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Food deprivation and prior anoxic coma have opposite effects on the activity of a visual interneuron in the locust

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ABSTRACT

We compared how different metabolic stressors, anoxic coma and food deprivation, affected signaling in neural tissue. We used the locust's Descending Contralateral Movement Detector (DCMD) interneuron because its large axon, high firing frequencies, and rapid conduction velocity make it energetically expensive. We exposed locusts to a 30 min anoxic coma or 1 day of food deprivation and found contrasting effects on signaling within the axon. After a prior anoxic coma, the DCMD fired fewer high-frequency (>200 Hz) action potentials (APs) (Control: 12.4 ± 1.6; Coma: 6.3 ± 0.9) with a reduction in axonal conduction velocity (CV) at all frequencies (~4–8%) when presented with a standard looming visual stimulus. Prior anoxic coma was also associated with a loss of supernormal conduction by reducing both the number of supernormal APs and the firing frequency with the highest CV. Initially, food deprivation caused a significant increase in the number of low- and high-frequency APs with no differences observed in CV. After controlling for isolation, food deprivation resulted in an increase in high-frequency APs (>200 Hz: Control: 17.1 ± 1.7; Food-deprived: 19.9 ± 1.3) and an increase in relative conduction velocity for frequencies >150 Hz (~2%). Action potentials of food-deprived animals had a smaller half-width (Control: 0.45 ± 0.02 ms; Food-deprived: 0.40 ± 0.01 ms) and decay time (Control: 0.62 ± 0.03 ms; Food-deprived: 0.54 ± 0.02 ms). Our data indicate that the effects of metabolic stress on neural signaling can be stressor-dependent.

1. Introduction

Accumulating evidence strongly suggests that energy consumption is a strong influence in how neurons encode information (For review see Niven et al., 2007, 2016). In particular, a common theme is how energy is traded-off for increased performance. For instance, homologous fly photoreceptors across species show a performance trade-off with energy consumption as species with higher signaling capacity consume more energy (Niven et al., 2007; Niven and Laughlin, 2008). Also, axons appear tuned to maximize reliability of transmitting high-frequency action potentials (APs) at the expense of increased metabolic cost (Hallermann et al., 2012). However, only a handful of studies have observed whether this trade-off of neural performance and energy consumption can be modulated by prior experience.

For anoxia-tolerant invertebrate species, such as *Locusta migratoria* (*L. migratoria*), exposure to anoxic environments can induce a coma, a state of complete loss of motor function (Wu et al., 2002; Rodgers et al., 2007, 2010; Rodgers-Garlick et al., 2011; Armstrong et al., 2009, 2012; Hou et al., 2014; Money et al., 2014). Entry into a coma is correlated with an abrupt increase in extracellular K+ in the central nervous system (CNS) and an associated cessation of neural activity (Rodgers et al., 2007, 2010; Hou et al., 2014). This is believed to be a mechanism to conserve energy in neural tissue by preventing a depletion of cellular ATP by the Na^+/K^+ ATPase (Rodgers et al., 2010). Within minutes of being removed from the anoxic environment, provided that the exposure time was brief enough (~6 h for *L. migratoria*; Wu et al., 2002), CNS extracellular K+ recovers, the animal begins ventilating (Rodgers et al., 2007; Hou et al., 2014), and over time completely recovers without any long-term injury (Wu et al., 2002; Armstrong et al., 2009; Hou et al., 2014). However, lingering effects of the anoxic coma can be detected for several hours post coma in neural recordings.

Abbreviations: DCMD, Descending Contralateral Movement Detector; LGMD, Lobula Giant Movement Detector; CV, conduction velocity; AP, action potential; CNS, central nervous system.

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Studying the axon of the Descending Contralateral Movement Detector (DCMD) interneuron of *L. migratoria,* Money et al., (2014) found a reduction in high-frequency signaling by the axon following an anoxic coma that lasted for ~5 h before returning to pre-stress levels, suggesting a trade-off of performance for energy conservation.

The DCMD's axon provides an excellent system to investigate the effects of metabolic stress on neural signaling. The DCMD faithfully transmits APs from the Lobula Giant Movement Detector (LGMD) interneuron with a 1:1 spike ratio (O'Shea and Rowell, 1975; Rind, 1984) to motor neurons and interneurons in the metathoracic ganglion (e.g. Burrows and Rowell, 1973; Simmons, 1980) via its larger diameter axon (~17 μm; O'Shea et al., 1974). Transmission of high-frequency APs (>150 Hz) by the axon is vital for survival as they are necessary to elicit activity in post-synaptic partners for escape behaviour and their removal diminishes the escape response (Santer et al., 2006). This requirement for high-frequency firing combined with its large axon diameter supports the idea that the axon is energetically expensive when active (Perge et al., 2012; Sengupta et al., 2013). Although activity in the DCMD axon can be suppressed by self-generated movements in its own visual hemi-field, like antenna cleaning (Rowell, 1971) or visual saccades (Zaretsky and Rowell, 1979), externally generated movements during flight (Santer et al., 2006) or peer interaction elicit a strong response. This suggests the axon would be continuously active in the wild or in a swarm, further supporting the idea that it is energetically expensive by virtue of continual activity.

The DCMD axon, as well as many others, including axons in the frog sciatic nerve (Bullock, 1951, giant fibers in the earthworm (Bullock, 1951), and crab motor axons (Ballo et al., 2012), exhibit subnormal and supernormal conduction, where APs within a high-frequency band conduct slower or faster, respectively, than their lower frequency counterparts. Explanation of subnormal conduction is quite intuitive, as the refractory period of transient Na+ channels diminishes the available pool of active Na+ channels, which is known to correlate with conduction velocity (CV) (De Col et al., 2008), thereby causing higher frequency APs to conduct more slowly. Stochastic opening of Na+ channels ahead of the depolarizing front of the AP can reduce conduction time along the axon (Faisal and Laughlin, 2007) and may explain supernormal conduction in thin axons where stochastic channel kinetics are a significant source of noise. However, for large diameter axons like the DCMD axon, stochastic channel kinetics have relatively little effect (Faisal et al., 2005) and so supernormal conduction relies on additional currents, including T-type calcium currents and persistent/resurgent Na+ currents, to provide a temporary depolarization immediately following an AP, a so called after-depolarization potential (ADP), that reduces the required threshold for subsequent APs (Bucher and Goaillard, 2011; Cross and Robertson, 2016). For the DCMD axon, it is likely a top priority to dampen the effects of subnormal conduction by employing mechanisms that induce supernormal conduction, because slowing of high frequency APs along the axon will increase the inter-spike interval (ISI) (and therefore decrease the frequency) of APs arriving at synapses. After depolarizing potentials have been recorded in the DCMD (Money et al., 2006) that have been predicted to be due a persistent/resurgent Na+ current (Money et al., 2006; Cross and Robertson, 2016). However, this increase in performance by the axon is likely at the cost of energy consumption. Previous work has shown a significant reduction in the DCMD axon's ability to conduct high-frequency APs following a prior anoxic coma (Money et al., 2014). Notably, the reduction in the axon's excitability is evident long after the energy shortage caused by anoxia, as ATP levels have been shown to return to pre-anoxic levels within 1 h of recovery in thoracic ganglion tissue (Rodgers et al., 2007). This suggests the axon can reallocate resources to minimize energy shortages in a trade-off of performance against energy conservation. Therefore, we hypothesized that a similar reduction in excitability to prevent an energy shortage would occur following food deprivation, which is likely to be a common stress encountered by locusts as they migrate from one food source to the next. Similar trade-offs in performance following food deprivation have been observed in other animals including flies (Longden et al., 2014) and fish (Sinnett and Markham, 2015).

To test this, we directly compared changes to the DCMD's signaling properties when locusts were food deprived for 1-day, which has been found to significantly diminish hemolymph concentration of carbohydrates (Goldsworthy, 1969; Moreau et al., 1984), and when they had experienced a prior anoxic coma. We found, contrary to our hypothesis that prior anoxic coma and food deprivation had opposite effects on the DCMD particularly for high-frequency signaling. A prior anoxic coma caused a significant reduction in conduction velocity along the axon and reduced the number of high-frequency APs elicited in response to a standard looming visual stimulus, thus confirming previous work with the DCMD and prior anoxic coma (Money et al., 2014). When compared to a prior anoxic coma and controls, we found that food deprivation caused an increase in conduction velocity for high-frequency APs and elicited more high-frequency APs in response to the same looming visual-stimulus. We also found a significant reduction in overall half-width of APs following food deprivation, which may explain the increased capacity for high-frequency signaling. However, following more prolonged food deprivation periods of 4 days, we found no significant changes to the DCMD axon. This was not likely due to an absence of metabolic stress as a Western blot for phosphorylated-AMPK (pAMPK), an indicator of metabolic stress (Hardie et al., 2012), was elevated in neural tissue after 4 days of food deprivation.

2. Materials and methods

Animal preparations. Adult male locusts (*L. migratoria)* aged 2–5 weeks past their imaginal ecdysis were raised in a crowded colony in the Biosciences Complex at Queen's University. They were raised on a 12 h:12 h photoperiod and fed wheat grass, yeast and bran daily *ad libitum*. The colony was maintained at 30 °C during the light cycle and 25 °C during the dark cycle.

Locusts were dissected as previously described (Robertson and Pearson, 1982). Briefly, the animal's legs and wings were removed and an incision was made caudal to rostral on the dorsal side of the animal. The digestive tract was cut posteriorly and used to pin forward the animal's head. Air sacs, fat, muscle and connective tissue were removed to reveal the mesothoracic and metathoracic ganglia. The exposed tissue was bathed with standard locust saline: 147 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 3 mM NaOH and 10 mM HEPES. All chemicals were procured from Sigma-Aldrich Canada. A grounded silver wire was placed in the animal's abdomen. All experiments were conducted at room temperature (22 ± 1 °C).

Animals undergoing an anoxic coma were submerged in deionized, room temperature water for 30 min. Initially, animals showed hyperexcitable behaviour when submerged as they sought a way to escape. Within ~3 min most animals entered a coma as indicated by lack of ventilation and movement, and remained in this state for the duration of the suffocation. They were then removed, patted dry with paper towel and allowed to recover for 1 h before experiments by which time all the animals were ventilating and had righted themselves. The choice of anoxic coma duration was to match previous studies on anoxic coma (Money et al., 2014, 2016; Rodgers-Garlick et al., 2011; Hou et al., 2014).

Animals undergoing food deprivation were placed in separate containers (~1 L) to prevent cannibalism. In our initial experiment,

for controls we used animals taken from a crowded colony on the day of the experiment, however, for follow up experiments we isolated control animals in containers and provided wheat grass and bran.

Electrophysiology. Extracellular electrodes were made from pulled glass pipettes that had their tips broken to produce a smooth rim suitable for suction onto a connective without damage. Extracellular electrodes were placed rostral to the mesothoracic ganglion and between the meso- and metathoracic ganglia where the DCMD activity was easily recorded. We chose these recording sites as they were used in other studies exploring environmental effects on axonal signaling in the DCMD (Money et al., 2005, 2014, 2016). Furthermore, given that we were interested in the effect of metabolic stress on axonal signaling rather than axonal membrane properties the choice of recordings along a segment of axon that includes branching into the mesothoracic ganglion as opposed to a homogenous axonal segment in the connective is appropriate. Signals from the extracellular electrodes were amplified and filtered with an AM-Systems AC Differential Amplifier model 1700 with the bandpass filter set at 300–5000 Hz.

Intracellular electrodes were pulled from borosilicate glass pipettes to a resistance of 20–40 MΩ when backfilled with 3 M KCl. Recordings were made using an AM-Systems Neuroprobe Amplifier model 1600 and the amplifier's DC offset was zeroed relative to the bath before penetration of the DCMD axon. A metal plate was inserted beneath the thoracic ganglia for stability and recordings were made along the thoracic connective, just caudal to the mesothoracic ganglion. We current-clamped the resting membrane potential to −60 mV during the duration of the recording as this is near the physiological potential at room temperature (Money et al., 2005) and enables accurate comparison of action potential parameters.

Extracellular and intracellular recordings were digitized with an Axon Instruments Digidata 1440 A digitizer at 83 kHz. AxoScope 10.3.0.2 (Molecular Devices) software was used to acquire the recordings. pClamp 10.2 (Molecular Devices) was used offline to analyze the data using Clampfit's threshold detection to determine AP timing.

Looming visual stimulus. To quantify activity in the DCMD axon, we used a looming visual stimulus as previously described (Money et al., 2014). It was back-projected onto a white, semi-transparent screen 7 cm away from the animal's eye and showed a black disc on a white background that expanded, giving the illusion of an approaching object. At its maximum, the disc was 3.8 cm in diameter and appeared to approach the viewer at a speed of 1 m/s. There were 300 frames in the visual stimulus lasting a total of 3 s and projected using a DV11 Optima digital projector or a Sharp Notevision XG-C556 with a refresh rate of 60 Hz. Although the refresh rate is too low to provide an illusion of a looming visual stimulus to the locust, our intention was to have a standard visual stimulus across trials to elicit high-frequency firing. As such, the DCMD's response was to the black disc increasing in diameter with each successive video frame.

During intracellular recordings, a hand-waving motion parallel to the animal's body was used instead of a looming visual stimulus, as quantifying the number of APs generated by a standard stimulus was no longer necessary because we had already quantified the looming response using extracellular electrodes.

Western Blot. We collected 3 samples from locusts that were food deprived for 4 days or isolated for the equivalent duration. For a single sample of neural tissue we collected metathoracic ganglia from 4 different locusts, while muscle was collected from 2 different locusts and fat body was collected from 1 locust. In total, control and food deprivation each used 12 locusts for the neural tissue, 6 for the muscle tissue and 3 for the fat body tissue. The Western blotting protocol has been described previously (Hou et al., 2014) with the exception that we used a mouse monoclonal antibody for pAMPK (Cell-Signalling). A protein of approximately 60 kDa was detected with the p-AMPKα antibody and was visualized using a chemiluminescent HRP substrate (Millipore) according to the manufacturer's protocol on the Cell Biosciences FluorChem HD2 system. The intensity of immunoreactivity was analyzed using the band analysis tool of the Image Studio Lite software as per the manufacturer's specifications. Each Western blot contained one sample from each condition (control and food-deprived) for all 3 tissues collected. Statistical comparisons were made between control and food-deprived within each tissue.

Data analysis. Interspike intervals recorded at the anterior electrode were used to calculate instantaneous firing frequency and delays between APs recorded at the two electrodes determined CVs. Relative CVs were calculated with respect to the first AP elicited by the visual stimulus. Frequencies and CV were extracted using Clampfit's threshold search function. Intracellular measurements were analyzed using custom Python scripts using the Stimfit library (Guzman et al., 2014) and quantified AP amplitude, half-width, rise time and decay time. Action potential amplitude was defined as the difference between the resting membrane potential and the peak potential of the AP. Half-width was defined as the full width at half the maximum amplitude of the AP. Rise time was defined as the duration from 10% to 90% of the AP peak during the rising phase of the AP and decay time was the equivalent measure during the decaying phase of the AP.

When comparing two groups, a *t*-test or a Mann-Whitney Rank Sum Test was performed. To compare multiple groups we used an ANOVA with Holm-Sidak pairwise multiple comparisons or ANOVA on Ranks with Student-Neuman-Keuls-Method pairwise multiple comparisons. To compare multiple groups over time we used a Two-way Repeated Measures ANOVA with Holm-Sidak pairwise multiple comparisons. Data are reported as mean ± standard error (SE) for normally distributed data or median (Mdn) and interquartile range (IQR) for non-parametric data.

3. Results

Prior anoxic coma and food deprivation result in opposite effects on high-frequency signaling in the DCMD. We investigated how different metabolic stresses could modulate axonal signaling in the DCMD axon by directly comparing the effects of food deprivation with the effects of a prior anoxic coma (30 min coma followed by 1 h recovery). Food–deprived animals were isolated for 1 day without food while control animals undergoing an anoxic coma were removed from a crowded cage the day of the experiment. Following their respective treatments, clear changes could be observed in the DCMD's response to a looming visual stimulus (Fig. 1A). Prior anoxic coma animals exhibited less activity in the DCMD axon and typically did not begin to respond until ~1 s to collision. Control and food-deprived animals exhibited some low-frequency activity between 1 and 3 s prior to collision and food-deprived animals seemed to show increased excitability. These observations were reflected in the total number of APs elicited (as measured from the start of the looming visual stimulus to the Time of Collison), with prior anoxic coma animals exhibiting significantly fewer APs (32.9 ± 1.6) compared to controls (49.7 ± 3.3), whereas food-deprived animals exhibited significantly more (58.6 ± 2.4) (Fig. 1B). When APs were binned according to their instantaneous frequency, we found that prior anoxic coma and food deprivation significantly diminished and increased the number of low-frequency (0–50 Hz: Control: 13.7 ± 1.6; Coma: 7.1 ± 0.6; Food-deprived: 18.4 ± 1.3) and high-frequency APs (>200 Hz: Control: 12.4 ± 1.6; Coma: 6.3 ± 0.9; Food-deprived: 18.8 ± 1.4) (Fig. 1C), respectively. The decline in high-frequency APs in prior anoxic

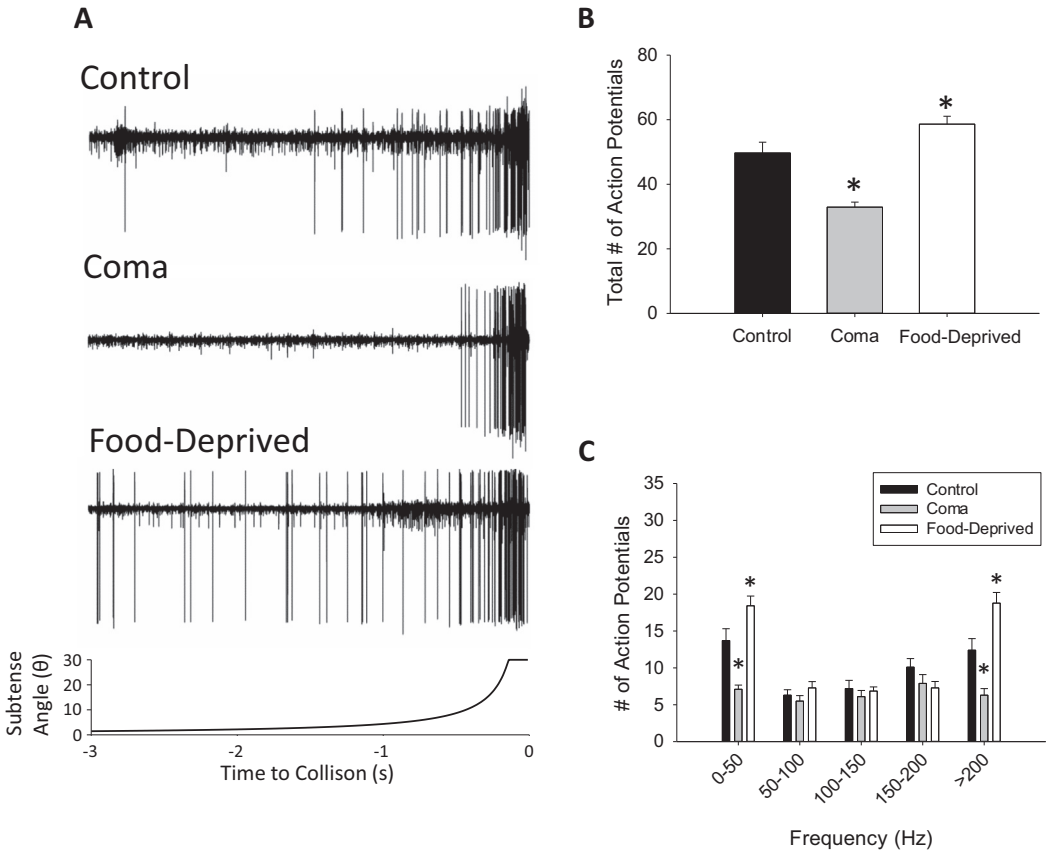

Fig. 1. *Prior anoxic coma and food deprivation have opposite effects on the DCMD's activity.* (A) Sample extracellular recordings from the DCMD axon for each experimental condition in response to a looming visual stimulus. Time is made relative to time of collision of the looming visual stimulus. Note the flat portion in the subtense angle graph is when the stimulus stops moving as we cannot simulate an actual collision. (B) Prior anoxic coma (n = 10) elicited fewer APs in response to a looming visual stimulus and food deprivation (n = 14) elicited more compared to controls (n = 10) (One-Way ANOVA with Holm-Sidak pairwise multiple comparisons, $p < 0.001$). (C) Prior anoxic coma also elicited fewer low- and high-frequency APs than controls and food deprivation elicited more at low- and high frequencies (Two-Way Repeated Measures ANOVA with Holm-Sidak pairwise multiple comparisons, $p < 0.001$). (B-C) Data are plotted as mean and SE. Asterisks indicate significant differences from control ($p < 0.05$).

coma animals was associated with a reduction in CV as observed in the relative CV profile (relative to the first AP elicited by the looming visual stimulus) in response to a looming visual stimulus (Fig. 2A). Prior anoxic coma resulted in a greater reduction of CV at higher frequencies compared to control and food-deprived animals. Across all frequency bins except the 0–50 Hz bin, there was a significant decline in relative CV for prior anoxic coma animals compared to controls with a difference of means between the two groups ranging from ~0.04 (50–100 Hz) to ~0.08 (150–200 Hz) (Fig. 2B). However, there was no significant difference between food deprivation and controls across any of the frequency bins.

Prior anoxic coma reduces supernormal conduction. As observed in the control example in Fig. 2A, the DCMD exhibits supernormal conduction across a band of frequencies and this is lost following a coma. To quantify the effect anoxic coma had on supernormal conduction, we determined the percentage of APs with relative CV >1.0 and found prior anoxic coma animals had significantly less (12.9 ± 4.0%) compared to controls (52.0 ± 3.0%) whereas food deprivation showed no effect (53.5 ± 6.2%) (Fig. 2C). The frequency with the largest relative CV (maximal velocity) was also significantly lower following a coma (Mdn = 20.3, IQR = 15.5, 54.2 Hz) as compared to controls (Mdn = 106.0, IQR = 69.8, 118.1 Hz) while food-deprived animals were not significantly different (Mdn = 66.7, IQR = 50.6, 77.3 Hz) (Fig. 2D).

The effects of food deprivation at high-frequencies are not due to isolation effects. Locusts can express two separate phenotypes: gregarious and solitarious, which arise when the animal is crowded or isolated, respectively. Gregarious locusts tend to seek out conspecifics while solitarious locusts tend to avoid their conspecifics. Phenotypic expression also impacts activity in the LGMD/DCMD circuit, as gregarious locusts show greater resistance to habituation of DCMD firing compared to solitarious locusts (Matheson, 2003; Rogers et al., 2010), have larger peak firing rates and elicit more APs in response to a looming visual stimulus (Rogers et al., 2007, 2010). Locusts can also be switched between behavioral phenotypes by either isolating gregarious or crowding solitarious locusts. Although differences in morphology due to switching between the two phenotypes do not arise for several generations, behavioral (Simpson et al., 2001) and biochemical changes can arise within hours (Ma et al., 2015).

We were interested in controlling for changes in the DCMD axon caused by phenotypic changes due to isolation of our food-deprived animals. We used control animals that were isolated for the same period of time as food-deprived animals and no longer included a prior anoxic coma group given the large contrast in effects between food deprivation and prior anoxic coma. In response to a looming visual stimulus, both control and food-deprived animals had similar relative CV profiles (Fig. 3A), and elicited the same number of action potentials (Control: n = 11, 66.7 ± 4.4; Food-deprived: n = 11, 70.3 ± 3.9) (Fig. 3B). Binning APs by instantaneous frequencies, revealed no significant effect at low-frequencies following food deprivation (0–50 Hz: Control: 17.1 ± 1.7; Food-deprived: 19.9 ± 1.25), however food deprivation

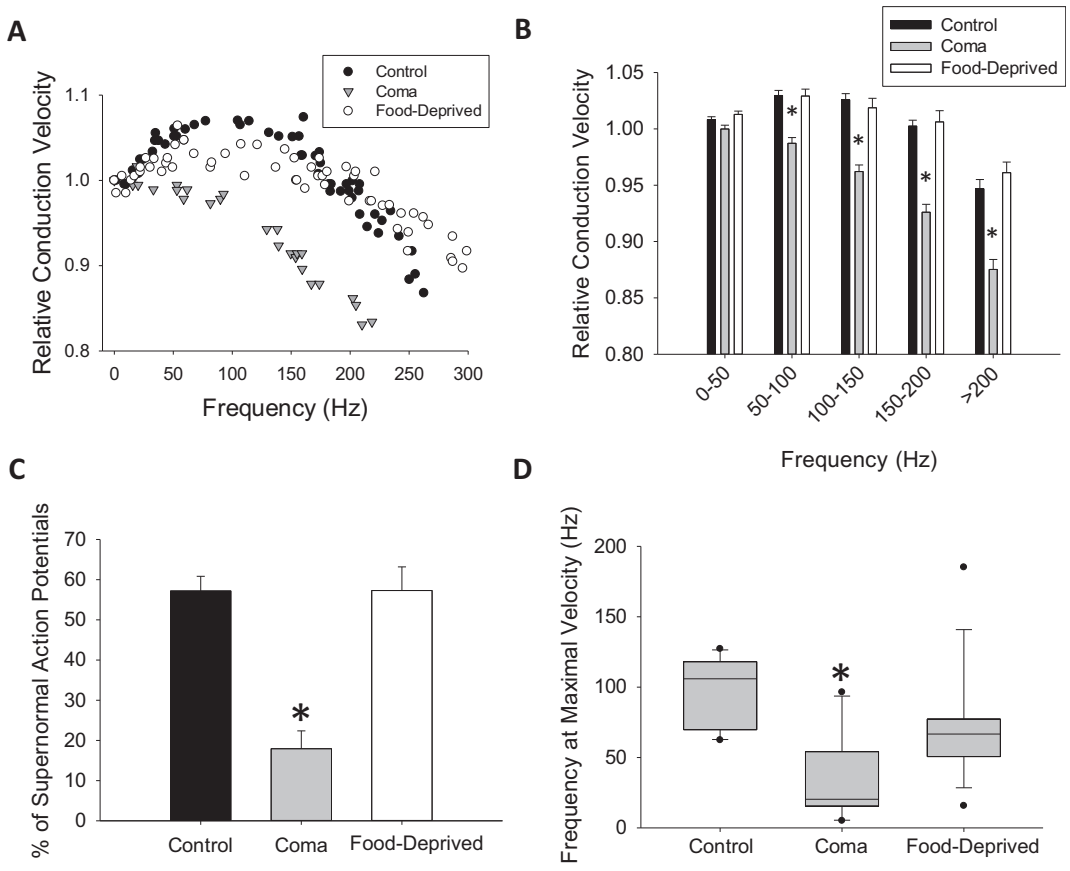

Fig. 2. *Prior anoxic coma reduces high-frequency CV in the DCMD's axon.* (A) Sample relative conduction velocity profiles of the DCMD for each experimental condition in response to a looming visual stimulus. In control and food-deprived animals, supernormal conduction is clearly seen as APs travelling with relative CV > 1.0. (B) Relative conduction velocities were significantly slower following an anoxic coma at almost all frequencies compared to controls while food-deprived were unchanged (Two-Way Repeated Measures ANOVA with Holm-Sidak pairwise multiple comparisons, $p < 0.001$). Conduction velocities were calculated relative to the first AP elicited by the looming visual stimulus. (C) After an anoxic coma, the percentage of supernormal APs was significantly fewer than controls (One-Way ANOVA with Holm-Sidak pairwise multiple comparisons, $p < 0.001$), (D) and the frequency at maximal velocity was significantly less than controls while food deprived were unchanged (One-Way ANOVA on Ranks with Dunn's pairwise multiple comparisons, $p < 0.001$). (B-D) Data are plotted as mean and SE. Asterisks indicate significant difference from controls ($p < 0.05$).

elicited significantly more high-frequency APs (>200 Hz: Control: 24.2 ± 2.1; Food-deprived: 30.6 ± 3.1) (Fig. 3C). Food deprivation also had a significant main effect on relative CV with the largest effect size occurring for the highest frequency bin (>200 Hz: Control: 0.95 ± 0.01; Food-deprived: 0.97 ± 0.01) (Fig. 3D). Food deprivation had no effect on supernormal conduction as the frequency with the largest relative CV was not significantly different (Control: Mdn = 81.4 Hz, IQR = 64.4, 101.9 Hz; Food-deprived: Mdn = 51.2 Hz, IQR = 27.9, 149.3 Hz; Mann-Whitney Rank Sum Test, $U = 57$, $p = 0.84$) and so was the percentage of supernormal APs (Control: 36.4 ± 6.5%; Food-deprived: 53.4 ± 6.0%), though a trend did exist for the latter (two tailed *t*-test, $p = 0.07$) (Fig. 3E). These data suggest some effects of food deprivation at high-frequencies were independent of isolation and not simply due to an increase in total number of APs elicited. They also suggest that the food-deprivation-induced increase in total number of APs and number of low-frequency APs we observed in our earlier experiment was dependent on isolation, not food deprivation. However, we could not directly compare our controls that were isolated with those directly from the cage to further confirm these results, as they were not collected at the same time. As a result, other factors unrelated to food deprivation may influence the comparison, including seasonal variations.

Food deprivation reduces AP half-width and decay time. Next we investigated whether the effects of food deprivation on the DCMD's CV was reflected in changes in axonal AP properties. Previous work has already explored how a prior anoxic coma affects AP properties in the DCMD axon (Money et al., 2014) so we chose to focus exclusively on food deprivation. We used control animals that were isolated alongside our food-deprived animals. We recorded APs from the DCMD axon routinely with amplitudes of ~100 mV and half-widths of ~0.45 ms, which are close to previous reported values for the DCMD (Money et al., 2005, 2014). We found clear changes to the AP shape between control and food-deprived animals (Fig. 4A), however there was no significant change of the AP amplitude (Two-Way Repeated Measures ANOVA, $p > 0.9$). Food-deprived animals had a significant reduction in AP half-width across all frequency bins (Two-Way Repeated Measures ANOVA, $p < 0.001$) (Fig. 4B) that occurred on both the rising phase and the hyperpolarizing phase of the AP. Differences in rise time were significant for all but the 0–50 Hz bin (Two-Way Repeated Measures ANOVA, $p < 0.001$) (Fig. 4C) and decay time was significantly decreased compared to controls for all bins (Two-Way Repeated Measures ANOVA, $p < 0.001$) (Fig. 4D).

Prolonged food deprivation has no effect on the DCMD. To determine if the increased high-frequency firing was present following more prolonged food deprivation, we food deprived locusts for 4 days and observed no changes in the number of APs elicited (Fig. 5 A: Control: $n = 8$; Food deprived: $n = 8$; $p > 0.9$) or the relative CV (Fig. 5B: $p > 0.3$). Prolonged food deprivation also had no effect on the AP properties as amplitude (Fig. 5C: Control $n = 7$; Food-deprived: $n = 8$; $p > 0.3$), half-width (Fig. 5D: $p > 0.3$) decay

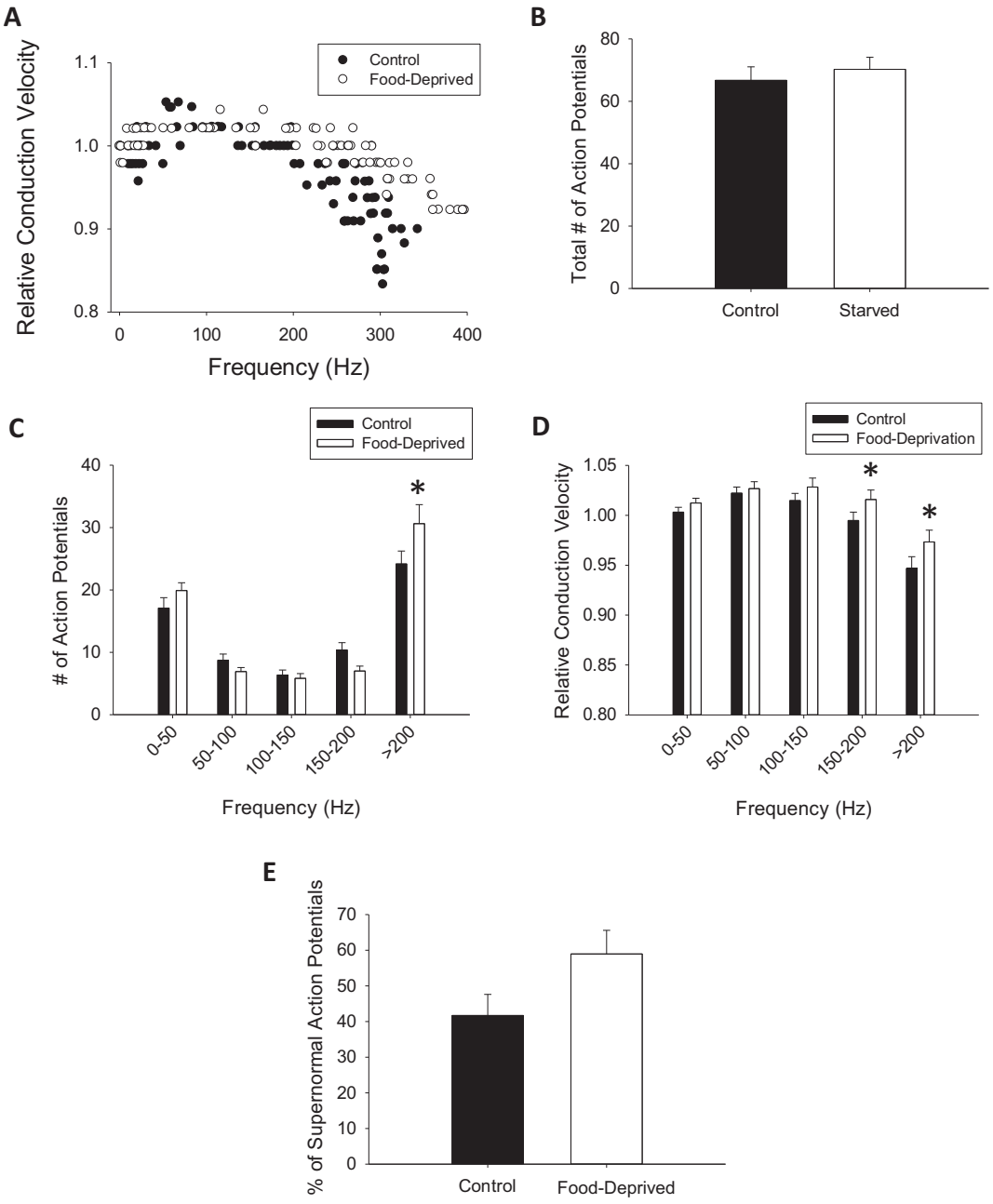

Fig. 3. *Increased high-frequency signaling after food deprivation is not due to isolation.* (A) Relative CV profiles of a control animal that was isolated and a food-deprived animal. (B) The total number of APs was not significantly different between control (n = 11) and food-deprived (n = 11) (*t*-test, p = 0.4) (C) nor was the number of low-frequency APs, however food deprivation still increased the number of high-frequency APs (Two-Way Repeated Measures ANOVA with Holm-Sidak pairwise multiple comparisons, p < 0.05). (D) Food deprivation also increased the relative CV of the high-frequency APs compared to controls (Two-Way Repeated Measures ANOVA with Holm-Sidak pairwise multiple comparisons, p < 0.001), (E) however it had no effect on the percentage of supernormal APs. Asterisks indicate significant difference from controls (p < 0.05).

time (data not shown: p > 0.9) or rise time (data not shown: p > 0.6) were not significantly different.

Prolonged food deprivation significantly increases the level of pAMPK. One reason we may not have seen any effects of prolonged food deprivation is that mechanisms (e.g. release of energy stores) to generate ATP may have been activated by the longer time without food to compensate for an early loss of energy. A hallmark of energy shortage at the cellular level is the phosphorylation of AMP-activated protein kinase (pAMPK) (Hardie et al., 2012). Phosphorylated-AMPK is sensitive to the ratio of AMP:ATP (Hardie et al., 2012) and can diminish expensive neural signaling (Potter et al., 2010; Ikematsu et al., 2011). We used a Western blot to assess levels of pAMPK in locust tissues after food deprivation for 4 days. We found significantly increased levels of pAMPK in fat body (*t*-test, p < 0.001), muscle (*t*-test, p < 0.01) and neural tissue (p < 0.05) when compared to their control counterparts (Fig. 6). Therefore, activation of pAMPK suggested that our prolonged food deprivation protocol caused an energy shortage in the locust's CNS.

4. Discussion

In the present study, we compared how different sources of metabolic stress impact neural performance. We hypothesized that

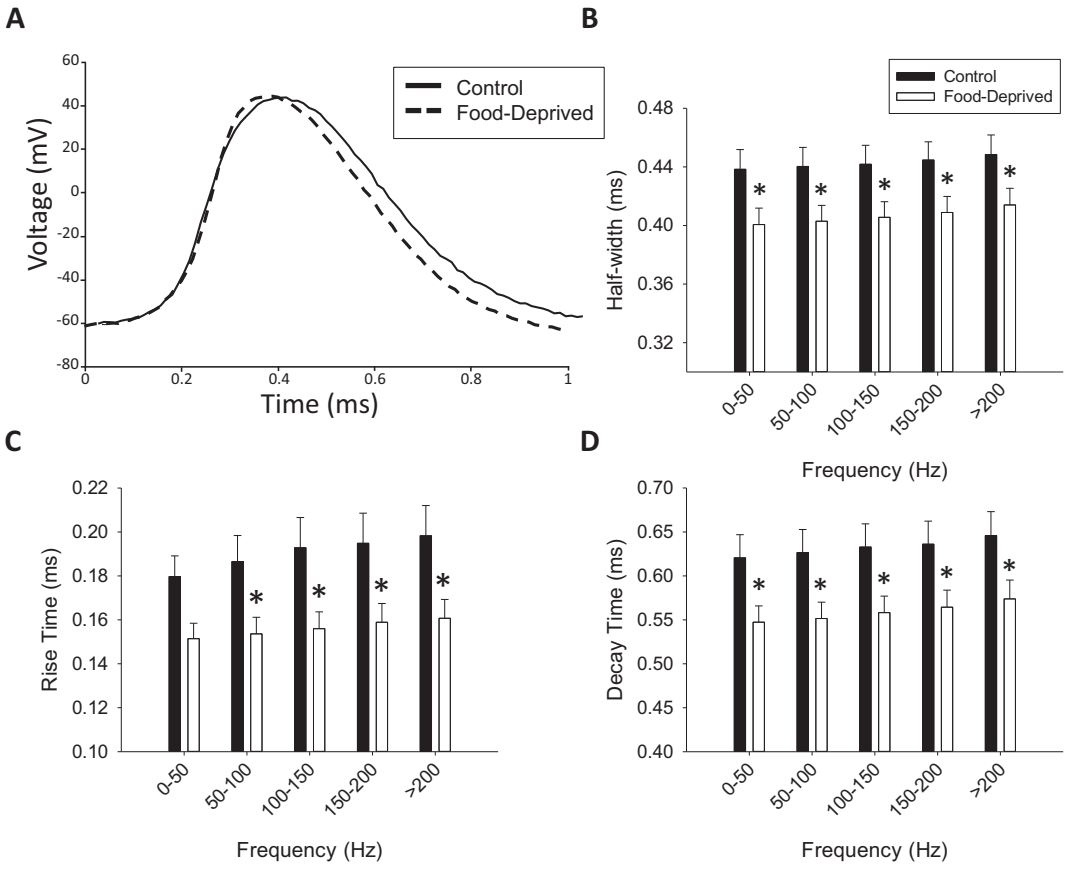

Fig. 4. *Food deprivation reduces the AP half-width and decay time.* (A) Sample recording of an AP from a control and food deprived animal. (B) Food deprivation (n = 8) significantly decreased half-width compared to controls (n = 7) (Two-Way Repeated Measures ANOVA, $p < 0.001$). (C) Food deprivation significantly decreased the decay time in all frequency bins (Two-Way Repeated Measures ANOVA, $p < 0.001$) (D) as well as rise time in all but the 0–50 Hz bin (Two-Way Repeated Measures ANOVA, $p < 0.001$). (B-D) Data are plotted as mean and SE. Asterisks indicate significant difference from controls ($p < 0.05$).

prior anoxic coma and food deprivation would exhibit similar reductions in signaling in the DCMD axon. Contrary to this, we found a differential effect between the two stressors, with prior anoxic coma diminishing and food deprivation increasing high-frequency signaling. This was reflected in the number of high-frequency APs elicited by a looming visual stimulus and their relative CVs. Our results with a prior anoxic coma confirm previously reported effects of the stressor on the DCMD (Money et al., 2014) and are included here to enable comparison with the effects of food deprivation. Furthermore, food deprivation resulted in changes to the AP shape, reducing the half-width and the decay time. However, changes caused by food deprivation were absent after more prolonged food deprivation.

Cellular mechanisms involved in the metabolic stress responses. Overall, our results suggest metabolic stresses modulate neural tissue in ways that depend on the stressor and duration of exposure. The effects of anoxia and hypoxia on neural tissue have been well studied in insects (Rodgers et al., 2010; Rodriguez and Robertson, 2012; Hou et al., 2014; Money et al., 2014) and mammals (Tymianski et al., 1993; Jarvis et al., 2001; Armstrong et al., 2010). Phosphorylated-AMPK is an important cellular messenger in mediating an anoxic response and is capable of modulating cellular processes to reduce energy expenditure (Hardie et al., 2012). Increased levels of pAMPK follows exposure to hypoxic environments (Gusarova et al., 2011; Mungai et al., 2011; Rousset et al., 2015), suppresses long-term potentiation at synapses (Potter et al., 2010) and directly phosphorylates ion channels to reduce excitability (Ikematsu et al., 2011). In the locust, a prior anoxic coma (Money et al., 2014) mimics the effects of elevated levels of pAMPK on coma onset times (Rodgers-Garlick et al., 2011). Our data also show that food deprivation increased levels of pAMPK in line with previous work (Ching et al., 2010).

Why would a prior anoxic coma, but not food deprivation, diminish the DCMD's performance? One possibility is that anoxic coma activates other cascades that may diminish the DCMD's performance such as the NO/cGMP/PKG pathway, which is known to exacerbate the disturbance to ionic homeostasis during anoxia (Armstrong et al., 2009, 2010). An alternative explanation is that the animal was not experiencing an energy shortage, due to energy reserves that it could utilize. We feel this is unlikely based on previous work involving prolonged food deprivation in locusts. Food deprivation for 1 day significantly diminishes hemolymph concentration of carbohydrates including trehalose (Goldsworthy, 1969; Moreau et al., 1984) and Moreau et al. (1984) showed that this trend continues for up to 6 days. This is accompanied by a significant elevation of hemolymph lipid concentrations (Mwangi and Goldsworthy, 1977a) that can be reversed by injection of trehalose (Mwangi and Goldsworthy, 1977b) or feeding (Mwangi and Goldsworthy, 1977b, Siegert, 1995). Also, as observed by Loveridge (1975), locusts die after ~7 days of food deprivation. Finally, our Western blot showing elevated pAMPK indicates that an energy shortage arises in the thoracic ganglia. Taken together, this strongly suggests that our food deprivation protocols were effective for inducing an energy shortage in the animal including in its neural tissue.

Interestingly, 1 day of food deprivation increased high-frequency signaling in the LGMD/DCMD circuit in spite of the fact that this treatment is sufficient to reduce the hemolymph

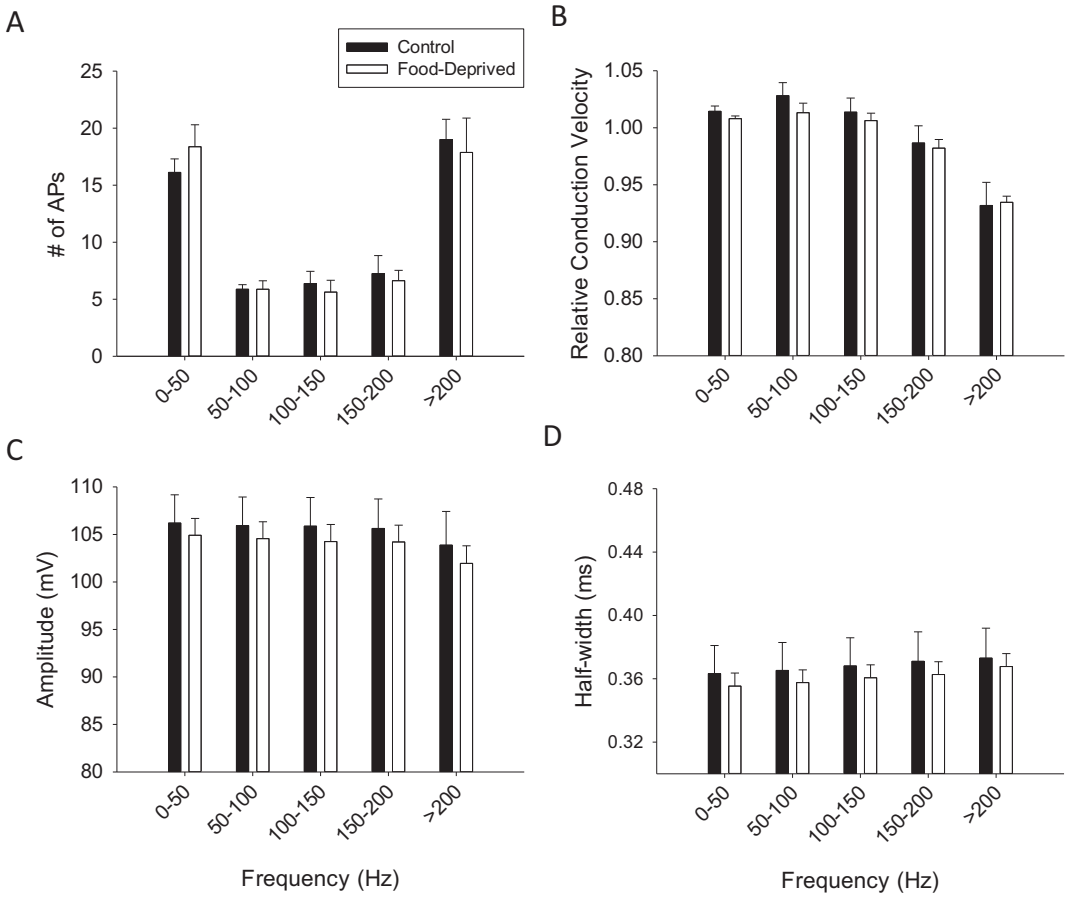

Fig. 5. *Prolonged food deprivation does not affect the DCMD.* (A) Number of APs elicited by the looming visual stimulus were not significantly different between control (n = 7) and food-deprived animals (n = 8) (Two-Way Repeated Measures ANOVA, $p > 0.9$). (B) Relative CV was similarly unaffected (Two-Way Repeated Measures ANOVA, $p > 0.3$). (C) AP amplitude and (D) half-width were also unaffected by food deprivation (Two-Way Repeated Measures ANOVA, $p > 0.3$).

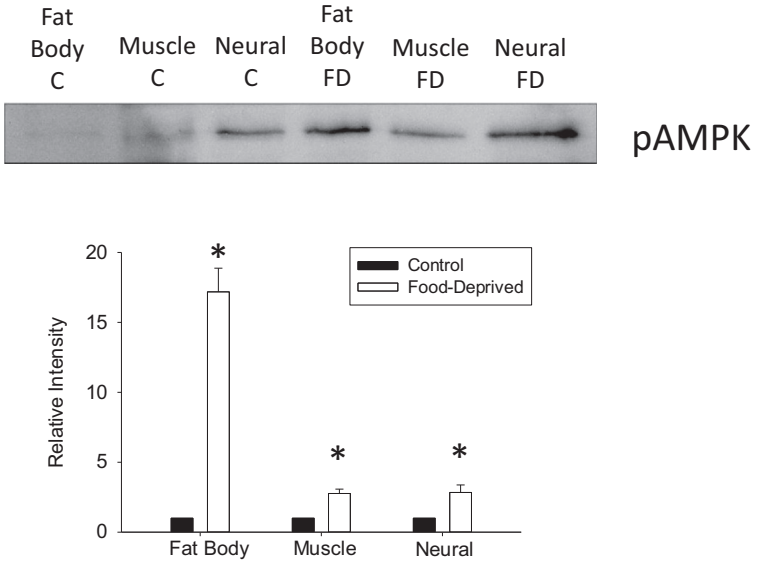

Fig. 6. *Prolonged food deprivation increases pAMPK levels in neural tissue.* We used a Western blot to identify phosphorylated AMPK (pAMPK) in fat body, muscle and neural tissue from control (C) and 4 day food-deprived animals (FD). Top are representative blots of each tissue under each stress condition. Bottom is the relative intensity from the Western blot. Relative intensities were calculated relative to control animals. Significantly increased levels of pAMPK were observed in all tissues (*t*-test $p < 0.05$; indicated by asterisk).

concentration of carbohydrates in locusts (Goldsworthy, 1969; Moreau et al., 1984). The effect of this loss of nutrients, expected to reduce signaling, may have been masked by octopamine, a biogenic amine analogous to norepinephrine in mammals. Octopamine can directly modulate the CNS of the locust (Armstrong et al., 2006), reduce hypoxia-induced conduction slowdown in

the DCMD axon (Money et al., 2016), increase excitability in the DCMD's presynaptic partner the LGMD (Rind et al., 2008) and stimulate the release of lipid by fat stores during long-term flight (Orchard et al., 1982, 1993). Levels of octopamine in hemolymph increase during a brief period of food deprivation (9 h) coinciding with increased foraging behaviour in locusts (Davenport and Evans, 1984). Octopamine also increases foraging behaviour in *Drosophila* after food deprivation (Yang et al., 2015). Dorsal unpaired medial (DUM) neurons (see Roeder, 2005 for review) are likely involved in the foraging response as they are octopaminergic neurons involved in the fight-or-flight response that can modulate neural activity (Evans and Siegler, 1982), fat stores (Orchard et al., 1982, 1993) and muscle properties (Malamud et al., 1988). However, the LGMD/DCMD circuit is best known for its role in predator evasion rather than foraging, so the effect of food deprivation on DCMD is more difficult to explain. It may be part of a general stress response caused by elevated octopamine in the hemolymph, including the startle response for which the LGMD/DCMD circuit is vital. Alternatively, foraging behaviour may increase the risk of predation, therefore requiring increased activity in predator-evasion circuits.

Octopamine may also be involved in the significant increase in relative CV for food-deprived animals in the isolation control experiments, which was absent in our initial experiments. We isolated control animals for the equivalent duration as our food-deprived animals, which may have started the transition from gregarious to solitarious physiology and behaviour. Within hours of isolation, gregarious animals begin to exhibit solitarious behaviour that may be partly dependent on a reduction in octopamine signaling (Ma et al., 2015) although an assortment of other neuromodulators also contribute to the phase change including serotonin and dopamine (Ma et al., 2011, 2015; Guo et al., 2013). In isolating our controls, we may have better separated the octopamine effect brought on by acute food deprivation by unintentionally reducing octopamine levels in our controls.

The increase of low-frequency APs and the subsequent increase (as compared to the food-deprived animals) of total number of APs in control animals that were isolated was not expected. This is particularly surprising given that gregarious locusts tend to elicit more APs in response to a looming visual stimulus than solitarious ones (Rogers et al., 2010). Given the relatively little importance low-frequency APs have on escape behaviour (Santer et al., 2006), it seems unlikely that the consequences of the increased low-frequency firing would be behaviorally important.

Ionic mechanisms of metabolic stress on excitability. Each metabolic stress appeared to be modulating different ion channels. We found that food deprivation reduced the AP decay time suggesting modulation of the hyperpolarizing K+ current. This would increase the capacity of the axon for conducting high-frequency APs as faster hyperpolarization will shorten the recovery time of the transient Na+ channels from inactivation and increase the number of available Na+ channels for subsequent, high-frequency APs. This could explain why food-deprived animals exhibited an increase in high-frequency CV as the shortened recovery time would diminish subnormal conduction by the axon.

In contrast, prior anoxic coma appeared to modulate Na+ currents. Supernormal conduction observed in the DCMD axon can arise due to several mechanisms, including a persistent or resurgent Na+ current (Bucher and Goaillard, 2011). In the DCMD, after-depolarizing potentials (ADP) have been recorded (Money et al., 2005) and more recently, we have found evidence for a current in the DCMD that shortens the afterhyperpolarization potential (AHP) (Cross and Robertson, 2016). Both phenomena (ADP production and AHP shortening) can be produced by a persistent or resurgent Na+ current. After an anoxic coma, we found a significant decline in the proportion of supernormal APs suggesting a reduction in the putative persistent or resurgent Na+ current. Also, a prior anoxic coma results in a loss of AP amplitude suggesting a modulation of the transient Na+ current (Money et al., 2014). This suggests food deprivation and prior anoxic coma's effects are mediated primarily through K+ and Na+ current modulation respectively, providing a possible explanation for the contrasting effects each stressor had on excitability.

Energetics and the DCMD axon. It is tempting to connect the reduction of the AP half-width and amplitude by food deprivation and prior anoxic coma, as mechanisms to decrease energy consumption. Decreases in AP amplitude are correlated with reductions in metabolic cost (Sengupta et al., 2010), however linking changes in half-width with energy consumption is more problematic. Carter and Bean (2009) found AP half-width correlated with energy consumption. However, more recently Sengupta et al. (2010) showed that the current overlap between the transient Na + and rectifying K+ currents was a better predictor for energy consumption and found no correlation of half-width with energy consumption. Despite this apparent conflict, our additional observation that food deprivation and prior anoxic coma increased and decreased high-frequency CV (i.e. performance) suggests that these changes in AP shape and size likely increased and decreased energy consumption. Money et al. (2014) has already discussed the consequences of decreased performance following prior anoxic coma and energy consumption in the DCMD axon and so we focus on food deprivation exclusively. As mentioned, the reduction in half-width associated with the decay phase of the AP suggests an increase of the rectifying K+ current. Two methods could cause this, either an increase in the steady-state K+ conductance or a reduction of its time constant, both of which increase not only the maximal firing rate but also the energetic cost per AP (Hausenstaub et al., 2010). Changes in the transient Na+ currents could also have occurred following food deprivation as we observed a small, but significant change to the rising phase of the APs. However, modulation of transient Na+ current to trade-off performance with metabolic cost is less clear. Decreasing the inactivation gate's rate constant for the transient Na+ current does increase the metabolic cost per AP, however this increase does not necessarily result in an increased maximal firing frequency, and the same could be said about increasing the activation gate's rate constant (Hausenstaub et al., 2010; Hallermann et al., 2012). Therefore, the possible modulation of the transient Na+ current may have improved CV in a frequency-dependent manner, as we observed, and not maximal firing frequency, at an increased metabolic cost.

For energetics, a more salient factor is the increased excitability of the axon in food-deprived animals. Conducting more APs per looming visual stimulus will increase the Na+ load and therefore increase the ATP required to re-establish the ionic gradients by the Na+/K+ ATPase. Given that the DCMD axon follows its presynaptic partner, the LGMD, with a 1:1 spike ratio, neural interactions upstream of DCMD likely cause increased excitability in the pathway. Photoreceptors are known to incur a large metabolic cost for the information that they carry (Niven et al., 2007, 2008; Laughlin et al., 1998) and it seems counter-intuitive for locusts to increase LGMD/DCMD excitability during short-term food deprivation but not for prolonged food deprivation. As suggested previously, perhaps food deprivation-induced foraging increases the risk of predation and escape circuitry needs to be protected.

Consequences of metabolic stress on behaviour. The DCMD axon is vital for reliably triggering motoneurons in the thoracic ganglion to initiate an escape/avoidance response. High-frequency APs (>150 Hz) from the DCMD axon elicit excitatory post-synaptic potentials in flight motoneurons that summate to trigger an AP (Santer et al., 2006). Similarly, activity in leg muscles involved in an escape-jump response appears to be time-locked with the peak of the DCMD's activity (i.e. the highest frequency APs) in response

to a looming visual stimulus (Fotowat and Gabbiani, 2007). However, ablating the DCMD does not inhibit the escape-response, but only increases the variability of the timing of the response (Fotowat et al., 2011). Short periods of food deprivation should then result in decreased variability in timing of the escape-response, increasing the likelihood of predator escape appropriate for increased foraging.

Recently, Longden et al. (2014) found that food deprivation for up to 3 days increased walking and reduced firing frequency in a blowfly visual neuron, likely impairing visual performance. They suggest a strategy by which the fly may reduce visual processing in favour of increased olfactory and gustatory systems. In comparison, locusts are known to experience food deprivation-induced hyperactivity (i.e. increased walking) following 1 day of food deprivation (Davenport and Evans, 1984). We found an increase in signaling, with more prolonged food deprivation of 4 days showing no modulation of neural activity. Available energy supply cannot account for this discrepancy as the total hemolymph concentration of carbohydrates were unchanged in the blowfly (Longden et al., 2014) whereas previous work with locusts show a significant decline following a single day (Goldsworthy, 1969; Moreau et al., 1984) and our Western blot results with pAMPK, suggest a reduction in available energy for locust neural tissue. Furthermore, the requirement by Longden et al. (2014) of their flies to walk on a surface, thereby requiring additional energy expenditure could not be a factor as both fed and food-deprived animals were required to do this. Rather, the differences observed between our study and theirs highlights separate strategies to deal with energy conservation. These results are similar to what is observed in the electric organ of two species of weakly electric fish: *E. virescens* reduces high-frequency signaling by the electric organ (Sinnett and Markham, 2015) following food deprivation while *E. gauderio* increases high-frequency signaling (Gavassa and Stoddard, 2012).

Conclusion. We directly compared how different metabolic stresses modulate neural performance and found a differential effect between prior anoxic coma and food deprivation. We confirmed that prior anoxic coma diminished signaling in the axon, conducting fewer and slower high-frequency APs. We also found, in contrast, food deprivation increased the number of high-frequency APs and their respective CVs. We suggest the increase in high-frequency signaling by food deprivation is due to elevated octopamine brought on by the metabolic stress and future studies should examine the interaction of food deprivation with octopamine signaling using pharmacological approaches.

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