J Neurophysiol 100: 2997–3008, 2008.
First published October 15, 2008; doi:10.1152/jn.91033.2008.

Effects of Temperature Acclimation on a Central Neural Circuit and Its Behavioral Output

Theresa M. Szabo,[1,2,3] Ted Brookings,[2] Thomas Preuss,[3] and Donald S. Faber[3]

[1]*Marine Biological Laboratories, Woods Hole;* [2]*Department of Biology, Brandeis University, Waltham, Massachusetts; and* [3]*Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York*

Submitted 11 September 2008; accepted in final form 14 October 2008

Szabo TM, Brookings T, Preuss T, Faber DS. Effects of temperature acclimation on a central neural circuit and its behavioral output. *J Neurophysiol* 100: 2997–3008, 2008. First published October 15, 2008; doi:10.1152/jn.91033.2008. In this study, we address the impact of temperature acclimation on neuronal properties in the Mauthner (M-) system, a brain stem network that initiates the startle-escape behavior in goldfish. The M-cell can be studied at cellular and behavioral levels, since it is uniquely identifiable physiologically within the intact vertebrate brain, and a single action potential in this neuron determines not only whether a startle response will occur but also the direction of the escape. Using animals acclimated to 15°C as a control, 25°C-acclimated fish showed a significant increase in escape probability and a decrease in the ability to discriminate escape directionality. Intracellular recordings demonstrated that M-cells in this population possessed decreased input resistance and reduced strength and duration of inhibitory inputs. In contrast, fish acclimated to 5°C were behaviorally similar to 15°C fish and had increased input resistance, increased strength of inhibitory transmission, and reduced excitatory transmission. We show here that alterations in the balance between excitatory and inhibitory synaptic transmission in the M-cell circuit underlie differences in behavioral responsiveness in acclimated populations. Specifically, during warm acclimation, synaptic inputs are weighted on the side of excitation and fish demonstrate hyperexcitability and reduced left–right discrimination during rapid escapes. In contrast, cold acclimation results in transmission weighted on the side of inhibition and these fish are less excitable and show improved directional discrimination.

INTRODUCTION

One major question regarding temperature acclimation concerns adaptive changes in the functional properties of neural circuits, including intrinsic membrane properties and excitatory and inhibitory transmission. Studies have demonstrated that compensatory processes occur during acclimation; for example, the fluidity of neuronal membranes is maintained at relatively constant levels in a process termed "homeoviscous adaptation" (coined by Sinesky 1974) and in *Carassius* temperature changes have been shown to produce alterations in fatty acid saturation (Cossins 1977; Cossins and Prosser 1978; Kakela et al. 2008). Behavioral studies have indicated acclimation alters capabilities for learning and memory (Borsook et al. 1978; Brezden et al. 1973; Riege and Cherkin 1972; Roussel et al. 1982; Shashoua 1973; Zerbolio 1973). However, the manner in which vertebrate neuronal circuitry is altered to preserve function is not well known, in part due to the difficulty of recording from CNS neurons in vivo.

To date, most studies examining temperature effects on neuronal properties have focused on acute or acclimated effects in invertebrate systems (Dierolf and McDonald 1969; Johnson et al. 1991; Neumeister et al. 2000; Rosenthal and Bezanilla 2000, 2002; Weight and Erulkar 1976; Zhurov and Brezina 2005) or the neuromuscular junction in "lower" vertebrates (Katz and Miledi 1965; Macdonald and Montgomery 1982). Although, in general, colder temperatures slow membrane processes whereas warmer temperatures speed them up (reviewed in Prosser and Nelson 1981), one study that examined acute temperature effects on the CNS of a vertebrate demonstrated hyperexcitability in cold (Preuss and Faber 2003). This suggests that some processes, such as synaptic transmission and membrane excitability, that involve the integrated functioning of multiple factors might be differentially influenced by acute or maintained temperature change (reviewed in Montgomery and Macdonald 1990).

To address the effects of temperature acclimation on a neuronal circuit and its behavioral correlate in the vertebrate CNS we used the well-described goldfish Mauthner (M-) cell circuit as a model (for review see Zottoli and Faber 2000). The M-cells are a pair of bilaterally symmetrical reticulospinal neurons that trigger the rapid escape response. The major excitatory synaptic inputs to the M-cell lateral dendrite arise from the auditory (VIIIth) nerve and contact the M-cell as large myelinated club endings (LMCEs). LMCEs form large synaptic terminals distally on the lateral dendrite of the M-cell that can be $\leq$12 μm in diameter (Nakajima and Kohno 1978) and have mixed synapses (i.e., containing both chemical and electrical synaptic components). Thus stimulation of presynaptic fibers results in a mixed excitatory postsynaptic potential (EPSP) in the M-cell with a characteristic shape that includes an initial rapid coupling potential (CP) followed by a slower chemical PSP (Lin and Faber 1988). Inhibitory inputs to the M-cell have also been well described and are important regulators of threshold for the M-cell. Threshold is maintained at relatively high levels in the M-cell, since otherwise soft sounds would produce an escape. Because M-cell firing initiates the startle response, or C-start, an examination of factors affecting initiation of the C-start in freely swimming fish is also an assessment of M-cell threshold properties. Also, because the M-cell is physiologically identifiable within the intact brain and therefore accessible for in vivo recordings (Furshpan and Furukawa 1962), it is a particularly valuable model for study-

Address for reprint requests and other correspondence: T. M. Szabo Volen Center MS 013, Brandeis University, Waltham, MA 02454 (E-mail: tszabo@brandeis.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

ing neuronal function and synaptic transmission under acclimated conditions.

We found that acclimation to 25°C is associated with 100% escape probability and a degradation in the ability to localize the direction of the stimulus compared with 5 and 15°C acclimated fish. On a cellular level, this corresponded to reduced strength and duration of M-cell inhibition relative to 5 and 15°C populations. In contrast, animals acclimated to 5°C have the same lower escape probability and improved directionality as those kept at 15°C, a finding which corresponded to increased inhibitory transmission and reduced strength of excitatory inputs onto the M-cell. These latter changes represent true acclimation, as acute chilling has been shown to demonstrate the same behavioral consequences and alterations in neurotransmission seen here with acclimation to 25°C (Preuss and Faber 2003).

METHODS

Animals and acclimation environment

All studies were performed at the Marine Biological Laboratories at Woods Hole, MA over the course of 4 mo, June to October 2007. Goldfish (*Carassius auratus*) ranging from 9.4 to 12 cm in length were obtained in June 2007 from Midwest Tropical Imports (North Kansas City, MO) and were maintained in three tanks containing about 300 gallons of water each at 5, 15, and 25°C. These temperatures are consistent with the natural range described for goldfish (Sohn et al. 1999). Fish at 25°C were fed daily, at 15°C every other day and at 5°C twice a week according to the metabolic needs of each group, and any food not eaten within 20–30 min was removed. Water temperature was monitored and recorded every 15 min over the course of the summer using a HOBO Pendant Temperature/Light Data Logger (MicroDAQ.com, Contoocook, NH). Water in each tank was aerated and fresh water continually flowed into the tanks via a system of pipes from a freshwater reservoir. Fresh water was made from tap water conditioned with (per 200 gallons): 54 g Sea Salt (Instant Ocean), 16 g bicarbonate, and 64 g sodium thiosulfate. The 25°C tank had an additional floating biofilter since bacterial buildup in this tank was extensive. The three tanks were exposed to the same ambient light conditions so that temperature was the only acclimation factor studied. It has been demonstrated that acclimation from 15 to 5 or 25°C occurs over the course of 3–4 wk in goldfish (Sidell et al. 1973; reviewed in Roots and Prosser 1962). For this reason, fish were acclimated ≥4 wk before use. All procedures were carried out in accordance with IACUC protocols approved at the Marine Biological Laboratories at Woods Hole.

Behavioral experiments

Nineteen animals were used to examine the effect of temperature acclimation on escape kinematics. The goldfish used for these studies had comparable body lengths (5°C: 10.93 ± 0.33 cm; 15°C: 11.52 ± 0.22 cm; 25°C: 10.58 ± 0.36; $P = 0.132$) and weights (5°C: 43.75 ± 2.62 g; 15°C: 46.99 ± 5.60 g; 25°C: 40.37 ± 2.42 g; $P = 0.491$). We examined kinematics of the C-start behavior in goldfish acclimated to three temperatures: 5, 15, and 25°C. The tank used to examine escape behavior was a 21 × 26-cm clear plastic container with a grid taped to the bottom outside for measuring distance (Fig. 1*A*). Each square on the grid represented 1 cm^2. Startle responses were elicited by a rubberized hammer contacting one side of the test container. A black sheet was taped on the side of the tank where the hammer hit so that the fish could not see the hammer approach. This behavioral setup was contained within a plastic tub covered by black sheeting so the fish were distracted as little as possible by movements and sounds in the laboratory. The stimulating hammer was drawn back by a string

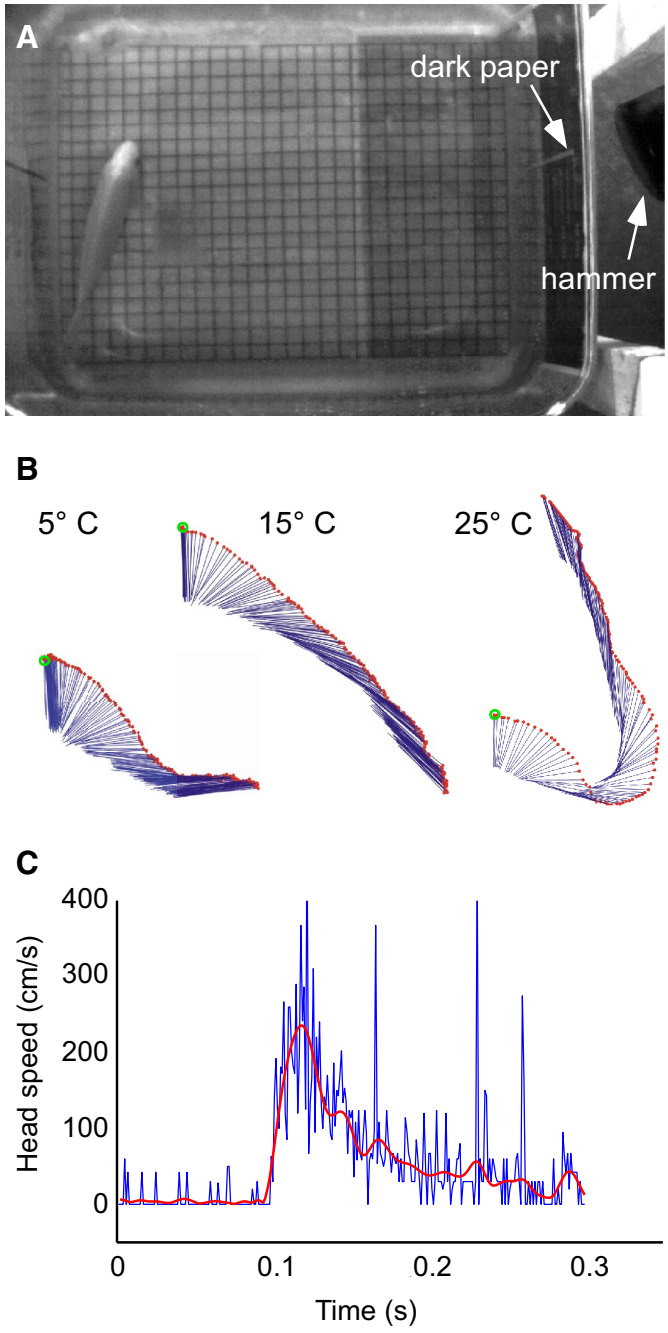

FIG. 1. Analysis of behavioral kinematics. *A*: still image of a fish in the behavioral test tank. A grid was taped underneath the tank with 1-cm^2 markings for calibration purposes. A hanging rubber hammer was pulled back by a string to contact the container and the corresponding side of the container was covered with dark paper so that the fish could not see the approach of the hammer. Escape responses were recorded for about 300 ms before, during, and after the hammer contacted the side of the tank. *B*: examples of the digitized escape behavior for 3 fish at 5, 15, and 25°C. For each frame, the position of the head of the fish was digitized by marking the nose (indicated by red dots) and the back of the skull; the blue line connects these 2 points. Green circles indicate the position of the nose of the fish at the onset of each recording. Recordings were made at 1 frame/ms so the distance between each line indicates the distance moved by the head/ms. *C*: example of smoothing analysis of the digitized data. The blue trace represents the quantification of the raw data (head speed in this example). The red trace represents the smoothed data used for peak amplitude measurements reported in these studies.

extending under the outer sheeting. A mirror was placed on a 45° angle above the tank and a high-speed video camera (1200 digital high-speed 10-bit CMOS camera system, PCO/Cooke, Romulus, MI) recorded the escape behavior from the reflection in the mirror at 1 frame/ms on a PC. Recordings started just before the hammer was released and captured the behavior of the fish prior to, during, and following contact of the hammer with the container. Two lights sitting near the camera illuminated the fish via the mirror.

Animals were placed in the recording chamber in their native tank water and taping of the escape behavior began within 5–10 min. Each fish was given 10 escape trials at its acclimated temperature, with about 3–4 min between trials. Fish acclimated to 5°C were then acutely warmed 10° over the course of 1 h in the same aerated tank water (a bubbler and heating unit were added to the water). After 1 h, they were then given an additional 10 trials at 15°C. Similarly, fish acclimated to 25° were acutely chilled (Arctica Titanium Chiller, Petsolutions.com, Beavercreek, OH) in their own aerated tank water over the course of 1 h following an initial 10 trials and then given another set of 10 trials at 15°C. In both cases, water was also circulated through a chiller that continuously monitored water temperature.

The escape response in the goldfish has been well described (Eaton et al. 1977). When one M-cell fires, it simultaneously triggers multiple motor neurons on the contralateral side of the fish's body so that muscles on that side contract and the fish forms a C-bend, or C-start. The C-start consists of two basic parts: Stage 1, which rotates the body around the center of mass; and Stage 2, which involves an axial acceleration that moves the center of mass 2–6 cm (Eaton et al. 1988). Only escapes that fit the definition of a C-start were examined in this study. Nonresponses and escapes immediately into the side of the container were also not included in the analysis of kinematics; i.e., maximum head speed, angular velocity, and angular acceleration were measured in cases where the fish started in the middle of the chamber and was able to execute a full turn. Only the kinematics of Stage 1 were examined because Stage 2 was often constrained, either by the size limitation of the container or by the reverberations of water off of the sides of the container. Escape probability and onset latency were measured for all escapes, since these factors were not influenced by limitations of the testing container. Escapes were analyzed using Image J (National Institutes of Health) by placing a dot in the middle of the fish's nose and the middle of the back of the head for each frame of the 300-ms trial. In this manner, the x and y coordinates were recorded and the location of the head in each frame was digitized (Fig. 1*B*). For each trial, distance was first calibrated to the grid on the bottom of the container.

Calculation of swimming kinematics: angular velocity, angular acceleration, and head speed

Velocities and accelerations were calculated in Matlab. From the head and body positions, body angle (θ) was determined with arctangent (allowing θ to exceed the interval $[-\pi, \pi]$ so as to remain continuous), then θ and the x and y components of head position were smoothed using a low-pass filter (fourth-order Butterworth forward and reverse digital filter with a cutoff frequency of 1/20 the sample frequency). Angular velocity (ω) and head velocity (v) were then calculated from the smoothed quantities using finite differences with respect to time (t) and a step size of two, i.e.

$$\omega_n = \frac{\theta_{n+2} - \theta_n}{t_{n+2} - t_n}$$

$$v_n = \frac{\sqrt{[x_{n+2} - x_n]^2 + [y_{n+2} - y_n]^2}}{t_{n+2} - t_n}$$

The step size for finite differences was chosen to balance reduction of noise with elimination of features (both increase as the step size is increased). For the purpose of calculating angular acceleration (α), a smoothed version of angular velocity (ω^*) was computed, using another low-pass filter (fourth-order Butterworth, but with a cutoff of 1/32 the sample frequency). Then angular acceleration was calculated using finite differences with a step size of four

$$\alpha_n = \frac{\omega^*_{n+4} - \omega^*_n}{t_{n+4} - t_n}$$

Electrophysiological experiments

Fish were initially anesthetized using 20 mg/l 3-aminobenzoic acid ethyl ester (MS-222; Sigma, St. Louis, MO). They were then injected intramuscularly with curare (1 μg/g body weight), to paralyze them for the duration of the experiment, and mounted in the recording chamber. They were respirated by flowing water from their acclimation tank through their mouth and over their gills. The water was maintained at the temperature of their acclimation tank with a chiller and contained 20 mg/l MS-222. The spinal cord was exposed for antidromic stimulation of the M-cell that, because the M-cell extracellular spike is distinctively recognizable, allows identification of the field in the intact brain. The fish medulla was exposed to permit intracellular recording from the M-cell. An additional opening was made lateral to the medulla over the inner ear of the fish so that the VIIIth nerve could be exposed for stimulation. Fish brains were then continually superfused with saline (in mM: 124.0 NaCl, 5.1 KCl, 2.8 $NaH_2PO_4 \cdot H_2O$, 0.9 $MgSO_4$, 1.6 $CaCl_2 \cdot 2H_2O$, 5.6 glucose, and 20.0 HEPES, pH 7.2) at the appropriate acclimation temperature (5, 15, or 25°C). Fish saline was maintained at the same temperature as the perfusion water by hanging a beaker in the perfusion water so that the rim was above water level, whereas the rest of the beaker was submerged. Saline temperature was then measured throughout each experiment.

Recording procedures and identification of the M-cell have been described previously (Faber and Korn 1978). M-cell intracellular responses to sound as well as to posterior eighth nerve (orthodromic) and spinal cord (antidromic) stimulation were recorded in the soma (50 μm lateral to the axon cap) as well as distally along the cell's lateral dendrite using 4- to 7-MΩ microelectrodes filled with 5 M potassium acetate (KAc) and 0.1 M potassium chloride (KCl). All recordings were performed in current clamp (Axoclamp 9A; Axon Instruments Foster City, CA). Data were digitized using a Digidata (Axon Instruments) and collected and analyzed using pCLAMP 10.2 software. A Master-8 (AMPI) was used to synchronize stimulation of the spinal cord or VIIIth nerve (Digidata) with recordings.

Data analysis

Data were analyzed using Clampfit 10.2 (Axon Instruments) software and SigmaStat 3.5 for statistical analysis. All data are reported as means ± SE, unless otherwise stated. P values were obtained using a one-way ANOVA (SigmaStat 3.5; Systat Software) and Holm–Sidak post hoc tests, unless otherwise noted. The number of animals are reported as "n," the number of trials as "N."

RESULTS

Behavioral kinematics are reduced in cold, greater in warm

Poikilotherms are exposed to many alterations in environmental temperature and the question of how these different conditions influence behavior and the underlying circuits in the CNS are difficult to address. The C-start is a well-described escape startle response in which a fish contracts the major muscles on one side of its body simultaneously in response to a stimulus such as a predator or loud sound (Eaton et al. 1977;

Zottoli 1977). The goldfish escape is well characterized and has been shown to be initiated by the M-cell (Eaton et al. 1981; Zottoli 1977); thus an examination of escape behavior is also an assessment of firing activity in a central neuron. Escape behavior was captured and digitized as described earlier (Fig. 1*A*; see METHODS). As indicated in the digitized representation of three sample escapes in Fig. 1*B*, cold-acclimated animals were slower and did not move as far as warm-acclimated animals in the same time period.

To determine whether there were differences in the kinematics of the escape behavior between acclimated populations, swimming trajectories were quantified and smoothed (see METHODS; Fig. 1*C*). Compared with 15°C-acclimated animals, 5°C animals had 37.6% slower maximum head speeds and 32.4% slower maximum angular velocities, whereas 25°C-acclimated animals had 25.7% faster maximum head speeds and 26.9% faster maximum angular velocities. All three populations were significantly different (max. head speed, $P < 0.001$; max. angular velocity, $P < 0.002$; Fig. 2, *A* and *C*). For maximum angular acceleration, 5°C-acclimated animals were 12% slower and 25°C animals 3% faster than 15°C animals, performances that were significantly different from each other but not from 15°C animals ($P = 0.032$; Fig. 2*B*). Comparison of the peak latencies for each of these factors, from time of hammer contact, indicated that they were also significantly different (all latency measurements, $P < 0.001$; Fig. 2, *A–C*). Finally, we examined escape onset latency, a measure that reflects the time needed for the neural network to process the stimulus and produce a motor output. Escape latencies measured from stimulus onset (hammer contact) to initial movement of the fish's head were significantly longer in 5°C than in 15°C (123.6%) and 25°C (198.4%) populations ($P < 0.001$; Fig. 3*A*), and the 15 and 25°C populations were not significantly different.

The studies described earlier were performed on fish tested in water at their acclimation temperature. To further separate out the acute effects of water temperature from long-term acclimatory changes, the populations were compared at 15°C; i.e., 5°C fish were acutely warmed 10°C and 25°C fish were acutely chilled 10°C over the course of 1 h and both groups were compared with the 15°C population. Except for a significant difference in maximum head speed between the two groups acclimated to the temperature extremes, there were no longer differences in the measures between these groups (max. head speed, $P = 0.012$; max. angular velocity, $P = 0.058$; max. angular acceleration, $P = 0.520$; time to max. head speed, $P = 0.106$; time to max. angular velocity, $P = 0.125$; time to max. angular acceleration, $P = 0.147$). Thus most of the differences in escape kinematics between populations were due to acute effects of water temperature.

Warm acclimation increases escape probability and reduces directionality

Since studies of acute temperature change have demonstrated that cellular properties such as input resistance can change with temperature, one might also expect the integration of information by the M-cell to be altered. One way to address this is by examining the effect of temperature acclimation on M-cell threshold properties. Since the M-cell triggers the escape response, a determination of escape probability is an indicator of successful M-cell firing. Probability of escape in animals acclimated to 25°C

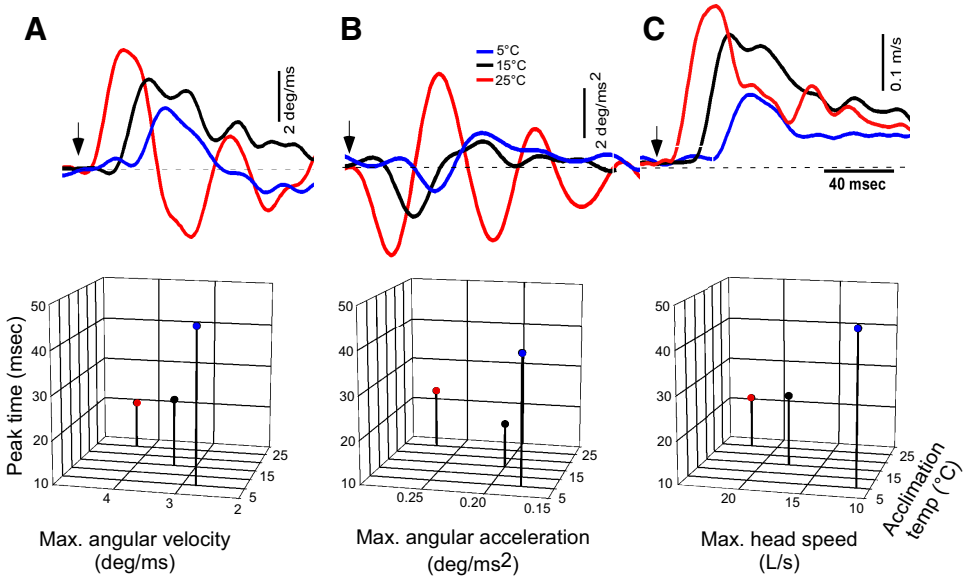

FIG. 2. Effect of temperature acclimation on the kinematics of the startle response (C-start). *A*, *top*: representative traces of angular velocity at the 3 acclimated temperatures: 5, 15, and 25°C. *Bottom*: plot of maximum angular velocity vs. time for each temperature group. Maximum angular velocity (deg/ms): 5°C = 2.28 ± 0.09 ($n = 6$); 15°C = 3.37 ± 0.05 ($n = 6$); 25°C = 4.28 ± 0.21 ($n = 7$) ($P < 0.002$). Time to maximum angular velocity (ms): 5°C = 45.48 ± 1.08 ($n = 6$); 15°C = 25.61 ± 1.52 ($n = 6$); 25°C = 20.91 ± 1.06 ($n = 7$) ($P < 0.001$). *B*, *top*: representative traces of angular acceleration at the 3 acclimation temperatures: 5, 15, and 25°C. *Bottom*: plot of maximum angular acceleration vs. time for each temperature group. Maximum angular acceleration (deg/ms^2): 5°C = 0.18 ± 0.04 ($n = 6$); 15°C = 0.20 ± 0.01 ($n = 6$); 25°C = 0.21 ± 0.01 ($n = 7$) ($P = 0.032$, 5 vs. 25°C only). Time to maximum angular acceleration (ms): 5°C = 39.76 ± 3.06 ($n = 6$); 15°C = 20.19 ± 2.74 ($n = 6$); 25°C = 23.69 ± 3.12 ($n = 7$) ($P < 0.001$). *C*, *top*: representative traces of head speed at the 3 acclimated temperatures: 5, 15, and 25°C. *Bottom*: plot of maximum head speed vs. time for each temperature group: 5°C = 11.07 ± 0.39 ($n = 6$); 15°C = 17.74 ± 0.41 ($n = 6$); 25°C = 22.31 ± 0.76 ($n = 7$) ($P < 0.001$). Time to maximum head speed (ms): 5°C = 45.29 ± 1.18 ($n = 6$); 15°C = 26.41 ± 1.64 ($n = 6$); 25°C = 22.01 ± 1.85 ($n = 7$) ($P < 0.001$).

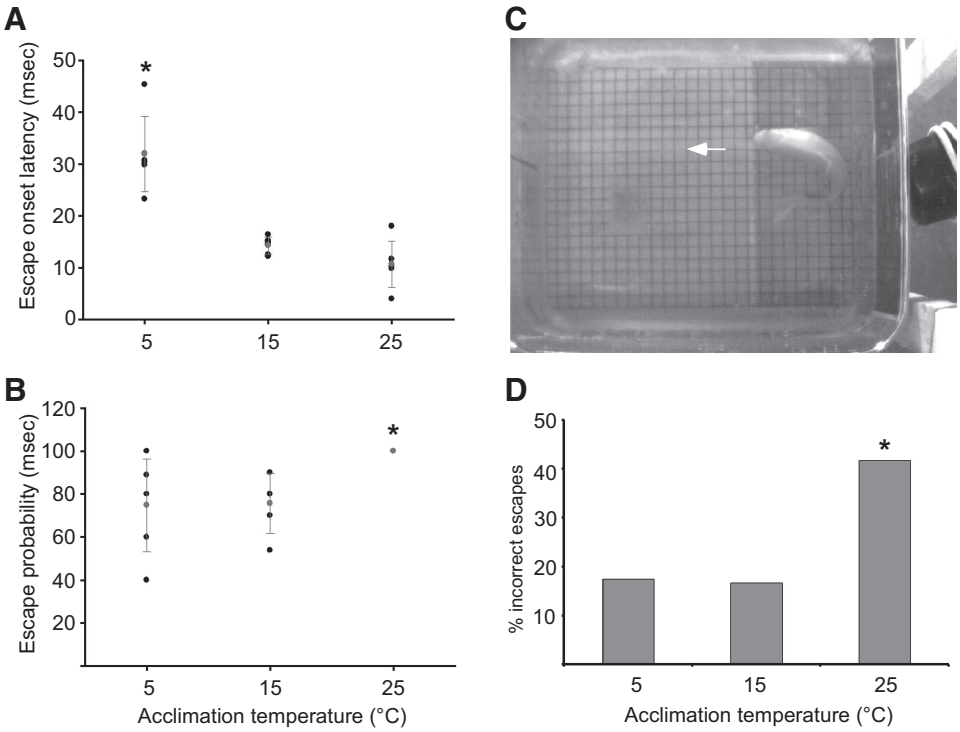

FIG. 3. Effect of temperature acclimation on escape onset latency, probability, and directionality. *A*: escape onset latencies for the 3 acclimated populations (5, 15, 25°C) from the time of hammer contact to the first movement of the head. Black points represent measurements for each fish; grey represents mean ± SD. Escape onset latency (ms): 5°C = 31.91 ± 2.96 (n = 6); 15°C = 14.27 ± 0.66 (n = 6); 25°C = 10.69 ± 1.83 (n = 7) (*P < 0.001; 5 vs. 15 and 25°C). *B*: escape probabilities for the 3 acclimation groups. Each fish was given 10 trials. Black points represent measurements for each fish; grey represents mean ± SD (*P = 0.015, 25 vs. 5 and 15°C). *C* and *D*: directionality of escape. *C*: example of a fish escaping in the "correct" direction, away from the hammer. Only escapes in which the fish was initially oriented parallel to the wall contacted by the hammer were included so that the escape was clearly "toward" or "away from" the hammer. *D*: percentage of "incorrect" escapes (i.e., toward the hammer). Comparison of cumulative trials for all animals at each temperature (*P = 0.045, χ^2 test; 25 vs. 5 and 15°C).

was 100.0 ± 0.0% (n = 7) for all animals, a value significantly greater (P = 0.015) than both the 5°C- (74.8 ± 8.8%, n = 6) and 15°C-acclimated populations (75.6 ± 5.7%, n = 6), respectively, which were not significantly different from each other (Fig. 2*B*). Therefore M-cell firing was greater in the population of animals acclimated to 25°C than those acclimated to 5 and 15°C.

Another measure that addresses neural processing by the M-cell circuit is the ability of an animal to escape in the appropriate direction; i.e., away from an aversive stimulus instead of toward it (e.g., Fig. 3*C*). This measure addresses the balance between excitatory and inhibitory inputs to the right versus left M-cell in the presence of an abrupt stimulus. Escapes used for these studies required that a fish initially be oriented perpendicular to a wall hit by a hammer so that a clear determination of "toward" and "away" could be made (Fig. 3*C*). A comparison of cumulative trials for all animals at each temperature demonstrated that 5 and 15°C populations possessed similar directionality at their acclimation temperatures, escaping away from the hammer 82.6% (N = 23) and 83.3% (N = 30) of the time, respectively. In contrast, animals acclimated to 25°C made only 58.3% (N = 36) correct escapes (P = 0.045, χ^2 test; Fig. 3*D*). Note that escape directionality for 25°C-acclimated animals is essentially the same as chance. When directionality percentages were first calculated for each animal, and then averaged for each temperature, similar results were seen [5°C = 85 ± 7% (n = 6); 15°C = 87 ± 8% (n = 6); 25°C = 61 ± 7% (n = 7); P = 0.06]. Therefore the ability to discriminate appropriate escape directionality (i.e., firing of the appropriate M-cell) is disrupted in the 25°C population.

M-cell action potentials are broader in cold, reduced in warm

To better understand the cellular properties underlying firing of the M-cell in the three populations, we recorded from the M-cell intracellularly in animals that had been acclimated to the same conditions as those used in behavioral experiments. In all three acclimation groups (5, 15, and 25°C), resting membrane potentials were in the range of −79 to −83 mV in both the soma and dendrite and did not differ significantly between populations (Kruskal–Wallis, one-way ANOVA on ranks, P = 0.10). We first examined characteristics of the action potential (AP), since this event reflects kinetics of sodium and potassium channels as well as cellular input resistance. Spike height was essentially the same for the three populations (Fig. 4*A*) and the antidromically evoked AP varied by no more than 5% in amplitude in each population.

We next took a closer look at the functioning of sodium and potassium channels during the AP by examining the kinetics of depolarization and repolarization. AP half-width varied significantly between populations, as it was 31.3% broader in 5°C and 26.9% narrower in 25°C- versus 15°C-acclimated animals (Fig. 4*B*). Compared with 15°C animals, the changes in 5°C-acclimated animals corresponded to a reduction in the slope of the 10–90% rising phase of the AP of 40.3% and a decrease of 29.8% in the 10–90% decay slope (Fig. 4, *D* and *E*). Animals at 25°C showed similar temperature-related changes in comparison to 15°C animals with a significant increase in rise slope of 27.2%; however, decay kinetics were not significantly different between 15 and 25°C populations.

We also examined the time from antidromic stimulation to AP onset, which reflects conduction velocity of the M-axon, since animals in different groups were not significantly different in length and all animals were stimulated antidromically in the same region of the spinal cord (see METHODS). AP onset latency was 25.5% faster in 25 than in 15°C animals, and 5°C populations were 15.7% slower (Fig. 4*C*), although only 5 and 25°C populations were significantly different. Increases in M-axon conduction velocity in warm-acclimated populations

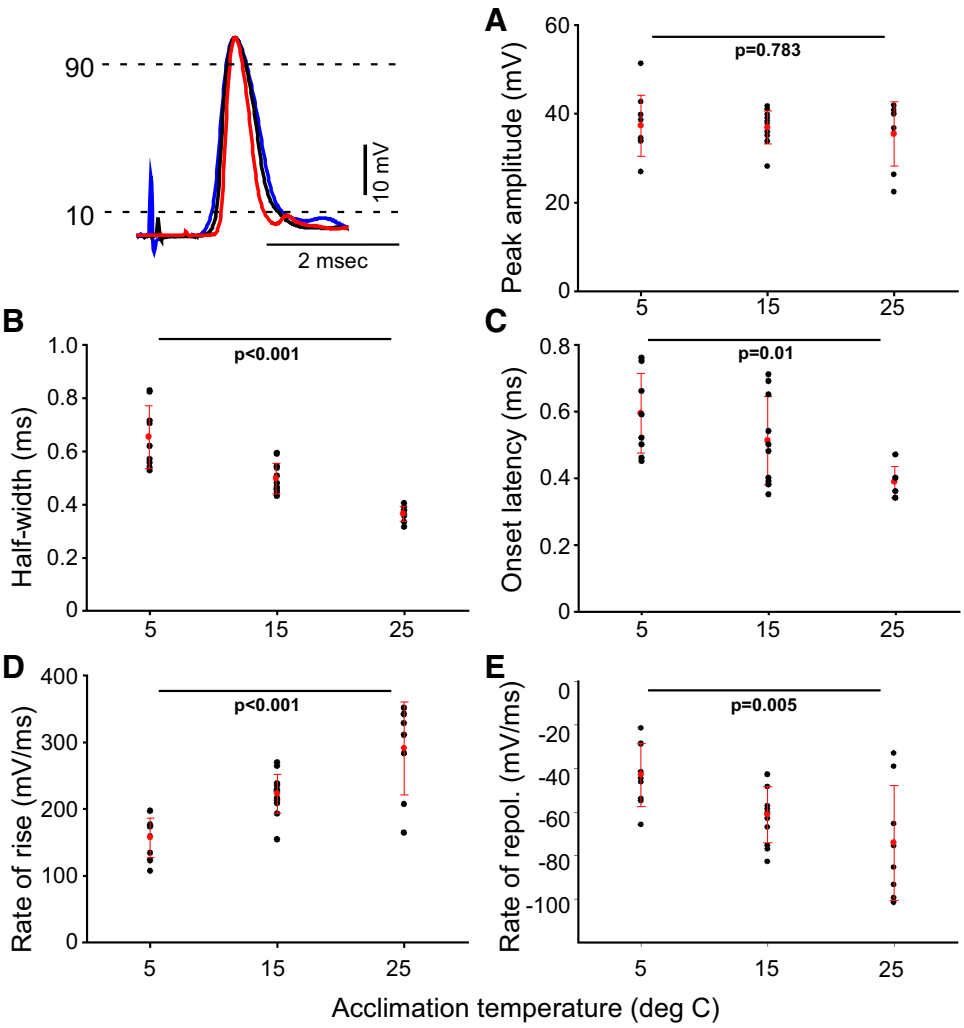

FIG. 4. Effect of temperature acclimation on M-cell action potential (AP) kinetics. For all plots, black points represent measurements for each fish; red represents mean ± SD. *Top left*: representative APs from 5°C (blue), 15°C (black), and 25°C (red) animals superimposed by aligning the peaks. Dashed lines represent the 10–90% rise and decay of the AP. Quantifications of AP kinetics were made for the 3 acclimated groups (5, 15, and 25°C). *A*: peak amplitude (mV): 5°C = 37.29 ± 2.29 (n = 9); 15°C = 36.88 ± 1.03 (n = 13); 25°C = 35.41 ± 2.55 (n = 8); no significant differences (NSD). *B*: width at half-amplitude (half-width) (ms): 5°C = 0.65 ± 0.04 (n = 9); 15°C = 0.49 ± 0.02 (n = 13); 25°C = 0.36 ± 0.01 (n = 8); all groups are significantly different. *C*: latency from antidromic stimulation to AP onset (ms): 5°C = 0.59 ± 0.04 (n = 9); 15°C = 0.51 ± 0.04 (n = 13); 25°C = 0.39 ± 0.02 (n = 8); 5 and 25°C groups are significantly different. *D*: rate of rise (mV/ms): 5°C = 129.74 ± 8.01 (n = 9); 15°C = 179.68 ± 6.49 (n = 13); 25°C = 228.59 ± 19.28 (n = 8); all groups are significantly different. *E*: rate of repolarization (mV/ms): 5°C = −42.95 ± 4.81 (n = 9); 15°C = −61.22 ± 3.58 (n = 13); 25°C = −74.18 ± 9.34 (n = 8); 5 and 25°C groups are significantly different.

should at least partially account for reduced C-start onset latencies.

M-cell input resistance is greater in cold, reduced in warm

Although the alterations in AP kinetics described earlier indicated that there were alterations in channel kinetics, it was likely that M-cell neuronal membrane properties differed between populations as well. We therefore first looked at input resistance, which should increase when more channels are closed and decrease when channels open. To determine the input resistance of the M-cell, we performed experiments using two electrodes where current was injected into the M-cell using one electrode while potential changes were recorded with the other (Fig. 5*A*). Compared with 15°C, values of M-cell input resistance were significantly greater in 5°C fish (43.0%) and lower in 25°C (12.7%; Fig. 5*B*), indicating changes in the number of open channels and therefore conductance. Injection of current ramps into the M-cell further demonstrated this (Fig. 5*D*) because, although the threshold membrane potential was not significantly different between these groups (Fig. 5*E*), the amount of time it took to reach threshold was significantly less in cold-acclimated groups (Fig. 5*F*). Thus the amount of current required to bring the cell to threshold is decreased in cold-acclimated animals with higher input resistances and a larger number of closed channels and increased in warm-acclimated animals with lower input resistances.

We also estimated changes in space constant in the three populations by examining the attenuation of antidromic spike height by recording sequentially from two sites with the same electrode (attenuation factor = V_{dend}/V_{soma}, where the average distance between recording sites was ~220 μm). From these values, we were able to estimate the space constant; i.e., distance required for decay of 63% of the signal (Fig. 5*C*). In contrast to acute temperature studies which showed significant differences in the space constant (Preuss and Faber 2003), there were no significant differences between groups, suggesting that acclimation of membrane properties compensated at least partially for temperature effects on cable properties.

Excitatory synaptic transmission is reduced in cold

The large myelinated club endings (LMCEs) that contact the lateral dendrite of the M-cell from the auditory (VIIIth) nerve form mixed electrical and chemical synaptic contacts onto the M-cell; thus acclimation could alter passage of current through both gap junctions as well as glutamate receptors at these sites. To determine the effects of temperature acclimation on these contacts, we stimulated the VIIIth nerve orthodromically using

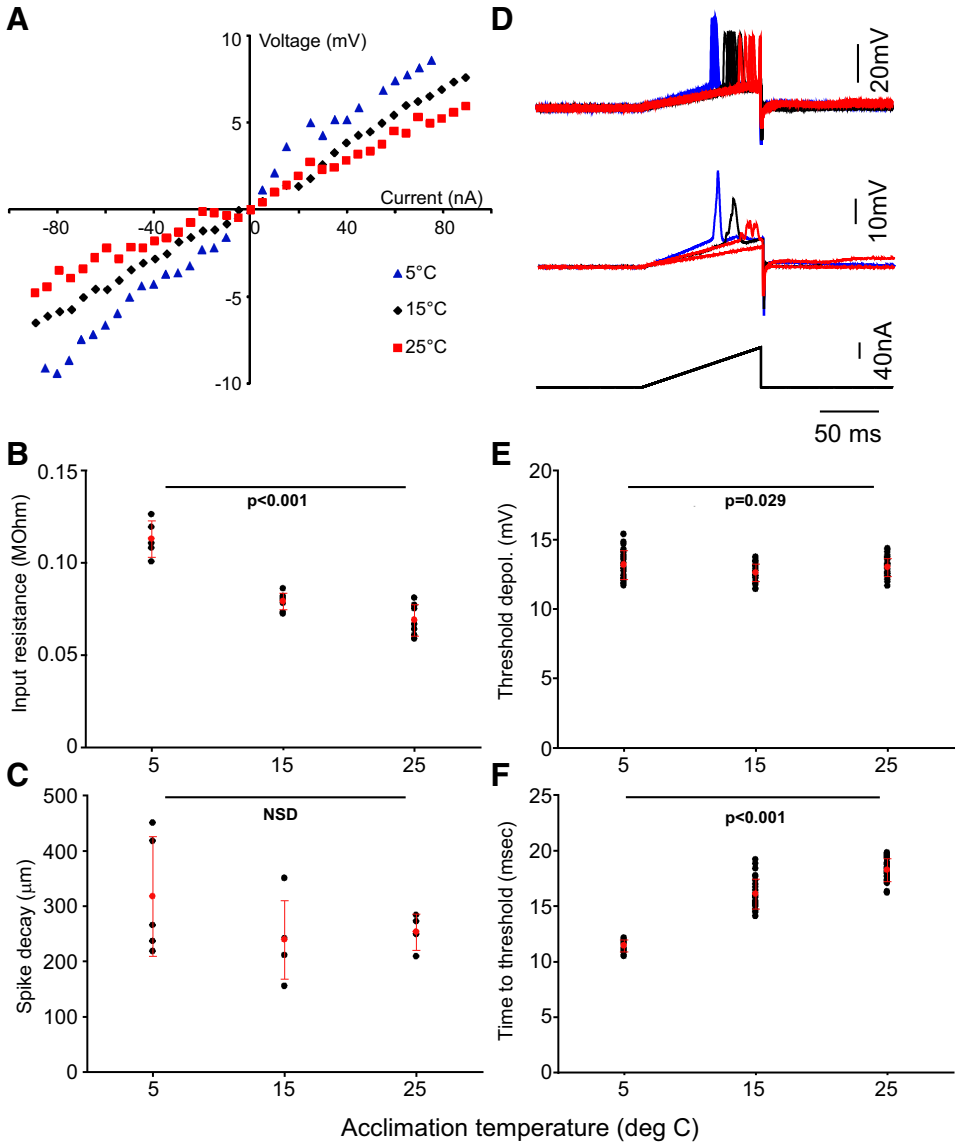

FIG. 5. Effect of temperature acclimation on M-cell membrane properties. For all plots, black points represent measurements for each fish; red represents mean ± SD. *A* and *B*: input resistance of the M-cell in acclimated fish. *A*: voltage changes during current injection in 3 fish: one acclimated to 5°C (blue), one to 15°C (black), and one to 25°C (red). The M-cell was penetrated with 2 electrodes while current steps were injected through one electrode and voltage was recorded with the other. *B*: measurements of input resistance from acclimated animals (MΩ): 5°C = 0.113 ± 0.005 (n = 2, N = 5); 15°C = 0.079 ± 0.002 (n = 2, N = 8); 25°C = 0.069 ± 0.003 (n = 2, N = 9). All groups are significantly different. *C*: 63% attenuation was seen at (μm): 5°C = 317.40 ± 31.76 (n = 5); 15°C = 239.37 ± 31.76 (n = 5); 25°C = 253.16 ± 16.48 (n = 4); P = 0.303. *D*: using 2 electrodes, current ramps were injected into the M-cell through one electrode (*bottom*, black trace), while voltage was recorded with the other (*top*: single traces from 5°C (blue), 15°C (black), and 25°C (red) animals; *middle*: averaged traces). *E*: measurements of threshold voltage (mV): 5°C = 13.17 ± 0.19 (N = 29); 15°C = 12.61 ± 0.12 (N = 27); 25°C = 13.01 ± 0.11 (N = 35); 5 and 25°C groups are significantly different. *F*: measurements of time to threshold (ms): 5°C = 11.43 ± 0.10 (n = 2, N = 29); 15°C = 16.09 ± 0.26 (n = 2, N = 27); 25°C = 18.24 ± 0.18 (n = 2, N = 35). All groups are significantly different.

an extracellular electrode while recording mixed EPSPs from the M-cell lateral dendrite near the LMCEs. We first measured coupling potential half-width at a peak amplitude of 20 mV, with the assumption being that similar coupling potential amplitudes represent stimulation of similar numbers of VIIIth nerve fibers across populations. This measure is also indicative of the half-width of the presynaptic (VIIIth nerve) action potentials, albeit with some filtering. Coupling potential half-width was 28.9% longer in 5°C- and 35.0% shorter in 25°C-acclimated animals than in 15°C animals and it varied significantly between the three populations (Fig. 6*A*). These results have implications for synaptic transmission from the VIIIth nerve onto the M-cell.

We also measured the time from the onset of the stimulus artifact to the peak of the coupling potential, which reflects the conduction velocity of VIIIth nerve fibers (Fig. 6*B*). Similar to the M-axon, the latency from stimulation onset to CP peak was shorter in warm-acclimated populations, indicating increased conduction velocity in these animals; similarly, longer latencies indicate slower conduction velocities in cold-acclimated populations. In general, increases in conduction velocity in warm-acclimated populations would reduce the temporal separation of inputs to the two M-cells, resulting in the reduced right–left discrimination and impaired directionality seen in this population.

We next examined the chemical component of the mixed EPSP onto the M-cell. The decay time constant of the PSP reflects glutamatergic channel properties and is an important factor in determining whether responses will summate with repetitive stimulation, a condition favorable to induction of long-term potentiation (LTP) at these synapses. In addition, chemical synaptic transmission has been shown to contribute to the long-term modulation of the club ending gap junctions (Smith and Pereda 2003). The decay of the chemical PSP was best fit with a double-exponential function (α = 0.01), which resulted in two values for τ : τ_1, which averaged 0.5–1 ms, and τ_2, which was about 5 ms (Fig. 6*C*). Although values for both τ_1 and τ_2 were somewhat reduced at 25°C, no significant differences between groups were apparent for either measure.

We also looked at the strength of excitatory chemical synaptic transmission onto the M-cell in each of the three populations by examining the ratio of chemical PSP amplitude to CP amplitude at various stimulation strengths. Since the amplitude of the chemical

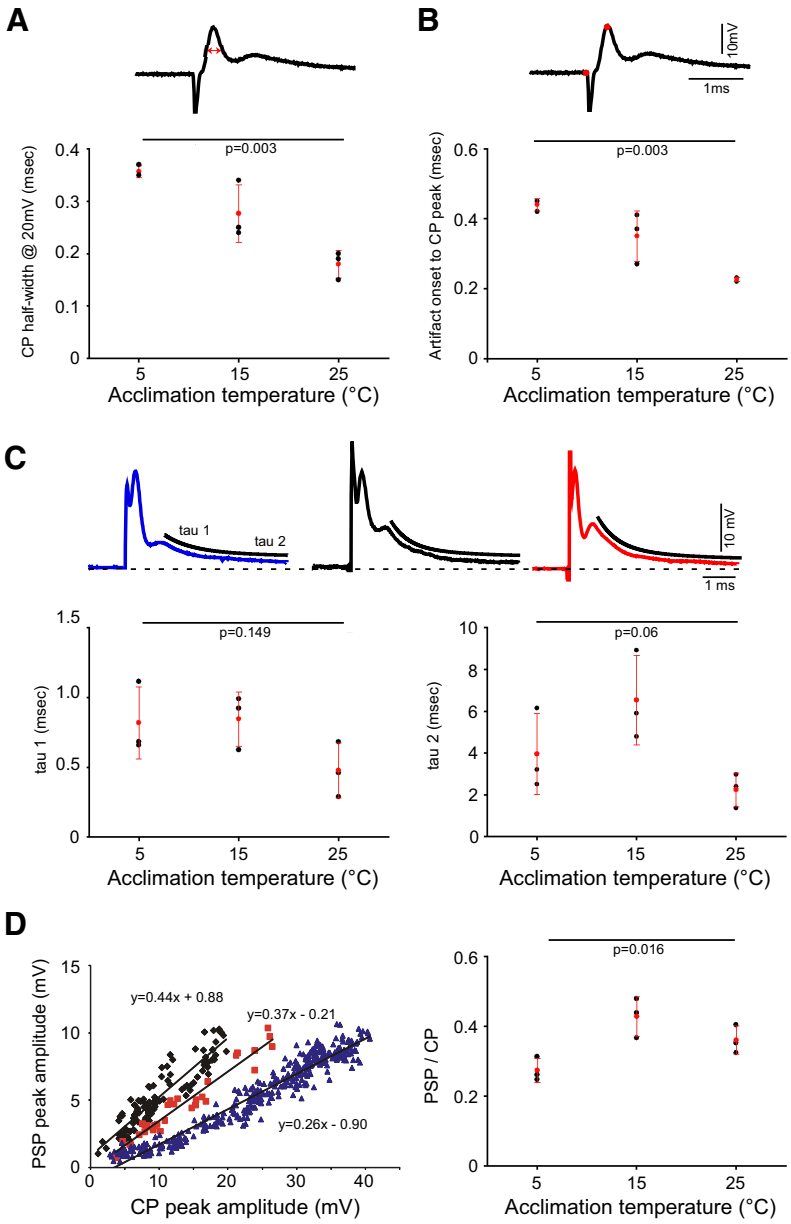

FIG. 6. Effects of temperature acclimation on excitatory neurotransmission: VIIIth nerve-M-cell mixed synapses. For all plots, black points represent measurements for each fish; red represents mean ± SD. *A*: coupling potential (CP) half-width. *Top*: representative excitatory postsynaptic potential (EPSP) in the M-cell in response to direct stimulation of fibers of the VIIIth nerve. Note the downward signal is the stimulus artifact, followed first by the rapid, large-amplitude CP then by the smaller-amplitude EPSP due to the chemical component of the mixed synapse. *Bottom*: CP half-width at 20 mV: 5°C = 0.36 ± 0.01 (n = 3); 15°C = 0.28 ± 0.03 (n = 3); 25°C = 0.18 ± 0.02 (n = 3); all groups are significantly different. *B*: latency from the onset of the stimulus artifact to the peak of the coupling potential. *Top*: representative EPSP in the M-cell in response to direct stimulation of fibers of the VIIIth nerve. Red dots on the M-cell EPSP indicate the artifact onset and CP peak, respectively. *Bottom*: latency for the 3 acclimated populations: 5°C = 0.44 ± 0.01 (n = 3); 15°C = 0.35 ± 0.04 (n = 3); 25°C = 0.23 ± 0.003 (n = 3); 5 and 25°C groups are significantly different. *C*: τ from a double-exponential fit of the decay of the chemical EPSP (α = 0.01). *Top*: representative traces of EPSPs in the M-cell at CP peak = 20 mV in animals acclimated to 5°C (blue), 15°C (black), and 25°C (red), and the corresponding fit of the decay of the chemical EPSP. τ_1 represents the early portion of the decay, τ_2 the later portion. *Bottom*: τ_1 and τ_2 (ms): 5°C: τ_1 = 0.82 ± 0.15, τ_2 = 3.95 ± 1.11 (n = 3); 15°C: τ_1 = 0.85 ± 0.11, τ_2 = 6.53 ± 1.23 (n = 3); 25°C: τ_1 = 0.48 ± 0.11, τ_2 = 2.24 ± 0.47 (n = 3). Both τ_1 and τ_2 are not significantly different between groups. *D*: measurements of synaptic strength. *Left*: CP peak/EPSP peak for many stimulation strengths in 3 fish, one acclimated to 5°C (blue), one to 15°C (black), and one to 25°C (red). *Right*: slopes from the relationship shown on the *left*. PSP/CP slope: 5°C = 0.27 ± 0.02 (n = 3); 15°C = 0.41 ± 0.06 (n = 3); 25°C = 0.36 ± 0.02 (n = 3); 5 and 15°C groups are significantly different.

PSP depends on coactivation of LMCEs (reviewed in Pereda et al. 2004), and the amplitude of the coupling potential presumably reflects the number of active presynaptic fibers, this ratio should reflect the strength of chemical synaptic transmission for VIIIth nerve fiber populations of similar size, or their input–output relationship. Therefore for each fish we plotted the amplitude of the chemical PSP versus the amplitude of the coupling potential over a range of stimulus intensities, determined the linear fit of this relationship, and then compared the slopes of the fits (Fig. 6*D*, *left*). The strength of chemical synaptic transmission in the 15°C population was 47.5% greater than that in the 5°C fish and 12.2% greater than that in the 25°C fish, and only differences between the 5 and 15°C populations were significant (Fig. 6*D*, *right*).

Inhibitory synaptic transmission increases in cold, decreases in warm

The effects of temperature acclimation on inhibitory synaptic transmission are not well known. Friedlander et al. (1976) and Preuss and Faber (2003) demonstrated that inhibitory transmission is reduced in response to acute cooling and the former also found that inhibitory transmission can be blocked more easily than excitatory transmission by low temperatures. One form of M-cell inhibition that can be quantified is the glycinergic feedback inhibition activated by antidromic stimulation of the M-axon in the spinal cord. Because the resting membrane potential of the M-cell is close to the inhibitory (I)PSP equilibrium potential, inhibitory inputs cannot be detected as hyperpolarizing changes. Rather, they can be most easily quantified by measuring the change they produce in membrane conductance. This conductance can be determined by comparing the amplitude of the M-cell AP in the presence or absence of inhibitory inputs (Fig. 7*A*). This can be done by stimulating the M-cell antidromically twice: once to trigger the inhibition and the second time to measure the shunt. In addition, varying the interstimulus interval between the two stimuli exposes the time course of that inhibition (Faber and Korn

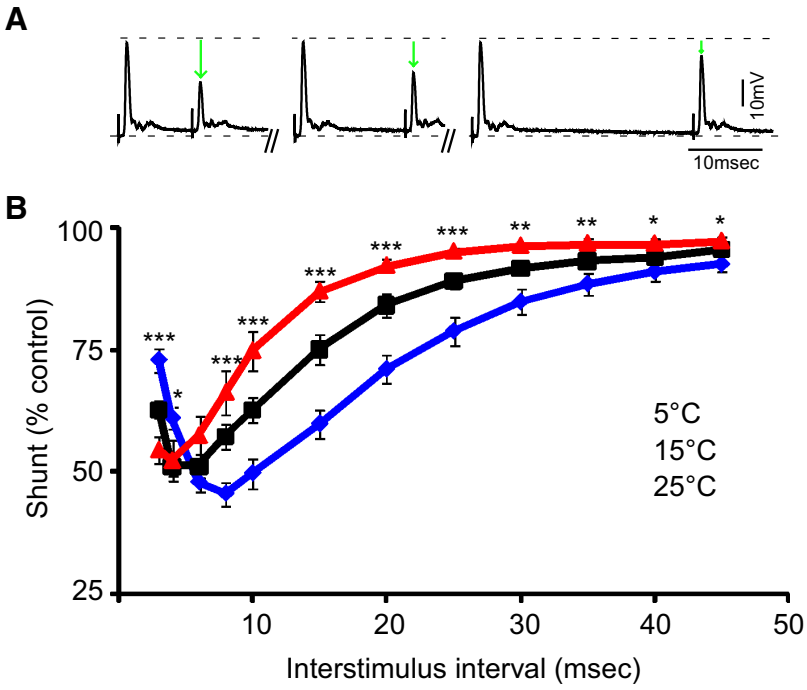

FIG. 7. Effects of temperature acclimation on feedback inhibition. *A*: feedback inhibition was measured by double antidromic stimulation of the M-cell. The first stimulus fires the M-cell, which produces a shunting inhibition of further excitatory events; in this case, a second AP. Triggering the second M-cell spike at various intervals following the first spike permits characterization of the temporal aspects of the shunt by measuring the ratio of the amplitude of the second AP to controls (shunt = green arrow). *B*: quantification of the shunt at various interstimulus intervals ≤50 ms for the 3 acclimated populations: 5°C (blue), 15°C (black), and 25°C (red): 3 ms: $P < 0.001$, 5°C population is significantly different from the 15 and 25°C; 4 ms: $P = 0.030$, 5 and 15°C populations are significantly different; 6 ms: $P = 0.134$; 8 ms: $P < 0.001$, 5°C population is significantly different from the 15 and 25°C; 10 ms: $P < 0.001$, all groups are significantly different; 15 ms: $P < 0.001$, all groups are significantly different; 20 ms: $P < 0.001$, 5°C population is significantly different from the 15 and 25°C; 25 ms: $P < 0.001$, 5°C population is significantly different from 25°C; 30 ms: $P = 0.002$, 5°C population is significantly different from 25°C; 35 ms: $P = 0.004$, 5°C population is significantly different from 25°C; 40 ms: $P = 0.025$, 5°C population is significantly different from 25°C; 45 ms: $P = 0.018$, 5°C population is significantly different from 25°C. $*P < 0.03$, $**P < 0.005$, $***P < 0.001$.

1982; Furukawa and Furshpan 1963; Hatta and Korn 1999). In this manner, we systematically examined the magnitude of feedback inhibition over the course of 50 ms for the three temperature acclimated populations (Fig. 7*B*: 5°C, $n = 9$; 15°C, $n = 10$; 25°C, $n = 6$). Feedback inhibition in the 5°C-acclimated population peaked 4 ms later than that in the 15 and 25°C populations, had a greater peak amplitude, and was significantly stronger for a longer duration than both of the other groups. Although peak amplitude and time of peak inhibition appeared to be the same for the 15 and 25°C populations, inhibition decayed more rapidly in the warm-acclimated population. Therefore inhibition was greatest in the cold-acclimated group and weakest in the warm-acclimated group, a result directly contrasting with the effect of acute chilling on inhibition (Preuss and Faber 2003).

DISCUSSION

In this study we examined the effects of temperature acclimation on the C-start behavior in goldfish as well as on the cellular physiology of the underlying M-cell circuit. We demonstrate that long-term exposure to high and low temperatures alters the balance between excitatory and inhibitory transmission onto the M-cell, resulting in alterations in M-cell excitability as deduced from analysis of escape behavior. In particular, the balance between excitatory and inhibitory transmission is weighted in favor of excitatory transmission in warm-acclimated animals, whereas it is weighted in favor of inhibitory transmission during long-term cold exposure, compared with 15°C populations (Fig. 8). The results seen in the 5°C population indicate that acclimation occurred; i.e., it is known that acute

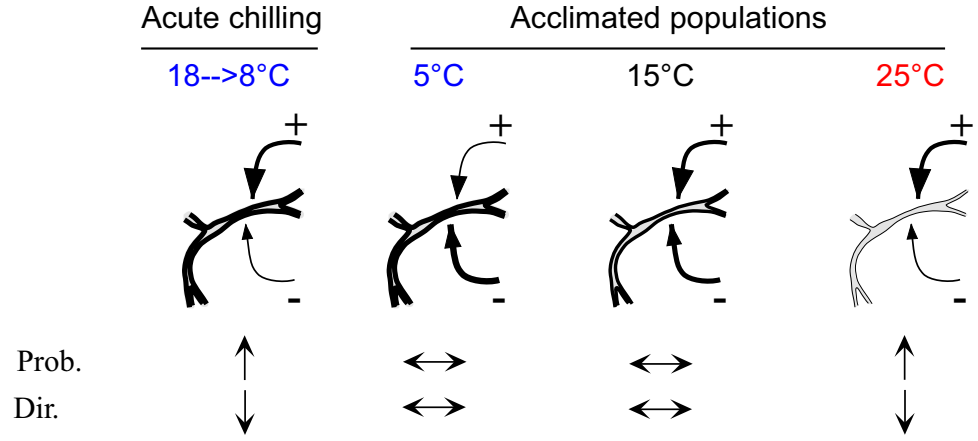

FIG. 8. Acclimation effects on the M-cell circuit and behavioral correlates. "Acute chilling" refers to the Preuss and Faber (2003) study, whereas "Acclimated populations" refers to the 3 groups in this study. For the schematic of each M-cell, the M-axon is at the *top left* with the lateral dendrite at the *top right* and the ventral dendrite at the *bottom*. M-cell input resistance is represented by the thickness of the M-cell membrane, whereas the strength of excitatory (+) and inhibitory transmission (−) are represented by arrow thickness. Strength of each variable is represented relative to 15°C. Probability of escape (Prob.) and escape directionality (Dir.) are also given relative to 15°C-acclimated animals; i.e., values equal to 15°C populations are indicated by horizontal arrows (↔); greater values are indicated by vertical arrows pointing up, lower by vertical arrows pointing down.

cold exposure reduces inhibition and maintains the strength of excitatory transmission, which coupled with increased input resistance, leads to an increased escape probability and reduced directionality (Fig. 8, "Acute Chilling"; Preuss and Faber 2003). Thus acclimation to the cold restores behavioral performance by increasing inhibition relative to excitatory drives to the M-cell. It is also noteworthy that we observed behavioral hyperexcitabililty with acclimation to warm temperatures, an effect similar to that seen with acute chilling (Fig. 8). Yet, these groups exhibited increased and decreased M-cell input resistances, respectively, indicating that changes in input resistance alone are unlikely to account for observed effects on behavioral output.

Direct effects of temperature on the M-cell and synaptic physiology

Acute and acclimated effects of temperature on synaptic transmission have been described for invertebrates as well as various vertebrate neuromuscular junctions (e.g., Hodgkin and Katz 1949; Macdonald and Montgomery 1986; Rosenthal and Bezanilla 2000; Zhurov and Brezina 2005). In general, these studies have demonstrated that synaptic transmission—a complex, multifactorial process—is more sensitive than membrane properties to extreme temperatures and that temperature effects on transmission are sometimes specific to the preparation in question. Few studies have examined the effects of temperature on synaptic transmission in the vertebrate CNS and those that have focused on acute temperature effects (Eccles et al. 1975; Friedlander et al. 1976; Kushmerick et al. 2006; Preuss and Faber 2003). Although in this study we observed spike broadening in cold, decreases in temperature have been associated with decreased amplitude and increased duration of PSPs at excitatory synapses (Cox and Macdonald 2008). Thus physiological processes that involve multiple factors, such as synaptic transmission, are likely to have complex relationships to temperature.

Temperature acclimation causes structural changes that can influence physiological function on a cellular level. Matheson and Roots (1988) demonstrated that optic nerves in goldfish acclimated to colder temperatures had larger-diameter fibers, a change that would counteract cooling-induced increases in input resistance. The fact that differences in input resistance were not matched by similar differences in the estimated space constant is likely attributable to our method of measuring the latter. The space constant would be more accurately measured using hyperpolarizing current injection at multiple locations along the M-cell and it is possible that there is a significant difference that was not detected using our method of measurement.

In this study, we recorded from the M-cell intracellularly and demonstrated that the direct impact of temperature in acclimated animals was less than that in acute studies. For example, Preuss and Faber (2003) showed a greater than threefold increase in the duration of repolarization following a 10°C drop in temperature. Hodgkin and Katz (1949) also demonstrated that acute temperature decreases have a greater effect on repolarization than depolarization kinetics and Carpenter (1981) showed that potassium channels are more sensitive than sodium channels to temperature. However, in this study, rising–falling phases were influenced approximately the same percentages in each temperature group, indicating some amelioration of direct temperature effects. The fact that acclimation to cold speeds up kinetics of potassium channels has important implications for signal processing; for example, spike broadening with cooling should enhance transmitter release and acclimation can reverse this process.

The inhibitory inputs onto teleost M-cells are well documented (Faber and Korn 1978; Furukawa and Furshpan 1963; Hatta and Korn 1998; Kimmel et al. 1985; Triller and Korn 1981; Zottoli and Faber 1980). Each M-cell is known to receive three types of inhibitory glycinergic input: recurrent, feedback, and feedforward. We therefore characterized the activity of glycinergic transmission by examining feedback inhibition, which should be predictive of effects on all three types of inhibition. Contrary to studies of acute temperature exposure that found reduced inhibition in response to acute cold exposure (Friedlander et al. 1974; Preuss and Faber 2003), we have shown that inhibition acclimates to cold in exactly the opposite manner—by increasing in magnitude. In both this study and studies of acute temperature changes, these alterations in inhibition appear to underlie differences in excitability because animals with reduced inhibition are hyperexcitable regardless of changes in input resistance.

The fact that inhibitory transmission is strongest at 5°C could contribute to the decreased amplitude and duration of excitatory synaptic transmission at this temperature if there is an associated increase in tonic inhibition with greater shunting of excitatory inputs to the M-cell. Since we did not observe a change in M-cell time constant, it is likely that increased glycinergic channel open times or repetitive firings of the inhibitory interneurons are responsible for the long duration of inhibition. However, the reduction of excitatory transmission seen in this population could also be due to the method of measuring the strength of chemical synapses. Since input resistance is greater in the cold-acclimated population, the amplitude of the coupling potential might not represent similar size fiber populations; i.e., at 5°C a 20-mV CP might represent a smaller number of fibers. Since chemical PSP amplitude depends on coactivation of LMCEs, coupling potentials might represent smaller fiber populations at 5°C.

Direct effects of temperature acclimation on escape behavior

Studies of the effects of temperature on behavior (reviewed in Bennett 1984; Montgomery and Macdonald 1990; Prosser and Nelson 1981) generally found that acute temperature changes act on physiological processes in a predictable manner: cold slows processes down, warm speeds them up. Acclimation, on the other hand, presumably acts to counteract these effects in a manner that permits normal behavior at extreme temperatures; i.e., extends an animal's functional temperature range to aid survival via resistance or adaptation to new temperatures (reviewed in Roots and Prosser 1962). Acclimation to cold temperatures has been shown to affect many aspects of behavior including: response time (Temple and Johnston 1997, 1998), muscle fiber distribution (Johnston and Lucking 1978), muscle fiber recruitment order (Rome 1990), muscle twitch contraction time (Johnston et al., 1990), and escape behavior kinematics (Beddow et al. 1995).

Escape probability and directionality are determined by neural circuit properties and, in this study, acclimation of these

two measures occurred in a manner consistent with the energetic state of the animal; i.e., fish acclimated to cold temperatures have a reduced probability of escape relative to warm-acclimated animals. This result contrasts with studies of acute temperature change that showed that acute cooling increased activity and probability of escape (Friedlander et al. 1976; Preuss and Faber 2003) and acute warming or heat shock decreased activity and increased vulnerability to predators (Webb and Zhang 1994; Yocom and Edsall 1974). Specifically, Preuss and Faber (2003) demonstrated that during acute cooling, an increase in escape probability was associated with a decrease in the percentage of "correct" escapes away from the stimulus. In contrast, cold-acclimated animals in our study showed the same number of incorrect escapes as 15°C fish, indicating that there was functional acclimation of neuronal processes. The acclimated population that did exhibit a similar behavioral hyperexcitability was at 25°C, a response that may be appropriate to increased metabolic demands and predator mobility in the warmer environment (Beddow et al. 1995; reviewed in Hazel and Prosser 1974; Prosser and Nelson 1981). However, these animals also demonstrated reduced directionality, presumably due to increases in spike propagation in the VIIIth nerve. These increases would reduce the amount of processing time the fish has to discriminate between left–right inputs resulting in the reduced temporal discrimination of inputs apparent in the diminished directional abilities of this group.

ACKNOWLEDGMENTS

We thank S. Lindell, E. Enos, W. Mebane, J. Simmons, M. Peterson, H. Luther, and R. Langill at the Marine Biological Laboratories in Woods Hole, MA for technical advice and support. We thank the Grass Foundation, Grass Fellows, and Fellowship Directors, C. Carr, G. Haspel, and D. Soares for valuable advice and conversation. We thank E. Marder, A. Taylor, H. Bierman, L. Tang, and especially A. Pereda for critical reading of this manuscript and/or helpful discussion and comments. We also thank E. Marder and L. and C. Szabo for generous support and assistance.

Present address of T. Preuss: Department of Psychology, Hunter College, New York, NY 10021.

GRANTS

This work was supported by a Fellowship from the Grass Foundation and a Minigrant from the Marine Biological Laboratory Neuroscience Institute to T. Szabo.

REFERENCES

Beddow TA, van Leeuwen JL, Johnston IA. Swimming kinematics of fast starts are altered by temperature acclimation in the marine fish *Myoxocephalus scorpius*. *J Exp Biol* 198: 203–208, 1995.

Bennett AF. Thermal dependence of muscle function. *Am J Physiol Regul Integr Comp Physiol* 247: R217–R229, 1984.

Borsook D, Woolf CJ, Vellet AD. Temperature acclimation and learning in fish. *Cell Mol Life Sci* 34: 70–71, 1978.

Brezden BL, Fenwick JC, Moon TW. The effects of acclimation temperature and conditioning temperature on the learning rate of the goldfish, *Carassius auratus*. *Comp Biochem Physiol A Mol Integr Physiol* 50: 373–377, 1975.

Carpenter DO. Ionic and metabolic bases of neuronal thermosensitivity. *Fed Proc* 40: 2808–2813, 1981.

Cossins AR. Adaptation of biological membranes to temperature. The effect of temperature acclimation of goldfish upon the viscosity of synaptosomal membranes. *Biochem Biophys Acta* 470: 395–411, 1977.

Cossins AR, Prosser CL. Evolutionary adaptation of membranes to temperature. *Proc Natl Acad Sci USA* 75: 2040–2043, 1978.

Cox SL, Macdonald JA. Temperature dependency of miniature end plate currents from the extraocular muscle of Antarctic teleost fishes. *Polar Biol* 31: 709–715, 2008.

Dierolf BM, McDonald HS. Effects of temperature acclimation on electrical properties of earthworm giant neurons. *J Comp Physiol A Sens Neural Behav Physiol* 62: 284–290, 1969.

Eaton RC, Bombardieri RA, Meyer DL. The Mauthner-initiated startle response in teleost fish. *J Exp Biol* 66: 65–81, 1977.

Eaton RC, DiDomenico R, Nissanov J. Flexible body dynamics of the goldfish C-start: implications for reticulospinal command mechanisms. *J Neurosci* 8: 2758–2768, 1988.

Eaton RC, Lavender WA, Wieland CM. Identification of Mauthner-initiated response patterns in goldfish: evidence from simultaneous cinematography and electrophysiology. *J Comp Physiol A Sens Neural Behav Physiol* 144: 521–531, 1981.

Eccles JC, Rosen I, Scheid P, Taborikova H. The differential effect of cooling on responses of cerebellar cortex. *J Physiol* 249: 119–138, 1975.

Faber DS, Korn H. Electrophysiology of the Mauthner cell: basic properties, synaptic mechanisms, and associated networks. In: *Neurobiology of the Mauthner Cell*, edited by Faber DS, Korn H. New York: Raven Press, 1978, p. 47–131.

Faber DS, Korn H. Transmission at a central inhibitory synapse. I. Magnitude of unitary postsynaptic conductance change and kinetics of channel activiation. *J Neurophysiol* 48: 654–678, 1982.

Friedlander MJ, Kotchabhakdi N, Prosser CL. Effects of cold and heat on behavior and cerebellar function in goldfish. *J Comp Physiol* 112: 19–45, 1976.

Furshpan EJ, Furukawa T. Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *J Neurophysiol* 25: 732–771, 1962.

Furukawa T, Furshpan EJ. Two inhibitory mechanisms in the Mauthner neurons of goldfish. *J Neurophysiol* 26: 140–176, 1963.

Hatta K, Korn H. Physiological properties of the Mauthner system in the adult zebrafish. *J Comp Neurol* 395: 493–509, 1998.

Hatta K, Korn H. Tonic inhibition alternates in paired neurons that set direction of fish escape reaction. *Proc Natl Acad Sci USA* 96: 12090–12095, 1999.

Hazel JR, Prosser CL. Molecular mechanisms of temperature compensation in poikilotherms. *Physiol Rev* 54: 620–677, 1974.

Hodgkin AL, Katz B. The effect of temperature on the electrical activity of the giant axon of the squid. *J Physiol* 109: 240–249, 1949.

Johnson BR, Peck JH, Harris-Warrick RM. Temperature sensitivity of graded synaptic transmission in the lobster stomatogastric ganglion. *J Exp Biol* 156: 267–285, 1991.

Johnston I, Lucking M. Temperature induced variation in the distribution of different types of muscle fibre in the goldfish (*Carassius auratus*). *J Comp Physiol* 124: 111–116, 1978.

Johnston IA, Fleming JD, Crockford T. Thermal acclimation and muscle contractile properties in cyprinid fish. *Am J Physiol Regul Integr Comp Physiol* 259: 231–236, 1990.

Kakela R, Mattila M, Hermansson M, Haimi P, Uphoff A, Paajanen V, Somerharju P, Vornanen M. Seasonal acclimatization of brain lipidome in a eurythermal fish (*Carassius carassius*) is mainly determined by temperature. *Am J Physiol Regul Integr Comp Physiol* 294: R1716–R1728, 2008.

Katz B, Miledi R. The effect of temperature on the synaptic delay at the neuromuscular junction. *J Physiol* 181: 656–670, 1965.

Kimmel CB, Metcalfe WK, Schabtach E. T reticular interneurons: a class of serially repeating cells in the zebrafish hindbrain. *J Comp Neurol* 233: 365–376, 1985.

Kushmerick C, Renden R, von Gersdorff H. Physiological temperatures reduce the rate of vesicle pool depletion and short-term depression via an acceleration of vesicle recruitment. *J Neurosci* 26: 1366–1377, 2006.

Lin JW, Faber DS. Synaptic transmission mediated by single club endings on the goldfish Mauthner cell. I. Characteristics of electrotonic and chemical postsynaptic potentials. *J Neurosci* 8: 1302–1312, 1988.

Macdonald JA, Montgomery JC. Thermal limits of neuromuscular function in an Antarctic fish. *J Comp Physiol A Sens Neural Behav Physiol* 147: 237–250, 1982.

Macdonald JA, Montgomery JC. Rate-compensated synaptic events in Antarctic fish: consequences of homeoviscous cold-adaptation. *Cell Mol Life Sci* 42: 806–808, 1986.

Matheson DF, Roots BI. Effect of acclimation temperature on the axon and fiber diameter spectra and thickness of myelin of fibers of the optic nerve of goldfish. *Exp Neurol* 101: 29–40, 1988.

Montgomery JC, Macdonald JA. Effects of temperature on nervous system: implications for behavioral performance. *Am J Physiol Regul Integr Comp Physiol* 259: R191–R196, 1990.

Nakajima Y, Kohno K. Fine structure of the Mauthner cell: synaptic topography and comparative study. In: *Neurobiology of the Mauthner Cell*, edited by Faber DS, Korn H. New York: Raven Press, 1978, p. 133–166.

Neumeister H, Ripley B, Preuss T, Gilly WF. Effects of temperature on escape jetting in the squid *Loligo opalescens*. *J Exp Biol* 203: 547–557, 2000.

Pereda AE, Rash JE, Nagy JI, Bennett MVL. Dynamics of electrical transmission at club ending of the Mauthner cells. *Br Res Rev* 47: 227–244, 2004.

Preuss T, Faber DS. Central cellular mechanisms underlying temperature-dependent changes in the goldfish startle-escape behavior. *J Neurosci* 23: 5617–5626, 2003.

Prosser CL, Nelson DO. The role of nervous systems in temperature adaptation of poikilotherms. *Ann Rev Physiol* 43: 281–300, 1981.

Riege WH, Cherkin A. One-trial learning in goldfish: temperature dependence. *Behav Biol* 7: 255–263, 1972.

Rome LC. Influence of temperature on muscle recruitment and muscle function in vivo. *Am J Physiol Regul Integr Comp Physiol* 259: R210–R222, 1990.

Roots BI, Prosser CL. Temperature acclimation and the nervous system in fish. *J Exp Biol* 39: 617–629, 1962.

Rosenthal JJC, Bezanilla F. Seasonal variation in conduction velocity of action potentials in squid giant axons. *Biol Bull* 199: 135–143, 2000.

Rosenthal JJC, Bezanilla F. A comparison of propagated action potentials from tropical and temperature squid axons: different durations and conduction velocities correlate with ionic conductance levels. *J Exp Biol* 205: 1819–1830, 2002.

Roussel B, Turrillot P, Kitahama K. Effect of ambient temperature on learning. *Phys Behav* 28: 991–993, 1982.

Shashoua VE. Seasonal changes in the learning and activity patterns of goldfish. *Science* 181: 572–574, 1973.

Sidell BD, Wilson FR, Hazel J, Prosser CL. Time course of thermal acclimation in goldfish. *J Comp Physiol* 84: 119–127, 1973.

Sinesky M. Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. *Proc Natl Acad Sci USA* 71: 522–525, 1974.

Smith M, Pereda AE. Chemical synaptic activity modulates nearby electrical synapses. *Proc Natl Acad Sci USA* 100: 4849–4854, 2003.

Sohn YC, Yoshiura Y, Kobayashi M, Aida K. Seasonal changes in mRNA levels of gonadotropin and thyrotropin subunits in the goldfish, *Carassius auratus*. *Gen Comp Endocrinol* 113: 436–444, 1999.

Temple GK, Johnston IA. The thermal dependence of fast-start performance in fish. *J Therm Biol* 22: 391–401, 1997.

Temple GK, Johnston IA. Testing hypotheses concerning the phenotypic plasticity of escape performance in fish of the family Cottidae. *J Exp Biol* 201: 317–331, 1998.

Triller A, Korn H. Morphologically distinct classes of inhibitory synapses arise from the same neurons: ultrastructural identification from crossed vestibular interneurons intracellularly stained with HRP. *J Comp Neurol* 203: 131–155, 1981.

Webb PW, Zhang H. The relationship between responsiveness and elusiveness of heat-shocked goldfish (*Carassius auratus*) to attacks by rainbow trout (*Oncorhynchus mykiss*). *Can J Zool* 72: 423–426, 1994.

Weight FF, Erulkar SD. Synaptic transmission and effects of temperature at squid giant synapses. *Nature* 261: 720–722, 1976.

Yocom TG, Edsall TA. Effects of acclimation temperature and heat shock on vulnerability of fry of lake whitefish (*Coregonus clupeaformis*) to predation. *J Fish Res Bd Can* 31: 1503–1506, 1974.

Zerbolio DJ. Temperature-dependent learning in goldfish: a multi-trial active avoidance situation. *Behav Biol* 8: 755–761, 1973.

Zhurov Y, Brezina V. Temperature compensation of neuromuscular modulation in *Aplysia*. *J Neurophysiol* 94: 3259–3277, 2005.

Zottoli SJ. Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish. *J Exp Biol* 66: 243–254, 1977.

Zottoli SJ, Faber DS. An identifiable class of statoacoustic interneurons with bilateral projections in the goldfish medulla. *Neuroscience* 5: 1287–1302, 1980.

Zottoli SJ, Faber DS. The Mauthner cell: what has it taught us? *Neuroscientist* 6: 26–38, 2000.