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A study of behavioural habituation to a feeding deterrent in nymphs of *Schistocerca gregaria*

Á. SZENTESI and E. A. BERNAYS* Research Institute for Plant Protection, Budapest, Hungary, and *Tropical Development and Research Institute, London

ABSTRACT. Behavioural habituation† of *Schistocerca gregaria* Forskål fifth instar larvae to a feeding deterrent, nicotine hydrogen tartrate (NHT), is described. An attempt was made to differentiate between various factors possibly involved in the induction of this behavioural habituation. Sensory stimulation (through maxillary palp), cannulation of NHT into the crop, and its administration by gelatine capsules placed into the oesophagus each resulted in behavioural habituation, but in the latter two cases a sole induction via post-ingestional input is not completely proven. Injection of NHT into the haemolymph did not induce habituation. It is concluded that habituation via sensory stimulation is a central phenomenon.

Key words. Habituation, deterrent, antifeedant, *Schistocerca gregaria*, nicotine, toxicity.

Introduction

In some insects, previous experience of deterrent-containing food may increase its acceptance during subsequent exposure (Strebl, 1928; Schoonhoven, 1969; Gill, 1972). Field studies also suggest that insect feeding deterrents sprayed onto plants to protect them from pests do not retain their effectiveness over a period of days (Bernays, 1983); habituation to such deterrents may occur (Chapman, 1974), although Schoonhoven & Jermy (1977) considered it unlikely with inorganic deterrent compounds.

Recently, experiments with plant-derived products have also shown behavioural habituation in two polyphagous insect species (Jermy *et al.*, 1982); *Schistocerca gregaria* Forskål, a polyphagous acridid, was found to ingest relatively more of the deterrent nicotine hydrogen tartrate (NHT) if there had been prior exposure to it, and the increased acceptability of the deterrent was shown to be due to habituation as defined by Thompson & Spencer (1966). That is, experience led to a waning of a behavioural response, there being no assumptions regarding neural processes.

Although habituation is considered to be a central nervous phenomenon (Groves & Thompson, 1970), little is known about the neurophysiological processes involved. Rowell (1970) assumes either synaptic changes in afferent pathways, or collateral inhibition, to be most generally important in insects. Habituation to a feeding deterrent which is also toxic to the insect is an added complication, since post-ingestional effects of the chemical may also influence behaviour.

The present study provides a detailed description of behavioural habituation to NHT in nymphs of *Schistocerca gregaria*, together with results of experiments designed to separate the effects of chemosensory input from

† For definitions see appendix.

Correspondence: Dr E. A. Bernays, Agricultural Experiment Station, University of California at Berkeley, 1050 San Pablo Avenue, Albany, California 94706, U.S.A.

any possible influence of physiological changes consequent upon ingestion.

Materials and Methods

Insects

Stock colonies of locusts originated in Saudi Arabia in 1974. They were reared in crowds in rectangular metal cages of 64-litre capacity (Hunter-Jones, 1961) and fed on seedling wheat (15–20 cm high), dry bran and hay occasionally supplemented with a mixture of pasture grasses or cabbage. Daylength was 15 h with temperatures ranging from 29 to 37°C across the cage. Night temperature ranged from 20 to 25°C. For experiments, insects reaching the fifth instar were removed from stock cages within 12 h of ecdysis and kept in groups for up to 24 h in 11-litre capacity perspex cylinders with ample food. The day of ecdysis was designated day 0.

On day 1, each insect was individually confined in a transparent plastic box (60 × 110 × 75 mm) having a small hole in one side. Strips of expanded aluminium lined three walls of the box, and provided perches. Insects were screened from one another with card between the boxes and kept in constant light at 31 ± 2°C. Food consisted of seedling wheat, *Poa* sp. or *Agrosis* sp., presented in water in small vials (10 ml). Where the water content of the food was high, hay was also provided.

Presentation of the deterrent

The feeding deterrent, NHT (nicotine hydrogen tartrate; B.D.H. Chemicals Ltd), was dissolved in 70% ethanol at 5% (w/v). Detergent (Cetavlon; I.C.I. Ltd), was added to give a concentration of 0.05% (w/v). Usually a new solution was made up for each experiment. Freshly cut, good quality expanded leaves of glasshouse-grown mature sorghum (*Sorghum bicolor* var. local Pachajona from ICRISAT in India) were dipped in the solution, briefly drained and then allowed to dry completely in a cool airstream before standing with cut ends in a vial with water and cotton wool. The material appeared to be evenly spread over the surface and the final quantity on the leaf was 9.8±2.0 (mean±SD) mg per g dry weight or 19.9±2.4 (mean±SD) mg per g. The differences appeared to be due to differences in the degree of hairiness on the two lots of sorghum used. This food was called deterrent diet. The initial water content of the leaves varied from 72% to 78% throughout the experiments, and to a much lesser extent in individual tests, but usually was further reduced during the experiment.

Behavioural habituation

A preliminary experiment was carried out to determine the approximate amounts of NHT consumed and to estimate the quantity of food, treated only with the Cetavlon solution, to give control insects in a more comprehensive experiment (Jermy *et al.*, 1982; and see below). Individuals were given the deterrent diet *ad libitum* for 19 h daily from the days 1 to 5. During the remaining 5 h, untreated wheat was provided *ad libitum* to prevent starvation due to reduced feeding over the 19 h period of exposure to the deterrent. Each individual was given ample treated food (the deterrent diet) which had been measured in an electronic area measurer (Li-Cor) accurate to 0.1 cm^2. At the end of the 19 h period, the leaf material remaining was remeasured so that the quantity ingested over the 19 h could be calculated in cm^2.

Since locusts eat progressively more over the first 5 days of the fifth instar (Davey, 1954), the data from the preliminary experiment were used to set up a more comprehensive experiment with controls for increasing age in the instar. Ten individuals received the treatment described above and are referred to as experienced. A large number of others received control sorghum leaves (dipped in solvent plus Cetavlon only) but each was limited to the average quantity ingested daily by the experienced insects in the preliminary experiment. This restricted amount of food was usually ingested during the first half of the 19 h period; in all cases wheat was given *ad libitum* for the remaining 5 h of each 24 h. On each day, ten of these insects on restricted diet were selected for testing on deterrent diet in parallel with experienced insects. These insects are referred to as naive since they experienced the deterrent only once and, after exposure, were discarded. Thus it was possible

to compare daily the ingestion of deterrent diet by experienced and naive insects of the same physiological age, and which had ingested similar quantities of food overall (see Jermy *et al.*, 1982). Earlier work showed that there were no gross differences in amounts eaten during the 5 h period by experienced and naive insects and that ecdysis to the adult stage took place after similar periods of time (Jermy *et al.*, 1982). The experiment was carried out once in a repeat of earlier work at a concentration of NHT on the leaves of about 1% dry weight. In a second experiment, the concentration of NHT on the leaves was approximately 2% and the comparison of experienced and naive insects made only on day 5, since on this day maximum differences occurred (in both the first experiment and in earlier work). Also, in this latter experiment, amounts eaten were measured by area difference and by estimated dry weight intake.

Behavioural studies of the habituation process

Time lapse filming of insects having leaves with NHT at *c.* 2% dry weight, as well as of insects having untreated leaves was carried out for a 4 day period. Six or eight nymphs in individual boxes were filmed simultaneously, three or four receiving each treatment. Boxes were screened from one another and photographed at 30 or 40 s intervals with a Bolex 16 mm cinecamera and Paillard-Wild timing equipment. Films were analysed using a Dynamic Frame IIMC, NAC, model DF-16B analysis projector; feeding and resting periods being recorded.

In order to establish whether the treated diet (i.e. deterrent diet) was rejected at palpation, after biting or following some feeding (Blaney & Chapman, 1970), continuous visual observations were carried out on four individuals. Parallel recordings were made on four specimens having untreated food. Insects were transferred to plastic boxes immediately after moulting, in order to precondition them. Care was taken to minimize handling and visual influence gained by the insects during 4 days. Treated or untreated sorghum was present between 13.00 and 08.00 hours, while wheat and hay were provided for the rest of the day. Records of palpation (= P), biting of the leaf (=B), and feeding (=F) were made during every 1 min interval from 13.00 to 21.00 hours daily. As the observed events clearly showed a hierarchy, i.e. P being involved in B, and this in turn in F, if one activity of the highest rank (=F) occurred, it naturally also involved P and B.

Experiments to investigate possible mechanisms involved in habituation

Habituation may result from the effect of mouthpart chemosensory information during palpation and feeding on the CNS, or from effects which follow ingestion of the deterrent. Experiments were carried out in an attempt to differentiate between these two possibilities. During the period prior to testing, all insects had access *ad libitum* to fresh wheat, *Poa* or *Agrostis*. On day 5, all insects were tested with deterrent diet and the amount eaten after 19 h measured by area and dry weight difference.

Sensory stimulation without ingestion was provided by holding solutions of NHT in water over the distal segment of the maxillary palps to ensure contact with the terminal sensilla which comprise 30% of the mouthpart chemoreceptors (Chapman, 1982). On day 1 each insect had a small nylon capillary (o.d. 816 μm, i.d. 539 μm, length *c.* 3 mm) pretreated with silicone (Sigmacote; Sigma Ltd) fitted over each maxillary palp, and sealed into position proximally using dental sticky wax (Cottrell Ltd) at just above its melting point of 50°C. This was done under a dissecting microscope and took 2–4 min. Since the nerve from the terminal sensilla is more or less in the centre of the palp (Blaney & Chapman, 1969), it was assumed that heat damage to the nerve did not occur. The distal end of the capillary was left open and experimental insects had approximately 1 μl of NHT solution (see below) injected into the capillary to cover the distal end of the palp. Water was used for control insects. Each individual was treated on days 1–4, and was restrained for 1–2 min. The time for which the solution was left in position was determined from the approximate average feeding time during the periods of experience as found from film analyses (see below and Table 1). These times were day 1: 30 min; day 2: 30 min; day 3: 120 min; and day 4: 150 min. At the end of the treatment period the NHT solution

and the water in each capillary was removed with small absorbent paper wicks. It is impossible to equate the sensory information entering the CNS during treatment with that entering during similar lengths of time feeding. Palpation is intermittent whereas the experimental treatment was continuous, so that sensory adaptation will be taking place though probably not completely (Blaney & Duckett, 1975).

Three experiments were carried out with the above palp treatments. Each experiment used approximately twenty insects for each treatment, with 1%, 5% and 20% aqueous (w/v) NHT solutions. With the highest concentration, the exposure period was lengthened to 180 mm per day for the 4 days. A very small quantity of amaranth was added to all solutions so that filling of the capillaries could be followed more easily, and removal of the solutions ensured. The colour was light pink. The capillaries on most insects were still in position on the test day (day 5).

Possible physiological feedbacks resulting from ingested NHT were tested in three experiments. First, insects were injected with NHT. This treatment ensured that effects occurred without mouthpart chemoreceptor input, but did not mimic naturally occurring effects resulting from absorption through the gut. Injection was made twice daily on days 1–4 with 5 μl of a 2% aqueous NHT solution using a micrometer syringe. Controls were injected with distilled water. This quantity of NHT (100 μg) causes obvious toxicity symptoms lasting about 1 h but is well below the LD_0 level of 500 μg for 3-day-old insects (P. Cottee, pers. comm.). Insects were tested with NHT treated diet as usual on day 5. Injections were into the abdomen through various intersegmental membranes laterally. In view of the toxic effects, these insects were kept until ecdysis to ensure that no gross difference in physiological age, which may itself have influenced intake, resulted from the treatment. There were twenty insects in each group.

A second experiment involved cannulation of aqueous NHT directly into the crop via the mouth. This treatment aimed to mimic postingestional effects with little or no chemoreceptor stimulation. The end of a Portex nylon intravenous cannula /200/300/010 was warmed, slightly drawn out, and bent about 5 mm from the tip to allow easy access to the crop, the entrance of which is approximately at right angles to the pharynx. The depth of insertion was approximately 1 cm, so that the tip reached about half way along the crop. Treated insects received a quantity equivalent to the average NHT level ingested by experienced insects on days 1–4 (Table 1), always in 10 μl of 0.2% agar. The agar (which is not digested) was used in an attempt to hold the solution in the gut for somewhat longer than water alone. The amounts given were respectively 3, 6, 10 and 13 mg on days 1, 2, 3 and 4. Controls were given 10 μl of 0.2% agar. Regurgitation during and following cannulation was common, and although the regurgitate was often re-swallowed, some insects were recannulated. It was assumed that little stimulation of the maxillary chemoreceptors occurred, and limited stimulation of other mouthpart chemoreceptors, but no measure of this was possible.

A third experiment involved dosing insects with solid NHT in gelatine capsules. This treatment was intended further to reduce the chance of chemoreception. Gelatine tubing was made by casting on a hypodermic needle of 1 mm diameter. Strips of 2–3 mm length were filled with NHT powder and sealed at each end with dental sticky wax. Such capsules could easily be placed in the oesophagus with forceps, and swallowing automatically followed. Experiments with dyes indicated that release of contents in the crop were complete within 30 min. Each capsule held only 0.4–0.6 mg NHT and thus two capsules per day (*c.* 1 mg) were given to each insect, while controls received empty capsules.

To assess whether contamination of the mouthpart receptors occurred as a consequence of regurgitation, gelatin capsules were made up with NHT as above, but with added U-^{14}C-glucose as a marker (1 μCi). Twenty individuals were dosed and after 5 min decapitated with a razor blade. The heads were kept frozen until later, when the mouthparts of each were removed and dissolved in concentrated sulphuric acid. The acid digest was added to Insta-Gel (Nuclear Enterprises) for counting in a Packard Tri Carb 2660 liquid scintillation counter. Mouthpart contamination by less than 1 part in 10,000 could be detected by this method.

TABLE 1. Consumption of NHT-treated diet (approx. 2% dry wt) by experienced insects on consecutive days, and mean contact time of insects with the leaves.

Day	Loss of NHT-treated sorghum during 19 h*			Approximate mean minutes of contact with treated leaves over 19 h†
	Area (cm^2 ± SD)	Dry weight (mg ± SD)	Estimated NHT ingested (mg ± SD)	
1	17.9 ± 9.7	47 ± 23	3 ± 2	30
2	33.3 ± 16.3	69 ± 45	6 ± 4	30
3	45.2 ± 18.4	115 ± 52	9 ± 4	120
4	50.9 ± 24.9	158 ± 74	12 ± 6	150

* n = 16.
† From film analysis, n = 4.

Results

Behavioural habituation

Nymphs with daily experience of NHT-treated leaf material ate significantly more of it than naive insects on most days of the instar (Fig. 1), with the greatest difference occurring on the fourth day of treatment (day 5 of instar). In a second experiment amounts eaten on day 5 were: experienced 65.4 ± 6.3; naive 39 ± 6.2 (mean cm^2 ± SE). Insects experiencing a high concentration in the test period ate less, but the difference between experienced and naive insects persisted: the areas eaten being 33.1 ± 6.9 and 19.9 ± 3.4 on day 5 respectively.

A frame-by-frame analysis of film recordings of sixteen individuals on NHT-treated diet, and ten on control (ethanol-treated) sorghum showed that, in addition to a progressive change in behaviour of insects experiencing NHT from day to day, their behaviour also changed each day, becoming progressively more like that of control insects over the 19 h exposure to treated food. More time was spent feeding on the NHT-treated diet on successive days of experience: 28% of controls on day 1 to 58% of controls on day 4. Insects on the control diet fed more or less uniformly throughout the day, except on day 1, whereas test insects showed an increase in time spent feeding throughout each of the four successive days (Fig. 2). In addition, feeding on the deterrent diet began earlier on successive days (median time before feeding 553, 510, 420 and 263 min on days 1 to 4 respectively), and an increasing proportion of the feeding time occurred in the first 9 h of the 19 h exposure period (8, 26, 38 and 40% on days 1 to 4 respectively).

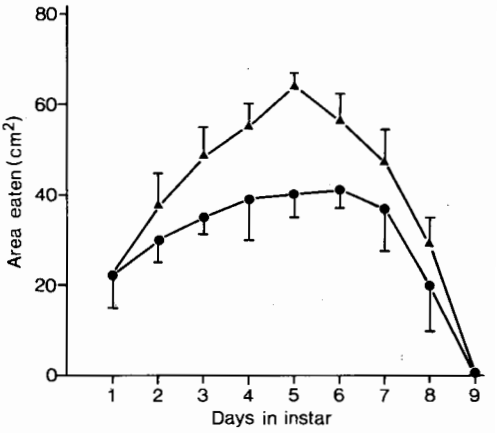

FIG. 1. Daily intake of sorghum leaves with *c.* 1% dry weight of NHT added throughout fifth instar: ▲ = experienced insects; ● = naive insects. For further explanation see text. In each case n = 10. Vertical bars represent SD.

The mean number of meals taken by control insects was always greater than that of the test insects, but there was no obvious changing pattern (mean number of meals/insect on days 1–4; controls 14, 33, 30, 26; treated 11, 18, 19, 16). However, examination of calculated meal length over the 19 h period each day showed a higher proportion of short meals in the treated insects. This proportion became gradually less over the 4 days (Fig. 3), but even on day 4 the majority of meals was less than 6 min in length in insects on deterrent diet, but more than 6 min in control insects. Differences between individuals were very pronounced. For example, two individuals on the deterrent diet ate nothing during their first day of exposure.

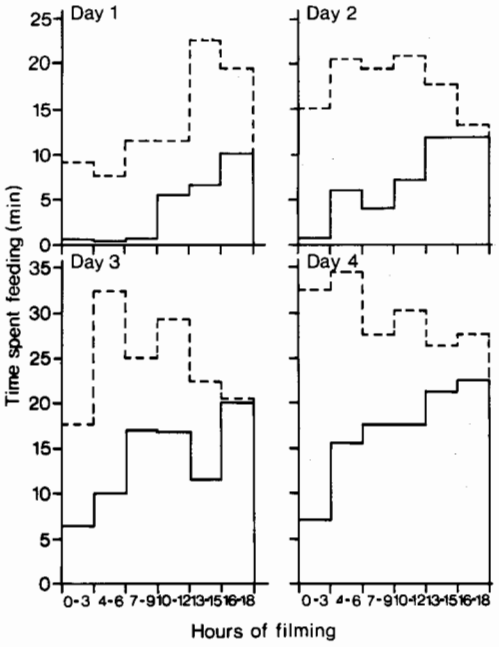

FIG. 2. Mean time spent feeding by individuals on NHT-treated (*c.* 2% dry weight) sorghum (solid lines), and control sorghum (broken lines) in successive 3 h periods on four successive days of experience. Treated, $n = 16$; controls, $n = 8$ or 10.

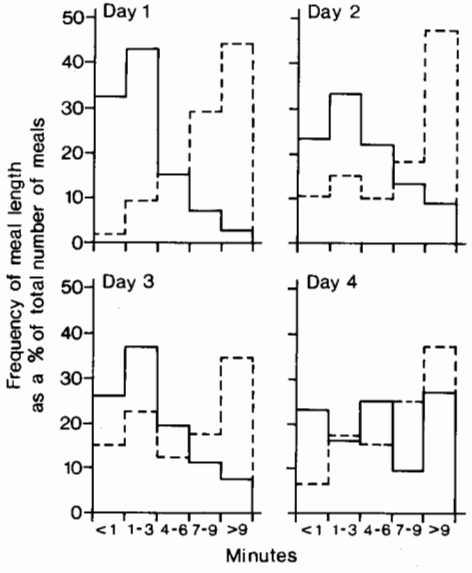

FIG. 3. Meal size distribution estimated from film analyses of insects having NHT-treated (*c.* 2% dry weight) sorghum (solid lines) or control sorghum (broken lines), for 19 h on four successive days. Numbers as in Fig. 2.

The relative frequency of interfeeds of different lengths also changed with time. On day 1 of exposure, insects on NHT-treated diet had significantly more interfeeds of short length (30 min) and of greater length (3 h) than control insects, while interfeeds in the range of 1–1.5 h were more frequent in the controls (Fig. 4). The differences disappeared by day 2, except for the preponderance of very long interfeeds in test insects. However, in these insects, interfeeds of more than 3 h gradually decreased from 18% of the total number on day 1 to 6% of the total number on day 4.

A closer examination of behaviour over the first 7 h of exposure to NHT-treated or control leaves on each day was made by direct observation and compared with data obtained from filming. The trends were similar although the direct observation revealed a larger number of small meals taken by treated insects. This is demonstrated in Fig. 5(a); the number

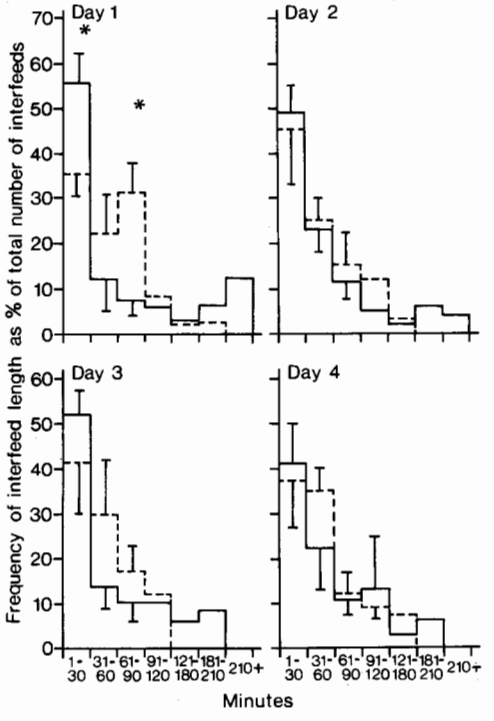

FIG. 4. Distribution of interfeed lengths of insects having NHT-treated (*c.* 2% dry weight) sorghum (solid lines) or control lines (broken lines) for 19 h on four successive days. Vertical bars represent SE; asterisk = significantly different at $P<0.05$ (*t*-test). Numbers as in Fig. 2.

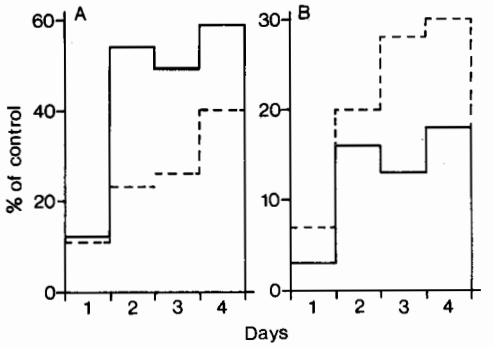

FIG. 5. Comparison of behavioural measurements by film analysis and direct observations over the first 7 h of exposure on four successive days. (A) Numbers of meals by test insects as a percentage of time spent feeding by controls. (B) Time spent feeding by test insects as a percentage of time spent feeding by controls. Solid line, direct observation; broken line, film analysis.

of meals taken by test insects as a percentage of controls is greater in the direct observations. Also, overall time spent feeding in the observation experiment increased as expected in the treated group, but calculating the time spent feeding by test insects as a percentage of times by control insects gave smaller values in the observation experiment compared with the filming experiments (Fig. 5b).

The continuous behavioural observations revealed that insects on the treated diet gained information about leaf quality soon after their introduction into the boxes. At first the insects avoided the treated leaves and tarsal contact often resulted in leg raising and movement away from the leaf, indicating that the NHT was detected by the tarsal sensilla: as a consequence, palpation did not occur on day 1 for over 240 min, whereas on control sorghum leaves palpation occurred from the outset (Fig. 6). Palpation and biting were rarely followed by feeding on treated leaves on day 1 in this 7 h period, but on days 2 and 3 palpation and biting led to feeding on about 20% of occasions, and by day 4 feeding was relatively frequent. Even on day 4, however, only 30% of mouthpart contacts led to feeding. On each day there was a change during the observation period, with more of the contacts leading to feeding in the latter half. On control leaves, palpation and biting usually resulted in feeding.

A combination of the data from direct observation with those from filming, shows the major changes over successive days of exposure. The most notable initial change concerns the time from exposure to NHT-treated diet to palpation; this time drops from 4 h on day 1, to 1 h or less on succeeding days (Fig. 7). Changes also occurred in the time taken between palpation and feeding; in the second half of the 19 h exposure it is notable that on day 1 a period during which most meals were of more than 5 min gave way in the last 5 h to a period during which shorter meals predominated. This phenomenon was progressively lost over the 4 day period and contributed to the overall habituation.

Studies on habituation induction

Insects fitted with capillaries containing 1% NHT solution on the maxillary palps for 30–150 min ate significantly more of the treated diet on the fifth day compared with controls (Table 2). At higher concentrations of NHT there were no significant differences between experienced and naive insects. About 10% of the insects had lost one of the maxillary palps by the time of the test.

Injection of NHT into the haemolymph failed to influence feeding behaviour: on the fifth day experienced and control groups consumed similar amounts of treated diet (10.2 ± 12.3 and $13.5 \pm 8.4\ cm^2$ respectively, mean ±SD). Although NHT-injected insects were knocked down for a short time after injection, mortality was low and times of subsequent ecdysis were similar to those of controls.

Introduction of 32 mg NHT over 4 days through the mouth into the crop increased consumption of the treated diet significantly compared with controls (Table 3). Administration of solid NHT in gelatin capsules had a similar effect (Table 3). Some regurgitation occurred before, during and following treatments but tended to be greatly reduced with repeated treatments. Experiments with ^{14}C-labelled glucose in the capsules showed that only 10% of the insects had measurable contamination of the mouthparts from a single placement. Since each insect had a total of eight capsules *c.* 50% of the insects might have tasted the NHT once in the course of the experiment.

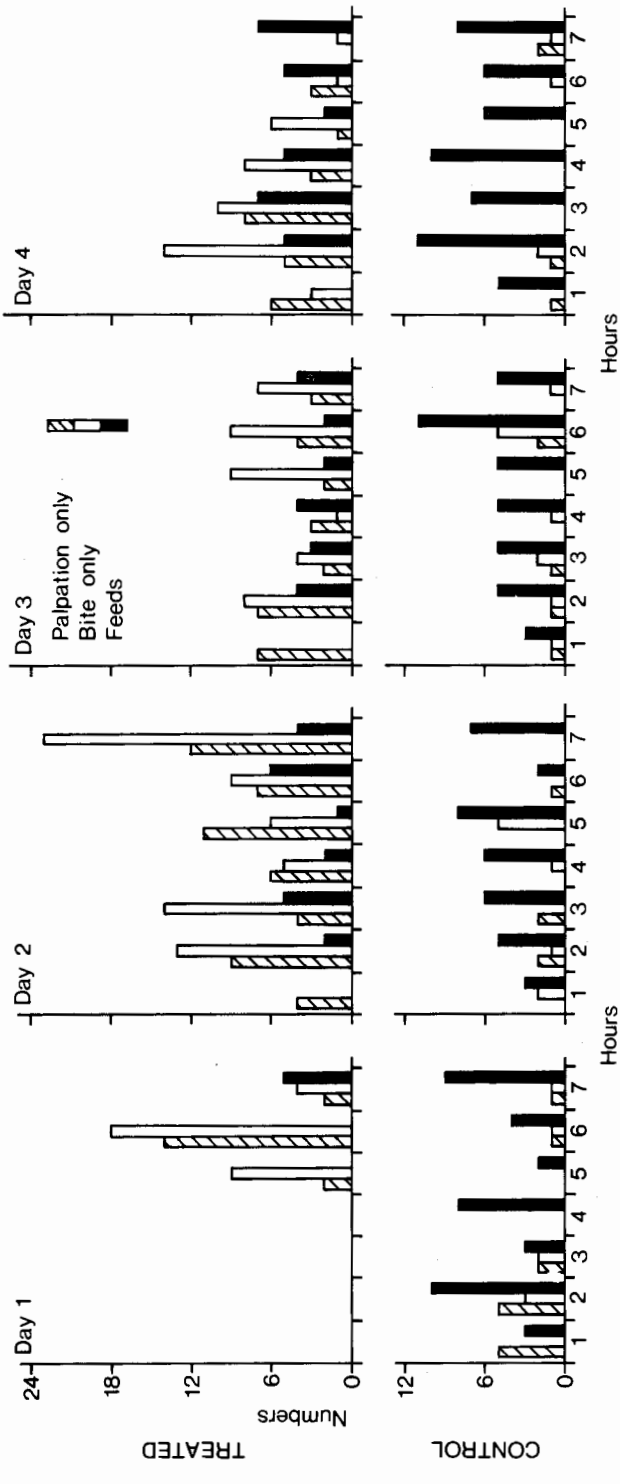

FIG. 6. Frequency distribution of separate palpations (P), bites (B) and feeds (F) of insects having NHT-treated diet or control sorghum over the first 7 h of experience on four successive days. In each case, $n = 4$.

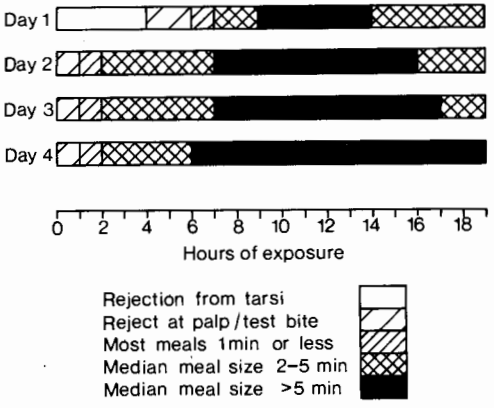

FIG. 7. Summary of the changing patterns in feeding behaviour over the 19 h period of exposure to NHT-treated diet. Hours 1–7 based on four individuals observed directly, plus sixteen insects filmed; hours 8–19 based on sixteen filmed insects.

Consumption was low in all these experiments and lowest of all in the capsule experiment, perhaps as a result of the stress from treatments and the effect of the gelatine. Mortality was above control levels in the NHT-cannulated group, though toxic effects were not apparent.

Discussion

The results described here show that, as shown by Jermy *et al.* (1982), behavioural habituation of *S.gregaria* to nicotine hydrogen tartrate occurs when the insects are forced to feed on contaminated food for long periods over several days. Habituation is usually considered to be a non-associative process and thus not true learning (Marlin & Miller, 1981) though it could perhaps be argued that the insect 'learns' to associate the deterrent with an otherwise suitable food. While it is likely to be centrally mediated, the neural basis of habituation is unknown, and whether or not associative and non-associative processes are neurally distinguishable is a controversial issue (Duerr & Quinn, 1982). Nevertheless, the stimulus environment is important, because when NHT is presented on dry, sucrose-impregnated glass fibre discs, experienced insects eat less (rather than more) of the deterrent than naive insects (Jermy *et al.*, 1982).

The change in behaviour with respect to food through each day, and on successive days, may be attributable to increasing 'hunger' driving acceptance of food which was initially unacceptable. Insects deprived of food for long periods without contact with a deterrent will then tolerate more of the deterrent in the food (e.g. Chapman & Bernays, 1977), so that the change throughout the day in the present experiments may not be true habituation, but rather an overriding of the deterrency input by the increased state of deprivation. However, such behaviour may be an essential feature of the habituation process since it results in increasing contact with the deterrent.

The differences in feeding behaviour between successive days probably do represent habituation. Especially important are those differences over the first few hours of each day, since at this time the insects have had access to untreated food in excess over the previous 5 h and are probably all in similar states of 'repletion'. The changes are summarized in Fig. 7, which shows that the greatest change occurred from day 1 to day 2.

The process of habituation to NHT is not simple. The deterrent input is from a variable number of chemosensory neurones. Rejection after tarsal contact involves input from a limited number of neurones on the tarsi, with a maximum of 2000 neurones which could be stimulated, but in practice probably many fewer (Chapman, 1982). If this deterrent input is overcome or tolerated, palpation and/or a bite test occurs. This involves palp sensilla and peripheral labral receptors, adding several thousand neurones to the possible chemosensory input. Finally if this input is tolerated, a fragment of leaf is removed and ingested, in which case up to 6 or 7×10^3 inner mouthpart chemoreceptor neurones may be stimulated. Thus, as successive steps are reached, the extent of sensory input, including information on both deterrents and phagostimulants, increases: once feeding starts a time factor is also involved. While overcoming these steps during the day is likely to be partly a result of the increased 'hunger', the fact that this behavioural hierarchy is brought forward on successive days, especially on day 2, suggests that habituation to the sensory input is occurring from day to day and perhaps more markedly between days 1 and 2. Although some of these changes may be caused by age-related changes in the insects, these

TABLE 2. Feeding response of *S.gregaria* nymphs on NHT-treated diet after covering of the maxillary palps with NHT solution for various periods on the previous 4 days (n = 24 or 25).

Concentration of NHT solution applied to the palps (%,w/v)	Sum of hours of exposure to NHT during 4 days	Area (mean cm^2 ±SD) of sorghum leaves eaten over 19 h		Dry weight of diet eaten (mean mg ±SD)*	
		Experienced	Naive	Experienced	Naive
1	5.5	23.1 ± 11.8^a	10.5 ± 7.9^b	88.8 ± 64.5^c	34.5 ± 34.3^d
5	5.5	9.8 ± 10.7^e	7.1 ± 7.4^e	28.3 ± 36.5^e	9.3 ± 22.8^e
20	12	14.9 ± 13.9^e	15.0 ± 13.8^e	68.9 ± 60.1^e	78.5 ± 47.5^e

a–b, $P<0.001$; c–d, $P<0.01$; e–e, NS (Wilcoxon two-sample test).
* NHT at *c.* 2% of the dry weight.

TABLE 3. Food consumption of *S.gregaria* nymphs on NHT-treated diet over 19 h, after insertion of NHT into the gut by cannula or within gelatine capsules for 4 days prior to the test.

Treatment	Area of sorghum leaves eaten (mean cm^2 ±SD)	Dry weight of diet eaten ¶ (mean mg ± SD)
NHT-cannulated*	37.2 ± 20.4^a	123.5 ± 72.3^c
Control†	13.8 ± 9.2^b	48.6 ± 37.0^d
NHT-dosed through gelatine capsules‡	10.7 ± 6.5^e	52.4 ± 34.1^c
Control§	6.7 ± 7.6^f	27.9 ± 30.7^d

a–b, $P<0.001$; c–d, $P<0.01$; e–f, $P<0.05$ (Wilcoxon two-sample test).
* 32 mg NHT in 40 µl 0.2% (w/v) agar solution cannulated into crop during 4 days (n = 14).
† 40 µl 0.2% agar only cannulated (n = 22).
‡ *c.* 0.5 mg NHT given in 3 × 1 mm size sealed gelatine capsules, twice a day for 4 days (n = 22).
§ Empty sealed gelatine capsules only given (n = 22).
¶ NHT at *c.* 2% of the dry weight.

are apparently much less marked than the habituation-related changes (see Fig. 1, naive, and Figs. 3 and 6, untreated controls).

In *S.gregaria,* the nature of the deterrent input to the central nervous system from receptors stimulated by NHT is not certain. In the presence of sucrose and salt, NHT induces an overall reduction in firing rates from the mouthpart sensilla in a non-specific manner (Blaney, 1980) but, as discussed by Chapman (1982), there are also likely to be specifically deterrent messages as indicated, for example, by the avoidance behaviour after tarsal contact. This is an important distinction, as habituation seems unlikely to occur in response to a non-specific reduction in input.

Experiments in which maxillary palp sensilla were treated with NHT solutions show clearly that habituation can result from sensory stimulation alone. In both experimental and control (naive) groups, maxillary sensilla could not have been stimulated during the final test because of the nylon sleeves placed over them. These sleeves were empty at the time of the test but will none the less have prevented contact of the palps with the substrate. Deterrent input during the test meal would have been from the remaining sensilla which had previously had no contact with the NHT. Those insects which had previously had the palp sensilla stimulated ate more of the NHT-treated diet. This demonstrates that sensory adaptation was not the basis for behavioural habituation, and that central nervous phenomena are involved. Additionally, any long-term decrease in sensitivity to the deterrent resulting from previous exposure is not an essential feature of the process. Such long-term changes which correlate with increased tolerance, have been shown in several caterpillars (Schoonhoven, 1976; Städler & Hanson, 1976) and may sometimes contribute to behavioural habituation, although it is usual to exclude sensory adaptation in explanations of such processes (Horn & Hinde, 1970).

Habituation occurred after treatment of the palps with 1% NHT solution, but not

with higher concentrations. There are at least two possible explanations: first, continuous exposure to high NHT concentrations probably damages chemoreceptor membranes (W. M. Blaney, pers. comm.) preventing the input of information to the central nervous system; second, strong stimuli are known to be less effective than weaker ones in eliciting habituation responses (Thompson & Spencer, 1966; Hinde, 1970).

Habituation may also be consequent upon ingestion of NHT, although the palp experiment shows that it is not essential. NHT is toxic to *S.gregaria* but with continuous exposure, it is excreted with increasing rapidity by the Malpighian tubules (P. Cottee, pers. comm.), a similar phenomenon to that described by Rafaeli-Bernstein & Mordue (1978) in *Zonocerus variegatus*. Similar high excretory rates are found in *Rhodnius*, and in larvae of *Manduca* and *Pieris* (Self *et al.*, 1964; Maddrell & Gardiner, 1976), following injection or oral administration of nicotine. Thus, after experiencing similar doses, haemolymph concentrations will decrease more rapidly in experienced insects than in naive insects.

In addition, with repeated exposure to NHT, a greater proportion of it is converted to the less toxic cotinine, because of the development of a mixed function oxidase system in the midgut epithelium (P. Cottee, pers. comm.). Such development has been described in other insect species (Brattsten, 1979). In our experiments, on day 1 of feeding on NHT-treated diets a period of relatively long meals is followed by one of shorter meals. Possibly, toxic effects resulting from a large intake of NHT lead to rejection after relatively short meals, but this phenomenon is progressively lost over the 4 day period, and this correlates with an increasing ability of the locusts to excrete and detoxify NHT (P. Cottee, pers. comm.). Injection of NHT into the haemolymph failed to induce habituation, suggesting that there is no physiological input via the haemolymph for habituation induction. However, the lack of response to injection of NHT may be due to its short half-life in the haemolymph.

Habituation to NHT occurs both after direct introduction into the crop in liquid form and in gelatine capsules. In neither treatment can chemosensory stimulation caused by regurgitation of crop content be totally discounted. The chemoreceptors of the epipharyngeal surface of the labrum and on the hypopharynx (Chapman & Thomas, 1978) will almost certainly have been stimulated in many cases by NHT during cannulation, and in a few cases by regurgition after capsule ingestion. Thus possible post-ingestional physiological effects which could result in behavioural habituation cannot be distinguished with certainty from those produced by sensory input.

Individual variation of food intake was considerable throughout our experiments. It may reflect variation in the habituation process, though other factors such as sex, body weight and detoxifying ability could be involved. Experienced individuals ingesting small quantities of treated diet on day 5 converted the smallest proportions to cotinine (P. Cottee, unpublished observation). In the experiments involving maxillary palp treatment, possible sources of variability are that some insects lost a maxillary palp , and that the stress on the insects during treatment and handling had a variable influence on food intake and mortality.

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Appendix

Definitions of terms used in this work

Habituation: Relatively long-lived waning of behavioural response as a result of repeated presentation of the same stimulus and not followed by any reinforcement.

Sensory adaptation: Rapid waning of neural activity at the receptor level with continued exposure to the stimulus.

Non-associative learning: An alteration in behaviour as a result of experience, but without any reinforcement due to 'rewards'. Habituation is the simplest non-associative learning.

Associative learning: Acquisition of the capacity to respond to a stimulus with the reaction to another stimulus, and resulting from their concurrent application a number of times.

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