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Short Communications

Rate-compensated synaptic events in antarctic fish: Consequences of homeoviscous cold-adaptation[1]

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Summary. At ambient sub-zero temperatures, muscles from antarctic fish produce spontaneous postsynaptic currents (MEPCs) of significantly shorter duration than those of temperate fishes. Fast decay of antarctic MEPCs is a predictable consequence of the increased membrane fluidity attributable to fatty acid unsaturation in cold-adapted animals.
Key words. Antarctic fish; synaptic currents; decay rates; cold adaptation.

A widespread feature of adaptation to low temperatures in living organisms is an increase in the proportion of unsaturated fatty acids, preserving optimum membrane fluidity (the Homeoviscous Hypothesis of Temperature Adaptation)[2-5]. Increased membrane fluidity is also thought to underly the action of many general anesthetics, which substantially shorten the duration of synaptic events, and reduce the mean lifetime of chemically-opened synaptic channels (the Lipid Fluidity Hypothesis of Anaesthesia)[6-8]. These two hypotheses can be combined to predict that low-temperature synaptic events in cold-adapted poikilotherms should be of relatively short duration compared with similar events in warm-adapted animals. This prediction may be tested by comparing miniature end plate currents (MEPCs) of an antarctic teleost, *Pagothenia borchgrevinki* (Family Nototheniidae), with those already reported for a temperate fish, *Trachurus novaezelandiae* (Family Carangidae)[9]. The results are consistent with the membrane fluidity hypothesis.

Specimens of *P. borchgrevinki* were taken from under the sea ice of McMurdo Sound, Antarctica, close to New Zealand's Scott Base (77° 51′ S, 166° 48′ E), where the mean annual seawater temperature is $-1.87 \pm 0.1\,^{\circ}C$[10]; fish used for neuromuscular recordings were either freshly caught, or were held in cages under the ice until required. Lipids extracted from brain and retina of *P. borchgrevinki* show the increased fatty acid unsaturation characteristic of cold-adapted poikilotherms[11,12]. *T. novaezelandiae* were collected in Port Jackson (Sydney), Australia (33° 51′ S, 151° 12′ E) from ambient summer water temperatures of 23 °C, and acclimated to 12–15 °C in the laboratory. Like *T. no-*

vaezelandiae, P. borchgrevinki is a free-swimming predator on zooplankton in the upper water column[13]. Similar experimental protocol was followed for recording and analysis of MEPCs from both species; further details of the *T. novaezelandiae* experimental methods can be found in earlier publications[9,14].

Focal extracellular recordings of MEPCs were made from large 'white' muscle fibers of isolated inferior oblique extraocular muscles with firepolished blunt (30 µm) glass electrodes, filled with physiological saline. Extraocular muscles were chosen because of their well established homologies throughout the Vertebrata. Dissection was carried out on ice in chilled saline. The isolated muscles remained contractile and produced large MEPCs for up to 8 h in physiological saline[15] made up to match major ion concentrations in *P. borchgrevinki* plasma[16].

Bath temperature was regulated between 0 °C and 25 °C with a Peltier-effect heat pump. MEPCs were amplified approximately 1000 times and stored as FM tape recordings. Replayed MEPCs were analyzed both by measurement from selected photographs, and by semilogarithmic least squares regression of digitized values from the exponential decay phase. Both methods of analysis produced comparable results, which served as a check on possible systematic analytical errors.

With the exception of their rates of decay, MEPCs from antarctic fish muscle closely resemble those from other vertebrates; they grow rapidly (200–250 µs) to a peak amplitude of 300–700 µV, and decay much more slowly, in a non-linear fashion (fig. 1). Decay is exponential over the experimental temperature range (fig. 2). The *P. borchgrevinki* MEPCs are pharmacologically cholinergic, being enlarged and prolonged by the cholinesterase inhibitor neostigmine, and abolished by the postsynaptic blocking agent tubocurarine.

The major, predictable, difference between *P. borchgrevinki* MEPCs and those of other vertebrates, including *T. novaezelandiae,* was their high rate of decay at low temperatures. At 0 °C, the time constant of exponential decay (τ) of antarctic MEPCs was between 2 and 3 ms (fig. 3), compared with extrapolated values of about 6 ms for *T. novaezelandiae,* 10 ms for 25 °C-acclimated goldfish *(Carassius auratus)*[17], and 20 ms for the tropical toad *Bufo marinus'.*

An Arrhenius plot of log decay rate (–ln τ) vs reciprocal of absolute temperature (1/K) produces a more-or-less straight line with a remarkably low 'activation energy' (E_A) of 21 kJ mol^{-1} deg^{-1} compared with values from other vertebrates (e.g. *Trachurus:* 78 kJ; *Bufo:* 64 kJ)[9] (weighted least squares regression: $-ln\ \tau = 15.723 - 2573/K$) (fig. 3). Above about 5 °C, decay of antarctic MEPCs may be prolonged by denaturation of acetylcholinesterase. Reduced activation energies have been reported or can be inferred for a number of enzyme-mediated processes in antarctic fishes[18–24]. Similarly, propagation of action potentials in peripheral nerves of antarctic fishes shows a reduction in sensitivity to temperature change[25]. The opening and closing of synaptic ion channels depends on both protein and lipid components; although the known changes in lipid composition are sufficient to predict the fast decay of antarctic MEPCs, the protein constituents of the synaptic channels presumably also play an important role in these effects.

Regardless of its underlying biophysical cause, the fast decay of synaptic currents has significant consequences for neural function in antarctic fishes. For example, it would tend to limit synaptic gain, particularly in cells which rely on spatial and temporal summation of junction potentials, and do not readily develop propagated action potentials. Many teleost muscles are of this sort[26], and action potentials were rarely observed in *P. borchgrevinki* muscle fibers at normal temperatures. Neuromuscular gain increases markedly at low temperatures[27], and would be even higher in antarctic fishes were it not for shortened synaptic currents. Mean lifetime of synaptic channels seems to be a conservative property, which in a variety of animals is regulated between values of about 5 ms and 0.5 ms over a wide range of natural cell temperatures[9] to preserve a balance between

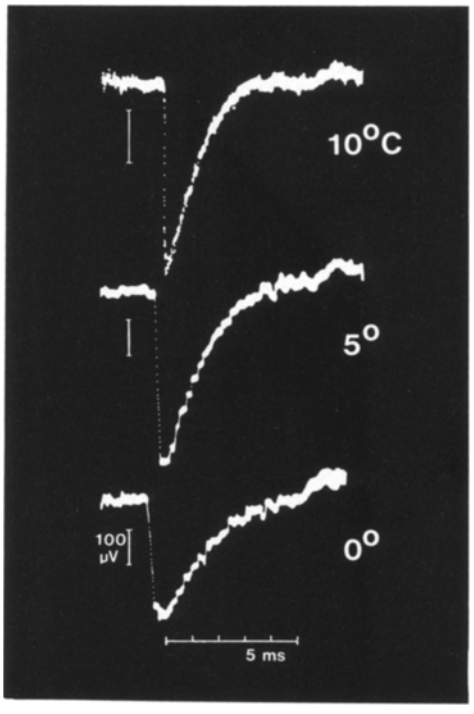

Figure 1. Miniature end plate currents (MEPCs) recorded from *Pagothenia borchgrevinki* extraocular muscle at three different temperatures. These are typical vertebrate MEPCs, with fast growth, sharp peaks, and slower exponential decay back to baseline. Rate of decay is accelerated by higher experimental temperatures. Vertical scale: 100 µV; horizontal scale 5 ms.

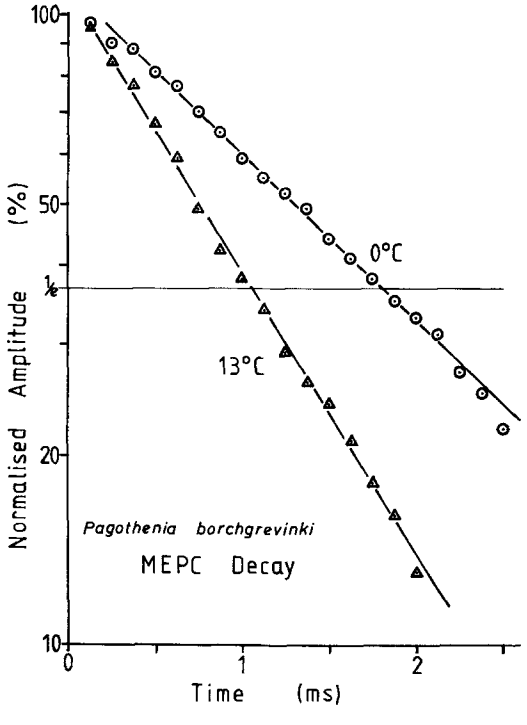

Figure 2. Semilogarithmic graph of decay phase of single *P. borchgrevinki* extraocular MEPCs at two temperatures. The logarithmically transformed data approximates a straight line, confirming that decay is an exponential function. Time constant (τ) is taken as the time required for each MEPC to drop to 1/e of the peak amplitude.

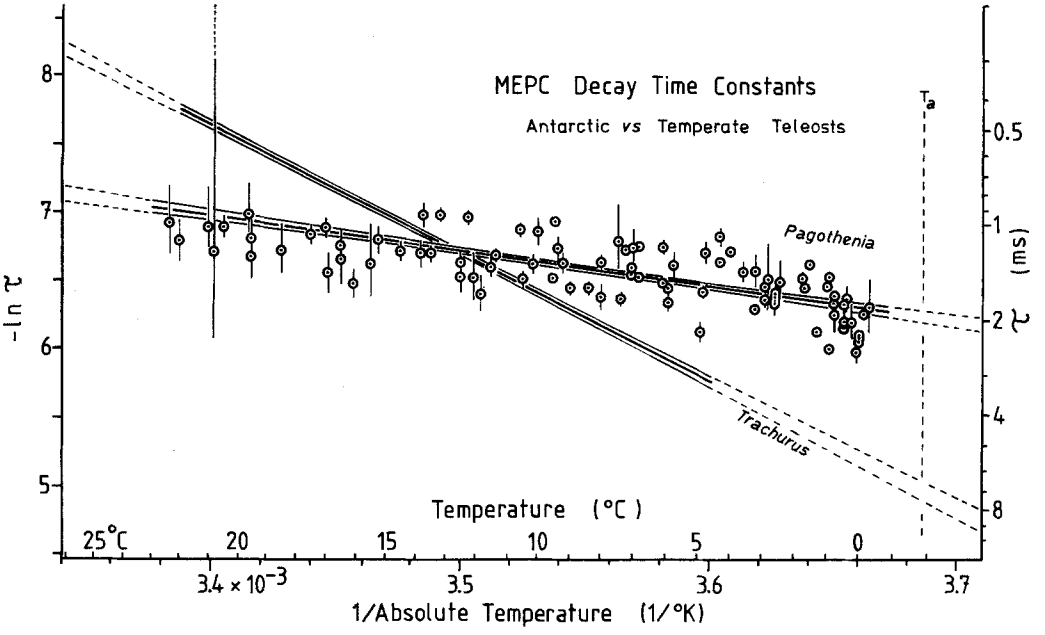

Figure 3. Arrhenius plot of natural logarithms of mean decay time constants ($-\ln \tau \pm 95\%$ confidence limits) of *P. borchgrevinki* MEPCs versus reciprocal of absolute temperature (1/K). The slope of the Arrhenius plot is a measure of the 'activation energy' (E_A) of MEPC decay. The weighted least squares regression line for all values ($-\ln \tau = 15.723 - 2573/K$) is shown as a heavy line, flanked by 99% confidence limits for the regression line. A decay time constant of about 2 ms is indicated for MEPCs at the ambient antarctic temperature of −1.9 °C. A similarly derived regression line ($-\ln \tau = 39.389 - 9337/K$) and confidence limits are also shown for the temperate teleost *Trachurus novaezelandiae*[9], for which a time constant 3–4 times greater is extrapolated for the same temperature. The decay of antarctic MEPCs is clearly faster at antarctic ambient temperatures.

gain and flexibility of control. Changes in lipid fluidity may be an important part of the mechanism responsible for such regulation.

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