Heat Shock-Induced Thermoprotection of Action Potentials in the Locust Flight System

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ABSTRACT: There is increasing evidence that heat shock (HS) has long-term effects on electrophysiological properties of neurons and synapses. Prior HS protects neural circuitry from a subsequent heat stress but little is known about the mechanisms that mediate this plasticity and induce thermotolerance. Exposure of *Locusta migratoria* to HS conditions of 45°C for 3 h results in thermotolerance to hitherto lethal temperatures. Locust flight motor patterns were recorded during tethered flight at room temperature, before and after HS. In addition, intracellular action potentials (APs) were recorded from control and HS motoneurons in a semi-intact preparation during a heat stress. HS did not alter the timing of representative depressor or elevator muscle activity, nor did it affect the ability of the locust to generate a steering motor pattern in response to a stimulus. However, HS did increase the duration of APs recorded from neuropil segments of depressor motoneurons. Increases in AP duration were associated with protection of AP generation against failure at subsequent elevated temperatures. Failure of AP generation at high temperatures was preceded by a concomitant burst of APs and depolarization of the membrane. The protective effects of HS were mimicked by pharmacological blockade of I_{K^+} with tetraethylammonium (TEA). Taken together, these findings are consistent with a hypothesis that HS protects neuronal survival and function via K^+ channel modulation. J Neurobiol 49: 188–199, 2001

Keywords: **heat stress; thermotolerance; neuronal action potential; tetraethylammonium; K^+ channel modulation**

INTRODUCTION

The ecology and molecular biology of the heat shock (HS) response has been well characterized (Becker and Craig, 1994; Buchner, 1996; Craig and Gross, 1991; Feder and Hofmann, 1999; Parsell and Lindquist, 1993), but the electrophysiological effects of HS on neuronal function are only beginning to be understood. Temperature can alter neuronal processes (Janssen, 1992) and neural circuitry can fail at high temperatures, impairing motor pattern generation and thus endangering organisms long before neurons start to die. Prior exposure to high, but sublethal, temperatures activates cellular HS responses that induce thermotolerance in all tissues, including nervous tissue (Marcuccilli and Miller, 1994). There is also increasing evidence that heat shock proteins (HSPs) can be cytoprotective agents against various neuronal pathologies, including stroke, ischemia, amyloidosis, and neurodegenerative diseases (Morimoto and Santoro, 1998; Sharp et al., 1999). It is surprising, therefore, that the extent to which the HS response might mitigate the potentially damaging effects of elevated body temperature on neural circuit function is for the most part unknown.

Neurons and synapses can be altered and protected from heat stress by prior HS. At *Drosophila* neuromuscular junctions, HS increases the upper temperature limit for synaptic transmission and stabilizes synaptic performance during a subsequent heat stress (Karunanithi et al., 1999). In African migratory locusts, a previous HS treatment can protect neuronal circuits responsible for generation of flight rhythms by increasing the temperature for heat-induced failure and by decreasing their thermosensitivity (Robertson

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et al., 1996b). HS also protects synaptic transmission in motor circuits from a subsequent heat stress by increasing the upper temperature limit for synaptic transmission by 6°C, reducing the thermosensitivity of synaptic delay, protecting the amplitude of postsynaptic potentials, and decreasing the time to recovery after heat-induced failure (Dawson-Scully and Robertson, 1998; Barclay and Robertson, 2000). Such thermotolerance of synaptic transmission may be mediated by HSPs (Karunanithi et al., 1999). HS also has long-term effects on channel conductance and activation kinetics. Voltage-dependent K^+ currents (I_{K^+}) recorded from neuronal somata in a locust thoracic ganglion slice are attenuated by prior HS and inactivate rapidly (Ramirez et al., 1999). This can be interpreted as a measure to minimize K^+ efflux and thus metabolic demands during stress, but it may also play an important part in the HS-induced protection of synaptic efficacy. Nevertheless, the causal link between I_{K^+} modulation, protection of circuit function, and behavior has not been established.

To investigate the electrophysiological consequences of HS on neuronal function, we recorded action potentials (APs) from locust flight muscles and motoneurons and examined the effects of HS on motor patterns during tethered flight and in response to heat stimuli. A steering response elicited by a heat stimulus is a well-characterized behavior in locusts (Robertson et al., 1996a), and is a useful measure of how well the nervous system is able to integrate information and coordinate and execute an appropriate behavior. Characterization of the protective effects of HS on AP signaling during heat stress and of similar protective effects after pharmacological blockade of K^+ channels is consistent with the contention that modulation of I_{K^+} has a role in the protection of neural function.

METHODS

Animals

Male *Locusta migratoria* L. at least 2 weeks past imaginal ecdysis were selected from a crowded colony maintained at 25°C on a 16 h:8 h light/dark cycle. Locusts were fed once daily with wheat grass, carrots, and dry food (a mixture of bran and milk powder).

Heat Shock Treatment

Locusts were placed in a 2 L container and heat shocked for 3 h at 45°C in a humid oven. Experiments were performed 1–5 h after HS treatment. Control animals were kept under similar conditions at room temperature (25 ± 1°C).

Electromyographic Recording

Whole Intact Animal. Motor patterns were collected from left and right first basalar muscles (m97) of tethered flying locusts at room temperature. Locusts were tethered with wax at the pronotum and placed in front of a wind tunnel (wind speed 3 m/s). Electromyographic (EMG) electrodes consisted of 24 gauge insulated copper wire with exposed tip. A single EMG electrode was inserted into each of the left and right forewing first basalar muscles (m97, depressor muscles). A ground electrode was inserted into the thorax through the cervical membrane. EMG signals were amplified with Grass P15 differential AC preamplifiers in single-ended configuration, displayed on a Gould DSO 630 digital oscilloscope, and simultaneously recorded onto videotape via Neuro-Corder (DR 890) VCR interface. After tethering, locusts were placed in front of the wind tunnel and allowed to fly for 1–2 min. To induce a steering response we alternately presented heat stimuli from lamps (Chauffa 250 W infrared heat lamps) placed on the left and right side of the locust. AP duration was determined from the prestimulus sequence and measured in depressor muscle activity only. To avoid complications resulting from overlapping signals, only signals that contained a single AP were used in the analysis of AP duration. Extracellular AP duration was recorded as the peak of the positive phase to the trough of the negative-going phase. These two points are easily distinguished and can be related to intracellular electrophysiological events (e.g., Pearson et al., 1970; Stein and Pearson, 1971). Analysis of EMG recordings revealed that there were no significant differences between AP peak to trough duration from the many APs within a flight sequence. Consequently, data presented represent AP peak to trough duration for a single AP trace for each animal. Depressor frequency was calculated from measurement of the period between consecutive m97 muscle AP peaks. Depressor-elevator interval was measured from the m97 spike to the beginning of elevator activity that was recorded by electrical spread from an elevator muscle (m83). Steering responses were recorded as shifts in the time of the peak of left and right m97 activity. Larger differences between forewing muscle asymmetry before and after the heat stimuli relate to larger steering responses. For analysis, responses recorded on videotape were digitized using Axoscope (10 kHz sampling rate; Axon Instruments).

Semi-Intact Animal. A semi-intact locust preparation that is capable of generating flight rhythms (Robertson and Pearson, 1982) was used to measure motor patterns. Motor patterns were induced by blowing on the head of the locust and were recorded from dorsal longitudinal muscle m81. The thoracic ganglia and muscle were bathed in temperature-controlled standard or 40 m*M* tetraethylammonium (TEA) saline (see below). Signals were recorded, amplified, and analyzed as described.

Intracellular Recording

For intracellular recordings, locusts were heat shocked and allowed 1 h recovery before recording. APs were recorded

intracellularly from the neuropil segments of motoneurons in a semi-intact locust preparation (Robertson and Pearson, 1982; same preparation as described above). The ventral nerve cord was exposed and superfused with standard saline containing (m*M*): 147 NaCl, 10 KCl, 4 $CaCl_2$, 3 NaOH, and 10 HEPES buffer (pH 7.2). In TEA preparations, 40 m*M* TEACl was added to the standard saline, and NaCl was reduced accordingly to maintain osmolarity. Although the TEA concentration appears relatively high, the concentration of TEA in the neuropil would be considerably lower because of the relative difficulty for substances to equilibrate across the ganglionic sheath (Parker, 1995; Xu and Robertson, 1996). We did not desheath the ganglia because such efforts generally disrupt the generation of flight motor patterns.

APs were evoked with a stimulating electrode on nerve 3 of the mesothoracic ganglion and recorded intracellularly with a glass microelectrode (1 *M* KAc, 40 MΩ; World Precision Instruments) from passive membrane in the dendritic region of forewing motoneurons. The temperature of the saline was controlled using a heating coil of Nichrome wire wrapped around the inlet pipette and was monitored with a thermocouple (Bat-12; Physitemp Instruments, Inc.) adjacent to the mesothoracic ganglion. The temperature of the saline flow bathing the thoracic ganglia was increased until an AP could no longer be generated (= failure). After heat-induced failure, increases in stimulus voltage were ineffective in restoring the AP. Subsequently, the saline was allowed to cool to 25°C to allow recovery. The time to recovery was measured at the time when there was a just noticeable deflection of the membrane potential at the appropriate latency from the stimulus artifact. Apparently complete recovery took several minutes longer. Signals were amplified (Getting, Model 5), recorded to video tape via Neuro-Corder (DR 890), and subsequently digitized and analyzed (Digidata 1200, Axoscope; Axon Instruments). Decremental conduction from the axon to the electrode resulted in APs of around 20 mV in control neurons at 25°C. We characterized APs by measuring time to peak, latency, amplitude, duration at half amplitude, and area under the curve. We selected for analysis only those APs with initial amplitudes between 10 and 30 mV.

Statistical Analysis

t tests, two-way ANOVA, and ANCOVA determined statistical significance among data. Statistical analysis was performed using SigmaStat 2.0 software (Jandel Scientific, San Rafael, CA). Data are presented as means ±S.E. and were plotted using SigmaPlot 4.0 graphing software (Jandel Scientific). Differences in means were considered significant when $p < 0.05$.

RESULTS

HS did not alter the depressor muscle (m97) firing frequency [Fig. 1(A,B); *t* test, $t = .42$, $p > .05$, $df = 14$] or the depressor (m97)/elevator (m83) muscle interval [Fig. 1(A,C); *t* test, $t = .62$, $p > .05$, $df = 14$] during tethered flight at room temperature. However, in HS animals the duration of the APs recorded from the forewing depressor muscle was significantly increased from an average of 2.2 ± 0.4 (S.E.) to 2.8 ± 0.5 ms [Fig. 1(D); *t* test, $t = -2.13$, $p = .027$, $df = 14$]. Steering responses were observed as asymmetries in the timing of left and right forewing muscle activity. During a steering response, the difference between left and right forewing muscle activity is increased as one wing is depressed earlier than the other. Both control and HS animals exhibited forewing asymmetry in response to heat stimuli [Fig. 1(E); control: *t* test, $t = 3.9$, $p = .006$, $df = 7$ HS: *t* test, $t = 2.9$, $p = .03$, $df = 6$], demonstrating that HS does not hinder the animal's ability to perform an adaptive steering maneuver (Robertson et al., 1996a).

Motoneuronal APs were evoked at 1 Hz and fired in time with the stimulus as temperature was increased except for a brief period just prior to heat-induced failure. Failure to generate an AP with the evoked stimulus at high temperatures was preceded by a burst of spontaneous (i.e., not firing with 1 Hz stimulus) APs in 22 of 29 cells [Fig. 2(A,B); control: 12/17; HS: 10/12]. The membrane potential depolarized prior to and/or during and after the burst of APs [Fig. 2(A)]. Prefailure spontaneous AP bursts were characterized for duration, change in membrane potential, and amplitude of first and last AP in burst. There were no statistically significant differences between prefailure burst and depolarization in control and HS neurons. Burst characteristics are presented in Table 1.

Increases in temperature caused a change in AP characteristics that ultimately led to AP failure and subsequent recovery [Fig. 2(C)]. HS increased the temperature at failure for generation of APs in locust motoneurons from 41.7 ± 1.1°C ($n = 17$) to 50.3 ± 1.5°C ($n = 12$) [Fig. 2(D); *t* test, $t = -4.76$, $p < .001$, $df = 28$]. HS did not affect the time to recover (i.e., reappear) after heat-induced failure [Fig. 2(E); control: 60.0 ± 15.9 s; HS: 54.6 ± 16.1 s; $df = 20$; *t* test, $t = .27$, $p = .79$, $df = 18$], and recovery appeared complete within several minutes [Fig. 2(C)]. Baseline membrane potential was around −45 mV upon penetration of the motoneuron and was variable between preparations such that statistical comparison revealed no difference between HS and control. Consistent with a previous study using the same preparation, we observed a slight hyperpolarization (~5 mV) of the membrane potential with increases in temperature (Xu and Robertson, 1994) that did not differ significantly between control and HS preparations.

Increases in temperature decreased latency, time to

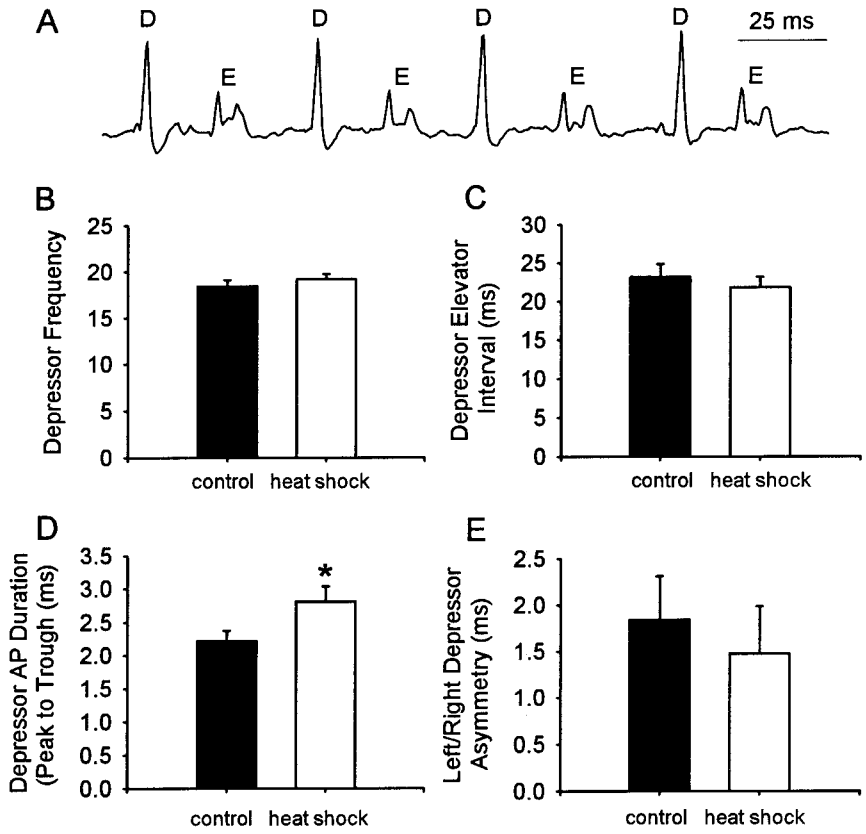

Figure 1 The effects of heat shock on flight motor patterns at room temperature. (A) Sample electromyographic (EMG) recording of flight muscle activity of a control animal in tethered flight. Large spikes (D) represent depressor muscle 97 activity and small spikes (E) represent elevator muscle 83 activity. (B) Heat shock did not affect the frequency of depressor muscle activity (*t* test, $t = -.83$, $p = .42$, $df = 14$). (C) Heat shock did not affect the interval between activity of depressor muscle 97 and elevator muscle 83 (*t* test, $t = .62$, $p = .54$, $df = 14$). (D) Heat shock increased the duration of depressor muscle action potentials (measured from peak to trough; *t* test, $t = -2.13$, $p = .027$, $df = 14$). (E) The effect of heat shock on steering during tethered flight. Control and heat shock locusts both exhibited steering responses as indicated by motor pattern asymmetry in response to a laterally placed infrared source (control: *t* test, $t = 3.9$, $p = .006$, $df = 7$; heat shock: *t* test, $t = 2.9$, $p = .03$, $df = 6$).

peak, amplitude, and duration in both control and HS animals [Figs. 2(C), 3]. HS did not change the value or thermosensitivity of AP time to peak [Fig. 3(C); two-way ANOVA, $F = 1.39$, $p = .24$, $df = 1$; two-way ANOVA, $F = .34$, $p = .89$, $df = 5$] or latency [Fig. 3(D); two-way ANOVA, $F = 1.73$, $p = .19$, $df = 1$; two-way ANOVA, $F = .04$, $p = .99$, $df = 5$]. HS treatment also did not significantly affect the amplitude of APs at moderate temperatures [Fig. 3(E)]. However, at elevated temperatures (>35°C) APs from HS animals were less sensitive to increases in temperature compared to controls (two-way ANOVA, $F = 2.93$, $p = .02$, $df = 5$). HS increased the duration at half amplitude of APs [Fig. 3(F); two-way ANOVA, $F = 10.56$, $p = .002$, $df = 1$], but this effect was not apparent at temperatures >35°C [Fig. 3(F)]. One possibility is that the difference in duration was a consequence of an alteration in the extent of passive decrement from the axon to the recording electrode. Such an effect would concomitantly reduce AP amplitude. To ensure that was not the case we compared the relationship between duration and amplitude (Fig. 4). There was no difference in the regression slopes fit through these relationships from eight control and eight HS preparations [Fig. 4(A); *t* test, $t = .73$, $p > .05$, $df = 12$], indicating that duration varies with amplitude in the same way for control and HS APs. However, the intercepts of the regressions were significantly different [Fig. 4(A); ANCOVA, $F = 90.9$, $p < .05$, $df = 1, 13$], and thus the absolute values of duration were different in HS APs across all amplitudes. The statistical analysis of Figure 3(F) was repeated after selecting APs from control and HS animals that were

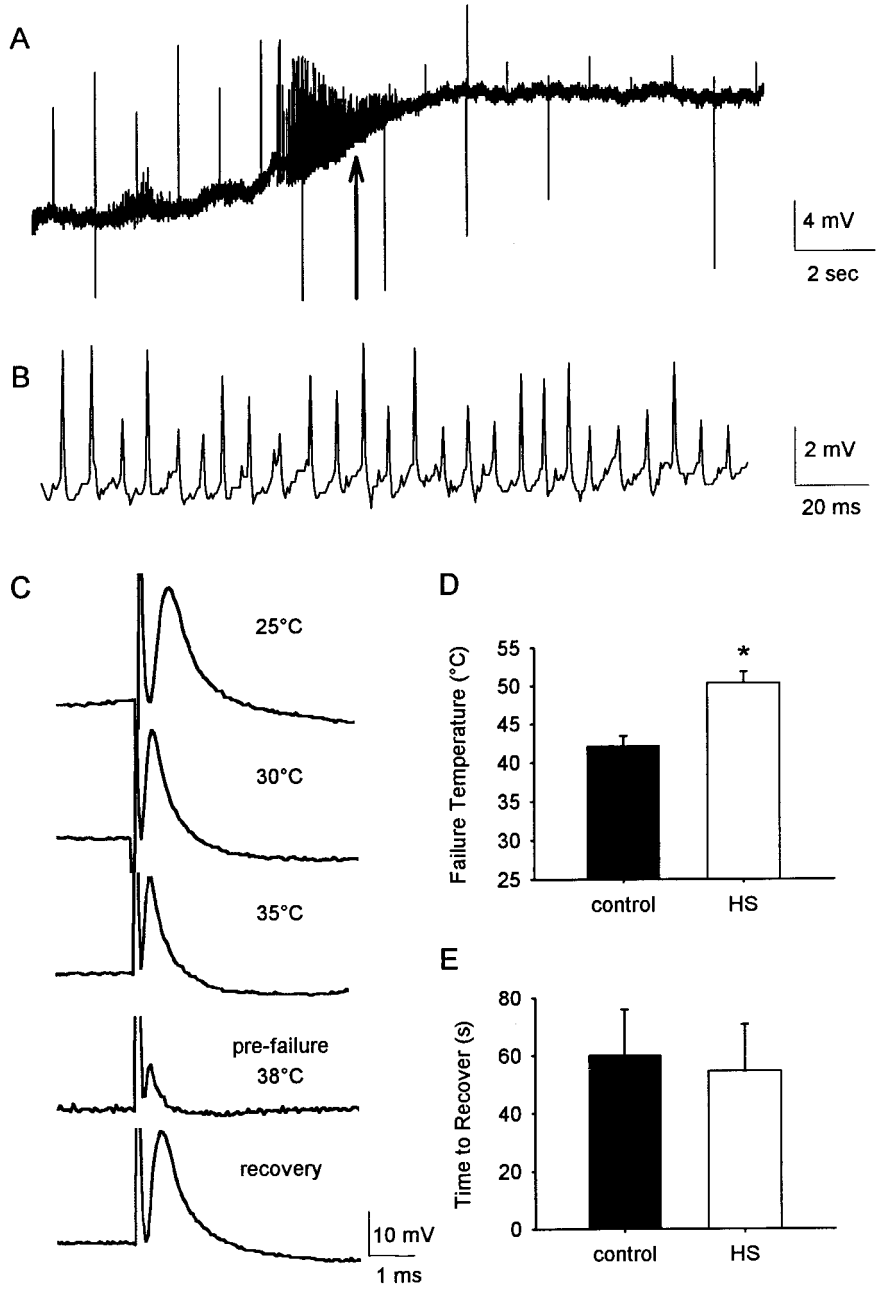

Figure 2(A–B) Failure of action potential (AP) generation by an evoked stimulus was preceded by a spontaneous burst of high frequency APs and a change in membrane potential. (A) Representative trace of a prefailure spontaneous burst of APs and the accompanying depolarization in membrane potential. Note the stimulus artifact that is present at 1 s intervals. The stimulus artifact is variable in size due to the sampling rate during digitization. The arrow indicates the location of the expanded trace in panel (B). (B) Representative trace of spontaneous AP bursting prior to AP failure (arrow in A). (C) Sample traces of an AP during heat-induced failure and subsequent recovery. Note the stimulus artifact that precedes the APs. (D–E) Heat shock (HS) increases the upper temperature limit at which AP generation fails but does not alter the time to recover after heat-induced failure. (D) APs in HS neurons failed at higher temperatures than those in control neurons (t test, $t = -4.76$, $p < .001$, $df = 28$). (E) Prior HS did not affect the time to recover for generation of an AP after heat-induced failure (t test, $t = .27$, $p = .79$, $df = 18$). For six neurons it was not possible to record the time to recover due to loss of the penetration.

Table 1 Characterization of Prefailure Spontaneous Action Potential Bursting

	Control $n = 13$	Heat Shock $n = 10$	TEA $n = 8$
Duration of burst (s)	5.41 ± 1.31	13.20 ± 4.99	11.00 ± 2.78
Change in membrane potential (mV)	10.43 ± 2.62	17.40 ± 4.77	12.93 ± 1.99
Amplitude of first action potential in burst (mV)	9.75 ± 1.11	8.04 ± 1.09	12.78 ± 2.99
Amplitude of last action potential in burst (mV)	3.67 ± 0.57	4.45 ± 1.14	3.35 ± 0.59

Data are presented as means ±S.E. Statistical analysis revealed that there were no significant differences in burst characteristics between any of the groups.

matched for amplitude at 25°C [Fig. 4(A,C)] to confirm that "outliers" were not biasing the statistical treatment. These analyses again demonstrated that HS increased AP duration independent of amplitude.

Measurement of area under the AP curve revealed that there were no significant differences between the area under control and HS spikes at 25°C (control: 437.15 ± 45.35 mV · ms; HS: 461.89 ± 33.85 mV · ms; *t* test, $t = -.44$, $p = .66$, $df = 15$), 30°C (control: 277.87 ± 32.81 mV · ms; HS: 331.66 ± 93.07 mV · ms; *t* test, $t = -1.19$, $p = .25$, $df = 15$), and 35°C (control: 186.43 ± 16.54 mV · ms; HS: 232.72 ± 40.03 mV · ms; *t* test, $t = -1.02$, $p = .32$, $df = 15$). Analysis of AP after-hyperpolarizations revealed that five out of eight control APs and four out of nine HS APs had after-hyperpolarizations. There was a tendency for HS APs to have reduced after-hyperpolarizations [Fig. 3(A,B); control amplitude: 21.2 ± 6.31 mV; HS amplitude: 7.0 ± 1.73; *t* test, $t = 1.94$, $p = .09$, $df = 7$; control duration at half amplitude: 21.11 ± 3.93 ms; HS duration at half amplitude: 13.17 ± 5.41 ms; *t* test, $t = 1.22$, $p = .26$, $df = 7$].

To test the hypothesis that a drug-induced decrease in I_{K^+} could mimic the effect of HS and thereby contribute to the thermotolerance of APs, the K^+ channel blocker TEA was added to the saline bathing the preparation. Consistent with this hypothesis, the presence of TEA altered AP shape [Fig. 5(A)], increased the percentage of APs being generated at elevated temperatures [Fig. 5(B)], and increased the upper temperature limit for generation of an AP by more than 4°C [Fig. 5(C); two-way ANOVA with Dunnett's multiple pairwise comparisons, $F = 12.90$, $p < .001$, $df = 2$], representing a substantial portion of the increase in failure temperature found in HS animals. In preparations with TEA, failure to generate an AP with the stimulating electrode was also preceded by a spontaneous burst of APs (8 of 10 cells) and depolarization of the membrane (Table 1). TEA increased the time to peak of APs compared to both control [Fig. 6(A); two-way ANOVA, $F = 17.10$, $p < .001$, $df = 1$] and HS (two-way ANOVA, $F = 14.89$, $p < .001$, $df = 1$) neurons. However, TEA did not alter the latency of APs when compared to control [Fig. 6(B); two-way ANOVA, $F = 1.94$, $p = .17$, $df = 1$] or HS (two-way ANOVA, $F = 1.08$, $p = .30$, $df = 1$) APs. TEA also did not significantly increase AP amplitude when compared to control APs [Fig. 6(C); two-way ANOVA, $F = 2.38$, $p = .12$, $df = 1$], but amplitude was significantly larger than in HS neurons [Fig. 6(C); two-way ANOVA, $F > 10^{10}$, $p < .001$, $df = 1$]. However, TEA increased AP duration [Figs. 5(A), 6(D); two-way ANOVA, $F = 16.82$, $p < .001$, $df = 1$] to a level that was indistinguishable from that observed in HS animals [Fig. 6(D); two-way ANOVA, $F = 2.82$, $p = .01$, $df = 1$].

To determine whether the drug-induced decrease in I_{K^+} can also contribute to the protection of motor patterns, EMG electrodes were inserted into the dorsal longitudinal muscle, m81, in a semi-intact locust preparation that was perfused with standard or 40 m*M* TEA saline. Preparations perfused with standard saline could produce flight rhythms up to 42.5 ± 4.4°C (mean ±S.E., $n = 5$), but preparations bathed in 40 m*M* TEA were not capable of generating a flight rhythm.

DISCUSSION

Timing of motor patterns for locusts in flight has been extensively characterized and it is thus a useful model to study the maintenance of nervous system function after HS treatment. In particular, locusts can be monitored for their ability to execute an avoidance maneuver (Robertson et al., 1996a). Here we compared motor patterns from control and HS animals.

Our results demonstrate that HS does not alter the timing of depressor and elevator muscle activity during flight. However, HS did increase the duration of APs recorded from depressor muscle 97. Although it is not known how I_{K^+} in muscle is affected by HS, we speculate that the broader duration of APs is a result of changes in I_{K^+} similar to those found in locust neurons (Ramirez et al., 1999). Both control and HS locusts performed steering maneuvers in response to a heat stimulus. Coupled with a previous study showing that HS does not alter the timing of muscle activity at

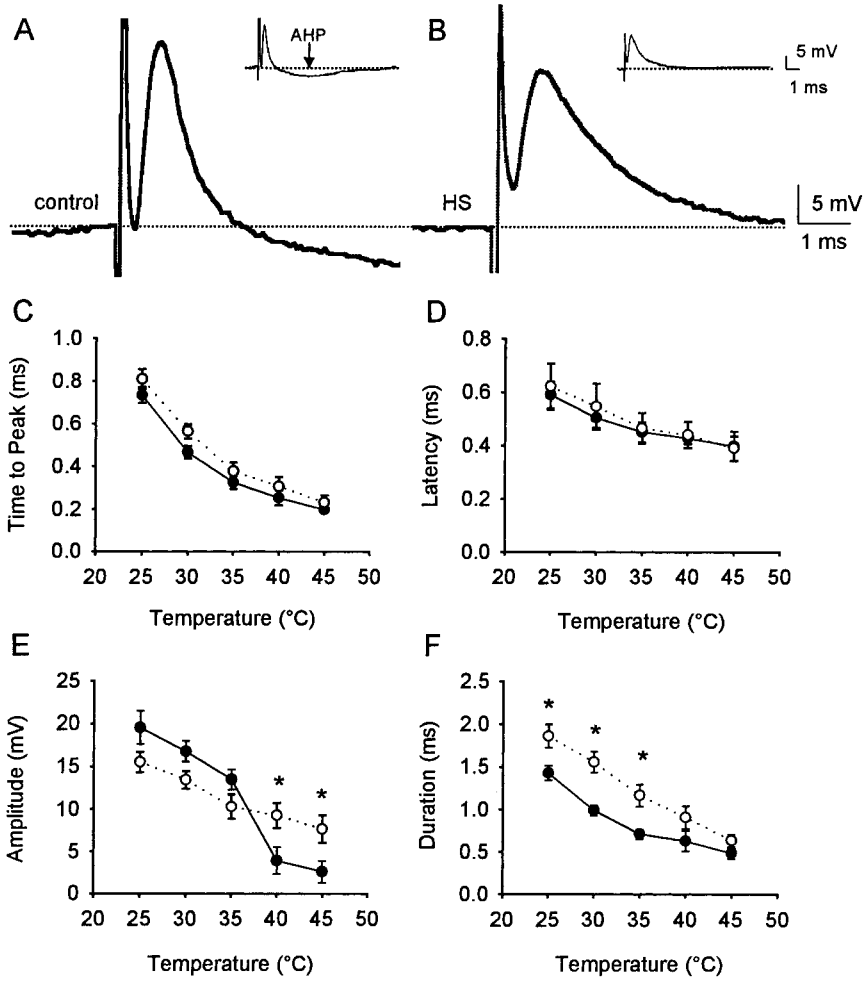

Figure 3(A–B) Representative traces of evoked action potentials (APs) recorded intracellularly from neuropil segments of locust forewing motoneurons. Note the stimulus artifact preceding the APs. (A) Sample recording of an AP recorded from a control motoneuron at 25°C. [(A) inset] Compressed trace of AP displayed in (A) to illustrate the after-hyperpolarization (AHP). (B) Compressed trace of an AP recorded from a heat shock (HS) motoneuron at 25°C. In (A) and (B) dotted lines indicate prestimulus membrane potential. [(B) inset] Extended recording of AP displayed in (B). (C–F) HS modifies the thermosensitivity of APs recorded intracellularly from locust forewing motoneurons. (C) Thermosensitivity of AP time to peak in control (filled circles) and HS neurons (open circles; $n = 8, 8$; error bars may be hidden in symbols). (D) Thermosensitivity of AP latency in control (filled circles) and HS neurons (open circles; $n = 8, 8$). (E) Thermosensitivity of AP amplitude in control (filled circles) and HS neurons (open circles; $n = 8, 8$). At 40 and 45°C, APs in HS neurons were significantly larger in amplitude compared with controls. (F) Thermosensitivity of AP duration in control (filled circles) and HS neurons (open circles; $n = 8, 8$). At 25, 30, and 35°C, APs were significantly longer in HS neurons compared with controls. Asterisks indicate a difference from control at $p < .05$ assessed by two-way ANOVA and Tukey multiple pairwise comparisons.

room temperature (Robertson et al., 1996b), these findings demonstrate that HS does not adversely affect the functional integrity of the nervous system at a behavioral level.

Intracellular recordings of evoked APs from locust motoneurons revealed that failure to generate an AP at high temperatures was preceded by a depolarization of the membrane potential and a concomitant burst of spontaneous AP activity. The burst of APs is likely a result of increased excitability due to the depolarization of the membrane potential. Changes in membrane potential could result from complications associated with the loss of the structural integrity of the membrane. At high temperatures the lipid bilayer may become dissociated as a result of direct effects of temperature on the intrinsic biophysical properties of

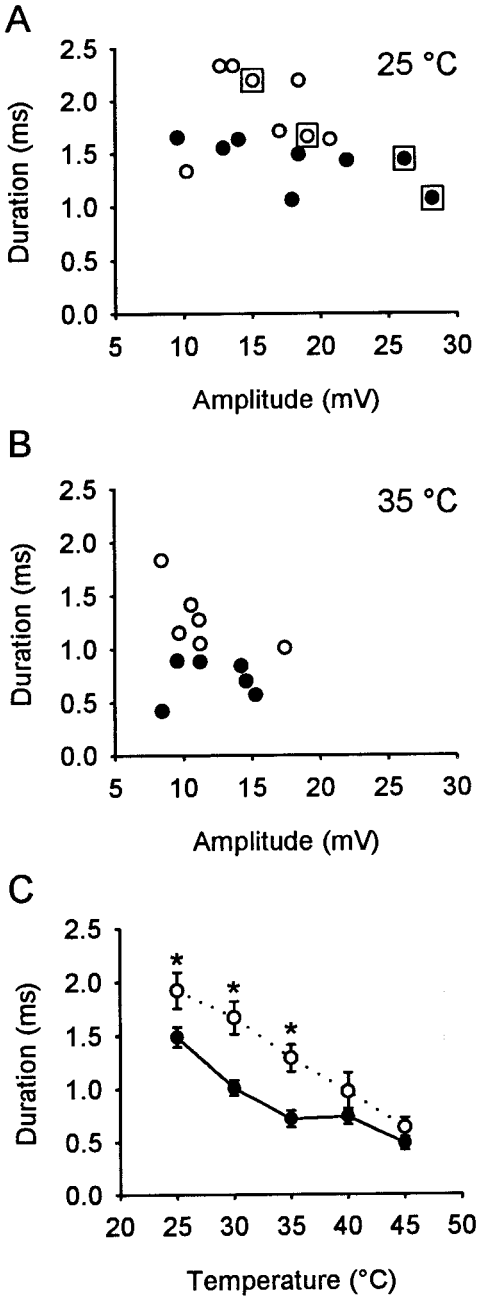

Figure 4 Heat shock (HS) increased action potential (AP) duration independent of AP amplitude. (A) Plot of AP duration against amplitude in control (filled circles, $n = 8$) and HS (open circles, $n = 8$) neurons at 25°C. APs from control and HS groups were matched for amplitude and unpaired values were excluded from the subsequent analysis. Unmatched values are inscribed within a square. (B) Plot of AP duration against amplitude in control (filled circles, $n = 6$) and HS (open circles, $n = 6$) neurons at 35°C. (C) Thermosensitivity of AP duration in control (filled circles) and HS (open circles) neurons ($n = 6, 6$). Duration in control neurons was significantly shorter compared to HS neurons (two-way ANOVA). In (C) asterisks indicate a difference from control at $p < .05$ assessed by two-way ANOVA and Tukey multiple pairwise comparisons.

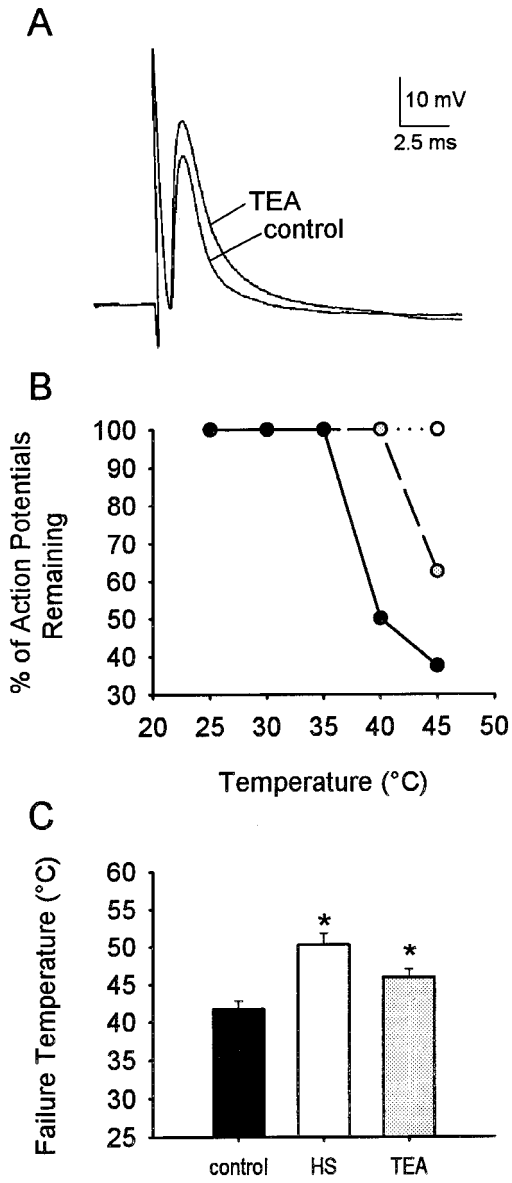

Figure 5 Blockade of K^+ currents with TEA increased the amplitude and duration of locust APs and mimicked the protective effect of heat shock (HS) on APs at high temperatures. (A) Raw traces of APs recorded from neurons bathed in standard (control) and TEA saline. Note the stimulus artifact preceding the APs. (B) Thermosensitivity of AP generation in control (filled circles), HS (open circles), and TEA (shaded circles) neurons represented by the percent of APs remaining at increasing temperatures. (C) Upper temperature limit for heat-induced failure of AP generation in control, HS, and TEA neurons ($n = 17, 12, 12$). HS and TEA increased the upper temperature limit for heat-induced failure. Asterisks indicate significant difference from control at $p < .05$ assessed by one-way ANOVA with Dunnett's multiple pairwise comparisons.

the membrane. In addition, proteins embedded in the membrane that are critical to maintaining the lipid bilayer may lose their native structure and cause the

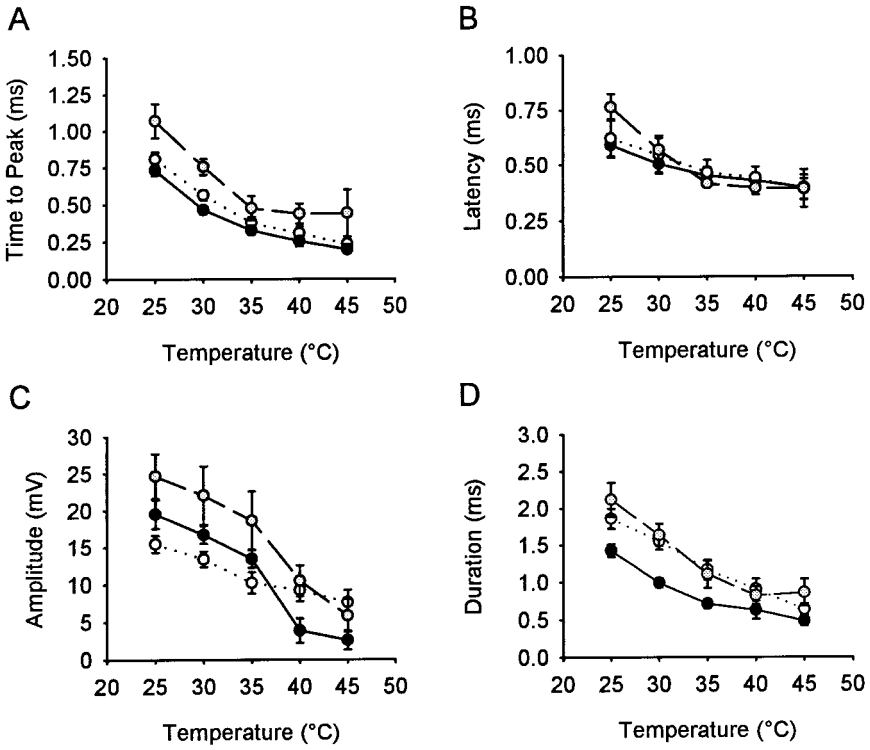

Figure 6 TEA modified the thermosensitivity of action potentials (APs) recorded intracellularly from locust forewing motoneurons. (A) Thermosensitivity of AP time to peak in control (filled circles), HS (open circles), and TEA (shaded circles) neurons. TEA increased the time to peak of APs compared to both control and HS neurons ($n = 8, 8, 10$; error bars may be hidden in the symbols). (B) Thermosensitivity of AP latency in control (filled circles), HS (open circles), and TEA (shaded circles) neurons. Latency in TEA-treated neurons was not significantly different from control or HS neurons ($n = 8, 8, 10$). (C) Thermosensitivity of AP amplitude in control (filled circles), HS (open circles), and TEA (shaded circles) neurons. TEA did not significantly increase AP amplitude compared to control APs. However, amplitude in TEA neurons was significantly larger than in HS neurons ($n = 8, 8, 10$). (D) Thermosensitivity of AP duration in control (filled circles), HS (open circles), and TEA (shaded circles) neurons. Duration in control neurons was significantly shorter compared with both HS and TEA neurons. Duration in HS and TEA neurons was not different. Significant difference at $p < .05$ was assessed by two-way ANOVA with Tukey multiple pairwise comparisons. Refer to text for further details regarding statistical analysis.

membrane to separate from cellular scaffolding (Luna and Hitt, 1992; Kusumi and Sako, 1996; Goni and Alonso, 1999). Alternatively, depolarization of the membrane, the burst of APs, and subsequent suppression of activity may result from changes in ion channel function leading to the build up of extracellular K^+ and/or failure of active transport pumps. Such changes are associated with spreading depression (SD) and there is a striking similarity between SD and the phenomenon described here (Wu and Fisher, 2000; Martins-Ferreira et al., 2000; Obeidat and Andrew, 1998).

Other stressors can also cause modulation of ion channels leading to depolarization of the membrane, increases in neuronal activity, and subsequent suppression of activity. Brief periods of hypoxia induce such changes in cockroach motoneurons that are associated with decreases in I_{K^+} (Le Corronc et al., 1999; Walter and Nelson, 1975). Membrane depolarization is believed to be associated with a decrease in Ca^{2+}-dependent K^+ currents (I_{KCa}) that limit the amplitude of depolarization in response to hypoxia (Le Corronc et al., 1999). In our findings, a similar modulation of I_{K^+} is consistent with the observed depolarization of the membrane during failure and the increase in AP duration. We suggest that modulation of K^+ channels and ion pumps that underlie the depolarization of the membrane, brief hyperexcitability, and subsequent depression of activity in neurons are general neuronal responses to acute stress.

Intracellular recordings show that HS protected the generation of APs in locust motoneurons from a subsequent exposure to high temperature stress. This result is consistent with previous studies showing that

prior HS has protective effects on circuit function (Robertson et al., 1996b) and synaptic transmission (Dawson-Scully and Robertson, 1998) at high temperatures. We found a significant and profound increase in the duration of APs after HS. One explanation for this result is that the heat pretreatment altered the passive cable properties of the neurite such that the back-propagating APs were increased in duration due to altered filtering properties of the passive dendritic membrane. We believe that this is unlikely because the increase in duration was independent of any alteration in AP amplitude and any passive filtering resulting in increased duration of such magnitude would be correlated with decreased amplitude. We favor the interpretation that the increased duration we recorded reflects an increase in duration of the APs at the axon and thus reflects changes in the kinetics of voltage-dependent ion channels. This effect is consistent with a reduction of neuronal I_{K^+} demonstrated previously (Ramirez et al., 1999). Differences in AP duration were not apparent at temperatures greater than 40°C. This may be because the thermoprotection afforded by HS is no longer effective above 40°C. However, given that about 20% of control animals can survive a normally lethal thermotolerance test (Robertson et al., 1996b), it is more likely that the few control preparations still capable of generating APs at elevated temperatures had characteristics associated with an intrinsic thermoprotection (i.e., APs with long durations). Measurement of area under the AP curve demonstrated that HS did not alter the net transfer of charge measured during an AP. Interpretation of this observation, however, must wait until more is known about how HS modifies the cable properties of the neurite.

The significant increase in duration of APs after heat treatment is consistent with modification of I_{K^+}, and thus the effects of a pharmacological blocker, TEA, were examined. TEA altered AP time to peak, amplitude, and duration. Time to peak in TEA-treated neurons changed due to a reduction in the amount of time before the depolarization phase of the AP started. Consequently, the time to peak in TEA-treated neurons was longer than in both control and HS neurons. Although HS increased AP duration with no significant effect on AP amplitude, TEA treatment increased both AP duration and amplitude. Despite these differences, TEA applications directly increased thermotolerance of a semi-intact preparation resulting in protection of AP generation. These findings demonstrate that modulation of I_{K^+} is sufficient for inducing thermotolerance. It is possible that Na^+ channels are also modulated by HS, accounting for the differences in AP amplitude in HS and TEA-treated neurons. Alternatively, HS may modulate only a subpopulation of K^+ channels in contrast to the more indiscriminate nature of TEA blockage on K^+ channels (Mathie et al., 1998; Yellen, 1987; Heginbotham and MacKinnon, 1992), which results in I_{K^+} that increases AP amplitude. Other possibilities also could be examined, including the modulation of Ca^{2+} currents (I_{Ca2+}) and consequently Ca^{2+}-dependent potassium currents (I_{KCa}) by HS. Changes in I_{KCa}, which can be involved in repolarization of the membrane potential during an AP (e.g., Mills and Pitman, 1999; Sah, 1996) and is largely responsible for the after-hyperpolarization of APs (Sah, 1996; Velumian and Carlen, 1999), may explain the observed differences in AP duration after HS. Changes in I_{KCa} may also underlie the trend towards larger prefailure depolarization of the membrane in HS and TEA neurons. Decreased I_{K^+} may account for both the observed changes in membrane potential prior to failure and the longer duration of APs in HS and TEA neurons. Although characterization of HS and TEA prefailure depolarizations did not detect any significant differences from controls, there was a tendency for the depolarizations to last longer in HS and TEA neurons. This idea is consistent with our model that reduced I_{K^+} is responsible for the observed increase in AP duration and plays a role in the prefailure depolarization of the membrane potential. The neuronal protective effects of TEA were not observed at the level of motor pattern generation as preparations perfused with 40 m*M* TEA saline were unable to generate motor patterns. This inability to generate motor patterns may be a consequence of the high concentration of TEA. The ganglionic sheath encloses the neuropil segments from which intracellular recordings were made, resulting in a lower actual concentration of the drug. Pattern generation may also be more sensitive to TEA due to unpredictable additive effects in a complex circuit. Such effects may include unmasking of non-K^+ outward currents that contribute to regenerative electrical properties in motoneurons (Cattaert et al., 1994) and shifts in spike threshold (Matzel et al., 1995).

AP failure in muscle cells and neurons at elevated temperature could result from the more rapid kinetics of voltage-dependent channels such that K^+ currents overwhelm Na^+ currents before they develop. Thermotolerant cells could continue to generate APs at high temperatures due to a HS- or TEA-induced decrease in I_{K^+} allowing sufficient Na^+ flux through sodium channels to depolarize the cell. In neurons, the concomitant increase in AP duration would also result in a greater influx of Ca^{2+} at presynaptic terminals that in turn would trigger the release of greater amounts of neurotransmitter (Augustine, 1990; Katz and Miledi, 1965). Higher concentrations of neurotransmitter could compensate for the decrease in syn-

aptic efficacy during temperature stress (Robertson, 1993) and thereby extend the functional temperature range for synaptic transmission and motor pattern generation. To support this suggestion, it is known that K^+ channel blockers (TEA or 4-aminopyridine) can restore axonal conduction after heat-induced failure in mammalian nerve roots (Eliasson et al., 1986), again indicating a possible role for I_{K^+} modulation in thermotolerance in higher organisms. Indeed, a heat pretreatment in neonatal mouse brainstem neurons suppresses I_{K^+} during exposure to elevated temperatures (unpublished observations). Because this phenomenon appears in organisms as diverse as locusts and mice, it may be an evolutionarily conserved mechanism for nervous system protection.

Our results suggest that the neuroprotective effects of HS are mediated in part via suppression of K^+ efflux. Although we postulate that HS induces thermotolerance by changes in I_{K^+}, the mechanism by which this arises is unknown. HSPs are involved in maintaining the general health of neurons during heat stress and manipulation of the levels of these proteins can alter ionic currents (Nanasi et al., 2000). Bimoclomol, a novel HSP coinducer, can change the amplitude of intracellular calcium transients and the duration of APs recorded from myocytes (Nanasi et al., 2000). Therefore, HSPs may have a direct role in the acquired thermotolerance of neuronal signaling, perhaps by stabilizing ion channels involved in setting the membrane potential. Alternatively, they may act indirectly by regulating cellular pathways that modulate ion channels. Neuromodulators may also be involved in acquired thermotolerance, as increases in temperature have been shown to induce the release of neuromodulators that have profound effects on ionic conductances and circuit function (Johnson et al., 1992). For example, serotonin (5-hydroxytryptamine) is known to decrease I_{K^+} via protein kinase A (Hochner and Kandel, 1992; Parker, 1995), increase AP duration (Hochner and Kandel, 1992; Parker, 1995), and increase excitatory postsynaptic potentials (Parker, 1995).

Much remains to be determined and future studies will more directly address the possible mechanism by which HSPs and neuromodulators may be involved in mediating neuronal plasticity, which results in thermotolerance. The findings presented here provide an important link between HS-induced changes in I_{K^+} and the protection of AP generation, synaptic transmission, and motor patterning.

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