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Research report

Neural parameters contributing to temperature compensation in the flight CPG of the locust, *Locusta migratoria*

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Abstract

Elevated thoracic temperature increases the wingbeat frequency of flying locusts. We investigated the extent to which temperature-induced changes in resting membrane potential and postsynaptic potential amplitude contribute to the effects of increased temperature on the frequency of the central flight rhythm. Flight neurons were hyperpolarized by changing the K^+ concentration of the superfusing saline from 10 mM to 2 mM. 5 min of low-K^+ superfusion hyperpolarized flight motoneurons from −42.8 mV to −50.1 mV with a concomitant decrease of the frequency of the central flight rhythm from 11.6 Hz to 10.5 Hz. The amplitude of postsynaptic potentials was halved after 10 min of zero Ca^{2+}/high Mg^{2+} superfusion, but the frequency of the central rhythm did not change significantly. GABAergic inhibitory connections were reduced in amplitude using picrotoxin. This treatment increased the frequency of the central rhythm from 11.6 Hz to 12.9 Hz, and increased the thermosensitivity of the rhythm frequency. We conclude that the excitatory effect of increased temperature on rhythm frequency is not mediated by temperature effects on membrane potential and/or synaptic potential amplitude. We propose that the inhibitory effect of temperature-induced hyperpolarization of the membrane potential compensates for the excitatory effect of temperature on rhythm frequency (e.g. via increased conduction velocity). We further suggest that some measure of temperature compensation is afforded by equal effects on the amplitudes of excitatory and inhibitory postsynaptic potentials, such that the net effect on the level of excitation is zero.

Keywords: Insect; Locust; Flight; Motor pattern; Temperature; Membrane potential; PSP amplitude

1. Introduction

Ambient temperature can have considerable impact on the operation of neuronal circuits and the form and existence of subsequent rhythmic behaviors, particularly in poikilotherms (e.g. heartbeat frequency in leeches [2], teleost and ascidian swimming [3], pyloric contractions in lobsters [16,17]). Therefore, a complete understanding of the adaptive operation of neuronal circuits is not possible without also considering the impact of the thermal regime of the organism under investigation.

The natural habitat of the African migratory locust, *Locusta migratoria*, is characterized by variable and high temperatures [45]. Although air density decreases at higher temperatures, many of the aerodynamic requirements of a flight system remain the same at different temperatures. A compelling question is therefore how the neuronal circuits controlling kinematic parameters of the wingbeat maintain appropriate output in spite of a wide range of operating temperatures. In the laboratory it has been demonstrated that the locust flight system is capable of operating in the range from 24°C to 42°C [24,46]. One parameter of the wingbeat, wingbeat frequency, changes minimally with ambient temperature in locusts [46] (0.27 Hz/°C, [9]) and one implication of this finding is that compensatory mechanisms exist within the flight system of locusts to stabilize the output frequency.

Flight motor patterns of locusts are generated by neuronal circuits that include peripheral as well as central components [25,31,47]. A number of flight interneurons have been identified (reviewed in [31,32]) and some have been shown to support plateau potentials which may contribute to rhythm generation in the system [27–29]. A simple circuit of flight interneurons which could account for several main features of the central motor pattern has been described [37], and recently a computer simulation

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has demonstrated that this circuit is capable of generating robust flight rhythms [11].

Temperature has profound effects on neuronal properties in the locust nervous system [1,5,14,21,22,26,40]. The effects of temperature on some cellular, synaptic and circuit properties of locust flight neurons have also been described [33,48]. Amongst other things, it has been proposed that: (1) hyperpolarization of the resting membrane potential is involved in automatic temperature compensation at the cellular level [48], and (2) the frequency of the rhythm is unaffected by the effects of temperature on the amplitude of postsynaptic potentials within permissive limits [33]. The main goal of the experiments described here was to test these ideas by mimicking particular changes in neural properties wrought by temperature, and assessing the effect on flight rhythm generation. Also, Miles [22] has suggested that temperature compensation in the afferent pathway from wind-sensitive head hairs of the grasshopper may be a result of equal effects of temperature on concurrent excitatory and inhibitory inputs. Effects of temperature on the different inputs may thus cancel each other out. We tested this idea in the flight circuitry by pharmacologically changing the relative strengths of excitatory and inhibitory synaptic connections.

The specific objectives of this study were: (1) to determine the influence of resting membrane potential (RMP) on the central flight rhythm by manipulating membrane potentials with reduced extracellular K^+ (e.g. [42]); (2) to study the effect of postsynaptic potential (PSP) amplitude on the central flight rhythm by manipulating PSP amplitude with reduced extracellular Ca^{2+} (e.g. [4]); and (3) to examine the response of the central flight rhythm to changes in the relative strength of excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) in the central circuitry by manipulating IPSP amplitude with picrotoxin (e.g. [37]).

2. Materials and methods

Adult male locusts (*Locusta migratoria*) were obtained from a colony maintained in the Department of Biology, Queen's University. Only mature animals (> 10 days after the imaginal moult) were used. Colony conditions were 31 ± 1°C, with a 16:8 h light/dark cycle.

2.1. Preparations

We used a standard preparation of the deafferented locust flight system [34]. The wings and legs were cut off and the locust was pinned to a cork board. The thoracic nervous system was exposed by a dorsal dissection that removed the gut and overlying tissue. Nerves 3, 4, and 5 of the mesothoracic and metathoracic ganglia [7] were severed. These two ganglia were supported on a stainless steel platform. The preparation was initially superfused with locust saline which could be replaced with ion-substitution or drug solutions. The temperature of the solutions was controlled with a heating coil around a glass pipette that directed the saline into the thoracic cavity. A copper/constantan thermocouple was placed adjacent to the mesothoracic ganglion to monitor thoracic temperature and the preparation was grounded through an Agar/electrolyte bridge. Most experiments were performed at room temperature (22–25°C). The experiments using picrotoxin also examined the thermosensitivity of the rhythm frequency in the 20–35°C range.

2.2. Intracellular and extracellular recordings

Short flight sequences were obtained by blowing air over the head of the animal. The time of depression in the flight rhythm was monitored using an electromyographic (EMG) electrode (50 μm copper wire, insulated except at the tip) inserted into one of the dorsal longitudinal (DL) muscles.

Spontaneous action potentials of the forewing stretch receptor or hindwing tegulae were recorded from nerve 1 of the mesothoracic or metathoracic ganglion with a monopolar silver wire electrode insulated from the saline with vaseline. Standard criteria for presumed monosynaptic connections were used: a constant latency between presynaptic spikes and postsynaptic potentials, and synaptic latency 1 ms or less after subtracting a conduction delay of 1 ms. This conduction delay was confirmed numerous times from recordings of afferent activity simultaneously with a peripheral extracellular electrode and a central intracellular electrode.

Intracellular recordings were made using 1 M KAc filled glass microelectrodes (resistance around 40 MΩ) inserted in the neuropile processes of flight motoneurons and interneurons in the mesothoracic and metathoracic ganglia. Recordings were maintained for at least 10 min, allowing sufficient time to change the superfusing solutions.

Electrical signals were amplified conventionally and all recordings were stored on VHS videotape using a Neurodata neurocorder (DR-886) for subsequent analysis.

2.3. Solutions

Locust saline contained (in mmol/l) NaCl, 147; KCl, 10; $CaCl_2$, 4; NaOH, 3; HEPES, 10 (pH 7.2). To reduce extracellular K^+ to 2 mM, 8 mM mannose replaced the KCl. The zero Ca^{2+} solution contained 20 mM $MgCl_2$ instead of $CaCl_2$ and 10 mM EDTA was added. For pharmacological experiments, 5×10^{-5} M picrotoxin (Sigma Chemical Co., St. Louis, MO) was added to locust saline. Stock solutions of picrotoxin were maintained in a refrigerator for up to a week while being used in experiments. The pH for all ion-substitution or drug solutions was adjusted to 7.2.

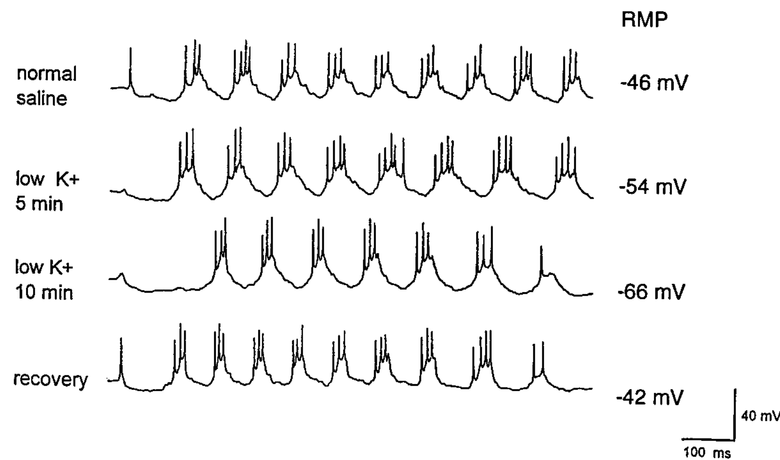

Fig. 1. Effects of low K^+ treatment on resting membrane potential and central flight rhythm. Intracellular recordings from a depressor motoneuron during expression of the flight rhythm before and after low K^+ treatment. Flight-initiating stimuli were delivered at the time of the brief depolarizing potential seen at the beginning of each trace (giving rise to action potentials in the top and bottom traces). Note the decrease in rhythm frequency after low K^+ treatment. Note also the gradual increase of the delay for initiation of bursting activity.

The concentrations of the ion-substitution and drug salines indicated above are higher than the concentrations that will actually be experienced by the neurons in the flight circuitry. The nature of saline substitution in a semi-intact preparation that contains crevices difficult to wash out, and tissues able to act as reservoirs is such that complete substitution of the superfusing saline and the extracellular fluid is impossible within the duration of these experiments. Also, these global treatments could have non-specific effects on neural parameters other than the ones we were targeting, especially at high concentrations. For these reasons we used only the minimum concentrations necessary to mimic the effects of temperature described previously [33,48] and did not examine an extended range of concentrations.

2.4. Data analysis

Data presented here were collected from experiments performed with at least seven animals for each treatment. Rhythm frequency was recorded as the mean instantaneous frequency (reciprocal of period) of five consecutive cycles from four flight sequences. EPSP amplitudes were measured from on-line averages of approximately 20 EPSPs. To test for differences, the data were tested for normality and equal variance and the appropriate statistical tests were performed using the statistical software package SigmaStat (Jandel Scientific, Corte Madera, CA). Significance was assessed at $P < 0.05$.

3. Results

3.1. Influence of resting membrane potential on rhythm frequency

Reduced extracellular K^+ gradually decreased the rhythm frequency although there was no obvious effect on the amplitude of action potentials or on the amplitude of the membrane potential oscillations (Fig. 1). After 5 min of superfusion with low K^+ saline, the resting membrane potential of flight motoneurons hyperpolarized by 7.3 mV, from -42.8 ± 1.6 mV to -50.1 ± 2.0 mV (mean ± S.E.M.). Simultaneously, the mean frequency of the cen-

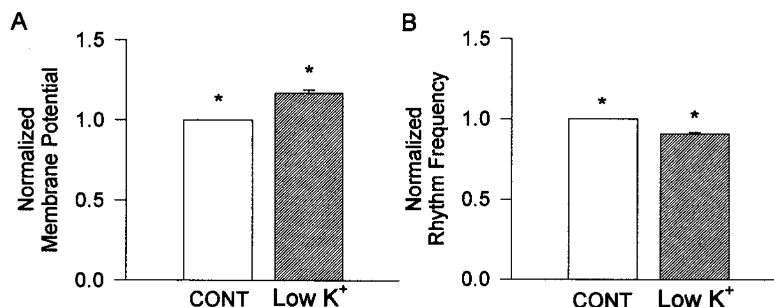

Fig. 2. Superfusion with low K^+ saline hyperpolarizes the membrane potential (A) and decreases the rhythm frequency (B) of flight motoneurons. Measurements of membrane potential and rhythm frequency were normalized to control values (CONT). Bars indicate means and standard errors before, and 5 min after, low K^+ treatment. Asterisks indicate significant differences.

Table 1
Absolute values of deafferented flight rhythm frequency before and after 5 min or less of different ion substitution and drug treatments

Treatments	Rhythm frequency (Hz)	
	Control	After treatment
Low K^+ ($n = 9$)	11.6 ± 1.6	10.5 ± 1.7 *
Zero Ca^{2+}/High Mg^{2+} ($n = 9$)	11.2 ± 1.3	11.0 ± 1.5
Picrotoxin ($n = 11$)	11.6 ± 1.2	12.9 ± 0.8 *

Values represent the mean ± S.E.M. * Significant difference from control values (paired t-tests; $P < 0.05$).

tral flight rhythm decreased from 11.6 ± 1.6 Hz to 10.5 ± 1.7 Hz (Table 1). To accommodate variability in the control (pre-treatment) values for membrane potential and rhythm frequency, post-treatment values were normalized by division with control values. Five min of superfusion with reduced extracellular K^+ significantly hyperpolarized the membrane to 1.2 ± 0.02 of the control membrane potential (paired t-test: $t = -8.81$, df = 8, $P < 0.0001$) (Fig. 2A), and significantly decreased the rhythm frequency to 0.9 ± 0.01 of the control frequency (paired t-test: $t = 9.06$, df = 8, $P < 0.0001$) (Fig. 2B).

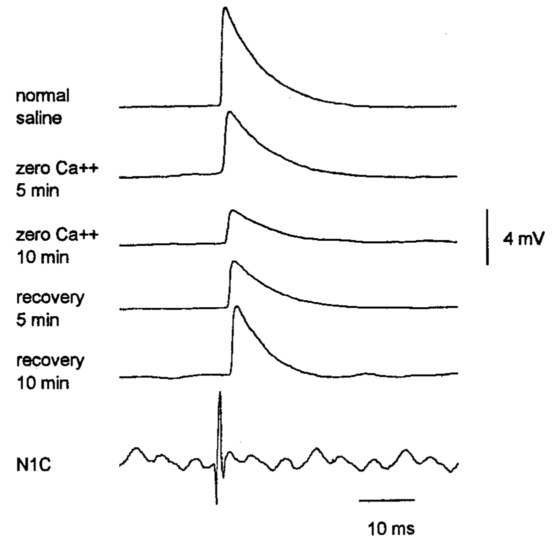

Fig. 3. Effects of zero Ca^{2+}/high Mg^{2+} treatment on EPSP amplitude recorded in an unidentified flight interneuron. Each of the five top traces is an average of 20 EPSPs triggered from the extracellular recorded action potentials of the hindwing tegulae (N1C, only one sweep shown). Note that EPSP amplitude decreased after zero Ca^{2+}/high Mg^{2+} treatment and that the effect was reversible.

3.2. Influence of PSP amplitude on rhythm frequency

The amplitudes of EPSPs generated by action potentials from a forewing stretch receptor (not shown) or from a hindwing tegula (e.g. Fig. 3) were reduced by superfusion with zero Ca^{2+}/high Mg^{2+} saline. However, there was little effect of the same treatment on the frequency of flight rhythm generation (Fig. 4). Ten recordings of affer-

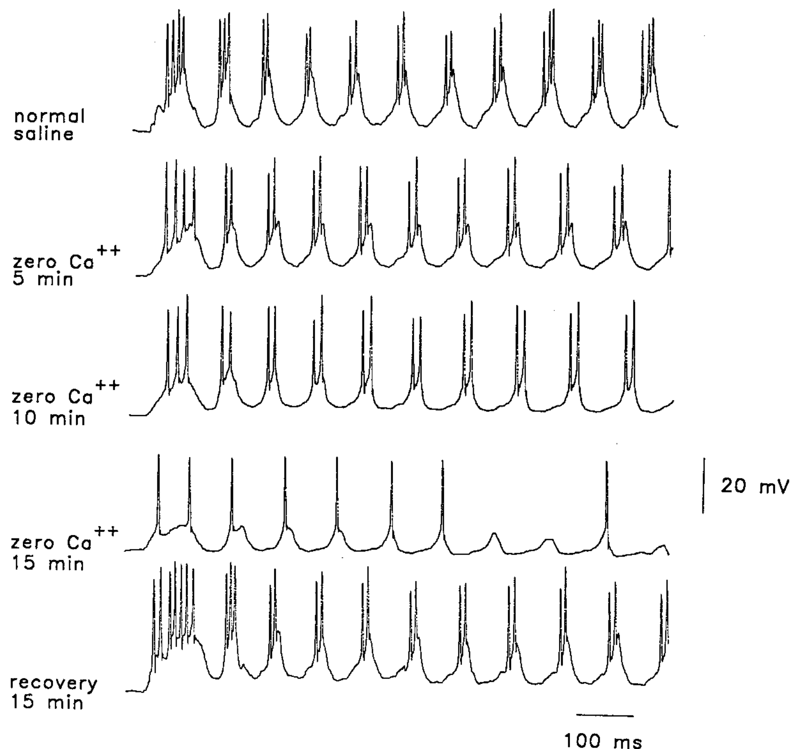

Fig. 4. Effects of zero Ca^{2+}/high Mg^{2+} treatment on the rhythmic bursting activity of a tergosternal elevator motoneuron. Although the waveform amplitude was attenuated, zero Ca^{2+}/high Mg^{2+} treatment had minimal effect on the rhythm frequency. Note the reduction in the intraburst firing frequency, particularly at the start of flight sequences.

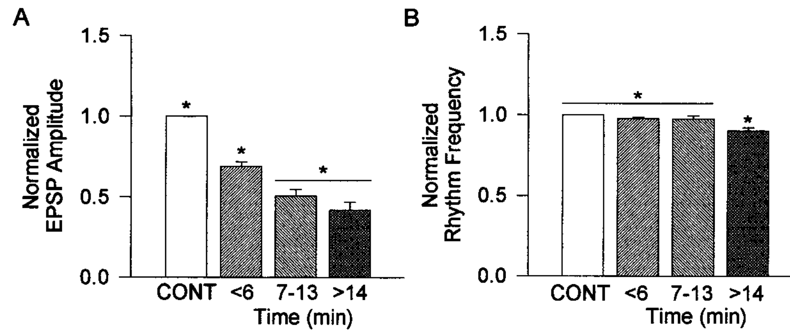

Fig. 5. Superfusion with zero Ca^{2+}/high Mg^{2+} saline reduced EPSP amplitude within 10 min (A) but had no significant effect on rhythm frequency within the same period (B). Rhythm frequency decreased minimally only after more than 14 min of treatment. Measures were normalized to control values. Bars indicate mean and standard error. Asterisks indicate significant differences and a line above the bars indicates no significant difference.

ent EPSPs in unidentified flight neurons in separate experiments provided the data set to quantify the reduction in EPSP amplitude. The effects of superfusion with zero Ca^{2+}/high Mg^{2+} saline on the rhythm frequency and the amplitude of membrane potential waveforms were established by recording flight sequences from 11 flight motoneurons and interneurons. Measurements were taken at different times after starting the superfusion and collected into three different time classes: less than 6 min (usually around 5 min), between 7 and 13 min (usually around 10 min), and greater than 14 min (usually around 15 min with one or two readings at 20 min). For technical reasons it was difficult to obtain data on both EPSP amplitude and rhythm frequency from each experiment. Nevertheless, four of the recordings were common to both sets of experiments, i.e., provided simultaneous data on EPSP amplitude and rhythm frequency. There was no difference between the data obtained from these four recordings and those from the other recordings. The data were normalized to control values and pooled. The amplitudes of the membrane potential waveforms of neurons during flight sequences (not shown) were significantly reduced by the zero Ca^{2+}/high Mg^{2+} superfusion to 0.85 ± 0.06, 0.75 ± 0.07 and 0.63 ± 0.1 of control values at the three different time periods (one-way ANOVA: $F = 6.32$, df = 27, $P = 0.003$, followed by Student-Newman-Keuls test for multiple comparisons). The last two values were significantly different from the control, but none of the post-treatment values was different from any other. The duration of superfusion had a significant effect on EPSP amplitude (Fig. 5A; one way ANOVA: $F = 79.8$, df = 22, $P < 0.0001$). EPSP amplitude dropped significantly to 0.7 ± 0.03 of control within 6 min. Longer superfusion (up to 13 min) reduced EPSP amplitude significantly further to 0.5 ± 0.04 of control. After more than 14 min of superfusion, EPSP amplitude had dropped to 0.4 ± 0.07 of control, but this was not significantly different from the value at the previous time period. Although the same treatment did have an effect on rhythm frequency, this effect was marginal and only apparent after 14 min of superfusion (Fig. 5B; one-way ANOVA: $F = 8.49$, df = 27, $P = 0.0005$). After more than 14 min of zero Ca^{2+}/high Mg^{2+}

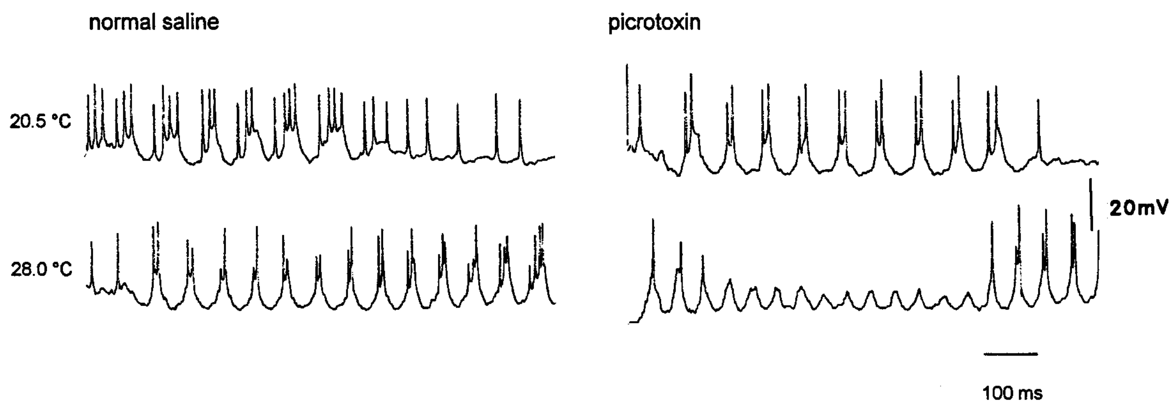

Fig. 6. Comparison of the difference in thermosensitivity of the central flight rhythm recorded intracellularly in a flight motoneuron before, and 2 min after, picrotoxin (5×10^{-5} M) treatment. Horizontal comparison shows the increase in rhythm frequency recorded in a flight motoneuron after picrotoxin treatment, and vertical comparison illustrates the increased thermosensitivity of rhythm frequency after picrotoxin treatment.

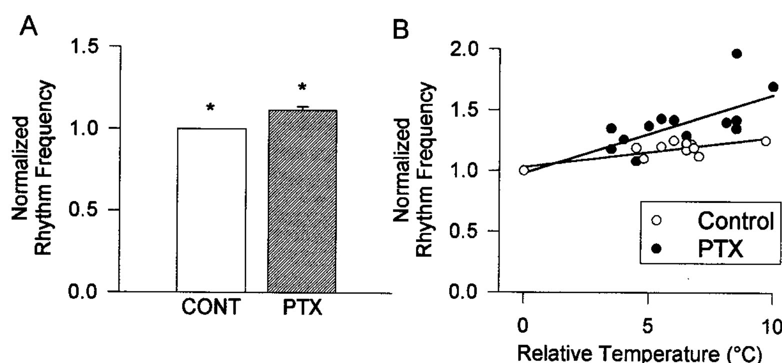

Fig. 7. Superfusion with picrotoxin increases rhythm frequency at room temperature (A) and increases the thermosensitivity of rhythm frequency (B). In A, bars indicate mean and standard error, and asterisks indicate a significant difference. In B, individual data points have been fitted with linear regressions (see text for slopes of the regressions). The first data point at 0°C relative to room temperature represents the initial values for both the control data and the picrotoxin data. Rhythm frequency was normalized to control values.

treatment, the frequency of the central rhythm had dropped to 0.9 ± 0.02 of control, with no significant change until that time (Fig. 5B; Table 1).

3.3. Influence of picrotoxin on rhythm frequency

We used picrotoxin to reduce the amplitude of GABAergic IPSPs in the flight circuitry. We measured the effect of this treatment on rhythm frequency and the thermosensitivity of the frequency (Fig. 6). Two min superfusion of 5×10^{-5} M picrotoxin significantly increased the mean rhythm frequency from 11.6 ± 0.4 Hz to 12.9 ± 0.2 Hz in 11 flight motoneurons (Table 1; paired *t*-test: $t = -6.75$, df = 10, $P < 0.0001$). This represents an increase to 1.2 ± 0.02 of control (Fig. 7A).

We also measured the thermosensitivity of rhythm frequency before and 2 min after administration of picrotoxin (5×10^{-5} M) in eight flight motoneurons. Rhythm frequencies were normalized to the value at room temperature and plotted against the temperature relative to room temperature (22–25°C) (Fig. 7B). Before picrotoxin treatment there was a significant linear relationship between normalized rhythm frequency and temperature ($r^2 = 0.61$; $P = 0.004$) with a slope of 0.025 ± 0.007. After two min of picrotoxin treatment there was a significant linear relationship between normalized rhythm frequency and temperature ($r^2 = 0.52$; $P = 0.003$) with a slope of 0.065 ± 0.02. The increase in the slope was significant (*t*-test; $t = 2.16$, df = 22, $P < 0.05$), indicating that picrotoxin treatment increased the thermosensitivity of rhythm frequency. This change in the slope represents an increase in the Q_{10} from 1.25 in normal saline to 1.65 in the presence of picrotoxin. Attempts to reverse the effects of picrotoxin by washing with fresh saline were unsuccessful.

4. Discussion

Our intention in this study was to mimic some of the effects of temperature on locust flight circuitry in order to test their role in determining the thermosensitivity of flight rhythm generation. By ion substitution and by manipulating saline composition pharmacologically, we tried to induce more widespread effects on the properties of the flight neurons and synapses than is possible using conventional intracellular recording and stimulation techniques. We have demonstrated that: (1) treatment with saline reduced in K^+ hyperpolarized neurons and decreased the frequency of central flight rhythms, (2) treatment with saline reduced in Ca^{2+} decreased the amplitude of PSPs but had little effect on the frequency of central flight rhythms, and (3) treatment with the Cl^- channel blocker, picrotoxin, increased the frequency of central flight rhythms and increased their thermosensitivity.

4.1. Technical considerations

One problem with the interpretation of these results is in determining the extent to which the flight circuitry was affected by the treatments. The ventral nerve cord of insects is ensheathed by perineurial glial cells which regulate the fluxes of ions and molecules across the sheath [39,44]. Nevertheless, ionic transfer between the haemolymph and the central nervous system does occur [43], and in our preparations, nerves 3, 4, and 5 of the mesothoracic and metathoracic ganglia were severed close to the ganglia allowing direct access of the saline to the neuropile through the cut ends of the roots. It is therefore reasonable to expect that the ion substitution and pharmacological treatments would affect the composition of extra-neuronal environment. This is clearly evident in the observations that each of the treatments had obvious physiological effects. A question remains as to the extent of each manipulation. For example, it is possible that the reduced Ca^{2+} saline decreased PSP amplitude of superficially located synapses from afferent axons but that the rhythm frequency was determined by neuronal interactions located deeper in the neuropile and more inaccessible to the treatment within the time course of the experiment. Thus differences in effects could simply be attributable to

the extent of spread of the experimental saline through the neuropile. Four observations suggest that this is not the case and that most, if not all, of the flight circuitry was affected by the modified salines. First, the known flight circuitry is located within a dorsal layer of superficial neuropile; the neuritic branching of flight interneurons is primarily confined to the 50 μm of neuropile just beneath the sheath [35,36]. Second, we monitored the effects of the reduced Ca^{2+} treatment on PSP amplitude recorded from a variety of interneurons and motoneurons and there was no obvious difference in the time taken to respond in different penetrations. More than half of the change was completed after 5 min and after 10 min there was little or no further reduction in PSP amplitude. The fact that PSP amplitude was not reduced to zero simply reflects the difficulty of reducing extracellular Ca^{2+} to zero in experiments with semi-intact preparations (see Section 2). Third, similar effects and time courses were observed when different PSPs (i.e., interneuron to motoneuron synapses) were monitored indirectly by measuring the amplitude of the membrane potential waveform of motoneurons during flight sequences. Fourth, treatments which did affect rhythm frequency had an effect in less than 10 min. We are confident that the effects of the saline substitutions described here reflect widespread actions within the flight circuitry occurring within the same approximate time period.

4.2. Resting membrane potential

Resting membrane potential is recognized as a critical factor that determines the rhythm frequency of neuronal oscillators (e.g. [20,41]). We found that 5 min treatment with low-K^+ saline hyperpolarized flight motoneurons from around -43 mV to -50 mV. This change is similar to the hyperpolarization caused by an increase in temperature from 25°C to 35°C (from around -41 mV to -48 mV [48]). Coincident with the hyperpolarization, rhythm frequency decreased by about 1 Hz. The low-K^+ saline could have had effects other than simply on the membrane potential, for example on voltage-dependent K^+ currents which could affect the shape of action potentials and subsequently the amplitude of postsynaptic potentials. However, the concentration of extracellular K^+ that we used did not produce obvious differences in intracellularly recorded action potentials or in the membrane potential waveforms of the rhythm. In similar experiments with neonatal rats, it has been reported that manipulation of extracellular K^+ changes membrane potential and consequently cycle period without affecting the generation of action potentials [42]. In addition, this finding is not surprising, as there is considerable precedent and a solid theoretical foundation for the suggestion that hyperpolarization reduces the frequency of oscillation [41]. We conclude that the excitatory effect of increased temperature on rhythm frequency described previously is not likely to have been mediated by the temperature-induced hyperpolarization of neurons in the flight circuitry. Rather, the present results support our proposal that such hyperpolarization acts to compensate for excitatory effects of increased temperature on other neural properties such as conduction velocity [48].

4.3. PSP amplitude and relative strength

Although increases in temperature cause a rise and fall in PSP amplitude with a transition plateau around room temperature, rhythm frequency monotonically increases over the same temperature range. Thus, it has been proposed that the frequency of the central flight rhythm is unaffected by the amplitude of unitary PSPs, within permissive limits [33]. Some circumstantial support for this proposal comes from the observation that during the 2-week maturation of the flight system, when wingbeat frequency almost doubles, EPSPs recorded in identified flight neurons do not change in amplitude [10].

In the present investigation we found that around 10 min superfusion with zero Ca^{2+}/high Mg^{2+} saline decreased the amplitude of unitary PSPs (see also [4]) and the membrane potential waveform during flight sequences. However, the same treatment had no significant effect on rhythm frequency. Longer periods of treatment did eventually reduce rhythm frequency in some preparations. It is conceivable that the reduction of extracellular calcium could have effects other than on PSP amplitude, especially given that Ca^{2+}-dependent plateau potentials play a major role in a wide variety of pattern-generating networks [6]. Ca^{2+}-Dependent regenerative potentials have been described in insect neurons [12] including locust thoracic interneurons [18]. Moreover, locust flight interneurons can be induced to generate plateau potentials although the ionic basis for these is not known [27,28]. However, the expression of endogenously generated bursts by flight interneurons is dependent upon octopamine treatment or upon large amplitude synaptic input from proprioceptive afferents [29]. Interneurons 566 and 567 have the capacity to generate bursts but do *not* do so in deafferented preparations in the absence of octopamine treatment [29], which were the conditions of our experiments. To date there is no evidence that Ca^{2+}-dependent regenerative potentials have any role in the generation of wind-induced flight rhythms by deafferented preparations.

The results would be more difficult to interpret if the treatment had affected the frequency of the rhythm; however, superfusion with zero Ca^{2+}/high Mg^{2+} saline had no discernible effect on rhythm generation in the deafferented preparation at a time when PSP amplitudes were significantly reduced. The results lead inescapably to the conclusion that rhythm frequency is resistant to large variations of PSP amplitude. Any suggestion to the contrary would have to propose that the experimental treatment also affected an unknown process in order to com-

pensate exactly for a presumed sensitivity of rhythm frequency to PSP amplitude. Although this is not impossible, it is improbable and it is certainly not the simplest explanation that fits all the facts. These observations support the contention that the excitatory effect of increased temperature on rhythm frequency is not mediated by effects of temperature on PSP amplitude. Computer simulations of the flight circuit have recently demonstrated that operation of the locust flight central rhythm generator is relatively insensitive to variations in the setting of the 'synaptic strength' parameter [11]. This insensitivity is a consequence of multiple oscillatory loops in the circuit, and the robustness of operation is unaffected by addition of plateau generating properties to the simulated flight neurons.

In contrast to the insensitivity to PSP amplitudes shown above, we have demonstrated that altering the relative strength of EPSPs and IPSPs in the circuitry did affect the rhythm frequency. To do this we used picrotoxin to reduce the amplitude of IPSPs in the circuitry. We did not directly monitor the effect of picrotoxin treatment on Cl^--mediated GABAergic IPSPs. However, it is established that most inhibitory flight interneurons are immunoreactive to antibodies raised against GABA [38], and that 1.5×10^{-4} M picrotoxin completely blocks IPSPs between elements of the flight central rhythm generator within 4 min with no observable non-specific effects [37]. We believe that the lower concentration of picrotoxin used in the present investigation (5×10^{-5} M) would have specifically reduced the amplitude of IPSPs in the flight circuitry without affecting excitatory transmission. Note that the effective concentration of picrotoxin reaching the neuropile is lower than the nominal concentration (see Section 2). The difficulty of reversing the effects of the picrotoxin saline by washing has been described [37] and is evident even with isolated neuronal somata from this species [19].

Low concentrations of picrotoxin to reduce IPSP amplitude increased the rhythm frequency from 11.6 Hz to 12.9 Hz after 2 min of superfusion. The same treatment also increased the temperature sensitivity of rhythm generation. The Q_{10} increased from 1.25 in normal saline to 1.65 in the presence of picrotoxin. Slopes of the normalized relationships convert to actual slopes of 0.29 Hz/°C in normal saline and 0.84 Hz/°C with picrotoxin. It is worth noting that the slope of the temperature relationship in normal saline exactly confirms the relationship described previously (0.29 Hz/°C for deafferented preparations [9]).

As for the previous treatments, a concern is the extent to which picrotoxin had non-specific effects in the circuitry. We believe this to be unlikely. It is well established that picrotoxin is a potent antagonist of insect GABA receptors [30], and there are few descriptions of it having other effects on insect neurons. Prolonged treatment with picrotoxin (20–40 min) can induce bursting activity in a cockroach motoneuron [13], but it is significant that even such prolonged treatment had no effect on membrane potential, effective membrane resistance or the ability to generate plateau potentials. Indeed, this bursting activity may have been induced by the relative enhancement of excitatory synaptic input [13]. We conclude that the excitatory effect of picrotoxin on rhythm frequency was a consequence of the reduction of IPSP amplitude in the flight circuitry and thus a shift in the balance of excitatory and inhibitory synaptic weights. A recently described model of the locomotor pattern generator of the *Xenopus* embryo demonstrates that the generation of swimming motor patterns is possible with a wide range of synaptic strengths [8]. In this experimentally derived model the cycle period is also dependent upon the balance of excitatory and inhibitory synaptic strengths, such that cycle period is shortened by increasing excitation or by decreasing inhibition.

The results described above support the contention that equal temperature effects on EPSPs and IPSPs automatically compensate each other, causing the rhythm frequency to show a minor thermosensitivity. This is a similar mechanism to the temperature compensation shown by the tritocerebral commissure giant interneuron due to the simultaneous, temperature-induced increases in the activities of both excitatory and inhibitory presynaptic sensory neurons [22]. However, for this wind-sensitive interneuron the EPSPs and IPSPs overlap in time, whereas during operation of the flight circuit the EPSPs and IPSPs generally occur at separate phases of the flight rhythm. Thus, in the flight system temperature compensation is unlikely to be a simple algebraic cancellation of effects. Nevertheless, the relative amplitudes of EPSPs and IPSPs that phasically alternate will determine the membrane potential level around which the oscillations occur and this would affect the rhythm frequency. Furthermore, the accepted model for the wingbeat frequency-stabilizing role of stretch receptor activity in intact animals [25] involves reduction in the amplitude of a hyperpolarization via the simultaneous excitatory feedback from the stretch receptor (i.e. overlapping EPSPs and IPSPs).

Poikilotherms maintain adaptive behavior over a range of temperatures indicating a need to compensate for the effects of temperature on neural function [15,23]. There have been some indications of temperature compensation in insect nervous systems [1,22]. The experiments described here suggest that at least two compensatory mechanisms may be incorporated into the locust flight nervous system. First, we propose that hyperpolarization with increasing temperature minimizes excitatory effects of temperature on cellular properties. Second, we propose that equal temperature effects on IPSPs and EPSPs cancel each other out to render the central flight circuit relatively temperature insensitive. It is important to remember that our experiments were performed with deafferented preparations and that temperature compensation in intact animals is likely to include other mechanisms. In particular, the expression of conditional burst generating properties during intact flight [29] would probably render the system

even more insensitive to PSP amplitude and under normal flying conditions synaptic input, such as from proprioceptive afferents, may serve simply to initiate and terminate endogenous mechanisms of burst generation. Finally, a significant general observation of the work described here is that relatively large changes in synaptic strength can have minimal effects on the cycling frequency of a circuit when the relative importance of excitatory and inhibitory interactions is balanced. This is supported by computer simulations of this [11] and other [8] central pattern generators. It will be interesting to determine to what extent this insensitivity to synaptic strength changes extends to the phasing of motor activity within each cycle.

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