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Exposure to high sub-lethal temperatures (heat shock) renders organisms thermotolerant of normally lethal temperatures and in mammalian nervous systems it protects neurons against a variety of subsequent neuropathological insults including ischemia/anoxia. Here I show that heat shock has important protective effects on synaptic transmission in an invertebrate model system. These data can explain the extension of the temperature operating range for the generation of motor patterns in this system. I suggest that an important role of heat shock proteins may be to stabilize the protein machinery underlying synaptic transmission and thus to allow neural function during and after stressful events.
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPG</td>
<td>central pattern generator</td>
</tr>
<tr>
<td>CSP</td>
<td>cysteine string protein</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory postsynaptic potential</td>
</tr>
<tr>
<td>fSR</td>
<td>forewing stretch receptor</td>
</tr>
<tr>
<td>HS</td>
<td>heat shock</td>
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<tr>
<td>HSP</td>
<td>heat shock protein</td>
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<tr>
<td>PSP</td>
<td>postsynaptic potential</td>
</tr>
<tr>
<td>Q₁₀</td>
<td>temperature coefficient</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature (21.1±0.2°C, N=42)</td>
</tr>
<tr>
<td>SR</td>
<td>stretch receptor</td>
</tr>
<tr>
<td>t-test</td>
<td>student t-test</td>
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<td>TTP</td>
<td>time to peak, or rise time</td>
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CHAPTER 1 INTRODUCTION

Most organisms that have been examined exhibit a heat shock response in which increased expression of a suite of heat shock proteins (HSPs), or stress proteins, is coincident with the induction of thermotolerance (the ability to survive normally lethal temperatures). There is considerable interest in the role of HSPs in nervous tissue because of the demonstrations that heat shock (HS) can protect neurons against ischemic damage (Kitagawa et al., 1990) and, recently, that this protection may be mediated by HSPs (Fink et al., 1997). Much is known about the molecular biology of HSPs and the HS response (Lindquist and Craig, 1988) and about the distribution of HSPs in nervous tissue (Mayer and Brown, 1994) yet surprisingly little is known of how HS, or HSPs, might affect the normal signaling operation of neurons during and after the stressful event. Given the ecological salience of temperature to poikilotherms it may be with these organisms that effects of HS on neural function can most profitably be investigated. An ecological perspective on the species distribution and role of HSPs can yield significant insight (Coleman et al., 1995) and it is clear that HSPs allow certain poikilotherms to behave and survive under extreme environmental conditions (Gehring and Wehner, 1995). I have investigated the effects of HS on the operation of the flight motor circuitry in the migratory locust, *Locusta migratoria*. 
1.1 Poikilotherms and Temperature

Unlike homeothermic organisms, poikilotherms lack thermoregulatory mechanisms for maintaining their internal temperature and are therefore profoundly dependent upon ambient temperatures (for review see Chappell and Whitman, 1990). In order for poikilotherms to function and survive, they must do so in the range of temperatures characteristic of their ecological niche. In some cases, this range can be wide, for example for desert or arctic adapted animals. Nonetheless, most of these animals exhibit thermoregulatory behaviours that can change their internal temperatures so as to promote optimal conditions for functioning.

Heterothermic insects, like the moth, are more active at higher temperatures. If the ambient temperature is too low, the moth can contract thoracic muscles in a mechanically inefficient manner to generate heat, thereby raising thoracic body temperature into the range suitable for flight (Hanegan and Heath, 1970). In ambient temperatures that are too high desert locusts can orient their bodies parallel to the sun's rays to reduce the amount of body surface exposed to the sun (Hardy, 1979). Evaporative cooling in insects also prevents internal temperatures from becoming dangerously high if the ambient temperature increases to extreme values (for review see Prange, 1995).
Physiological acclimation is a form of phenotypic plasticity that modifies the organism in minutes to months, and the modifications are rarely reversible within the organism's lifetime (Huey and Berrigan, 1996). Acclimatory shifts in heat tolerance or metabolic rate in response to photoperiod and/or temperature may cause phenotypic modifications (Levins, 1968). This may constitute a reactive response to experienced environments or anticipatory responses to future environments (e.g. seasonal weather).

Poikilotherms can function and survive within their ecological niches, and it is interesting how most of these organisms can deal with environmental extremes of heat and cold. An important question is how do these poikilotherms cope with threatening changes in the environment that adapted behaviours or acclimation cannot overcome.

1.2 Neuronal Properties and Temperature

Neuronal processes that can adapt to fluctuations of an organism's internal temperature are essential for function and survival. Homeotherms maintain their internal temperatures at a constant "set point" with little variability, and even minor fluctuations of internal temperatures can be lethal. However, for poikilotherms, fluctuation of body temperature is a normal part of their existence. Changes in internal temperature affect the rates of neuronal processes, including the membrane channel ion kinetics, conduction velocity and amplitude of action
potentials, and synaptic activation and neurotransmitter release.

Therefore, understanding how temperature affects the nervous system is difficult but necessary.

Neurons within the central nervous system (CNS) are affected by temperature in several ways and it is important to determine the nature of the changes at different levels (cellular to circuits).

i) Cellular Properties

The membrane lipid bilayer becomes more fluid with increases in temperature (Hazel, 1995; Janssen, 1992). For neurons, increasing membrane fluidity can cause a decrease in membrane resistance (Thompson et al., 1985; Griffin and Boulant, 1995). This leads to a decreased membrane time constant and an increased length constant, both of which affect neuronal signaling (Jack et al., 1983).

When examining post synaptic potentials, it has been shown that temporal parameters caused by passive cellular properties, like rise time and duration, decreased with increases in temperature due in part to decreases in the membrane time constant (Robertson, 1993). In addition, these changes are due to ion channel kinetics evoked by increases in temperature which can be predicted from the Hodgkin-Huxley equation (Joyner, 1981). It is clear that ion channel properties are highly dependent on membrane structure because this is
where they reside and operate. Therefore, changes in membrane fluidity may directly affect the kinetics and conductance of ion channels.

The conduction velocity of an axon increases as temperature increases (Huxley, 1959). Action potential amplitude and duration are also reduced with increases in temperature, and this has been shown in several different organisms (e.g. in the leech, Janssen, 1992; rat hippocampus, Thompson et al., 1985; and in the locust, Burrows, 1989). These effects of temperature can be explained, in part, by the temperature dependence of the time course of sodium and potassium currents, which are included in the Hodgkin-Huxley equation (Joyner, 1987).

**ii) Synaptic Properties**

Increases or decreases of temperature from room temperature cause excitatory post-synaptic potential (EPSP) amplitude to decrease in locust flight interneurons (Robertson, 1993), suggesting that ion channel conductance may be set for optimal operation in a specific temperature range. However, changes in EPSP amplitude have also been shown to have little effect on rhythm generation in the locust flight circuit suggesting an adaptive resistance to the effects of temperature fluctuation in this system (Xu and Robertson, 1996). As temperature increased, calcium (Ca$^{2+}$) channel currents recorded from dorsal raphe neurons in the adult rat exhibited an increase in current amplitude up to a maximum at 25°C, and then current amplitude leveled off up to 30°C (McAllister-
Williams and Kelly, 1995). Similar studies have also shown increased intracellular Ca\(^{2+}\) concentrations with increased temperature suggesting an adaptive mechanism for depolarizing the presynaptic membrane so as to oppose the reduction of action potential amplitude (Charlton and Atwood, 1979; Llinas et al., 1987).

The temporal parameters of EPSPs such as latency, time-to-peak (TTP), and duration, decrease exponentially with increases in temperature (Burrows, 1989; Robertson, 1993). The rate at which the different temporal parameters decrease are different from one another and therefore as temperature changes, the conflicting rates of change may cause the “shape” of the EPSP to distort. Robertson (1993) showed that the temperature coefficients (Q\(_{10}\)) for latency, TTP, and duration were different between 24-34°C (Q\(_{10}\)s = 0.51, 0.70, and 0.68). It has been suggested that poikilothermic nervous systems have adapted to have similar Q\(_{10}\)s for different neuronal processes so as to maintain the shape of the evoked EPSP, thereby allowing for normal functioning over a range of temperatures (Jaslove and Brink, 1986). The reduction in synaptic temporal parameters should correlate with increased speed of motor output from a neuronal circuit (Goodman and Heitler, 1977). However, in many poikilotherms, increases in temperature only mildly affect motor rhythm generation (e.g. in the locust, Foster and Robertson, 1992).
iii) Circuitry

Rates of neural processes controlling behaviours in poikilotherms have exhibited linear relationships with changes in temperature within certain limits (Walker, 1975; Janiszewski and Otto, 1988). The increased neural activity associated with increasing temperature has been shown to be a result of increased conduction velocity and reductions in synaptic delays thereby speeding up the neuronal circuit (Fraser, 1990; Burrows, 1989). This will therefore not only change the rhythm generation of the motor circuitry, but for many organisms, profoundly change behavioural performance (Montgomery and Macdonald, 1990). Therefore, it is important to understand the aforementioned effect on cellular mechanisms in the CNS.

1.3 Tolerance to extreme temperatures

Animals must sometimes deal with extreme temperatures in order to survive and function. Interestingly in poikilotherms, acclimation and learned adaptive behaviours can not always compensate for temperature extremes in their environment. A dramatic example of this is the Pompeii worm which periodically experiences extreme temperatures above 80°C and survives in its normal habitat in a temperature gradient of 22°C to 60°C along its body length (Cary et al., 1998). Another example is the Sahara desert ant, Cataglyphis, which forages terrestrially at midday on the desert surface, reaching internal
temperatures of 55°C; during the rest of the day, it resides underground where the temperature rarely drops below 20°C (Gehring and Wehner, 1995). In these examples, both of the animals do not continuously experience extreme temperatures, but are exposed to them for short periods of time. During times of extreme heat stress, several things can occur that could be detrimental to the animal. Suggested mechanisms for severe heat damage are: (1) denaturing of proteins which is also known as thermal coagulation; (2) thermal inactivation of enzymes exceeding rates of formation; (3) different temperature effects on independent metabolic reactions; and (4) temperature effects on membrane structure (Schmidt-Neilson, 1990). Interestingly, most organisms develop thermotolerance and demonstrate a heat shock (HS) response to extreme temperatures which is followed by the increased expression of heat shock proteins (HSPs) (for review see Moseley, 1994).

HSPs have been termed “molecular chaperones” and play important roles in cellular transportation and cell survival (Ellis, 1987). After a stress, for example extreme heat, the dominant product of protein synthesis in the organism becomes HSPs. In most organisms HSPs are expressed as early as 15 min following exposure to high temperatures. There have been observations that several HSPs are induced during normal cellular development and in the absence of heat stress (Schlesinger, 1986). A number of HSPs have been described from a variety of organisms. Most HSPs are known only by their
subunit molecular weights: HSP90, HSP70, and HSP20-30. Across organisms the molecular weight of HSPs vary slightly (Lindquist, 1986).

Interestingly, all organisms possess a type of HSP70 protein which is known for its role in stabilization and transportation (Locke and Noble, 1995). Studies have shown that the nervous systems in a number of organisms express HSP70 following exposure to physiologically stressful conditions (Burdon and Cutmore, 1982; Carr et al., 1994; Greenberg and Lasek, 1985; Ruder et al., 1988). HSP expression in the nervous system could be protective against excitotoxic injury (Kitagawa et al., 1990) and against cell death (Mailhos et al., 1993).

In E. coli, the HSP70-like protein is the product of the DnaK gene which has been shown to interact sequentially with other proteins (DnaJ and GrpE) (McCarty and Walker, 1991). The DnaJ proteins are also known as cysteine string proteins (CSPs) defined by an N-terminus ‘J’-domain and a central string of cysteine string residues (Buchner and Gunderson, 1997). It has been suggested that the thermotolerance induced by HSP70 sometimes requires these CSPs, and is shown by the temperature-sensitive breakdown of Ca^{2+} channels in Drosophila CSP null mutants. Therefore, CSPs could play an integral role in HSP70 interactions. The literature suggests that the major role of HSPs is primarily as a structural chaperone, yet the physiological mechanisms by which HSPs allow cells to tolerate extreme heat are still unclear.
1.4 Locust Flight

Locusts have been described as one of the best organisms for studying the neural basis of behaviour (Kammer, 1985). There are about 40 muscles and 80 motoneurons used by the locust for flight (Robertson, 1986). Since locusts are neurogenic insects, the flight motor activity is generated and controlled directly by neurons in the thoracic ganglia. The motor pattern from an intact locust has been characterized as having an alternation of activity in elevator and depressor flight muscles controlling the cyclical movement of the forewings and hindwings (Kammer, 1985; Weis-Fogh, 1956). The two pairs of wings beat at the same frequency which is in the range of 15-25 Hz. The activity of the hindwings leads the forewings, and the motor pattern is robust enough to function in a dissected preparation.

In deafferented locusts, it has been shown that flight rhythms are still produced but have a lower rhythm frequency than intact preparations (Wilson, 1961). However, deafferentation does not significantly alter the basic motor pattern. Also, the flight rhythm in the locust is a product of network oscillators rather than endogenous oscillators (for a review of neural mechanisms of oscillators see Marder and Calabrese, 1996). The operating circuit in the locust can be modified to the current condition of the animal and its flight circuit (Robertson, 1995). The locust's nervous system has a wide range of temperatures with which it can operate, being able to generate flight rhythms
between 24-42°C (Weis-Fogh, 1956; Neville and Weis-Fogh, 1963), therefore the locust flight model is ideal for examining the effects of changing temperature on neural parameters and how this modifies motor output. Foster and Robertson (1992) showed that the intact locust’s wingbeat frequency (WBF) increases with temperature increases. Similar results were shown for deafferented rhythm frequency with increases in temperature, where the only difference from intact animals was that deafferented preparations were consistently slower, which was to be expected (Foster and Robertson, 1992; Wilson, 1961). The change of WBF frequency can thus be explained purely by changes in the flight central circuitry and the underlying synaptic mechanisms.

Robertson (1993) examined the effects of temperature on synaptic potentials in the locust flight system. He found that increases in temperature reduced latency, time-to-peak and duration while increasing the slope of the EPSP (Robertson, 1993). It was also reported that amplitude decreased both above and below room temperature. Therefore, neuronal and synaptic function were affected by temperature and the changes were predictable due to thermal effects on channel conductance and kinetics, and on membrane properties such as time and length constants (Robertson, 1993). From this study, Robertson (1993) suggested that rhythm frequency was unaffected by changes observed in amplitude within permissive limits. This was later verified by observing a lack of effect on rhythm frequency using zero Ca\(^{2+}\) saline (Xu and Robertson, 1996). It was also suggested that the increased conduction velocity and decreased
membrane time constant could account for any changes observed in rhythm frequency with changes in temperature. The hyperpolarization of the membrane resting potential and a reduction in input resistance may be involved with compensation of temperature effects (Xu and Robertson, 1994).

HSPs, which have been previously shown to be overexpressed with the induction of thermotolerance, have been found in locusts. Whyard et al. (1986) showed that locusts, *L. migratoria*, when exposed to 45°C for 0.5 to 4.5 hours exhibit thermotolerance by surviving at temperatures of 50°C or higher, which is normally lethal. There was also a large correlation between the molecular weight of the proteins expressed in fat body after heat shock and HSPs that exist in *Drosophila* (Whyard et al., 1986). It has also been shown that *L. migratoria* overexpresses HSPs in their epidermis after HS treatment (Baldaia et al., 1987). These studies suggest that the thermotolerant effects observed in HS locusts are a result of the overexpression of HSPs. However, it was not known whether thermotolerance observed in HS locusts had consequences for their behaviour, or more specifically their flight circuitry, before or during subsequent heat stress.

An interesting question that Robertson et al. (1996) investigated was how did HS affect the rhythm frequency of the locust during changes in temperature. Similar studies to Foster and Robertson (1992) were performed on HS and control locusts in order to determine how HS affected motor rhythm output of the locust flight circuit. It was found that at an upper temperature limit, in both intact and deafferented locusts, they could no longer produce flight rhythms.
Interestingly there was a 5-6°C higher upper temperature limit for HS locusts compared with that of controls. Also, when comparing WBF and rhythm frequency with increases in temperature, HS animals showed significantly reduced thermosensitivity from that of controls (Robertson et al., 1996). It was concluded that prior HS conditioned the flight system, including the central circuitry, so that it was able to function at higher temperatures with minimal thermosensitivity. Gray and Robertson (1998) examined the effects of HS on axonal conduction. They found reduced thermosensitivity of the amplitude and conduction velocity in the stimulated fSR axon of the deafferented HS locust. These changes in thermosensitivity however, were dissimilar from those observed for flight rhythm frequency of HS locusts. Nothing is known of the effects of HS on neural physiology during heat stress. The locust flight circuit thus provides a unique and valuable opportunity to investigate this important phenomenon at the cellular level.

1.5 Hypothesis

I propose that the effects of HS demonstrated by the increased operating temperature limit with decreased thermosensitivity of the rhythm frequency in the locust is a result of changes in axonal conduction and/or synaptic transmission. To test this hypothesis, I performed a similar study to that of Robertson (1993)
and examined the effects of HS on synaptic properties in the locust flight system with increases in temperature.
CHAPTER 2  MATERIALS AND METHODS

2.1 Animals

Adult male locusts were obtained from a crowded colony maintained at the Department of Biology, Queen's University (25°C, 18:6 hours light:dark). Animals were mature and at least thirteen days past the imaginal moult when they were collected for experiments.

2.2 Heat Shock Treatment

Locusts were heat shocked in a small ventilated plastic container within a humid incubator set at 45°C for 3 hours. Control animals were also placed in a ventilated plastic container for the same duration of time at room temperature (25°C for 3 hours). Locusts were given at least one hour to recover from the treatment before each experiment and all experiments were performed within 12 hours of the heat shock treatment.
2.3 Dissection and Preparation

To make a semi-intact preparation, an animal's legs and wings were removed and a dorsal midline incision of the thorax was made. Air sacs were removed, the gut was cut posteriorly and pulled to one side and the salivary glands were removed. The thoracic ganglia were thus exposed (Figure 1) and a stainless steel plate was slid beneath them to provide stability (Robertson and Pearson, 1982). The body cavity was filled with saline at room temperature [147mM NaCl, 10mM KCl, 4mM CaCl₂, 3mM NaOH, 10mM HEPES buffer pH=6.8-8.2].

2.4 Extracellular Recording

To stimulate the forewing stretch receptor (fSR) a suction electrode was placed on mesothoracic nerve 1D/1D₂ (nomenclature of Campbell, 1961) (Figure 2). Nerve 1D₂ was cut distal to the suction electrode to eliminate spontaneous fSR activity, to control the fSR action potential frequency by stimulating it electrically, and to prevent activation of the dorsal longitudinal muscle. The fSR axon was stimulated with suprathreshold, short duration (0.01 ms) voltage pulses at 5Hz. In some experiments an additional recording suction electrode was placed at the nerve 1D/1C proximal to the stimulating electrode. The distance between the two suction electrodes was measured with fine calipers to enable calculation of axonal
Figure 1. The thoracic nervous system of *Locusta migratoria*. Nerve 1D$_2$ carries the forewing stretch receptor (fSR) axon from the periphery towards the mesothoracic ganglion (Meso). The fSR also extends into the prothoracic ganglion (Pro) through the recurrent nerve. The metathoracic (Meta) dorsal longitudinal muscle (DLM) is also indicated. The first three abdominal ganglia are fused to the metathoracic ganglion; three more abdominal ganglia can be seen toward the posterior end. Taken from Gee (1993; redrawn from Stevenson and Kutsch, 1987).
Thoracic ganglia and electrode placement. The prothoracic, mesothoracic, and metathoracic (PRO, MESO, META) were exposed in a semi-intact preparation (not shown) and intracellular recordings were taken (INT) from neuropil segments of flight interneurons. The axon of the forewing stretch receptor (fSR) was stimulated with a suction electrode (STIM) at the junction of nerves 1d1 and 1d2. fSR action potentials were recorded with a second suction electrode (SR) located proximal to the stimulating electrode.
conduction velocity. Nerves 3 and 4 of both meso- and metathoracic ganglia were cut to increase stability of the preparation (labeled in Figure 1). In some preparations all thoracic nerves were cut or crushed and the pro-meso and abdominal connectives were crushed to reduce background synaptic activity that could obscure or influence the fSR EPSP.

2.5 Intracellular Recording

Intracellular recordings were taken from the neuropil segments of flight interneurons ipsilateral to the stimulating electrode in the mesothoracic ganglion (Figure 2). Recordings were made with glass (borosilicate or sodium borosilicate) microelectrodes (~40 megohms) filled to the tip with 2M potassium acetate (KAc). The interneurons recorded have been previously identified as having monosynaptic excitatory connections from the ipsilateral and/or contralateral fSR (Reye and Pearson, 1987). The excitatory postsynaptic potentials (EPSPs) were judged to be monosynaptic using standard criteria (e.g. short constant latency) (Robertson, 1993). The neurons recorded from are all spiking interneurons and in some cases it was necessary to inject hyperpolarizing current to stop action potentials from being generated. The amount of current injected was always the minimum required to stop action potentials from forming in response to fSR EPSPs.
2.6 Temperature Manipulation

The preparation was superfused with saline which was temperature controlled with a Nichrome heating coil around an inlet pipette that led through an electrically shielded mesh from a reservoir maintained at room temperature (RT). The tip was placed on the left side of the animal, located 0.5 cm above the mesothoracic ganglion, and approximately 1 cm lateral to the ganglion. A flow rate of 0.1 ml/s ensured that the preparation was flushed with fresh saline within 10s. Saline temperature was monitored with a copper/constantan thermocouple, with an epoxy coating on the tip, (0.2 mm diameter, BAT-12, Sensortek, Clifton, NJ) located adjacent to the mesothoracic ganglion. A voltage output proportional to temperature was continuously recorded.

Temperature was increased in a ramp-like fashion in which it took approximately 3 minutes for the temperature to rise from room temperature to 50°C. Temperature was increased until the EPSP could no longer be observed; this event was classified as synaptic failure and the temperature was recorded. At failure, the heater was turned off, and if the EPSP recovered, the time taken to observe a consistent deflection following the stimulus was denoted as time to recovery.
2.7 Waveform Analysis

Electrophysiological and temperature traces were digitized on VHS video tape. They were subsequently analyzed off line using the Brainwave (Datawave Technologies, Longmont, CO) analysis software package. 25 consecutive EPSPs (approx. every 5 seconds) were averaged and I measured amplitude, time to peak, duration at ½ height, area, latency, and initial slope (Figure 3). For each averaged EPSP, the extracellular action potential was averaged in a similar fashion and I measured latency (stimulus artifact to peak of action potential) and amplitude (second peak of triphasic waveform). Conduction velocity and synaptic delay were calculated from the direct measures. Data were collected into 5°C bins increasing from room temperature. Each bin contains only one data point from each preparation to avoid pseudoreplication and is plotted as mean ± standard error. Parameters such as latency, amplitude, and area are presented relative to the value at room temperature since the initial values of these parameters differed between penetrations. For example, EPSP amplitude would differ because my penetrations were in different interneurons. Also, since the stimulating electrode was not always placed in the same spot, latency differed. Nonetheless, synaptic delay was independent of these factors.
Figure 3  EPSP parameters (25 sweeps averaged) that were measured (L - latency from the stimulus, T - rise time or time to peak, S - slope, A - amplitude, D - duration at half amplitude, and AREA – area under the curve shown as shaded area).
A set of criteria was used to exclude portions of amplitude data because most preparations were hyperpolarized (and therefore "noisy" due to current passage through a high resistance electrode), some penetrations were poor, the stimulus artifact sometimes summated on the EPSP, and at synaptic failure the penetrations usually began to depolarize giving an incorrect amplitude. Therefore, because of the last point (see above) if the amplitude was greater just before failure than it was at room temperature, it was excluded. This criterion is appropriate since it has been previously shown that amplitude of an EPSP decreases significantly with increases in temperature (Robertson, 1993; Burrows, 1989).

2.8 Statistics

The first dataset contained 8 control and 10 heat shocked animals. For these experiments I did not measure conduction velocity using a separate suction electrode. To confirm my results and to obtain values for conduction velocity in each preparation a second dataset of 12 controls and 12 heat shocked animals was collected using the second suction electrode. A preliminary dataset of 10 penetrations in 6 control animals and 20 penetrations in 11 heat shocked animals, collected the year previously showed the same qualitative effects but are not presented here. Data for statistical comparison were tested for normality
and equal variance and appropriate parametric or non-parametric tests were applied using commercial software (Sigmastat, Jandel Scientific, Corte Madera, CA). All data were found to be distributed normally and have equal variance except for conduction velocity, so a Mann-Whitney rank sum statistical test was used for this non-parametric data. Significance was assessed at $P<0.05$. 
CHAPTER 3 RESULTS

Increases in temperature from room temperature caused a reduction in amplitude and duration of the extracellular action potential of the fSR axon and a reduction in the amplitude of evoked EPSPs in flight interneurons (Figure 4).

3.1 Synaptic Failure and Recovery

For both data sets collected, the temperature of the preparations was increased until synaptic failure occurred. Heat shock increased the temperature at which the EPSPs, evoked by stimulating the fSR, failed and reduced the time to recover from failure. EPSPs in control animals failed at 38±0.4 °C (N=19) whereas heat shocked animals failed at 44.4±0.8 °C (N=16). The 5.6 °C difference (figure 5A) was found to be significant (student t-test: t=6.58, DF=33, P<0.0001).

The time it took for the EPSPs to recover after failure was 129.1±15.0s (N=11) for control animals, but only 38.0±15.7s (N=7) for heat shocked animals. The difference of 91.1s (Figure 5B) was significant between the two groups (student t-test: t=4.03, DF=16, P<0.001). For 8 control and 9 heat shocked animals, it was not possible to record the time to recovery since the penetration was lost during the recovery period.
Figure 4  Sample recordings (25 sweeps averaged) of the triphasic extracellular fSR action potential (SR) and an EPSP recorded in a flight interneuron (INT) at 20°C (upper panel) and at 35°C (lower panel). The events were evoked by the stimulus (STIM).
Figure 5 Failure and recovery time of EPSPs. Heat shock increased the temperature at which EPSPs from the fSR fail, and reduced the time to recover from failure. Asterisks indicate significant differences between control and heat shocked values. (A) EPSPs in control animals failed at 38.8±0.4°C whereas EPSPs in heat shocked animals failed at 44.4±0.8°C. (B) EPSPs took 129.1±15.0s to recover in control animals but only 38.0±15.7s in heat shocked animals.
In all the following results there is an additional temperature bin for HS preparations reflecting the fact that control preparations failed at lower temperatures.

3.2 Axonal Conduction

High temperatures that resulted in failure of synaptic transmission did not result in conduction failure of the fSR axon in either control or HS preparations.

i) Conduction Delay and Velocity

The conduction velocity of the fSR action potentials increased with increases in temperature for both control and heat shocked animals (Figure 6). The plot of control and heat shock data binned in 5 °C increments shows a significant difference between the two groups using a paired t-test on the means (t=7.13, DF=5, P=0.0008). Comparing the individual 5°C bins for control (N's control = 12, 12, 12, 12, 8, 2; in bins of increasing temperature from zero) and heat shocked animals (N's heat shocked = 12, 12, 11, 11, 10, 6, 3) showed significant differences between heat shocked and control animals at 0°C (room temperature) (Mann-Whitney rank sum test: U=200.5, DF=22, P=0.004). 0-5°C
Figure 6  Heat shock and conduction velocity. In each graph the results have been collected into bins of 5°C relative to RT (21.1±0.2°C, N=42) and are presented as means±SE (error bars may be hidden in the symbol). Asterisks indicate significant differences between control and heat shocked values. Heat shock animals exhibit conduction velocities along the fSR significantly slower than that of control preparations.
Conduction Velocity (m/s)

- Control
- Heat Shocked

Temp Above Room (°C)
(Mann-Whitney rank sum test: U=197, DF=22, P=0.007) and 5-10°C (Mann-Whitney rank sum test: U=99, DF=21, P=0.045).

**ii) Relative Action Potential Amplitude**

The relative amplitude of the fSR action potentials decreased with increasing temperatures above room temperature in control animals (Figure 7). In HS preparations however, relative amplitude increased up to 5-10°C above room temperature and then began to decrease at 10-15°C above room temperature. The relationships of relative amplitude with increasing temperature between heat shocked and control animals were significantly different using a paired t-test (t=3.42, DF=5, P=0.019). The sample sizes for control and heat shocked locusts were the same as for conduction velocity (see above). Comparison of individual 5°C bins for each group showed significant differences at 5-10°C above RT (student t-test: t=2.88, DF=21, P=0.008) and 10-15°C (student t-test: t=2.32, DF=21, P=0.003).
Figure 7  Heat shock and action potential amplitude. The relative action potential amplitude of signals evoked along the fSR are less sensitive to temperature change above room temperature in heat shocked preparations. In each graph the results have been collected into bins of 5°C relative to RT (21.1±0.2°C, N=42) and are presented as means±SE. Asterisks indicate significant differences between control and heat shocked values. Heat shock animals exhibit relative action potential amplitudes along the fSR significantly greater than that of control preparations.
3.3 Synaptic Parameters

i) Relative Slope, Relative Time to Peak, and Relative Duration at ½ Amplitude

For the relative time to peak, relative slope, and relative duration at ½ amplitude, no significant differences were found between heat shocked and control preparations.

As temperature increased, relative time to peak decreased to 60% at 20-25°C above room temperature (Figure 8A). Also, with increases in temperature, relative slope showed negligible thermosensitivity (Figure 8B). There was an exponential-like decay of relative duration at ½ amplitude with increases of temperature (Figure 8C). At 20-25°C above RT, relative duration at ½ amplitude fell to 20% of original values.

ii) Amplitude and Area

As temperature increased EPSPs in both control and heat shocked preparations showed a reduction in relative amplitude, however, heat shocked EPSPs were less thermosensitive (Figure 9A). The temperature sensitivity of relative EPSP amplitude in control and heat shocked animals (N’s control = 11, 11, 10, 9, 4; heat shocked = 13, 13, 13, 10, 7, 5) showed significantly different relationships (paired t-test: t=2.94, DF=4, P=0.04) and the individual bins in the
Figure 8  EPSP time to peak, slope and duration at 1/2 amplitude. There is no
difference in the thermosensitivity of heat shocked and control
animals (A) for relative time to peak; (B) relative slope; and (C)
relative duration at 1/2 amplitude. In each graph the results have
been collected into bins of 5°C relative to RT (21.1±0.2°C, N=42)
and are presented as means±SE.
ControI

Heat Shocked

Temp Above Room (°C)

Relative TTP

0 5 10 15 20 25 30

Temp Above Room (°C)

Relative Slope

0 5 10 15 20 25 30

Temp Above Room (°C)

Relative 1/2 Amp. Dur.

0 5 10 15 20 25 30

Temp Above Room (°C)
Figure 9  Heat shock and EPSP amplitude and area. In each graph the
results have been collected into bins of 5°C relative to RT
(21.1 ± 0.2°C, N=42) and are presented as means ± SE. Asterisks
indicate significant differences between control and heat shocked
values. (A) EPSP amplitudes could not always be measured to
failure because of the tendency of the interneuron to generate
action potentials that obscured the underlying EPSP. The
relationships are significantly different. (B) No significant
differences were found for relative area in heat shocked and control
EPSPs.
Heat Shocked
Temp Above Room (°C)

A

Relative Amplitude

- Control
- Heat Shocked

B

Relative Area

- Control
- Heat Shocked

Temp Above Room (°C)
10-15°C range were also significantly different (student t-test: t=2.29, DF=17, P=0.04).

Relative EPSP area exhibited a decay with increasing temperatures from RT (Figure 9B). There was no significant difference in thermosensitivity between heat shocked and control locusts, nor is there a difference at any 5°C bins.

**iii) Relative Latency and Synaptic Delay**

For relative synaptic latency, as temperature increased from RT there was an exponential decrease for control animals such that at 20-25°C above RT, relative latency was 50% of its original value (Figure 10A). In heat shocked animals however, at 0-5°C above RT there is no thermosensitivity of relative latency with increasing temperature up to failure (Figure 10A)(N's control = 20, 20, 20, 15, 10, 2; N's heat shocked = 22, 22, 21, 19, 13, 6, 3). The temperature sensitivity of relative synaptic latency was significantly different between heat shocked and control preparations (paired t-test: t=3.2, DF=5, P=0.024). The 5°C bins at temperatures higher than 0-5°C showed significant differences using student t-tests between control and heat shocked animals, including 5-10°C (t=2.36, DF=39, P=0.023), 10-15°C (t=2.94, DF=32, P=0.006), 15-20°C (t=3.66, DF=26, P=0.0015), and 20-25°C range (t=7.08, DF=6, P=0.0004).

The relationships between synaptic delay in control and heat shocked animals were not statistically significant due in part to the cross over of the graph (Figure 10B). The sample sizes for synaptic delay are the same as in conduction
Figure 10  Heat shock and EPSP latency and delay. In each graph the results have been collected into bins of 5°C relative to RT (21.1±0.2°C, N=42) and are presented as means±SE (error bars may be hidden in the symbol). Asterisks indicate significant differences between control and heat shocked values. (A) Temperature sensitivity of synaptic latency in control and heat shocked animals. The relationships are significantly different. (B) Temperature sensitivity of synaptic delay in control and heat shocked animals. Because the relationships cross over, a paired T-test on the means does not show significance. However, individual bins are different shown by asterisks.
A

Relative Latency

- - Control
- - Heat Shocked

B

Synaptic Delay (ms)

Temp Above Room (°C)
velocity. In control preparations, I found that increasing temperature from RT caused synaptic delay to decrease (e.g. to 20% at 20-25°C above RT). In heat shocked preparations, synaptic delay exhibited an initial decrease, parallel with controls, between RT and 5°C, however, between the 0-5°C and 5-10°C bin, synaptic delay increased 10% and became insensitive to further increases in temperature up to failure. In four of the six 5°C bins for synaptic delay, I found significant differences between heat shocked and control animals using a student t-test. More specifically, significant differences were found between the RT bins (t=3.63, DF=22, P=0.001), the 0-5°C bins (t=2.34, DF=22, P=0.03), the 15-20°C bins (t=2.47, DF=16, P=0.025), and the 20-25°C bins (t=2.72, DF=6, P=0.034).
CHAPTER 4 DISCUSSION

In the natural habitat of *Locusta migratoria*, the temperature of the terrestrial substrate can reach 80°C (Hardy, 1979). The internal temperature of these poikilotherms, when they are on the ground, has been recorded at 45°C (Uvarov, 1966). It has also been shown that internal temperatures of locusts can be 6-10°C higher in flight due to the heat produced by working muscles (Weis-Fogh, 1956, 1964). Locusts will therefore be challenged with heat stresses throughout their life, and must be able to compensate both biologically and neuronally in order to operate with these extreme internal temperatures. One way in which organisms cope with extreme thermal conditions is via a heat shock (HS) response. The HS response is the upregulation of protective molecular “chaperones” called heat shock proteins (HSPs) that prevent the denaturing and aggregation of native proteins thereby inducing thermotolerance within an organism (for review see Moseley, 1994; Marimoto *et al.*, 1994; Lindquist and Craig, 1988). The HS response is a valuable adaptive phenomenon enabling survival of animals like the locust which have little or no control over internal temperatures. In some organisms there is constitutive expression of HSPs prior to heat shock, depending on their ecological niche (Ulmasov *et al.*, 1992; Gehring and Wehner, 1995). Whyard *et al.* (1986), showed that when locusts, *L. migratoria*, were exposed to ambient temperatures of 39°C for 3 hrs (heat shocked), thermotolerance was induced with the simultaneous increased
expression of heat shock proteins (HSPs). Whereas much is known of the molecular biology of HSPs, little is known of the changes in neurophysiological function during or after heat stress in any organism. It is clear that organisms adapted to extreme environments can continue to behave and recent studies have shown that there are profound differences both in behaviour and the survival of HS locusts during extreme temperature increases compared to those of controls (Robertson et al., 1996).

Previous investigations have described the effects of HS on rhythm generation and wingbeat frequency (WBF) in locusts (Robertson et al., 1996). Increases in deafferented rhythm frequency similar to those from control animals were observed in HS animals up to 30°C. At higher temperatures HS animals showed no thermosensitivity to increases in temperature up to failure. These effects of HS on rhythm frequency cannot be explained by effects on axonal conduction since conduction velocity increases monotonically in both HS and control animals, and never shows the thermal insensitivity evident for rhythm frequency and wingbeat frequency (Gray and Robertson, 1998). A possible explanation for the reduced thermosensitivity observed for rhythm frequency and WBF is that synaptic transmission within the locust flight circuit is protected.

In this study, I investigated parameters of synaptic transmission in locust flight circuitry to determine whether transmission is protected by HS treatment. Briefly, I found that (1) HS locusts maintain synaptic transmission at temperatures 5.6°C higher than controls with a significantly shorter recovery
period after failure. (2) Conduction velocity and action potential amplitude of the forewing stretch receptor axon (fSR) in HS animals showed similar changes in thermosensitivity with increasing temperature as the results of Gray and Robertson (1998), and (3) significant differences were found between the thermosensitivities of EPSP amplitude, and synaptic delay of HS and control locusts.

An important question is whether HS may have had damaging effects in my experiments. For several reasons I believe that HS did not damage the locusts. (1) After treatment they looked healthy. Their colour was similar to that of controls and their appendages (e.g. legs and antennae) were fully operational. (2) They also behaved normally being highly active and exhibited normal mating behaviour. (3) The locusts were able to fly in a wind tunnel tethered to a stick with wax. Moreover they were rendered thermotolerant by the HS treatment.

HS has two interesting and arguably adaptive effects on rhythm generation in the flight system. First, the rhythm generation in deafferented HS locusts fails 5 to 6°C higher than that of controls (Robertson et al., 1996). Second, with increasing temperature, HS locusts show increased rhythm frequency similar to that of controls up to 30°C; however above this, HS locusts, in contrast to controls, exhibit no thermosensitivity to the increasing temperature up to failure (Robertson et al., 1996). My results can help to explain these differences in rhythm frequency exhibited by HS animals at a synaptic level and how HS may be protecting circuit function.
4.1 Failure

Rhythmical circuits operate in two basic ways, either via endogenous mechanisms of membrane oscillators or as a result of synaptic interaction in a circuit (for review see Marder and Calabrese, 1996). Reversible failure of an endogenous oscillator neuron at high temperatures is shown by the R15 bursting neuron in Aplysia, which fails at 35°C (Fletcher and Ram, 1991). The locust flight circuit however, operates by way of a network mechanism dependent on synaptic transmission between neurons in the pattern generator (Robertson and Pearson, 1985; for review see Robertson, 1991). Even though plateau potentials contributing to oscillation within the locust flight circuit have been described, it has also been shown that synaptic connections are essential for these potentials to be activated (Ramirez and Pearson, 1991). Failure of such a network oscillator would occur if either axonal conduction or synaptic transmission failed. My results suggest the latter. First, throughout my experiments there were no observations of conduction failure along the fSR axon in control or HS animals, even at temperatures in excess of 55°C. Conduction failure has been previously predicted to occur in the squid giant axon at approximately 44.5°C (Hodgkin and Katz, 1949) and conduction failure of an afferent from the cockroach tactile spine has been shown to occur at 35°C (French, 1985). It may be that temperature of
Conduction failure in the locust was higher because it is adapted to semi-arid conditions. Second, the wingbeat frequency (WBF) and the flight motor rhythm in intact and deafferented locusts have both shown a 5-6°C increase in the upper temperature limit for operation in HS animals before failing (Robertson et al., 1996). These findings are strikingly similar to synaptic transmission in HS animals, which fails 5.6°C higher than in control animals. Given the nature of the flight circuit, failure of synaptic transmission would result in failure of rhythm generation.

These EPSPs recovered after the temperature was reduced from the failure temperature. In comparing the recovery times between HS and control animals, the amount of time required for synaptic transmission to recover after failure was 129±15.0 seconds for control animals, and 38±15.7 seconds for HS (less than a third of the control value). The faster recovery exhibited by HS locusts would allow for improved survival and suggests an adaptive response to heat stress in its environment.

4.2 Thermosensitivity

The thermosensitivity of WBF and rhythm generation is relatively mild (Foster and Robertson, 1992) and current hypotheses suggest that thermosensitivity is mediated by effects on communication delays around the
flight circuit (Xu and Robertson, 1996). Since direct manipulation of EPSP amplitude using zero Ca\(^{2+}\) saline has no significant effect on frequency of rhythms expressed by the flight circuit, any changes in EPSP amplitude due to temperature would not affect the flight rhythm frequency of the locust (Xu and Robertson, 1996). Communication delays in a circuit are determined by conduction velocity and synaptic delays. Conduction velocity represents the speed at which neuronal signals travel along the axon, whereas synaptic delay represents the time it takes for synaptic transmission to occur between the pre- and postsynaptic membrane (for review see Sudhoff, 1995). This is the time taken for transmission after arrival of the action potential at the terminals and includes presynaptic calcium entry, vesicle release, transmitter diffusion and postsynaptic receptor activation. It is unlikely that conduction velocity plays a role in the reduced thermosensitivity and failure of the rhythm frequency of HS locusts. Both Gray et al. (1998) and I showed a lack of direct correlation between conduction velocity and rhythm frequency with increasing temperature. The conduction velocity of HS locusts was slower than that of controls and there was a trend for conduction velocity to increase up to synaptic failure. However, as previously described, rhythm frequency in HS animals is the same as in controls at room temperature and increases up to 30°C. However, at higher temperatures it plateaus becoming thermally insensitive up to failure (Robertson et al., 1996).

It is also unlikely that EPSP slope, TTP, or duration at ½ amplitude were involved with the effects of HS on rhythm frequency and WBF since they all
showed no significant differences between HS and control animals. Robertson (1993) has suggested that the increase in the EPSP slope with increasing temperature is primarily due to an increased channel opening rate (kinetics) and/or a decreased membrane time constant caused by reduced membrane resistance (e.g. Lev-Tov et al., 1983). He also suggested that TTP and duration at ½ amplitude would be influenced by the same passive properties of the membrane and by similar ion channel kinetics. Here I confirm findings of Robertson (1993), who showed exponential decreases in time to peak (TTP) and duration at ½ amplitude. I found no differences in the thermosensitivities of these synaptic parameters in HS locusts compared with controls. This suggests that channel kinetics and the passive properties of the membrane are not responsible for the reduced thermosensitivity and thermotolerant effects observed in HS locusts. However, my results with synaptic delay can provide an explanation.

It has been shown that conduction velocity becomes faster and synaptic delay shortens with increases in temperature (Burrows, 1989; Robertson, 1993). In HS locusts however, relative EPSP latency exhibits almost no thermosensitivity with increases in temperature higher than 5°C above room temperature. This suggests a stronger effect of HS on synaptic delay compared with the effect on latency and motivated the collection of the second data set with which conduction delays could be calculated (synaptic delay = latency - conduction delay). For control animals the synaptic delay decayed exponentially and confirmed studies done by Burrows (1989) who showed a 50% reduction by
35°C (approx. 15°C above room temp). However, for HS locusts, synaptic delay was found to be 0.19 ms shorter at room temperature and the effect of increased temperature paralleled the reduction in synaptic delay for control animals up to 5°C above room temperature. At this point synaptic delay abruptly increased from 0.73±0.07ms to 0.85±0.10ms and then leveled off showing no thermosensitivity from 10°C above room temperature to failure.

The thermosensitivity of synaptic delay I have described is remarkably similar to the thermosensitivity of the rhythm in deafferented locusts. For both rhythm period (1/frequency) and synaptic delay, HS introduces a discontinuity in their relationships with temperature whereby they are insensitive to temperatures above 30°C (10°C above room). I propose that the reduced thermosensitivity in rhythm generation, and consequently WBF, is caused by the reduced thermosensitivity observed in synaptic delay in the motor circuitry of the locust.

4.3 Underlying Mechanisms

It is difficult to come to any conclusion about the cellular mechanisms underlying the effects of HS on failure and thermosensitivity. One reason is because temperature affects most cellular processes and my experiments were not designed to address the cellular mechanisms for the effects. Another is that this is the first description of HS affecting neural function and thus there are no previous studies for comparison. Nevertheless, I have
shown that HS affects conduction velocity, fSR amplitude, EPSP amplitude, and synaptic delay. Therefore some brief discussion on possible mechanisms is warranted.

It is possible to calculate the magnitude of parameters of intracellular action potentials (e.g. amplitude) from a good extracellular recording (Pearson et al., 1970). In my experiments the large diameter of the fSR axon resulted in excellent recordings. Thus, the reductions in extracellular action potential parameters I observed are directly correlated with intracellular parameters. A reduction in fSR action potential amplitude with increases in temperature, would cause reductions in evoked EPSP amplitudes. This is because reduced action potential amplitude causes reduced intracellular Ca\(^{2+}\) increases in the presynaptic terminal and the concentration of Ca\(^{2+}\) determines the amount of transmitter released (Burrows, 1989; Janssen, 1992; Robertson, 1993).

However, this could be directly opposed by presynaptic calcium flux, which can be increased with rises in temperature and thereby increase transmitter release (Charlton and Atwood, 1979). The existence of these opposing forces indicates that synaptic failure may not be from the reduction in amplitude of fSR action potentials. HS animals may have protective effects on the Ca\(^{2+}\) channels thus increasing the upper temperature limit of this automatic compensatory mechanism.

I found that relative EPSP amplitude in control animals decreased with increasing heat above room temperature. HS animals showed a significant
difference with reduced thermosensitivity of amplitude as temperature increased compared with control animals. The relative EPSP amplitude in HS animals is always greater than that of controls by approx. 15% above room temperature. Changes in EPSP amplitude, of the same magnitude, would not affect rhythm frequency as has been previously shown in deafferented animals (Xu and Robertson, 1996) and therefore they would not contribute to the changes in thermosensitivity observed in the rhythm frequency in HS locusts (Robertson et al., 1996). Relative EPSP amplitudes evoked by action potentials from the fSR have previously shown decreases in size above and below room temperature (Robertson, 1993). Several reasons have been given to explain why amplitude is affected by temperature.

In the pre- and postsynaptic membrane, suggested mechanisms include: (1) increases in ion channel conductances with increasing temperatures (Correa et al., 1992; Edman and Grampp, 1991; Hille, 1992); (2) increases in channel opening and closing kinetics (Edman and Grampp, 1991; Hille, 1992); (3) decreases in membrane resistance (Burrows, 1989; Janssen, 1992); and (4) kinetic or conformational changes in synaptic machinery.

Relative EPSP amplitudes from HS and control animals show significant differences between their thermosensitivities, whereas time to peak, duration at ½ amplitude, and slope which mainly represent changes in ion channel kinetics and the reduction of membrane resistance (Robertson, 1993) do not. I suggest that ion channel kinetics and the reduction of membrane resistance therefore
contribute minimally to the reduced thermosensitivity observed in HS EPSP amplitude with increasing temperatures. Therefore, the differences observed in HS animals for EPSP amplitude are most likely caused by changes in ion channel conductances and their effects on synaptic transmission.

Relative EPSP area for both HS and control animals showed no significant differences with increasing temperatures. This was unexpected since the main components of EPSP area are most likely to be amplitude which showed significant differences between HS and controls, and duration which did not. An observable trend in the reduced thermosensitivity of relative area can be seen below 15°C (above room temp) which is similar to that of relative EPSP amplitude. Conceivably future experiments with a large sample size would be able to establish a difference.

The difference in synaptic delay after HS, observed at room temperature suggests synaptic delay may be able to resolve the discrepancy with differences in conduction velocity between HS and control animals which show no differences in rhythm frequency (Robertson et al., 1996). Conduction velocity is slower in HS animals but synaptic delay is shorter. Thus effects on synaptic delay may contribute to maintaining rhythm frequency in HS animals.
4.4 Conclusion and Speculation

Since these studies did not address whether the reduced thermosensitivity observed in HS synaptic delay was pre- or postsynaptic, it is difficult to determine what mechanism may underlie this phenomenon. An interesting hypothesis is that for such a rapid response to occur then the site of the observed effect is likely to be close to the cell body since HSPs are produced in the nuclei (for review see Lindquist and Craig, 1988). The postsynaptic membrane may therefore be a candidate for the location of the initial effects of HS (H.L. Atwood and R.M. Robertson, personal communication). Nonetheless, the reduced thermosensitivity observed in synaptic delay indicates that presynaptic mechanisms may be affected by HS. Also, the reduction in thermosensitivity of EPSP amplitude in HS animals may be a result of presynaptic Ca$^{2+}$ protection (see above) which may thereby increase the upper temperature limit of synaptic transmission.

Several suggested roles of HSP70, an HSP which is expressed in Locusta migratoria (Whyard et al., 1986), indicate it could be involved in interactions within the synaptic machinery responsible for neurotransmission in the presynaptic membrane. For example, cytosolic members of HSP70 were discovered using various biological assays. Bovine clathrin uncoating ATPase, which promotes the disassembling of clathrin lattices during neurotransmitter re-uptake, is a member of the HSP70 family (Schlossman et al., 1984; Chappell et
Cysteine string proteins (CSPs), which are known to interact with HSP70, have been shown to regulate Ca$^{2+}$-dependent secretion of neurotransmitter at *Drosophila* neuromuscular junctions (Buchner and Gundersen, 1997). This relationship between CSPs and Ca$^{2+}$ channels shows that CSPs participate in a regulatory interaction involving presynaptic Ca$^{2+}$ channels at elevated temperatures. In CSP null mutants, HSP70 (known to have an affinity with CSPs J domain (Caplan *et al*., 1993)), may not be able to interact with Ca$^{2+}$ channels to prevent denaturing of the ion channel complex with increases in temperature. Unfortunately, in my study, the link between HS induced thermotolerance and the expression of HSPs is hypothetical. I propose that the effect of HS is caused by the increased expression of HSPs shown by previous studies with animals from the Queen's Biology colony (Whyard *et al*., 1986). However, to verify this, I recommend that a similar investigation on synaptic transmission be performed on the larval neuromuscular junction of *Drosophila* which is amenable to modern molecular genetic techniques (Karunanithi *et al*., 1997). With this preparation it would be possible to study genetically engineered flies expressing varying levels of different HSPs (Feder *et al*., 1996).

The ideas presented for the mechanisms involved in both thermotolerance and reduced thermosensitivity in synaptic transmission involve both the pre- and postsynaptic membrane, and therefore suggest the hypothesis that HSPs stabilize the protein machinery underlying synaptic transmission. It will be
important to use quantal analysis of synaptic transmission in HS and control locusts in order to determine whether the effect is pre- or postsynaptic.

The reversibility of synaptic failure in the locust demonstrates that a conformational change is most likely taking place and is reversible over a period of time. Time to recovery was faster in HS locusts suggesting that proteins may be more stabilized or "chaperoned" by the induction of HSPs (Mailhos et al., 1993).

To my knowledge, this is the first evidence that HS protects neuronal function at the level of synaptic transmission undergoing temperature stresses. I therefore conclude that heat shock is a probable consequence of the locust’s environment and in its natural habitat it is most likely expressing HSPs constitutively. The HSPs could condition synaptic transmission within the flight circuitry to operate at high temperatures with minimal temperature sensitivity as shown in this study. I believe that this neural ecological phenomenon would be conserved for most poikilotherms.

For poikilotherms, HS is beneficial since their internal temperature is susceptible to changes in the environment. However, homeotherms, like mammals, have the ability to regulate and maintain their internal temperatures. Interestingly, there have been reports that mammals express HSPs and there have been suggestions that medical applications through the use of these HSPs may be possible for the protection of different tissues under stress. An example of this is that HSPs can protect mammalian nervous tissue from ischemia, a
consequence of stroke (Fink et al., 1997). I believe my study has a large potential impact on the scientific community. As previously mentioned, this is the only study to date which has examined the effects of heat shock on synaptic transmission. Moreover, information obtained from this study shows us that changes in HS locusts' behaviour and survival at extreme temperatures are probably the result of changes in its flight circuitry due to changes in synaptic transmission.


Kitagawa, K., Matsumoto, M., Tagaya, M., Hata, R., Ueda, H., Niinobe, M.,
Ischemic tolerance phenomenon found in the brain. Brain Res. 528:21-28.

Press. Princeton, N.J.


1191.

release coupling in squid giant synapse. In: *Molecular Mechanisms of
Neuronal Responsiveness*, Ed: Y.H. Ehrlich, R.H. Lenox, E. Kornecki, and


IMAGE EVALUATION TEST TARGET (QA-3)