J Comp Physiol A (2005) 191: 559–573
DOI 10.1007/s00359-005-0621-0

ORIGINAL PAPER

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Effects of neck and circumoesophageal connective lesions on posture and locomotion in the cockroach

Received: 23 November 2004 / Revised: 9 February 2005 / Accepted: 24 February 2005 / Published online: 30 April 2005

Abstract Few studies in arthropods have documented to what extent local control centers in the thorax can support locomotion in absence of inputs from head ganglia. Posture, walking, and leg motor activity was examined in cockroaches with lesions of neck or circumoesophageal connectives. Early in recovery, cockroaches with neck lesions had hyper-extended postures and did not walk. After recovery, posture was less hyper-extended and animals initiated slow leg movements for multiple cycles. Neck lesioned individuals showed an increase in walking after injection of either octopamine or pilocarpine. The phase of leg movement between segments was reduced in neck lesioned cockroaches from that seen in intact animals, while phases in the same segment remained constant. Neither octopamine nor pilocarpine initiated changes in coordination between segments in neck lesioned individuals. Animals with lesions of the circumoesophageal connectives had postures similar to intact individuals but walked in a tripod gait for extended periods of time. Changes in activity of slow tibial extensor and coxal depressor motor neurons and concomitant changes in leg joint angles were present after the lesions. This suggests that thoracic circuits are sufficient to produce leg movements but coordinated walking with normal motor patterns requires descending input from head ganglia.

Keywords Brain · Suboesophageal ganglion · Walking · Arthropod · Descending control

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00359-005-0621-0

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Abbreviations SOG: Suboesophageal ganglion · NL: Neck connective lesion · CoCL: Circumoesophageal connective lesion · Ds: Slow depressor of the trochanter motor neuron · SETi: Slow extensor of the tibia motor neuron · Fti: Femur- tibia joint · CTr: Coxa- trochanter joint

Introduction

The basic pattern of muscular contractions that occurs during walking is generated at the spinal or nerve cord level. However, successful locomotion requires muscle activity that is accompanied by forceful propulsion, proper leg coordination and control of posture (Grillner 1997). Research in vertebrates and arthropods suggests that inputs from neural centers in the brain modulate, coordinate and adapt the motor pattern to produce effective walking (Kien and Altman 1992; Grillner et al. 2000).

The interactions among neurons in the brain, descending neural tracts and circuits in the spinal cord or nerve cord is an area of intensive study (Buchanan 2001; Schaefer and Ritzmann 2001; Kozlov et al. 2002; Comer et al. 2003; Drew et al. 2004; Ritzmann et al. 2005). These studies have shown that locomotor behavior can occur in the absence of descending inputs. For example, decapitated cockroaches are able to make directionally appropriate escape turns (Schaefer and Ritzmann 2001). In addition, cats with spinal cord injuries can regain the ability to walk with step-training and drug therapy (Belanger et al. 1996; Rossignol 1996; De Leon et al. 1998a, 1999). However, neither the kinematics nor the motor patterns of lesioned animals are the same as an intact preparation. Normal walking behavior requires that the animal moves its legs in a coordinated fashion and extends its leg joints through normal excursions. A complete evaluation of post-lesion walking requires detailed kinematic and physiological analysis. Recent advances in motion recording simplify kinematic analysis. Data about physiological properties of individual neurons in the vertebrate walking circuit are limited because it is difficult to identify and to record from single neurons in vivo (Kiehn and Eken 1997). However, arthropods provide useful model systems with relatively limited circuits and identifiable neurons (Kien and Altman 1992; Selverston 1999; Comer and Robertson 2001)

However, even in the experimentally more-favorable arthropod preparations, the specific roles played by circuits in the head ganglia (brain and suboesophageal ganglia) in walking behavior have been studied very little. Most of the work that has examined motor and sensory control during walking in insects has focused on the properties of the thoracic ganglia, the central nervous system location of the central pattern generators for walking locomotion, as well as local reflexes that shape those patterns (Pearson and Iles 1970; Pearson and Fourtner 1975; Foth and Bässler 1985; Bässler and Büschges 1998). In one of the most studied species, the stick insects, pattern generation circuits for each leg joint have been identified in the thoracic ganglia (Büschges et al. 1995). Nevertheless, decapitated insects do not walk well (Roeder 1937; Graham 1979). Studies in locust found that descending interneurons originating in the suboesophageal ganglion (SOG) are active during leg movements, but show extensive variability in their response properties (Altman and Kien 1987). The abnormalities in posture and locomotion present in decapitated animals could be due to either the lack of descending input from the brain and/or the SOG or trauma associated with decapitation. If animals with the neck connectives lesioned are maintained over several weeks, we can differentiate between these two possibilities. Specifically, we can document: (1) changes in walking behavior as the animal recovers from the trauma associated with the surgery and (2) the time course of adjustments that may occur in the lower neural circuits found in the thoracic ganglia. Animals in various periods post-trauma, that have normal posture but limited walking activity, can also provide a useful model for investigating the neural circuitry underlying walking behavior.

Studies in arthropods and vertebrates have shown that certain pharmacological agents can be effective in eliciting locomotory behavior in restrained or reduced preparations (Ryckebusch and Laurent 1993; Büschges et al. 1995; Belanger et al. 1996; Johnston and Levine 1996; Zhang and Grillner 2000; Barbeau and Norman 2003; Giroux et al. 2003; McLean and Sillar 2003; Dasari and Cooper 2004). These chemicals are believed to mimic or enhance the roles of endogenous chemicals that act on the ventral nerve cord or the spinal cord via descending inputs from the brain (Johnston and Levine 1996; Chau et al. 1998a, 1998b; Johnston et al. 1999). Therefore, it may be possible to compensate for the lack of descending modulatory pathways and to elevate overall neural activity in lesioned animals by injecting these chemicals into the open circulatory system.

In this paper, we examine the effects of removing higher-order sensory and pre-motor processing on cockroach locomotion and document to what extent the local circuitry can support behavior in absence of inputs from the head ganglia. We describe kinematic and neurobiological observations on animals that had experienced bilateral lesions of either the neck connectives (between the SOG and the prothoracic ganglion) or the circumoesophageal connectives (between the SOG and the brain). The circumoesophageal connective lesion removes brain information and control but retains whatever circuitry that resides in the suboesophageal ganglion, while the neck lesion removes all descending input and all ascending feedback. Neck lesioned animals were then maintained for extended periods of time to examine the extent of their recovery. Circumoesophageal lesioned animals will not feed and, therefore, could not be maintained for long periods of time. Because these animals were more immediately active than neck lesioned animals, long-term recovery was not critical. These data suggest that although thoracic circuits directly control walking movements, the release and expression of normal behavior requires input from circuits within the head ganglia in cockroaches.

Methods

Lesions of the neck connectives

Adult cockroaches, *Blaberus discoidalis*, were anesthetized with CO_2 and were placed, ventral side up, on a resin platform. The legs were then restrained with staples made from insect pins. The neck was ligated with nylon suture prior to surgery to prevent excessive loss of hemolymph. A small longitudinal incision was made in the ventral cuticle of the neck with a razor blade and both neck connectives were cut with fine scissors. While the animal was under anesthesia, dots were painted on all six legs with a white paint pen to facilitate examination of leg movements. After clotting of the wound occurred, the ligature was removed from the neck. The wings were clipped with scissors so that the legs could be seen from the side. Success of the surgery was confirmed by post-mortem dissection of the neck area.

Lesions of the circumoesophageal connectives

Adult *B. discoidalis* were anesthetized and restrained, dorsal side up, as described above. In cockroaches, the brain and the suboesophageal ganglion are separated by the esophagus, which facilitates the lesion of the circumoesophageal connectives. A small longitudinal incision was made in the head capsule between the antennae sockets. This incision allowed us to see the connectives branching off of the brain and they were cut with a small pair of scissors. The wound was then sealed with dental wax. The wings were clipped with scissors so that the legs could be seen from the side.

Animal care

NL (neck connective lesion) animals will readily eat if food is placed near the mouth. Individuals were fed soy baby formula using a syringe every 2–3 days. This technique can be used to keep NL animals alive up to 3 months, because it provides both nutrients and water to the animal. The presence of fecal pellets was used to confirm that food was being ingested and processed. CoCL (circumoesophageal connective lesion) animals do not feed and could, therefore, not be maintained for long periods post surgery. However, a glass Petri dish filled with aquarium gravel and water added to the cages minimized desiccation of the animals. Typical survival periods were 7–10 days. Animals were housed in plastic containers under a 12 h-light: 12 h-dark circadian cycle.

Kinematic analysis

Video images of animals standing still, free-walking and under 'oil-plate' tether conditions (Tryba and Ritzmann 2000a) were recorded with a Redlake high-speed video PCI system from the side and from below, via a mirror mounted at 45° under the glass plate. Images were stored as AVI files on a PC computer. For the posture analysis, video sequences were taken when the animal was completely still and individual frames were captured to measure the height of the head and the body above the substrate using computer software. Walking was stimulated by stroking the thorax and abdomen with a paintbrush. To standardize the force of stimulation, we used the same paintbrush in all trials. Each animal was stimulated five times and subsequent stimulations were not performed until all movement stopped. Stimulation was initiated at the first thoracic segment and ended at the last segment of the abdomen. Care was taken to move the paintbrush slowly for a total of 2 s from anterior to posterior. One can use calibrated brush hairs to generate stimulation at a known force. In our case, we only sought to have a reasonably reproducible way of initiating locomotion. Since we used the same brush each time, we feel that we accomplished that goal.

Walking behavior was documented by analyzing the number of steps per stimulus, gait pattern for all six legs and leg joint angles of the right rear leg. A step was defined as movement of a leg from posterior to anterior (from the end of stance to swing and then back to the end of stance). The position of the leg dots from below was digitized using motion analysis software (WINanalyze, Mikromak, Berlin). The leg dots were connected using line segments and angles of the coxa-trochanter (CTr) and femur-tibia (FTi) joints of the right metathoracic leg were calculated from the line segments. Joint angle data were imported into Datapac 2002 software (Run Technologies, Mission Viejo, CA) and were smoothed linearly using an 8 ms time constant. In addition, points at the metathoracic body-coxa joint and the head (at level of antennae sockets) were digitized to calculate height above the substrate. With these two points, we could determine if the whole body is elevated or the front end is either pitched upward or downward.

Myographic analysis

Animals were restrained, ventral side up, in a resin dish and pairs of fine wire (42 gauge) electrodes, insulated to the tip, were inserted in the left metathoracic leg. Wires were positioned to record discharges in the slow extensor of the tibia (SETi) and the slow coxal depressor (Ds), following techniques and placement described in Watson and Ritzmann (1998). All recording wires were tied to the leg and secured with cyanoacrylate adhesive. Myogram data were captured with a Digidata 1322A data acquisition system (Axon Instruments, Union City, CA) at a sampling rate of 10 kHz and stored on a PC computer. Data were analyzed using Datapac 2002 software and filtered with a Butterworth high pass filter at 150 Hz. Physiological data were synchronized with kinematic sequences and merged into a single file. In order to quantify motor activity during the stance phase, normalized histograms of discharges were constructed, as in several previous studies (Akay et al. 2001; Noah et al. 2004). The onset and termination of stance were determined from the video data and the bursts were divided into 20 bin intervals. Burst onset and offset were defined as 0.5 ms before the first spike and 0.5 ms after the last spike (Watson and Ritzmann 1998). The total number of spikes for each bin was calculated and trials for all animals were added together and graphed.

Effects of modulators

To examine the effects of modulators on the locomotor ability of neck lesioned cockroaches, separate solutions of 10^{-5} M or 10^{-3} M (diluted with cockroach saline) of both pilocarpine and octopamine were prepared. seventy to hundred micro microlitres of octopamine or pilocarpine was injected into the abdomen or through the soft tissue of the metathoracic body-coxa joint. Each chemical was injected separately into different animals. As control experiments, 80 μl of saline was injected into NL animals ($n = 5$). Furthermore, 80 μl of 10^{-3} M of either octopamine or pilocarpine was administered to intact individuals ($n = 5$, each chemical).

Experimental protocol

To examine the changes in walking behavior of NL cockroaches with time, test animals were placed in a plexiglass arena every 2–3 days. Leg movements were

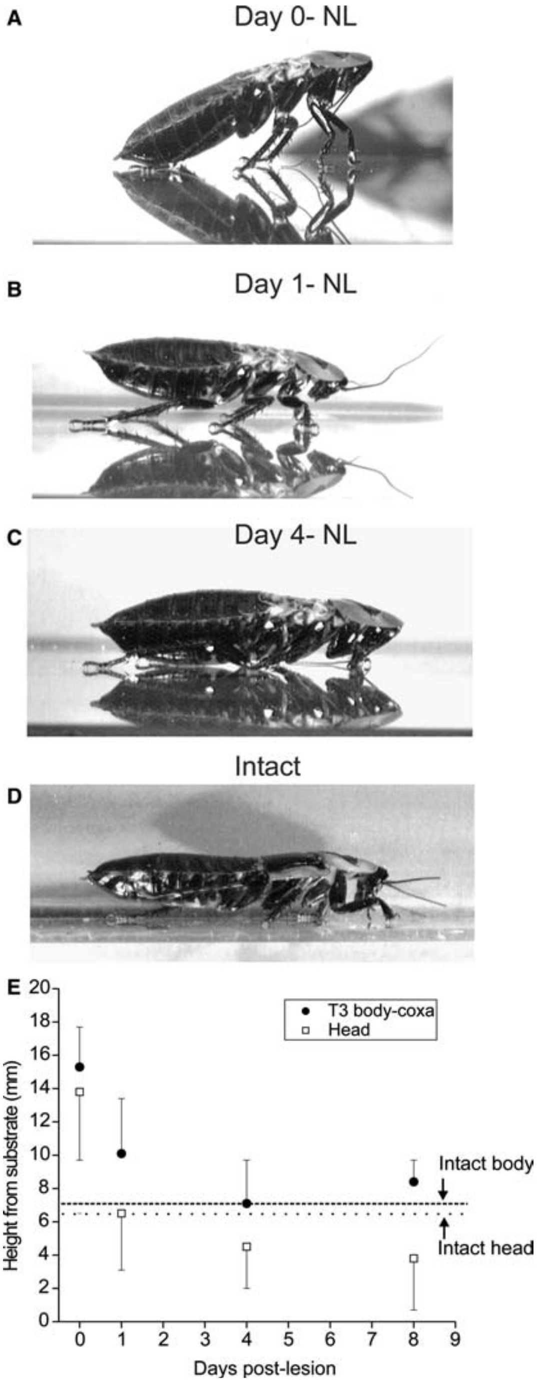


Fig. 1 **a–e** Changes in posture with time after a neck lesion. Both neck connectives were cut and animals were kept alive for several days. **a** Immediately following the lesion, the legs were hyper-extended and the body appeared to be rigid. **b** One day post lesion, the hind legs are still hyper-extended, but the head is now lower than the body. **c** By day 4, body posture looks similar to an intact animal. **d** Body posture of intact animal. **e** Quantitative analysis of the position of the head and metathoracic body-coxa joint above the substrate shows that there is a significant decrease in the height of the body posture after 1 day post-lesion (ANOVA, $P < 0.05$). The height of the body and the head further decreases in subsequent days. Furthermore, the head is significantly lower than the T3 body-coxa joint in lesioned animals at day 8 post-lesion (*T*-test, $P < 0.05$, "head-down" posture). The lines represent the average height of the body (*dashed*) and the head (*dotted*) in intact animals

recorded with high-speed video (125 frames/s). After neuromodulators were injected, animals were immediately released into the arena and their movements were recorded.

CoCL animals were tested in the walking arena either on the day of the lesion or 1–2 days afterwards. Stimulation was not typically required to evoke walking in these animals. In order to obtain precise joint angle data in conjunction with the electromyograms, these data were collected from animals that were tethered over a lightly oiled glass plate. Cockroaches placed in this tether preparation generate leg movements that are similar to those seen in animals walking on a treadmill (Tryba and Ritzmann 2000a).

Statistical analysis

All statistical analysis (One-way ANOVA and Independent *t*-Test) was performed using Origin 7.0. Data are reported in the text as mean ± standard deviation. Tukey Means Comparison test was used to determine statistical significance between data pairs (Neter et al. 1996).

Results

Posture in NL cockroaches

Normal posture is altered in the absence of descending input from the brain and the suboesophageal ganglion. Immediately after lesion of the neck connectives, animals ($n = 9$) show hyper-extended posture (Fig. 1a). Specifically, the height of the head and the metathoracic body-coxa joint above the substrate was greater in NL cockroaches (head, 13.8 ± 4.1 mm; body-coxa joint, 15.3 ± 2.4 mm; ANOVA, $P < 0.01$) than that recorded in intact animals ($n = 10$, head, 7.1 ± 1.3 mm; body-coxa joint, 6.5 ± 1.2 mm; Fig. 1d, dotted and dashed lines in Fig. 1e). Within 1 day after the lesion, there is a significant decrease in both the head and body-coxa joint height (ANOVA, $P < 0.01$, Figs. 1b, e). Indeed, now the head is lower than the thorax. Within 4 days, height of the body-coxa joint is similar to intact animals (lines in Fig. 1e) but the head is still pitched downward ("head-down" posture, Figs. 1c, e). Further significant changes in posture were not detectable after 4 days post-lesion. However at Day 8 post-lesion, the difference between height of the T3 body-coxa joint and the head above the substrate was significant (*t*-test, $P < 0.05$).

Walking behavior in NL cockroaches

Cockroaches with bilateral lesions of the neck connectives do not walk without direct tactile stimulation (i.e., spontaneous walking is never observed). This lack of movement suggests that either descending activity from the head ganglia is necessary for normal walking or that decapitation limits walking for other reasons. It is also possible that lack of coordinated walking is caused by an immediate response to injury. To test this hypothesis, we maintained NL animals (*n* = 18) for several weeks after the lesion and monitored their ability to initiate stepping and to walk in a coordinated fashion. We averaged the number of steps per stimulation in each thoracic segment (left and right sides were combined) and plotted them against time post surgery. Early in the recovery period, cockroaches did not move forward but sometimes showed leg movements after manual stimulation (Figs. 2a–c). For these studies, walking was defined as leg movement combined with forward progression of the center of mass. For all legs, there was a gradual increase in the number of steps taken by lesioned animals up to day 10. Furthermore, 10-day animals could initiate slow leg movements for multiple cycles and could move forward (Movie 1). After 10 days, step number declined, but remained elevated over the day-1 values. The prothoracic legs (Fig. 2a) often took more steps than the mesothoracic (Fig. 2b) or metathoracic (Fig. 2c) legs, but this difference was not significant across the population.

In addition to step number, we also examined changes in gait pattern with time after the lesion. Early in the recovery period (Day 4), leg movements were rapid and rarely coordinated as metachronal or tripod gaits (Fig. 3a). After 9–10 days, elements of metachronal and tripod gaits were sometimes present. For example, modified metachronal gaits (legs on one side move in a posterior to anterior wave, solid line) and single tripod cycles (e.g. L3, L1 and R2 move in synchrony,Fig. 3b) or partial tripod cycles (e.g. L3 and L1 swing together) were detectable in some animals. However, individual tripod cycles were not maintained for long periods. Righting behavior was readily initiated by placing the animals on their dorsal side. In a few cases (*n* = 2 animals), individuals would walk 2–3 cycles spontaneously after righting behavior, suggesting that effects associated with righting behavior may be sufficient for excitation of the neurons that are important in walking. However, walking in these animals was not consistently coordinated.

To further address the changes in leg coordination in NL animals, we calculated the phase of leg swing between thoracic segments (intersegmental; metathoracic/mesothoracic, mesothoracic/prothoracic) and within segments (intrasegmental; R3/L3, R2/L2, R1/L1). In tripod walking, each leg enters swing in antiphase with the contralateral leg of the same segment and the ipsilateral legs in adjacent segments. Therefore, intersegmental and intrasegmental phase values in an intact animal walking with a tripod gait are close to 0.5 (Fig. 4a, b). In NL animals at 9–10 days post-lesion, intersegmental phase values are significantly reduced (ANOVA, $P <$ 0.01; Fig. 4a) but intrasegmental values are close to 0.5 (Fig. 4b). Thus, coordination within each thoracic segment is normal, but between segments, it is not.

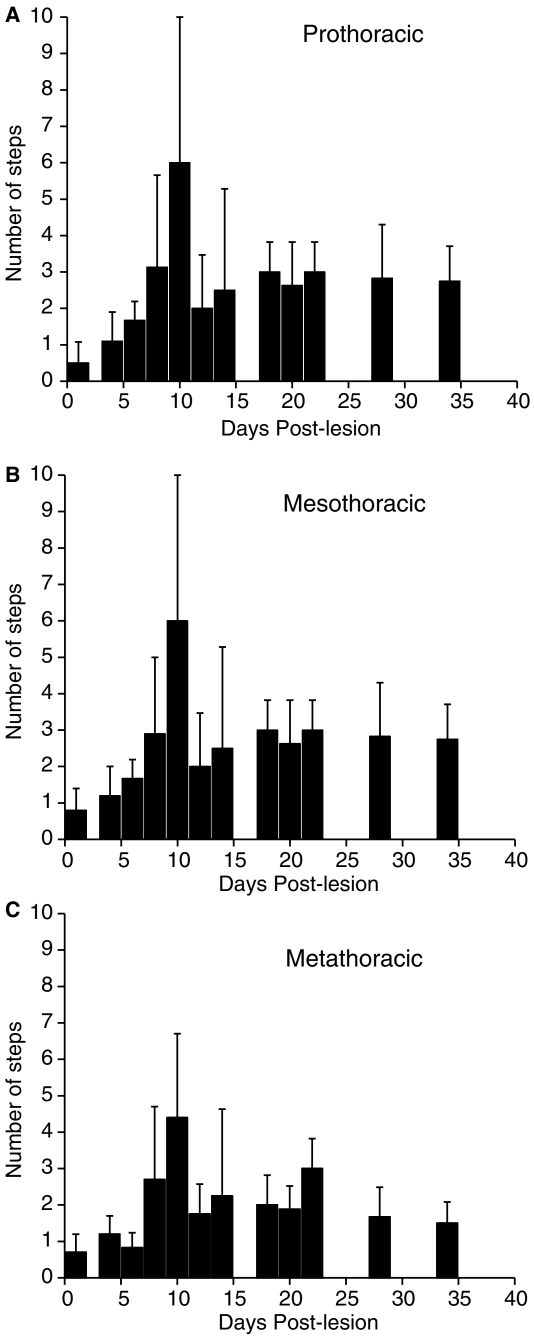


Fig. 2 a–c Changes in the ability of neck-lesioned animals to walk with time. Immediately following the lesion, animals did not walk when stimulated. However, the number of steps in prothoracic (**a**), mesothoracic (**b**) and metathoracic (**c**) legs increased to a maximum around Day 10 and then decreased with time to a sustained level (~2 steps). Legs on the left and right sides of each segment were combined in this analysis

Neck lesions result in generally depressed activity. It is possible that descending activity simply serves to increase the tonic level of motor activity in the thoracic

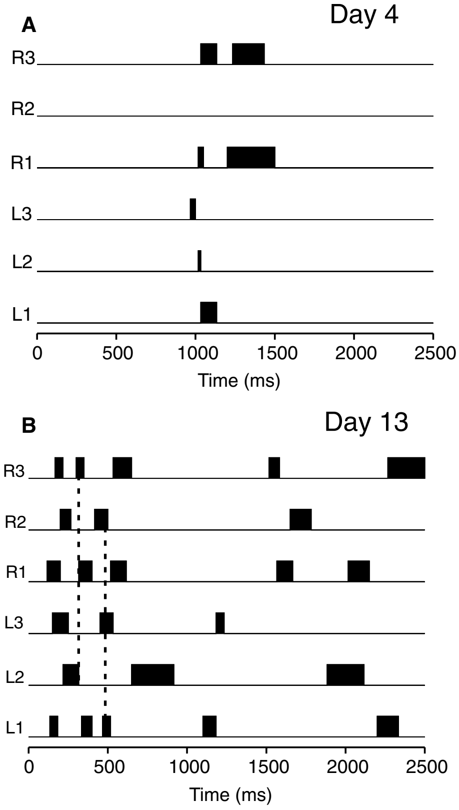


Fig. 3 a–b Changes in gait pattern in a neck-lesioned animal with time. **a** Four days post-lesion. Rapid and synchronous leg movements could be elicited with stimulation, but multiple leg cycles were not present in this animal. R and L indicate right and left legs and 1, 2 and 3 the fore, middle and rear legs of the thoracic ganglia respectively. *Bars* swing phase (leg is in the air and moving forward) **b** Thirteen days post-lesion. Single tripod cycles (*dotted line*) and slow leg movements could be elicited in this animal

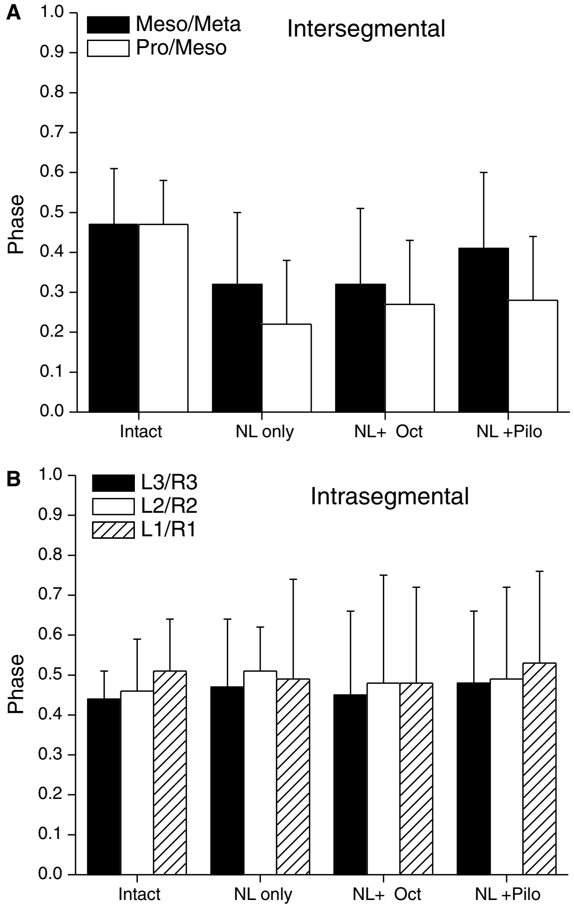


Fig. 4 a–b Changes in leg coordination after NL and the injection of octopamine and pilocarpine into the hemolymph. **a** NL animals show significantly reduced intersegmental phase for both Meso/Meta and Pro/Meso. Although octopamine and pilocarpine cause an increase in number of steps taken by neck-lesioned animals, the overall phases of the anterior leg movements are still significantly (ANOVA, $P < 0.01$) less than intact individuals and similar to late-stage neck lesioned animals (Day 9–10 post lesion). **b** In contrast, the phase relationships of leg movements within each segment are similar under all conditions. These results suggest that octopamine and pilocarpine are sufficient to elicit leg movements in these individuals but coordination among the leg segments is still limited

ganglia. If this is the case, the changes in intersegmental coordination could be, at least in part, attributed to a decrease in walking speed associated with the decline in tonic excitation (Wilson 1966; Delcomyn 1971). With an increase in this tonic activity, coordination might return through actions of the thoracic neural circuitry. To examine this possibility, we administered excitatory neuromodulators, octopamine ($n = 18$) or pilocarpine ($n = 10$) to the animals at different days post-lesion (0–28 days). With the exception of animals in hyper-extended postures (Day 0 post-lesion), all individuals increased the number of steps taken and their excitability after injection of either drug as compared to their condition before drugs were administered (Figs. 5a). For example, in a 1 day post-lesion animal, the gait pattern was generally uncoordinated before injection of octopamine (Fig. 5b). After injection, this individual greatly increased the number of steps taken per stimulation and showed evidence of metachronal gait. However, most leg cycles remained uncoordinated (Fig. 5c). That is, neither octopamine nor pilocarpine resulted in a significant increase in the intersegmental phase values from the levels calculated in untreated NL individuals ($P > 0.1$, ANOVA, Fig. 4a). Intrasegmental phase values remained near 0.05 (Fig. 4b). In addition, excessive grooming behavior could be elicited by octopamine and flight behavior (rapid movement of the base of the wings) could be evoked with pilocarpine. No significant differences in the effects of these two neuromodulators were present among animals at different days post-lesion ($P > 0.1$, ANOVA). Furthermore, administration of saline to NL animals ($N = 5$) does not cause an increase in step number.

Post-mortem dissection showed that all the experimental animals had severed neck connectives and no evidence of regeneration was detected. These data indicate that walking in cockroaches with severed neck connectives can improve with time post-surgery. However, coordination between leg segments does not return to normal levels, even if prolonged bouts of walking are elicited using pharmacological manipulation

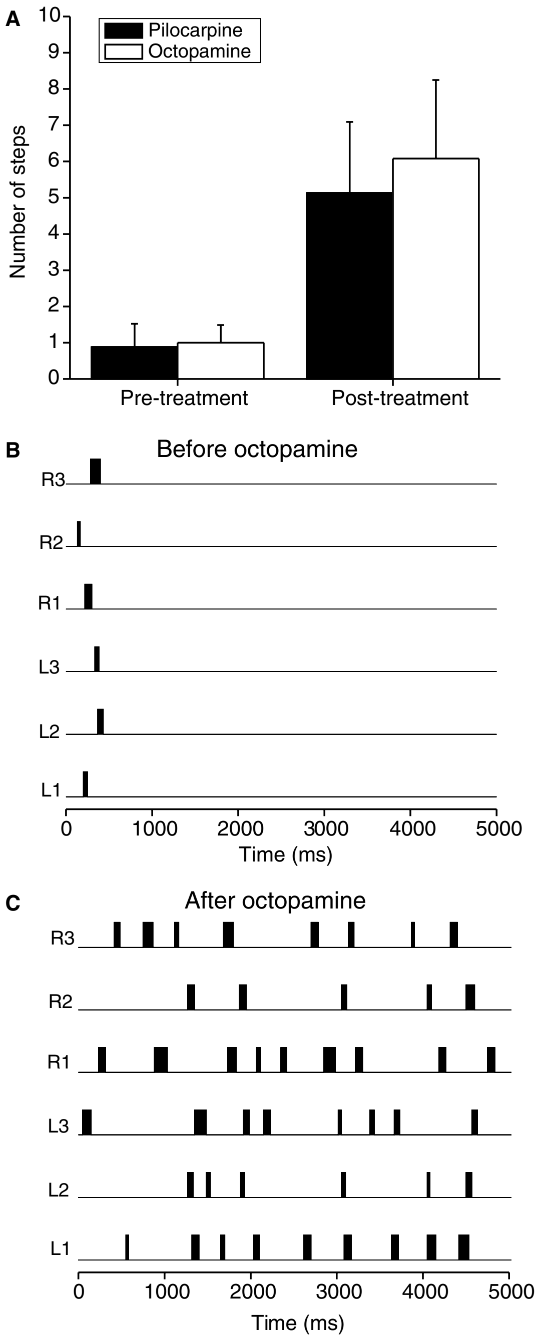


Fig. 5 a–c Changes in leg movement after injection of octopamine or pilocarpine into the hemolymph of NL animals. **a** There was a significant increase in the number of steps taken after injection of octopamine and pilocarpine. **b** Gait plot in a Day 3 post-lesion animal. Prior to injection of octopamine, stimulation elicited a response in which the legs moved a single cycle and were not coordinated. Multiple leg cycles could not be elicited in this individual. **c** Immediately after injection, the animal walked five cycles (L3) that were not regularly coordinated. *Bars* swing phase

Walking behavior in CoCL cockroaches

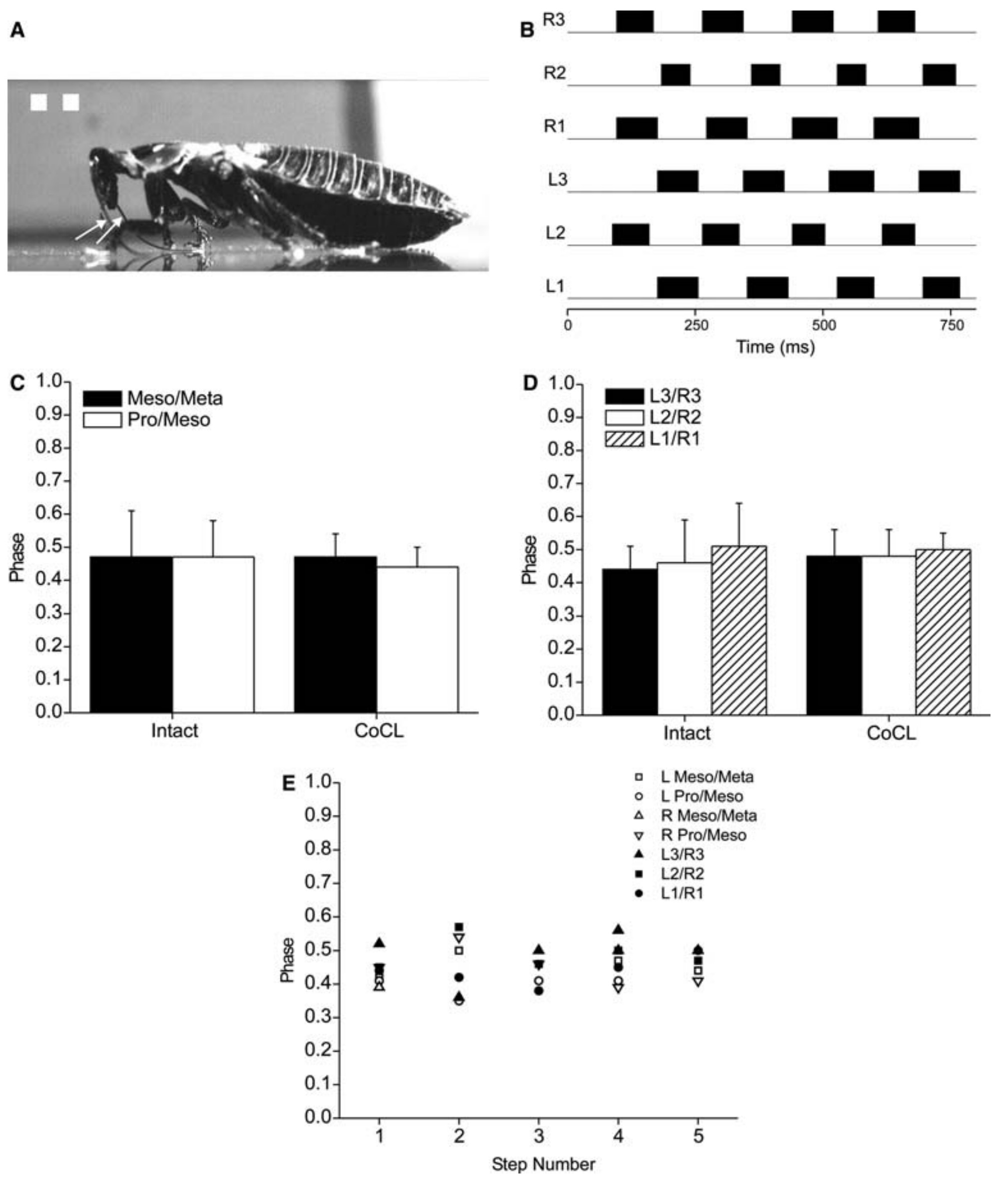
Examination of neck lesion animals shows that the head ganglia are important for coordinated walking, but it does not address the specific roles of the brain and the SOG. Therefore, we performed lesions on the circumoesophageal connectives (CoCL) between the brain and the SOG. This allows for signals to flow between the SOG and the thoracic ganglia but signals from the brain are absent. Head (7.8 ± 1.3 mm) and body posture (8.1 ± 1.2 mm) in CoCL animals are not significantly different (ANOVA, $P > 0.05$) from intact cockroaches, but the antennae drag on the ground (Fig. 6a). These cockroaches walk continuously for long periods of time in a tripod gait (Fig. 6b, Movie 2). The maximum recorded time of continuous walking in these animals was 2 min, but casual observation suggests that much longer periods often occurred. In contrast to NLs, both inter- and intra-segmental phase values of CoCLs are close to the 0.5 value observed from normal intact cockroaches (Figs. 6c, d) and changes in leg coordination with time were not detected (Fig. 6e).

Kinematics and electromyograms in CoCL and NL animals

We examined the changes in joint angles of the coxa-trochanter (CTr) joint, the femur-tibia (FTi) joint, and their related motor activity, i.e. the activity in the slow depressor of the trochanter (Ds) and the slow extensor of the tibia (SETi) in intact, CoCL and NL cockroaches. During tethered walking in intact animals, Ds is active at the beginning of extension of the CTr joint (Fig. 7a). A histogram is shown of Ds activity that was normalized to burst duration in intact cockroaches (Fig. 7b, $n = 5$ animals, 69 bursts). There are some particularly high frequency bins at the beginning of the burst and activity decreases slowly throughout the step. These findings are consistent with other studies that have examined walking in tethered animals (Tryba and Ritzmann 2000a, b), as well as in cockroaches walking on a treadmill (Watson and Ritzmann 1998). Figure 7c is an example of a Ds recording in a CoCL individual. CTr joint excursion and Ds activity is similar between CoCL and intact animals (Figs. 7a–d, 9a). The firing frequency of Ds is high at the beginning of the burst, again with a significant decrease in the last quarter ($n = 4$ animals, 84 bursts, ANOVA, $P < 0.05$, Figs. 7d, 9c). In contrast, leg movements in NL cockroaches were quite different from CoCL or intact individuals. CTr joint angle excursions were significantly reduced in NL cockroaches to half that seen in intact and CoCL animals (ANOVA, $P < 0.05$, Figs. 7e, 9a). A significant decrease in the number of potentials throughout the burst was also present in the Ds motor neuron in NL cockroaches ($n = 5$ animals, 27 bursts, ANOVA, $P < 0.05$, Figs. 7f, 9d). Interestingly, Ds was also tonically active (Mean frequency- 106 ± 15.5 spikes/s) in 60% of NL individuals when standing still. This was not observed from normal intact individuals.

The FTi joint and its related motor activity are also affected by the lesions. Figure 8a shows an example of a SETi recording and FTi joint angle in an intact animal.

Fig. 6 **a–e** Posture and leg coordination in CoCL animals. **a** Posture of the legs and body segments in a CoCL animal is similar to an intact cockroach, however their antennal posture is abnormal resulting in these individuals dragging their antennae (*arrows*) on the ground when they walk. **b** CoCL animals walk with a tripod gait. **c** Intersegmental phase values in CoCL animals are very similar to intact cockroaches (0.5). **d** CoCL animals also show intrasegmental phase values that match intact individuals (0.5). **e** intersegmental and intrasegmental phase values in a CoCL animal are consistent among five sequential steps. Each open symbol represents intersegmental (Right and Left Meso/meta, Pro/meso) and closed symbols show intrasegmental, phase values (L3/R3, L2/R2 and L1/R1)



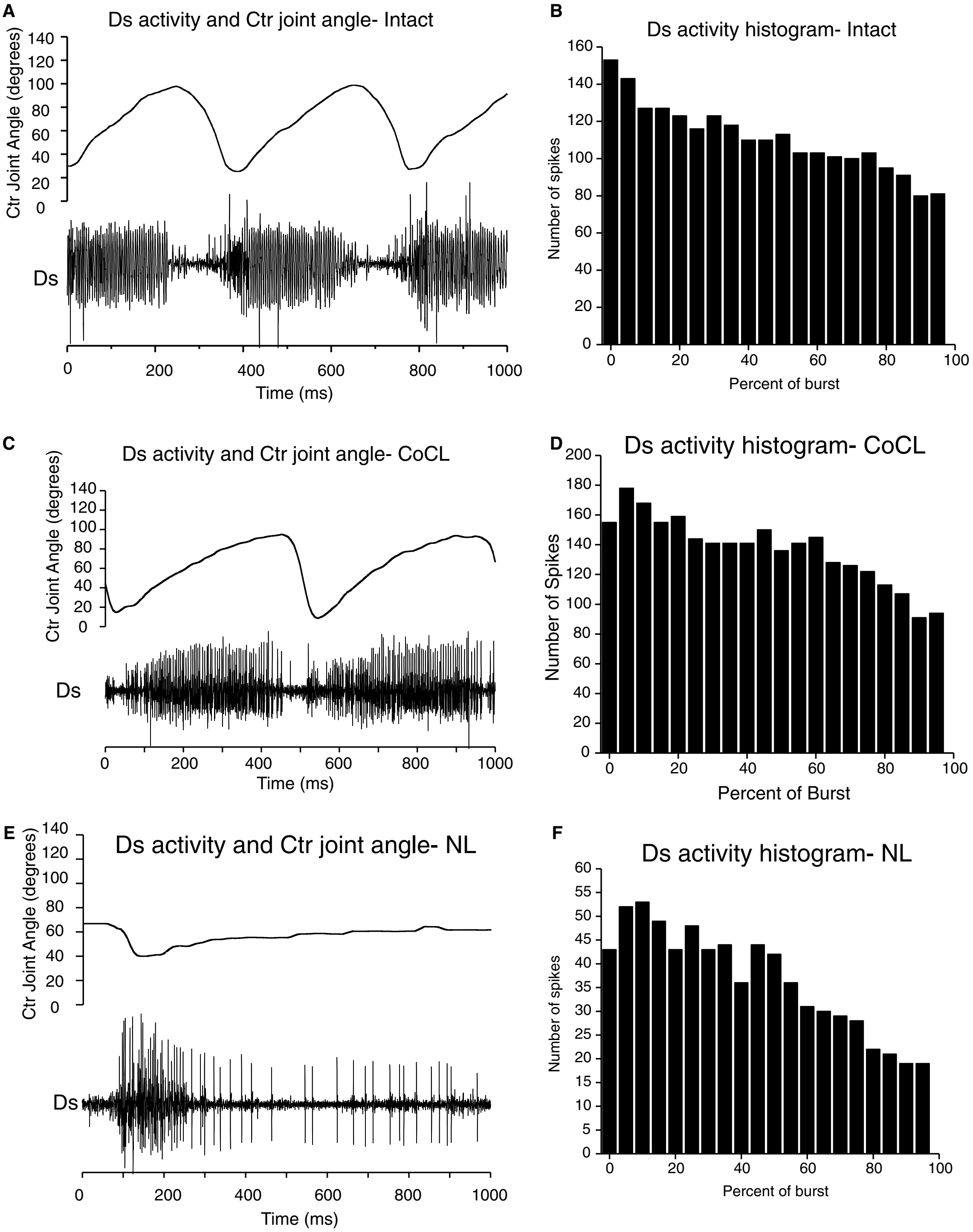
SETi is activated during joint extension and has a high-frequency component at the end of the burst ($n = 5$ animals, 69 bursts, Figs. 8b, 9b). This pattern is typical for SETi during walking (Watson and Ritzmann 1998; Tryba and Ritzmann 2000a, b). Although the kinematics of the FTi joint are similar in CoCL's (Fig. 9a), the activity pattern of SETi during the burst in CoCL animals ($n = 5$ animals, 75 bursts, Fig. 8b) is the mirror image of that recorded in intact cockroaches. Where intact recordings typically show a high-frequency component at the end (Fig. 9b), CoCL's SETi shows a high-frequency component at the beginning of the burst followed by a decrease in firing to a stable rate that is maintained throughout the burst (Fig. 9c). Again in NL individuals, FTi joint angle excursions are significantly reduced (ANOVA, $P < 0.05$, Figs. 8e, 9a). The activity pattern of SETi in NL animals ($n = 5$ animals, 23 bursts, Figs. 8F, 9d) is similar to that seen in CoCL cockroaches

▶

Fig. 7 **a–f** CTr joint angle and Ds motor neuron recordings during walking in intact, CoCL and NL animals. **a** Example of joint angle excursion and motor neuron activity in an intact animal. Ds is activated just prior to joint extension and is maintained throughout. **b** Histogram of Ds spike activity during a normalized burst in intact cockroaches. Each burst was divided into 20 bins and the total number of spikes was added across trials and individuals. The coxal depressor initially shows high frequency activity at the beginning of the burst with a gradual decline. **c** Example of joint angle excursion and motor neuron activity in a CoCL animal. Ds is activated at the beginning of joint extension and is maintained throughout. **d** Histogram of Ds spike activity during a normalized burst in CoCL animals. The coxal depressor shows activity similar to intact animals. **e** Example of joint angle excursion and motor neuron activity in a NL animal. Ds is activated just prior to joint extension and quickly decreases in activity. **f** Histogram of Ds spike activity during a normalized burst in NL animals. The coxal depressor initially shows high frequency activity at the beginning of the burst with a rapid decline to low levels of activity

(Figs. 8d, 9c), with a high-frequency component at the onset, rather than at the end as in intact animals. However, SETi was also tonically active (50.7 ± 20.5 spikes/s) in 66 % of NL individuals when standing still. As with Ds, this is rarely observed in intact standing cockroaches that have not walked recently.

Analysis of burst duration shows that there is a significant decrease in the length of SETi in NL animals when compared to intact and CoCL animals (Fig. 9e, ANOVA, $P < 0.05$). In addition, there is a significant increase in the burst duration of Ds in CoCL cockroaches that is not seen in NL's (Fig. 9e, ANOVA, $P < 0.05$). Burst duration was calculated from the first spike to the time when the frequency of activity returned to baseline levels, as seen in non-walking animals. As expected, burst duration is related to the speed of walking in intact and CoCL animals (Watson and Ritzmann 1998). NL animals sometimes move with rapid leg movements but



the burst duration is low at all speeds of leg movement. CoCL individuals walk fairly slowly at an even pace. The higher speed of NL's may be due to the fact that they had to be tapped to get any movement and may have been in escape mode. If this is the case, the fact that they rarely showed activity in fast motor neurons suggests that the system is suppressed. Ideally, it would be best to normalize the burst duration using the cycle period. However, NL animals often show long periods of time without leg movement so it is difficult to calculate an accurate cycle period.

Discussion

Although early work in insects tested the effects of brain lesions on behavior (Roeder 1937; Graham 1979), this is the first study, of which we are aware, to combine an examination of gait pattern, muscle activity and leg joint angles in cockroaches with lesions of either the neck or circumoesophageal connectives. Furthermore, early experiments did not examine long-term recovery or the effects of exogenous neuromodulators on lesioned individuals. In the following section, we discuss our observations in the context of testable hypotheses about the organization of the locomotor system of the cockroach. We also address common principles regarding descending control of locomotion for a variety of animals moving through a range of environmental situations.

Neck lesioned animals show postural and locomotor deficits

Neck lesioned cockroaches showed hyper-extended postures immediately after the lesion. Similar behavior was noted in decapitated mantids (Roeder 1937) and stick insects with a lesion of the neck connectives (Kien 1983; Graham 1979). Our studies take these observations a step further by observing changes in posture with time. Posture in neck lesioned animals relaxed within four days, upon which the animal assumed a slanted orientation where the head was lower than the rest of the body. A center for regulating posture was postulated to be in the SOG of locusts (Altman and Kien 1979). Lesion of the neck connectives removes input from the SOG to the thoracic ganglia, compromising the effect of such a center and could cause the head-down posture that we reported for NLs. In locusts, motor neurons for neck muscles are located in SOG and the nerve axons run down the length of cervical connectives (Altman and Kien 1979). Disruption of innervation to neck muscles in NL cockroaches could also contribute to the "head down" posture. However, the angle of the body after the neck lesion is more consistent with effects on the front legs. Fore limbs are often associated with exploratory movements and steering during locomotion in multi-legged walking animals (Cruse 1976; Full and Tu 1991; Jindrich and Full 1999). Therefore, the forelegs may receive more control inputs from higher brain centers.

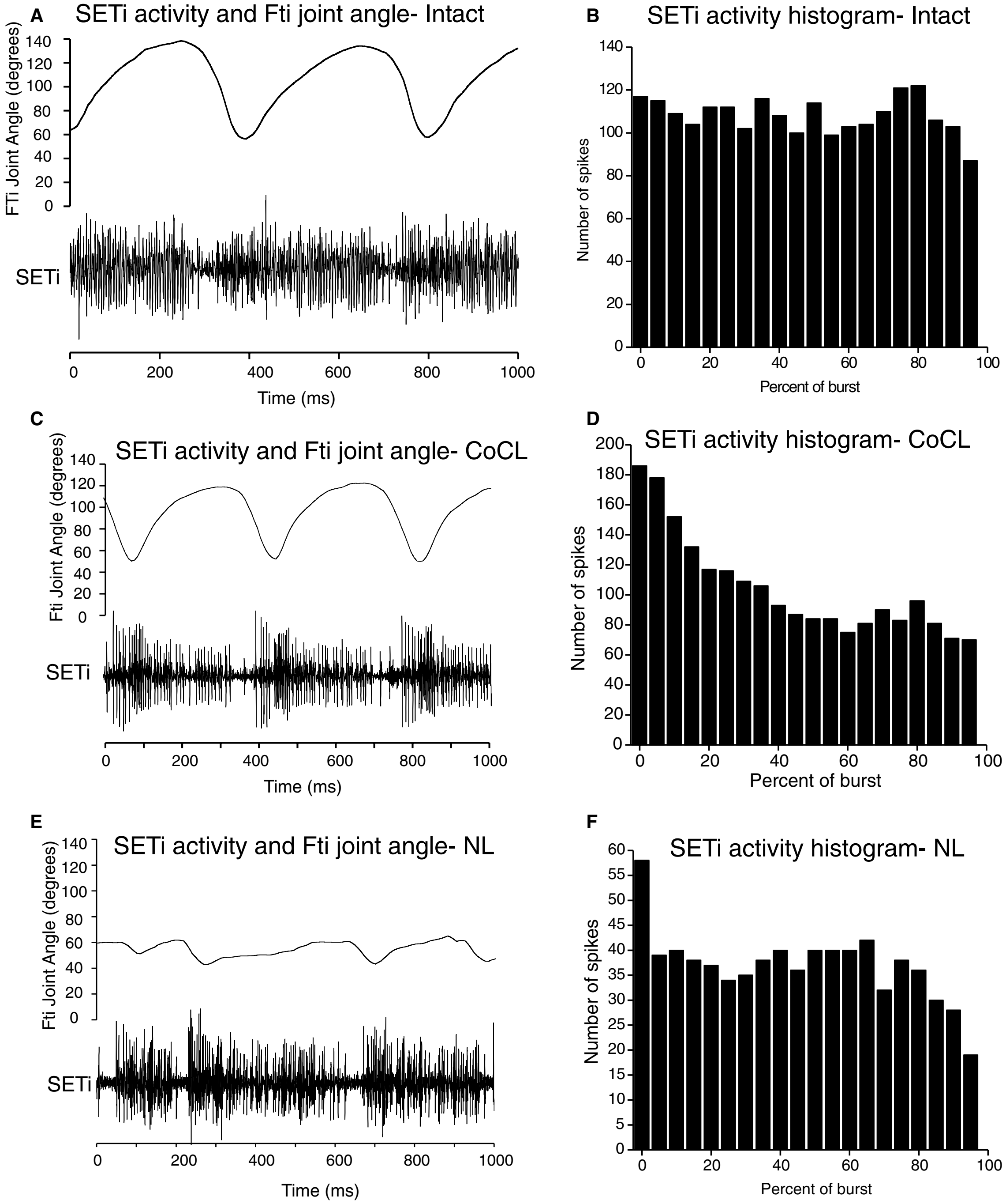
▶

Fig. 8 a–f FTijoint angle and SETi recordings during walking in Intact, CoCL and NL animals. **a** Example of joint angle excursion and motor neuron activity in an intact animal. SETi is activated at the beginning of joint extension and is maintained throughout. **b** Histogram of SETi spike activity during a normalized burst in intact cockroaches. The tibial extensors show high frequency activity at the end of the burst. **c** Example of joint angle excursion and motor neuron activity in a CoCL animal. SETi is activated at the beginning of joint extension (small unit) **d** Histogram of SETi spike activity during a normalized burst in CoCL animals. In contrast to intact animals, the tibial extensors show high frequency activity at the beginning of the burst which quickly declines to a level that is maintained throughout. **e** Example of joint angle excursion and muscle activity in a NL animal. SETi is activated during joint extension. **f** Histogram of SETi spike activity during a normalized burst in NL animals. The tibial extensors initially show high frequency activity at the beginning of the burst with a rapid decline to moderate levels of activity

Although NLs show reduced walking ability, it is not clear whether the immediate effect is due to a loss of activation or a temporary blockage resulting from the traumatic lesion. Recovery of locomotion with time has not been previously examined in insects but several studies in cats have documented a similar increase in the number of steps and changes in EMG activity in animals at 4–6 weeks after spinalization (Barbeau and Rossignol 1987; Belanger et al. 1996; De Leon et al. 1998a, b). These studies provide evidence that time-dependent plasticity in the circuitry of the spinal cord can result in changes in the stepping ability of spinal animals (De Leon et al. 1999). Indeed, locomotion in spinal cats improves after treadmill training (Lovely et al. 1986; Barbeau et al. 1993; De Leon et al. 1999). However, spinal cats have to be supported and lateral stability must be provided in order for the legs to show walking movements. Because of their sprawled hexapod structure, neck lesioned cockroaches are able to support their body weight at all times.

CoCL cockroaches show normal posture and walk with a tripod gait

Animals with lesions of the circumoesophageal connectives behave quite differently than NL cockroaches. Posture in these animals is similar to intact cockroaches with the exception of the position of the antennae. Antennae are dragged as they are in crickets after similar lesions (Horseman et al. 1997). These findings suggest that the circuits in the isolated brain are not sufficient to generate complex antennal movement. Furthermore, complete deafferentation of an antenna did not have an effect on antennal movements during the first 24 h (Horseman et al. 1997). Based on these findings, Horseman et al. (1997) concluded that the pattern generator for antennal movement resides in the SOG. Stick insects with circumoesophageal lesions also show posture that is indistinguishable from intact animals but again the antennae drag on the ground (Graham 1979).



However, Graham noted that this lesion actually cuts the musculature for the antennae. Dissections of the head capsule of CoCL cockroaches do not show damage to the antennal muscles.

Of greater significance to our study is the observation that CoCL animals walk in a perfect tripod gait for very long periods. This observation is very different from our findings on NL's. Similar results were also found in mantids (Roeder 1937) and stick insects (Graham 1979). Although the walking movements of CoCL's seem very normal, more detailed observations suggest that this is not the case. Even at the behavioral level, the loss of inputs from sensors mounted upon the head and the ability to integrate these inputs and adapt the pre-motor commands to local environments cause CoCL animals to bump into objects more often and results in a rigid stereotyped gait pattern.

Hypotheses for the role of the brain and the SOG in complex locomotion

Our data clearly indicate that removal of the head ganglia adversely affects walking behavior in insects.

However, an important question remains. How do the neural circuits within the head ganglia impact local control within the thoracic ganglia? Previous lesion studies suggested that brain regions simply turn on/off motor patterns or provide tonic activation that could influence speed of walking (Roeder 1937). However, our data suggest a more subtle but important role for descending activity.

The observations on NLs, coupled with the perfect tripod gaits seen in CoCLs, imply an important role for SOG circuitry in actively maintaining intersegmental coordination. The circuitry that is necessary for generating the tripod gait clearly resides in the thoracic ganglia, because occasional tripod steps were observed even in NLs. However, in the absence of functional connections with the SOG, expression of that pattern is limited. The exact role of the SOG in this process is unclear. It could simply provide tonic signals that control stepping speed. If the SOG's primary role involves speed control, coordination could be a secondary effect. As the animal moves slower, the tripod gait gives way to metachronal patterns and at very slow speeds random stepping could occur. However, if this is the case, one would expect that enhanced activation with modulators would not only increase step number but also return coordination. Step number increased without a change in coordination when animals were injected with pilocarpine or octopamine.

The fact that intersegmental coordination did not recover with increased activity suggests that descending interneurons play a greater role than simply increasing tonic activation of thoracic neurons. A more likely

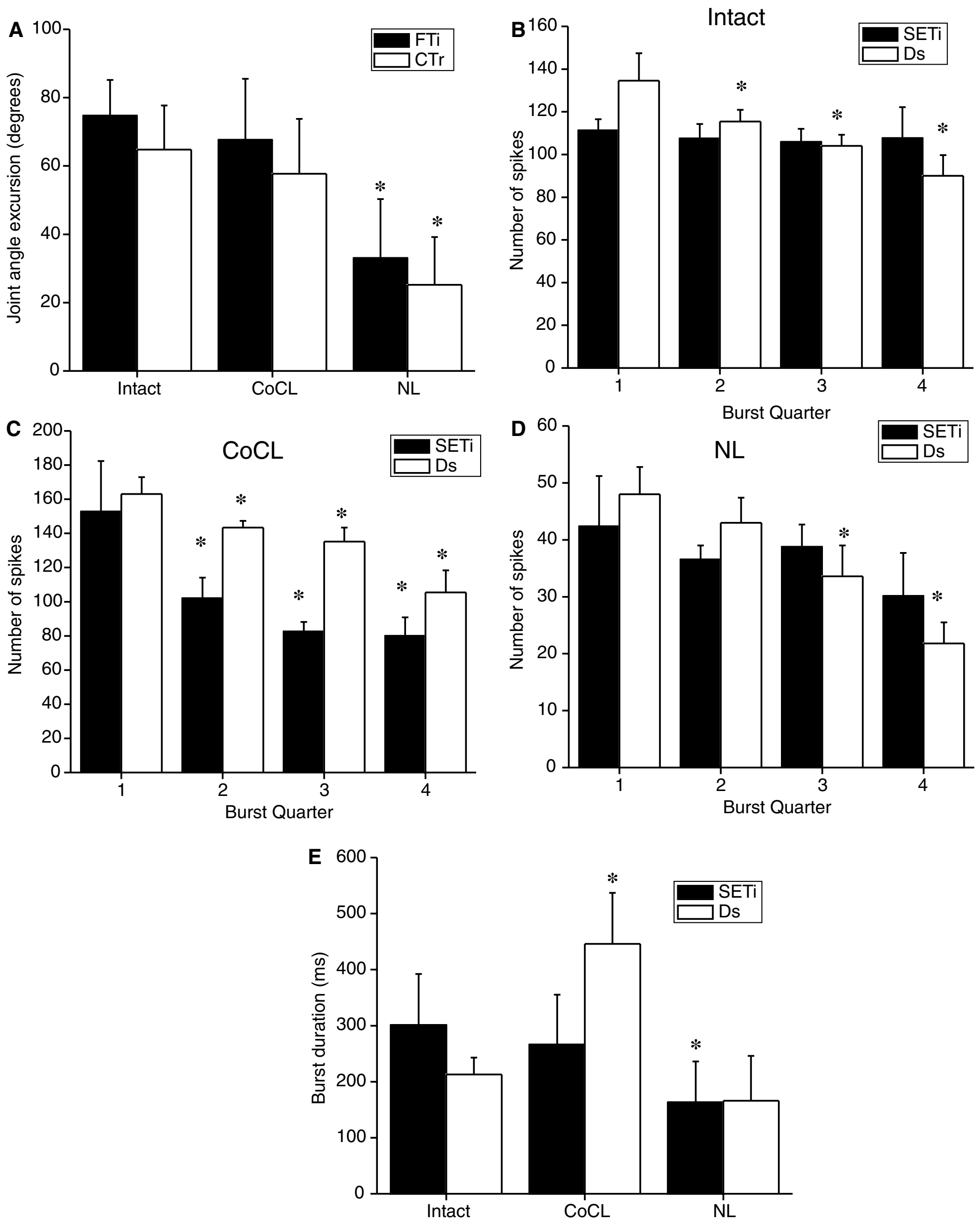


Fig. 9 a–e Joint angle excursions and motor activity during walking in Intact, CoCL and NL animals. **a** Excursions of the FTi and CTr joints in the metathoracic leg are similar between intact ($n = 5$ animals, 33 cycles) and CoCL individuals ($n = 9$ animals, 123 cycles) but significantly reduced in NL ($n = 12$ animals, 76 cycles) cockroaches. **b–d** Each burst of muscle activity was divided into four equal quarters and the average number of spikes was calculated during each of these quarters. In intact animals (**b**), there is a significant reduction in the activity of Ds after the first quarter. SETi activity remains fairly constant throughout the burst. In CoCL individuals (**c**) Ds activity followed a similar pattern. However, SETi fires at a high frequency during the first 25% of the burst followed by a significant decrease after first quarter. In NL animals (**d**), SETi activity gradually declines throughout the burst while Ds shows a rapid and significant decrease during the last half of the burst. **e** Burst duration in Ds is significantly increased in CoCL animals when compared with intact animals. In addition, there is a significant decrease in the burst duration of SETi in NL animals when compared to CoCL and intact cockroaches (in all cases $*P < 0.05$, ANOVA) possibility is that the SOG provides descending activity that potentiates (or even makes possible) proprioceptive reflexes. A loss of reflex activation in neck lesioned animals could also impact the failure to recover intersegmental coordination. The influences that have been described for intersegmental coordination in stick insects (Cruse 1985; Cruse and Schwarze 1988) rely upon reflex circuitry and could be reduced to such a level that normal swing times would be lost. Further studies are planned that will record activity of leg proprioceptors, such as campaniform sensilla, in NL animals to examine the influences of the brain and SOG on these reflex pathways during locomotion.

The changes in motor patterns and joint kinematics in NL animals also imply a deficit in proprioceptive reflex modulation. In walking cockroaches, the typical pattern of Ds activity includes a high-frequency burst that begins prior to foot touchdown (Watson and Ritzmann 1998). This activity is followed by a more variable period that, on a flat horizontal surface, declines toward the end of the burst. During climbing, this period appears to be enhanced, presumably through local reflexes, in order to deal with increased force requirements to push the animal upward (Watson et al. 2002). This modifiable period of the stance cycle appears to be lost in the NLs, resulting in a reduced burst period and limited joint extension. That observation could be explained if the latter part of the Ds burst is not only influenced by reflexes generated by sensors on the legs, but requires some descending activity in order to reach threshold and influence motor activation.

Consider the possibility that descending signals from the head ganglia impose subtle changes in the motor patterns through modulation of these reflexes. It is unlikely that changes in locomotion bypass the local circuits within the thoracic ganglia. Rather, it is probable that they impart changes through modifying of them. Detection of a barrier could generate descending signals that place the animal in the appropriate posture to climb over or tunnel under the object, or could alter leg movements sufficiently to evoke a turn. By completely severing the cervical connectives, the reflex activation of leg motor neurons could be lost, leaving the animal to move simply through its pattern generation circuitry. Such a change would allow movements, but would reduce effectiveness and provide for little adjustment in response to barriers or changes in substrate. Indeed, the animal would be reduced to an automaton with shallow movements, similar to what we observed in the NL animals.

The variability seen in SETi burst pattern could occur through a similar change. In intact animals SETi typically increases to a high-frequency period near the end of the burst. In CoCLs and NLs SETi fires in a high frequency burst at the onset that declines through stance phase. It is possible that the distal joints that are controlled by SETi require some reflex activation to prevent collapse as the animal moves through stance. In both cats and cockroaches, extensor activity after the initial burst is mediated by loading and muscle force (Hiebert and Pearson 1999; Noah et al. 2001). Activity of the proximal campaniform sensilla on the tibia excites SETi in restrained and freely moving cockroaches (Zill et al. 1981; Ridgel et al. 2001). Sensory discharges early in stance result from forces generated by muscle contractions that press the leg against the substrate. At the end of stance, as the leg is unweighted, force feedback may also adjust motor output to changes in leg loading (Noah et al. 2001). Therefore, a loss of reflex activity could leave the pattern seen in the lesioned animals.

There is precedence for similar coordinating roles in descending activity of other animals. Intersegmental coordination in the lamprey has been extensively studied. During swimming, bursts of activity in the body wall muscles alternate between left and right sides and a constant phase lag occurs among muscle bursts in successive segments (Wallen and Williams 1984). Nevertheless, swimming is believed to be initiated in the basal ganglia that projects to areas in the brain stem (El Manira et al. 1997). These studies in lamprey suggest that initiation of locomotion occurs through disinhibition of the brainstem areas by the brain (Grillner 2003). While the isolated spinal cord can produce intersegmental coordination with a constant rostro-caudal lag (Grillner et al. 1995), studies using the brain–spinal cord preparation have shown that feedback from the brain to the spinal cord plays an important role in the generation and adjustment of locomotion (Wang and Jung 2002). Specifically, supraspino-spinal feedforward-feedback loops provide modulation and dynamic control of spinal neural circuits by increasing variability in the rhythmic motor output of the neurons in the spinal cord. These loops have been shown to alter intersegmental delay of locomotor activity in the brain-spinal cord preparation of the lamprey (Wang and Jung 2002). In the intact lamprey, variability in interaction between the brain and the spinal cord would provide the nervous system with a fast response to environmental perturbations. Although the exact mechanisms of this descending control in lamprey are not known, acetylcholine appears to be important in intersegmental coupling because it can modulate phase lag between segments (Quinlan et al. 2004). Acetylcholine-producing neurons have been found in the mesencephalic region of the brainstem that sends descending fibers to the spinal cord (Pombal et al. 2001).

Another observation that supports this notion that descending signals modulate local reflexes is the ‘head-down’ posture seen in NLs. It is not surprising that cervical lesions would have a particularly strong effect on front leg posture. After cervical lesion, signals that normally evoke escape responses still generate movements in hind and middle legs, but fail to drive fast motor neurons of the front legs to threshold (Schaefer and Ritzmann 2001). This could occur because the total loss of descending signals renders local reflexes in the prothoracic ganglion ineffective. A similar effect is observed in decerebrate or spinal cats walking on a treadmill. In these animals, the rear limbs can show walking movements but the front legs are not active (Rossignol et al. 1996). Cortical signals seem to be important in voluntary gait modifications in the forelimbs (Drew 1988, 1993; Drew et al. 2004). The motor cortex does not act independently to alter gait, but acts through interneurons that are part of, or are influenced by, the central pattern generator in the spinal cord.

Overall, these results provide evidence that descending input from the head ganglia can influence locomotor patterns beyond initiation of activity. This study suggests that both the brain and the SOG can modulate thoracic circuits and reflex pathways to alter the motor patterns of the legs, making them more effective in immediate situations. Experiments such as these will bring us closer to understanding how the neural circuits within the head impact local control within the thoracic ganglia and provide important insight into the neural control of locomotion.

Movie 1. Video of walking in a 9 day post-lesion NL animal as viewed from below via a mirror. Note that movement is slow but animal is moving forward and the legs are moving through swing and stance phases

Movie 2. Video of CoCL animal walking in a treadmill. Note the tripod gait and the antennae position of this animal

Acknowledgements Special thanks to S.N. Zill and A.J. Pollack for their advice and help on this project. We would also like to thank Dr. Mark Willis and two anonymous reviewers for helpful comments on the manuscript. This work was supported by NIH Grant NRSA F32-NS43004 to ALR and Eglin AFB Grant F08630-01-C-0023 to RER.

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