20% in both regions 4 days after the end of a repeated treatment for 21 days with haloperidol but returned to control level after 8 or 12 days of haloperidol withdrawal.

Previous studies have shown that under in vivo conditions, (^{3}H) spiperone labels mainly DA receptor sites in the rat hippocampal formation[4]. The increase in (^{3}H) spiperone binding observed in the rat hippocampal formation after repeated administration of haloperidol suggests that sustained blockade of dopaminergic transmission results in an increase in DA receptors. This has been found not only in striatum (as previously demonstrated in ex-vivo experiments by Burt et al. and confirmed in vivo in the present study) but also in the hippocampal formation as well as in other limbic areas such as the septum and tuberculum olfactorium. It was not, however, seen in frontal cortex or retina (Bischoff, in preparation). The effect of chronic treatment with haloperidol on in vivo (^{3}H) spiperone binding in hippocampus is time-dependent. A significant effect was first observed after 10 days of treatment, and a further gradual increase was seen up to 28 days (data not shown). With our experimental design it is not possible, however, to decide whether the increase in (^{3}H) spiperone binding reflects a change in the affinity or in the number of DA receptor sites, or both. Although a direct effect of haloperidol on the bioavailability of the radioligand cannot be entirely excluded, it is unlikely in view of the almost total disappearance of haloperidol (as judged by the only weak antagonistic activity against apomorphine-induced stereotyped behaviour in the rat (Delini-Stula, personal communication)) from the brain 4 days after administration. More extensive experiments (dose dependence, chronic treatment with neuroleptics of other types such as phenothiazines) are underway.

In conclusion, the present data suggest that a prolonged blockade of hippocampal dopaminergic transmission by neuroleptics results in the development of a supersensitivity of hippocampal target cells probably related to an increase in DA receptor density and/or affinity. This might account for the occurence of tolerance to the increase in hippocampal DOPAC levels during repeated treatment with haloperidol[9].

Effects of repeated treatment with haloperidol on in vivo (^{3}H) spiperone binding in rat striatum and hippocampus

Duration of treatment (days)	Duration of wash-out period (days)	Specific (^{3}H) spiperone binding Striatum (% of control)	Hippocampus (% of control)
Experiment A			
10	4	116 ± 6(n.s.)	118 ± 5*
Experiment B			
21	4	123 ± 7*	124 ± 3***
21	8	108 ± 5	110 ± 6
21	12	113 ± 8	104 ± 7

In vivo specific (^{3}H) spiperone binding was measured in rat striatum and hippocampus 4–12 days following the withdrawal of a repeated treatment for 10 days (experiment A) or 21 days (experiment B) with haloperidol (1 mg/kg s.c.). Results are means ± SEM of data from 8–12 animals and are expressed as a percentage of control values. *$p < 0.05$; ***$p < 0.001$ vs control (Student's t-test).

1 Acknowledgments. We wish to thank Mrs J. Krauss for skillful technical assistance, Drs Helmut Bittiger and Rainer Ortmann for helpful discussions.
2 S. Bischoff, B. Scatton and J. Korf, Brain Res. *165*, 161 (1979).
3 S. Bischoff, B. Scatton and J. Korf, Brain Res. *179*, 77 (1979).
4 S. Bischoff, H. Bittiger and J. Krauss, Eur. J. Pharmac. *68*, 305 (1980).
5 R. Jork, G. Grecksch, M. Jirka, B. Lössner and H. Matthies, Pharmac. Biochem. Behav. *12*, 317 (1980).
6 P. L. Herrling, Brain Res. *212*, 331 (1981).
7 B. Scatton, H. Simon, M. Le Moal and S. Bischoff, Neurosci. Lett. *18*, 125 (1980).
8 H. Simon, M. Le Moal and A. Calas, Brain Res. *178*, 17 (1979).
9 B. Scatton, Eur. J. Pharmac. *71*, 499 (1981).
10 D. R. Burt, I. Creese and S. H. Snyder, Science *196*, 326 (1977).
11 J. C. Schwartz, M. Baudry, M. P. Martres, J. Costentin and P. Protais, Life Sci. *23*, 1785 (1978).

Immunohistochemical localization of pancreatic polypeptide (PP) in the brain of the larval instar of the hoverfly, *Eristalis aenus* (Diptera)[1]

M. El-Salhy

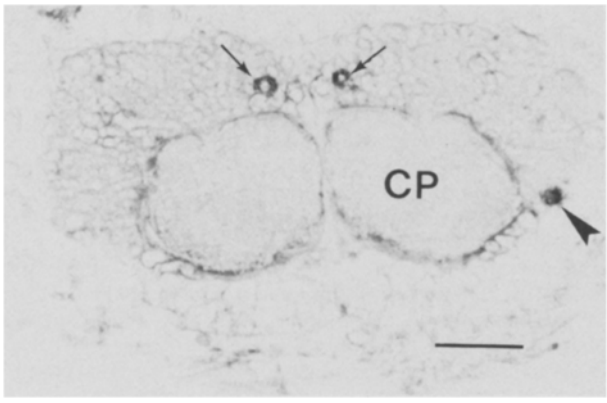
Department of Pathology, University of Uppsala, Box 553, S-75122 Uppsala (Sweden), 5 February 1981

Summary. 2 pancreatic polypeptide (PP) immunoreactive cells were observed in each half of the protocerebrum of the last (5th) larval instar of the hoverfly, *Eristalis aenus*. No PP immunoreactive nerve fibers could be detected in the brain.

Pancreatic polypeptide (PP) immunoreactive cells have been reported to occur in the brain of the 5th larval instar and adults of the silkworm *(Bombyx mori)*[2] and of the adults of blowfly *(Calliphora erythrocephala)*[3]. As described in a previous report[4], PP immunoreactive cells have been observed in the fused ventral ganglia of the last (5th) larval instar of the hoverfly *(Eristalis aenus)*. In order to test whether or not PP cells are also present in the brain of this larval instar of the hoverfly, the present study has been performed.

The brain of the 5th (last) larval instar of the hoverfly, *E. aenus* (provided by Dr R. Abou-Elella, Department of Entomology, Cairo University, Egypt) was dissected out as described previously[4] and fixed for 24 h in Bouin's fluid, embedded in paraffin and cut serially at 5 µm. For the demonstration of PP cells, the peroxidase-antiperoxidase (PAP) method of Sternberger[5] was applied. The anti-BPP (No. 615-R-110-146-10, donated by Dr R. E. Chance, Lilly Res. Lab., Indianapolis, USA) was used at the dilution of 1:1600. In addition to the controls described previously[4], the first-layer antiserum was pre-incubated with rabbit anti-human Clq complement (Dako, Lot No. 038B) for 24 h at 4 °C.

In the brain of the larval hoverfly 2 PP immunoreactive cells were observed in each protocerebrum hemisphere. One cell was located in the mid-anterior and the other was in a latero-posterior position to the corpora pedunculata. They were small round or polygonal cells (fig.). No PP



Horizontal section of the brain of the 5th larval instar of the hoverfly. 2 PP immunoreactive cells (arrows), 1 in each protocerebrum hemisphere, located in the mid-anterior position to the corpora pedunculata (CP). At the bottom to the right (arrow head) another PP immunoreactive cell is seen in latero-posterior situation to the corpora pedunculata. The outer nerve fiber layers of the corpora pedunculata show nonspecific staining. Scale bar = 21 µm.

immunoreactive nerve fibers could be detected. However, the outer nerve fiber layers of the corpora pedunculata showed a weak staining. The specificity of the immunostaining of these cells was confirmed by the following observations: a) no positive staining was seen when the first-layer antiserum was replaced by normal rabbit serum; b) the pre-incubation of anti-BPP with rabbit anti-Clq complement had no effect on the positive result obtained; and c) the immunostaining was abolished completely after preincubation of the antiserum with 125 µg of BPP (a gift from Dr R.E. Chance) per ml of the diluted antiserum. On the other hand, the weak staining of the outer nerve fibers of the corpora pedunculata appeared to be nonspecific, being observed after the previously mentioned negative controls.

The present observations confirm previous reports[2,3] on the occurrence of PP cells in the brain of insects. Furthermore, the absence of PP immunoreactive nerve fibers to convey the secretory products of the PP cells to the distant neurohaemol organ or other organs is in agreement with the assumption[3] that these cells may have a local function, acting on the neighbouring nerve tissues.

1 Support by a grant from the Swedish Medical Research Council (project No. 12X-102) and by a Swedish Institute scholarship.
2 R. Yui, T. Fujita and S. Ito, Biomed. Res. *1*, 42 (1980).
3 H. Duve and A. Thorpe, Cell Tissue Res. *210*, 101 (1980).
4 M. El-Salhy, R. Abou-El-Ela, S. Falkmer, L. Grimelius and E. Wilander, Regul. Peptides *1*, 187 (1980).
5 L.A. Sternberger, Immunocytochemistry. 2nd edn. John Wiley, New York 1979.

Stereological analysis of thyroid follicle structure

C. Penel, J.-B. Rognoni, D. Durieu and C. Simon[1]

Laboratoire d'Endocrinologie Cellulaire, Université de Provence, F-13331 Marseille Cedex 3 (France), 19 May 1980

Summary. Besides a possible bias induced by experimental design, some difficulties inherent in stereological analysis appear during thyroid morphometric investigations. These technical considerations are discussed in the light of the follicle size distribution of the 2 lobes of a rat thyroid.

The thyroid gland is composed of a set of convex entities of different diameters; the follicles, which consist of a shell of epithelial cells surrounding a colloid lumen. In order to study the action of these follicles as functional units in the mechanism of iodine turnover and hormone secretion, their size distribution must be determined. Owing to technical difficulties, little work has been carried out to determine thyroid follicle size distribution[2-4]. With the exception of fixation artefacts recently outlined by Denef et al.[5], most of the bias and difficulties appearing during thyroid stereological analysis have not yet been considered or quantified; bias is caused by the non-sphericity of the follicles, underestimation of the number of small diameter follicle sections, statistical fluctuations resulting from the sampling procedures, and the various merits and limits of parametric and nonparametric methods. These technical points will be developed in this note together with the stereological analysis of the 2 lobes of a rat thyroid.

Experimental design. The 2 thyroid lobes of a rat fed on an iodine-rich diet of the industrial type were fixed in Bouin's fluid, dehydrated in alcohol and embedded in paraplast. From each lobe, 9 slices of thickness t = 5 µm, separated by a distance of 300 µm, were systematically taken. The 1777 (left lobe) and 1772 (right lobe) areas of the follicle sections were then measured by planimetry.

Stereological analysis. As a general rule, large follicles are found at the periphery of each lobe of the thyroid whereas all the other sizes are located inside the gland. It is assumed that the centers of these follicles are positioned, inside their respective spaces of distribution, according to the Poisson process[6]. To obtain information about the average follicle structure with minimal sample variance, slices were chosen perpendicular to the greatest axis of each lobe[7,8].

The thyroid follicles are convex entities of tetrakaidecaedral shape. In order to determine their size distribution, a simple

Mean diameter x (µm) and standard deviation Sx (µm) of the follicle size distribution of the 2 lobes of a rat thyroid obtained by the nonparametric and the parametric methods

	Left lobe		Right lobe	
	$\bar{x} \pm \sqrt{\text{var } \bar{x}}$	$Sx \pm \sqrt{\text{var } Sx}$	$\bar{x} \pm \sqrt{\text{var } \bar{x}}$	$Sx \pm \sqrt{\text{var} Sx}$
Nonparametric method	89.0 ± 3.7	35.0 ± 2.2	78.8 ± 3.2	34.4 ± 1.8
Parametric method	87.5 ± 1.7	34.5 ± 1.0	77.5 ± 1.5	32.27 ± 0.9