

Fig. S1. Probability density distributions (Gaussian error) of muscle resting potentials measured in the extensor tibialis (*in vivo*) of *Locusta migratoria* during acute temperature exposure. Resting potentials were binned by 10°C increments to determine characteristic potentials (vertical lines) that were used to discriminate between P1 (red) and P2 (blue) fibres. Distributions of resting potentials were bimodal regardless of temperature and the proportions of fibres classified as P1 and P2 were relatively stable (% values inside distributions). *n*, number of fibres in each group at a given temperature. Cooling significantly depolarized muscle resting potential (shifted the entire distribution to the right; see Results for details of statistics).

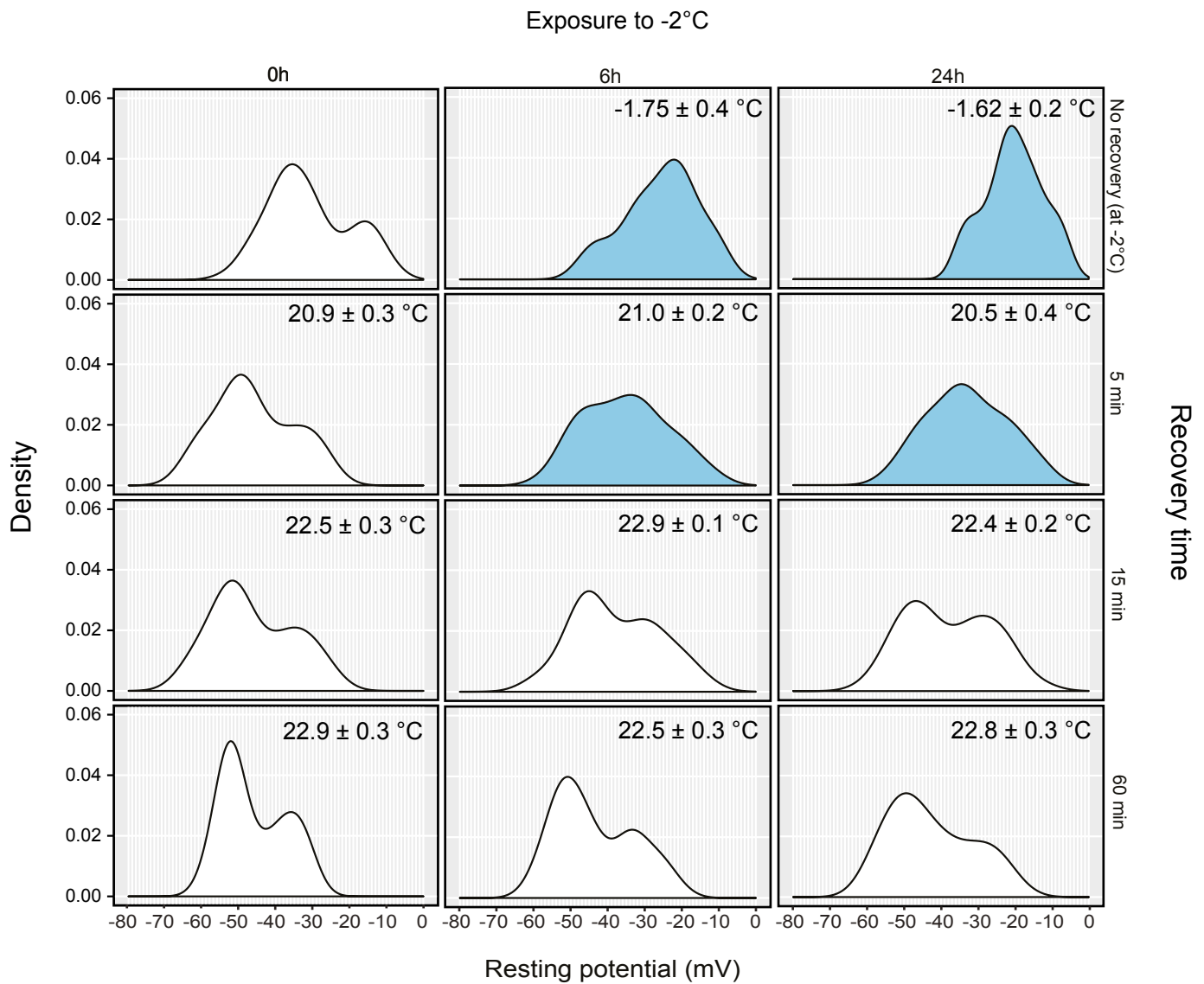


Fig. S2. Probability density distributions (Gaussian error) of muscle resting potentials (V_m) measured *in vivo* in the extensor tibialis of *Locusta migratoria* during prolonged cold exposure and recovery from cold exposure at 22°C . Temperatures in the upper right corner of each panel are the mean (\pm s.e.m.) temperature measured inside the femur, measured immediately after resting potential measurements were made. Distributions of V_m remained bimodal in locusts that were cooled to -2°C and immediately rewarmed (left). By contrast, locusts that had experienced prolonged cold exposure had unimodal V_m distributions (blue) that remained unimodal even after rewarming. These distributions became bimodal again after 15 or 60 min of recovery at 22°C .

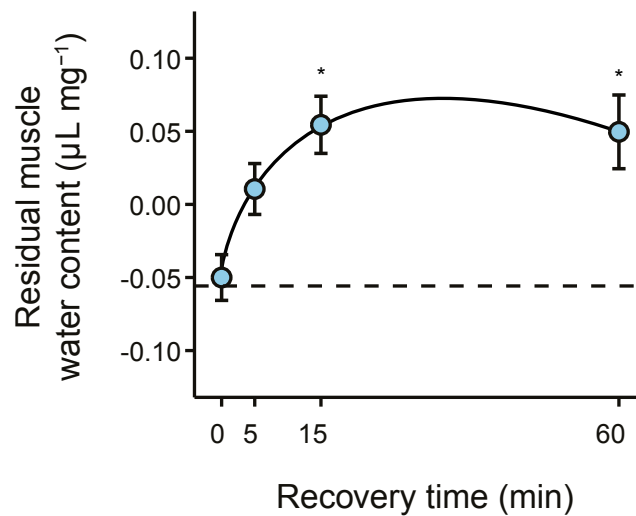


Fig. S3. Residual water content (expressed as per mg mean muscle wet mass) of muscle tissue from the femur of *Locusta migratoria* under control conditions (dashed line = mean) and during recovery from 6 h at -2°C (blue circles; mean \pm s.e.m.). Tissue dry mass was a strong predictor of water content ($R^2=0.96$, $P<0.001$), so we tested for changes in water content during chill-coma recovery using residuals of the regression of water content against dry mass. Locust muscle did not accumulate water during cold exposure (data not shown) but significantly accumulated water during recovery ($F_{4,55}=7.0$, $P<0.001$). Stars denote a significant difference in water content relative to control locusts at room temperature (based on Tukey's HSD). Solid black line is for illustrative purposes only.

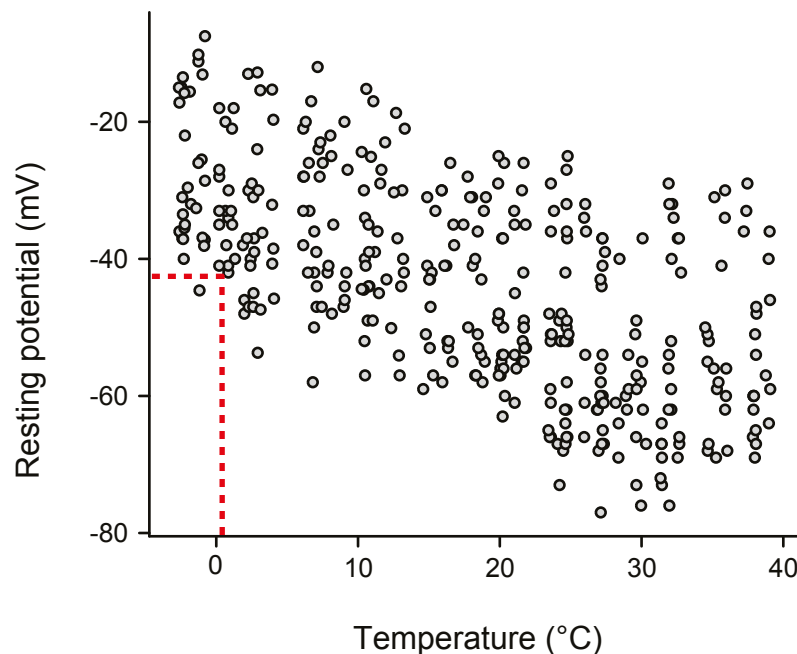


Fig. S4. Individual resting potentials ($n=370$ fibres in 62 animals) in the extensor tibialis of *Locusta migratoria* measured at -2 to 40°C . Vertical and horizontal dashed red lines denote the chill-coma onset temperature of *L. migratoria* [0.5°C (Findsen et al., 2014)] and the approximate theoretical excitability threshold of insect muscles [-40 to -45 mV (Hosler et al., 2000)]. Only the most polarized of fibres in the extensor tibialis were observed to cross this threshold at the chill-coma onset temperature.