Neuroscience

Learning and cognition in a decision made at reflex speed

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eLife Assessment

This **valuable** study investigates prey capture by archer fish, showing that even though the visuomotor behavior unfolds very rapidly (within 40-70 ms), it is not hardwired; it can adapt to different simulated physics and different prey shapes. Although there was agreement that the model system, experimental design, and main hypothesis are certainly interesting, opinions were divided on whether the evidence supporting the central claims is **incomplete**. A more rigorous definition and assessment of "reflex speed", more detailed evidence of stimulus control, and a more detailed analysis of individual subjects could potentially increase confidence in the main conclusions.

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Abstract

In recent years it has become clear that many decisions do not obey the rule that more time yields better decisions. These decisions can be made remarkably fast and yet accurately, sometimes based on very limited information. It is presently unclear whether such ‘blink’ or high-speed decisions lack cognitive aspects that only much slower ‘deliberative’ decision-making can support. Here we demonstrate an unexpected degree of flexibility and cognition in a decision made by a hunting animal at reflex-like speed. Based on observing initial speed, direction, and height of falling prey archerfish decide in just 40 milliseconds on a turn toward the later ballistic landing point. This enables the fish to dash off to arrive simultaneously with prey and to secure it against numerous competitors. We established an approach that allowed us to replace ballistics, the rule that governs the turn decisions, with a novel rule of how to connect the input variables with the rewarded turns. This approach revealed that the fish are not using a hardwired circuit but were able to reprogram their decision in efficient ways that allowed them to immediately generalize to untrained settings. Training even allowed the decision to simultaneously use two distinct sets of rules, one for each of two distinct objects. The flexibility of the decision and the occurrence of high-level cognitive features are counterintuitive for a reflex-like decision made faster than an Olympic sprinter can respond to the start gun. However, they imply that combining speed and accuracy in rapid decisions does not generally make them less smart than decisions made over far longer timescales.

Introduction

Everyday experience suggests that the quality of our decisions depends on how much time and effort went into them. However, many decisions drastically defy this rule (e.g. 1-7). Although diverse, they have in common that they all can be made surprisingly fast, often based on limited information and yet can be accurate, often with no increase in accuracy when allowed more time. For instance, rats categorize odors reliably within one sniff, i.e. within about 300 ms. Allowing for longer sampling time did not increase accuracy even for difficult tasks (3,8,9). Similar short sampling has been described for visual perceptual decisions. Humans can reliably classify natural images after surprisingly brief viewing times of only 20 to 40 ms (1,10) and it has long been known, though for comparatively simple tasks, that extending viewing time did not affect the thresholds in visual decisions that are based on stereoscopic depth perception (11,12), velocity-discrimination (13,14), or contrast sensitivity (15,16). This suggests that a natural sampling interval – the time it takes to produce a sniff, or a saccade – is the elementary interval in many visual and olfactory perceptual decisions (3). Adding urgency into laboratory decision tasks by having the subject respond to sensory information after it has already launched movement (e.g. a saccade) has allowed to dissect perceptual and motor aspects of decision-making and thereby allowed the actual speed of perceptual decisions to be measured with high temporal accuracy (5,17). Outside the laboratories, the use of rapid decisions is probably very common in sports. For instance, baseball hitters have a mere 400 ms to move their bat to an ideal hit point based on an estimated 70 ms of visual processing to inform the movement of the bat (18–20), with a mere 40 ms of more or lesser sampling deciding between the best achievable and a bad motor choice (5). For some much slower and more complex human 'blink' decisions, such as which product to buy, it has been shown that blink decisions can be superior compared to those made much slower and based on more information (6,7,21–23).

An intriguing question about these diverse but impressively fast and accurate decisions is whether they lack aspects that only slower decisions can offer. For instance, high-speed decisions might be fast because they make clever use of simplifying heuristics and of hardwired neuronal machinery so that they work only under the specific conditions for which they were optimized. Are slower 'deliberate' decisions therefore needed when flexibility and perhaps higher cognitive aspects are required? While this is presently poorly understood, many behavioral findings – from the movement of baseball outfielders and frisbee-catching dogs (24, 25) to the chase of hoverflies (26) – are available that could be (and have been 6,7) interpreted as supporting such a conclusion. For instance, to intercept a passing female, male hoverflies do not continuously monitor the female to guide their own movement but rapidly decide on an interception course immediately when a passing female comes into sighting distance (26). Based on information taken at this time the male immediately selects a specific turn so that moving in the chosen direction at fixed acceleration leads straight to the point of catch. This decision is based on hardwired assumptions that appear to be genetically imprinted in the neural circuits that govern the initial turn (**Fig. 1A**). When any of these assumptions were experimentally violated, for example when the target had unusual speed or sighting distance, then the turns were massively in error just as was predicted (26). So, the hoverflies solution affords a striking degree of speed and accuracy but clearly comes at the cost of flexibility. Nevertheless, there appear to be no selective pressures for the decision to afford more flexibility, in fact, the apparent limitations are potentially even to the advantage of the hoverfly males (26).

Here we addressed the question of limits in flexibility in a high-speed decision that is made faster than an Olympic sprinter responds to the start gun. Like the decisions of hoverflies (26), batters (18–20) or outfielders (24,25) it is also a decision based on visual information and it is also about being at the right spot at the right time. It operates under interesting constraints and has properties that make it amenable to experimental dissection in the lab (27). The decision is

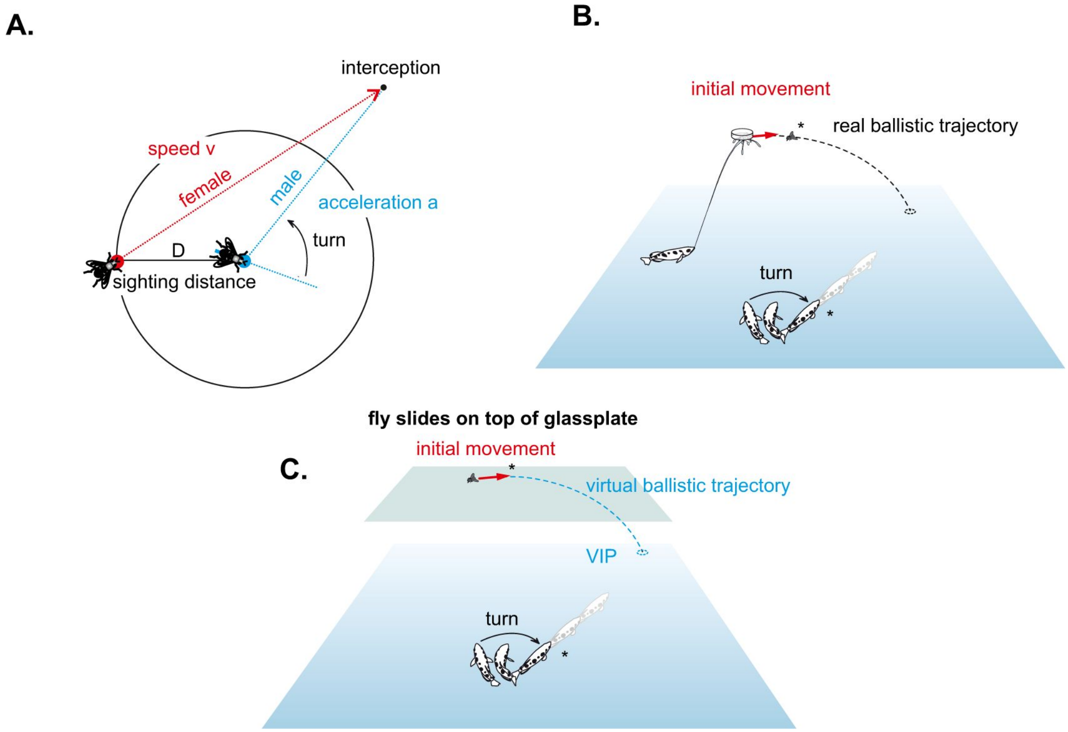

Fig. 1.

High-speed decisions used by animals to catch objects.

(**A**) Male hoverflies use a rapid open loop-strategy to catch passing females: When sighting a female moving at speed ‘v’ at fixed distance ‘D’ the male rapidly turns in a direction in which it will, always accelerating at a fixed rate ‘a’, be led to a point at which it can intercept the female. Here, information is taken only once. To make this decision, the male uses hardwired circuitry that assumes fixed values for D, v and a, which allows to simplify the complex problem considerably (6). (**B**) To secure downed prey hunting archerfish also use an open-loop strategy: Based only on visual information sampled after prey starts moving the fish turn to the later impact point and then (corresponding points in time indicated by an asterisk) take off at a speed that makes them arrive at full speed very slightly after their prey. The turns are produced in a so-called C-start maneuver and can be initiated in as little as 40 ms. The hunting context does not allow similar simplifications as in the hoverfly and fish can respond from a large range of distances and for any orientation. They are also, for instance, not limited to targets of an expected size or speed. (**C**) When prey is made to slide purely horizontally on a glass plate – without actually falling downwards – then the fish still elicit turn decisions. These are not aimed at the fly’s actual position and also not in the direction of the fly’s movement, but at the ‘virtual’ impact point (VIP), where ballistically falling prey would have landed given its initial speed, direction and height.

an essential part of the hunting behavior of archerfish, that are renowned for shooting down aerial prey with jets of water (28–30) (**Fig. 1B**): As soon as they have dislodged a prey insect with their shot, the fish (shooter and bystander archerfish) have the little time that remains till prey hits the water surface to be at the point of catch. A failure to arrive at this spot simultaneously with their prey means losing it to one of their numerous competitors, large crowds of other surface-feeding fish that also can launch pre-impact starts based on visually observing how the target falls (31). To outperform these competitors, archerfish watch the initial movement of a falling prey – which is typically hit from the side (see **Fig. 1B**) – and then select a turn right to the later landing point (e.g. 27, 32): Additionally, they then dart off with the speed needed to arrive just in time at the right spot (33,34). Because aerial prey is scarce (31), archerfish engage prey over a wide range of distances (30,31) and, hence, must also be able to select the correct turns for a wide range of sighting distances of prey, vertical and horizontal initial speed levels of prey, prey sizes. Archerfish can therefore not use similar simplifications that work so well for hoverflies (26). Indeed, the fish can decide correctly even when these variables were varied independently from each other over large ranges (27,35,36). Of key importance for our present study is this interesting property of the decision (**Fig. 1C**): Suppose a prey object is made, by a stream of air from a blastpipe (32), to slide on top of a glass plate. Faced with such a condition, hunting archerfish do not follow the trajectory of the object. Instead, they still make their turn decisions just as they would in absence of the glass plate and accurately turn towards the ballistic landing point predicted from the initial height, initial direction and initial speed of the sliding target (32). It should be stressed and will become clear in the results, that the accuracy of the turn decisions allows to decide whether the turns were to the ballistic landing point, or another point, such as the last sighting of the fly before turn onset (32) or the landing point appropriate for another estimate of initial height (35, 36). The glass plate experiments therefore show that the turn decision maps the initial values of motion of prey to the turn the fish must make and that this mapping is appropriate for ballistically falling objects. In these experiments prey movement was independent of shooting, and it turned out later, that the decision does not use prior information – i.e. on target position, height, timing of motion onset – that would be available already before prey starts its trajectory. This was tested in experiments in which size, initial position and height were changed relative to what the fish could have expected (27,35,36). Furthermore, accuracy and latency of the turn decisions are independent from viewing distance and the orientation of target motion relative to the responding fish (35,36).

Although exhibiting impressive features, the ‘machinery’ behind the turn decisions could still be hardwired and somehow restricted to work only for ballistically falling prey, such that any combination of input variables (speed, direction, height) and coordinates of the fish leads to the turn that would be appropriate for an object that falls ballistically. Such a hardwired mechanism could be acquired over the course of evolution or could be acquired or refined during early ontogeny. But once established it would be fixed. This would explain why all archerfish studied in our lab during the last 20 years consistently showed robust turn decisions to ballistic landing points (27,31–37). It would also be according to what intuition suggests: reflex-like speed should exclude any further flexibility. Finally, smaller prey is completely wetted by the shots and falls ballistically (32,35), so that no further flexibility would seem to be required, ecologically, from the decision. However, as we report here, adult fish readily learn to extract a new artificially introduced rule of how to connect the three independent input variables to the rewarded turns and to drive their high-speed decisions according to this new rule, ignoring all their ballistics. Moreover, they re-programmed their decisions in surprisingly clever ways that not only allowed them to immediately generalize their high-speed decisions to untrained constellations but even to simultaneously handle two different rules.

Results

A technique to substitute ballistics with a new rule

If the archerfish's high-speed decision was hardwired it would fail after a sudden and drastic change in how the rewarded output needs to be linked to the input. Adult archerfish would then continue to turn to the appropriate ballistic impact point and would have to learn ways to correct their error later on their approach path. Testing this idea requires a way to exchange the ballistic rule with a new rule and to reward the fish at locations that are dictated by this new rule. The responses to purely planar movement (33) (**Fig. 1C**) and the independence of the decision of the shooting context (28,33,36) suggests that LCD monitors could, in principle, be used to provide all information archerfish use for their decision. To reward the fish at defined virtual impact points without additional confounding motion cues we developed automatic feeders that can deliver food according to experimenter-defined rules of how to link place and time of food delivery to initial target motion (**Fig. 2A**, Fig. S2). However, these feeders are prominent landmarks and as such could substantially affect the decision. Surprisingly, a series of experiments showed, that the feeders did not influence the decision at all and that it was still exclusively based on initial target motion (**Fig. 2B**). Operating the feeders without showing motion on the screen failed to elicit decisions. Most importantly, when we created a direct conflict between aiming at a feeder or aiming at the ballistic virtual impact point (VIP) – appropriate for the fish's position and the target's movement – then the fish aimed at the VIPs, not at the feeders even though this was where they got their reward (**Fig. 2B**; 15° offset, n=353; 30° offset, n=198). Moreover, because these tests were conducted so that the VIPs also differed relative to other potential landmarks in the tank, the fish were also not starting to positions marked by landmarks other than the feeders. In other words, even in presence of the feeders the decisions were only based on the movement information on the screen and were not influenced by cues from the feeders or from any other landmarks in the tank.

Using this system, it should be possible to achieve our aim of presenting fish with novel non-ballistic rules that connect the input variables and the rewarded locations. However, one problem remains that needs to be examined: Given the short time in which the fish must respond and given the comparably low frame rate of the screen, it is unclear whether the decisions made with target movement displayed on the LCD screens would be comparable to those made with real prey. For instance, they could be much less accurate, be slower, or only possible from specific positions. To address this, we directly compared the performance of the same fish in response to either real falling objects (see Movie S1, that illustrates the speed of the response) or to the VR stimuli (Movie S2). Given the limitations in the temporal resolution of the screen it is striking that no aspect of the decisions was statistically different under real and virtual conditions (**Fig. 2C-G**, n=101 (real) and n=85 (virtual) starts). The accuracy of the aim achieved at the end of the turns was not statistically different (p=0.506, Mann-Whitney; **Fig. 2C**) and always minimized the error to the impact point, regardless of whether it was real or virtual (difference from zero: p=0.903 (real), p=0.455 (virtual); One-Sample Signed Rank) with no significant difference in variance (p=0.589, Brown-Forsythe). The turns also had the same latency (p=0.297; Mann-Whitney; **Fig. 2D**) and kinematics (**Fig. 2E**: no difference in bending duration under real and VR conditions, p=0.071, Mann-Whitney). Furthermore, responses came from a large range of orientations and distances and, most importantly, were equally accurate across all distances (**Fig. 2F**) and turn sizes (**Fig. 2G**). In summary, despite the limited frame rate of the screens and despite the need to use feeders in most presentations, the turn decisions can be studied exclusively with virtual stimuli. This sets the stage for now changing the relation between initial movement and the rewarded virtual landing point.

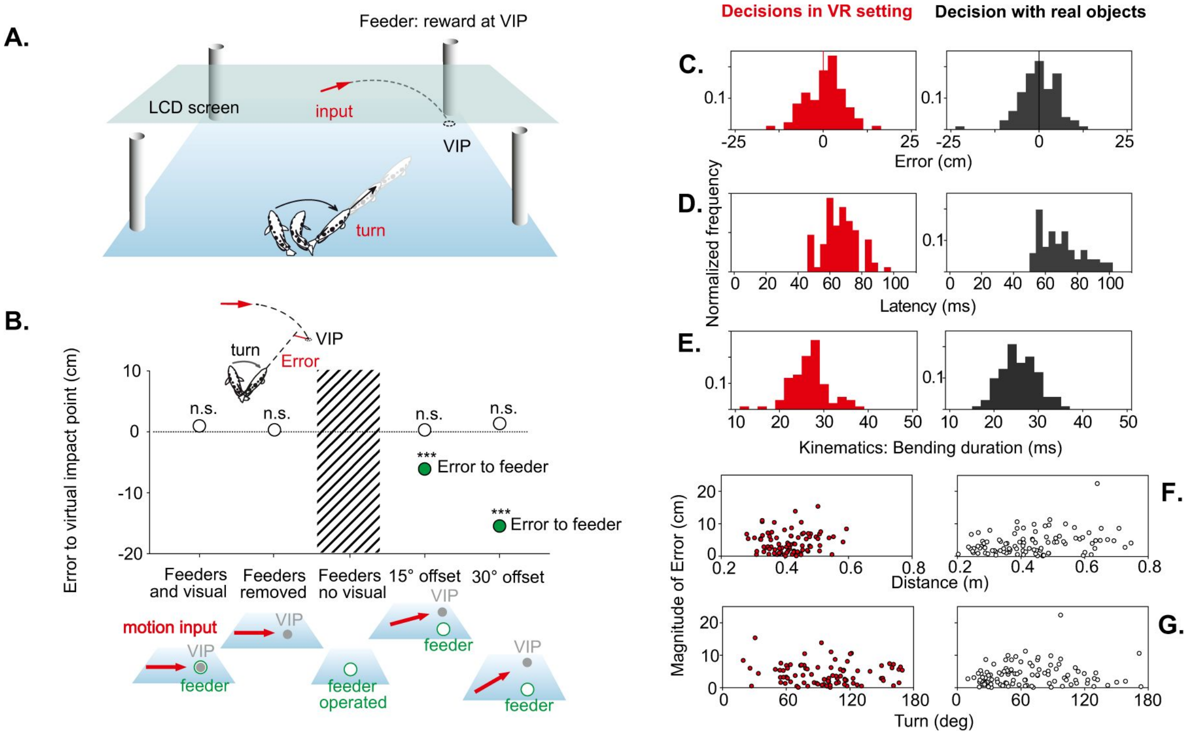

Fig. 2.

Establishing a technique that allows to replace ballistics.

To define arbitrary relations between input and the rewarded turns, as required in this study, we first established and calibrated a virtual reality approach. (A) Sketch of setup with motion presented on a screen and feeders operated to deliver food in time at the VIPs. Evidence that the decisions are not influenced by the feeders: Same errors to VIPs with feeders present or absent. Inset illustrates how error of turn decision is defined (for details see Fig. S1). No rapid turns are elicited when feeders are operated without movement on screen. When motion directions are chosen to create a conflict between turning either to the VIPs or to the feeder positions (offset either 11.2 or 22.3 cm), then turns minimized the error to the VIPs but not to the feeder positions. Data are represented as medians. (C-G) Comparing the nature of the turn decisions with real and virtual impact points. Same errors (C) to virtual and real impact point, same latency (D), same kinematics (E, duration of bending phase). (F, G) Under virtual conditions accurate starts are also possible across wide ranges of distance (F) and turning angle (G). See Figs. S1, S2, Movies S1, S2 and text for detailed statistics. ***p<0.001, n.s. not significant.

Challenging the decision with a new rule to link input and rewarded output

The setup now allowed us to study the decision after a systematic change in the rule that connects the input and the turn the fish needs to make to reach the virtual impact points. **Figure 3A** introduces the model behind the new rule that we used to generate a systematic large deviation of 14.9 cm between the new VIPs and the previous ballistic VIPs. After having travelled straight throughout what was the previous decision time, the direction was deflected by 39.8° (to the left, s. Methods) and the reward was now presented at the appropriate 'deflected' VIP. Hence, there is still a fixed relation between the input (initial target movement on the screen) and the rewarded point on the water surface. However, the relation is now different from the one the fish had previously been using. It is important to stress, that learning to turn according to this new rule is not at all simple and would require the fish to learn non-trivial corrections (**Fig. 3B**): Relative to the turn they previously made for a given input constellation they would be required to add a correction that is not constant but depends sensitively on the position of the responding fish. This map of corrections is, furthermore, not the same for all input constellations but a new map as in **Fig. 3B** is required for each combination of input variables (as illustrated in Fig. S3). The predictions were clear for adult archerfish: should the decision use a circuit that was hardwired to ballistic movement, then the fish would never be able to turn to any other than the ballistic VIPs. The fish would still have several options to efficiently adapt to the situation, for instance by learning how to modify their subsequent approach path, by employing slower responses (not the so-called C-starts employed in the turn decision) or by waiting until the deflection had occurred to launch their turns only after that time. But they would not be able to adjust their rapid turn decisions.

We now exclusively challenged the fish with the deflected trajectories and the new rewarded VIPs over the course of several weeks. During this time, we continuously sampled occurrence and error of all turn decisions (**Figs. 3C**, D). Interestingly, the fish never ceased to respond with their characteristic turns (response probability remained constant at all stages, p>0.08, Pairwise Chi-square) of unchanged kinematics and never significantly increased their latency (bending and straightening phase of their C-starts both p>0.28, latency p>0.07; Kruskal-Wallis). Furthermore, their aims were systematically in error for the initial stages of training (deviation from zero error to the deflected VIPs p≤0.001, One-Sample Signed Rank) as we had predicted (**Fig. 3D**; deviation of aim to ballistic VIPs from zero error p>0.06; One-Sample Signed Rank; Movie S3). However, gradually the errors made to the new 'deflected' VIPs became smaller and, after more than 1750 presentations, the turns clearly minimized the error to the appropriate new 'deflected' VIPs (**Figs. 3C, E, F**; Movie S4) and no longer to the ballistic ones.

This surprising finding does by no means show that the decision has now been based on the new rule. For example, the fish could have learned to feed information about the deflection of the prey's trajectory into their unfolding turns and so achieve their new final turning angles. To control for this, we interspersed tests in which the trained fish were only shown the initial linear part (without the deflection) among many presentations with the full trajectory (including the deflection). However, the errors made in the interspersed tests (n=68) were not different from those made when the full trajectories were visible (**Fig. 3G**). This shows that the starts were still only made using the initial motion and did not require seeing the deflection. A second explanation for the new turns would be that the fish could have learned to now take the feeder positions into account. They would then use initial movement merely as an indicator which feeder to approach. To test this, we interspersed tests in which the direction of initial target movement was always either 15° or 72° off from the feeder position and in which, additionally, exclusively the initial movement (before deflection) was shown. Moreover, in these tests feeders were offset both from the ballistic and the new VIPs. Under these conditions, the turns the fish made were still to the new deflected VIPs (differences from zero error p=0.721 at 15° (n=197) and p=0.113 at 72° (n=145); One-Sample t-tests) but not to the feeders or to the ballistic VIPs (Kruskal-Wallis: pσ0.001 at 15°;

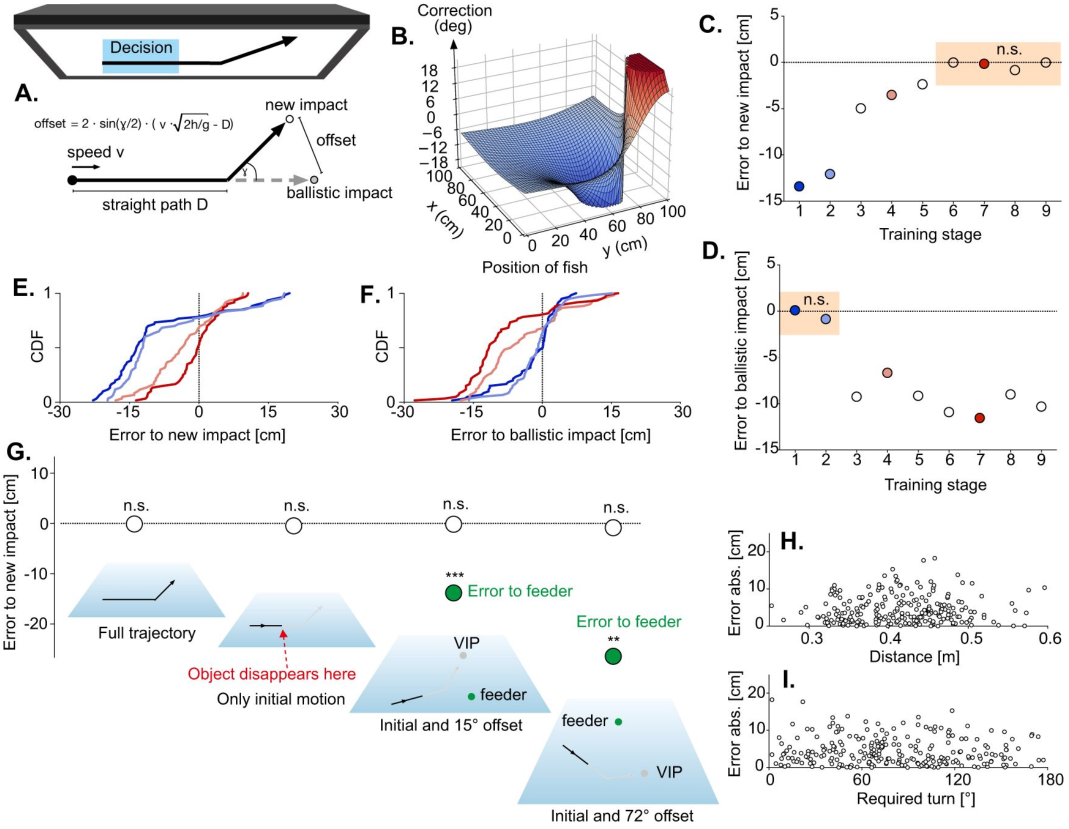

Fig. 3.

The high-speed decision learns a new rule of how to connect input and output.

(**A**) Sketch of how we employed virtual reality to challenge the fish with a new rule of how they must turn based on initial target motion. For all objects shown, the trajectories were always straight during the time it takes the fish to make its decision but then were deflected as illustrated in the inset. Reward is then given at the corresponding new 'deflected' impact point but no longer at the ballistic impact point (that ballistics would predict from the initial movement). (**B**) Despite its apparent simplicity, learning to adjust the turns to this type of motion would require non-trivial corrections to the previous (ballistic) turning angles. These corrections depend on the fish's initial position. Furthermore, a different such map would be needed for each set of input variables (see Fig. S3). (**C**, **D**) Median errors of the turns relative to the new and to the old ballistic (**D**) VIPs in successive training stages 1 to 9 (each with 60 evaluated turn decisions), with food always delivered at the new VIPs. Colored background highlights when turns were oriented towards the new (**C**) or to the old ballistic (**D**) VIPs. (**E**, **F**) Cumulative density functions (CDFs) at the stages indicated by colored circles in (**C**, **D**) to show how systematic error to the new landing point decreased (**E**) whereas errors to the ballistic landing points increased (**F**). (**G**) Errors made in several critical tests – from left to right: Deflection of trajectory visible and feeders present at the new VIPs (n=240), interspersed tests with only the initial straight trajectory (but not the later deflection) shown (n=68), interspersed tests (rate: 1 in 8) with also only the initial straight trajectory shown but direction of motion offset from the direction of the feeders by either 15° (n=197) or 72° (n=145) to create a clear conflict between turning to the feeder position or to the VIP derived according to the new rule. These controls show that the fish had indeed learned to base their turn decision on the initial movement. (**H**, **I**) After training the fish were able to again respond appropriately over a wide range of distances (**H**) and required angles of turn (**I**). Data are represented as median (**C**, **D**, **G**). ***p<0.001, **p<0.01, n.s. not significant.

p=0.005 at 72°; Dunn's p<0.05 for comparison of errors; (Movie S5)). Furthermore, the turns made when only the linear initial motion was visible and when the VIPs were spatially offset from the feeders did not differ in latency (p=0.686, Kruskal-Wallis) nor kinematics (bending duration: p=0.242, Kruskal-Wallis) from the turns made without offset and with the full trajectory visible (n=240).

Finally, it is important to stress that the fish had not learned to directly turn to the new VIPs just for a few restricted constellations (**Fig. 3H, I**). Instead, turns came from a broad range of distances and turn angles and across those had equal accuracy. So, the high-speed decisions are, surprisingly, not hardwired to a ballistic relation between the input variables and the turn the fish must make but can be reprogrammed according to a new rule

After restricted training to the new rule the decision immediately generalizes to untrained input constellations

Because all training to the new rule had occurred with targets presented at only one height level it is now possible to run generalization tests to examine the level of abstraction at which the decision was re-wired. Suppose the fish had adjusted its ballistic decision network in a way that can be formally represented as overwriting a 'lookup table'. Such a table (or its mechanistic realization) would store the past successful turn for a given orientation and position of the fish, speed, direction, and height of moving prey. Because training was restricted and had exclusively occurred at only one height level, the previously ballistic 'lookup table' would have been only partially changed and predictable problems should occur in tests with targets presented at untrained heights. For these untrained heights the table would either only contain entries for ballistic motion (prediction A) or the ballistic entries might be substituted with entries that were appropriate at the training height (prediction B) during retraining. Both predictions allow us to calculate the turns the fish would have made when they first encountered targets at untrained height levels (**Fig. 4A**): Turns should either be to the ballistic landing point as appropriate for the novel height level (prediction A). Or, according to prediction B, the turns should be to the 'deflected' landing point but appropriate for the training height and not the actual height level. To test these predictions, we challenged the trained fish with movement that occurred at either higher or lower than the training height. Testing heights were chosen such that the fish would make an error of at least 6 cm relative to one of the predicted points. **Figs. 4B**, C show the error of the first 30 turns made when the trained fish encountered movement at the untrained height levels. The error of the turn is shown relative to the VIP that would be appropriate for the now larger (**Fig. 4B**) or lower (**Fig. 4C**) height level. This error was around zero with no tendency to change over the trials (linear regression: all p>0.1). A detailed analysis of the errors (**Figs. 4D**, E) showed that the turns minimized the error to the deflected landing point at the true (novel) height (difference from zero: both p>0.07; One-Sample t-test), but neither towards the ballistic landing point (prediction A; both p<0.001; One-Sample Signed Rank) nor towards the deflected landing point at the training height (prediction B; both p<0.001; One-Sample Signed Rank). Again, none of the characteristics of the turns were different in the novel situation than in the situation the fish had encountered during training (also n=30 right before these tests): latency: p=0.098, kinematics: p=0.177 (Kruskal-Wallis), variance of errors: p=0.188 (Brown-Forsythe). In other words, the new rule is represented in a way that allowed immediate generalization to the new untrained heights.

We next tested whether the fish would also generalize to new untrained target size and untrained speed levels. **Fig. 4F** shows that after a change in absolute target size, the trained fish still turned to the deflected landing points (differences from zero error: each p>0.4, One-Sample Signed Rank; difference in aim before and after size change p=0.764, Mann-Whitney) and the error distributions were not significantly different (difference in distributions in **Fig. 4F**: p=0.278, Brown-Forsythe). This shows that learning the new (deflected rule) did not involve simplifying *a priori* assumptions about the absolute target size. Learning also allowed immediate generalization

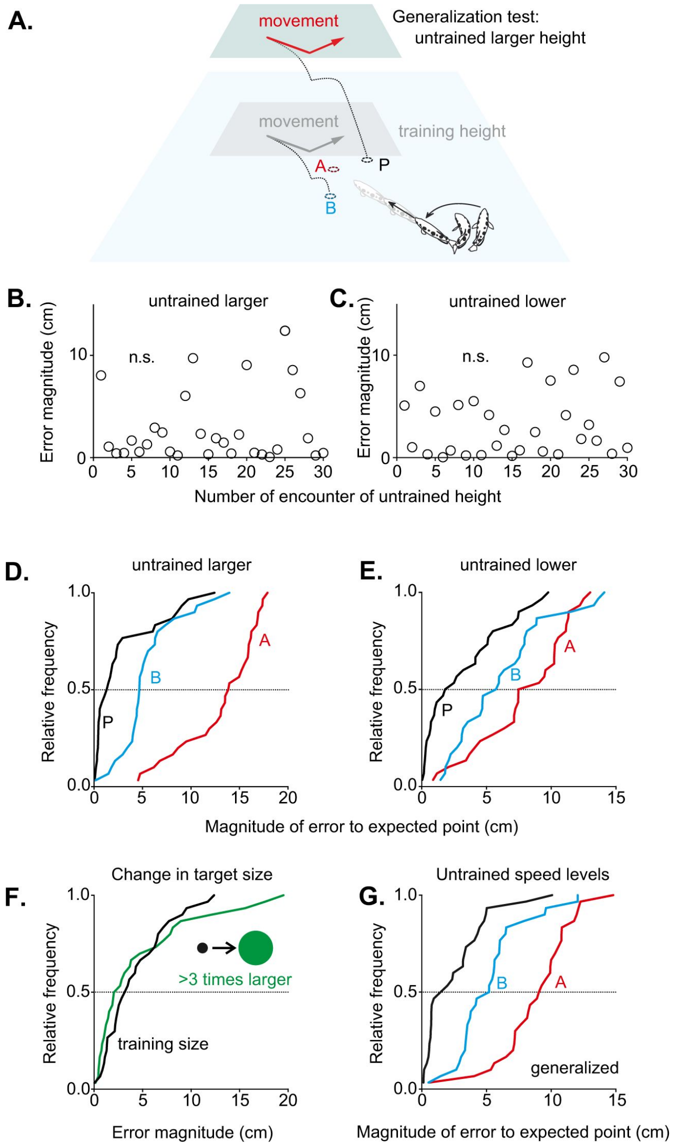

Fig. 4.

The new rule is represented in a way that allows immediate generalization.

(A) Idea for generalization tests that are possible because training to the 'deflected' trajectories (**Fig. 3**) was only for one level of target height. When faced with a larger than training height, fish would only be able to turn to the corresponding impact point P if they had acquired a more general rule of how to connect input (height, direction, speed) to the rewarding turn. Had they only substituted those input-output experiences that were no longer rewarded, then they should still use the previously rewarded input-output relations and turn to the ballistic impact point A (red, see text). Had they substituted the old input-output relation for all height levels with the trained new ones, then they should turn to point B (blue, see text) that would be appropriate for the training height. (**B**, **C**) When the fish were shown the deflected trajectories at larger (**B**) or lower (**C**) height than experienced during training, then already their first turns minimized the errors to the VIPs that are appropriate for the new rule at the untrained new height level with no indication that errors would initially be large and then decrease. (**D**, **E**) Closer analysis, using cumulative density functions (CDFs), for the errors made to the predicted points based on hypotheses introduced in (**A**), both for the larger (**D**) and for the lower (**E**) height (both n=30). (**F**) Evidence that learning the new rule had not used prior assumptions on target size. Errors of the turns made in the first 30 tests in which absolute target size was more than three-fold (13 mm) than the target size (4 mm) encountered throughout the training to the new rule. (**G**) CDFs of the first 30 tests with a low target speed (1.425 m/s) that the fish never encountered during training to the new rule. Again, the fish immediately chose turns to minimize the error to the predicted VIP based on the new rule but not the error to the points predicted by hypotheses A and B.

to a lower target speed, that was not encountered during the training period. To examine this, tests with lower target speed were interspersed among presentations in which targets had the same speed as during training. **Fig. 4G** shows that already the first 30 turns minimized the error to the deflected VIP for the actual speed (difference from zero p=0.102; One-Sample t-test). They also did not minimize the error to the VIPs given by either ballistics (analogous to prediction A above; p<0.001; One-Sample Signed Rank) or by using predictions for the deflected VIPs that are based on the trained speed levels (analogous to B; p<0.001; One-Sample Signed Rank). Comparing latency, bending duration and the variance of errors (speed: last 30 presentations with only training speed; size: last 30 presentations with target size during training) showed that changing target size and speed also did not affect the nature of the turns. Latency and bending duration were not significantly different in the changed versus training conditions (At novel untrained speed: Latency: p=0.280, Mann-Whitney; Stage 1: p=0.076, Mann-Whitney. At novel untrained size: Latency: p=0.305, t-test; Stage 1: p=0.997, Mann-Whitney). Variance was even slightly reduced (and not increased) at the untrained target speed (p=0.021, Brown-Forsythe) and remained unchanged after the increase in target size (p=0.742, Brown-Forsythe). In summary, all tests show that the high-speed decisions had been reprogrammed in much more clever ways than predicted. Rather than independently changing the size of an unrewarding turn for each individual constellation – as would seem required because the input variables themselves are varied independently from each other – learning allowed immediate generalization and thus must have in some way captured the new rule.

The decision can conditionally operate on two different rules

We next asked whether the fish would – at any given time – only be able to use one rule for the turn they must make. To explore this, we randomly showed the trained fish two types of objects that differed in shape: Either (i) a disk that moved on the 'deflected' trajectory and to which they responded with turns toward the rewarded deflected VIPs, or (ii) a 'ballistic' object (triangle) that moved straight and for which a reward was given at the ballistic VIP (**Fig. 5A**). In the experiments, one of the two objects was chosen randomly, appeared in stationary position for 2 s, and then started moving. Initially, the turns made in response to the ballistic object still minimized the error to the deflected VIP (difference from zero error p=0.256; One-Sample Signed Rank; same variance as for disk p=0.343; Brown-Forsythe; first 160 responses to ballistic object) and not to the ballistic VIP point (difference to ballistic landing point: p=0.038, Mann-Whitney; **Fig. 5B, C**). However, after 500 additional starts in response to the ballistic objects, turns were equally in error to the deflected and the ballistic VIPs (p=0.648, Mann-Whitney). During the same period, in contrast, the turns to the moving disks remained perfectly matched to the deflected landing point (difference from zero error p=0.273; One-Sample Signed Rank; **Fig. 5C**). After about 300 additional exposures to the moving triangle the turns minimized the error to the ballistic impact point when the triangle was shown (error to ballistic p=0.836, error to deflected p=0.020; One-Sample Signed Rank; **Fig. 5B**) but continued to minimize the error to the deflected impact point whenever the disk was shown (error to ballistic p≤0.001, error to deflected p=0.084, n=187; One-Sample Signed Rank; **Fig. 5C**).

Next, we interspersed tests that only showed the initial straight paths both for the disk (n=60) and for the triangle (n=187). So, picking either the 'ballistic' or the 'deflected' rule to choose the appropriate turn for a given set of input variables could now exclusively be based on target shape. Again, the turns were toward the ballistic landing point when the triangle was shown (difference from ballistic p=0.075, from deflected p<0.001; One-Sample Signed Rank) and to the deflected landing point when the disk was shown (difference from ballistic p=0.008, from deflected p=0.412; One-Sample Signed Rank) (**Fig. 5D**). Also, latency (p=0.189, Mann-Whitney; **Fig. 5E**) and kinematics of the turns (bending duration; p=0.984; Mann-Whitney; **Fig. 5F**) were the same in the responses to triangles and disks. In other words, the nature of the turn decision was the same, but object shape determined which rule was used to determine the appropriate turn given the input data.

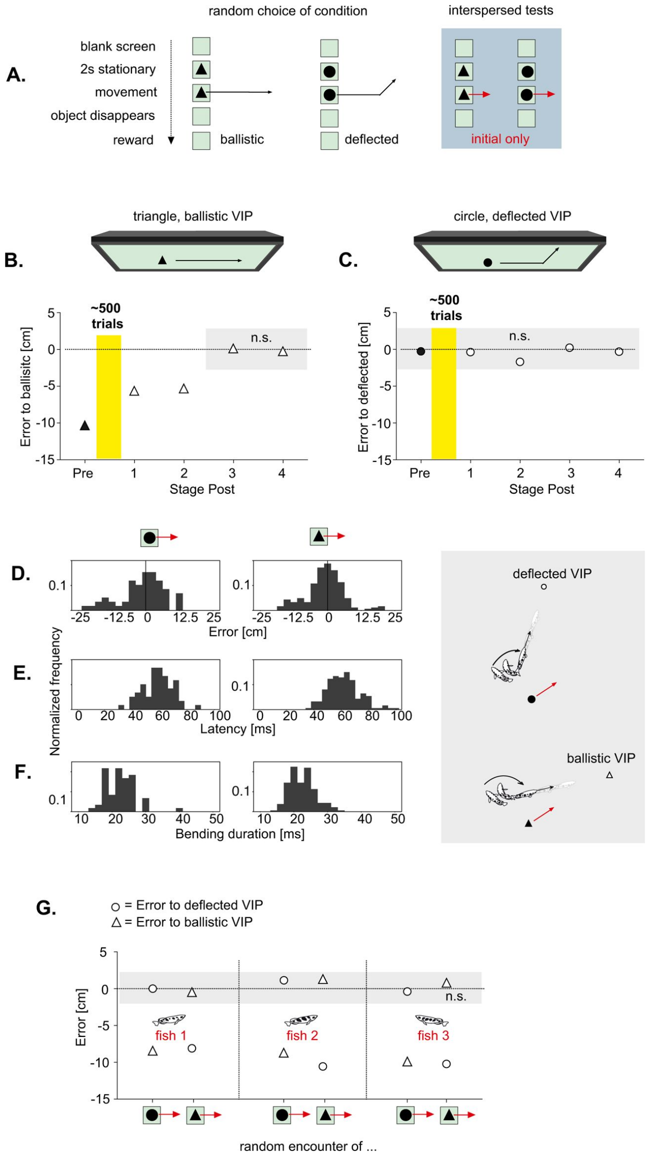

Fig. 5.

The high-speed decision can conditionally use two different rules of how to connect input and output.

(**A**) After successful training to infer the new (deflected) virtual impact points (VIPs), fish were randomly shown either the disks with the deflected trajectories (reward at deflected VIPs) or new non-disk objects that moved on straight trajectories and were rewarded at the corresponding ballistic VIPs. (**B**, **C**) Error in the turns as determined in interspersed tests that only showed the straight initial movement both for non-disk objects (triangle, **B**) and for the familiar disk objects (**C**). Objects were chosen randomly. ‘Pre’ (filled symbols) denotes baseline before fish were exposed to a total of 500 presentations of the non-disk objects with reward at their ballistic VIPs. Errors were determined in subsequent test phases. As the fish changed their turn decision to minimize the error to the non-disk VIPs (**B**) they continued to aim to the deflected VIPs whenever they encountered moving disks (**C**) showing that they had not reversed to generally using ballistics again. (**D**-**F**) Interspersed tests that only showed the same initial linear trajectory but with either a disk (n=60) or a triangle moving (n=187). Graphs on right side to illustrate choice situation as seen from above: For the same set of input variables (speed, height, direction) the fish must turn to the ballistic VIP when a triangle is moving, but to the deflected VIP when a circle is moving. Detailed analysis shows that turns were appropriate to the correct VIP with no difference in error (**D**), latency (**E**) or kinematics (bending duration) (**F**). (**G**) Individual fish were able to select the appropriate turn to the deflected VIPs when encountering moving disks and to the ballistic VIP when encountering triangles. Aims were equally appropriate and did not differ in variability. Data are represented as medians (B, C, G). n.s. not significant.

In principle these results could have arisen rather simply: some fish could have only responded to disks and others only to triangles, so that no single given individual would have to be able to handle both rules. Analyzing the contributions of the individuals showed that this was not the case, and that individual fish could respond appropriately to both conditions (**Fig. 5G**). Each of the three individual fish that contributed most starts had minimized the error to the appropriate VIP (difference from zero error always p>0.1; One-Sample Signed Rank) and the variability of the errors in applying the two rules did also not differ in any of the individuals (p=0.966; Brown-Forsythe). This shows clearly that individual archerfish are indeed capable of simultaneously operating on two different rules – one for each type of prey – of how to connect the input with the turn the fish must make.

A shape cue is needed to select which rule to use but is not needed prior to target movement

The previous series of experiments presented target shape as a potential prior that would inform the decision circuit as to which of the two rules will have to be used when target movement starts. To test whether it was necessary to instruct the circuit prior to the onset of motion, we interspersed tests in which the target object (disk or triangle) revealed its shape only at motion onset (**Fig. 6A**). In each of these interspersed tests, first a neutral shape (a cross) appeared, remained stationary for 2s, and then was exchanged randomly with either the moving triangle or the moving disk. Moreover, only the straight initial path was shown so that shape after motion onset was the only cue for the decision on whether to choose a turn based on the 'ballistic' or the 'deflected' rule (Movies S6, S7). Under these conditions, the decisions remained appropriate to the ballistic (triangle; n=109 starts) or deflected (disk; n=46 starts) VIPs (difference from zero error to appropriate VIP: p=0.451 (triangle) and p=0.671 (disk); p ≤ 0.001 for the alternatives, all One-Sample Signed Rank). Furthermore, also the distribution of errors was not statistically different from those obtained with the shape cue available 2s prior to the decision (p=0.269; Brown-Forsythe). Hence the information that is needed to select which rule is to be used can also be extracted in the very brief interval (as little as 40 ms) after motion onset, together with all other decision-relevant information but does not need to be available to instruct the decision circuit before target movement starts

Discussion

In their turn decisions, archerfish quickly observe the initial values of motion of falling prey and then turn at the ballistic landing point. When shown purely planar initial movement they still turn at the 'virtual' ballistic landing point, that specific point at which ballistics predicts it to impact at the water surface. Based on this property we established a paradigm that allowed us to exchange the rules of ballistics with a new one. In this paradigm, the fish can no longer use ballistics to connect the independent variables (height, speed, and direction) with the appropriate turn towards the point of reward. However, a new rule exists that would, in principle, allow the fish to connect the rewarded turn to the combination of input variables. We show here, that, against all expectations the fish learn to extract the new rule in a way that allows them immediate generalization to variable combinations (such us untrained new height levels) they have not been trained with. The reprogramming of the decision does not change its kinematic aspects or latency, showing that the high-speed decisions were indeed reprogrammed when a new relation exists that connects prey initial movement with the point of reward.

Our findings demonstrate that reflex-like speed of a decision does not rule out the involvement of learning and of cognitive aspects that are seen in much slower decisions and in behaviors shown at more than 100 times lesser speed. They thus provide a counterexample against everyday wisdom that what occurs at reflex speed must be a simple reflex and limited to fixed or mapped inputs and responses. Although we demonstrate this in a highly specialized decision that has

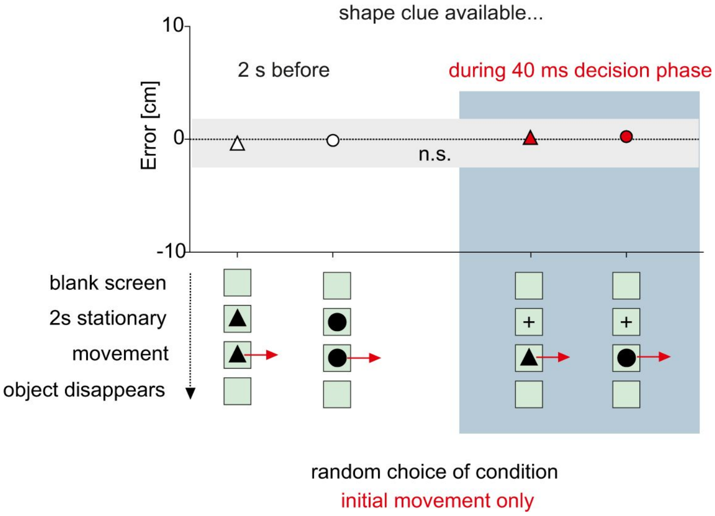

Fig. 6.

The clue indicating which rule to use needs not to be known before target movement starts.

Schematic illustration of the idea to examine whether shape information was required before movement information, giving the decision circuit some time to adjust which rule was subsequently to be used. Either the randomly chosen object or a neutral symbol (cross) was shown for 2s and then the chosen object (disk or triangle) started moving. In all tests, only initial straight movement was shown. Errors were the same, regardless of whether object identity was revealed before or only during the very brief decision time (see Movies S6, S7). Data are represented as medians. n.s. not significant.

evolved under particularly heavy constraints, it is not unlikely that similarly flexible and adaptive decisions at reflex speed might be much more widespread. They may be common in hunting contexts and employed whenever flexibility and the efficient re-programming of rapid decisions would be a major advantage. Nontrivial elements of flexibility could also be part of rapid perceptual decision making. Given the high temporal precision obtainable in urgent perceptual decision-making (5,17) using this approach to compare cognitive aspects in the 'classic' slower (38,39) and in rapid (3,5) perceptual decision making could be very rewarding.

Comparing cognitive aspects of high-speed decision making with those of much slower behaviors

We would like to stress that none of the cognitive aspects we describe here is in any way simpler when compared to its slower versions. The capacity to generalize efficiently has been demonstrated in the ability of archerfish to learn of how to engage moving targets and to judge their absolute size (40,41). Also, recognizing and assigning a value to different visual shapes has been demonstrated much earlier (38,42). Such aspects are also not unique to archerfish and have been described across the animal kingdom, including animals with far smaller brains (43–45). What is surprising is, of course, clearly not to find cognitive features in archerfish, but rather to find such features of similar complexity in a decision that is made at reflex-like speed on a timescale of milliseconds rather than one of minutes. Also, the complexity of the task that the fish needs to master to conditionally adjust their high-speed decisions is certainly not easier than what otherwise is considered a demanding task in many laboratory animals (e.g. 46-48). Here a much-studied task is to have an animal under condition 'a' respond to a stimulus 'α' by turning left and right to stimulus 'α'. Under condition 'b', however, the animal must do the opposite and turn right to stimulus 'α' and left to stimulus 'α'. In comparison, the task the decision circuit of archerfish handles in just 40 milliseconds is not only to conditionally switch between two motor outputs but to switch between two rules of how to connect a three-dimensional set of input data to a continuum of possible motor outputs.

Highspeed decision-making and circuit size

The plausible assumption that reflex-speed implies reduced complexity and flexibility (48,49) could have a similar foundation as the assertion that smaller brains have reduced cognitive capacities. Substantial progress has been made on this latter assertion, resulting in an impressive and continuously growing list of sophisticated cognitive features that were found in animals with bird-and fish brains (50–52). Most impressively, it has been demonstrated that insect brains that operate with a millionth of the neurons of a human brain can handle a wide range of cognitive tasks (43–45,53–55) that even include social or observational learning (55–57). The reflex-speed of some decisions also will likely limit the size of the underlying neuronal circuitry, allowing only a subset of the brain to be used, a subset that could be considered a 'functional mini-brain'. The assertion that everything that is fast as a reflex must be simple might therefore suffer the same fate as did the assertion that brains with fewer neurons must be simple. In the turn decisions of archerfish, specifically, it is known that the kinematics of their turn decisions is completely equivalent to the so-called C-starts that the fish produce to escape (37). Both the turn decisions (also called predictive C-starts) and the escape C-starts are among the fastest C-start known in fish (37) and so at least the motor circuits are restricted to the well-known powerful hindbrain circuits of a comparably small number of tractable numbers of neurons, organized around the giant Mauthner neuron (59–62). Its rapidly conducting axon would be suited to initiate the turn but it would clearly have to work together with other hindbrain neurons to account for the precision and adjusted straightening speed of the fish (37).

Ecological considerations: Why did archerfish evolve flexible highspeed decision-making?

The rapid and precise turn decisions are required because of severe competition with other surface-feeding fish that are not only more numerous – and therefore likely to be closer at the landing point of the archerfish's prey – but that are also capable of using vision to guide pre-impact turns and therefore need to be outperformed (31). We suggest that the flexibility of the archerfish's decision was required because of scarcity of aerial prey. If the fish must catch whatever scarce prey is available and if they are in serious competition with other fish, then they must address the following problem: While small prey will be completely wetted by the archerfish's shot and will fall ballistically (32,35), larger prey will face considerable frictional forces and – all other conditions being equal – will therefore not fall equally far. Simply using a hardwired circuitry for ballistically falling prey also for such larger prey would produce wrong initial turns which would require subsequent corrections that cost precious time and might mean losing the advantage over the competitors. The flexibility we found and the ability to operate on two types of prey that fall according to two different rules would allow archerfish to efficiently handle two types of prey that co-occur during a specific time of the year. When one is replaced by another type of prey with yet another way in which its initial movement relates to its impact point, the decision will be able to adjust, again, to the new prey type, being still able to accurately respond to the prey that remained. So, we suggest that the ecological constraints and the severe competition that archerfish face in the wild could have favored the evolution of a decision that needed to go right to the limits of combining speed, accuracy, and flexibility.

We hope that our findings, obtained in a special case, stimulate further studies to better understand whether there is a cognitive divide between slow and fast decision making. If no such divide could be found, then this would be a strong signal to exploit rapid and flexible high-speed decisions in technological applications.

Materials and methods

Animals

Experiments were conducted on a group of six adult archerfish (*Toxotes chatareus*). The experimental fish were randomly chosen from a larger group of (male and female) archerfish. They had been imported from Thailand (Aquarium Glaser, Rodgau, Germany) and were kept at 26°C on a 12h:12h light/dark cycle in the large experimental tank (1m x 1m x 0.6 m, filled to a height of 34 cm) in brackish water (3.5 mS cm^{-1}). All procedures were conducted in accordance with the German Animal Welfare Act (Tierschutzgesetz) as well as the German Regulation for the protection of animals used for experimental purposes or other scientific purposes (Tierschutz-Versuchstierordnung) and were approved by the government of Lower Franconia (Regierung Unterfranken, Würzburg, Germany).

General setup

To facilitate recording the fish from below – through the transparent bottom of the tank – together with the visual stimuli shown on the LCD monitor above, the tank was placed on steel frame stands, 110 cm above ground. A construction made from 3 cm aluminum profiles (item Industrietechnik GmbH, Germany) was used to hold the monitor on top of the tank as well as to position the feeders that were employed to reward the fish. To increase the contrast in the recordings of the fish, three conventional 500 W halogen floodlights, located around the tank, illuminated a square of white cloth (2x2 m) that was placed parallel to the water surface and 106 cm above it.

Monitors and stimuli

To present visual stimuli a 27’’ LCD monitor (Samsung SyncMaster S27A750D, 120 fps, max. intensity 300 cd/m^2) was used. A glass plate in front of the screen protected it against shots of brackish water fired by the fish. All objects used in this study had a Michelsen contrast of 0.96, as determined with a luminance meter (LS-110 Konica Minolta Sensing Europe) under the conditions of the actual experiments. The surface of the LCD monitor was parallel to the water surface, h = 28.8 cm above it and its center was directly above the tank’s center. Stimuli were generated using Apple Motion 5.4.7 and stored as 120 fps FullHD ProRes movies. We presented the movies with the presentation mode of Quicktime Player 10.4 (Apple Inc.) running on a MacPro 5.1 under MacOS 10.11. An AMD 4GB Radeon RX 480 graphic card produced the monitor signal and was connected via DisplayPort with the LCD monitor. Unless stated otherwise each presentation started with an object appearing in the center of the screen. After 2s the object starts moving in one of four pre-assigned, randomly chosen directions. Objects displayed were either disks of 4 mm diameter, disks of 13 mm diameter or equilateral triangles (height 13 mm). Objects that mimicked ballistically falling motion moved at a horizontal speed v_h of $v_h = 1.775$ m s^{-1} and their virtual landing occurred 243 ms later, after a (virtual) path of 43 cm. On the screen, the movement of the ballistic objects was visible for 116 ms. Objects on the ‘deflected’ trajectories moved at the same horizontal speed but changed the direction of motion after being visible for 116 ms (or 20.5 cm) at a (virtual) time of 118.7 ms (i.e. after an initial straight path of $d_r = 21.1$ cm) by an angle $\gamma = 39.8°$ to the left (see **Fig. 3A**). They were then visible for a further 58 ms. The systematic change in direction causes the virtual impact point, the one inferred from initial motion, to be systematically offset by $o = 2*(v_h*\mathrm{sqrt}(2h/g) - d_r) * \sin(\gamma/2)$ where h is the height at motion onset, g is the gravitational constant and d_r is the distance travelled prior to the change in direction. In our experiments we selected conditions so that the offset was large enough (14.9 cm) to allow us detecting any errors in the bearing of the C-starts. In critical tests only the straight initial movement (i.e. the initial path of 20.5 cm) was shown both for ballistic and deflected trajectories.

Stimulus-coupled feeders

Unless stated otherwise 4 feeders were typically present. During training they were located at the places of possible virtual impact points. In many tests, the virtual impact points were systematically offset from the feeders by changing the direction of motion shown on the screen. This way the virtual landing points differed both from the position of the feeders as well as from the position relative to landmarks the fish might have been using during training. The feeders were custom-made (Fig S2A) and consisted of a tube (2 cm inner diameter, 44 cm long) with a sliding plate inserted 20 cm below its upper end to block the passage of a food pellet (one half of a Sera Cichlid stick). Each feeder was equipped with an electromagnet that can retract the slider to allow the passage of the pellet. All feeders were pre-loaded at random intervals before the tests, using a funnel (6 cm diameter) on the top side of each feeder (Fig S2A, B). Which of the four electromagnets was activated at which time was defined by trigger pules on the channels of the audio track of the movies shown on the LCD monitor so as to achieve an impact of the pellet at the virtual impact point and after the expected time T=sqrt(2h/g) of falling (h=initial height, g=gravitational acceleration). Four audio-channels were used to control which of the four electromagnets was to be activated. To achieve the required number of channels we connected an AV-receiver (Sony STR-DH520) to the computer via HDMI. Four output channels of the AV-receiver delivered the trigger signals to a custom-made amplifier which activated the electromagnets by means of a 200 ms pulse (12 V/300 mA). An additional audio channel triggered a pulse generator (TGP110; TTi, England) that started the high-speed video recording.

High-speed imaging

All recordings were made from below through the tanks transparent bottom (Fig S2C, D). A Fastcam MC2 color 500 (Photron, Japan) high-speed camera, operated at 500 fps (512 x 512 pixels) and equipped with a CVM0411ND f1.6/4.4-11 mm lens (Lensation, Germany) was used to monitor the complete tank. The pre-trigger option of the camera was always used and set to record 4 s before and 4 s after the trigger signal (i.e. the onset of target motion). An Apple Macbook Pro 6.2 running Microsoft Windows 7 controlled the setup via Fastcam viewer 332. Recordings were stored as Quicktime movies and contained the appearance of the object on the screen, its motion, and the impact of food delivered by one of the feeders.

Discriminating the responses of individual group members

Long-term studies of the archerfish start decisions require the competition in a group (27) and cannot be carried out in fish kept individually. It is therefore important to find ways to identify the individual group members that contributed a decision. Here, individuals were recognized based on the contour of their frontal body as seen from below. A template-contour was used for each individual fish that was drawn starting at one pectoral fin, over the snout and ending at the other pectoral fin. Respective images of the high-speed recordings were extracted using Image J (63) just when the straightening phase (so-called stage 2) of the so-called C-start (Fig. S1A) that leads to the turn was terminated. Note that the turns are made right below the water surface and thus in a fixed distance from the camera. The contours taken at the end of stage 2 were specific for each individual fish in the experimental group. Discrimination performed this way coincided with one obtained independently by additionally recording the responses from the side (Photron Fastcam MC2 color 2K, equipped with same type of lens and also operated at 500 fps, view orthogonal to the front side and 50 cm away from it) in which the individuals could be identified by means of their individual stripe-patterns.

Natural stimuli used to test the virtual reality approach

A non-transparent platform (4.5 cm in diameter) was mounted above the center of the tank. This allowed to blow food pellets off the platform from a similar initial height as the virtual objects shown on the screen. Movement was started by an airflow (as in 27,35) and pellets fell ballistically toward the water surface.

Quantitative analysis of the turn responses

Image J 1.50 (63) was used to analyze the recorded videos. To ensure that the decisions could not be influenced by those of other fish, we exclusively analyzed the turn of the fish that responded first in a given test. Latency was the time between motion onset and onset of the C-start. Error in aim relative to a point of interest (e.g., the virtual impact point) was determined from the last frame at the end of the straightening phase (stage 2) of the C-start, i.e., before the fish actually starts its approach trajectory (Fig. S1A). Orientation of the fish’s rigid front end was determined from the tip of the snout and the point midway between the two pectoral fins. If a line drawn along this orientation did cut a second line drawn between the start of motion and the point of interest (e.g., the virtual impact point), both projected to the water surface[4], then the error was defined negative, otherwise it was defined positive (Fig. S1B). Additionally, we determined kinematic aspects of the C-starts, such as the duration of its first bending stage (stage 1 of the C-start), the mean angular speed (i.e., turn angle / turn duration), and the initial orientation and distance the fish had before the onset of their C-start. Only C-starts that were completed before prey impact were included in our analysis, to ensure that the C-start could not have used information from the impact of the food pellets. Furthermore, only C-starts were included in which the subsequent approach path of the responding fish was not blocked by another fish. When aims had to be evaluated relative to two points of interest (e.g., virtual landing point and feeder position) then (i) both possible approach paths had to be free and not blocked by any group member and (ii) the responding fish had not to be oriented in a line with both points. Analysis of the coordinates and frame numbers determined with ImageJ was carried out using Excel (14.0 & 15.11).

Statistics

Statistics were run on GraphPadPrism 9.5. (GraphPad Software, USA). Tests referred to in the main text were as follows: Normality of data was assayed using Shapiro–Wilk tests. For single data sets (error of aim), difference from zero was checked using One-Sample t-tests (parametric) or One-Sample Signed Rank tests (non-parametric). Comparisons of two data sets (latency, duration of stage 1, error of aim) were performed with unpaired t-tests or Mann-Whitney tests. Comparisons of three or more data sets (latency, duration of stage 1, error of aim) were performed with Kruskal– Wallis One-Way ANOVA on ranks, post hoc tested with Dunn's method (non-parametric). Equal variance between data sets (error of aim) was checked using Brown-Forsythe tests (non-parametric). Stability of aim was tested using linear regression. Tests for changes in C-start probability employed pairwise comparisons with χ^2 tests. All respective tests were performed two-sided. ***p<0.001, **p<0.01, n.s. not significant. All data are available in the main text or the supplemental information.

Acknowledgements

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Supporting Information

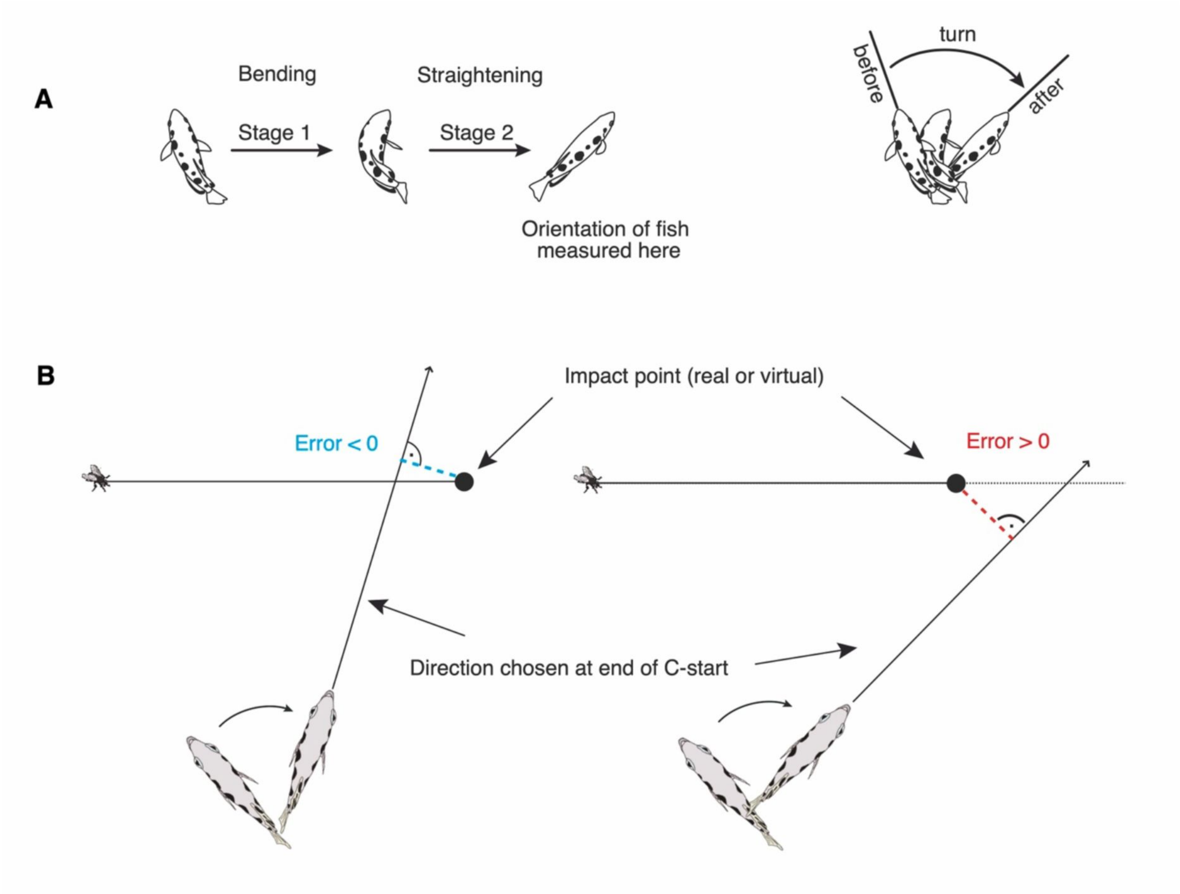

Fig. S1.

Analyzing the accuracy of the turn decisions.

Related to **Figs 1** and **2**. (A) Illustration of the two phases of the C-start that produced the turn: Initial bending into the shape of a letter ‘C’ (so-called stage 1 or bending phase) and subsequent straightening phase (so-called stage 2) at the end of which the aim of the fish is measured and the accuracy of the turn is evaluated. The maneuver takes place directly below the water surface and turns the fish from its previous (before) to its appropriate new (after) orientation, typically to the later landing point of falling prey. (B) Quantifying the accuracy of the C-start decision: As soon as the fish is straightened (end of stage 2 of the C-start), the orientation of its stiff front end is determined (using the bases of the two pectoral fins and the tip of the snout as markers). A line is then considered along this orientation and the minimum distance from the later landing point (real or calculated virtual) is determined. In case the line determined by the orientation at stage 2 cuts the projection of the (real or virtual) trajectory before the landing point, the error is defined negative, and positive otherwise. Note that this definition is needed to allow a direct comparison of the errors made across all possible initial distances and orientations of the responding fish relative to the various prey trajectories.

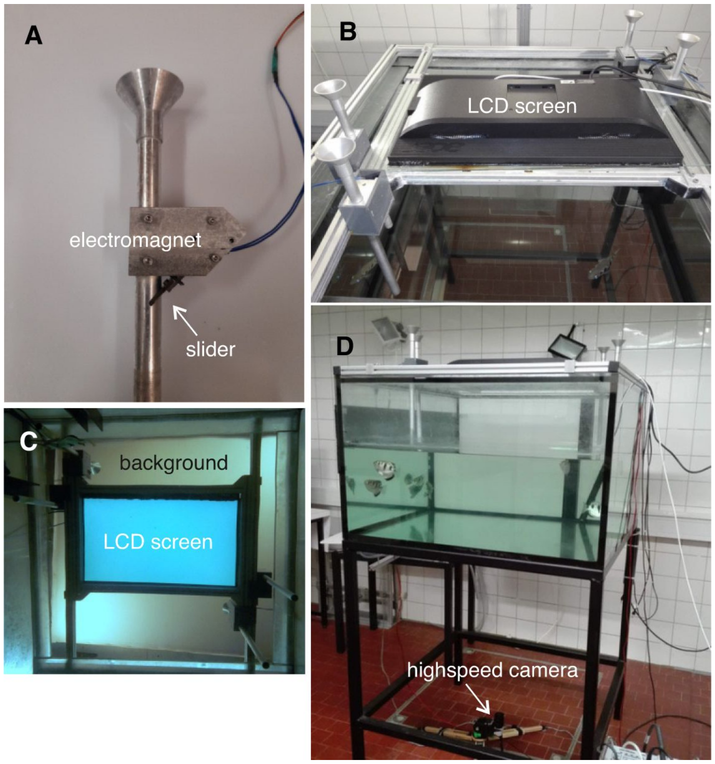

Fig. S2.

Apparatus for changing the rule that connects data on initial target movement to the turn the fish must make.

Related to **Fig. 2**. (A) Photo of feeder with slider that can be moved at the appropriate time by an electromagnet to allow the passage of a piece of food within the tube. (B) Top view of experimental tank with 4 feeders and LCD screen. (C) View from below through transparent bottom of the tank with LCD screen and background. (D) Side view of the tank (sized 1m x 1m x 0.6 m) with highspeed camera below.

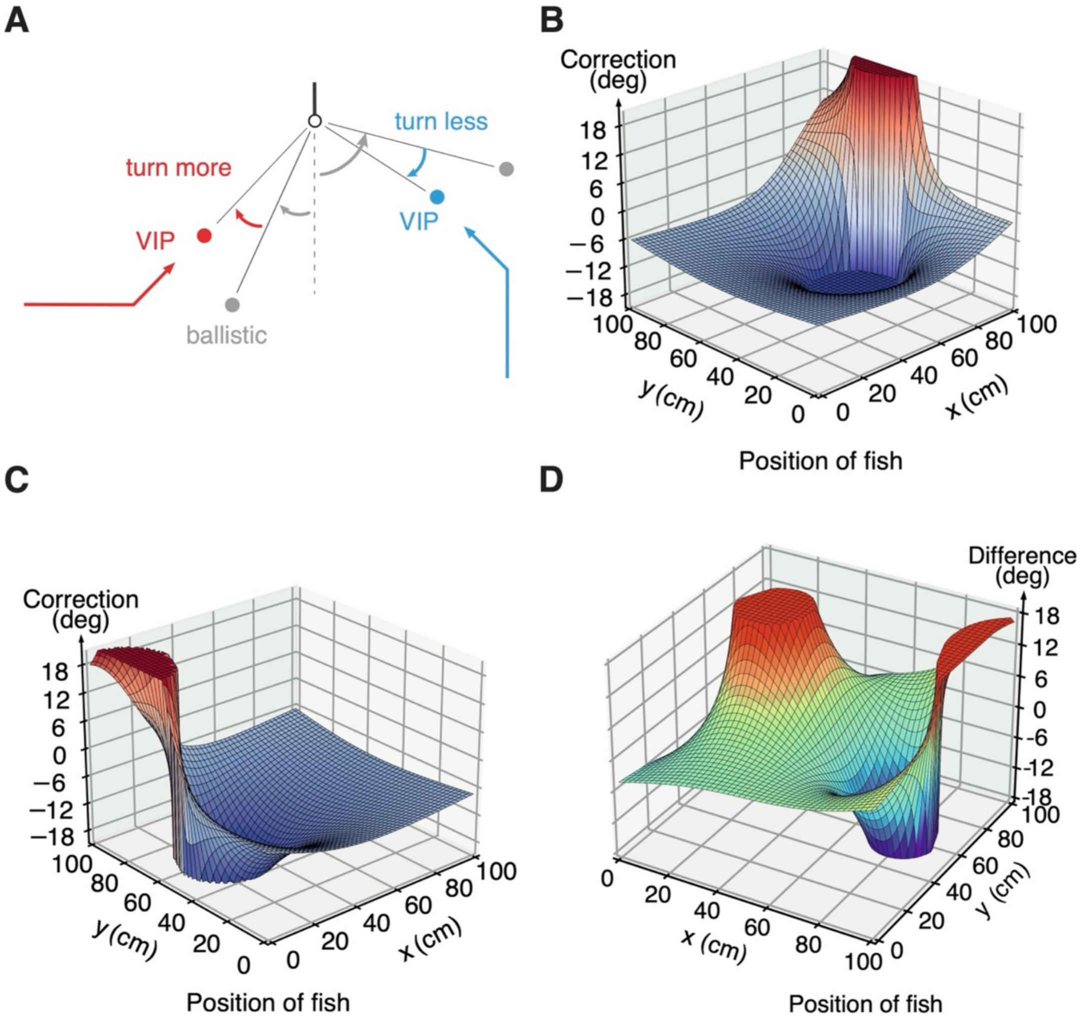

Fig. S3.

Learning the new rule requires position-dependent corrections to the ballistic turns with the pattern of corrections different for each input constellation.

Related to **Fig. 3**. It is important to understand that the apparently simple motion model (**Fig. 3A**) translates to rather complex corrections of the turns the fish must make. To illustrate this, we show the corrections the fish must make to its prior ballistic choices for only two different directions of initial target movement. (A) Definition of terms used. Sketch of top view of a responding fish that turns in response to either the motion shown in red or in blue on the screen above. The respective virtual (deflected) impact points are shown in red and in blue. The corresponding ballistic virtual impact points are shown in grey. ‘Correction’ is the angle that would have to be added to the turns toward the ballistic impact point. For two different directions of target movement (red versus blue) a fish located at the same spot would need to apply a different correction as indicated. shows the corrections required at each position in the tank (sized 100 cm x 100 cm) to ensure that turns at the rewarded new ‘deflected’ impact points. Note that this map of corrections applies for one direction of target movement only, with the (’deflected’) virtual impact point at x=89 cm, y=68 cm. Corrections > +18 deg and < -18 deg are assigned the values +18 and -18 deg, respectively. (C) Simply changing the direction of initial target motion requires a different map of corrections. Shown are the corrections the fish would have to apply, at each position, in response to a target that moves in a different initial direction, leading to another virtual impact point (x=11 cm, y=62 cm. (D) Quantitative analysis of the difference in the maps of corrections (B, C) that are required for the two different initial directions of target movement. This illustrates that accounting only for two possible types of initial prey movement (speed and height left constant) would require an extensive look-up table with stored corrections for every position and condition of target movement. Trained fish that randomly faced many different types of initial motion were able to respond appropriately to each of them from any location, without an increase in delay (see **Fig. 3**) and could apply the rule the extracted to generalize to untrained conditions (see **Fig. 4**).

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Reviewer #1 (Public review):

Summary:

The authors test whether the archerfish can modulate the fast response to a falling target. By manipulating the trajectory of the target, they claim that the fish can modulate the fast response. While it is clear from the result that the fish can modulate the fast response, the experimental support for the argument that the fish can do it for a reflex-like behavior is inadequate.

Strengths:

Overall, the question that the authors raised in the manuscript is interesting.

Weaknesses:

(1) The argument that the fish can modulate reflex-like behavior relies on the claim that the archerfish makes the decision in 40 ms. There is little support for the 40 ms reaction time. The reaction time for the same behavior in Schlegel 2008, is 60-70 ms, and in Tsvilling 2012 about 75 ms, if we take the half height of the maximum as the estimated reaction time in both cases. If we take the peak (or average) of the distribution as an estimation of reaction time, the reaction time is even longer. This number is critical for the analysis the authors perform since if the reaction time is longer, maybe this is not a reflex as claimed. In addition, mentioning the 40 ms in the abstract is overselling the result. The title is also not supported by the results.

(2) A critical technical issue of the stimulus delivery is not clear. The frame rate is 120 FPS and the target horizontal speed can be up to 1.775 m/s. This produces a target jumping on the screen 15 mm in each frame. This is not a continuous motion. Thus, the similarity between the natural system where the target experiences ballistic trajectory and the experiment here is not clear. Ideally, another type of stimulus delivery system is needed for a project of this kind that requires fast-moving targets (e.g. Reiser, J. Neurosci.Meth. 2008). In addition, the screen is rectangular and not circular, so in some directions, the target vanishes earlier than others. It must produce a bias in the fish response but there is no analysis of this type.

(3) The results here rely on the ability to measure the error of response in the case of a virtual experiment. It is not clear how this is done since the virtual target does not fall. How do the authors validate that the fish indeed perceives the virtual target as the falling target? Since the deflection is at a later stage of the virtual trajectory, it is not clear what is the actual physics that governs the world of the experiment. Overall, the experimental setup is not well designed.

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Reviewer #2 (Public review):

Summary:

This manuscript studies prey capture by archer fish, which observe the initial values of motion of aerial prey they made fall by spitting on them, and then rapidly turn to reach the ballistic landing point on the water surface. The question raised by the article is whether this incredibly fast decision-making process is hardwired and thus unmodifiable or can be adjusted by experience to follow a new rule, namely that the landing point is deflected from a certain amount of the expected ballistic landing point. The results show that the fish learn the new rule and use it afterward in a variety of novel situations that include height, side, and speed of the prey, and which preserve the speed of the fish's decision. Moreover, a remarkable finding presented in this work is the fact that fish that have learned to use the new rule can relearn to use the ballistic landing point for an object based on its shape (a triangle) while keeping simultaneously the 'deflected rule' for an object differing in shape (a disc); in other words, fish can master simultaneously two decision-making rules based on the different shape of objects.

Strengths:

The manuscript relies on a sophisticated and clever experimental design that allows changing the apparent landing point of a virtual prey using a virtual reality system. Several robust controls are provided to demonstrate the reliability and usefulness of the experimental setup.

Overall, I very much like the idea conveyed by the authors that even stimuli triggering apparently hardwired responses can be relearned in order to be associated with a different response, thus showing the impressive flexibility of circuits that are sometimes considered mediating pure reflexive responses. This is the case - as an additional example - of the main component of the Nasanov pheromone of bees (geraniol), which triggers immediate reflexive attraction and appetitive responses, and which can, nevertheless, be learned by bees in association with an electric shock so that bees end up exhibiting avoidance and the aversive response of sting extension to this odorant (1), which is a fully unnatural situation, and which shows that associative aversive learning is strong enough to override preprogrammed responding, thus reflecting an impressive behavioral flexibility.

Weaknesses:

As a general remark, there is some information that I missed and that is mandatory in the analysis of behavioral changes.

Firstly, the variability in the performances displayed. The authors mentioned that the results reported come from 6 fish (which is a low sample size). How were the individual performances in terms of consistency? Were all fish equally good in adjusting/learning the new rule? How did errors vary according to individual identity? It seems to me that this kind of information should be available as the authors reported that individual fish could be recognized and tracked (see lines 620-635) and is essential for appreciating the flexibility of the system under study.

Secondly, the speed of the learning process is not properly explained. Admittedly, fish learn in an impressive way the new rule and even two rules simultaneously; yet, how long did they need to achieve this? In the article, Figure 2 mentions that at least 6 training stages (each defined as a block of 60 evaluated turn decisions, which actually shows that the standard term 'Training Block' would be more appropriate) were required for the fish to learn the 'deflected rule'. While this means 360 trials (turning starts), I was left with the question of how long this process lasted. How many hours, days, and weeks were needed for the fish to learn? And as mentioned above, were all fish equally fast in learning? I would appreciate explaining this very important point because learning dynamics is relevant to understanding the flexibility of the system.

Reference:

(1) Roussel, E., Padie, S. & Giurfa, M. Aversive learning overcomes appetitive innate responding in honeybees. Anim Cogn 15, 135-141, doi:10.1007/s10071-011-0426-1 (2012).

https://doi.org/10.7554/eLife.99634.1.sa1

Author response:

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *The authors test whether the archerfish can modulate the fast response to a falling target.*

We have not tested whether archerfish can 'modulate the fast response'. We quantitatively test specific hypotheses on the rules used by the fish. For this the accuracy of the decisions is analyzed with respect to specific points that can be calculated precisely in each experiment. The ill-defined term 'modulate' does in no way capture what is done here. This assessment might explain the question, raised by the reviewer, of 'what is the difference of this study and Reinel, 2016' (i.e. Reinel and Schuster, 2016). In that study, all objects were strictly falling ballistically, and latency and accuracy of the turn decisions were determined when the initial motion was not only horizontal but had an additional vertical component of speed. The question of that study was if the need to account to an additional variable (vertical speed) in the decision would affect its latency or accuracy. The study showed that also then archerfish rapidly turn to the later impact point. It also showed that accuracy and latency (defined in this study exactly as in the present study) were not changed by the added degree of freedom. This is a completely different question and by its very nature does not leave the realm of ballistics.

> *By manipulating the trajectory of the target, they claim*
>
> *that the fish can modulate the fast response.*
>
> *While it is clear from the result that the fish can modulate the fast response, the experimental support for the argument that the fish can do it for a reflex-like behavior is inadequate.*

This is disturbing: The manuscript is full of data that directly report response latency (a parameter that's critical in all experiments) and there are even graphical displays of the distribution of latency (Figs. 2, 5). How fast the responses are, can also already be seen in the first video. Most importantly, the nature of the 40 ms limit has been discovered and has been reported by our group in 2008 (Schlegel and Schuster, 2008, Fig. 4). For easy reference, we attach Schlegel and Schuster, 2008 with the relevant passages marked in yellow. But later studies also using high speed video (ie. typically 500 fps) and simultaneously evaluating accuracy and kinematics (in the same ways as used here!) to address various questions repeatedly report and even graphically represent minimum latencies of 40 ms, e.g. Krupczynski and Schuster, 2013 (e.g. Fig. 2); Reinel and Schuster, 2014; Reinel and Schuster, 2016; Reinel and Schuster, 2018a, b (e.g. see Fig. 7 in the first part) and report how latency is increased as urgency is decreased (if the fish are too close or time of falling is increased), as temperature is decreased or as viewing conditions and their homogeneity across the tank change. Moreover, even a field study is available (Rischawy, Blum and Schuster, 2015) that shows why the speed is needed. This is because of massive competition with at least some of the competitor fish also be able to turn to the later impact point. So, speed is an absolute necessity if competitors are around. Interestingly, when the fish are isolated, latency goes up and eventually the fish will no longer respond with C-starts (Schlegel and Schuster, 2008).

Another aspect: considering the introduction it would not even have mattered if not 40 ms but instead 150 ms were the time needed for an accurate start (which is not the case). That would still be faster than an Olympic sprinter responds to a gun shot. Moreoever, please also note that we carefully talk of reflex-speed not of a reflex-behavior (which is as easy to verify as any other if the false statements made).

> *Strengths:*
>
> *Overall, the question that the authors raised in the manuscript is interesting.*

Given the statement of no difference between the present study and Reinel and Schuster, 2016, it is not clear what this assessment refers to.

> *Weaknesses:*
>
> *(1) The argument that the fish can modulate reflex-like behavior relies on the claim that the archerfish makes the decision in 40 ms. There is little support for the 40 ms reaction time.*

The 'little support' is a paper in Science in which this important aspect is directly analyzed (Fig. 4 of that paper) and that has been praised by folks like Yadin Dudai (e.g . in Faculty 1000). The support is also data on latency as presented in the present paper. Furthermore, additional publications are available on the reaction time (see above).

> *The reaction time for the same behavior in Schlegel 2008, is 60-70 ms, and in Tsvilling 2012 about 75 ms, if we take the half height of the maximum as the estimated reaction time in both cases. If we take the peak (or average) of the distribution as an estimation of reaction time, the reaction time is even longer. This number is critical for the analysis the authors perform since if the reaction time is longer, maybe this is not a reflex as claimed.*

See above.

> *In addition, mentioning the 40 ms in the abstract is overselling the result.*

See above.

Just for completeness: Considering a very interesting point raised by reviewer 2 we add an additional panel to further emphasize the exciting point that accuracy and latency are unrelated in the start decisions. That point was already made in Fig.4 of the paper in Science but can be directly addressed.

> *The title is also not supported by the results.*

No: the title is clearly supported by the results that are reported in the paper.

> *(2) A critical technical issue of the stimulus delivery is not clear.*

The stimulus delivery is described in detail. Most importantly we emphasize (even mentioning frame rate) that all VR setups require experimental confirmation that they work for the species and for the behavior at hand. Ideally, they should elicit the same behavior (in all aspects) as a real stimulus does that the VR approach intends to mimic. Whether VR works in a given animal and for the behavior at hand in that animal cannot be known or postulated a priori. It must be shown in direct critical experiments. Such experiments and the need to perform them are described in detail in Figure 2 and in the text that is associated with that figure.

> *The frame rate is 120 FPS and the target horizontal speed can be up to 1.775 m/s. This produces a target jumping on the screen 15 mm in each frame. This is not a continuous motion. Thus, the similarity between the natural system where the target experiences ballistic trajectory and the experiment here is not clear. Ideally, another type of stimulus delivery system is needed for a project of this kind that requires fast-moving targets (e.g. Reiser, J. Neurosci.Meth. 2008).*

See above. It is quite funny that one of the authors of the present study had been involved in developing a setup with a complete panorama of 6000 LEDs (Strauss, Schuster and Götz, 1997; and appropriately cited in Reiser) that has been the basis for Reiser. This panorama was also used to successfully implement VR in freely walking Drosophila (Schuster et al., Curr. Biol., 2002). However, an LED based approach was abandoned because of insufficient spatial resolution (that, in archerfish, is very different from that of Drosophila).

But the crucial point really is this: Just looking at Figure 2 shows that our approach could not have worked better in any way - it provided the input needed to cause turn decisions that are in all aspects just as those with real objects. Achieving this was not at all trivial and required enormous effort and many failed attempts. But it allows addressing our questions for the first time after 20 years of studying these interesting decisions.

> *In addition, the screen is rectangular and not circular, so in some directions, the target vanishes earlier than others. It must produce a bias in the fish response but there is no analysis of this type.*

Why 'must' it produce a bias? Is it not conceivable that you can only use a circular part of the screen? Briefly, and as could have been checked by quickly looking into the methods section, this is what we did. But still, why would it have mattered in our strictly randomized design? It could have mattered only in a completely silly way of running the experiments in which exclusively long trajectories are shown in one condition and exclusively short ones in another.

> *(3) The results here rely on the ability to measure the error of response in the case of a virtual experiment. It is not clear how this is done since the virtual target does not fall.*

Well, of course it does not fall!!! That is the whole point that enables the study, and this is explained in connection with the glass plate experiment of Fig. 1 and quite some text is devoted to say that this is the starting point for the present analysis. The ballistic impact point is calculated (just as explained in our very first paper on the start decisions, Rossel, Corlija and Schuster, 2002) from the initial speed and height of the target, using simple high-school physics and the justification for that is also in that paper. This has been done already more than 20 years ago. How else could that paper have arrived at the conclusion that the fish turned to the virtual impact point even though nothing is falling? We also describe this for the readers of the present study, illustrate how accuracy is determined in Figures, in all videos and in an additional Supplementary Figure. Consulting the paper reveals that orientation of the fish is determined immediately at the end of stage 2 of its C-start and the error directly reports how close continuing in that direction would lead the fish to the (real or virtual) impact point. This measure has also been used since the first paper in 2002 in our lab and it is very useful because it provides an invariant measure that allows pooling all the different conditions (orientation and position of responding fish as well as direction, speed and height of target).

> *How do the authors validate that the fish indeed perceives the virtual target as the falling target?*

See above. The fish produce C-starts (whose kinematics are analyzed and reported in Figures), whose latency is measured (from onset of target motion to onset of C-start) and whose accuracy in aligning them to the calculated virtual impact point is measured (see above). Additionally, the errors are also analyzed to other points of interest, for instance landmarks, the ballistic landing point in the re-trained fish or points calculated on the basis of specific hypotheses in the generalization experiments.

> *Since the deflection is at a later stage of the virtual trajectory, it is not clear what is the actual physics that governs the world of the experiment.*

As explained in the text what we need is substituting the ballistic connection with another fixed relation between initial target motion and the landing point. This other relation needs to produce a large error in the aims when they remain based on the ballistic virtual landing point. It is directly shown in the key experiments that the fish need not see the deflection but can respond appropriately to the initial motion after training (Figs. 3, 5 and corresponding paragraphs in the text as well as additional movies). Please also note that after training the decision is based on the initial movement. This is shown in the interspersed experiments in which nothing than the initial (pre-deflection) movement was shown.

> *Overall, the experimental setup is not well designed.*

It is obviously designed well enough to mimic the natural situation in every aspect needed (see Fig. 2) and well enough to answer the questions we have asked.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *This manuscript studies prey capture by archer fish, which observe the initial values of motion of aerial prey they made fall by spitting on them, and then rapidly turn to reach the ballistic landing point on the water surface. The question raised by the article is whether this incredibly fast decision-making process is hardwired and thus unmodifiable or can be adjusted by experience to follow a new rule, namely that the landing point is deflected from a certain amount of the expected ballistic landing point. The results show that the fish learn the new rule and use it afterward in a variety of novel situations that include height, side, and speed of the prey, and which preserve the speed of the fish's decision. Moreover, a remarkable finding presented in this work is the fact that fish that have learned to use the new rule can relearn to use the ballistic landing point for an object based on its shape (a triangle) while keeping simultaneously the 'deflected rule' for an object differing in shape (a disc); in other words, fish can master simultaneously two decision-making rules based on the different shape of objects.*
>
> *Strengths:*
>
> *The manuscript relies on a sophisticated and clever experimental design that allows changing the apparent landing point of a virtual prey using a virtual reality system.*

> *Several robust controls are provided to demonstrate the reliability and usefulness of the experimental setup.*
>
> *Overall, I very much like the idea conveyed by the authors that even stimuli triggering apparently hardwired responses can be relearned in order to be associated with a different response, thus showing the impressive flexibility of circuits that are sometimes considered mediating pure reflexive responses.*

Thank you so much for this precise assessment of what we have shown!

> *This is the case - as an additional example - of the main component of the Nasanov pheromone of bees (geraniol), which triggers immediate reflexive attraction and appetitive responses, and which can, nevertheless, be learned by bees in association with an electric shock so that bees end up exhibiting avoidance and the aversive response of sting extension to this odorant (1), which is a fully unnatural situation, and which shows that associative aversive learning is strong enough to override preprogrammed responding, thus reflecting an impressive behavioral flexibility.*

That's very interesting, thanks.

> *Weaknesses:*
>
> *As a general remark, there is some information that I missed and that is mandatory in the analysis of behavioral changes.*
>
> *Firstly, the variability in the performances displayed. The authors mentioned that the results reported come from 6 fish (which is a low sample size). How were the individual performances in terms of consistency? Were all fish equally good in adjusting/learning the new rule? How did errors vary according to individual identity? It seems to me that this kind of information should be available as the authors reported that individual fish could be recognized and tracked (see lines 620-635) and is essential for appreciating the flexibility of the system under study.*
>
> *Secondly, the speed of the learning process is not properly explained. Admittedly, fish learn in an impressive way the new rule and even two rules simultaneously; yet, how long did they need to achieve this? In the article, Figure 2 mentions that at least 6 training stages (each defined as a block of 60 evaluated turn decisions, which actually shows that the standard term 'Training Block' would be more appropriate) were required for the fish to learn the 'deflected rule'. While this means 360 trials (turning starts), I was left with the question of how long this process lasted. How many hours, days, and weeks were needed for the fish to learn? And as mentioned above, were all fish equally fast in learning? I would appreciate explaining this very important point because learning dynamics is relevant to understanding the flexibility of the system.*

First, it is very important to keep the question in mind that we wanted to clarify: Does the system have the potential to re-tune the decisions to other non-ballistic relations between the input variables and the output? This would have been established if one fish was found capable of doing that. However, we do have sufficient evidence to say that all six fish learned the new law and that at least one (actually four) individual was capable of simultaneously handling the two laws. We will explain this much better (hopefully) in our revised version. We also have to stress that not all archerfish might actually be able to do this and that not all archerfish might learn in the same way, at the same speed, or using the same strategies. These questions are extremely interesting and we therefore definitely will include all evidence that we have. If some individuals are better than others in quickly adjusting, then even observational learning could become a part of the story. However, we needed to make and document the first steps. Understanding these is essential and apparently is difficult enough.

> *Reference:*
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> *(1) Roussel, E., Padie, S. & Giurfa, M. Aversive learning overcomes appetitive innate responding in honeybees. Anim Cogn 15, 135-141, doi:10.1007/s10071-011-0426-1 (2012).*

Thanks for this reference!

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