

3 M KCl. Previous experiments demonstrated that this surgical treatment did not modify the survival capabilities of the insect in a significant way. The experimental set up used allowed us to test sensitivity of the same chemosensilla of the same flies before (1st measurement) and 24 h after JH administration (2nd measurement).

As regards the hormone administration, 0.25 µl of a JH analogue (JHA, Altosid, Zoecon Corporation, Palo Alto, Ca., USA) were applied topically on the ventral abdomen of each insect. The oily consistency of this chemical allowed easy application. Its ability to reproduce the JH biological effect on ovary maturation in *Phormia* when administered using the above procedure has already been demonstrated<sup>3</sup>. The same experiments (without JH treatment) were also performed on a group of control flies.

Results obtained in the 1st measurement (A in the fig.) showed no significant differences in response frequency ( $0.6 \leq p \leq 0.7$  with the Student t-test) between control flies and those in the group to be treated with JHA. The electrophysiological activity proved to be quite changed in both groups of flies at the 2nd measurement (B in the fig., 24 h later) as compared to the 1st one, the firing frequency in each group being remarkably increased. Differences were statistically significant ( $p \leq 0.001$  in both cases). In other words, responsiveness of chemosensilla in both groups was higher in 3-day-old than in 2-day-old flies; we

recall at this point that age-related changes in chemosensillar sensitivity (depending – at least in part – on ovarian cycles) seem to be normal physiological events in blowflies<sup>1,2</sup>. In addition, spike firing frequency was significantly higher in the JHA treated flies than in the control flies ( $p \leq 0.001$ ). In short, JHA administration enhanced chemosensillar sensitivity. This latter observation leads us to suggest that physiologically-occurring increases in chemosensillar sensitivity during ovarian cycles<sup>1,2</sup> – a sample of which is provided in this research by the increased response in control flies at the 2nd measurement as compared to the 1st one – may also be due to JH influence. Thus, this hormone could play a role in controlling both ovarian and chemosensory function.

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### Cyclic sensitivity variations in the labellar chemosensilla of *Calliphora*

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**Summary.** Cyclic variations in the sensitivity of labellar chemosensilla are time-related to ovarian development in *Calliphora*. It is assumed that a single endocrine mechanism controls both sensitivity of chemosensilla and ovarian function.

Electrical resistance of labellar chemosensilla in the female blowfly varies with age according to a pattern that has been supposed to be related in some way to ovarian cycles<sup>1</sup>. Since previous research strongly suggests that variations in the electrical resistance of chemosensilla may account for variations in their sensitivity<sup>2</sup>, it seemed to be of interest to investigate directly whether some ovarian cycle-related variations in sensitivity of chemosensilla do indeed exist. 500 adult female blowflies, *Calliphora vomitoria* L., were used. The insects, kept at 26 °C and 70% relative humidity were divided on emergence into 2 groups. The 1st group ('M' flies) was fed on a standard, protein-containing diet (minced beef, sucrose, and water); the 2nd group ('WM' flies) was provided with a protein-free diet (sucrose and water) that hindered full ovarian development<sup>3</sup>. 10–12 insects were taken from each group daily from day 3 to 9 after their emergence. Ovarian development was evaluated by measuring transverse as well as longitudinal ovarian diameters, and assessing the appearance of yolk (i.e., the beginning of vitellogenesis) in the follicles, according to the method described by Stoffolano<sup>4</sup>. Since these diameters showed very similar variation patterns (correlation coefficient  $r = +0.995$ ;  $p < 0.001$ ), only the values of the longitudinal diameter will be reported here. Electrophysiological recordings were taken from the 'largest' labellar chemosensilla<sup>5</sup> by using the tip-recording technique<sup>6</sup>. A 0.5 M NaCl solution was adopted as a stimulus. Spike firing frequency (in the 1st sec. after stimulation onset) and electrical resistance (according to the method used by Stürckow<sup>7</sup>) were measured on 4–5 chemosensilla in each insect used.

Results on the 'M' flies are reported in figure 1. Two increasing phases in ovarian length were present. That means (considering the ovarian length as an index of follicle maturation<sup>4</sup>) that 2 subsequent ovarian cycles took place. This observation is in full agreement with previous results, according to which ovarian cycles in this insect follow each other without delay<sup>8</sup>. The sensitivity of the labellar chemosensilla attained maximum levels (maximum and minimum peaks in frequency and resistance respectively; these parameters were inversely correlated at  $r = -0.840$ ;  $p < 0.001$ ) at the beginning of the vitellogenic period of the 1st as well as of the 2nd ovarian cycle (on days 4 and 7 respectively). Except in these peaks, sensitivity of chemosensilla showed a tendency to decrease toward the low level observed at the beginning of the experiments (or, in other words, to the level observed before vitellogenesis took place). The firing frequency and the electrical resistance (i.e. chemosensitivity) varied with time in a very similar way in the 'WM' insects also, despite their incomplete ovarian development (fig. 2). Still, the 2 parameters were inversely correlated ( $r = -0.800$ ;  $p < 0.001$ ).

In short, chemosensitivity showed a similar and cyclic variation pattern in flies either with regular or with incomplete ovarian function. On the basis of the time-related changes in both chemosensory and ovarian function in the 'M' flies, we suggest that a single control mechanism may act on both functions. This suggestion is further endorsed by the cyclic chemosensitivity changes we observed in the 'WM' flies, in which ovarian function was not achieved owing to the lack of protein in the diet. Our results do not

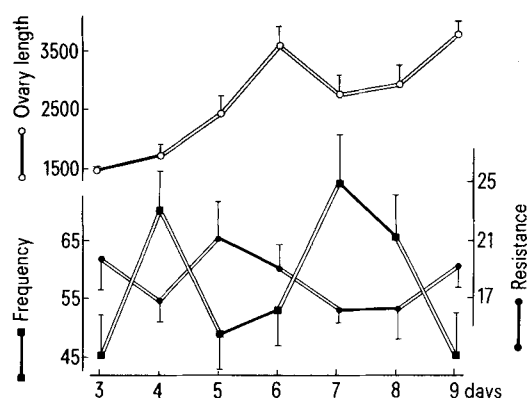


Figure 1. Firing frequency (imp/sec) and electrical resistance ( $M\Omega$ ) of labellar chemosensilla when tested with 0.5 M NaCl in protein-fed ('M') adult *Calliphora* females 3-9 day-old. Ovarian length ( $\mu m$ ) of these insects is also reported. Yolk was present from day 4 to day 6 in the primary follicles, and from day 7 to day 9 in the secondary follicles. Experimental points are mean values  $\pm$  SEM (vertical bars) of 40 (electrical resistance and firing frequency) and 10 (ovarian length) experiments. Experimental points joined by a double line are statistically different (Student's t-test;  $p < 0.001$ ).

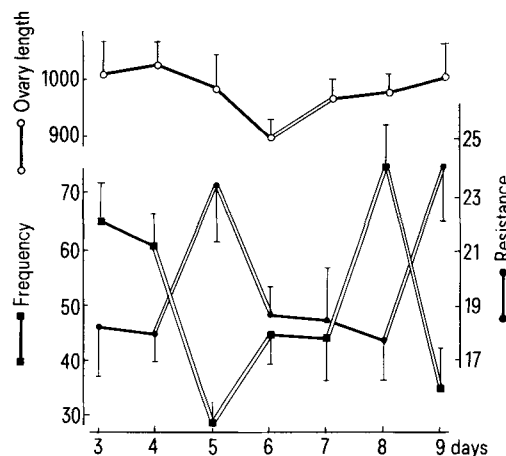


Figure 2. Firing frequency (imp/sec) and electrical resistance ( $M\Omega$ ) of labellar chemosensilla when tested with 0.5 M NaCl in protein-deprived ('WM') adult *Calliphora* females 3-9-day-old. Ovarian length ( $\mu m$ ) of these insects is also reported. Yolk was never present. Experimental points are mean values  $\pm$  SEM (vertical bars) of 40 (electrical resistance and firing frequency) and 10 (ovarian length) experiments. Experimental points joined by a double line are statistically different (Student's t-test;  $p < 0.001$ ).

provide direct information about the nature of this mechanism. Bearing in mind, however, that humoral control of chemosensory activity in other insect species<sup>9-11</sup> has already been demonstrated, it is likely to assume that a single endocrine mechanism controlling the ovarian function<sup>12</sup> also influences the sensitivity of taste chemosensilla in *Calliphora*.

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## Alterations in the morphology of the neuromuscular junctions following experimental immobilization in cats

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**Summary.** Immobilization of the hindlimbs in cats, in the neutral position, by applying a plaster cast for 4 weeks, led to paler and larger neuromuscular junctions. Beyond 8 weeks, this procedure caused elaborately branched-out and paler junctions which were significantly larger in their diameters than those of the contralateral control limbs.

The neuromuscular junction, the important functional link between the nerve and the muscle, has been a subject of study for a long time. The morphology of the neuromuscular junction<sup>2</sup> and its enzyme histochemistry in the normal muscle<sup>3</sup> and in the functionally overloaded muscle<sup>4,5</sup> have been described in detail. Though the effects of immobilization on the morphology<sup>6</sup>, physiology<sup>7,8</sup> and chemistry<sup>9</sup> of the skeletal muscle are very well known, detailed reports on the morphology of the neuromuscular junctions of an immobilized muscle are lacking.

Since it is known from tissue culture observations that the functional activity of a synapse influences its morphology<sup>10</sup>, it was decided to immobilize the hindlimbs of cats and

study in vivo the influence of function on the morphology of the neuromuscular junctions. Cole<sup>11</sup> attempted to study the morphology of the neuromuscular junctions after pinning the limb, and Vrbova<sup>8</sup> and Dias<sup>12</sup> studied them after tenotomy. The procedures of bone pinning and tenotomy, apart from causing immobilization, exert added strain on the muscle. Tenotomy produces contracture of the muscle and an increased excitability of the motoneurons, and later on connective tissue attachments are noticed between the tenotomized muscle and its bony attachment, resulting in increased tension<sup>13,14</sup>. The problem associated with the fixation of joints with bone pins is that an invasive object is implanted in the body, the effect of which on the muscular