frequency of 5 Hz is sufficient to cause closing of the dactyl when the middle band is intact (fig. C1) but 10 Hz is required after it is cut (fig. C2). In other experiments the dactyl was held partially open by stimulating the excitor motoneuron to the opener muscle (fig. D). Under these conditions firing the SCE at 5 Hz caused the dactyl to close (fig. D2). But when the middle bundle was cut, closing only occurred at 20 Hz (fig. D3). These experiments show that the middle band of fibers close the dactyl at low (5 Hz) frequencies of firing of the SCE axon. Consequently the middle band may be regarded as a distinct functional sub-unit within the SCE motor unit.

The discovery of such sub-units in the isolated claw closer muscle leads to the equally interesting question of their role in the intact animal. Because of the location of the middle band, it was possible to obtain myograms from it via paired extracellular wire electrodes in intact lobsters. There was a background activity of muscle EJPs at an average frequency of 1-5 Hz in a quiescent state with the claw held in a partially closed position. During closing movement, firing frequencies were much higher. The middle band therefore appears to maintain the posture of the dactyl with a low firing frequency. Furthermore it is strategically located within the muscle to maintain posture with a minimal amount of tension since it inserts on the tendon at the pivotal point of the dactyl (fig.). Functional subdivision of the SCE motor unit in the lobster closer muscle therefore extends the contractile capabilities of this muscle and may have evolved for this reason in a large muscle with few motor units.

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Trigeminal stimulation modulates vestibular unitary activity[1]

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Summary. 139 vestibular neurons were analyzed in 38 guinea-pigs after the stimulation of trigeminal fibers: increase or decrease of discharge rate and also rhythmic modulation of vestibular unitary activity were observed.

Previous reports have provided indications of the involvement of trigeminal sensory afferent fibers in the vestibular system. Unilateral neurotomy of the trigeminal nerve in the compensated guinea-pig after hemilabyrinthectomy induced reappearance of labyrinthine postural asymmetry; curvature of the trunk, head torsion, eye nystagmus, extension of the forelimb and impairment of righting reflexes[2]. Other research[3] on vestibular compensation demonstrated, after trigeminal neurotomy, an increment in amplitude of N_1- and N_2-waves of vestibular field potentials recorded from the non-deafferented vestibular nuclei. These findings showed that the trigeminal system contributes to reestablishing a condition of balance between the 2 vestibular nuclear groups through a mechanism of inhibition of the hyperactive vestibular nuclei of the intact side. Furthermore a role for the trigeminal system in the control of posture and orientation has been documented in animals with poor vision such as rats, guinea-pigs, nocturnal and burrowing animals[4]. In fact in recent research[5] a trigeminal reflex leading to the righting of the head was described both in normal and bilateral labyrinthectomized animals when placed on their sides on the ground: the involvement of the vestibular neuronal pool for the actuation of this reflex was suggested. Thus, on the basis of the above reported data, an analysis of trigeminal influence on vestibular unitary discharge has been undertaken.

38 guinea-pigs were anesthetized (ketamine hydrochloride: 24 mg/kg), tracheotomized, curarized and artificially ventilated. Then the animals were surgically prepared to expose bilaterally the ampulla of the lateral semicircular canals and the trunk of the trigeminal nerves immediately distal to the semilunar ganglion. Labyrinthine stimulation was carried out by introducing into each ampulla bipolar copper electrodes insulated except at the tip (0.05 msec, 1-100 Hz, 0.2-2.0 mA). The left and the right trigeminal nerves were stimulated through bipolar tungsten microelectrodes (0.05 msec, 10-100 Hz, 50-200 μA). The stimulation period lasted 200-1000 msec. Non-nociceptive mechanical cutaneous stimulations of the areas innervated by the ophthalmic, maxillary and mandibular branches of the trigeminal nerves were separately performed with a hand-held plastic probe and included light touch with wisps of cotton and gentle pressure with small wooden dowels. Mechanical displacement of the vibrissae was also carried out. A tungsten insulated microelectrode (tip diameter 1-8 μm; resistance 900-1500 kΩ) was advanced by means of a Transvertex micromanipulator towards the region of the vestibular nuclear complex to record evoked potentials and single-unit discharges. The signals from the microelectrode were amplified by a conventional AC preamplifier then recorded on tape (HP 3960) and displayed on an oscilloscope (Tektronix 565). The signals were also led to a signal analyzer (HP 5480 A) for field potential analysis (average of potentials 64 times). The extracellular signals from the tape recorder were directed into a window discriminator for spike amplitude selection. Each selected spike was displayed on an oscilloscope channel to verify the constancy of its morphology. Then, the discriminated spike was used to trigger a rectangular pulse of constant amplitude and width (0.1 msec) which was directed to an EEG channel. Spike frequency was determined by counting at intervals of 250-500 msec. In each experiment the stereotaxic coordinates of each recorded unit were noted and, at the end of the experiment, at least 1 site of recording was marked by an electrolytic lesion. The animal was given a lethal dose of anesthesia and the brain was removed, fixed in Carnoy

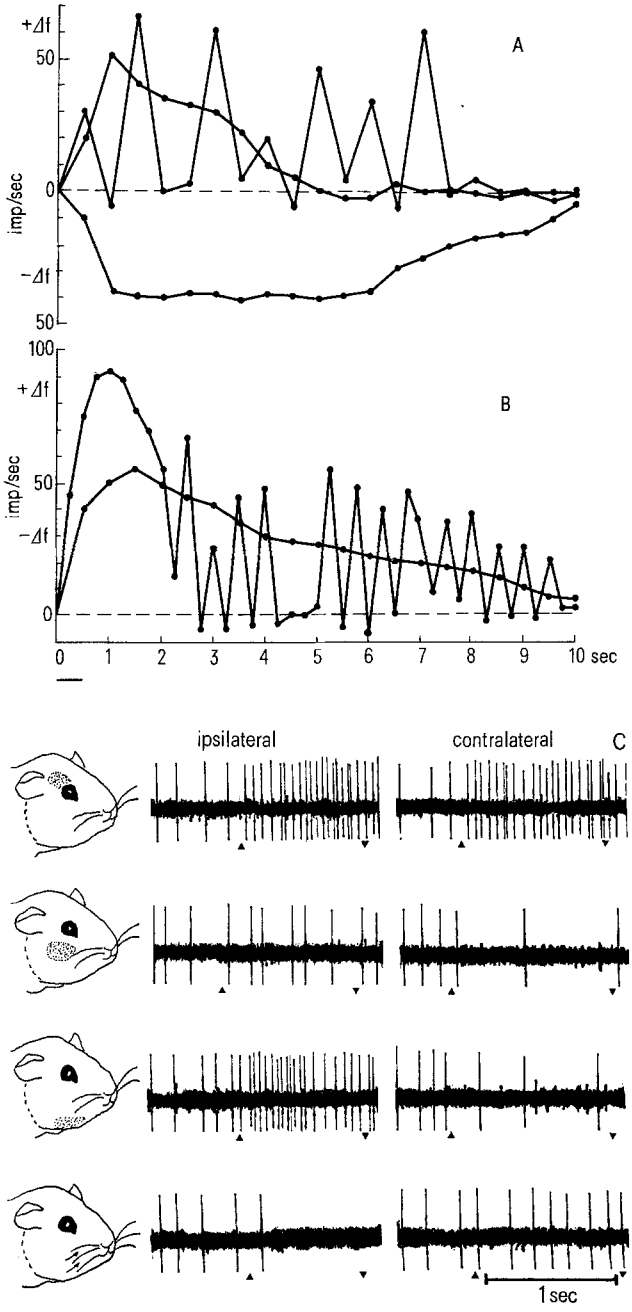
solution, embedded in paraffin, sectioned serially and stained according to the Nissl method.

On the basis of the histological verifications of the electrolytic lesions and on the basis of the stereotaxic coordinates, the exact sites of recordings were ascertained to be always sited in the core of the vestibular nuclei, and precisely either in the lateral (LVN) or in the medial (MVN) vestibular nuclei. During the experiments the boundaries of the vestibular nuclear complex were electrophysiologically delimited by evoking the characteristic vestibular field potential ($P\text{-}N_1\text{-}N_2$ waves) after stimulation of the ipsilateral lateral ampulla.

490 vestibular units were recorded, but only 131 neurons were affected by trigeminal stimulation (fig. A); results from these will be analyzed in the present report. The spontaneous resting activity ranged from 3 to 40 spikes/sec with mean $\pm$ SD frequency of 17.77 ± 8.84 spikes/sec. These units were identified as mono- or polysynaptically activated by primary vestibular afferent fibers according to Precht[6]. Following labyrinthine stimulation, most units showed an increase (25–90 spikes/sec) of firing rate whereas a small group of units exhibited rhythmic firing patterns during and/or after ampullar stimulation (fig. B). Out of 131 vestibular units, 86 were influenced by the electrical stimulation of both trigeminal nerves while the remaining 45 units were modulated only by ipsilateral trigeminal stimulation. The trigeminal effect consisted most frequently of an increase in discharge rate reaching values of 40 to 80 spikes/sec: the peak response occurred during the stimulation period and the discharge frequency returned to resting values (14–20 spikes/sec) within 2–6 sec. Some units showed a decrease of discharge frequency achieving a time block of unitary activity (0–12 spikes/sec); prestimulus values were observed after 7–12 sec. The responses of a small group of units consisted of bursts of high-frequency activity (60–90 spikes/sec) lasting 800–1000 msec and recurring regularly with intervals of 200–500 msec for a period of 6–10 sec (fig. A). These effects were constant during all stimulation trials and did not significantly change with varying the frequency of trigeminal stimulation (10–50–100 Hz). Out of 86 units receiving bilateral trigeminal modulation, most units exhibited similar sign patterns of response, whereas a minor group showed opposing sign patterns of response. By comparing the responses of vestibular units to trigeminal stimulation with those to labyrinthine stimuli, differences in behaviour were observed: some units showed a similar pattern of response, while others showed a different type of modulation. The most commonly observed pattern consisted of an activatory modulation of discharge frequency in response to both stimulations. Furthermore, it must be noted that only some of those vestibular units exhibiting a rhythmic modulation of discharge rate following labyrinthine stimulation could also show the same pattern of behaviour following trigeminal stimulation.

41% of the 131 units were also influenced by cutaneous trigeminal stimulation; most neurons were modulated by both ipsi- and contralateral stimulations, none being selectively driven by a single trigeminal branch. When the effects of one hemiface area stimulation were compared with the effect elicited by the homologous contralateral area, the great majority of units exhibited qualitatively equivalent responses, whereas a minor group showed opposing sign responses. Recruitment of previously silent units was at times observed. The discharge frequency of responses (both of excitatory and inhibitory sign) was quantitatively related to the extension, intensity, and duration of the stimulus; in some cases, the stimulation of a single vibrissa or of a small area provoked a phasic response only at the onset of stimulus, whereas the displacement of several vibrissae together or the stimulation of a larger area induced a sustained discharge persisting briefly even after the end of the stimulus (fig. C).

Definite modulation of vestibular neuronal discharge was thus demonstrated after both electrical and mechanical stimulation of trigeminal afferents. Convergence of trigeminal inflows on vestibular neurons was also observed, most vestibular units being influenced by symmetrical trigeminal inputs of both hemifaces.



A and *B*. Examples of vestibular responses to trigeminal (A: 0.05 msec, 80 μA, 50 Hz) and vestibular (B: 0.05 msec, 1.5 mA, 50 Hz) stimulations. Discharge frequency was determined by counting within intervals of 250 or 500 msec. Black line indicates the stimulation period. Ordinate: frequency increase (+ Δf) and decrease (− Δf). Abscissa: time in sec. *C*. Discharge pattern of a neuron recorded in LVN after non-nociceptive cutaneous stimulation. Dotted areas and arrows indicate stimulation fields, triangles stimulation time.

Concerning the rhythmic unitary activity induced in vestibular units by electrical trigeminal stimulation, the possibility that trigeminal inputs may influence the nystagmus rhythm-generating circuits must be suggested. In fact other authors have reported a modification in the intensity of vestibular nystagmus by stimulating the nasal mucosa[7], by evoking the corneal reflex[8] and after meningeal lesions[9]. In addition, ocular nystagmus following trigeminal stimulation or after trigeminal neurotomy can occur in compensated animals after hemilabyrinthectomy[2,10].

As regards the trigeminal tonic influence on the vestibular discharge, the hypothesis that there is a vestibular involvement in the actuation and coordination of trigeminal reflexes, through vestibulo-spinal pathways[11-13], can be put forward. Furthermore, the vestibular neurons exhibiting opposing sign responses to trigeminal stimulations of both sides could be involved in the avoiding and feeding reflexes, whose final effect is represented by moving the head away from or towards the stimulus[14]. The remaining vestibular neurons with equal sign responses could be implicated in the trigeminal reflexes which have as motor effects a dorsal extension or a ventral flexion of the head.

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Correlation between ovarian growth, vitellogenin titer, and yolk polypeptide pattern in the haemolymph of *Calliphora vicina*[1]

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Summary. During the first egg maturation cycle of *Calliphora vicina* changes in the vitellogenin titer and yolk polypeptide pattern of the haemolymph are correlated with the intensity of follicular growth, and the rate of yolk deposition.

Vitellogenesis, the process in which rapid oocyte growth and yolk deposition occurs, is one of the crucial periods in insect oogenesis. Formation of vitellogenins (egg yolk protein precursors) is a hormonally controlled process[2-5] involving vitellogenin production and secretion by the fat body[6-8], its transport via the haemolymph, and its sequestration by the cooperative action of the oocyte and the follicle cells[9, 10]. Vitellogenesis has been studied in several insect species, and essential differences in its biochemical course have been observed by different authors[11-13].

Previous work on the vitellogenin in *Calliphora vicina* has dealt with its ultrastructural localization in the fat body[8], and its purification and antigenic characterization[13].

This paper presents the results of the determination of the levels of vitellogenin in the haemolymph of *Calliphora vicina* during the 1st egg maturation cycle, its correlation with ovarian size and yolk deposition, and the yolk polypeptide pattern of the haemolymph.

Materials and methods. Haemolymph samples. Newly emerged female blowflies, *Calliphora vicina,* were kept on pig's heart, sucrose and water at 25 °C. The flies were anaesthetized with CO_2, the neck membrane punctured and two 1-µl haemolymph samples were collected, one for rocket immunoelectrophoresis, and one for SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The length of the terminal follicle and the yolk-containing part of the oocyte was measured in each fly. Flies 2, 3, 4, 5 and 6 days old were used. If the ovaries of 3-, 4- or 5-day-old flies did not contain yolk, the haemolymph samples were discarded.

Rocket immunoelectrophoresis. Rocket immunoelectrophoresis[14] was used for determination of the vitellogenin levels in the haemolymph samples. Glass plates (10 × 10 cm, 1.1 mm thick) were covered half-and-half with 1% agarose containing 10 µl/cm^2 of antivitellogenin, and 1% agarose without antivitellogenin respectively. The agarose (HSB, Mr = 0.1, Litex, Glostrup, Denmark) was dissolved in 36 mM Tris-HCl, 12 mM sodium barbital buffer containing 0.3 mM calcium lactate and 1% Triton X-100 (pH 8.6.) The antivitellogenin (0.86%) was the same as used by Jensen et al.[13]. Haemolymph samples were diluted 3 times with 0.1 M Tris-HCl pH 7.0, and applied to holes in the antivitellogenin-free zone. They were allowed to diffuse 1 h at 4 °C before electrophoresis was run at 2 V/cm for 18 h at 16 °C. Standards: 1 µl (4), 2 µl (8), 5 µl (7), 10 µl (7), and 20 µl (6) of purified vitellogenin (0.72 mg/ml) were applied to the plates. The numbers in parentheses are the numbers of samples run. The immunoprecipitates were visualized by staining with Coomassie Brilliant Blue.

Immunoprecipitation of fat body vitellogenin. Fat body pieces from 5 to 10 flies were prepared as described by