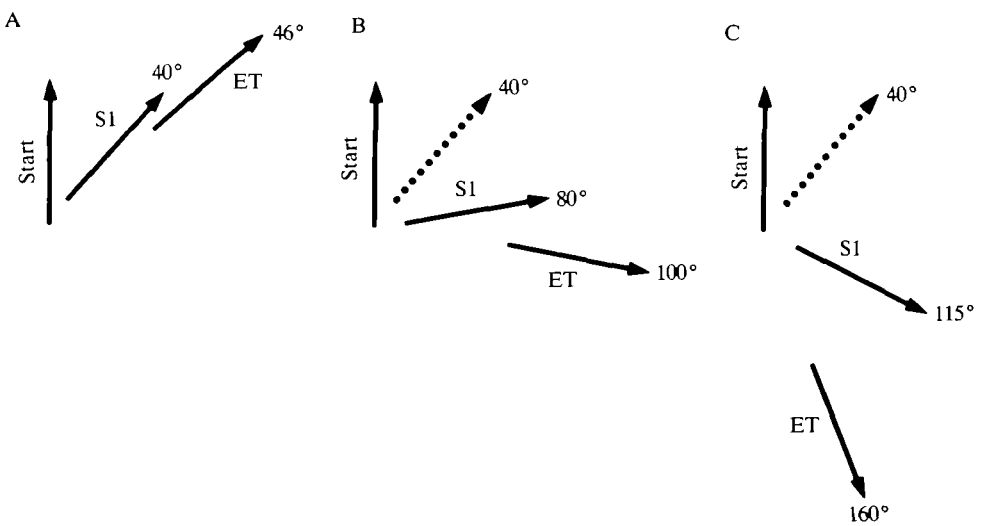

Fig. 2. Comparison of stage 1 angles according to the Mauthner reflex hypothesis and the contemporary concept. Solid arrows are the same as in Fig. 1. (A) For escape trajectories of about 30–50°, the Mauthner reflex concept correctly held that the stage 1 angle would be about 40°. (B,C) For escape trajectories larger than 30–50° the previous concept underestimates stage 1 angle. Dotted arrows are drawn at 40° according to the Mauthner reflex hypothesis and are shown in contrast to actual stage 1 angles of 80° and 115° that occur with escape trajectories of 100° and 160°, respectively. For escape trajectories smaller than 30–50° the previous concept overestimates stage 1 angle.

the underlying stage 1 turn (Fig. 2). The new concept predicts that the magnitude of the stage 1 turn should vary inversely with the angle of the threat approaching the fish. Stimuli behind the fish should produce small stage 1 turns so that the fish can accelerate forward; stimuli from the front should produce large stage 1 turns so that the fish can reverse its orientation (Eaton *et al.* 1991). These predictions could not be evaluated in our studies based on digital imaging because we used an acoustic stimulus for which we did not know the direction perceived by the fish.

Here we report findings from an analysis in which we emulated a predatory attack by dropping a ball into the water above the fish. Our study confirms the prediction that stage 1 angle varies with the position of the stimulus. We also found that obstacles in the surrounding environment alter the relationship between stimulus angle and stage 1 angle. Because the behavior appears to be ballistically produced, it is likely that the fish continuously updates sensory information on the location of nearby obstacles *prior to* the onset of the trigger stimulus.

Materials and methods

This study is based on behavioral data originally obtained in an earlier experiment. Here we extend the analysis to incorporate contemporary concepts of the biomechanics and to discover how the direction of the stimulus influences the C-start components. In the previous study, we made chronic electrophysiological recordings from the Mauthner neurons while simultaneously filming C-starts with a high-speed cinematographic camera (Eaton *et al.* 1981). The earlier publication covers procedural details and the neurophysiological findings. We gathered these data to show the temporal relationship between Mauthner cell firing and the various stages of the C-start. When a C-start occurred on the side opposite to the recorded Mauthner cell, the behavioral response was always preceded by a single Mauthner cell action potential. We have observed no exceptions to this rule (Eaton *et al.* 1981, 1982, 1988). Therefore, we combined all behavioral trials into one data set, whether the responses were opposite the monitored Mauthner cell or not. With this assumption, we consider all responses in the present study to be Mauthner-initiated.

Animals, behavioral testing and electrophysiological recording

We tested seven adult goldfish *Carassius auratus* (L.) (10–13 cm standard length) in an aquarium (30 cm×40 cm) filled to a depth of 10 cm with water between 19–23°C. The ciné camera (Redlake Locam) recorded the animal's movements from below the aquarium. The camera was equipped with an electronic speed calibration circuit. All behavioral responses were recorded at 500 frames s^{-1} (0.9-ms exposure) and we analyzed the films frame-by-frame. The initial data set contained 85 trials. Of these, we could analyze 50. The remainder were not studied for various technical reasons (e.g. there was no C-start, the fish moved before the ball dropped and so on). Reported measurements are rounded to the nearest degree and means are given as ±standard error of the mean.

The stimulus

The stimulus was produced by electronically releasing a 4.25-cm diameter ball from about 15 cm above the water surface over the fish. This stimulus was very effective in eliciting C-starts. From its release point, the ball took about 165 ms to reach the water surface. The ball had a plastic plate attached on its upper surface. This prevented it from rapidly penetrating the water surface and striking the fish. Approximately 12 ball-drop stimuli were given at 15-min intervals to each of the seven fish in this study. We made no attempt to prevent the fish from seeing the ball before it was dropped.

Definitions of response variables

Our cinematic measurements are similar to earlier studies (Eaton *et al.* 1988) except that the end of stage 1 was more precisely defined as the frame in which the center of mass (CM) became displaced by 0.3 cm from the rotation point. We show our conventions for analyzing the angular components of the C-start in Fig. 1C. To define the relationship between the stimulus and the components of the turn, we drew a line between the CM of the fish and the point on the water surface where the ball struck (Stimulus). Stimulus angle (SA) was the angle formed by the intersection of this line and the rostral midline of the fish at the start position (Fig. 1C). We chose this angle because it is believed that many predators aim for the CM, located in the thickest part of the body (Webb, 1986). Moreover, this is the point about which the propulsive forces develop as the fish turns away from the aversive stimulus.

We also analyzed our data using a stimulus angle defined by the intersection of the line drawn between the impact point of the ball and the midline of the head of the fish. We picked a point on the head at 19 % of the body length from the nose. This is the approximate position of the inner ear. Our statistical analyses were less significant, however, when using this angle than when using the stimulus angle relative to the CM. Therefore, these data are not reported.

We wanted to know the heading of the fish relative to the stimulus at a particular point in time after the start of movement. To do this we introduce the concept of *escape trajectory angle* (ETA). This is operationally defined as the angle formed by the intersection of starting position midline with the midline at 70 ms after the start of movement. In this interval the goldfish moves its CM by about 4 cm. This interval is of interest because it is within the realm of predator closing times (Lauder, 1983; Webb and Skadsen, 1980).

In this paper we refer to *stage 2 angle* as the difference between the escape trajectory angle and stage 1 angle. For those familiar with our earlier work, it is important to note that this differs from our previous definition of the term (Eaton *et al.* 1988). Previously, stage 2 angle was the angle turned in a 50-ms interval after the end of stage 1. Thus, because stage 1 varies in duration and extent with stimulus angle, stage 2 angle does not reveal the orientation of the fish at a consistent point in time after the start of movement. Our measurements of escape

trajectory angle at a fixed point in time (70 ms), therefore, include varying portions of the stage 2 component. It turns out, however, that because the average stage 1 turn is 25 ms in duration, the average of all escape trajectory angles will be about 45 ms after the end of the stage 1 turn (e.g. 70 ms−25 ms=45 ms). This is, on average, within 5 ms of the 50 ms over which stage 2 angle was previously measured.

Experimental design

In this study, we wanted to find out the relationship, under ideal conditions, between stimulus angle and the component turns of the C-start. Previously, we examined the relationship between stimulus angle and escape trajectory, but we did not study the quantitative relationship between stimulus angle and stage 1 angle, nor did we analyze complicating influences of nearby walls. In addition, we grouped all the responses into a single data set whether the turns were away from, or towards, the ball (Eaton *et al.* 1981). Thus, for the present study we first divided the data set of 50 trials into two groups according to whether the animal turned away from the ball (41 trials) or towards it (nine trials). The nine responses towards the ball are analyzed separately at the end of the Results section because they reveal additional important features of the behavioral response. The 41 responses away from the ball were further categorized according to whether the fish was in open water or whether there were walls nearby that might obstruct the escape route. To distinguish responses likely to be influenced by walls, we calculated the average distance travelled in 70 ms during responses in which the fish started in the center of the tank. This distance plus one standard deviation (total 76 mm) forms the radius of a circle (curved line; Fig. 3) extending from the

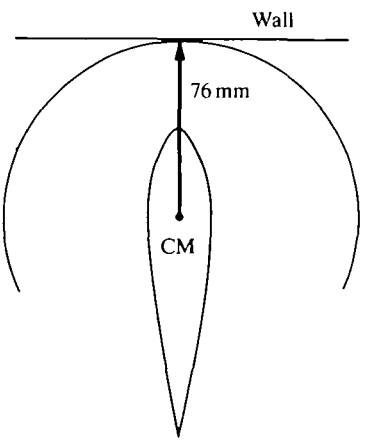

Fig. 3. Drawing to illustrate the criterion radius (arrow) used to separate trials having obstructed and unobstructed escape routes. The radius was equal to the mean distance travelled, as measured by the displacement from the center of mass (CM) at the start position to the tip of the fish's nose at 70 ms plus one standard deviation of the mean of this distance (N=41). If a wall intersected the circle described by the radius, the trial was considered to have an obstructed escape route.

CM. In trials when the fish was near a wall of the tank, the escape path was considered *obstructed* if a wall intersected the circle defined by the radius on the side towards which the fish turned. Of the 41 responses, 28 were *unobstructed* and 13 were *obstructed* by one or two walls. For the unobstructed trials, the average distance from the center of mass to the nearest wall was 88±5 mm; for the obstructed trials the distance was 49±7 mm.

Results

Stage 1 angle varies inversely with stimulus direction

The first major finding is that stage 1 angle varied systematically with respect to where the ball struck the water. For example, in Fig. 4A, the stimulus was from a caudal direction at 171°, the fish had a stage 1 turn of 59° and accelerated forwards in the direction of its initial orientation. When the stimulus was from the side at 95°, the fish made an 87° turn to the opposite side (Fig. 4B). When the stimulus was from the rostral left at 51°, the fish made a large turn of 95° in the right caudal direction (Fig. 4C). Thus, larger stimulus angles elicited responses with smaller stage 1 and escape trajectory angles; smaller stimulus angles elicited larger stage 1 and escape trajectory angles.

The relationship between individual stimulus angles and stage 1 response angles was statistically significant over the range we tested from 47° to 177°. Fig. 5A shows the position of all points of impact of the ball relative to the starting position of the fish. Line segments emanating from the CM are the corresponding midlines at the end of stage 1. The data in Fig. 5B provide the quantitative relationship

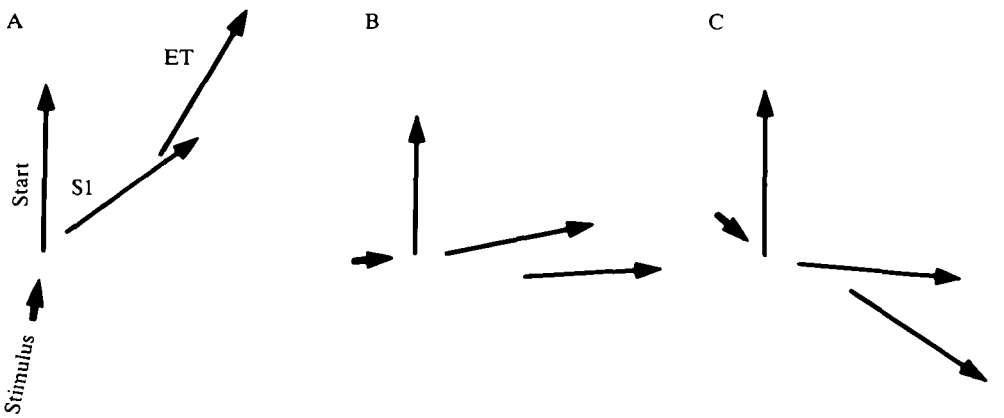

Fig. 4. Three examples of individual responses showing the relationship between stimulus angle and response components. The short arrow (Stimulus) indicates the direction of the impact point of the ball relative to the center of mass. Other arrows are the same as in Fig. 1. (A,B,C) As the stimulus approaches more from the rostral direction, stage 1 angle and escape trajectory angle increase to produce turns increasingly towards the caudal direction.

between stage 1 angle and stimulus angle for the points in Fig. 5A. From this evidence we conclude that information on stimulus angle is incorporated into the very earliest movement component, stage 1, of the C-start. Thus, by implication, the stage 1 contraction of the trunk muscle is coded by the angular variables of the stimulus.

We were also interested in the escape trajectory angle *at a particular point in time*, 70 ms after the start of movement. In the interval from the end of stage 1 to 70 ms after movement onset, the animal is in the propulsive component, stage 2. Our data show that stimulus angle and escape trajectory angle are also significantly correlated (Fig. 6). This means not only that is stage 1 coded by the angular variables of the stimulus, but that the animal was still oriented away from the stimulus well into stage 2.

The relationship between stimulus angle and escape trajectory angle in Fig. 6B was clearly more variable than the relationship between stimulus angle and stage 1 angle in Fig. 5B. Indeed, the standard error about the regression line increased from 10.0 for stage 1 angle to 18.5 for escape trajectory angle. This increase was due to an increase in variability of the stage 2 turn subsequent to stage 1. When we calculated the regression of this portion of the stage 2 turn (e.g. ETA−S1A, Fig. 1C) relative to the stimulus angle, the relationship was not statistically significant. However, because there was no statistically significant relationship between stage 1 and stage 2 angle in these data (r=0.37), we suspect that stage 2 variability may have been an effect of nearby walls (see Discussion).

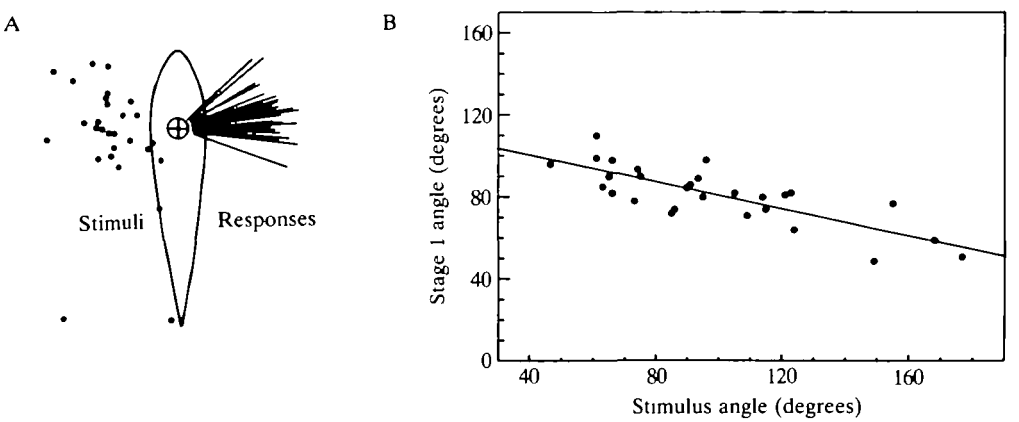

Fig. 5. The quantitative relationship between stimulus angle and stage 1 angle. (A) The entire data set showing all points of impact of the ball above the fish and the stage 1 midlines of the resulting responses. The ball was dropped on both the left and the right over a 120° range, but, for purposes of analysis, stimuli on the right and responses to the left are shown reflected onto the opposite side of the diagram. Stimuli near the tail should not be considered as *outliers* in the data set because the analysis included only stimulus angle and ignored distance as a variable. (B) Scatterplot of stimulus angle *versus* stage 1 angle. Regression analysis showed that the relationship was significant: $P<0.01$, $r=-0.79$, $y=-0.33x+113$.

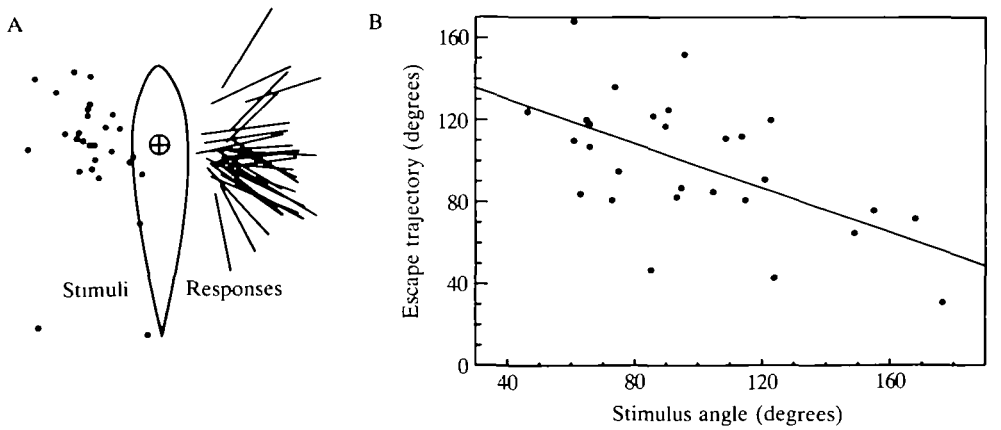

Fig. 6. The quantitative relationship between stimulus angle and escape trajectory angle. (A,B) Conditions as for Fig. 5. Regression analysis showed that the relationship was significant: $P<0.01$, $r=-0.58$, $y=-0.54x+152$.

Nearby obstacles modify stage 1 and escape trajectory angles

We found a significant increase in the magnitude of stage 1 and escape trajectory angles when we analyzed responses that began closer to a wall than in the above analysis. In other words, the animal moved farther from the wall than we would have expected from data obtained when it started closer to the center of the tank. This is our second major finding. Examples are shown in Fig. 7 where the solid arrows follow the same conventions as before and the dashed arrows are the stage 1 and escape trajectory angles *predicted* from the regression equations for the data in Figs 5 and 6.

Responses in Fig. 7A–C are examples of cases with one obstructing wall. For all eight responses away from the direction of the ball, stage 1 and escape trajectory angles were *larger* than expected (compare solid and dashed arrows) and the actual trajectory was farther from the wall than the predicted trajectory. The large percentage of responses greater than the prediction would not be expected if there were no effect of the wall. Instead, we would anticipate that half of the measurements should be smaller than the prediction and half should be larger. There is less than a 0.005 probability of all 16 measurements being too large (χ^2 analysis). Furthermore, the difference between the expected and the actual angles should differ by an average of about 0°, but the deviations averaged 27±5° for stage 1 and 42±9° for the escape trajectory angle. From these findings we conclude that physical barriers have a significant influence on the production of the stage 1 turn.

Except for a higher incidence of responses *towards* the ball (presented later), the effects of two walls were similar to those of one wall. Three examples of the five responses away from the ball are shown in Fig. 7D–F. As with one wall, the actual stage 1 and escape trajectory angles had average values larger than

predicted from stimulus angle. For the 10 measurements, only a single stage 1 angle was smaller than expected (by 10°); this fish exceeded the expected escape trajectory angle by 17°. The probability of observing this high percentage of large responses was only 0.025 if there were no difference between trajectories in open water and near a wall (χ^2 analysis). The differences between the expected and the actual angles were 25±10° and 62±16° for stage 1 and escape trajectory angle, respectively, whereas we would expect these values to be very close to 0° if the wall had had no effect.

In Fig. 8 we summarize the regression data for the 13 trials in which the fish started near a wall and turned away from the direction of the ball. Here we show the relationship between stimulus angle and stage 1 and escape trajectory angles. Even though we included only responses away from the ball, the relationships between stimulus angle and stage 1 and escape trajectory angles were not statistically significant. Thus, when a wall was present within the defined radius, the escape trajectory could not be predicted from stimulus angle alone. In summary, on the basis of these analyses we conclude that in the presence of a wall the animal turned farther than was anticipated from data taken from escapes in open water.

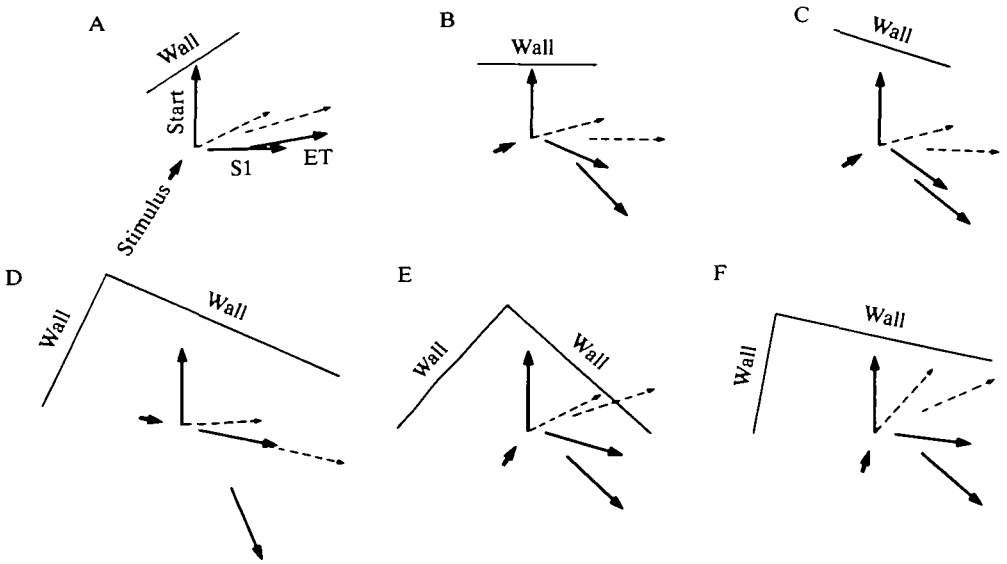

Fig. 7. Examples showing the effect of nearby walls. Dashed arrows are the predicted midlines at the end of stage 1 (S1) and escape trajectory angle (ET). Solid arrows are midlines of the actual responses. The distance moved by the predicted ET midline was the same distance as the actual ET midline for each case. (A,B,C) The orientation and distance of a single wall is shown by the straight line in front of the fish at the start. In all such cases the escapes carried the fish farther from the wall than the angles predicted from the stimulus direction. (D,E,F) Responses with the start near a corner (two walls). As with the above examples, the actual S1 and ET angles exceeded the predicted values and in E, and possibly F, prevented a collision with a wall.

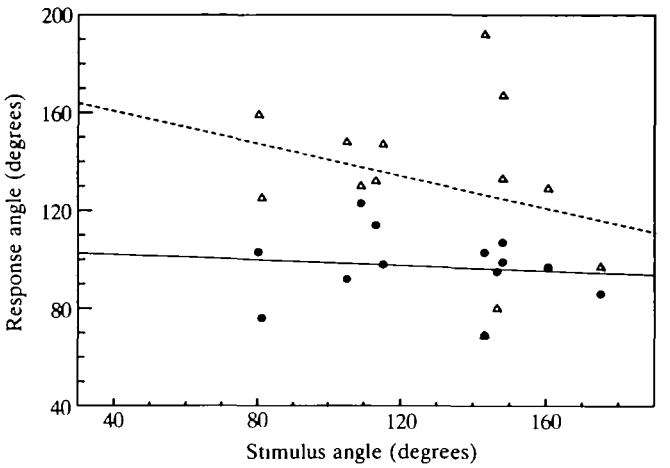

Fig. 8. Relationship between stimulus angle and S1 angle (●) and escape trajectory angle (△) for trials in which the escape trajectory angle was obstructed by one or two walls. Neither linear regression was significant. Thus, the presence of walls appears to disrupt the relationship seen in open water.

Apparent tactical errors

The observations described in the two sections above are the major findings of this study. The analysis of the remainder of the data was especially suggestive, however, of additional properties of the behavior and its underlying neural processes. These observations are presented in this and the following section.

In 12.5 % of the trials with unobstructed escape routes (N=4), the fish made an apparent tactical error and turned towards the stimulus (Table 1). Shown in Fig. 9 are the cases we observed. These are important because they provide evidence of whether the C-start is produced ballistically, or whether the animal relies on sensory feedback during the turn to correct its trajectory. If the fish could detect that it had turned towards the ball, and if it could correct its trajectory accordingly, we would expect the angles in Fig. 9 to be *larger* in magnitude than the negative of the usual angles. For example, if the predicted stage 1 angle is a 30° turn to the right, the fish should make a turn larger than a 30° to the left (or −30°). By producing larger turns, the fish would increase its distance from the stimulus in the case of an initial error in the left–right axis. However, the examples in Fig. 9 are remarkable because there is no suggestion that the fish could made such a correction.

In Fig. 9 the solid and dashed arrows are the actual and predicted angles. For the four responses, stage 1 angles were less than the negative value of the predicted stage 1 angles by a mean of 19±4°. The mean escape trajectory angle was less than the predicted one by 41±22°. In summary, for all eight angular measurements of the four responses, the actual angles were *smaller* than the negative of the expected angles. Although turns towards the ball were rare in open

Table 1. *Analysis of the percentage of responses in which the fish turned towards or away from the ball under different initial conditions*

		Obstructed	
	Unobstructed	Non-conflicting choice	Conflicting choice
Percentage of responses away from stimulus	87.5	100	28.6
Percentage of responses towards stimulus	12.5	0	71.4
Total trials	32	11	7
Probability of difference from random	<0.005	<0.005	NS
Probability of difference from unobstructed trials		NS	<0.005

Probabilities were calculated using a χ^2 analysis; NS indicates that the difference was not significant.

water, their consistently small turn angles are strongly suggestive behavioral evidence that the underlying neural commands for the C-start are ballistic and do not rely on sensory information from the stimulus once the movement begins.

Obstructed pathways influence initial escape direction

In some cases with an obstructing wall nearby at the start, the fish could turn either towards the ball or towards the wall. We categorized these stimulus situations according to whether a wall intersected the predicted escape route (Table 1). Cases with a wall intersecting the criterion radius, but not blocking the predicted escape trajectory (such as Fig. 7D), were operationally defined as *non-conflicting*; those with a wall across the predicted escape trajectory (Fig. 7E) were operationally defined as *conflicting.*

When the fish was presented with conflicting trajectory choices, there was a significant increase in the percentage of responses directed towards the stimulus (Table 1). In all 11 non-conflicting cases, the fish turned away from the ball much as it did in open water. There were seven trials where the predicted escape trajectory would lead the fish into a wall. Two responses were turns away from the ball but towards the wall (e.g. Fig. 7E; the fish did not hit the wall). Five cases were towards the ball and away from the wall. Three of these examples are shown in Fig. 10. Two occurred in trials 2 and 4 so it is unlikely that such maneuvers require practice. These findings suggest that the modulatory effect of walls is so strong that it can override directional information from the threatening stimulus.

Discussion

At least four general types of sensory information are needed to coordinate the C-start successfully. The fish needs to know *what* and *where* the stimulus is, *when*

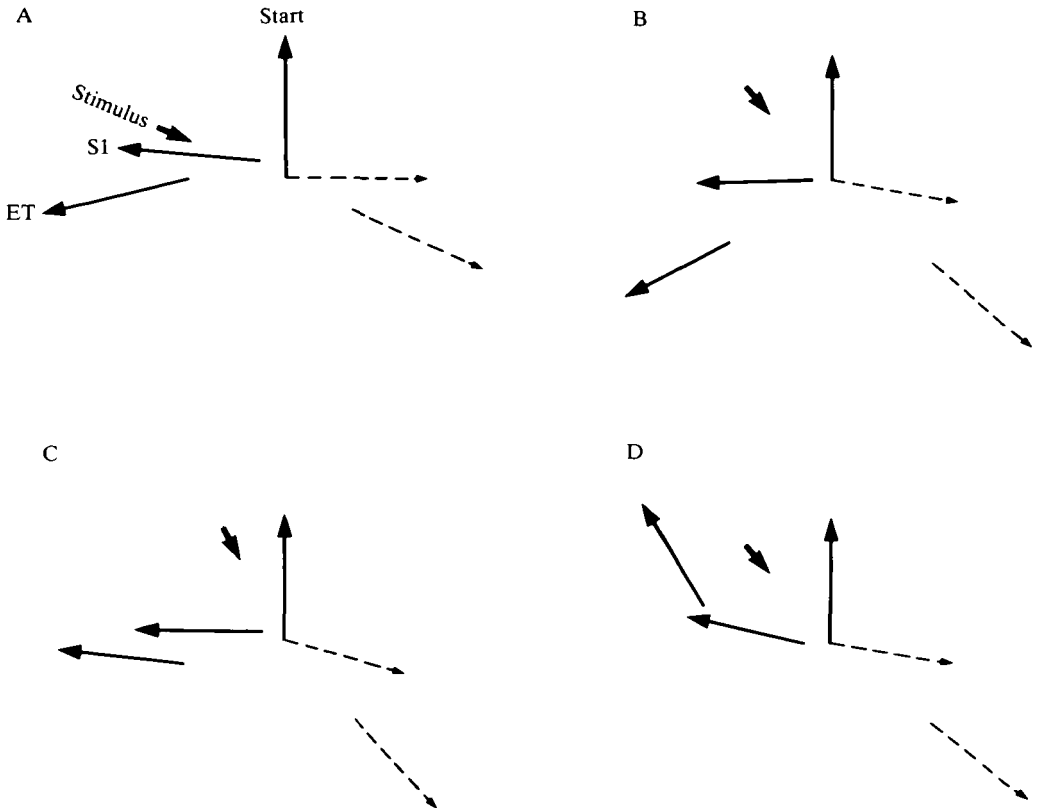

Fig. 9. Analysis of trials in which the fish turned towards the stimulus. Dashed arrows are the predicted midlines and solid arrows are the actual responses. In each case, the actual stage 1 angles are negative reflections of the predicted angles. (A,B,C) The stage 1 and ET angles appear to be due to errors of symmetry in the left–right direction. In D, the initial turn was to the left instead of to the right, but the fish turned to the right in stage 2. This brought it even closer to the direction of the stimulus. The lack of trajectory corrections in these cases suggests that the C-start is ballistic.

to begin the escape sequence and *where* to go (DiDomenico and Eaton, 1988). The present study reveals how stimulus location (the *where* of the stimulus) is related to escape path (the *where* to go). Recently, we have also provided information on how the C-start is triggered (the *when* to go; Canfield and Eaton, 1990). Taken together, these findings fill in some of the major gaps in our understanding of C-start neuroethology. This is important because the C-start is mediated by one of the few cases of a vertebrate neural network whose elements are well known and accessible for neurophysiological recordings (Faber *et al.* 1991; Fetcho, 1991; Lee and Eaton, 1991).

Relationship between stimulus angle and the stage 1 turn

In this study we show how the angular components of the threatening stimulus

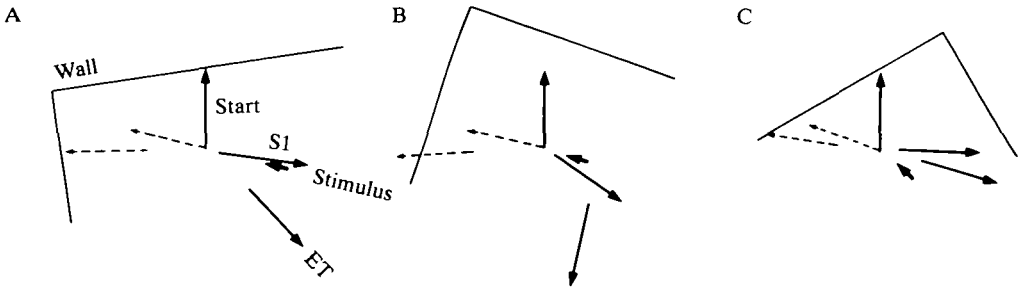

Fig. 10. Analysis of trials in which the fish turned towards the stimulus from starting positions near corners. Dashed arrows are the predicted midlines. In all cases, the predicted angles would have carried the fish closer to a wall than the actual angles. Thus, in each instance it could be argued that this was the correct strategy. These responses suggest a powerful influence of stationary objects on the neural processes mediating the decision to initiate a C-start.

influence stage 1 of the C-start so that the fish achieves the variety of turn angles required to escape in any direction. To do this the animal uses graded information on stimulus location to produce the muscle contractions of the trunk during stage 1. Thus, the combination of the direction and extent of the stage 1 turn plays a major role in producing the wide range of escape trajectories that enable the animal to turn in any direction.

We have postulated that stage 1 angle is controlled by a *brainstem escape network* consisting of the Mauthner neurons and other reticulospinal cells that fire in concert with each other (Nissanov and Eaton, 1989; Eaton *et al.* 1991). We propose that this network varies motoneuron recruitment of the trunk muscle to vary stage 1 angle in response to different stimulus angles. In the absence of such a network it is difficult to explain how stage 1 angle could be controlled by a single action potential in one of the Mauthner cells. Members of the brainstem escape network have not been demonstrated from functional studies. However, neuroanatomical work shows that the Mauthner cell is only one of at least two other pairs of morphologically similar neurons (Metcalfe *et al.* 1986). These cells share a variety of developmental and immunocytological features that suggest a functional relationship as well (Lee and Eaton, 1991).

Recent findings support the brainstem escape network hypothesis. First, recordings of stage 1 electromyograms (EMGs) suggest progressive motoneuron recruitment as stage 1 angle increases (Eaton *et al.* 1988; Foreman and Eaton, 1990). Second, artificial activation of a single Mauthner cell results in a movement that is weaker and with a smaller range of trajectories than stage 1 of the C-start evoked by sensory stimuli (Nissanov *et al.* 1990). We have suggested that activation of just the Mauthner cell fails to turn on other members of the brainstem escape network responsible for controlling the extent of the stage 1 turn. Finally, functional substitution by members of the brainstem escape network could also

explain how C-starts are produced in the absence of the Mauthner cells (DiDomenico *et al.* 1988).

Our current findings imply that the brainstem escape network is activated in a way that depends on stimulus angle. We cannot yet be explicit about how this activation occurs, but one can now imagine a model in which the brainstem escape network produces its smallest effect on the stage 1 motoneurons when the animal responds to stimuli approaching from behind. This results in a reduced motoneuron recruitment giving the small stage 1 contraction required to produce escape movements to the front. Correspondingly, by being most activated by stimuli from in front, the brainstem escape network triggers large stage 1 angles for large turns that reverse the initial direction of the fish.

The relationship between the stimulus direction and the stage 1 body contraction is quantitatively described by a single linear equation that is a function of the stimulus angle (SA). This equation is derived as the regression in the legend to Fig. 5:

$$\mathrm{S1A} = -0.33\mathrm{SA} + 113°, \quad (1)$$

where S1A is stage 1 angle. In effect, this equation is a quantitative description of the behavioral input–output relationship between stimulus angle and muscle contractions producing the stage 1 muscle contraction.

For the fish to produce the correct stage 1 angle, it must have sensory information telling it the angle of the stimulus relative to its orientation. By supplying this information to the brainstem escape network, the correct magnitude of the stage 1 contraction can be computed by the brainstem escape network. This is equal to minus one-third of the stimulus angle (SA) plus a constant. Thus, the equation gives a fundamental framework for future neuroethological studies intended to relate the sensory coding of afferent fibers to the production of motoneuron outputs for stage 1.

Sensory activation and modulation of the C-start

Which sensory elements of the ball-drop stimulus determine the onset time and the directionality of the C-start? We suggest that the behavior is triggered by the acoustic pressure of the impact of the ball on the water. In response to the ball, the fish appeared to use the 'matador strategy', suggested by Blaxter and Fuiman (1990), and usually did not start until *after* the ball had hit the water, even though it had about 165 ms to see the ball coming (Eaton *et al.* 1981). Response latencies from the ball impact to Mauthner cell firing (7.4±6.7 ms, N=46) were comparable to latencies of goldfish recorded in two other studies using purely acoustic stimuli (4.9±0.5 ms, Zottoli, 1977; 3.4±0.3 ms, Eaton *et al.* 1988). Thus, the triggering stimulus seems to be the acoustic signal caused by the impact of the ball on the water surface.

With acoustic stimuli, the goldfish Mauthner system uses the pressure component of the underwater sound to trigger the behavioral response (Canfield and Eaton, 1990). This implies that, in the absence of directional information from

other modalities, acoustic particle motion determines which Mauthner cell can reach threshold. This may be mediated *via* the PHP inhibitory interneurons (Canfield and Eaton, 1990). However, for the ball stimulus we cannot rule out the directional influence of visual or lateral line input, or both.

Our present findings show that the relationship in equation 1 is subject to modification by stationary obstacles. A physical barrier can cause the animal to take a different escape trajectory from that used in open water. Some swimming movements close to walls might be influenced by the hydrodynamic *ground effect* resulting from compression of the water between the fish body and the wall. Webb (1981) has shown, however, that this is unlikely for fast-starts from rest. We therefore conclude that, when the fish was near a wall, the increase in trajectory angle was a behavioral effect that enabled it to avoid collisions. Given the high acceleration during the C-start (Harper and Blake, 1990), such a collision would be expected to increase the probability of capture by the predator. Here, it seems likely that visual or lateral line stimulation may mediate this effect.

Our findings support the provisional conclusion that the influence of stationary obstacles can be so strong as to determine which Mauthner neuron fires in response to the trigger stimulus. When the fish was given a choice of turning into a wall or towards the ball, most responses were towards the ball in cases where it appeared that the fish would hit the wall if it turned away from the ball. In open water, a significantly smaller proportion of the turns were towards the ball. Clearly, therefore, whether the fish turned towards or away from the ball depended strongly on the surrounding environment. Crucial variables are probably the distance and angle of the fish relative to the wall and ball. These relationships would be quite informative if worked out quantitatively.

Under natural conditions, when the fish does not always have advance warning of an attack, it would be logical for the modulatory effects of obstacles to pre-set the brainstem escape network for possible and impossible trajectories. The information could be continuously fed into the brainstem escape network and Mauthner neurons. Thus, when the trigger stimulus arrived, the fish would not fire the Mauthner cell and other neurons that would cause a turn resulting in a collision with a stationary object. Preliminary reports of similar findings have been made for tadpole and frog evasive maneuvers (Hoff, 1988; Hoff and Ingle, 1988). Because of the monosynaptic connection between the acoustic afferents and the Mauthner cell (Faber *et al.* 1991), it is likely that the modulation influences either the afferents (*via* a presynaptic mechanism) or the Mauthner cell itself (Mintz *et al.* 1989). Such a pre-modulation also makes sense in terms of the ballistic production of the behavior, described next.

C-start neural commands are ballistic

We observed that the fish did not appear to correct their trajectories if they turned towards the ball. This behavioral evidence supports previous conclusions from EMG recordings that the C-start command is ballistic: onset of the stage 2 EMG can begin as early as the stage 1 movement and, therefore, could not be

coded by movement-induced sensory feedback (Eaton *et al.* 1988). From these two lines of evidence, behavioral and neurophysiological, we suspect that sensory feedback plays only a minor role in determining trajectory angle once the movement has begun. This means that before the end of stage 1 there is a discrete time after which the network no longer uses sensory information to compute its trajectory. This makes sense because the high acceleration during the movement could readily distort new incoming sensory information (Russell, 1976).

Is there a relationship between stimulus angle and stage 2 angle?

Because of the high variability in stage 2 angle, we did not see a significant relationship between stage 2 angle and stimulus angle. This observation may lead to the conclusion that stage 2 angle is unimportant in orienting the fish away from the stimulus. We think that this would be an incorrect conclusion because the variability in the stage 2 turn was probably due to the influence of nearby walls. In our recent digital analyses of free-swimming animals, the initial position of the fish averaged 132±3 mm from the nearest wall (retrospective analysis of 50 responses of 10 fish from Eaton *et al.* 1988). For these fish there was a significant linear regression between stage 1 and stage 2 angles (r=0.6, N=148, P<0.0001). For these responses stage 1 and 2 were coordinated to produce the escape trajectory. In the present study, the initial positions were closer to the wall (average 88 mm), and there was no significant relationship between stage 1 and stage 2 angles. Given the clear modulatory effect of walls on the behavioral response, it is likely that the stage 2 variability in the present analysis was artificially high owing to the initial positions of the fish. Thus, our present findings are inconclusive as to whether stage 2 angle is significantly related to stimulus angle. This point could be readily evaluated by analyzing stage 2 angles of fish with initial starting positions farther from a wall than in the present study.

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