

With the exception of **VII**, these compounds occur widely in the plant kingdom¹⁹. In addition, compounds **I**^{20a}, **II**^{20b} and **IV**^{4b, 20c} have been isolated from various insect species. Until now, compounds **III**, **V** and **VI** do not appear to have been identified in insects.

The binate hairpencils of *A. ochlea* consist of 5 different hair types (cf. figure 2)^{15, 16}. Black hairs (A) lie on the outer lateral sides of bundles of white hairs. A layer of another hair type (B) separates black hairs from the long white hairs (C) which make up the major part of the organ. The long white hairs produce pheromone-transfer-particles which are disseminated onto the female during courtship flight²¹. Enclosed by the long white hairs are the central hairs (E), which are separated from the rest of the organ by a tightly closing cone made of another hair type (D).

In view of the morphological complexity of this organ, it seemed desirable to determine whether some of the chemical components were localized in particular parts of the hairpencils. Interestingly, a spacial distribution of the chemicals was found. Preparations analyzed were a) black hairs (A), b) total white hairs (B-E), and c) central hairs (E). Chromatograms of extracts of these preparations are represented schematically in figure 3. The biological meaning of the topochemical distribution of the different compounds is under investigation.

Electrophysiological experiments, now in progress, show the olfactory effectiveness of all 7 characterized com-

pounds. Although the compounds have not been tested in behavioral experiments, their stimulatory power and their distinct distribution on the complex scent organ supports the idea that at least certain ones act as pheromones. In addition, it is quite possible that they play a major role in species recognition.

Analyses in progress with indoor raised *A. ochlea* indicate that the production of some of the volatile hairpencil components depends on the ingestion by males of precursors from plants. This, as well as the influence of contacts between abdominal and alar scent organs on the chemical components found in the hairpencils, shows similarity to *Danaus* species^{14, 22}.

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Increased membrane-bound polyribosome fraction in the brains of rats with hereditary diabetes insipidus

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Summary. Rats homozygous for hypothalamic diabetes insipidus were found to have a significantly higher concentration of membrane-bound polyribosomes than their heterozygous littermates. There was no difference in the concentration of free polyribosomes.

Hereditary hypothalamic diabetes (D.I.) occurs in rats of the Brattleboro strain. Animals that are homozygous for D.I. lack the ability to synthesize vasopressin, in contrast to their heterozygous littermates^{3, 4}. Vasopressin has been reported to be implicated in memory processes⁵. Support for this notion comes from findings suggesting that homozygous D.I. rats have memory deficits in comparison with their heterozygous littermates⁵. Since membrane-bound brain polyribosomes may also play a role in memory consolidation⁶, we decided to examine the polyribosome distribution between brain cytoplasm and brain membranes of homozygous and heterozygous D.I. rats.

Materials and methods. 9 homozygous and 10 heterozygous male D.I. rats of the Brattleboro strain (obtained from TNO, Zeist, The Netherlands) were housed in groups of 4-5. The rats were 3 months old. The homozygous rats weighed approximately 250 g, whereas the heterozygous ones weighed 350 g. The experiment was conducted according to a randomized block design. The rats were killed by decapitation, and the brains were rapidly removed and placed on dry ice. Cerebellum and pineal gland were removed and discarded. The brain was weighed and rinsed in ice-cold homogenization buffer. All subsequent procedures were performed at 0-4°C.

Free polyribosomes were prepared according to the method of Bloemendal et al.⁷. The brains were homogenized separately and centrifuged at 12,000 × g for 20 min. In the resulting supernatant, protein was determined according to the method of Lowry et al.⁸. For the preparation of membrane-bound polyribosomes, the initial 12,000 × g sediment was rehomogenized in 2/3 of the original homogenization volume, after which deoxycholate and Triton X-100 were added to a final concentration of 0.5% (w/v) and 1% (v/v), respectively, followed by the procedure of Bloemendal et al.⁷. Absorbances (A) of polyribosome suspensions at 236, 260 and 280 nm were measured with a Zeiss Spectrophotometer Q II using 1 cm quartz cuvettes. 0.3 ml fractions of the polyribosomal preparations were analyzed on 10 ml 15-35% (w/v) linear sucrose gradients, which were centrifuged at 4°C for 1 h at 40,000 rpm in a SB 283 rotor. After centrifugation, the absorbance of the gradients at 260 nm was continuously monitored using a Gilford Spectrophotometer 240 (2-mm-cuvet). The results were analyzed by means of a randomized blocks analysis of variance.

Results and discussion. The brains of homozygous D.I. rats were lighter than those of heterozygous rats (mean ± SEM: 1.36 ± 0.01 and 1.55 ± 0.03, respectively; U = 5, p < 0.002, 2tailed Mann-Whitney U-test).

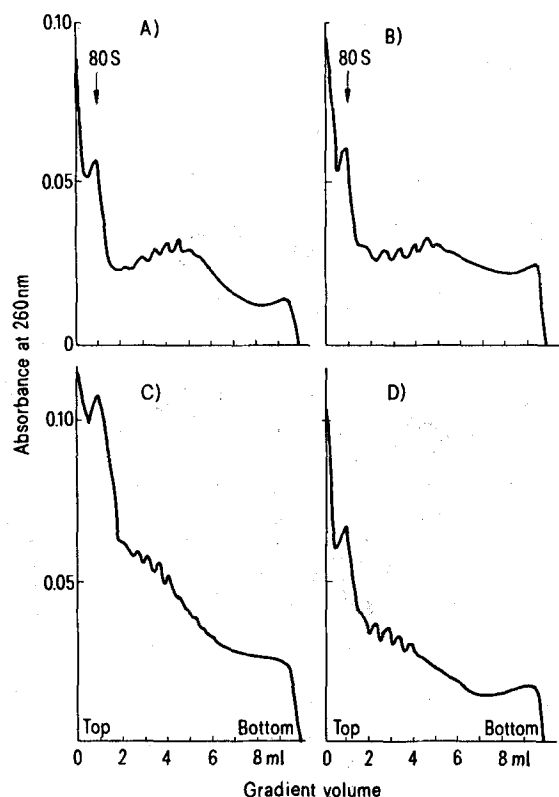
Polyribosome concentrations and corresponding polysome/monosome ratios in brains of rats homozygous or heterozygous for diabetes insipidus

	A Heterozygotes	Homozygotes	B Heterozygotes	Homozygotes
Free polyribosomes	1.11 ± 0.05	1.09 ± 0.05	4.19 ± 0.15	4.07 ± 0.09
Membrane-bound polyribosomes	1.07 ± 0.04	1.35* ± 0.03	2.71 ± 0.08	2.81 ± 0.12

A: Polyribosome concentrations. Each value represents the mean (as mg/g wet wt). Heterozygotes: 10 animals; homozygotes: 9 animals. The SE of the mean are also shown. B: Polysome/monosome ratios. Each value represents the mean of 6 estimations. Monosome = 80S. * Significance of difference with respect to the heterozygous group $p < 0.05$.

Protein contents of the initial 12,000 × g supernatant were not statistically different for heterozygous and homozygous rats (mean ± SEM: 21.73 ± 0.80 and 23.01 ± 0.74 mg protein/g wet wt, respectively; $U = 27.5$). The biochemical findings are summarized in the table, and the figure. The A_{260}/A_{280} - and A_{260}/A_{236} -ratios of the isolated free polyribosomes were found to be minimally 1.81 and 1.63, respectively; the corresponding values for the membrane-bound material were 1.77 and 1.40, respectively, indicating a high degree of purity. The table shows that in the brains of the homozygous rats 26% more polyribosomes were attached to the membrane fraction than in the brains of the heterozygous rats. There was no such difference for the free polysome fraction.

The difference in membrane-bound polyribosomes is also apparent in the sucrose profiles of these polyribosomes (figure).



Polyribosome profiles from brains of rats homozygous or heterozygous for diabetes insipidus. A Free polyribosomes from brains of homozygous rats. B Free polyribosomes from brains of heterozygous rats. C Membrane-bound polyribosomes from brains of homozygous rats. D Membrane-bound polyribosomes from brains of heterozygous rats. The large peak near the top of the gradient is the 80S monomer peak, verified in control gradients following mild ribonuclease treatment.

The table further shows that there were no significant differences between homozygous and heterozygous D.I. rats in the polysome/monosome ratio calculated from the profile.

Only 5% of the polyribosomes in the postmitochondrial supernatant (12,000 × g sup) was membrane-bound, as judged by the effect of treatment of this preparation with the DOC/Triton X-100 mixture (results not shown). Zomzely et al.⁹ showed that the DOC-treatment has a disaggregating effect on the polyribosomes. After detergent-free isolation, relatively more large polyribosomal aggregates were found than after an isolation procedure using DOC. At a 0.5% DOC-concentration, a loss of 16% in the free ribosomal fraction (polysomes and monosomes) was observed. This disaggregating effect can explain the differences in the profiles between free and membrane-bound polyribosomes (figure) and the low polysome/monosome ratios for the membrane-bound fraction. DOC also attacks monosomes, giving a RNA-fraction and a detergent-protein complex¹⁰.

In a previous investigation¹¹, we have found that urinary excretion of tyrosine and tryptophan, among others, was markedly elevated in homozygous D.I. rats, while the concentration of these amino acids in the brain did not differ much between homozygous and heterozygous rats. One explanation of the elevated excretion of amino acids is that protein turnover may be increased in homozygous D.I. rats. Similarly, the increase in membrane-bound brain polyribosomes that we found in the present study can be explained in terms of an enhanced overall protein turnover. Further investigations are needed to evaluate this possibility.

Both vasopressin and membrane-bound polyribosomes have been implicated in memory consolidation^{5,6}. One would therefore expect a decreased concentration of membrane-bound polyribosomes in the brains of homozygous D.I. rats. The present finding of an increased rather than a decreased concentration of whole-brain membrane-bound polyribosomes in homozygous D.I. rats suggests that there is no straightforward relation between the 2 variables as far as their possible involvement in memory consolidation is concerned.

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