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A ROLE FOR SENSORY INPUTS IN THE GENERATION OF THE FLIGHT MOTOR PATTERN
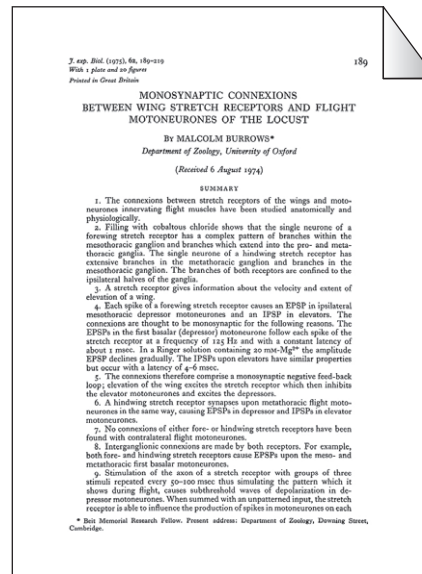
Jeremy Niven discusses Malcolm Burrows' 1975 paper entitled: 'Monosynaptic connexions between wing stretch receptors and flight motoneurones of the locust'.

A copy of the paper can be obtained from http://jeb.biologists.org/cgi/content/62/1/189.abstract

Rhythmic behaviours such as breathing, walking or flying are now understood to be generated by the interplay between patterns of neural activity generated in the central nervous system and sensory inputs. Many of the pioneering insights into the generation and control of rhythmic motor patterns were made by studying the neural circuits generating the locust flight motor pattern. Malcolm Burrows' classic 1975 paper on the role of the wing stretch receptors in patterning the activity of flight motor neurons in the locust was highly influential in determining both how sensory feedback influences centrally generated rhythmic motor activity and the structure of reflex arcs.

Early work on rhythmic motor pattern generation suggested that sensory feedback from proprioceptors during one breath, step or wing stroke initiates and shapes the motor pattern for the next. By the beginning of the 1960s, work on several systems, including beetle, dogfish and locust, had established that intrinsic central patterning played some role in generating rhythmic movements (Adrian, 1931; Lissman, 1946a,b; Miller, 1960). Donald Wilson demonstrated the presence of a central pattern generator for flight in locusts by eliminating sensory inputs to the thoracic ganglia whilst recording the motor output (Wilson, 1961) [reviewed for JEB

Classics by Edwards (Edwards, 2006)]. Although this work showed that in the absence of sensory inputs the frequency of the flight rhythm was lower than that of the intact system, it did not explain the role of phasic feedback from sensory neurons monitoring the wings in the flight motor pattern.

In the wake of Wilson's 1961 paper, numerous studies attempted to determine the precise influence of sensory inputs from the wings on the flight motor pattern (e.g. Gettrup, 1962; Wilson and Gettrup, 1963; Pabst, 1965; Wilson and Wyman, 1965; Gettrup, 1966; Iwasaki and Wilson, 1966). These studies used various techniques, such as cauterisation of sensory receptors, gluing the wings together and stimulating wing nerves, to try to infer the role of sensory feedback. Despite the profusion of sensory neurons at the base of the wings, several of the studies ostensibly focused on one sensory neuron type in particular, the stretch receptor, of which there are four, one at the base of each wing (Gettrup, 1962; Gettrup, 1963; Pabst, 1965). These studies concluded that even if timing cues were provided by the stretch receptor, they did not influence the motor pattern. Instead, it was proposed that sensory inputs were integrated over many wing beat cycles to increase wing beat frequency.

As Burrows eloquently argued in the Introduction of his 1975 paper, there were flaws in the execution of these experiments and, therefore, in the interpretation of the role of sensory inputs during flight based upon them. For example, although it was assumed that cauterisation selectively ablated the stretch receptor, numerous other sensory neurons were also likely to have been ablated. Moreover, imposed rhythmic movements of a locust's wings had been shown to entrain the flight rhythm, casting doubt on the idea that timing cues provided by sensory inputs were not important (Wendler, 1974). To circumvent the problems with previous experiments and resolve the function of sensory feedback in the generation of the locust flight motor pattern, Burrows focused precisely on the role of the stretch receptors (Burrows, 1975). He analysed the activity of these neurons and determined their connections to flight motor neurons combining anatomy, behaviour, and extracellular and intracellular electrophysiological recordings in a technical tour de force.

Burrows began by mapping and identifying the motor neurons innervating the flight muscles of the locust by placing extracellular electrodes in the flight muscles and intracellular electrodes into the somata

of individual motor neurons. Each motor neuron was identified in three ways: (1) by stimulating the muscle to evoke an antidromic spike that travelled from the muscle back along the axon to the soma, (2) by correlating the spikes of motor neurons and muscles, and (3) by depolarising the soma to evoke spikes that could be observed in the muscle recordings. The result was a map of the flight motor neurons in all three thoracic ganglia, which combined Burrows' results with those from Bentley (Bentley, 1970).

Next, Burrows determined the activity, branching pattern and anatomy of the stretch receptors themselves. Each stretch receptor branches in the periphery, one axon travelling in nerve 1 and the other in nerve 6, so by making paired extracellular recordings of these two peripheral nerves whilst moving the wings he was able to identify spikes from a single stretch receptor. These recordings showed that the stretch receptor produced spikes in response to an elevation of the wing but not to a depression of the wing. Further extracellular recordings of the connectives linking the thoracic ganglia showed that spikes from a forewing stretch receptor were transmitted to the ipsilateral side of the prothoracic, mesothoracic and metathoracic ganglia whereas those of a hindwing stretch receptor were transmitted only to the mesothoracic and metathoracic ganglia. By making use of a recently developed technique for staining neurons with cobalt chloride (Pitman et al., 1972), Burrows obtained the detailed projections of the forewing and hindwing stretch receptors within the thoracic ganglia. The forewing stretch receptor axon in nerve 6 branched in the prothoracic ganglion and, as had been shown by Bentlage (Bentlage, 1973), the axon in nerve 1 branched in the mesothoracic and metathoracic ganglia. The hindwing stretch receptor axon in nerve 6 branched in the mesothoracic ganglion and the axon in nerve 1 branched in the metathoracic ganglion. This anatomy confirmed the results of the extracellular recordings. Crucially, these experiments showed that each stretch receptor branched in the periphery, one axon travelling in nerve 1 and the other in nerve 6.

Making use of the unique morphology of the stretch receptor axon, Burrows was able to either record the activity of the stretch receptor in nerve 6 or stimulate this branch of the axon, triggering a spike that propagated along the axon and down the branch of the stretch receptor in nerve 1. Using these techniques it was possible to record stretch-receptor spikes during imposed wing movements or reliably elicit spikes in only the stretch-receptor axon. When combined with intracellular recordings of the flight motor neurons, these techniques allowed the connections made by the stretch receptor to the flight motor neurons to be established. Applying the techniques, Burrows found short-latency, reliable depolarising connections from the stretch receptor to the ipsilateral depressor motor neurons controlling the depressor muscles of both the forewings and hindwings (Burrows, 1975). He then showed that these depolarisations were chemically mediated monosynaptic excitatory post-synaptic potentials by replacing calcium with magnesium ions in the saline, injecting small current pulses during the depolarisations to detect resistance changes, and showing that the depolarisations could evoke spikes (Burrows, 1975).

These experiments also showed that the stretch receptors made inhibitory connections to the ipsilateral elevator motor neurons, which control the muscles lifting the wing. However, no connections were found to the motor neurons controlling the contralateral muscles. Thus, Burrows was able to show how the stretch receptors influence the flight motor pattern *via* two negative feedback loops to ipsilateral motor neurons: wing elevation caused by activity in the elevator motor neurons elicited spikes in the stretch receptor that inhibited their activity and simultaneously excited depressor motor neurons that depressed the wing stopping the spikes in the stretch receptor. As Burrows mentions in the paper's Discussion, remarkably, this was the first time a two-neuron reflex arc had been described in an insect.

Subsequent research has shown that the stretch receptor makes connections to interneurons in addition to those made to the motor neurons (Reye and Pearson, 1987). Recordings from rhythmically active preparations have also shown that the stretch receptor can modulate ongoing rhythmic activity in flight motor neurons (Pearson et al., 1983). The direct excitatory connection between the stretch receptor and the depressor motor neurons, which probably involves the release of several hundred vesicles during each excitatory post-synaptic potential, is modulated by octopamine and may also be modulated by pre-synaptic GABAergic inputs (Judge and Leitch, 1999; Simmons, 2001; Leitch et al., 2003). Yet these studies have not altered the role proposed by Burrows for the stretch receptor (Burrows, 1975).

Beyond understanding the generation of the locusts' flight motor pattern, Burrows' 1975 paper provided new insights into the role of sensory inputs in the patterning of rhythmic activity. Determining the neural mechanisms underpinning such activity is essential for understanding many behaviours, such as breathing, chewing, flying, swimming, walking, etc. Today's prevailing view that centrally generated patterns and sensory inputs make important contributions to the production of most rhythmic behaviours is at least partly attributable to the insights provided by Burrows' 1975 paper. Finally, the paper showed that it was possible to understand the generation of behaviour at the level of neurons and neural circuits using the anatomy of individual neurons combined with intracellular and extracellular electrophysiology. Although techniques may have changed a little in the intervening 36 years, this approach remains essential for understanding the neural basis of behaviour.

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