

CENTRAL CONTROL OF FEEDING IN THE DIAPAUSING ADULT BLOWFLY *PHORMIA REGINA*

BY JOHN G. STOFFOLANO JR.

*Department of Entomology, University of Massachusetts,
Amherst, Massachusetts 01002, U.S.A.*

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SUMMARY

1. The tarsal acceptance threshold of non-diapausing adult blowflies rises with age.
2. Diapausing flies have a non-significantly elevated tarsal acceptance threshold, but drink significantly less sucrose solution than their non-diapausing counterparts.
3. Removal of diapausing flies to non-diapause inducing conditions resulted in a lowering of the threshold.
4. Failure to demonstrate a significant difference between the input signal from the peripheral receptors of non-diapausing and diapausing flies showed that the inhibition of feeding in diapausing flies was not due to peripheral inhibition but was controlled centrally.

INTRODUCTION

Two or three generations of the black blowfly *Phormia regina* (Meigen), are produced during the summer months in temperate regions. Under these non-diapause inducing conditions there is no arrest of development in the life-cycle of this fly. Following eclosion, adult females alter their feeding preference, feeding on either carbohydrates or proteins, depending on the stage of ovarian development (Dethier, 1961). Both sexes have been shown to require a protein meal prior to normal reproductive activities (mating, vitellogenesis and ovipositing) (Stoffolano, 1974*a*). A protein feeding peak precedes each gonadotrophic cycle of the female (Dethier, 1969) while males, on the other hand, show only one peak period of protein feeding and thereafter feed mainly on carbohydrates (nectar) (Belzer, 1970). This blowfly, in common with most other insects, has evolved feeding preferences that coincide with the physiological demands placed on it by mating and egg production.

The shortening autumn daylengths provide the primary environmental cue that switches the adult blowfly endocrines to their overwintering programme. During this period adult behaviour changes: the flies refuse to mate, they seek shelter and their feeding patterns alter. The present report concerns experiments to determine whether failure of diapausing adult *Phormia* to feed is due to central or peripheral control mechanisms.

MATERIALS AND METHODS

A stock colony of *P. regina* was reared in non-diapause inducing conditions (27 °C and a 15 h photophase) using the techniques and diet described earlier (Stoffolano *et al.* 1974).

1. *Diapause induction*

In order to compare the feeding behaviour of non-diapausing with diapausing adults, pupae were placed in photoperiod control chambers under either diapause inducing conditions (18 °C and 9 h photophase) or non-diapause inducing conditions (18 °C and 16 h photophase). Each fly was dissected at the end of the experiment and was classified as either non-diapausing or diapausing. Diapausing females were characterized by lack of ovarian development and by fat body hypertrophy while males had only fat body hypertrophy. Non-diapausing flies lacked these characteristics.

2. *Determining tarsal acceptance thresholds*

Adult flies were removed from the various environmental conditions and placed at 27 °C and a 16 h photophase for a 3-day starvation period. During this period they had access to distilled water only. Flies were then anaesthetized using CO₂ and placed on applicator sticks with their wings stuck in Tackiwax. Once on the stick, each fly was given at least 20 min to adjust before testing. During this period, and immediately prior to testing, the flies were permitted to drink all of the distilled water they would imbibe. Flies were then separated into groups of 25 and by using a random selection technique, each group was tested on a different concentration of sucrose. Once a fly had been tested on one solution it was not tested on another. The tarsi of the fly were touched to the testing solution. Proboscis extension was recorded as a positive response and non-extension as a negative response.

3. *Determining intakes and duration of drink*

Adults in this experiment were treated identically to those used in determining the tarsal acceptance threshold, except that the flies were permitted to drink. The duration of one uninterrupted drink began when the proboscis was extended and ended when the proboscis was withdrawn. Flies were weighed before and after the drink to obtain intake.

4. *Electrophysiological recordings*

A glass capillary which served as the indifferent electrode was placed into the proboscival sinus of a fly of the desired age and physiological condition. The physiological saline in this electrode was adapted from Normann & Duve (1969). The stimulating electrode contained a 0.5 M sucrose solution. Stimulating time was from 5 to 10 sec. Action potentials were conducted to a Bioelectric type NF1 neutralized input capacity amplifier which led to a Tektronix no. 502 oscilloscope for visual display and then to a no. 531 oscilloscope with a Nikon-Kohden continuous-recording Linagraph camera for permanent recording. The oscilloscopes were set at 0.5 gain and the camera speed was set at 20 cm/sec. The chemoreceptors tested on both the left and right labellar aboral lobes were numbers 1, 2, 3, 4 and 8, 9, 10, 11 (cf. Wilzek, 1967).

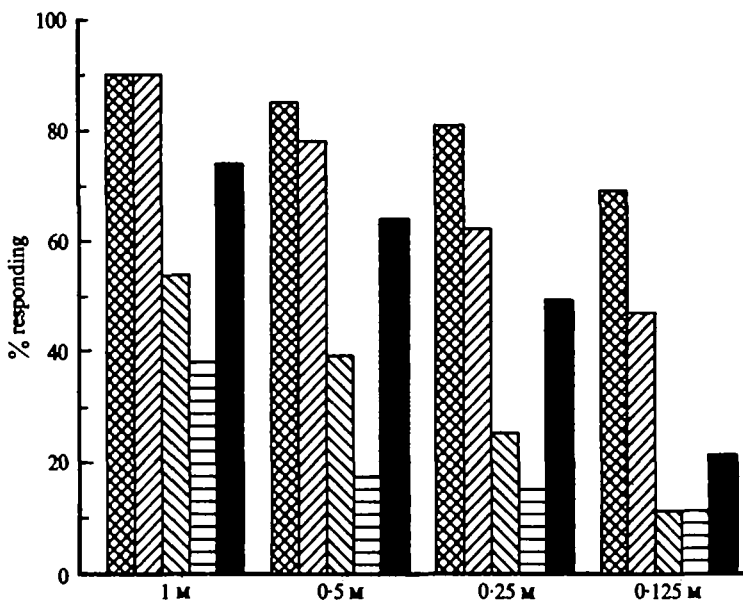


Fig. 1. The percentage of *P. regina* adults responding to various concentrations of sucrose at different ages following eclosion and at different physiological conditions. ▨, 3½ days old and non-diapausing; ▩, 39 days old and non-diapausing; ▤, 39 days old and diapausing; ▥, 10 days old and non-diapausing; ■, 41 days at diapausing inducing conditions and 14 days at non-diapausing inducing conditions. The smallest number of flies tested for each group was 162 for the 10-days-old, non-diapausing group, and the maximum number was 355 for the 39-days-old, diapausing group.

In order to evaluate the tarsal acceptance thresholds, potency probit analyses were run using the computer program developed by Dr Daum to determine if responses were significantly different (Daum, 1970).

RESULTS

1. Tarsal acceptance thresholds

Before comparing the tarsal acceptance thresholds of diapausing with non-diapausing adults, it was necessary to examine what changes, if any, occurred with age. Non-diapausing adults, 3.5 days old were very responsive to various concentrations of sucrose. However, as the flies aged, the tarsal acceptance threshold increased and fewer flies responded (Fig. 1).

Comparison of the tarsal acceptance threshold of non-diapausing flies, 39 days old, with diapausing flies of the same age showed that fewer diapausing flies responded to the different solutions tested (Fig. 1). Potency probit analysis, however, showed no significant difference between these two groups at the mean or 50% level.

In an attempt to separate an ageing effect from a diapausing one, flies were removed from diapause inducing conditions on day 41 and placed in non-diapause inducing conditions for 14 days. When tested on day 58 (i.e. allowing 3 days for starvation) the results showed that these flies now had a considerably lower acceptance threshold, in fact, lower than both the diapausing and non-diapausing flies 39 days old ($P < 0.05$ for the difference).

Table 1. *Mean (\pm S.D.) intake and duration of drink of diapausing and non-diapausing P. regina fed a 0.125 M sucrose solution*

Treatment	Mean intake (μ l)	Mean duration (sec)	No. tested
N-D*	24.7 (\pm 4.2)	140.6 (\pm 30.5)	29
D*	6.0 (\pm 4.8)	37.5 (\pm 29.8)	33
D, 41 days; N-D, 8 days	22.0 (\pm 4.6)	168.1 (\pm 64.5)	20

N-D = non-diapausing, D = diapausing.

* Forty days old.

Table 2. *Effect of diapause on the mean impulse frequency of labellar sugar chemoreceptor sensilla of P. regina using 0.5 M sucrose as the testing solution*

Age (days)	Frequency (imp/sec)	No. of flies tested	No. of sensilla recorded from
N-D, 60	34.0	6	70
D, 60	27.3	11	70

N-D = non-diapausing, D = diapausing.

2. Intake and duration of drink

In order to determine what the adult fly would actually imbibe at various physiological conditions and ages, intake and duration of drinking were measured. Non-diapausing, 40-day-old flies had a significantly greater intake (24.7 μ l) of 0.125 M sucrose solution than diapausing flies of the same age (6.0 μ l) ($P < 0.05$). They also drank for a longer period of time – 140.6 sec compared with 37.5 sec (Table 1). When returned to non-diapause inducing conditions, however, the flies increased their intake (22.0 μ l) and drank for a period (168.1 sec) comparable with that of the non-diapausing flies.

3. Electrophysiological recordings

Recordings from the labellar chemoreceptor sensilla of non-diapausing and diapausing adults of the same age failed to show any significant difference in the mean impulse frequency (Table 2) ($P < 0.05$).

DISCUSSION

1. Tarsal acceptance threshold

The data (Fig. 1) showing the effects of age and diapause on the tarsal acceptance threshold are in agreement with the previous work of Dethier and his co-workers (Dethier & Chadwick, 1948; Dethier, 1969). The important question was *how* age influences the gustatory threshold. It has been recently demonstrated on two closely related blowflies that as the flies aged the percentages of sugar sensilla that were operative decreased (Rees, 1970; Stoffolano, 1973). Earlier work by Dethier (1969) showed that the input from these receptors was additive, thus explaining how a decrease in the number of operative or functional sensilla could result in an increase in the acceptance threshold with increased age.

Comparison of the tarsal acceptance thresholds between diapausing and non-diapausing, 39-day-old flies revealed no significant differences, so it was important

to determine whether their elevated thresholds relative to that of younger flies was due to an ageing effect or to some other mechanism.

When non-diapausing 41-day-old flies were starved 3 days prior to testing they all died, whereas similarly aged diapausing flies removed to non-diapausing inducing conditions for 14 days survived the 3-day starvation period. Tested on day 58, these flies showed a much lower tarsal acceptance threshold relative to both the 39-day-old groups. Evidently something happened to the diapausing flies when they were returned to non-diapause inducing conditions. It is suggested that the elevated threshold of the non-diapausing, 39-day-old flies was due to degeneration of the peripheral receptors, whereas the elevated threshold of diapausing flies was due to a diapause factor that was removed when the flies were removed to non-diapause conditions.

2. Intake and duration of drink

The intake data reported in Table 2 support the data obtained for the tarsal acceptance thresholds. Since extension of the proboscis is dependent on the acceptance threshold of tarsal receptors, it is not surprising that flies with a high threshold failed to imbibe much sucrose solution. Closer examination of Fig. 1 shows that at 0.125 M sucrose both non-diapausing and diapausing, 39-day-old flies had identical tarsal acceptance thresholds. However, Table 1 shows that these two groups were considerably different in their intakes. The elevated tarsal threshold reported in Fig. 1 for diapausing flies explains why these flies failed to imbibe much liquid but does not explain the difference in their intakes from their non-diapausing contemporaries.

So far, both the tarsal threshold and the intake data reveal that some inhibitory factor was removed when diapausing flies were removed to non-diapause inducing conditions. The results, however, do not reveal whether the inhibition was central and/or peripheral.

3. Central control of feeding

In order to establish whether failure to feed in diapausing blowflies was either central and/or peripheral it had to be determined whether or not behavioural differences were reflected by electrophysiological differences in the peripheral chemoreceptors – that is, in afferent information to the motor command centres.

Comparisons of electrical recordings between the labellar receptor responses of non-diapausing and diapausing 60-day-old flies showed that there were no significant differences between the frequencies when stimulated with a 0.5 M sucrose solution: there was no difference between the input signal provided by the peripheral labellar chemoreceptors. Thus, the input signal from the peripheral receptors (i.e. sugar chemoreceptor sensilla) did not differ between the diapause and non-diapausing flies. This means that the difference in the behavioural motor output (i.e. proboscis extension and drinking) of these two groups was not due to peripheral inhibition but was due to some centrally controlled process, the mechanism of which still remains unknown.

4. Feeding during diapause

Reports in the literature as to whether or not diapausing insects feed are contradictory. Some authors report that diapausing insects cease feeding (Wallis, 1959;

Guerra & Bishop, 1962; Hodek, 1967; McMullen, 1967) while others state that feeding continues during diapause, is reduced or is intermittent (Davey, 1956; Burges, 1960; Ankersmit, 1964; Siew, 1966). In a recent review of dormancy in insects, Mansingh (1971) stated that 'no diapausing individuals ever feed during dormancy'. It seems probable that this generalization is too sweeping to apply to all insects. However, feeding may be absent in insects diapausing in areas where they experience freezing temperatures, since starvation promotes their supercooling ability (Salt, 1961). This is apparently due to the fact that food and its contaminants act as nucleators for ice crystal formation (Salt, 1953). Whether or not diapausing insects will drink (cf. Stoffolano, 1974*b*) has not so far been considered.

The influence of hormones on insect behaviour is well established (Truman & Riddiford, 1974) and de Wilde (1970) pointed out that diapause represents control not only over extrinsic growth, differentiation and reproduction, but also over behaviour. As stated by Highnam (1964), 'variability may be introduced into a behaviour pattern by a changing series of environmental stimuli coupled with a variable response by the nervous system to the stimuli'. Whether the variable response to feeding stimuli in all diapausing insects is due to changes in the central or peripheral nervous system is not known. It is evident from this work, however, that in diapausing blowflies the change is centrally controlled.

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