ance of soluble β-adrenergic receptors in the cytoplasm of frog erythrocytes (*6*) may be directly related to the phenomenon observed in astrocytoma cells.

A small number of β-adrenergic receptors (usually < 10 percent of the total) appear in the light gradient fractions of cells that have not been exposed to isoproterenol (Fig. 1B). Under control conditions the agonist-binding properties of the β-adrenergic receptors in light gradient fractions are characteristic of uncoupled receptors; that is, they exhibit only low-affinity binding for isoproterenol in the absence or presence of GTP (Fig. 2). The existence of β-adrenergic receptors in the light fraction from control cells could represent a normal transient population of receptors that were recently synthesized, that are about to be degraded, or that are simply undergoing a process of membrane recyclization (*14*). It is not unreasonable to speculate that the natural turnover of the β-adrenergic receptor would involve a population of vesicle-bound receptors in the cytosol; desensitization, by changing the properties of the β-adrenergic receptor, could accelerate the degradative phase of such a process.

T. KENDALL HARDEN
CALVIN U. COTTON, GARY L. WALDO
JOHN K. LUTTON, JOHN P. PERKINS
Department of Pharmacology,
University of North Carolina,
School of Medicine,
Chapel Hill 27514

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17. For experiments in which agonist binding curves were generated, fractions from several gradients were combined as follows. Light gradient fractions (fractions 6 and 7) and heavy gradient fractions (fractions 14, 15, and 16) were pooled from two control gradients and two gradients from desensitized cells. The pooled fractions were diluted five- to tenfold with 1 m*M* tris, *p*H 7.5, at 0°C and centrifuged in a SW27 rotor at 110,000*g* for 30 minutes. The supernatant was aspirated, the pellet was resuspended in 1 m*M* tris, *p*H 7.5, and the centrifugation was repeated. The pellet from this centrifugation was resuspended in 0.145*M* NaCl, 20 m*M* tris, *p*H 7.5, at 0°C, and centrifuged a third time before final resuspension in the same buffer.
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Flight Activity Initiated via Giant Interneurons of the Cockroach: Evidence for Bifunctional Trigger Interneurons

Abstract. *Activity in dorsal giant interneurons of the cockroach initiates flight movements if leg contact with a substrate is prevented. The same interneurons initiate activity associated with running when leg contact is maintained. Thus, which one of two completely different behaviors the giant interneurons evoke depends on the presence or absence of leg contact.*

Many behaviors are gated or triggered by activity in single interneurons or in discrete populations of interneurons. Such command or trigger interneurons have been identified in a wide variety of animals including annelids, arthropods, mollusks, and turtles (*1*).

An implication of these findings is that the excitation of a given command interneuron (or set of interneurons) automatically determines which behavior will be generated. In other words, each interneuron controls one and only one pattern of motor outputs. Although this might be the simplest way to control behavior, in many cases a much more efficient system would use multifunctional command-trigger interneurons; that is, the particular behavior initiated by an interneuron would depend on conditions that exist at that time.

One type of multifunctional command interneuron can evoke different behaviors by varying the frequency of its action potentials (*2*). Alternatively, a command interneuron producing a single pattern of activity could evoke multiple behaviors if existing physiological conditions, other than the command input, determine the appropriate behavior. Under the second system, a particular sensory stimulus consistently evokes different behaviors under different circumstances. For example, in the cockroach *Periplaneta americana*, wind puffs directed at the cerci (two antenna-like projections on the abdomen) initiate either running or flying, the type of movement depending on whether or not leg contact is made with a substrate (*3*). If the wind puff is presented while leg contact is maintained, the cockroach turns away from the wind source and runs (*4*, *5*). However, when leg contact is absent, a wind puff initiates flight (*3*) (Fig. 1B); that is, the wings unfold and beat at about 20 Hz. The legs are held up against the animal's abdomen rather than making running movements.

The giant interneurons (GI's) are prime candidates for triggering both of these behaviors. They are excited by sensory neurons that innervate wind receptive hairs on the cerci (*6*). Moreover, an excitatory pathway exists between many of the GI's and the leg neurons involved in walking movements (*7*). The motor activity initiated when each GI is excited fits well with movements observed in behavioral experiments with free-ranging animals (*5*).

All of these data provide strong circumstantial evidence supporting the role of the GI's in initiating running movements. If the same GI's that initiate these patterns also generate flight movements when leg contact is removed, the GI's will be established as bifunctional trigger

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interneurons (*8*). The experiments reported here demonstrate that one subgroup of GI's (the dorsal GI's) can initiate flight activity. As expected, a critical factor is leg contact.

To test the ability of the GI's to generate flight, we repeated earlier experiments; we intracellularly stimulated GI's (*7*), this time under conditions in which leg contact was prevented. Leg contact was eliminated by cutting off each leg, except the right metathoracic, at the joint between the coxa and the thorax. The right metathoracic leg was left intact to allow for recording from leg nerves; however, the main leg nerve, nerve 5, was severed in the midcoxal region. Under these conditions the cockroach would perform flight movements even when secured to a cork.

Nerve 5 root 1 (5r1) and nerve 6 branch 4 (6Br4) of the right metathoracic leg were cut and the proximal stumps were drawn into suction electrodes for extracellular recording. Branches 5r1 and 6Br4 contain, respectively, the main depressor and levator motor neurons of the leg. In some experiments, copper wires insulated to the tips were used to record electromyograms (EMG's) from elevator wing muscles. In addition, hook electrodes were placed under the whole ventral nerve cord to establish that each stimulus pulse delivered intracellularly to a GI resulted in one and only one GI action potential in the nerve cord. As in earlier experiments (*7*), a metal platform covered with vinyl wax was positioned under the connectives between abdominal ganglia 4 and 5 to stabilize them for intracellular penetration of GI's. Microelectrodes filled with 4 percent Procion yellow M4-RS in 0.2*M* KCl were used to impale and stimulate the GI's (*9*).

At the end of an experiment, Procion yellow was injected iontophoretically into the putative GI, and the cord was prepared for histology. Sections were cut 10 μm thick and observed in a fluorescence microscope. This procedure allowed the stimulated GI to be identified on the basis of its position within the abdominal ganglia (*10*).

Stimulus trains 73 msec long with interstimulus intervals of 2.0 msec (similar to those used in earlier studies to evoke motor patterns consistent with walking movements) (Fig. 1A) (*7*) were again presented to the dorsal GI's. However, with afferent input from the legs abolished, these same stimulus trains evoked movements characteristic of flight (Fig. 1C); that is, the wing stubs performed movements similar to those associated with flight, and the EMG's recorded from flight muscles were similar in waveform and frequency to those seen in tethered flight (*11*). Moreover, no obvious differences could be detected between the responses evoked by stimulating individual GI's and those evoked by wind puffs. Three ventral GI's were also stimulated in animals in which flight had been evoked by stimulation of a dorsal GI, wind puffs, or both. In no case did stimulation of ventral GI's evoke flight.

Walking and flying are also represented by very different motor patterns in the axons of 6Br4 and 5r1. With leg contact, stimulation of a dorsal GI initiated a burst of action potentials in 6Br4, 5r1, or both (depending on which dorsal GI was stimulated) (*7*). In particularly good preparations, these potentials were occasionally followed by low-frequency alternating bursts of action potentials in depressors and levators (Fig. 1A). The alternating levator and depressor burst pattern is reminiscent of activity recorded in the walking cockroach (*12*). Without leg contact, either a wind puff or stimulation of a dorsal GI (ten GI's in seven animals) elicited a long series of brief levator bursts with interburst intervals of about 10 msec (Figs. 1 and 2). During this entire period, the slow depressor (D_s) remains silent (Fig. 2). In more than 100 preparations in which dorsal GI's were stimulated while leg contact was maintained, this response pattern was never observed.

During flight, the metathoracic legs are held up against the animal's abdomen, and the femurs are held flexed against the coxae. The rapid bursting activity in levator motor neurons of 6Br4 contributes to maintaining this leg posture (*13*). There are two bursts of action potentials in 6Br4 for every cycle of activity in the flight muscles (Fig. 1, B and C). One burst occurs at the same time as a muscle potential from the wing elevator, and the second approximately in phase with a

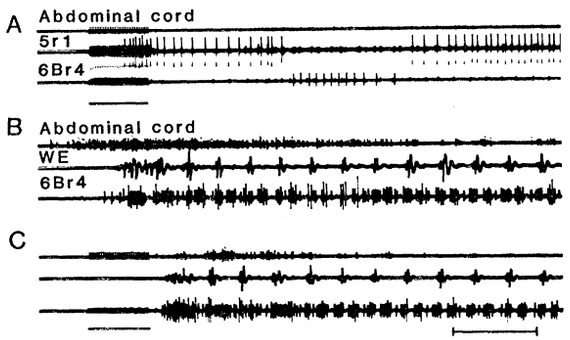

Fig. 1. Electrical activity associated with walking and flying. (A) Response to intracellular stimulation of GI-5 when the legs are making contact with a substrate. Top trace: Extracellular recording from the whole abdominal nerve cord. Middle trace: Extracellular recording from nerve branch 5r1, showing activity from the slow depressor motor neuron D_s. Bottom trace: Extracellular recording from nerve branch 6Br4, showing activity from levator motor neuron L_5. Stimulation of the GI is indicated by a line under the bottom trace. The stimulus produced a train of action potentials recorded in the abdominal cord, and stimulus artifacts in the 5r1 and 6Br4 recordings. Note the reciprocating bursts of activity from D_s and L_5 reminiscent of walking. (B and C) Recordings from a different animal from which the legs had been removed. Top and bottom traces are the same as in (A). Middle traces are EMG's from the wing elevator (*WE*) muscle. (B) Flight activity generated by blowing on the animal's cerci. The regularly occurring potentials in the wing muscle are characteristic of flight. In addition, axons in 6Br4 were excited in bursts that are approximately in time with these muscle potentials and those from the wing depressors (which would be in antiphase with the elevator muscles). The 6Br4 bursts tend to run together at the onset of the flight activity, but become distinct later. (C) Flight activity initiated by intracellular stimulation (as in A) of GI-6. The resulting flight activity is essentially the same as that seen in (B). Calibration represents 100 msec and applies to all three traces.

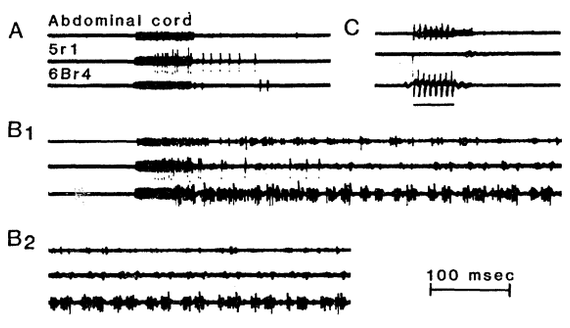

Fig. 2. The effect of N_5 activity on flight initiation. (A) The prothoracic and mesothoracic legs were removed but both metathoracic N_5's were still intact. (B) The order of traces is as in (A). (B_1) and (B_2) are continuous records. (C) From the same preparation as in Fig. 1, B and C (traces are as labeled in Fig. 1B). The same stimulus was presented to GI-6 as in Fig. 1C. However, at the same time N_5 was stimulated with eight pulses at a 7-msec interval. This was sufficient to prevent the flight activity from occurring. A bar under the bottom trace indicates the time of N_5 stimulation. The calibration applies to all records.

wing depressor. Thus, during flight the leg motor neurons in 6Br4 appear to be driven by both the wing depressor and elevator phases of the flight pattern generator. This establishes a near tonic level of 6Br4 activity, which helps maintain the proper leg posture.

Axons from sensory structures whose activity inhibits flight are located in leg nerve 5 (N_5) (*14*). To test whether these structures also provide the information for switching the GI-evoked motor output, we monitored levator axons in 6Br4.

We removed both pairs of prothoracic and mesothoracic legs, but left the metathoracic legs intact. Under these conditions, intracellular stimulation of a dorsal GI did not elicit motor outputs characteristic of flight (high-frequency levator bursts or wing movements) (Fig. 2A). Rather, the motor responses associated with turning and running were observed. Next, we cut the contralateral N_5 and again stimulated the same GI. Under these conditions, we occasionally observed slight wing movements and perhaps one or two weak bursts in 6Br4 motor neurons. Just as often there was no change. Finally, we cut the ipsilateral N_5 and again stimulated the GI. With both N_5's cut, the GI stimulation resulted in vigorous flight activity indistinguishable from that described above (Fig. 2B).

We have performed this experiment three times, twice with GI-5 and once with GI-7. In each case both N_5's had to be cut before vigorous flight could be evoked. This result suggests that sensory activity in N_5 prevents the dorsal GI's from initiating flight.

To further substantiate the role of activity in N_5, we again stimulated a GI after severing both N_5's, but this time the proximal stump of the ipsilateral N_5 had been drawn into a suction electrode for extracellular stimulation. As before, stimulation of the dorsal GI evoked flight activity. However, delivering four or more stimulus pulses to N_5 as the GI was stimulated prevented flight activity (Fig. 2C). Moreover, once flight was initiated, either by GI stimulation or by wind puffs, it could be immediately terminated by N_5 stimulation.

We conclude that the dorsal GI's are bifunctional interneurons that can initiate either flying or running; which movement occurs depends on whether any leg makes contact when the GI's are excited. During leg contact, sensory input via N_5 either allows or actively promotes one of the motor outputs generated by the GI's—turning and running. Removing this input promotes the alternative behavior—flight. This type of multifunctional interneuron provides an economical way for an animal to control diverse behaviors initiated by similar sensory cues but under different conditions. We expect that similar patterns of neural organization will be found in other systems.

ROY E. RITZMANN
MARTHA L. TOBIAS
Department of Biology,
Case Western Reserve University,
Cleveland, Ohio 44106
CHARLES R. FOURTNER
Department of Biology,
State University of New York,
Buffalo 14214

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Thermoregulation in Doves (Columbidae): A Novel Esophageal Heat Exchanger

Abstract. *Key elements in avian thermoregulation at high temperatures are panting and gular flutter. Although these mechanisms are important, they are not sufficient to maintain body temperature below high ambient temperatures in doves. In the Columbidae, evaporative cooling from an inflated esophagus, driven by heat from a vascular plexus, is also essential.*

Many studies of heat stress in birds, including doves, have demonstrated that temperature regulation is accomplished by evaporative water loss from the upper respiratory tract (panting) and, in many species, from the pharynx and anterior esophagus (gular flutter) (*1*). Panting typically precedes, but is replaced by, the more energy-efficient gular flutter in species that use both. While engaged in an unrelated study involving surgery on the cervical region of the ringdove *Streptopelia risoria* (*2*), I noticed that the entire esophagus was rhythmically inflated when the doves were under the heat of surgical lights and that there was an unusual vascular plexus in the integument adjacent to the esophagus. These observations led me to reconsider the mechanisms of temperature regulation in doves.

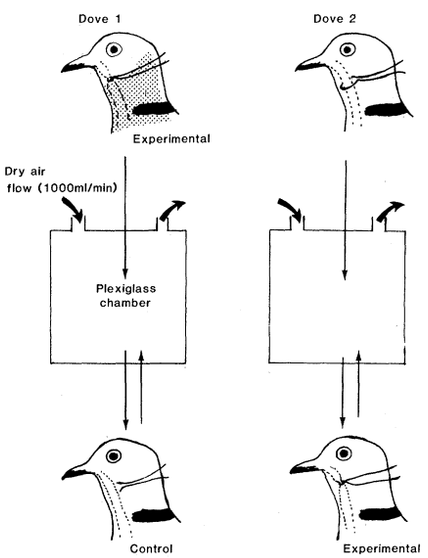

Fig. 1. The experimental design. Both chambers are submerged in a water bath (32° to 44°C). The shaded area on experimental dove 1 represents the vascular plexus in the integument. When inflated, the esophagus underlies this area.

Although the integument of birds in general and doves in particular has been extensively studied (*3*), the circumcervical plexus has been mentioned (*4*) but not described. The plexus is located in the cervical subcutaneous fascia and forms a collar (incomplete at the dorsal midline) around the neck. It extends from at least the hyoid bone cranially to the crop caudally. Arterial supply is by way of the carotid arteries; venous drain-

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