

According to GRIFFITHS<sup>16</sup> and MACDONALD and M'CREA<sup>17</sup> unilateral bladder responses in dogs and cats are obtained only when the distal ends of cut pelvic nerves are stimulated, while stimulation of intact nerves leads to bilateral contractions more pronounced on the stimulated side. The response in the opposite half was suggested to be dependent on a sacral reflex initiated by stimulation of afferent fibres in the ipsilateral pelvic nerve, the efferent impulses passing through the intact nerve of the contralateral side. The present results in the rat, however, are in agreement with those of INGERSOLL et al.<sup>12,13</sup> in the cat and dog showing bilateral responses to unilateral stimulation of the distal ends of cut pelvic nerves, with or without section of the opposite pelvic nerve.

The responses to pelvic nerve stimulation found in this study were not changed by section of the hypogastric nerves or vice versa, which was also found by INGERSOLL et al.<sup>13</sup> in the dog and by EDVARDSEN<sup>18</sup> in the cat. The contractile response to hypogastric nerve stimulation in the cat is caused via adrenergic fibres<sup>19</sup>. EDVARDSEN<sup>18</sup> found no synergistic effects of concomitant pelvic and hypogastric nerve stimulation in the cat but only reduction of the detrusor contraction as compared to the response to stimulation of the pelvic or hypogastric nerves separately. Sympathetic inhibition of intramural parasympathetic ganglia was suggested, since adrenergic nerve terminals forming synaptic structures around non-adrenergic cells have been demonstrated histochemically in the bladder wall of the cat<sup>20</sup>. The urinary bladder of the rat in contrast to other species contains no intramural ganglion cells<sup>2</sup>. In the present investigation in the rat the pelvic and hypogastric responses were instead added at simultaneous stimulation and in some rats this response

was even bigger than the sum of the responses produced by the nerves stimulated separately. This might indicate a spatial summation of effector cells innervated by cholinergic fibres from the pelvic and hypogastric nerves, suggesting that some muscle fibres are dependent on impulses from both nerves for contraction. This postulates a convergence by some of the fibres from the pelvic and hypogastric nerves. In the rat bladder cholinergic neuro-terminals are in contact with every muscle cell<sup>21</sup>.

**Zusammenfassung.** In der Harnblase von Ratten wurde der Druck während einseitiger und zweiseitiger Pelvicusreizung gemessen. Nach Injektion von Eserin wurde bereits bei einseitiger Reizung eine maximale Blasenkontraktion erhalten. Zusätzliche Reizung der Nn. hypogastrici führt – ohne funktionelle Überlappung – zu weiterer Kontraktionssteigerung.

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<sup>16</sup> J. GRIFFITHS, *J. anat. Physiol.* 29, 61 (1895).

<sup>17</sup> A. D. MACDONALD and E. D. M'CREA, *Q. Jl. exp. Physiol.* 20, 379 (1930).

<sup>18</sup> P. EDVARDSEN, *Acta physiol. scand.* 72, 234 (1968).

<sup>19</sup> P. EDVARDSEN and J. SETEKLEIV, *Acta pharmac. toxic.* 26, 437 (1968).

<sup>20</sup> B. HAMBERGER and K.-A. NORBERG, *Int. J. Neuropharmac.* 4, 41 (1965).

<sup>21</sup> A. EL-BADAWI and E. A. SCHENK, *Am. J. Anat.* 179, 405 (1966).

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### Digestive Enzymes in the Excreta of the Larvae of *Sesamia inferens* Walker and *Chiloatraea infuscatellus* Snell. (Lepidoptera: Insecta)

The fate of the digestive enzymes is unknown<sup>1</sup> but they are generally thought to be inactivated or resorbed<sup>2</sup>. HOBSON<sup>3</sup> detected trypsin, and glycyl-glycine dipeptidase in the excreta of *Lucilia sericata*, ENGELMAN<sup>4</sup> casually observed the activity of trypsin in the excreta of *Leucophaea maderae*, and YANG and DAVIS<sup>5</sup> found chymotrypsin, trypsin and amylase in the excreta of *Aedes aegypti*. During the course of studies of digestive physiology, various digestive enzymes were detected in the hindgut contents of the larvae of *Sesamia inferens*<sup>6</sup> and *Chiloatraea infuscatellus*<sup>7</sup>. To find out the fate of these enzymes, a study of the excreta was undertaken.

**Materials and methods.** 20 mg (wet weight) of fresh excreta of the last larval instar of each insect was collected after feeding sugarcane slices. It was homogenized in 0.5 ml of 0.1 M phosphate buffer (pH 8.2, the pH of the midgut contents), centrifuged at 3000 g for 15 min and the supernatant was used as the enzyme source. The enzymes were tested as described elsewhere<sup>7</sup>.

**Results and conclusion.** The excreta of the larva of *S. inferens* showed appreciable activity of aminotripeptidase, leucine aminopeptidase and glycyl-L-leucine dipeptidase, and a very weak activity of trypsin, prolinase and glycyl-glycine dipeptidase. All these enzymes were present in the hindgut contents of the larva.

In the excreta of the larva of *C. infuscatellus* appreciable activity of aminotripeptidase, leucine aminopeptidase and prolinase was seen, and a very weak activity of trypsin and glycyl-glycine dipeptidase. All these enzymes

were detected in the hindgut contents of the larva; and besides these, the contents also contained chymotrypsin and glycyl-L-leucine dipeptidase.

The activity of amylase, cellulase,  $\alpha$ -glucosidases (maltase, mellezitase, sucrase and trehalase),  $\beta$ -glucosidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -fructosidase, carboxypeptidase and prolidase was absent in the excreta; although sucrase, trehalase and  $\beta$ -glucosidase were present in the hindgut contents, while in the hindgut contents of *C. infuscatellus* a very weak activity of  $\alpha$ -galactosidase and  $\beta$ -fructosidase was also detected.

The presence of the enzymes in the hindgut contents but their absence in its tissue and excreta suggests that they are either denatured, self-hydrolyzed or digested. The absence of the enzymes in the excreta but their presence in the hindgut tissue and its contents reflects the possibility of their resorption in the hindgut. EVANS and PAYNE<sup>2</sup> have also suggested the absorption of the enzymes in the posterior gut regions.

<sup>1</sup> M. F. DAY and P. F. WATERHOUSE, in *Insect Physiology* (Ed. K. D. ROEDER; John Wiley & Sons, New York 1953), p. 318.

<sup>2</sup> W. A. L. EVANS and D. W. PAYNE, *J. Insect Physiol.* 10, 657 (1964).

<sup>3</sup> R. P. HOBSON, *J. exp. Biol.* 8, 109 (1931).

<sup>4</sup> F. ENGELMAN, *J. Insect Physiol.* 15, 217 (1969).

<sup>5</sup> Y. J. YANG and D. M. DAVIES, *J. Insect Physiol.* 17, 2119 (1971).

<sup>6</sup> A. K. AGARWAL, submitted for publication (1974).

<sup>7</sup> A. K. AGARWAL, in preparation.

The presence of the digestive enzymes in the excreta of insects, appears at first, to be a wasteful process. In *S. inferens* and *C. infuscatellus* they may have the function of keeping the microenvironment congenial, since the larval and pupal life of these insects is passed in a tunnel in sugarcane in which the excreta is also discharged.

**Zusammenfassung.** In den Fäces von *S. inferens*-Larven wurde eine beträchtliche Aktivität von Aminotripeptidase, Leucin-Aminopeptidase und Glycyl-L-Leucin-Dipeptidase, sowie eine sehr schwache Aktivität von Trypsin, Prolinase und Glycyl-Glycin-Dipeptidase festgestellt. In den Fäces von *C. infuscatellus*-Larven wurde eine Aktivität von Aminotripeptidase, Leucin-Amino-

peptidase und Prolinase, jedoch nur eine geringe Aktivität von Trypsin und Glycyl-Glycin-Dipeptidase gefunden. Es konnten keine Carbohydrasen festgestellt werden.

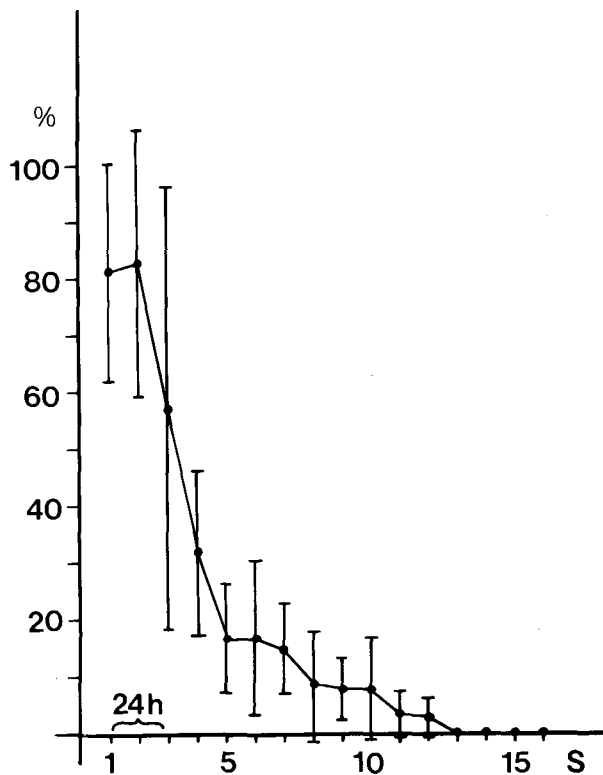
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### Long-Term Habituation to Species-Specific Alarm Calls in a Songbird (*Fringilla coelebs* L.)

When their species-specific alarm call, the 'pink' vocalization, is played back to chaffinches (*Fringilla coelebs* L.), they respond with a variety of behaviour patterns, one of which is 'freezing'<sup>1,2</sup>. Our study is concerned with how such a behaviour pattern changes, from a quantitative point of view, when captive birds are presented with a constant alarm call pattern stimulus twice daily. We also determined to what extent the changes could be considered as 'stimulus specific'. — This investigation was conducted in order to elucidate the effects of natural signal variation on the behaviour of conspecifics.



Long-term habituation to the species-specific alarm calls in the chaffinch (*Fringilla coelebs*): Changes in the response 'freezing' after repetitive stimulation twice daily with a 'pink' call pattern. Ordinate: Duration of 'freezing' (mean and standard deviation of the mean for  $n = 4$ , in % of the maximal value). Abscissa: sequence of stimulus presentations (S).

The birds were placed in a visually and acoustically isolated room 3 days before the experiments started. Locomotory reactions as well as freezing were registered by perch contact and transcribed directly on a multi-channel event recorder. Other responses were recorded on the same equipment over a keyboard by a hidden observer. The illumination was a 12:12 h on/off light cycle. The birds were fed every other day. The stimulus consisted of a series of 10 trisyllabic alarm calls at normal intensity with an interval of 1 sec between them. These were played at a constant volume through a loudspeaker placed near the cage. The length of 'freezing' brought about by the stimulus decreased with the number of stimulus exposures (Figure). Other behavioural parameters showed a similar decrease. This decrease was more marked at the beginning than at the end of the experimental series. A 'warming-up-phase' was only observable in a few of the experimental animals. After approximately 12 stimulations, the animals showed none of the responses observed at the start of the experiments. Only some of the orientation components, which are not included here, remained.

The question as to whether it was the one specific 'pink' alarm pattern which no longer acted as a releaser, or whether the response to other types of stimulus constellations was affected, was tested on conclusion of the above experiments. The animals were presented with stimuli varying both qualitatively and temporally (Table). Practically every change in the original stimulus resulted in a response from the birds (exception: decreased volume, Table, B). The response strength in the tests, however, never reached the maximum or original level. When the original stimulus pattern was presented daily throughout the above test series, the response to it was always nil (Table, K). The 'inhibition' of the releasing effect was thus shown to be stimulus specific and of long duration.

This 'inhibition' can be interpreted as long-term habituation. Operationally defined, this type of habituation is considered as a relatively long-term stimulus specific decrease in response to a repetitive uniform stimulus, not followed by a reinforcement<sup>3,4,1</sup>. Owing to its long-term effects, habituation is considered to be a form of learning. Here specific stimulus parameters, which act as releasers at first, are stored with a negative prefix and computed in the functional context of the releasing

<sup>1</sup> P. MARLER, *Ibis* 98, 231 (1956).

<sup>2</sup> W. FLEUSTER, *J. Ornith.* 114, 417 (1973).

<sup>3</sup> W. H. THORPE, *Learning and Instinct in Animals* (Methuen, London 1963).

<sup>4</sup> S. E. FILE, *Anim. Behav.* 21, 585 (1973).