Enhancement of Short-Term Synaptic Plasticity by Prior Environmental Stress

J. W. BARCLAY AND R. M. ROBERTSON
Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

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Barclay, J. W. and R. M. Robertson. Enhancement of short-term synaptic plasticity by prior environmental stress. *J Neurophysiol* 85: 1332–1335, 2001. All chemical synapses can rapidly up- or down-regulate the strength of their connections to reshape the postsynaptic signal, thereby stressing the informational importance of specific neural pathways. It is also true that an organism's environment can exert a powerful influence on all aspects of neural circuitry. We investigated the effect of a prior high-temperature stress on the short-term plasticity of a neuromuscular synapse in the hindleg tibial extensor muscle of *Locusta migratoria*. We found that the prior stress acted to precondition the synapse by increasing the upper temperature limit for synaptic transmission during a subsequent stressful exposure. As well, preexposure to a stressful high-temperature environment increased short-term facilitation of excitatory junction potentials concurrent with a decrease in excitatory junction potential amplitude and a reduction in its temporal parameters. We conclude that a stressful environment can modify synaptic physiological properties resulting in an enhancement of short-term plasticity of the synapse.

INTRODUCTION

Synaptic facilitation, a short-term strengthening of synaptic connections, plays an integral role in the functional reorganization of synaptic information processing and provides important physiological insight into learning-related synaptic modulation (Byrne and Kandel 1996; Davis and Murphey 1994; Dittman et al. 2000; Fisher et al. 1997; Zucker 1989, 1999). Stressful environmental conditions can influence and detrimentally affect synaptic transmission through modulation of ionic diffusion and flux within the active zone and structural stability of proteins intrinsic to the functional integrity of the synapse (Hu et al. 2000; Le Corronc et al. 1999; Robertson 1993). Within a natural ecology, stressful conditions come and go, and the prior history of an organism's environment can alter how neural circuitry operates in the long term (Barclay and Robertson 2000; Karunanithi et al. 1999; Robertson et al. 1996).

The locust provides an excellent in vivo model system for examining the effect of a prior environmental stress on synaptic facilitation and, more generally, the properties of synaptic transmission. An extended exposure to elevated temperatures (45°C for 3 h) is a potent environmental stress, inducing natural cellular stress responses (Whyard et al. 1986) and mimicking ecologically relevant conditions experienced by the organism (Cloudsley-Thompson 1975; Uvarov 1966). Here, we show that prior exposure to a stressful condition acts to enhance short-term synaptic facilitation and increase the limit for functional survival during repeated exposure to the environmental stress. This enhancement in plasticity is concurrent with a decrease in all other synaptic parameters (excitatory junction potential latency, rise time, duration and amplitude), regardless of the subsequent temperature stress.

METHODS

Experimental preparation

Mature locusts *Locusta migratoria* (at least 3 wk past the imaginal molt) were obtained from a crowded colony (25–30°C; 16 h:8 h light:dark photoperiod) maintained at Queen's University. The experimental setup was as previously described (Barclay and Robertson 2000). The axon of the slow extensor tibia (SETi) motoneuron, originating in the metathoracic ganglion and innervating the tibial extensor muscle in the locust hindleg, was stimulated with bursts of just-suprathreshold square voltage pulses (0.3 ms duration) at 40 Hz (burst frequency = 0.1 Hz; burst duration = 250 ms) using a suction electrode placed on the severed end of nerve 3 within the thorax. The stimulation protocol was chosen to ensure adequate facilitation reliably within the physiological range of SETi firing frequency (Burrows 1996). Intracellular recordings were made using glass microelectrodes (filled with 2 mol/l potassium acetate; resistance, 60–80 MΩ). Prior environmental stress was experimentally induced by exposing locusts to heat-shock (HS) conditions (45°C for 3 h). These conditions are known to be stressful to locusts, rapidly inducing up-regulation of heat shock proteins (Whyard et al. 1986). Control locusts were kept in a similar environment for the same period of time at room temperature (20–25°C). Experiments were performed within 1–3 h following the HS treatment.

Procedure and analysis

Excitatory junction potentials (EJPs) were recorded from SETi-innervated muscle fibers at the proximal end of the hindleg, located just distally to the "fan" region of muscle fibers (Hoyle 1978). This cluster of fibers is innervated by SETi and the common inhibitor (CI) motoneuron. However, in our recordings, there was no evidence that the CI was recruited by varying the stimulus strength. Recordings were made initially at room temperature (20–25°C) and then continuously as the saline temperature was gradually increased (approximately 5°/min) until complete failure of synaptic transmission. Intracellular recordings at their corresponding temperature were digitized and recorded onto VHS videotape for analysis using Brainwave analysis software (Datawave Technologies, Longmont, CO). In each

Address reprint requests to J. W. Barclay (E-mail: barclayj@biology.queensu.ca).

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animal, EJPs were analyzed for amplitude (measured from baseline to the peak of the 1st event), duration (event measured at half-maximal amplitude), rise time (measured from the beginning to the event peak), and latency (measured from the stimulus artifact to the event onset). An EJP facilitation index was calculated as the ratio of the amplitudes of the first and last EJP within each stimulus train. For each parameter, the results from one fiber per animal were averaged in 5°C bins such that each animal contributed only one value per temperature bin. The dataset consisted of nine control and seven HS animals. Significance ($P < 0.05$) between control and HS animals was assessed with two-way ANOVA and unpaired *t*-test where applicable. All values are reported as means ± SE.

RESULTS

EJPs in the locust hindleg extensor muscle were recorded intracellularly as temperature was continually increased, eliciting consistent changes to the individual parameters of the EJP in both control and previously stressed (HS) animals. As temperature increased, there was a decrease in all temporal parameters (EJP latency, rise time, and duration) and an increase in EJP amplitude (Fig. 1*A*). With an increase in temperature, the extent of EJP facilitation also decreased (Fig. 1*A*). The temperature at which synaptic transmission failed was increased by 3.9°C in animals exposed to prior HS stress (Fig. 1*B*). The improvement in upper temperature limit from 46.6 ± 1.5°C in controls to 50.5 ± 0.9°C in HS animals was found to be significant (*t*-test, $t = 2.25$, d.f. = 15, $P = 0.04$). Resting membrane potential was monitored throughout the experiment and was not significantly different between control and HS animals.

Individual EJP parameters were altered in HS animals. Prior exposure to HS stress caused a reduction in EJP latency (Fig. 2*A*), rise time (Fig. 2*B*), and duration (Fig. 2*C*). Using two-way ANOVA assessment, this effect was found to be significant for both latency ($F = 23.45$, d.f. = 1, $P < 0.01$) and rise time ($F = 5.82$, d.f. = 1, $P = 0.02$). Although HS stress did not have a significant effect on EJP duration ($F = 3.514$, d.f. = 1, $P = 0.07$), the observable trend was similar to that for latency and rise time. An increase in temperature decreased all temporal parameters (2-way ANOVA, $P < 0.01$); however, there were no significant differences in the effect of temperature between control and HS animals (2-way ANOVA, $P > 0.80$).

Prior HS stress also affected the amplitude of the EJP (Fig. 3*A*), which was reduced by 63% at room temperature (20–25°C). This effect of HS was found to be significant (2-way ANOVA, $F = 33.67$, d.f. = 1, $P < 0.01$). Temperature also had a significant effect (2-way ANOVA, $F = 2.58$, d.f. = 6, $P = 0.03$), increasing EJP amplitude with an increase in temperature. The effect of temperature on EJP amplitude was not found to be different between control and HS animals (2-way ANOVA, $F = 0.79$, d.f. = 6, $P = 0.58$). This dampening effect of HS on EJP amplitude was evident for each response to the stimulus train (data not shown). Although prior HS stress reduced EJP amplitude and all temporal parameters, it increased EJP facilitation (Fig. 3*B*). In HS animals, there was significantly increased synaptic facilitation by a factor of 1.85 at room temperature (2-way ANOVA, $F = 21.57$, d.f. = 1, $P < 0.01$). An increase in temperature significantly reduced the amount of facilitation (2-way ANOVA, $F = 3.14$, d.f. = 6, $P < 0.01$); however, temperature did not affect control and HS animals differently (2-way ANOVA, $F = 0.44$, d.f. = 6, $P = 0.69$).

DISCUSSION

Increases in temperature can have deleterious effects on synaptic transmission, which is of considerable importance to poikilothermic animals. Whereas prior exposure to heat stress is known to reduce the thermosensitivity of synaptic transmission (Barclay and Robertson 2000; Dawson-Scully and Robertson 1998; Karunanithi et al. 1999), in this paper, we have demonstrated that following stressful environmental conditions, neuromuscular transmission at the locust SETi synapse is altered (temporal parameters and EJP amplitude are reduced) but that the thermosensitivity of transmission is unaffected. It is thus becoming increasingly apparent that exposure to prior HS can exert strikingly different effects at different synapses. Previous work has shown that relative EJP (Barclay and Robertson 2000; Dawson-Scully and Robertson 1998) and excitatory junctional current (EJC) (Karunanithi et al. 1999) amplitudes decrease with increasing temperature and that this decrease is mitigated by prior HS. The opposite result occurs at the SETi synapse; EJP amplitude increases with temperature, which is similar to that seen for mEJC amplitudes in *Drosoph-*

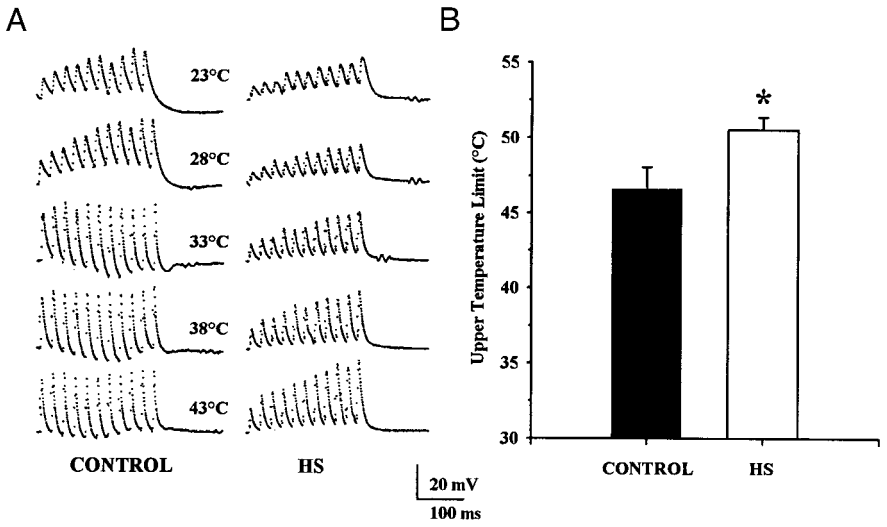

FIG. 1. Synaptic transmission is altered in animals exposed to a prior environmental stress, induced by exposure to heat shock (HS) conditions. Excitatory junction potentials (EJPs) were evoked via extracellular stimulation of the slow extensor tibia (SETi) motoneuron. *A*: representative EJP traces recorded from hindleg extensor muscle fibers of the locust at indicated temperatures. *B*: the upper temperature limit for synaptic transmission was significantly (*, $P < 0.05$) increased in HS animals. Reported values are means ± SE.

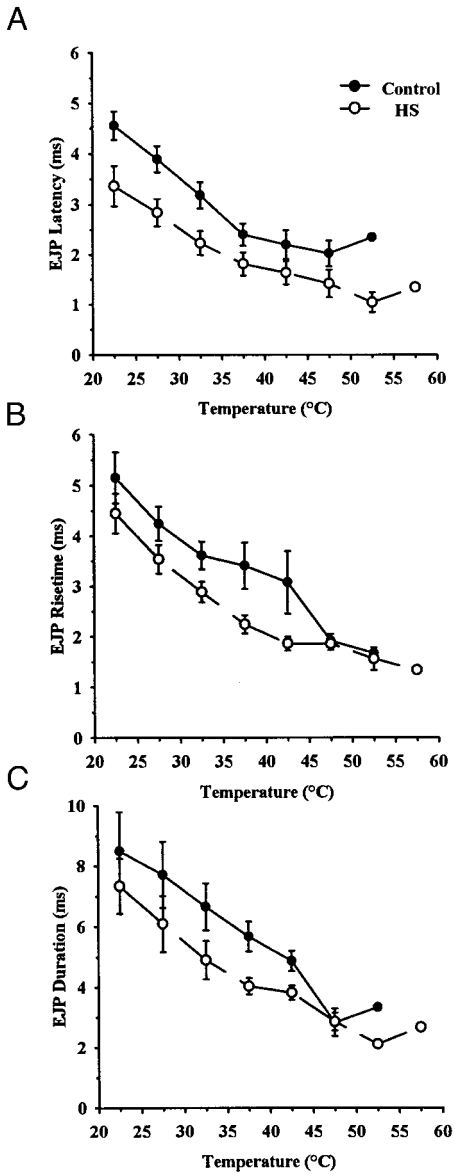

FIG. 2. Preexposure to an environmental stress (HS) decreases temporal parameters of EJPs. *A*: EJP latency was significantly reduced in HS preparations in comparison to controls. *B*: EJP rise time was significantly reduced in HS preparations. *C*: there was a nonsignificant trend for a reduction in EJP duration in HS preparations. Values are means ± SE.

ila (Karunanithi et al. 1999). Furthermore the reduction of absolute EJP amplitudes at the SETi synapse in HS animals is not evident at other synapses. This reduction in EJP amplitude could be due to a stress-induced alteration to postsynaptic input resistance or resting membrane potential. While both control and HS animals had similar membrane potentials, a difference in input resistance following HS cannot be ruled out. However, at *Drosophila* neuromuscular junctions, HS had no significant effect on either membrane potential or input resistance (S. Karunanithi and J. W. Barclay, unpublished observations). The reductions in EJP rise time and duration at the SETi synapse in HS animals are also in contrast with previous work describing no such effect in *Drosophila* (Karunanithi et al. 1999) and at the FETi synapse in the locust hindleg (Barclay and Robertson 2000). The effects of HS on latency are even more varied, ranging from no effect whatsoever (Barclay and Robertson 2000; Karunanithi et al. 1999) to an increase in latency (Dawson-Scully and Robertson 1998) and finally to our reported decrease in latency at the SETi synapse. Although our measure for latency does not differentiate between axonal conduction velocity or synaptic delay, previous work in the locust has indicated that HS has the opposite effect, slowing both conduction velocity (Gray and Robertson 1998) and synaptic delay (Dawson-Scully and Robertson 1998). The varied effects of heat stress at different synapses may indicate multiple pathways for HS alteration to synaptic performance or a single mechanism acting on different cellular targets depending on the synapse.

The ability of a synapse to regulate the relative strength of individual connections is absolutely critical for modulation of neural circuitry. During periods of stress, synaptic plasticity could become even more consequential by rapidly and appropriately modifying the postsynaptic message as environmental conditions change. It is therefore interesting that the preexposure of the animal to stressful conditions also increased the short-term plasticity of the neuromuscular synapse regardless of the subsequent temperature stress. Enhancement of synaptic facilitation most likely indicates an increase in presynaptic residual calcium levels during repetitive synaptic transmission (Dittman et al. 2000; Kamiya and Zucker 1994). Short-term facilitation could be achieved by axonal spike broadening increasing calcium influx, such as is seen in serotonin-induced short-term facilitation in the locust (Parker 1995) and in *Aplysia* (Byrne and Kandel 1996). It is unknown whether the effects

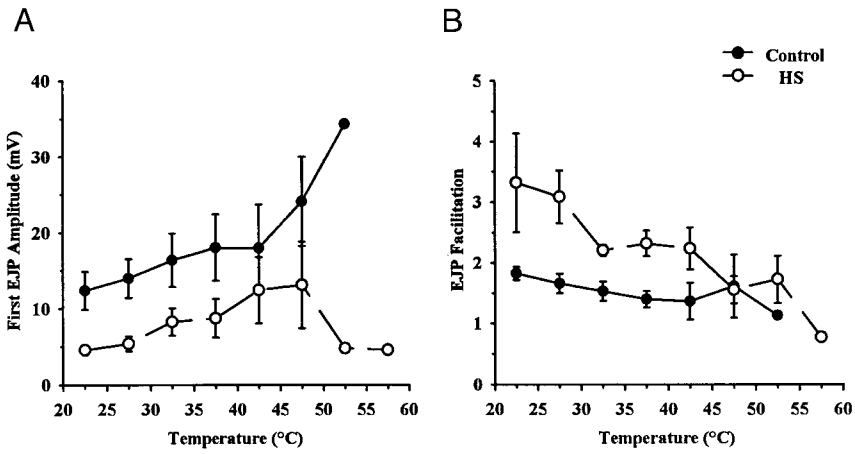

FIG. 3. Short-term synaptic plasticity is enhanced by prior exposure to environmental stress (HS). *A*: EJP amplitude was significantly reduced in HS preparations compared with controls. *B*: short-term EJP facilitation was significantly increased in HS preparations. Values are means ± SE.

seen here are achieved via a stress-activated release of neuromodulators, such as serotonin, that are known to enhance facilitation via cAMP pathways (Chen and Regehr 1997; Sugita et al. 1997). However, previous studies have linked a prior exposure to environmental stress with alterations to potassium channel dynamics and spike broadening (Nicol et al. 1997; Ramirez et al. 1999; Saad and Hahn 1992). Alternatively, the enhancement of synaptic facilitation could occur through spike broadening-independent effects on the synaptic machinery for transmitter release. This could be achieved via an upregulation in heat shock protein synthesis (Parsell and Lindquist 1993), protecting proteins integral to short-term facilitation, or by enhancing the persistent action of residual calcium within the presynaptic nerve terminal. It also cannot be ruled out that the effects of HS to alter the plasticity of the SETi synapse could be the result of chronic activity of the intact SETi motor axon activated during the heat stress. For example, similar changes to synaptic function (depression of EJP amplitude, potentiated EJP amplitude at higher frequencies) were found following long-term tonic stimulation of crayfish muscle fibers (Lnenicka and Atwood 1985; Mercier and Atwood 1989). An increase in neural activity during the 3-h HS period could induce a substantial modification to the performance of the synapse. Although much remains to be determined, it is an important and novel result that the short-term ability of a synapse to reorganize its connective strength is enhanced following a period of environmental stress.

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