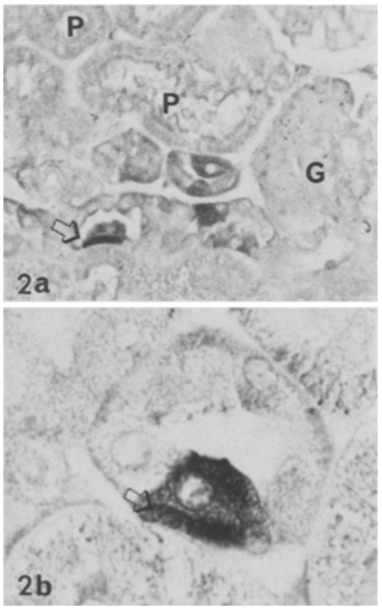

Figure 2. Renal cortex. Immunoperoxidase staining for BB. *a* ×200; *b* ×1000. Strong staining can be seen at the cellular basal part (arrow). G, glomerulus; P, proximal tubule.

ly localized in the epithelial cells of the thick ascending limb of the Henle's loop and collecting tubule, and certain epithelial cells in cortical tubules. Although the present immunohistochemistry does not discriminate BB and MB isoenzymes, both containing B-CK, it is likely that immunoreactivity to CK observed here largely corresponds to that of BB, since this isoenzyme is predominant (97%) in renal tissue homogenate [5]. Preliminary studies on the limited sections of human and mouse kidney revealed a similar distribution of immunoreactivity to B-CK. It is, therefore, highly likely that brain type of creatine kinase shows a defined cellular localization in mammalian renal tissue. In muscle, sperm cells, electric organ of *Torpedo marmorata*, or retina, CK has been postulated to be functionally coupled to the ATP-producing system in mitochondria and also to the ATP-regenerating system of intracellular components that require immediate supply of ATP [1, 8 – 11]. In heart cells, for example, a close functional coupling of CK with Na^+/K^+-ATPase has been demonstrated on their plasma membrane [12]. The present results, thus, raise the possibility that CK may also take a part in certain renal tubular function(s), by supplying ATP to the membrane-bound ATPase(s) present in these cell cubpopulations.

Note added in proof: After submission of the manuscript, I encountered an immunohistochemical study of human tissues for CK-B (Wold, L. E., Li, C.-Y., and Homburger, H. A., Am. J. clin. Path. *75* (1981) 327), in which the presence of the BB form in renal epithelial cells has been briefly mentioned.

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A comparison of the activity of flight interneurones in locusts, crickets, dragonflies and mayflies

R. M. Robertson and R. M. Olberg

Department of Biology, McGill University, 1205 Avenue Dr. Penfield, Montreal (Quebec H3A 1B1, Canada)
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Summary. The activity patterns of interneurones in the flight systems of dragonflies and mayflies were investigated using standard intracellular recording and staining techniques, and were compared with those of crickets and locusts. The results show several basic similarities in the operation of a central motor pattern generator for flight in all four groups of insects. These similarities can be explained as resulting from conservative evolution of flight pattern generating circuitry within the central nervous system.
Key words. Insect; flight; evolution.

Many features of nervous systems defy adequate explanation in terms of optimal design for some presumed function. One reason for this is that some features exist due to nonadaptive determinants of the current form and function of nervous systems [1]. A means of attacking this problem is to study homologous systems to distinguish adaptive features from those related to a common history. Several studies have shown that the identification of putative homologues in nervous systems is feasible [2 – 4] although dissimilar characteristics, in the absence of developmental evidence, could obscure the homologous relationship of neurones [5, 6].

There is now considerable evidence to support the conclusion that the insect wing is monophyletic and that it originated as a primitively articulated wing appendage [7]. Although the evolutionary precursor to the behaviour of flight is still unclear [8 – 11], similarities in the structure and organization

of the motor neurones innervating the flight muscles of many different insects[12] suggest that these motor neurones are homologous, and that the insect flight system is suitable for a comparative approach.

Intracellular recordings have been taken from neurones expressing neurogenic flight rhythms in a variety of different insects[13-16]. These studies have shown that the flight motor pattern is generated among interneurones. Only in orthoptera have flight interneurones been identified morphologically and physiologically[17-19]. In this paper we present results obtained by recording from interneurones during the expression of the flight rhythm in dragonflies and mayflies and comment on their similarities with each other and with published results from the locust and cricket.

Materials and methods. Dragonflies, *Anax junius* and Libellulid species, were reared from nymphs or caught in the wild. Mayflies, *Heptagenia* species, were caught in the wild, usually around the time they moulted from the subimago to the imago. The experimental approach to these insects was essentially the same, and similar to the approach used to develop a flight preparation of the locust[20]. Each was restrained on a cork board after removing the legs, and the thorax was opened with a dorsal midline incision. The preparations were held open by pulling on their wings and fixing them to pieces of 'tacky-wax'. The gut and overlying tissue were removed to gain access to the thoracic nervous system which was raised and stabilized on a stainless steel plate. The preparations were flooded with saline[21]. In dragonflies a monopolar, silver wire hook electrode was placed under root 1 of the metathoracic ganglion which contains the axons of the motor neurones innervating hindwing depressors[22]. The electrode and nerve were insulated with a mixture of mineral oil and vaseline and the nerve root was cut distal to this point. No extracellular monitor of the flight motor pattern was made in mayflies. The other nerve roots of the mesothoracic and metathoracic ganglia were cut to reduce movement of the preparation and to eradicate sensory feedback from the wings and wing hinges. Intracellular recordings were taken from the neuropil processes of different neurones, and these neurones were filled with Lucifer Yellow CH to determine their structures using techniques that are described in full elsewhere[20].

Results and discussion. The deafferented locust preparation will express a flight rhythm readily in response to wind stimulation of the head hairs although each flight sequence is of short duration and does not usually outlast the stimulation[20]. Crickets are initially reluctant to fly but after sufficient coaxing a flight rhythm can be produced which often persists much longer than the initiating stimulus[19]. Dragonflies were less eager than crickets in that relatively short flight sequences (less than 1 min) were produced only rarely and in response to a variety of stimuli (visual, mechanical etc.). The response to each different stimulus rapidly habituated. Despite its small size the mayfly proved the easiest to work with, both because of the ease of dissection and because the preparation produced long flight sequences (more than 10 min) in response to a single light puff of air directed at the animal. In the mayfly preparations we judged the rhythms recorded to be flight rhythms because, before cutting the nerve roots in restrained preparations, the same stimuli caused beating of the wings which appeared indistinguishable from wing movements during flight. Additional evidence in the dragon-

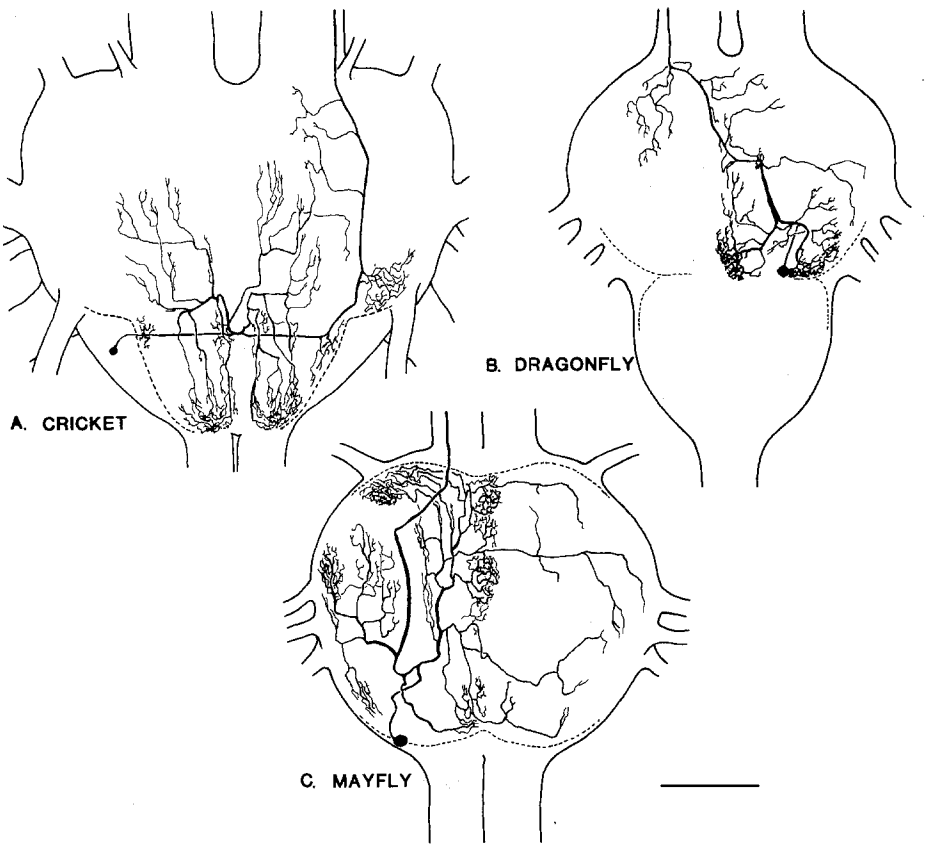

Figure 1. Dorsal aspects of the structures of interneurones phasically active with an expression of the centrally generated flight rhythm in three orders of insect. *A* An interneurone in the first, fused, abdominal neuromere of the cricket. *B* An interneurone in the metathoracic ganglion of the dragonfly. *C* An interneurone in the mesothoracic ganglion of the mayfly. In all three cases the cell somata are ventrally located whereas the neuronal branching is confined to a dorsal layer of neuropil. Anterior is towards the top of the figure. Scale – 200 μm for *A* and *B*; 100 μm for *C*.

fly preparations came from penetrations of elevator and depressor motoneurones. These were active at phases appropriate for flight when compared with the extracellular monitor of wing depressor activity. Furthermore, in all cases the rhythms recorded were brisk, at a repeatable frequency, and showed no signs of being non-specific, unpatterned responses to the stimuli. The first conclusion that we reached was that, in common with the other neurogenic flying insects that have been studied, a significant proportion of the mayfly flight motor pattern is generated by central interactions.

At present nothing can be concluded from consideration of the structures of the interneurones from which recordings were taken (e.g. fig. 1, which includes a drawing of a cricket interneurone for comparison). The structures shown in figure 1 are of the interneurones from which the recordings shown in figure 2 were taken. One feature that was common to all of the flight interneurones in the different species was that the branching of their neuronal processes was confined to a dorsal layer of neuropil just under the sheath of the ganglion.

A striking feature of the recordings taken from flight interneurones in dragonflies and mayflies was their similarity with each other and with previously published recordings from locusts [17,18] and crickets [19] (fig. 2A–C, which includes a recording from a cricket interneurone for comparison). One dissimilarity is with the frequency of the deafferented locust flight rhythm which lies around 12 cycles/s. The frequency of the flight rhythm recorded from the cricket is around 20 cycles/s [19] and those of the dragonfly and mayfly preparations reported here were also around 20 cycles/s (dragonfly: 17.5 cycles/s, n = 7, SD = 3.3; mayfly: 23 cycles/s, n = 3, SD = 2). In the intact, tethered animals the normal wingbeat frequency is much more variable both within a species and between species (locust: 22–26 cycles/s [23]; cricket: 30–36 cycles/s [19]; dragonfly: 10–40 cycles/s [16]; mayfly: 25 cycles/s for a species similar to the one used here [24]).

Recordings from flight interneurones share at least three consistent features. First, the oscillations of the membrane potential underlying bursts of action potentials were produced by at least three phases of excitatory and inhibitory synaptic input (E and I in fig. 2). Multiple phases of excitatory and inhibitory synaptic input are also seen in recordings from locust interneurones [18,20]. It is unlikely that all flight interneurones will show the same pattern of synaptic input (i.e. one phase of excitation followed by two of inhibition). Rather the inference to be taken from this is that the rhythm was generated as a result of the connections among a network of excitatory and inhibitory interneurones in each case (in none of the recordings was there any indication that the rhythm might be the result of endogenous activity in a single neurone or a population of similar neurones). Also, each network did not operate by simple reciprocal inhibition be-

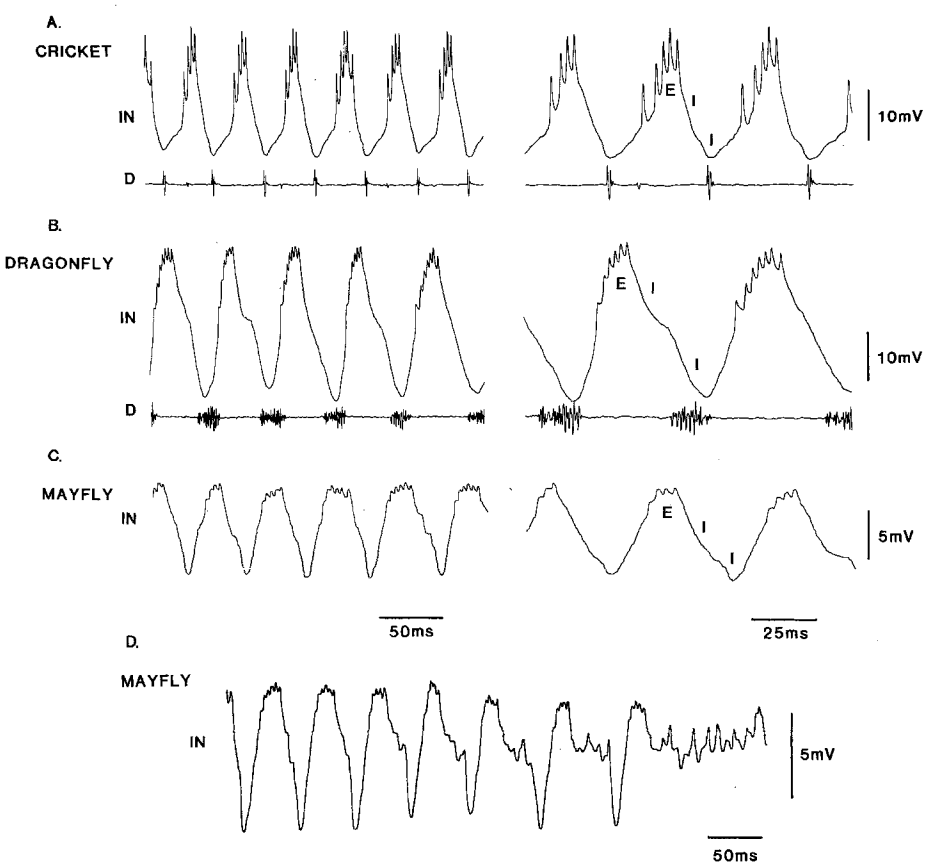

Figure 2. Intracellular recordings from interneurones phasically active with an expression of the centrally generated flight rhythm in three orders of insect. *A* An interneurone in the cricket metathoracic ganglion. *B* An interneurone in the dragonfly metathoracic ganglion. *C* An interneurone in the mayfly mesothoracic ganglion. A portion of each trace has been expanded to illustrate that at least three phases of synaptic input (indicated by E for excitatory, and I for inhibitory) underlie the membrane potential oscillation of each neurone. Note the similarity in the waveforms, cycle frequency and intraburst spiking frequency of each neurone. The time scale under the traces in *C* refer also to the traces of *A* and *B*. *D* Intracellular recording from a mayfly mesothoracic interneurone as the cycle frequency decreases at the end of a flight sequence. Note that the increase in period is accomplished by the selective increase of the duration of one particular phase of the cycle. Similar phenomena were observed in recordings from cricket and dragonfly interneurones. IN, interneuronal recording; D, extracellular recording of depressor motoneuronal activity (see text for details).

tween interneurones active during elevation and depression to produce a 2-phase symmetrical pattern. Second, intraburst spike frequency was similarly high in all instances (dragonfly: 280–480 Hz; mayfly: 250–350 Hz). Locust and cricket flight interneurones discharge at a frequency greater than 200 Hz during a burst whereas the flight motor neurones have an intraburst frequency of only around 70 Hz[19,20]. Third, all the flight systems exhibited an asymmetry such that a slowing of the rhythm (seen at the end of flight sequences) is produced by an increase in the duration of one particular phase of a cycle (e.g. fig. 2D) rather than by proportional increases in the durations of all phases of a cycle. This is evident in the deafferented flight motor pattern of locusts as a relatively constant elevator to depressor latency[25] which can be accounted for by the known interneuronal circuitry[18]. The possession of these three features results in recordings from each of the insects that can appear almost identical, and it also suggests a basic similarity in how the flight motor pattern is generated in the different insects.

The insects studied here differ markedly in the structure and function of their flight systems. In light of the differences and given that insect wings are thought to have a common evolutionary heritage[7], the simplest explanation for the similarity demonstrated here is that it results from a conservative evolution within the central nervous system[1]. Other explanations which are not ruled out by our results include divergence from a common stock followed by evolutionary convergence, or convergence after a polyphyletic evolution of flight and its neural control system (although the wings themselves may be monophyletic[7]). If the simplest explanation is correct and evolution of flight circuitry in the central nervous system is conservative, then it may be at the level of sensory modification of the centrally generated flight rhythm that one should search for adaptive features of organization.

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Address reprints requests to R. M. Robertson, Dept of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.

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Two serotonin-sensitive potassium channels in the identified heart excitatory neurone of the African giant snail, *Achatina fulica* Fèrussac

Y. Furukawa and M. Kobayashi

Physiological Laboratory, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima (Japan)
Received 21 April 1988; accepted 25 May 1988

Summary. By the patch clamp experiments, two serotonin-sensitive K^+ channels (SL-channel and SS-channel) were demonstrated in the identified heart excitatory neurone (PON) of the African giant snail, *Achatina fulica* Fèrussac. The activities of both channels could be recorded in the steady state and those activities disappeared on application of 5-HT.
Key words. Potassium channel; serotonin; modulation; snail neurone; patch clamp.

In the identified heart excitatory neurone (PON) of the African giant snail, *Achatina fulica* Fèrussac, serotonin (5-HT) produces slow depolarization via a decrease of the K^+ conductance[1]. The properties of the 5-HT-sensitive K^+ current in PON are similar to those of the S-channel (serotonin-sensitive K^+ channel) current in sensory neurones of *Aplysia*[2]. Both currents are a background K^+ current and contribute to the resting and action potentials. In *Aplysia* neurones, a single S-channel current has already been identified[3–5]. In the present study, single channel recordings by the patch-clamp technique were made to clarify the characteristics of the 5-HT-sensitive K^+ channel in PON. We found that the 5-HT-sensitive K^+ current in PON was due to the activities of two different 5-HT-sensitive K^+ channels, and that one of them had similar properties to the S-channel.

The subesophageal ganglia were dissected out of the animal and the dorsal surface of the right parietal ganglion was desheathed to expose the nerve cells. The preparation was pinned to the bottom of a chamber and treated by 0.5% trypsin dissolved in the normal solution for 15–20 min. The composition of the normal solution was (in mM): NaCl 61, KCl 3.3, $CaCl_2$ 10.7, $MgCl_2$ 13, glucose 5, HEPES 10, pH 7.5. After the enzymatic treatment, the preparation was perfused with Tris solution (all NaCl of the normal solution being replaced with TrisCl) to block the bursting activity of PON (see below). The temperature of the perfusate was