Reconfiguration of the Respiratory Network at the Onset of Locust Flight

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Ramirez, Jan-Marino. Reconfiguration of the respiratory network at the onset of locust flight. J. Neurophysiol. 80: 3137–3147, 1998. The respiratory interneurons 377, 378, 379 and 576 were identified within the suboesophageal ganglion (SOG) of the locust. Intracellular stimulation of these neurons excited the auxiliary muscle 59 (M59), a muscle that is involved in the control of thoracic pumping in the locust. Like M59, these interneurons did not discharge during each respiratory cycle. However, the SOG interneurons were part of the respiratory rhythm generator because brief intracellular stimulation of these interneurons reset the respiratory rhythm and tonic stimulation increased the frequency of respiratory activity. At the onset of flight, the respiratory input into M59 and the SOG interneurons was suppressed, and these neurons discharged in phase with wing depression while abdominal pumping movements remained rhythmically active in phase with the slower respiratory rhythm (Fig. 9). The suppression of the respiratory input during flight seems to be mediated by the SOG interneuron 388. This interneuron was tonically activated during flight, and intracellular current injection suppressed the respiratory rhythmic input into M59. We conclude that the respiratory rhythm generator is reconfigured at flight onset. As part of the rhythm-generating network, the interneurons in the SOG are uncoupled from the rest of the respiratory network and discharge in phase with the flight rhythm. Because these SOG interneurons have a strong influence on thoracic pumping, we propose that this neural reconfiguration leads to a behavioral reconfiguration. In the quiescent state, thoracic pumping is coupled to the abdominal pumping movements and has auxiliary functions. During flight, thoracic pumping is coupled to the flight rhythm and provides the major ventilatory movements during this energy-demanding locomotor behavior.

INTRODUCTION

The respiratory control system of invertebrates and vertebrates must be capable of interacting with other neuronal networks in a state-dependent manner. It has to adapt to an alteration in the animal’s behavioral state to 1) maintain constant oxygen supply and 2) coordinate the muscles that control the airflow with muscles that are activated during the new behavioral act. Oxygen demand is particularly high during locomotion. Thus two strategies were described in various respiratory systems: 1) the recruitment of additional auxiliary muscles, which increases the airflow (van Lunteren and Dick 1997), and 2) the synchronous activation of muscles involved in respiration and locomotion, which is an energy-efficient mechanism to utilize locomotor activity for controlling airflow (Bramble and Jenkins 1993; Funk et al. 1992a,b; Paterson et al. 1987). Adaptive mechanisms are also critical during behaviors that involve the activation of respiratory muscles in a multifunctional manner. Respiratory muscles are activated during vocalization, vomiting, coughing, sneezing, and swallowing, and it is essential to control the airflow by resetting the respiratory system (Dick et al. 1993; Jean et al. 1997; Larson et al. 1994; Martin et al. 1994; Miller and Yates 1993; Shannon et al. 1997; Widdicombe 1995). This raises the general issue of how the coordination of the respiratory system is controlled by the nervous system.

In mammals the same neurons in the ventral respiratory group (VRG) of the medulla are activated during breathing and nonrespiratory behaviors (Chiao et al. 1994; Dawid-Milner et al. 1993; Fukuda and Koga 1995; Gestreau et al. 1996; Yajima and Larson 1993). The activation pattern of respiratory neurons changes considerably during these nonrespiratory behaviors, suggesting that the respiratory network is reconfigured (Grelot et al. 1993; Koga and Fukuda 1994). Therefore it was proposed that principles of network reconfiguration as demonstrated in detail for the stomatogastric system of crustacean should also apply for the respiratory system (Dickinson 1995; Dickinson et al. 1990; Meyrand et al. 1991, 1994; Weimann et al. 1991). However, in a mammalian network it is difficult to prove that the same neurons that are activated during breathing and nonrespiratory behaviors are also part of the rhythm-generating network. Although there was great progress in localizing the site for respiratory rhythm generation in mammals (Ramirez et al. 1998; Rekling and Feldman 1998; Smith et al. 1991) and lower vertebrates (Togerson et al. 1997), it remains unknown which neurons are part of the oscillator and which are not. This level of investigation is more easily achieved in invertebrates.

Here the respiratory system of the locust was used to address at the single cell level the issue of how the respiratory rhythm-generating network behaves during a state-dependent alteration. Although the locust respiratory system is not homologous with the vertebrate respiratory network, it has to fulfill similar functional roles. It has to maintain constant oxygen supply and coordinate respiratory and nonrespiratory muscles during a behavioral change. As in mammals auxiliary respiratory muscles are recruited in a state-dependent manner.

In the quiescent locust auxiliary muscles in the prothorax and neck can enhance the airflow, which is normally provided by abdominal pumping movements (Miller 1960a). However, the relative importance of abdominal and auxiliary pumping movements changes during flight. Abdominal pumping movements alone become insufficient and even nonessential for oxygen supply, whereas prothoracic and neck muscles become the major respiratory muscles during flight (Miller 1960a). A muscle that is critical for protho-
Auxiliary pumping is the muscle 59 (Miller 1960a; Snodgrass 1929). So far it is unknown how auxiliary muscles such as motoneuron 59 are controlled by the CNS. However, it was suggested that the head ganglia play a major role in controlling these muscles. Miller (1960a) demonstrated that carbon dioxide receptors on the head increase abdominal pumping, induce neck and prothoracic ventilation, and cause contraction of spiracle I (Miller and Mills 1976). This effect might be mediated by neurons in the suboesophageal ganglion (SOG). We previously reported one neuron in this ganglion which was part of the respiratory oscillator (Ramirez and Pearson 1989a), and similar neurons were also identified in crickets (Otto and Campan 1978; Otto and Hennig 1993; Otto and Weber 1982). Furthermore, lesion experiments have shown that the SOG strongly affects the ventilation frequency (Huber 1960). In this study, additional respiratory neurons are characterized within the SOG, and it will be demonstrated that these neurons control the activity of auxiliary motoneuron 59. To address the issue of how these SOG neurons behave during a behavioral change their activity was examined at the onset of flight. As will be demonstrated the SOG neurons, which are elements of the respiratory oscillator, are also activated in the flight rhythm, indicating that portions of the respiratory oscillator must be reconfigured in a state-dependent manner. This neural reconfiguration seems to adapt the prothoracic pumping movements to a different functional role during flight.

METHODS

Adult male and female Locusta migratoria were kept in crowded colonies with a light/dark cycle of 12 h. Experiments were performed at room temperature (22–25°C).

**FIG. 1.** Auxiliary muscle 59 is activated in phase with expiratory activity as demonstrated by simultaneous electromyography (EMG) recordings from muscle 59 (*top traces*) and abdominal muscle 179 (active in phase with expiration, *bottom traces*, A). However, even in the same animal, activity in M59 can cease for several cycles (B). Thus M59 is not always activated during each respiratory cycle. C: camera lucida drawing from the motoneuron innervating muscle 59. The neuron was stained intracellularly with Lucifer yellow. D: intracellular recording from motoneuron 59 (*top trace*) reveals a rapid onset of activity (*top trace*) that coincides with the activation of the abdominal, expiratory muscle 179 (*bottom trace*).
and labial neuromere. All neurons of this type \( n \) had a cell body on the ventral midline of the anterior labial neuromere and a contralateral axon descending at least into the mesothoracic ganglion. The bilateral arborizations extended through the maxillary, mandibulary, and labial neuromere. All neurons of this type \( (n = 14) \) had a very thin process ascending into the anterior connective (Fig. 2A). Another secondary process projected into the ipsilateral connective, which however did not continue into the prothoracic ganglion.

Interneurons within the suboesophageal ganglion

The suboesophageal ganglion contained some neurons that discharged in phase with expiration. Interneuron 378 (Fig. 2A) had a cell body on the ventral midline of the anterior labial neuromere and a contralateral axon descending at least into the mesothoracic ganglion. The bilateral arborizations extended through the maxillary, mandibulary, and labial neuromere. All neurons of this type \( (n = 14) \) had

FIG. 2. Suboesophageal ganglion interneuron 378 is activated in a variable manner. A: camera lucida drawing of the interneuron 378. B: intracellularly recorded interneuron 378 (top trace) is activated in phase with expiration as indicated by the extracellular recording from abdominal muscle 179 (Exp, bottom trace). C: interneuron 378 (top trace) received 2 components of phasic synaptic input. A weak plateau-like depolarization in phase with abdominal expiration (M179, Exp, bottom trace), this component was also seen in the absence of activity in muscle 59 (middle trace); an additional depolarization that correlated with the activation of muscle 59. D: in some instances, during weak ventilation, interneuron 378 (top trace) was only rhythmically active during muscle 59 discharge (bottom trace). The recordings in this B–D were obtained from different preparations.
Interneuron 377 (Ramirez and Pearson 1989a), interneuron 378 was sometimes not activated in a pronounced rhythmic manner during weak ventilation (Fig. 2D). Only when muscle 59 was activated vigorously did interneuron 378 discharge in phase with this auxiliary muscle (Fig. 2D, bottom trace).

Interneuron 379 (Fig. 3A) was also activated in a variable manner. The soma of this interneuron lies laterally in the maxillary neuromere of the SOG. The axon descends contralaterally at least to the metathoracic ganglion. The bilateral arborization extends through all SOG neuromeres. This neuron was mostly inactive during weak respiration and only activated during increased activity in muscle 59 and increased activity in the expiratory muscle (Fig. 3B).

Interneuron 576 was characterized by a cell body localized at the ventral midline of the labial neuromere. Its axon ascends in the connectives contralateral to the cell body at least into the tritocerebrum. The processes of 576 are symmetrically distributed within the labial and maxillary neuromeres (Fig. 3C). In 1 of 19 cases, a physiologically and anatomically similar neuron was stained with an ascending and descending axon. Interneuron 576 discharged in phase with expiration (Fig. 3D). The neuron was activated in a decrementing pattern, i.e., its major activity occurred at the onset of expiration. Maximal inhibition of the neuron occurred just preceding expiration.

**SOG neurons affect the activity of muscle 59**

All interneurons in the SOG had not only an activity pattern that corresponded with the activity of the auxiliary muscle 59 but they also affected the activity of this muscle. Examples for each identified SOG neuron are shown in Fig. 4. The bridge balance of the amplifier was not adjusted properly; thus a 0.1-Hz filter was used to show the evoked activity in these neurons. During this stimulation respiratory-related modulation of rhythmic activity persisted, but the neurons discharged also during the inspiratory phase. In all examined cases with a simultaneous recording of the muscle 59 and abdominal muscle (576, n = 2; 377, n = 4; 378, n = 3; 379, n = 1), 1–3 nA of depolarizing current injection led to the activation of muscle 59 as well as to an increased activity in abdominal expiratory muscles (Fig. 4, A–D). However, the number of experiments was too low to allow a quantitative evaluation of this phenomenon. However, as qualitatively shown in Fig. 4B, the activity of the inspiratory muscle was decreased during the activation of the SOG neuron. Note also that the frequency of respiratory rhythmic activity increased, indicating that these SOG neurons affected the respiratory rhythm generator. The excitatory effect on motoneuron 59 was most likely indirect. Simultaneous recordings from 378 and motoneuron 59 revealed that action potentials in SOG neurons were not followed in a 1:1 manner by excitatory postsynaptic potentials in motoneuron 59. However, this simultaneous recording was successful in only one experiment and was therefore not further evaluated.

**SOG interneurons as elements of the respiratory rhythm generator**

In accordance with previous studies (e.g., Friesen et al. 1978; Ramirez and Pearson 1989a; Weeks 1981) a neuron can be considered an element of the respiratory rhythm generator if 1) it is rhythmically polarized in phase with the respiratory rhythm and 2) current injection into this neuron can shift the phase of the respiratory rhythm (i.e., reset the rhythm).
Alteration in the activity pattern of SOG neurons and motoneuron 59 at the onset of flight

At the onset of flight, motoneuron 59 changed its activity pattern and started to discharge in phase with wing depression (Fig. 6A). Each wingbeat cycle led to a rhythmic depolarization in the motoneuron, which was accompanied by the generation of two to four action potentials. Thus thoracic pumping switched from being in phase with abdominal pumping in the quiescent locust to an activity occurring in phase with the wing depressor rhythm.

The SOG interneurons 377 (Fig. 6B), 378 (Fig. 6C), 379 (Fig. 6D), and 576 (Fig. 6E) exhibited a similar change in their activity and locked on to the flight rhythm. In all neurons the phase-locked depolarization started 0–7 ms before and ceased 25–40 ms after subalar EMG activity (muscle 129, a depressor; Fig. 6). Simultaneous intracellular recordings from suboesophageal ganglion interneurons (377, 378, and 379; Fig. 7) and thoracic depressor motoneurons reveal that the SOG interneurons reached the peak of rhythmic depolarizations after the peak of depolarizations in thoracic motoneurons. The first action potential in the interneurons often coincided with the last action potentials in the depressor motoneurons (Fig. 7, A and B). Thus, based on the activation time, it is unlikely that these interneurons could contribute to the activation of depressor activity in the flight rhythm generator. Simultaneous intracellular recordings with thoracic elevator motoneurons reveal that the last action potentials in the SOG interneurons often coincided with the first action potentials in elevator motoneurons (shown for IN 379, Fig. 7C). Thus these neurons are activated during depression but discharge into the interphase between wing depression and elevation, an activation pattern that is distinct from the known flight interneurons within the thoracic flight rhythm generator (compare, e.g., Robertson and Pearson 1982). Each wingbeat led to a rhythmic depolarization with one to six interneuronal action potentials. However, the activation pattern of these neurons differed at the onset of wind-induced flight rhythmic activity. The interneurons 377 and 576 were depolarized just after the onset of wind (presumably by the wind sensory stimulus). The activity started before the first elevator activity and also before interneuron 379. Interneuron 379 was strongly depolarized 40–60 ms after wind onset. This latency was remarkably long and variable. Thus the flight initiating wind information must have reached the thoracic ganglia before this interneuron was excited. The activation of interneuron 379 coincided with the activation of elevator neurons (Fig. 7C).

The ascending mesothoracic interneuron 404, which is known to be involved in the initiation of flight (Pearson et al. 1985; Ramirez 1988), was excited 10–12 ms before the activation of interneuron 379 as revealed by simultaneous intracellular recordings (not shown). Interneuron 378 was hyperpolarized before the onset of flight (Fig. 7A).

After a flight sequence SOG neurons resumed respiratory rhythmic activity. However, the onset of the first expiratory burst, which followed flight termination, was variable. As shown in Fig. 8, the first expiratory burst followed either shortly after flight termination (Fig. 8A), directly after flight termination (Fig. 8B), or relatively late after flight termination (Fig. 8C). Thus there seems to be no strict coupling between both motor patterns at the offset of flight. This differs from the onset of flight, which was always associated with a reset of the respiratory rhythm in the abdomen (Ramirez and Pearson 1989b).
FIG. 5. Stimulation of the suboesophageal ganglion interneurons 378, 576, and 379 reset the respiratory rhythm. A: intracellular current injection into the identified interneuron 378 (top trace) causes a shortening of the respiratory cycle as indicated by a simultaneous EMG recording of muscle 179 (Exp, middle trace). The bottom trace represents unaffected respiratory cycles to better demonstrate the evoked shortening effect. B: reset curve obtained from 3 individual animals. The procedure to obtain these curves was described previously by Ramirez and Pearson (1989a,b). C: reset curve obtained from interneuron 379. D: reset curve obtained from 2 individual neurons 576.

Suppression of the respiratory rhythmic input into the SOG neurons during flight

In the SOG neurons, the respiratory rhythmic depolarizations that occurred in the quiescent animal were in all cases suppressed at the onset of flight. As exemplified for interneuron 576 (Fig. 9A), there was an inhibition of the respiratory depolarization after the wind stimulus (arrow). The interneuron was then rhythmically active in phase with wing depression. The initial suppression of the respiratory activity was most likely induced already by the wind stimulus. In some cases as shown in Fig. 9B the respiratory rhythmic input was reduced even if no flight was initiated. Toward the end of the flight sequence the respiratory and flight rhythmic depolarizations overlapped as indicated by the gray shading (Fig. 9A). Each filled block indicates a wing depression.

Tonic interneuron 388 suppresses the respiratory rhythmic input to motoneuron 59

A possible candidate for a neuron that might be involved in the suppression of the respiratory rhythmic input in these neurons is interneuron 388 (Ramirez 1988) (Fig. 10). As described previously, this interneuron received indirect ex-
At the onset of flight the respiratory rhythm driving these abdominal pumping movements is reset, i.e., expiratory interneurons and motoneurons located within the abdominal and thoracic ganglia are initially inhibited (Fig. 11B, bottom left panel) and inspiratory interneurons are excited (Ramirez and Pearson 1989b). Neurons in the abdominal and thoracic ganglia maintain respiratory rhythmic activity at a higher frequency (Fig. 11A) throughout the entire flight sequence (Fig. 11B, bottom left panel) (Ramirez and Pearson 1989b). The interneurons controlling the abdominal pumping movements are part of the respiratory rhythm generator (Ramirez and Pearson 1989a). The reset of the respiratory rhythm and the increase in the respiratory rate is, at least partly, due to a feed-forward mechanism. Interneurons involved in controlling the alterations of the respiratory system also contribute to the initiation of flight (Ramirez 1988; Ramirez and Pearson 1989b). A similar strategy, i.e., an increase in the respiratory rate in anticipation of need, was also described in the respiratory system of mammals (DiMarco et al. 1983; Eldridge et al. 1981, 1985; Feldman 1986).

In this study I demonstrated that the respiratory rhythm generator is not only reset at the onset of flight but is also reconfigured. A group of identified interneurons within the...
suboesophageal ganglion was shown to participate in the generation of the respiratory rhythm in the quiescent locust. When electrically stimulated, these neurons were able to reset the respiratory rhythm and increase the frequency of respiratory rhythmic activity. At the onset of flight the respiratory rhythmic input into these interneurons was suppressed and they discharged in phase with the flight rhythm. Although these interneurons were able to excite thoracic flight interneurons (Ramirez, unpublished observation) it was not possible to show that they became elements of the flight rhythm generator itself. In no case was it possible to reset the flight rhythm by stimulating a SOG neuron. Thus these SOG neurons seem not to be involved in the generation of the flight rhythm. However, because stimulation of these neurons affects the activity of motoneuron 59, they seem to have an important function in the control of thoracic pumping movements. These thoracic pumping movements are

**FIG. 10.** A likely candidate mediating the suppression of respiratory rhythmic input, the suboesophageal ganglion interneuron 388. Simultaneous intracellular recordings revealed that action potentials in interneuron 388 were not followed in a 1:1 manner by excitatory postsynaptic potentials in motoneuron 59, suggesting that both neurons are connected indirectly. This indirect excitatory connection is symbolized in A by a broken line. B: during flight, which was evoked by a wind stimulus (bottom trace), interneuron 388 is tonically activated (top trace), whereas the simultaneously recorded motoneuron 59 (2nd trace) is rhythmically active in phase with wing depression as indicated by the simultaneous EMG recording from subalar muscle 129 (3rd trace). C: simultaneous intracellular recording obtained from interneuron 388 (top trace) and motoneuron 59 (bottom trace) in a quiescent locust. D: intracellular stimulation of 388 (top trace) induced a complete suppression of the respiratory depolarizing input in motoneuron 59 (bottom trace). The bridge in interneuron 388 was not balanced properly. Thus no action potentials are visible during positive current injection (+DC and arrow) into interneuron 388. E: slight current-induced increase (+DC and arrow) in the spontaneous activity of interneuron 388 (top trace) decreased significantly the respiratory rhythmic input into motoneuron 59 (bottom trace).

**Fig. 9.** Respiratory rhythmic input into the interneuron 576 is suppressed during flight, which was induced by a wind puff (arrow). A: intracellular recording from interneuron 576 obtained subsequently in the quiescent locust (A, top trace, shading indicates respiratory rhythmic depolarization), at the onset of flight (A, 2nd trace, shading indicates the beginning of the respiratory rhythmic depolarization that preceded the onset of flight), during flight (A, 3rd trace), and at the end of flight (A, 4th trace). Fourth trace: each wing depression as reflected in a fast depolarization in interneuron 576 is indicated by a black block to better illustrate the overlap between respiratory (shaded area) and flight rhythmic input (blocks). B: initial suppression of the respiratory depolarizing input in interneuron 576 (top trace) was most likely elicited by the wind stimulus (bottom trace, recording from the magnetic valve that controlled the air stream). Note that a suppression of the respiratory input was also evident if a wind stimulus did not induce flight rhythmic activity (middle trace, EMG from muscle 129).

It was previously described that the muscle 59 is involved in the control of thoracic pumping, which has an auxiliary function in the quiescent animal (Miller 1960a; Miller and Mills 1976). It was demonstrated in this study that its activation is consistent with its auxiliary role. During weak ventilation, this muscle was often inactive but was recruited during vigorous ventilation. A recruitment of additional respiratory muscles during increased oxygen demand is also a common principle in the control of mammalian breathing (e.g., Ainsworth et al. 1989).
In locusts, the recruitment of additional muscles is accompanied by the recruitment of additional elements of the respiratory rhythm generator, a principle that was previously demonstrated (Ramirez and Pearson 1989a) and that was also described in this study (e.g., for the interneuron 378). This leads to the important conclusion that the number of active elements in a rhythm-generating network is state dependent, a principle that was described in various rhythm-generating networks, including Clione swimming system (Arshavsky et al. 1989) and lobster stomatogastric system (Hooper et al. 1990). The principle that the number of active elements of a rhythm generator may be state dependent has interesting implications for mammalian respiration. For this system it was demonstrated that a relatively small portion...
of the ventrolateral medulla (the pre-Bötzinger complex) is essential and sufficient for the generation of the respiratory rhythm (Ramirez and Richter 1996; Ramirez et al. 1998; Rekling and Feldman 1998; Richter et al. 1997; Smith et al. 1991). However, it is unknown whether the extension of the respiratory rhythm generator changes in a state-dependent manner in an intact behaving mammal. This is a likely possibility because we know that respiratory neurons are widely distributed in the nervous system. Such neurons were described, e.g., in the so-called VRG around the nucleus ambiguous, the dorsal respiratory group within the nucleus of the solitary tract, and the Kölliker-Fuse region in the pons (Bianchi et al. 1995; Dick et al. 1994).

Another important aspect of this study is that the change in the rhythmicity of auxiliary muscles, such as muscle 59, involves a reconfiguration of the respiratory rhythm-generating network itself. Here it was demonstrated that the SOG neurons that contributed in the quiescent animal to the generation of the respiratory rhythm became rhythmically active in phase with the flight rhythm. This is a different strategy than that found in the control of bifunctional muscles that are activated during walking and flight in the locust (Ramirez and Pearson 1988). In the control of these muscles there was neither a reconfiguration of portions of the flight nor a walking-generating network. Instead, both rhythm-generating networks were functionally independent and were converging only at the motoneuronal level to activate the bifunctional muscles during the two different behaviors. Thus, with respect to the control of bi- or multifunctional muscles, it is important to examine at the level of the rhythm-generating network whether portions of the rhythm generator are reconfigured or whether independent rhythm-generating networks converge at the motoneuronal level. As mentioned in the introduction, this is a critical issue in the control of mammalian respiratory muscles that are commonly activated in a variety of nonrespiratory behaviors. In the future it should be possible to dissect the different portions of the mammalian respiratory network to solve one of the most exciting problems in the field, i.e., the interaction of various mammalian neural networks known to be localized within the brain stem, including the neural networks for breathing, gasping, brate cat.