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RESEARCH ARTICLE

The Mauthner cell in a fish with top-performance and yet flexibly tuned C-starts. II. Physiology

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ABSTRACT

The parallel occurrence in archerfish of fine-tuned and yet powerful predictive C-starts as well as of kinematically identical escape C-starts makes archerfish an interesting system to test hypotheses on the roles played by the Mauthner cells, a pair of giant reticulospinal neurons. In this study, we show that the archerfish Mauthner cell shares all hallmark physiological properties with that of goldfish. Visual and acoustic inputs are received by the ventral and lateral dendrite, respectively, and cause complex postsynaptic potentials (PSPs) even in surgically anaesthetised fish. PSP shape did not indicate major differences between the species, but simple light flashes caused larger PSPs in archerfish, often driving the cell to fire an action potential. Probing archerfish in the classical tests for feedback inhibition, established in the Mauthner-associated networks in goldfish, revealed no differences between the two species, including the indications for electrical and chemical synaptic components. Also, the established hallmark experiments on feed-forward inhibition showed no differences between the goldfish and archerfish Mauthner system. Extending these experiments to visual stimuli also failed to detect any differences between the two species and suggested that acoustical and visual input cause feed-forward inhibition, the magnitude, time course and duration of which match that of the respective PSPs in both archerfish and goldfish. Our findings question simple views on the role of the Mauthner cell and suggest that the archerfish Mauthner cell should be a good system to explore the function of these giant neurons in more sophisticated C-start behaviours.

KEY WORDS: Reticulospinal system, Mauthner system, Feed-forward inhibition, Visual response, Neuroethology

INTRODUCTION

Within the hindbrain reticulospinal network of fish and amphibians, two identified neurons, the Mauthner cells, are notable because of their enormous size and for sending axons down the spinal cord that have a diameter which is by far larger than that of any other neuron (e.g. Zottoli, 1978; Korn and Faber, 2005; Sillar, 2009). Characteristics of the Mauthner cell have facilitated the discovery of many fundamental insights in neuroscience, arguably more than any other vertebrate neuron (see Zottoli and Faber, 2000; Korn and Faber, 2005; Sillar et al., 2016). The accessibility of the Mauthner cell in goldfish has led to many remarkable neuroethological insights in which *in vivo* recordings have allowed cellular and synaptic properties to be linked to detailed aspects of motor behaviour (Oda et al., 1998; Preuss and Faber, 2003; Preuss et al., 2006; Szabo et al., 2008; Neumeister et al., 2008). In the intact reticulospinal network of goldfish, the Mauthner cell is always the first to fire whenever a rapid and powerful C-start manoeuvre is initiated. Conversely, firing one of the Mauthner neurons elicits rapid body bending into the shape of a letter 'C' towards the side contralateral to the cell body (e.g. Zottoli, 1977; Zottoli and Faber, 2000). Nevertheless, the actual roles of the Mauthner cells within the reticulospinal network are still discussed controversially. One view, for instance, is that correlated Mauthner cell firing does not actually drive the C-start, but instead shuts off other, potentially conflicting, motor behaviours (Eaton et al., 1995). This view was motivated by early findings in which ablation of the Mauthner cell soma (e.g. Eaton et al., 1982) or removal of the complete Mauthner cell (Kimmel et al., 1980) did not eliminate short-latency high-power C-starts. However, removal of the two Mauthner neurons without also destroying a class of inhibitory interneurons did show drastic effects (Zottoli et al., 1999), suggesting that the Mauthner cell is necessary to ensure that other reticulospinal neurons reach threshold. Liu and Fetcho (1999), working in zebrafish larvae, showed that eliminating the Mauthner cells together with their two pairs of serially homologous reticulospinal cells, MiD2cm and MiD3cm (located in the hindbrain rhombomeres r5 and r6, respectively), leads to a distinct decrease in performance and increase in latency. Hence, any compensation for the absence of a Mauthner cell should occur among the remaining cells of this so-called Mauthner series. Intracellular recordings from the Mauthner homologues MiD2cm and MiD3cm showed that all cells of the Mauthner series utilise the same mechanosensory information, but process it very differently (Nakayama and Oda, 2004). This finding suggests that the Mauthner series operates on the basis of the same information, but the different cells contribute differently to escape behaviour, with the Mauthner cell presumably triggering it and the homologues providing more detailed, slightly delayed commands to the motor system. Kohashi and Oda (2008) showed that the Mauthner cell inhibits the MiD3cm neurons and that MiD3cm fires (while the Mauthner neuron remains silent) in slower C-starts. A recent study simultaneously monitored behavioural variability and activity in the reticulospinal system of zebrafish larvae (Bhattacharyya et al., 2017). Interestingly, the Mauthner cells were always recruited in rapid but stereotyped C-starts, but never in the more variable fast-starts of the larvae. Based on these findings, the authors suggested an evolutionary scenario in which Mauthner cells were efficient at driving rapid but inflexible 'robust' C-starts. As the demand for producing more variable responses increased in 'higher' vertebrates, the giant Mauthner cells should have become obsolete in them.

In this context, the present paper – as the second of two connected studies – examines the physiology of the Mauthner cell in archerfish, a fish that performs two equally powerful, kinematically equivalent C-starts (Wöhl and Schuster, 2007).

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One of them, used in hunting, is remarkably flexible and can be precisely tuned, whereas the other is used in escapes (where fine tuning is difficult to show; see Krupczynski and Schuster, 2013). In the preceding study (Machnik et al., 2018), we demonstrated that the size and morphology of the archerfish Mauthner cell are surprisingly similar to those of equally sized goldfish. The only major deviations were that the ventral dendrite was longer in archerfish and its lateral dendrite thinner. The similarity between the Mauthner cells of the two species is surprising: if the Mauthner cell cannot be used in the variable C-starts of archerfish, then it should be reduced relative to that of the goldfish or even absent, as in many adult anurans (e.g. Stefanelli, 1951: reduction in adult *Xenopus*). However, even if it was involved in any of the two archerfish C-start manoeuvres, it would still be expected to be different from a more standard teleost Mauthner cell to accommodate the functions that allow it to be used in variable and fine-tuned C-starts. Here, we used hallmark experiments on the functionality of the Mauthner cell and its associated inhibitory circuits, established over half a century in goldfish, to probe for any physiological peculiarities in the archerfish Mauthner cell. Specifically, we set two goals for the present study. First, we needed to clarify whether the archerfish Mauthner cell allows robust and stable intracellular *in vivo* recordings. In particular, given the importance of visual information in the selection of the appropriate predictive C-start (e.g. Rossel et al., 2002; Schlegel and Schuster, 2008; Schuster, 2011; Reinel and Schuster, 2016) and given that Mauthner recordings in archerfish would be invaluable for deciding whether it is linked to the known behavioural properties of the predictive starts (e.g. Krupczynski and Schuster, 2013), it was crucial to establish conditions under which visual responses could reliably be obtained. Second, we then wanted to directly compare hallmark physiological properties of the goldfish and the archerfish Mauthner cell, using equally sized fish of the two species treated in the same way to clarify whether the archerfish Mauthner cell differs from that of goldfish in its physiology.

MATERIALS AND METHODS

Experimental animals and surgery

Banded archerfish [*Toxotes jaculatrix* (Pallas 1767), Perciformes] (N=30) and goldfish [*Carassius auratus* (Linnaeus 1758), Cypriniformes] (N=14) were used in the experiments of this and the preceding study (which described how the fish were kept; Machnik et al., 2018). Archerfish and goldfish were chosen of the same standard length (7–8 cm). Animal care procedures, surgical procedures and experimentation were in accordance with all relevant guidelines and regulations of the German animal protection law and explicitly approved by state councils. Anaesthesia and surgical procedures were as described in the preceding paper (Machnik et al., 2018). We would like to emphasise that it was crucial not to use the customary MS-222 as general anaesthetic. Instead, we used 2-phenoxyethanol (2-PE; Sigma-Aldrich, Steinheim, Germany). This allowed us to reliably record both visual and mechanosensory postsynaptic potentials (PSPs) in both archerfish and goldfish. MS-222, which is the most commonly used general anaesthetic in ectothermic vertebrates (Treves-Brown, 2000) and also the commonly used anaesthetic in Mauthner research, massively interferes with retinal function and therefore significantly affects visual information processing (Hoffman and Basinger, 1977), whereas 2-PE does not.

Because of the greater simplicity and because we only used simple visual flash stimuli, we kept the opened brain above the water level, which meant that the eyes were above the water surface (in both goldfish and archerfish). This required great care to prevent the otherwise rapid dehydration of the eyes. We covered the eyes with strips of thin wet filter paper, the lower ends of which were submerged in the water. In view of future experiments, in which it will be important to mimic the complex optics at the water–air interface, we would like to stress that we have also established ways to work with fish that have their eyes submerged. The best way was raising the outer edge of the skull preparation with two-component silicone (Detaseal hydroflow lite fast, DETAX GmbH & Co. KG, Ettlingen, Germany) additionally sealed by a tissue adhesive (Surgibond, SMI AG, Steinerberg, Belgium), so as to create an edging that prevents water from entering the recording site. This requires extra preparation, but works very well so that all properties described here could be reproduced.

Electrophysiological measurements

In both species, all measurements were made at the same controlled temperature of 20.0±0.1°C. We used a bridge-mode amplifier (BA-01X, npi electronic GmbH, Tamm, Germany) in current-clamp mode for intracellular recordings. Recording electrodes were pulled from 3 mm glass (G-3, Narishige Scientific Instrument Lab, Tokyo, Japan) by using a vertical electrode puller (PE-22; Narishige International Limited, London, UK) and then broken to obtain sharp electrodes with an electrode resistance between 4 and 7 MΩ. Electrodes were filled with 5 mol l^{-1} potassium acetate except in the experiments on feedback inhibition in which electrodes contained 3 mol l^{-1} potassium chloride. Electrodes were positioned and moved by using a motorised micromanipulator (MP-285, Sutter Instrument, Novato, CA, USA). Recordings were filtered (Hum Bug Noise Eliminator, Quest Scientific, North Vancouver, BC, Canada) and digitised using an A/D converter (Micro1401, Cambridge Electronic Design Ltd, Cambridge, UK) at 50 kHz sampling rate and the acquisition software package Spike2 (version 6; Cambridge Electronic Design Ltd), which was also used for data analysis.

In each preparation, we first determined conduction velocity (Machnik et al., 2018) and confirmed for the archerfish (but also for the control goldfish) the low input resistance and the extremely short time constant that have consistently been reported for the goldfish Mauthner cell. As in the literature, the range of variation was large, so that these characteristics could not profitably be used as sensitive indicators for any deviations between archerfish and goldfish. Nevertheless, we confirmed that also in archerfish input resistance of the Mauthner cell is low, ranging from 107 and 210 kΩ, and the time constant is short, ranging from 0.19 to 0.52 ms, and that the ranges overlap both with our own control measurements in goldfish and with the ranges reported in earlier work (Furshpan and Furukawa, 1962; Fukami et al., 1965; Faber and Korn, 1986). Our determinations are based on the injection of sufficient current to hyperpolarise the cell by 2 mV.

To reconstruct the actual transmembrane potential, we penetrated the cell, measured the action potential amplitude and then moved the recording electrode out to measure the local amplitude of the extracellular field (which is not seen by the distant reference electrode) in the direct vicinity of the Mauthner cell.

Sensory stimuli

Short acoustic broadband pulses were delivered using an active loudspeaker (the box pro Achat 115 MA, Thomann GmbH, Burgebrach, Germany) driven by a 4.5 V rectangular pulse of 1 ms duration, generated by a pulse generator (Master-8, A.M.P.I., Jerusalem, Israel). Sound pressure level was 145 dB re. 1 µPa measured underwater close to the fish using a hydrophone

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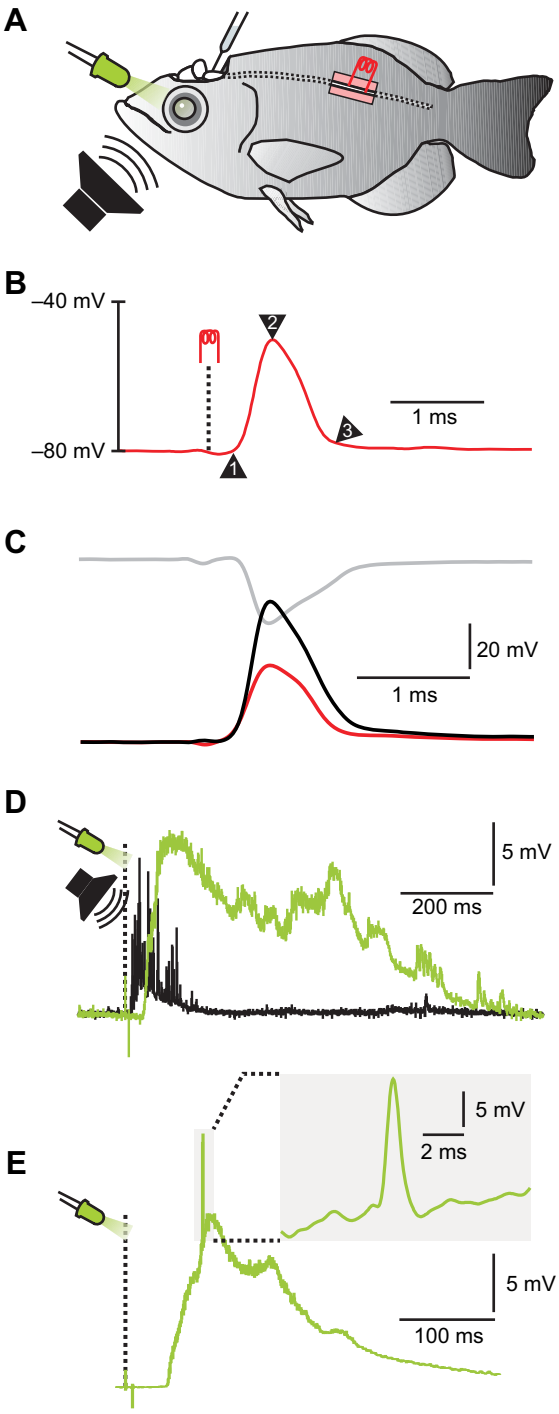

Fig. 1. The archerfish Mauthner cell as an experimental system. (A) Sketch of the preparation with the electrode in place to record intracellularly from the Mauthner cell, located about 1 mm below the surface of the medulla, while the cell was stimulated antidromically (red; electrical stimulation of spinal cord), visually (green; light-emitting diode) or acoustically (black; loudspeaker). (B) Spinal cord stimuli cause one and only one action potential in the archerfish Mauthner cell with a shape that is typical for teleost Mauthner cells. Arrowheads emphasise the short latency (1) and absence of both overshoot (2) and undershoot (3). (C) Reconstruction of the transmembrane potential (black) from the recorded action potential (red) and the negative all-or-none field potential (grey) recorded outside the Mauthner cell. (D) Examples of postsynaptic potentials (PSPs) recorded in response to a brief 7 ms light flash (green PSP) and to a loud sound burst (black PSP). Examples were chosen to illustrate the larger delay and amplitude of the visual PSP and its remarkably long duration. (E) The light flash triggered an action potential in the Mauthner cell in one-third of the anaesthetised archerfish. This action potential (inset) generally arose in the rising phase of the PSP (main trace).

(Type 8106, Brüel & Kjær, Nærum, Denmark). The frequency distribution of the sound pulses ranged from 25 to 1000 Hz with peak amplitude at 300 Hz. Visual stimuli were light flashes of 7 ms duration delivered by calibrated LEDs (RS Components GmbH, Mörenfelden-Walldorf, Germany). The LEDs were selected on the basis of their emission spectrum (measured at 1 cm distance; Minolta CL-500A, Konica Minolta, Tokyo, Japan), because they would stimulate both the red and green cones of goldfish (Neumeyer, 1992) and of archerfish (Temple et al., 2010). Peak radiation (at about 569 nm) was 700 µW m^{-2} nm^{-1} and width at 100 µW m^{-2} nm^{-1} was 56 nm (range: 543–599 nm). Single LEDs were positioned directly in front of both eyes and used to stimulate the ipsilateral Mauthner cell. Behind the wet filter paper, which was used to keep the eyes wet (see above), the luminance of the light flashes was still high (100 cd m^{-2}).

Quantifying inhibition in the archerfish Mauthner neuron

Here, we replicated the classical experiments of Furukawa and Furshpan (1963) (see also Faber and Korn, 1978) on the goldfish Mauthner cell to explore any differences that may exist in archerfish in, for example, time course, strength or other hallmark characteristics of feedback and feed-forward inhibition. To ensure that any differences were not introduced by our slightly different techniques (including, for instance, the different anaesthetic), we also repeated the original experiments in our control goldfish, using the same procedures as in archerfish. In the tests on feedback inhibition, we stimulated the Mauthner neuron antidromically, with two spinal cord stimuli with inter-stimulus intervals ΔT between 1 and 100 ms. In goldfish, the first action potential is known to trigger inhibitory neurons, which are part of a recurrent inhibitory network. The effect of the recurrent inhibitory network can then be seen from the reduction in amplitude of the second action potential. To analyse the time course of the inhibition, we fitted the function $f(\Delta T)=a_1\exp(-\Delta T/\tau_1)+a_2\exp(-\Delta T/\tau_2)$ (where where τ_1 and τ_2 are the time constants and a_1 and a_2 are constants) using GraphPad Prism 5.0f (GraphPad Software, Inc., La Jolla, CA, USA). To examine the characteristics of the feed-forward inhibition (which is well characterised in goldfish) in the archerfish Mauthner neuron, we again followed the established protocol and elicited a subthreshold PSP in the Mauthner neuron by sensory (acoustic or visual) stimulation. During various phases of the PSP, we elicited an action potential (by spinal cord stimulation) and determined the reduction in spike amplitude (relative to an action potential elicited in the absence of a PSP) as a measure of sensory-driven inhibition on the Mauthner neuron using exactly the same analysis as in all earlier work on goldfish (e.g. Faber and Korn, 1978).

Controls employed during the recordings

To ensure that only data from healthy preparations were included, we regularly checked the resting potential, antidromic activation latency and action potential amplitude of each recorded Mauthner cell, at least every 20 min. When a change occurred in any of these values, no more data were taken. A tolerance of 10% was allowed for variations in the resting potential and the amplitude of the action potential. Antidromic activation latency was required to remain below 0.4 ms.

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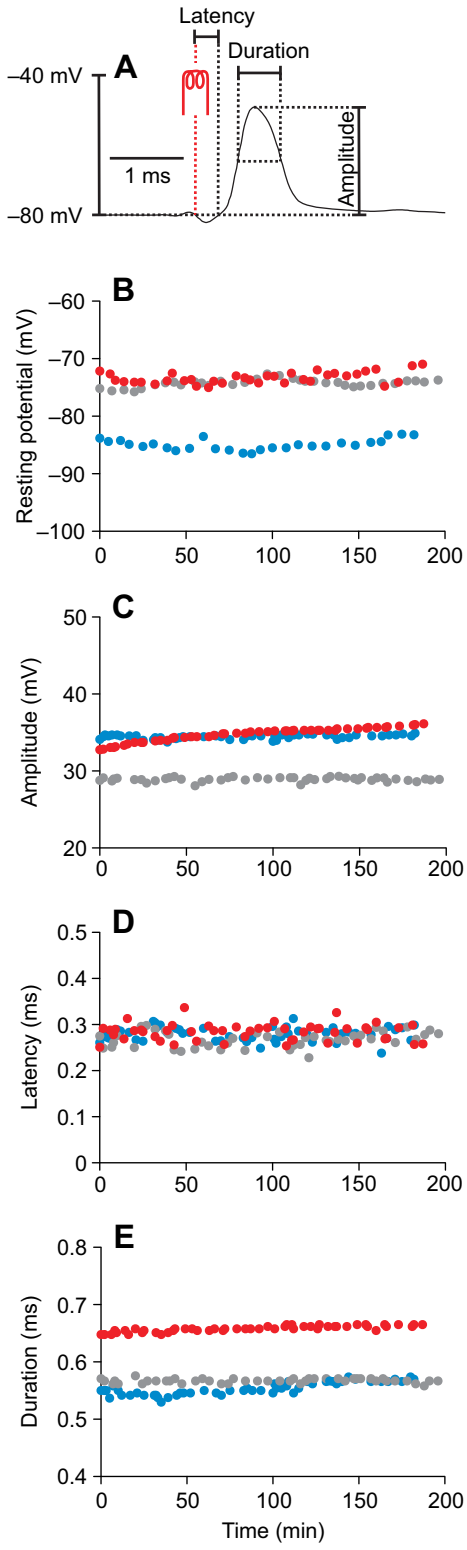

Fig. 2. Robust *in vivo* intracellular recording from the archerfish Mauthner neuron is possible. (A) Sketch of measurements taken on individual action potentials that were elicited by spinal cord stimulation (red). Latency was time from stimulus onset to the rise of the membrane potential above the resting potential. Duration was taken as the time between the two points where amplitude was half-maximal. (B–D) Examples of recordings made in three fish (indicated by different colours) to illustrate the stability and small variability within any given fish. Typical for all fish analysed in this study, resting potential (B), amplitude (C), latency (D) and duration of the action potential (E) remained stable over sufficient time to allow detailed experimentation.

Statistical analyses

Statistical tests were run using the software package GraphPad Prism 5.0f (GraphPad Software, Inc.) and performed two-tailed with α=0.05. Means±s.e.m. are given; N and n denote the number of animals and measurements, respectively.

RESULTS

The action potential

Archerfish Mauthner cells were localised as described in the preceding paper (Machnik et al., 2018) by tracking the characteristic all-or-none field potential that accompanies Mauthner cell activation. The identification was then based on unique features of the Mauthner cell action potential (Fig. 1). Antidromic stimulation of sufficient amplitude caused one and only one action potential and this held over the full range of amplitudes of the antidromic stimuli that we were able to deliver (up to 8 times threshold amplitude; see fig. 2 of Machnik et al., 2018). Note that the presence of a field potential in the vicinity of the Mauthner cell means that the recorded action potential (relative to a distant reference electrode) is not directly the transmembrane potential, but that this can be reconstructed if measurements of the field potential are also available (Fig. 1C). To assay the stability of the preparation and to estimate the duration of stable intracellular recording that our preparation would allow, we first checked the stability of several characteristics of the action potential. Fig. 2B–E illustrates typical examples from three archerfish (grey, blue and red circles) to demonstrate the remarkable stability of the resting potential and all measured properties of the action potential over the indicated recording time of up to 3 h. The data obtained from all 30 archerfish were similar, confirming the stability and robustness of our recordings. It is more difficult to maintain stable recordings from the archerfish than from the goldfish Mauthner cell and the recording time available is shorter for archerfish under the same experimental conditions. However, an interval of 2–3 h of intracellular recording in the archerfish Mauthner cell is realistic. Across all our experimental animals, the scatter in the recorded resting potential and action potentials was small, but clearly larger than that within any given animal (Fig. 3). Resting potentials were consistently low with an average of −79.5±0.6 mV (N=30). Latency of the onset of the action potential after antidromic stimulation was short, on average 0.265 ±0.007 ms (N=30). All recorded action potentials closely resembled the Mauthner action potentials of goldfish (e.g. Furshpan and Furukawa, 1962; Faber and Korn, 1978). In all 30 archerfish, we consistently observed a lack of any apparent after-hyperpolarisation and an apparently low amplitude without an overshoot. Measured in the soma, the Mauthner action potential had an amplitude of only 36.7 ±0.9 mV (N=30) and its half-maximal duration was 0.54±0.01 ms (N=30). Fig. 3 shows the same measurements in N=14 goldfish, analysed for comparison under the same conditions. The similarity in all aspects is striking: conduction speed did not differ (see Machnik et al., 2018), and nor did amplitude (35.6±2.4 mV; N=14 goldfish; difference from archerfish P=0.58, t-test) or half-maximal duration (0.54±0.01 ms; N=14 goldfish; difference from archerfish P=0.97, t-test).

Simple light flashes elicit strong and long-lasting PSPs

Our simple standardised light flashes (duration 7 ms, intensity 100 cd m^{-2}, maximum intensity at 569 nm) caused remarkably large and long-lasting PSPs, in both archerfish and goldfish (Fig. 4A). Although the initial rising part of the visual PSPs generally was stereotyped, the much longer later part showed

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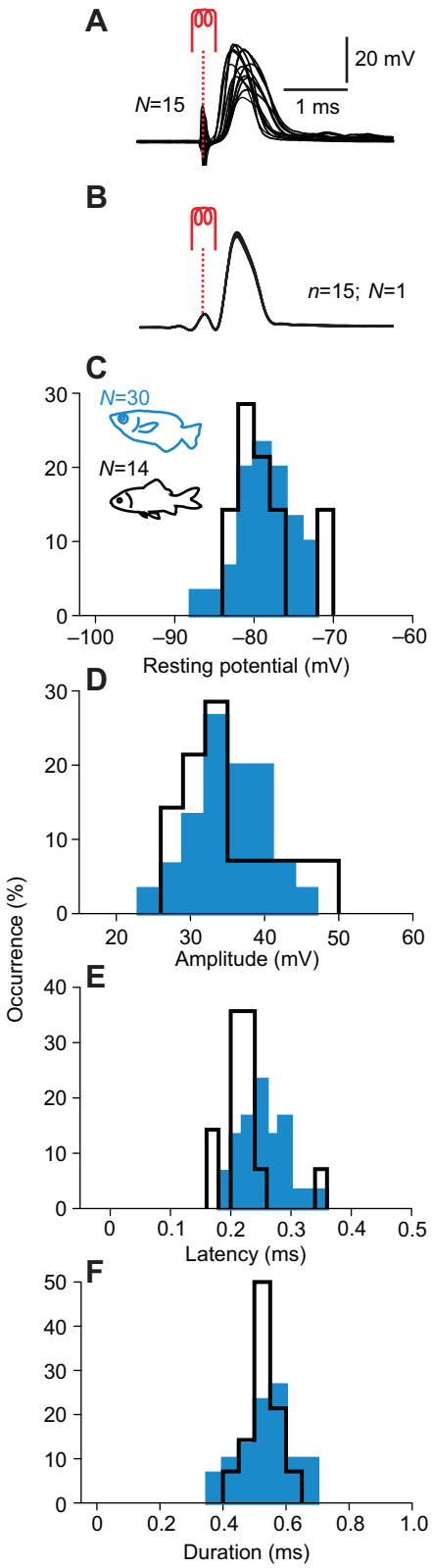

Fig. 3. Distribution of action potential characteristics across all preparations. (A) Examples of action potentials triggered by spinal cord stimulation in *N*=15 of the 30 archerfish to illustrate large differences between fish. (B) Example of 15 successive action potentials recorded from one of these 15 fish to illustrate that action potentials were consistent within any given fish (see also Fig. 2). *n* is the number of measurements. (C–F) Distribution of resting potential (C), amplitude (D), latency (E) and duration (F) across the *N*=30 archerfish used in this study (blue filled area) and *N*=14 goldfish (black line). See Fig. 2A for illustration of these values. All action potentials were recorded at the soma at a distance of not more than 50 µm from the axon hillock. As indicated, ranges and variations in archerfish were similar to those in goldfish Mauthner cells measured under the same conditions.

remarkable variation from one response to the next, but did so in both archerfish and goldfish. This is illustrated in Fig. 4A with examples of 10 visually elicited PSPs (five PSPs measured consecutively with an inter-stimulus interval of 10 s and five PSPs measured in the same way, but 90 min later) in an archerfish and a goldfish. The shape of the long-duration PSPs cannot be described by a single exponential decay suggesting – for archerfish and goldfish alike – that visually driven, delayed polysynaptic excitatory inputs cause the later parts of the PSP. Interestingly, the average duration of the visual PSPs was not statistically different between the two species, and this was irrespective of the chosen threshold level: half-maximal duration was 243.5±21.7 ms (*N*=30) in archerfish and 225.0±24.9 ms (*N*=14) in goldfish (Mann–Whitney test, *P*=0.4). Taking duration at 10% of maximum PSP amplitude yielded 775.5±78.9 ms (*N*=30) in archerfish and 713.2±193.1 ms (*N*=14) in goldfish (Mann–Whitney test, *P*=0.27).

Similar to the resting and action potential (Fig. 2), amplitude and latency of the visual PSPs were remarkably stable over the measurement period. Variability was small in each given animal and did not depend on the number of stimuli previously experienced. Fig. 4A shows this in one typical fish for five PSPs induced with an inter-stimulus interval of 10 s (linear regression analysis; r^2=0.03, *P*=0.68 amplitude; r^2=0.01, *P*=0.80 latency). Five further PSPs recorded 90 min later are superimposed. The PSPs that were measured later did not differ significantly in amplitude and latency from those that were measured first (*t*-test; *P*=0.17 amplitude; *P*=0.18 latency). In archerfish, the amplitude of the visually induced PSPs ranged from 5.3 to 28.2 mV across the *N*=30 fish (17.1±1.1 mV; Fig. 5A,B). The latency of PSP onset after onset of the light flashes ranged from 27.5 to 54.2 ms across archerfish (39.4±1.3 ms, *N*=30; Fig. 5B), but for each given animal, latency, measured with the same standard flash stimulus, was remarkably constant (as illustrated with the example shown in Fig. 4A). In 26 of the 30 archerfish, the light flashes caused PSPs with an amplitude larger than 10 mV and in 10 of these fish the flash even caused a spike in the Mauthner cell. The flash-driven spikes were elicited during the rising phase of the PSP (Fig. 1D). In comparison, the same light flash delivered to goldfish under otherwise identical conditions caused PSPs with an amplitude of 7.0±0.4 mV (*N*=14; Fig. 5A,C). In goldfish, we never observed flash-induced Mauthner cell spikes and average PSP amplitude was significantly lower in goldfish than in archerfish (*t*-test; *P*<0.0001).

The PSPs elicited by acoustic stimuli

The time course of the acoustically evoked PSPs was distinctly different from that of the visually evoked PSPs (Fig. 4B). It was impressive that the loud acoustic bursts of 145 dB re. 1 µPa were much less efficient in causing PSPs in the Mauthner cell of archerfish than were the brief light flashes. Across all archerfish, the amplitude of the acoustically induced PSPs ranged from zero to about 18 mV (4.3±0.8 mV, *N*=30; Fig. 5A,B). In the 23 animals with detectable acoustic burst-induced PSPs, latency was – as expected – much shorter than for the visual stimuli. It ranged from 7.4 to 15.1 ms (9.8±0.5 ms, *N*=23; Fig. 5B) but, again, varied little for each given animal.

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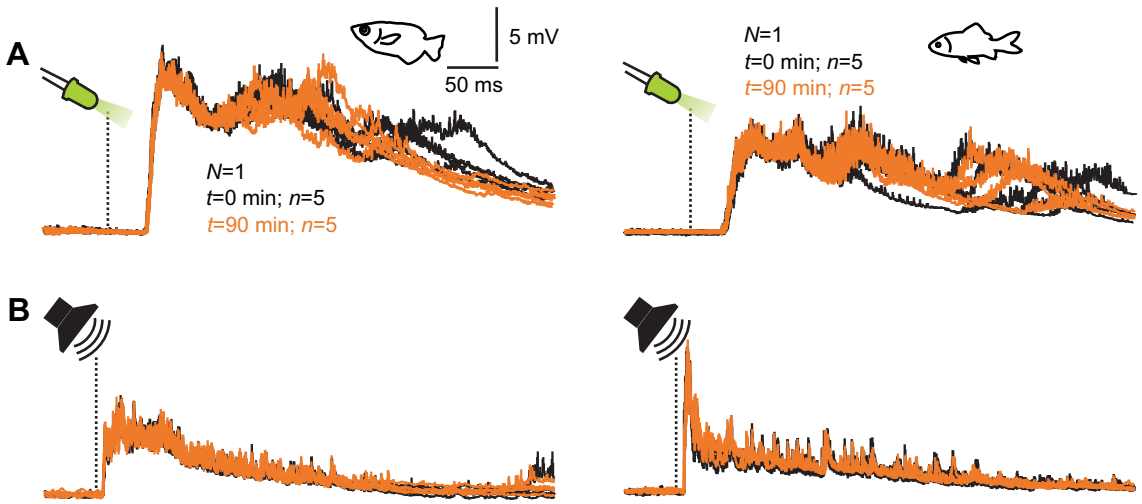

Fig. 4. Postsynaptic potentials in response to visual and acoustic stimuli in archerfish and goldfish. (A) Postsynaptic potentials (PSPs) in response to light flashes recorded in the soma of one archerfish (left) and one goldfish (right) Mauthner cell to illustrate the extent of consistency in visually induced PSPs for both species. For each of the two individuals, 10 PSPs are shown: five PSPs (black) were recorded consecutively with an inter-stimulus interval of 10 s; the other five PSPs (orange) were also recorded consecutively, but about 90 min later. In both species, the initial part of the visually induced PSPs was relatively constant, whereas the long later part of the PSP showed considerable variability. The measurements also illustrate the stability of the recording of visual PSPs in both archerfish and goldfish. Note that the same stimulus and the same recording conditions were used in the two species. (B) As in A, but for PSPs in response to the loud acoustic bursts recorded in the same two fish as shown in A.

PSP amplitude was significantly smaller (on average about 25%) than for the light flashes (Fig. 5A; paired *t*-test; $P<0.0001$). In almost two-thirds (19 of 30) of the fish, the acoustically induced PSPs had an amplitude below 5 mV, less than the smallest amplitude (5.3 mV) recorded in any light flash-induced response in the same animals. In goldfish, the relative importance was reversed, with significantly lower amplitude (7.0±0.4 mV; $N=14$) in the visually compared with the acoustically induced PSPs (8.2±0.5 mV, $N=14$; difference $P<0.05$, paired *t*-test; Fig. 5A,C). Fig. 5 directly compares the amplitudes of visually and acoustically induced PSPs measured in all individual archerfish and goldfish. In 29 of 30 individual archerfish, the acoustically induced PSPs were of lower amplitude, whereas in 10 of 14 goldfish they were of larger amplitude than visually induced PSPs. In seven of the 30 archerfish in which the light flashes had caused strong PSPs, the acoustic bursts even failed to elicit detectable PSPs (Fig. 5A,B). Because the same stimuli were used

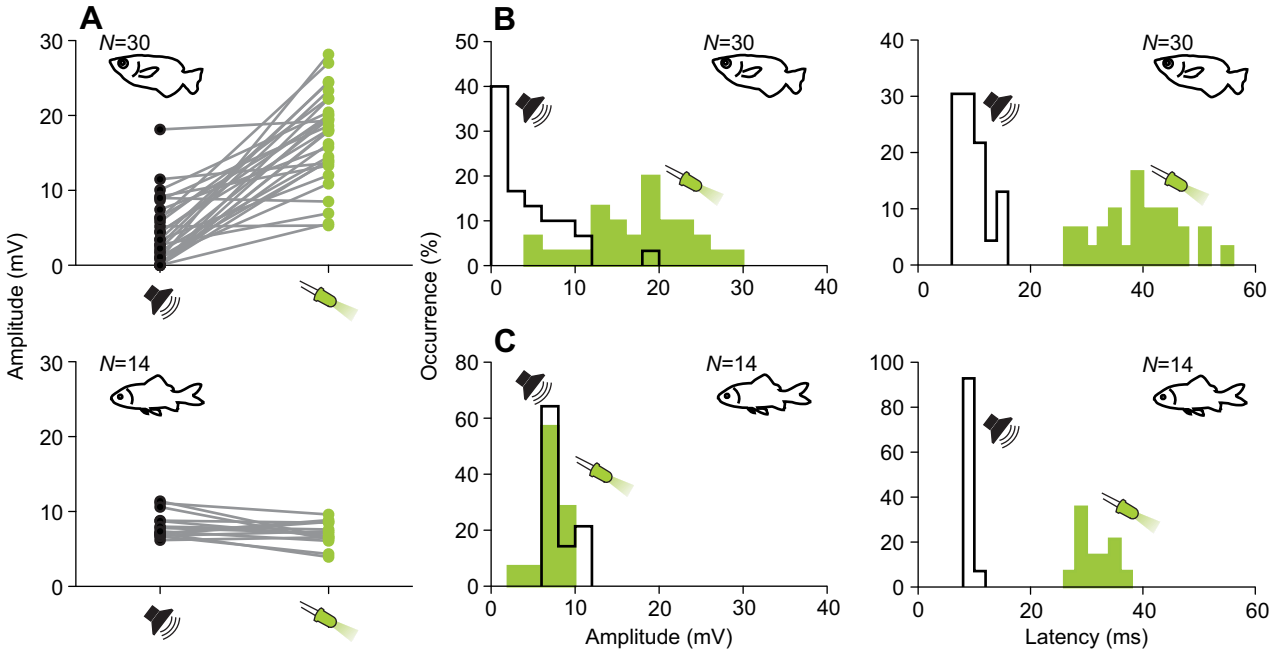

Fig. 5. Comparison of visually and acoustically induced PSPs in archerfish and goldfish. (A) Relationship between PSP amplitudes in $N=30$ archerfish and $N=14$ goldfish in which PSPs had been recorded in response to both the visual and acoustical stimulus in the same individuals. Note that in archerfish, the efficiency of the visual stimulus was larger ($P<0.0001$; paired *t*-test), whereas the acoustical stimulus was more efficient in goldfish ($P<0.05$; paired *t*-test). (B) Distribution of amplitude and latency of the visually (green filled area) and acoustically (black line) induced PSPs recorded in the Mauthner cell soma in the $N=30$ archerfish of this study. (C) As in B, but for the $N=14$ goldfish used in the current study.

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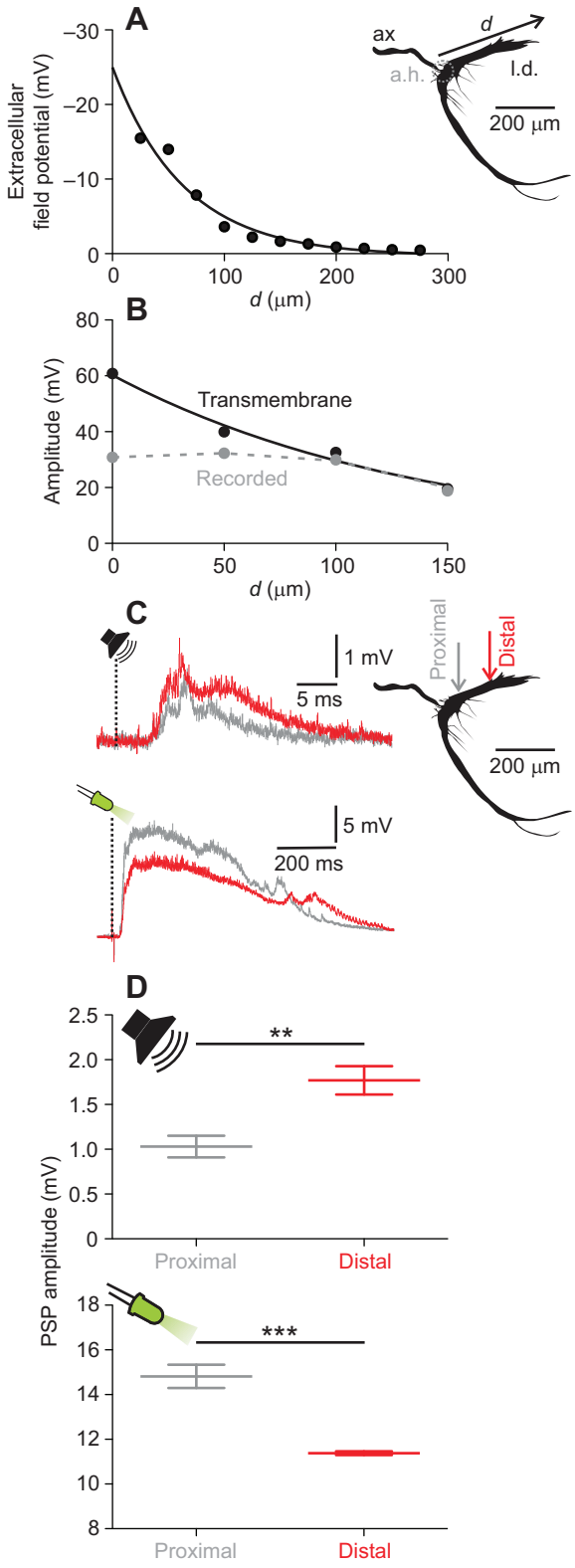

Fig. 6. Signal propagation along the lateral dendrite of the archerfish Mauthner cell and reconstruction of the sites of visual and mechanosensory input. (A) Amplitude of the extracellular field potential accompanying Mauthner activation recorded at various positions along the lateral dendrite (l.d.) at a fixed distance (10 µm) from its surface. The field potential decreased exponentially with horizontal distance *d* from the Mauthner axon hillock (a.h.) with a characteristic length constant of 60 µm (r^2=0.96; exponential decay). ax, initial part of the Mauthner axon. (B) The decay of the action potential along the lateral dendrite can be reconstructed based on measurements in A. While the recorded amplitude of the action potential (grey dashed line) appears to be almost constant, the reconstructed real transmembrane potential (black line; see Fig. 1C) decays exponentially with distance, with a length constant of about 140 µm (r^2=0.98). (C,D) The decay properties can be used to show that mechanosensory input arrives at the lateral dendrite, but visual input does not. (C) Examples of acoustic (top) and visual (bottom) PSPs recorded in the same animal at the indicated two positions in the lateral dendrite. Note that acoustical PSPs were larger in the distal recording position, but visual ones were larger in the proximal position. Proximal position was 50 µm lateral to the axon hillock; distal position was 150 µm from the axon hillock. (D) The amplitude of acoustically induced PSPs was significantly larger in the distal position (top; ***P*=0.006, *t*-test). In contrast, the visually induced PSPs decreased with distance from the soma (bottom; ****P*<0.0001, *t*-test).

in the two species, it is appropriate to say that in archerfish the visual stimuli were more efficient, compared to our standard acoustic stimulus, in causing large PSPs in the Mauthner cell. Average duration of the acoustic PSPs was not statistically different in the two species. The duration between the points of half-maximal PSP amplitude was 196.6±26.7 ms (*N*=23) in archerfish and 185.2±32.2 ms (*N*=14) in goldfish (Mann–Whitney test, *P*=0.84). Between the points where amplitude was 10% of the maximum value, duration was 384.8±58.6 ms (*N*=23) in archerfish and 372.1±52.1 ms (*N*=14) in goldfish (Mann–Whitney test, *P*=0.67).

Decay of the Mauthner cell spike along the lateral dendrite

We next studied the propagation of the antidromically evoked action potential along the lateral dendrite. As described in the preceding paper (Machnik et al., 2018), we first searched the focus point of the all-or-none field potential that accompanies the Mauthner cell spike (see fig. 4 of Machnik et al., 2018). To measure the amplitude of the extracellular field potential along the lateral dendrite, at a fixed distance from it, we first penetrated the dendrite and then moved the recording electrode out in dorsal direction so that it was always located 10 µm away from the surface of the dendrite (Fig. 6A). While the external potential decayed exponentially, the amplitude of the Mauthner action potential, recorded between the intracellular electrode and the distant reference electrode, appeared to be constant (Fig. 6B). However, when the actual transmembrane potential was reconstructed in the way illustrated in Fig. 1C, its exponential decay along the lateral dendrite became apparent (Fig. 6B). We examined the decay in three archerfish. Each fish showed similar properties to those shown in Fig. 6B. The length constants of the lateral dendrite ranged from 141 to 164 µm (149±7 µm, *N*=3) and are thus close to measurements obtained in goldfish (e.g. Faber and Korn, 1978). Hence, in archerfish and goldfish alike, signals spread passively along the lateral dendrite with similar length constants.

The passive decay along the lateral dendrite enables a simple check for the sites of input of the visual and acoustic stimuli to the archerfish Mauthner cell, just by measuring PSP amplitude at a proximal and a more distal position on the lateral dendrite (Fig. 6C). For sensory input at the distal part of the lateral dendrite, PSPs should be large at the distal position and smaller close to the soma. In contrast, input to the ventral dendrite (or to the soma) should cause PSPs that decay along the lateral dendrite with increasing distance from the soma. The former condition held for the acoustically induced PSPs, which were significantly (*P*=0.0059, *t*-test) larger in the distal part of the lateral dendrite (Fig. 6D). In contrast, the amplitude of visually induced PSPs decreased

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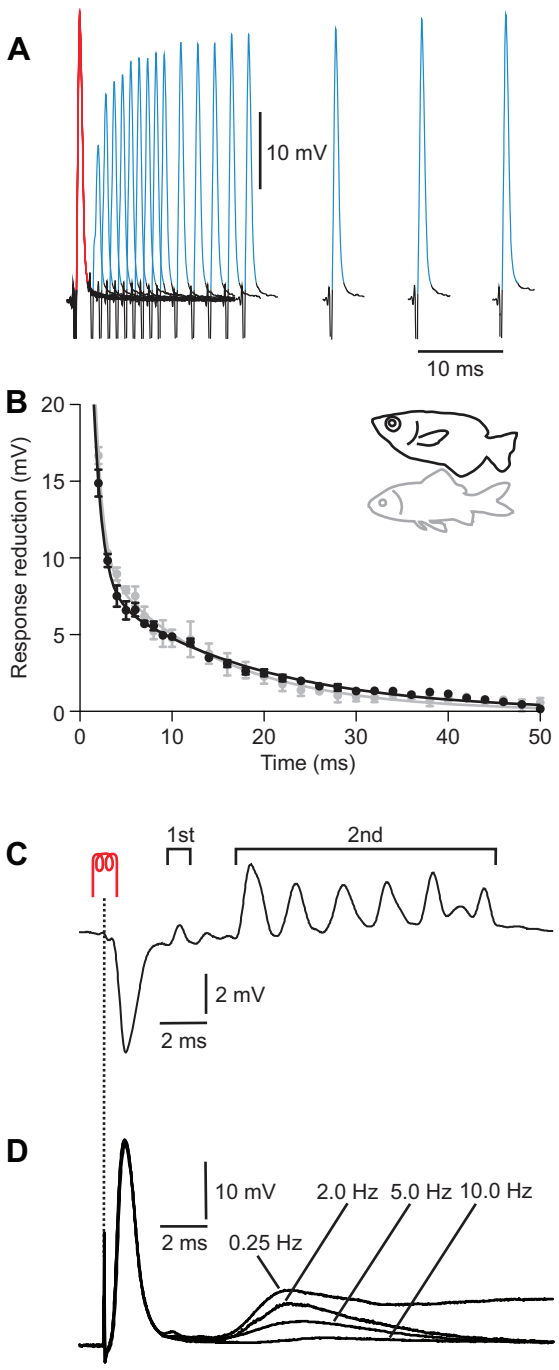

Fig. 7. All hallmarks of feedback inhibition are shared by the goldfish and archerfish Mauthner system. (A) In the now classical experiment of Furukawa and Furshpan (1963), a second action potential (blue) is elicited a defined short interval after the first one (red), typically of smaller amplitude, which allowed quantification in goldfish of the inhibitory effects triggered by the first action potential. Recordings (overlaid; measured in one archerfish) were made in different trials with pairs of antidromically elicited action potentials, but with different temporal spacing between the two action potentials. (B) Quantitative comparative analysis of the decay of the inhibitory effect in goldfish and archerfish. Data are reported as means±s.e.m. for N=3 archerfish (black) and N=3 goldfish (grey) tested under identical conditions. In both species, the decay of the inhibition is best described by a two-phase decay (goldfish r^2=0.95; archerfish r^2=0.98). Time constants of the fast component were 0.9 ms in archerfish and 0.8 ms in goldfish and are presumably linked to sodium channel inactivation. Time constants of the slow component were 16.5 ms in archerfish and 12.8 ms in goldfish. In both species, inhibition was long lasting and can be seen even after 40 ms. (C) Field potential recorded within the axon cap, 10 µm above the surface of the Mauthner cell, after spinal cord stimulation (timing as indicated). Note the positive potential (marked 1st) that follows the Mauthner spike-accompanying negative field potential after a short delay. Also note the barrage (marked 2nd) of later positive potentials. The findings are strikingly similar to the classical results in the goldfish. (D) Superimposed intracellular recordings from a chloride-loaded Mauthner cell (i.e. recorded with an electrode filled with 3 mol l^{-1} KCl) aligned to the extracellular recording in C. Just as in the goldfish (Furukawa and Furshpan, 1963), the second (inhibitory) component can now be seen as apparently positive potentials (because the chloride content has shifted the reversal potential). The four recordings were made in the Mauthner soma of the same archerfish, but during continuous spinal cord stimulation at various stimulation frequencies (from 0.25 to 10 Hz) as indicated. Amplitude and latency of the Mauthner action potential amplitude were unaffected by stimulation frequency, but amplitude and latency of subsequent PSPs were affected as in goldfish, suggesting at least functionally equivalent inhibitory networks in archerfish and goldfish.

significantly (P<0.0001; t-test) from the soma to the distal part of the lateral dendrite (Fig. 6D).

All hallmarks of feedback inhibition are shared by the goldfish and archerfish Mauthner system

Efficient inhibitory feedback ensures that only one of the two Mauthner cells fires (so that the fish is bent by unilateral activation of trunk muscles and not compressed by simultaneous bilateral contraction) and that it fires only once. This so-called recurrent inhibition was discovered early in Mauthner research (Furukawa and Furshpan, 1963) and has been thoroughly studied in goldfish. In goldfish, it can be detected and characterised in experiments in which two action potentials are elicited antidromically with a fixed interval between them. The inhibition triggered by the first action potential can then be measured by the reduction in amplitude of the second action potential. By increasing – in successive experiments – the interval between the pair of antidromic stimuli, it has been shown in goldfish that it is possible to map the time course of the inhibition. Running this classical experiment on the archerfish Mauthner cell should provide a sensitive assay for any functional differences in feedback inhibition. However, we found that the time course of the inhibition is strikingly similar to that known in goldfish (Furukawa and Furshpan, 1963). To directly compare the two species, Fig. 7B reports the decay for archerfish and goldfish measured under the same conditions (including temperature). A two-phase exponential decay described the data very well, with time constants of 0.9 ms (archerfish) and 0.8 ms (goldfish) for the fast component and 16.5 ms (archerfish) and 12.8 ms (goldfish) for the slower component. In goldfish, feedback inhibition involves electrical and chemical synaptic components. Recurrent inhibition causes a positive-going field potential in the axon cap immediately after a Mauthner spike. This field potential shows a short-latency component and one that occurs later. The two components respond distinctly differently to repeated stimulation. While the rapid early component does not fatigue, the second does. In goldfish, this has been traced back to the first component being caused by an electrical synapse, whereas the fatigue in the later response is due to chemical synaptic transmission. Fig. 7C,D shows an example of tests in which the classical goldfish experiments were repeated in archerfish. The example shown was typical for every one of the four archerfish examined in this respect. The findings are so strikingly similar to those of Furukawa and Furshpan (1963) in goldfish that it would seem impossible to say which was conducted in which of the two species.

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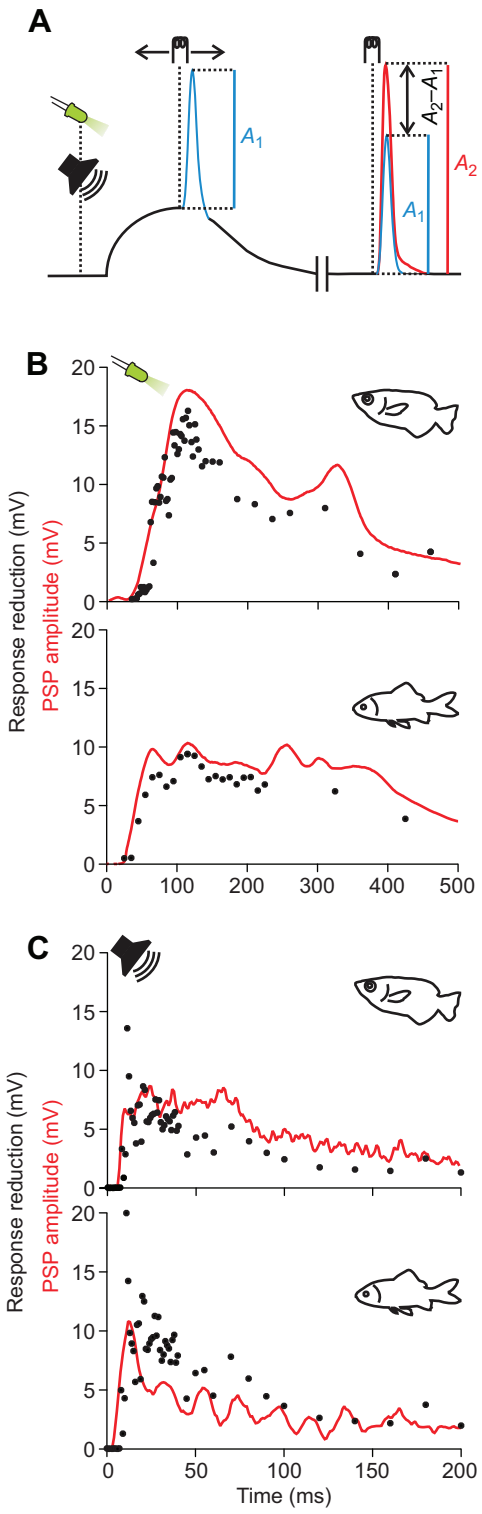

Fig. 8. The classical experiments on feed-forward inhibition detect no specialisation in the archerfish Mauthner system. (A) Outline of the experimental approach used by Furukawa and Furshpan (1963) to infer feed-forward inhibition in goldfish. Sensory stimulation (visual or acoustic) causes a subthreshold PSP. A set time after the onset of the PSP, a first Mauthner spike (blue, amplitude A_1) is elicited by spinal cord stimulation. A second action potential (red) is elicited later after decay of the PSP to yield baseline amplitude A_2. In goldfish, the difference A_2–A_1 can profitably be used as a measure for sensory-driven feed-forward inhibition. (B,C) Feed-forward inhibition for visual (B) and acoustic (C) stimulation in archerfish and goldfish. Each diagram shows a typical PSP (in red) after stimulation at time zero (t=0) together with measurements of the reduction in amplitude of the action potential in the same experimental animal and measured as illustrated in A.

Classical experiments on feed-forward inhibition detect no specialisation in the archerfish Mauthner system

In the goldfish Mauthner system, a further important type of inhibition is driven by the sensory stimulus itself. In addition to exciting the Mauthner cell, sensory input also drives feed-forward inhibition. The properties of the feed-forward network have been worked out in detail in goldfish, but, to our knowledge, only for mechanosensory stimuli (Furukawa and Furshpan, 1963; Faber and Korn, 1978). To probe for any deviations in feed-forward inhibition in archerfish and goldfish, we analysed the apparent reduction in the amplitude of the action potential during a PSP – using the goldfish results with mechanosensory stimuli to confirm the compatibility with the earlier studies. Running these tests (Fig. 8A) on archerfish and goldfish under the same conditions showed (in four out of four fish of either species) no difference between the two species – not only for the mechanosensory stimuli but also for the visual stimuli. In the two species, the duration of the inhibitory effect seems equally well matched to PSP duration. While the functional hallmarks of inhibition are strikingly similar, we need to emphasise that they could be realised differently. The simplest explanation would, however, appear to be that (i) visual and mechanosensory stimuli drive the same inhibitory network in goldfish and (ii) the same network is also used in the archerfish Mauthner system.

DISCUSSION

We have demonstrated that archerfish Mauthner neurons allow robust *in vivo* intracellular recordings of responses to both mechanosensory and visual stimuli. Because the available evidence suggests that all neurons of the Mauthner series (i.e. the Mauthner neuron and its serial homologues) receive the same information (Nakayama and Oda, 2004), our findings establish an important window of opportunity to investigate the flow of visual information into the archerfish's fast-start circuitry. In the present study, we used this accessibility to check all hallmark physiological properties known for the goldfish Mauthner cell in archerfish, with the intention of probing for any differences between the Mauthner cells of goldfish and archerfish. Such differences could be linked to the occurrence of the archerfish's fine-tuned predictive C-starts and/or the variable escape C-starts, regardless of whether these starts actually do recruit the Mauthner cell or not. If the Mauthner cell was not recruited in the variable archerfish C-starts, then the evolutionary pressures of keeping a giant Mauthner cell with all its hallmark physiological properties should be reduced. Conversely, if the archerfish Mauthner neuron was involved in the archerfish C-start behaviours then its physiology might require some amendment to be used in variable C-starts, provided that 'typical' Mauthner neurons are not useful in more sophisticated, variably tuned C-starts. Quite surprisingly, we found that the archerfish Mauthner cell resembles that of the less specialised goldfish in every examined aspect, including all hallmark properties of its feedback and feed-forward inhibitory circuits. Although the question remains open whether and how the archerfish Mauthner cell is involved in triggering the archerfish C-starts, our findings are clearly not in line with recent suggestions on the evolution of the Mauthner neuron.

The archerfish Mauthner cell shares all hallmark physiological characteristics with that of goldfish

In the companion paper (Machnik et al., 2018), we described the remarkable similarity in size and morphology between the goldfish and the archerfish Mauthner neuron. Here, we extend this similarity to hallmark physiological characteristics that have been established for over half a century in goldfish (see Zottoli and Faber, 2000; Korn and Faber, 2005). Even the organisation of sensory input, with the lateral dendrite receiving mechanosensory input and the ventral dendrite receiving visual input (Zottoli, 1977, 1978; Zottoli et al., 1987), and even the length constants of the lateral dendrite appear to be similar to those reported for goldfish (Fig. 6). Although archerfish consistently produced larger PSPs in response to the visual stimuli (Fig. 5), the overall structure of the archerfish visually and acoustically induced PSPs was not different from that found in the control goldfish. The similarity extends even to the occurrence of variations in the long later phase of the visual PSPs (Fig. 4). This phase occurs too late to be of importance in directly triggering a C-start (though it can allow other stimuli to reach threshold), and we first thought that its variability could have been indicative of a specialisation of the archerfish Mauthner system. However, this aspect is also shared by the goldfish visual PSPs (Fig. 4). The late inputs would modulate response threshold and would be particularly important when the variations are not matched to the time course of the feed-forward inhibition. Additionally, they could be important in the responses of the MiD2cm and the MiD3cm neurons that are thought to receive the same inputs. In this regard, we note that MiD2cm and MiD3cm are also present in archerfish (they can be filled with Calcium Green dextran to visualise them in archerfish larvae; P.M., in preparation). Regardless, the effects of the long PSPs are shared by goldfish and archerfish alike.

Feedback inhibition is a key hallmark of the goldfish Mauthner system and was discovered and characterised in remarkable depth in the classical work of Furukawa and Furshpan (1963) (also see Faber and Korn, 1978). In goldfish, the network responsible for the long-lasting feedback inhibition has been worked out in detail. It is striking that repeating the hallmark experiments on feedback inhibition in archerfish produced the same results as in the classical goldfish study. Functionally, feedback inhibition is remarkably similar in the two species (Fig. 7) and – when tested under identical conditions (including an identical temperature of 20°C) – even shows the same two-phase decay with a similar time constants of about 1 ms for the fast component (probably due to sodium channel inactivation) and 13–16 ms for the slow component that in goldfish indicates the effect of the inhibitory network. Clearly, we cannot exclude that the functional similarity might be brought about by different physiological mechanisms, but the similarity of the recordings obtained with the KCl electrode (that in goldfish is known to invert the inhibitory PSPs) and of the effects of repetitive stimulation with the classical findings is so striking that shared mechanisms seem likely. Another important characteristic of the Mauthner cell-associated networks in goldfish is feed-forward inhibition. The underlying circuitry has been characterised in detail for mechanosensory-driven inputs (Faber and Korn, 1978; Faber et al., 1989), but (to our knowledge) not for visual input. Briefly, in goldfish, mechanosensory input affects the Mauthner neuron via excitatory input to its lateral dendrite and via commissural interneurons that inhibit the Mauthner cell. Repeating the classical experiments in archerfish again failed to detect any differences from the properties found in goldfish and this also includes the effect of the visual stimuli. In both species, the time course of the inhibition matched the waveform of the respective PSPs remarkably well (Fig. 8). Again, we cannot exclude that archerfish attain this by using different networks, but clearly the result is strikingly similar.

The only major difference we found between the Mauthner cells of the two species is that the archerfish Mauthner cell has a longer ventral and a thinner lateral dendrite (Machnik et al., 2018) and appears to be affected more strongly by visual and less by acoustic (mechanosensory) input (Fig. 5). Although we can presently only speculate about any actual involvement of the Mauthner cell in the predictive C-starts, it is clearly tempting to link this higher visual sensitivity to the importance of visual information in driving these starts (e.g. Schuster et al., 2004, 2006; Schlegel et al., 2006; Rischawy and Schuster, 2013).

Potential roles of the archerfish Mauthner neuron and its associated networks

Suppose the equivalence of the archerfish and goldfish Mauthner neurons indicates that they use their Mauthner cell similarly, to trigger their escape C-starts. Because archerfish escape C-starts are – in both phases – kinematically as variable as the predictive C-starts (Wöhl and Schuster, 2007), this would directly disprove that Mauthner neurons cannot be recruited to trigger variable C-starts (Bhattacharyya et al., 2017). This would, however, raise the question, why they then cannot also be used to trigger the predictive C-starts. An alternative view would be that the archerfish Mauthner neuron is not used in any of the archerfish C-starts. If so, our findings make it difficult to see which evolutionary pressures would have stabilised the archerfish Mauthner cell to be so remarkably similar to that of goldfish. Our hypotheses, based on the present findings, therefore are: (i) that archerfish do use their Mauthner neurons in both types of C-starts and (ii) that even the Mauthner cell of (adult) goldfish is fully capable of triggering sophisticated and flexibly tuned C-starts, although this is more difficult to show in standard escape trials. We suggest that the most convincing test of the first hypothesis (i.e. of any involvement of the archerfish Mauthner cell) would be to modify the characteristics of the escape and/or predictive C-starts in suitable behavioural (e.g. training) experiments. By then stimulating the fish during *in vivo* recording of the Mauthner cell in the same way as in the behavioural experiments, it would be possible to check for correlated changes in the properties of the Mauthner neuron. Response probability, latency and also directionality of the predictive C-start can be varied in many ways. For instance, luminance, visual contrast and temperature strongly affect response probability and latency, but not accuracy of the predictive start (e.g. Schlegel and Schuster, 2008; Krupczynski and Schuster, 2013; Reinel and Schuster, 2014). Conversely, fish can be trained to systematically start differently as they normally would, but without changing response probability and latency. In the light of the rich behavioural background available for archerfish, we hope that our present findings on the archerfish Mauthner neuron will allow a fresh look at the role of a typical teleost Mauthner neuron in variable and fine-tuned C-start manoeuvres.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.M., S.S.; Methodology: P.M., W.S.; Formal analysis: S.S.; Investigation: P.M., K.L., S.F.; Resources: W.S.; Data curation: P.M.; Writing - original draft: P.M., S.S.; Writing - review & editing: P.M., S.S.; Visualization: P.M.; Project administration: P.M., S.S.

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