The Neuropeptides
Pharmacology Biochemistry and Behavior, Vol. 5, Suppl. 1, pp. 29–33. ANKHO International Inc., 1976.

Dorsal Hippocampus: A Site of Action of Neuropeptides on Avoidance Behavior?

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WIMERSMA GREIDANUS, TJ.B. VAN AND D. DE WIED. *Dorsal hippocampus: a site of action of neuropeptides on avoidance behavior?* PHARMAC. BIOCHEM. BEHAV. *5*: SUPPL. 1, 29–33, 1976. – Vasopressin and ACTH 4-10 induce a dose dependent long-term, respectively short-term inhibition of extinction of a pole jumping avoidance response in animals with sham lesions in the antero-dorsal hippocampus. Small lesions, causing a restricted damage in this area of the brain, partly inhibit the behavioral effect of vasopressin. Extensive lesions in the antero-dorsal hippocampus completely prevent the inhibitory effects of vasopressin and of ACTH 4-10 on extinction of the avoidance response. The extensive lesions in the dorsal hippocampus complex do not interfere with the rate of extinction, but acquisition of the response is retarded. These observations do not allow the conclusion that the hippocampal complex is the locus of action of neuropeptides in relation to avoidance behavior; it is more likely that this brain region is but one site of behavioral action of these hormones, and that the limbic system needs to be intact to permit the neuropeptides to exert their behavioral effects.

Vasopressin ACTH 4-10 Antero-dorsal hippocampus Avoidance behavior

THE NEUROHYPOPHYSIAL system is involved in the formation and maintenance of avoidance behavior. Administration of vasopressin and of vasopressin analogs increases resistance to extinction of active and of passive avoidance behavior for a relatively long period of time [1, 18, 24]. Moreover absence of vasopressin results in a marked deficit in avoidance conditioning. Brattleboro rats homozygous for hereditary hypothalamic diabetes insipidus display a severe disturbance in retention of active and passive avoidance responses in comparison with their heterozygous littermates [5,20].

Intracerebroventricular administration of antivasopressin serum, resulting in a blockade of centrally available vasopressin, also interferes with passive avoidance behavior. In contrast, intravenous administration of 100 times as much antiserum which results in a temporary diabetes insipidus is ineffective on behavior [26]. These results, together with the observation that intracerebroventricular administration of vasopressin itself needs approximately 200 times less of the peptide than a subcutaneous injection for a behavioral effect of the same magnitude (De Wied, unpublished data) points to an important role of the brain-ventricular system in the distribution of vasopressin within the brain [23]. Anatomical evidence exists for a direct release of neurosecretory material into the cerebrospinal fluid (CSF). Axons of the supraoptic tract, filled with neurosecretory substances have been shown to end in the infundibular recess [29]. In addition vasopressin has been demonstrated to be present in the CSF [7,17]. Thus the CSF may be an efficient way of transport of vasopressin to brain structures which are the site of action of this nonapeptide in relation to avoidance behavior. However, it is also possible that ascending neurosecretory fibers carry their material directly to different structures of the limbic system ([15], Pfaff, personal communications).

On the other hand not only peptides from posterior pituitary origin, but also ACTH and MSH, present in the anterior and intermediate lobe of the pituitary, as well as the analogs ACTH 1-10 and ACTH 4-10 inhibit extinction of active and passive avoidance behavior [19]. These peptides, however, exhibit a short-term effect on extinction of avoidance responses. Depending on the dose the inhibitory effect on avoidance extinction lasts from several hours to maximally one day [22,24].

Whether the brain-ventricular system plays a role in the distribution of ACTH or MSH related peptides as well is uncertain, but not impossible. ACTH has been reported to be present in the CSF and a special system has been suggested for a retrograde transport of anterior pituitary peptides from the pituitary to the brain and more particular to the brain ventricles [2].

It has been assumed that ACTH and MSH and related peptides affect arousal in the central nervous system, which may lead to an alteration in the motivational strength of specific environmental stimuli, which in turn may be reflected in behavioral changes [19,21]. The long-term effect of vasopressin on avoidance behavior suggests that this peptide affects brain processes which are related to memory consolidation [27]. This difference between the effects of vasopressin one one hand and those of ACTH related peptides on the other made it desirable to further investigate the sites of the behavioral action of these peptides.

Implantation studies as well as lesion experiments point to limbic midbrain structures like the posterior thalamic area and the septal region as important structures in this

respect [22, 24, 25, 28]. Since the hippocampal complex is frequently reported as an essential brain structure in avoidance conditioning the present study was designed to further explore the role of this brain region in the effect of vasopressin and of ACTH 4-10 on avoidance behavior.

METHOD

Male rats of an inbred Wistar strain, weighing 120–140 g were operated 4–5 days before being submitted to avoidance training. By use of a stereotaxic instrument bilateral lesions were made in the antero-dorsal hippocampus, resulting in either restricted or more extensive damage of this area. For a restricted damage a single lesion was made on each side, for more extensive damage two adjacent lesions were made on either side of the midline (Fig. 1).

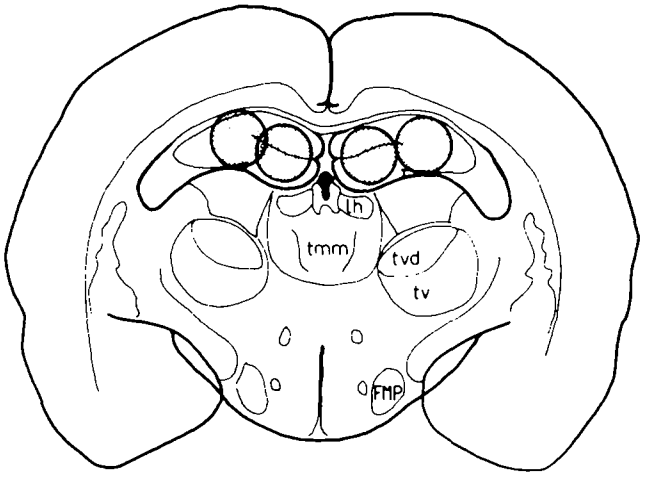

FIG. 1. Rat brain diagram illustrating the size and localization of extensive lesions in the antero-dorsal hippocampus. FMP: Fasciculus medialis prosencephali; lh: nucleus habenulae lateralis; tmm: nucleus medialis thalami, pars medialis; tv: nucleus ventralis thalami; tvd: nucleus ventralis thalami, pars dorsalis.

When animals had recovered from the operation avoidance conditioning was started in the pole jumping apparatus. Animals were trained to jump onto a pole which was placed in the middle of the box, within 5 sec after the onset of the conditioned stimulus (CS), which was a light emitted by a 40 W bulb placed on the transparent top of the box. Rats which failed to jump onto the pole within the 5 sec period received the unconditioned stimulus (US) of scrambled electric footshock (0.25 mA). Ten conditioning trials were given each day with an average intertrial interval of 60 sec. Animals which made 8 or more positive responses on the last acquisition day were injected subcutaneously (SC) with graded amounts of lysine-8-vasopressin (LVP (90 IU/mg) in 0.5 ml. On the next day extinction trials were run in which the US was not presented.

For the ACTH studies the heptapeptide ACTH 4-10 was injected SC in graded doses 1 hr prior to each extinction session. Each treatment group contained 7–11 animals.

At the end of the experiment the brain was fixated by formaldehyde perfusion, removed and cut into slices for histological examination of the size and the exact localization of the lesions.

RESULTS

Training in sham operated animals and in animals with small lesions in the antero-dorsal hippocampus lasted for 4 days. Rats with small lesions showed a tendency towards a retarded acquisition. However, on Day 4 the criterion of 80% conditioned avoidance responses (CAR's) was reached.

In sham operated animals a dose dependent long-term inhibition of extinction was observed following 1 μg or 3 μg LVP. At the 6th extinction session high response levels were still present in the LVP treated groups whereas no response occurred anymore in the placebo group (Fig. 2).

In animals with small lesions in the dorsal hippocampal area no effect was found on the rate of extinction following administration of 1 μg or 3 μg LVP. Only a high dose of 9 μg LVP was able to inhibit extinction of the CAR (Fig. 3).

Animals with extensive lesions in the antero-dorsal hippocampus showed retarded acquisition of the avoidance response. Therefore, acquisition training was continued for 6 sessions. At that session, which was performed on Day 8, the learning criterion was reached and animals were injected with either 1 μg or 9 μg LVP. As indicated in Fig. 4 no effect of LVP was observed on extinction of the CAR. Neither 1 μg nor 9 μg of the peptide resulted in an inhibition of the avoidance response. On the 4th extinction session all treatment groups, including the 9 μg LVP group, were almost completely extinguished.

In the ACTH experiments only sham operated animals and rats bearing extensive lesions in the antero-dorsal hippocampus were used. Again the latter animals showed a retarded acquisition as compared with the sham operated controls.

Daily injection of ACTH 4-10 in doses of 1 μg and 3 μg at 1 hr prior to each extinction session resulted in a dose-dependent inhibition of extinction of the CAR in sham operated rats (Fig. 5). However, doses of 9 μg ACTH 4-10 failed to delay extinction of the avoidance response in animals bearing extensive lesions in the antero-dorsal hippocampus (Fig. 6).

Moreover it appeared that neither in the LVP nor in the ACTH 4-10 experiments the lesions themselves affected significantly the rats of extinction of the CAR, although in the experiments a tendency towards a facilitation of extinction was observed in the lesioned animals.

DISCUSSION

Extensive lesions in the antero-dorsal hippocampal area prevent vasopressin- and ACTH-induced preservation of the pole jumping avoidance response, whereas the lesions themselves do not interfere significantly with the rate of extinction of the response. However, acquisition of the CAR is retarded in animals with extensive lesions in the dorsal hippocampal complex. Smaller lesions, causing a more restricted damage of the antero-dorsal hippocampus only partly inhibited the behavioral effect of vasopressin.

The hippocampal complex has been reported to play an important role in behavioral performance [6,14]. Although the variability in lesion size makes it difficult to compare the results from different laboratories, the literature agrees that hippocampal lesions affect behavior, albeit that smaller lesions may sometimes have different effects than larger ones. In particular the effect of hippocampectomy on one way conditioning is not clear. Small lesions have no effect, but large lesions produce deficits [9,13]. Retardation of acquisition as observed in our experiments was also found

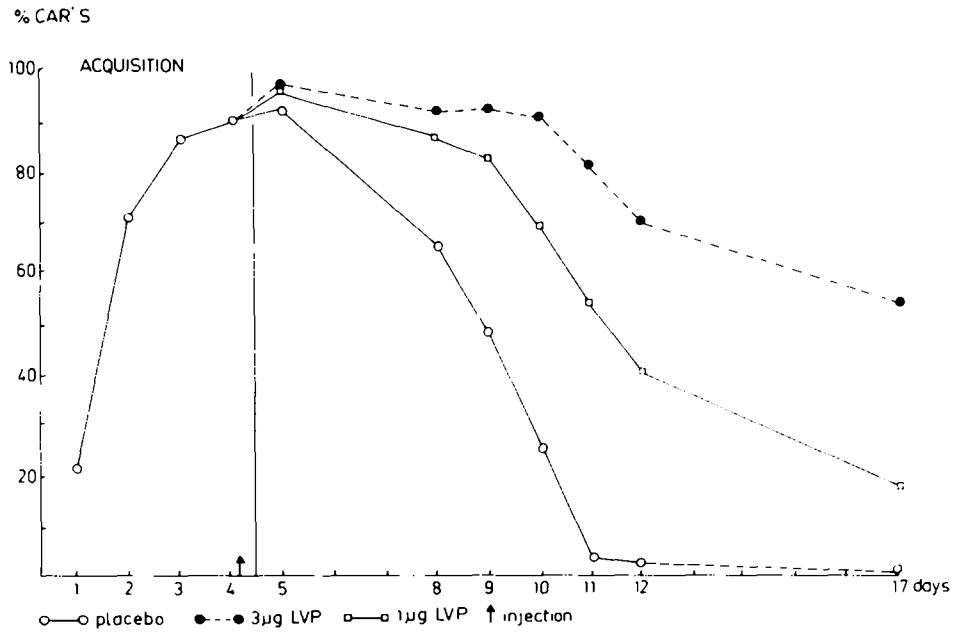

FIG. 2. Effect of a single injection of LVP on extinction of a pole jumping avoidance response in sham hippocampectomized rats.

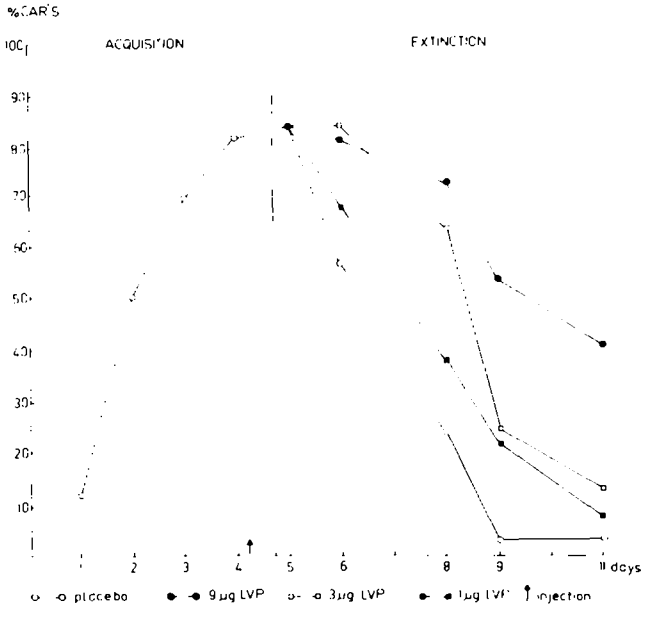

FIG. 3. Effect of a single injection of LVP on extinction of a pole jumping avoidance response in rats with small lesions in the antero-dorsal hippocampus.

by Nadel [11], who suggested that the dorsal hippocampal area may function in the modulation of responses to motivating stimuli. It has also been suggested that in situations with high arousal, hippocampectomized animals show a tendency of repeating previous responses due to either greater response preservation or to deficient response inhibition [6,8]. This tendency is frequently observed in two way active avoidance situations. In the one way avoidance situation as used in the present experiments, however, no effect of dorsal hippocampal lesions on extinction could be observed.

Previous experiments from our group [4, 25, 28] showed that other brain areas such as the posterior thalamic area, including the parafascicular nuclei, and the rostral septal area are also important for the behavioral effects of neuropeptides like vasopressin and ACTH- and MSH-analogs. Those results, together with the present data, suggest that vasopressin and ACTH analogs act on midbrain limbic structures.

It may well be that we are not dealing with a more or less restricted and well defined region in the brain as the site of action of neuropeptides in relation to avoidance behavior. It is more likely that limbic system structures need to be intact in order to allow neuropeptides to exert their behavioral effects, and that the hippocampus is but one substrate of this action of the hormones [3]. In this respect it is worthwhile to mention that administration of ACTH 4-10 results in alterations in electrical activity in both hippocampus and posterior thalamus as induced by the electrical stimulation of the reticular formation in freely moving rats [16]. Thus, ACTH 4-10 may facilitate transmission of midbrain limbic structures or increase the excitability of the theta-generating system, thereby enhancing the arousal state of certain brain regions.

It may be that under circumstances related to specific stimuli endogenous vasopressin, ACTH and MSH and probably their analogs as well, are (directly) released into the CSF and transported to midbrain limbic structures. It has been found by Pelletier *et al.* [12] that the choroid plexus, ependymal cells and meninges are strongly labelled following intracarotic injection of radioactive α-MSH, suggesting that MSH and/or its metabolites penetrate into the CSF. Moreover they observed a specific uptake in the striatum and the reticular nucleus of the thalamus. This preferential localization of radioactivity, particularly in the thalamus could probably be correlated with modifications

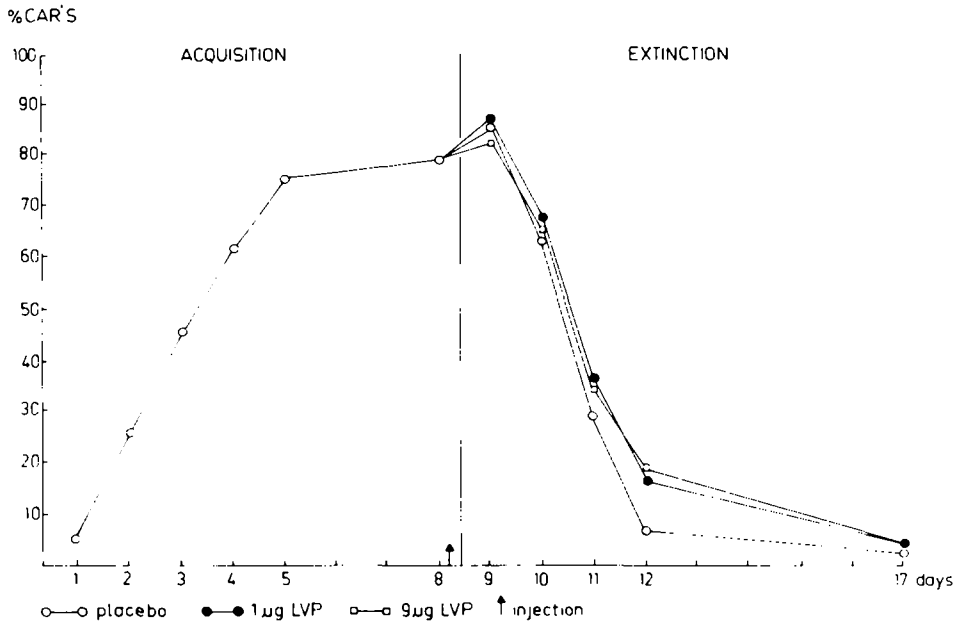

FIG. 4. Effect of a single injection of LVP on extinction of a pole jumping avoidance response in rats with extensive lesions in the antero-dorsal hippocampus.

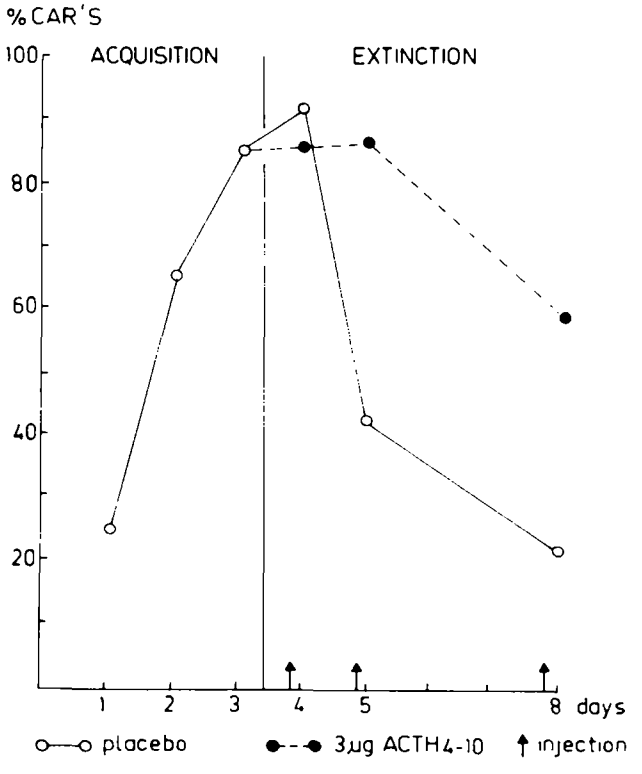

FIG. 5. Effect of repeated injections of ACTH 4-10 on extinction of a pole jumping avoidance response in sham hippocampectomized rats.

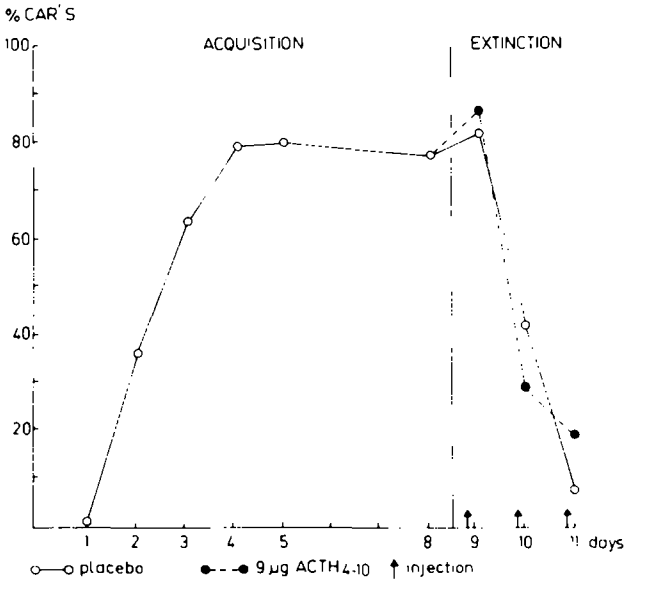

FIG. 6. Effect of repeated injections of ACTH 4-10 on extinction of a pole jumping avoidance response in rats with extensive lesions in the antero-dorsal hippocampus.

of behavior [12]. However, as stated before it is also possible that direct neuronal connections exist between the sites of synthesis and the sites of action of vasopressin ([15], Pfaff, personal communication). In addition a variety of hypothalamic peptides have been found in many brain regions and the release of these peptides from peptidergic nerve terminals into extra-hypothalamic limbic structures may be of biological importance in the regulation of behavior [10].

ACKNOWLEDGEMENTS

The skilful assistance and enthusiastic participation of Angèle Balvers, Anneke Bakker, Ruud Wielaard and Maud Rapis is gratefully acknowledged.

REFERENCES

1. Ader, R. and D. de Wied. Effects of lysine vasopressin on passive avoidance learning. *Psychon. Sci.* **29:** 46–48, 1972.
2. Allen, J. P., J. W. Kendall, R. McGilvra and C. Vancura. Immunoreactive ACTH in cerebrospinal fluid. *J. clin. Endocr. Met.* **38:** 586–593, 1974.
3. Bohus, B. The hippocampus and the pituitary-adrenal system hormones. In: *The Hippocampus*, Volume 1, edited by R. L. Isaacson and K. H. Pribram. New York: Plenum Press, 1975, pp. 323–353.
4. Bohus, B. and D. de Wied. Failure of α-MSH to delay extinction of conditioned avoidance behavior in rats with lesions in the parafascicular nuclei of the thalamus. *Physiol. Behav.* **2:** 221–223, 1967.
5. Bohus, B., Tj.B. van Wimersma Greidanus and D. de Wied. Behavioral and endocrine responses of rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain). *Physiol. Behav.* **14:** 609–615, 1975.
6. Douglas, R. J. The hippocampus and behavior. *Psychol. Bull.* **67:** 416–442, 1967.
7. Heller, H., S. H. Hasan and A. Q. Saifi. Antidiuretic activity in the cerebrospinal fluid. *J. Endocr.* **41:** 273–280, 1968.
8. Kimble, D. P. Hippocampus and internal inhibition. *Psychol. Bull.* **70:** 285–295, 1968.
9. Liss, Ph. Avoidance and freezing behavior following damage to the hippocampus or fornix. *J. comp. physiol. Psychol.* **66:** 193–197, 1968.
10. Martin, J. B., L. P. Renaud and P. Brazeau. Hypothalamic peptides: New evidence for "peptidergic" pathways in the CNS. *Lancet* II, **7931:** 393–395, 1975.
11. Nadel, L. Dorsal and ventral hippocampal lesions and behavior. *Physiol. Behav.* **3:** 891–900, 1968.
12. Pelletier, G., F. Labrie, A. J. Kastin and A. V. Schally. Radioautographic localization of radioactivity in rat brain after intracarotid injection of ^{125}I-α-melanocyte-stimulating hormone. *Pharmac. Biochem. Behav.* **3:** 671–674, 1975.
13. Rich, I. and R. Thompson. The role of hippocampo-septal system, thalamus and hypothalamus in avoidance conditioning. *J. comp. physiol. Psychol.* **59:** 66–72, 1965.
14. Segal, M. and J. Olds. Behavior of units in hippocampal circuit of the rat during learning. *J. Neurophysiol.* **35:** 680–690, 1972.
15. Sterba, G. Das oxytocinerge neurosekretorische system der Wirbeltiere, Beitrag zu einem erweiterten konzept. *Zool. Jb. Physiol.* **78:** 409–423, 1974.
16. Urban, I. and D. de Wied. Changes in excitability in the theta activity generating substrate by ACTH 4-10 in the rat. *Expl Brain Res.* **24:** 325–334, 1976.
17. Vorherr, H., M. W. B. Bradbury, M. Hoghoughi and C. R. Kleeman. Antidiuretic hormone in cerebrospinal fluid during endogenous and exogenous changes in its blood level. *Endocrinology* **83:** 246–250, 1968.
18. Wied, D. de. Long term effect of vasopressin on the maintenance of a conditioned avoidance response. *Nature (London)* **232:** 58–60, 1971.
19. Wied, D. de. Pituitary-adrenal system hormones and behavior. In: *The Neurosciences Third Study Program*, edited by F. O. Schmitt and F. G. Worden. Cambridge: MIT Press, 1974, pp. 653–666.
20. Wied, D. de, B. Bohus and Tj.B. van Wimersma Greidanus. Memory deficit in rats with hereditary diabetes insipidus. *Brain Res.* **85:** 152–156, 1975.
21. Wied, D. de, B. Bohus, I. Urban, Tj.B. van Wimersma Greidanus and W. H. Gispen. Pituitary peptides and memory. In: *Chemistry, Structure and Biology of Peptides*, Proc. 4th American Peptide Symposium, edited by R. Walter and J. Meienhofer. Ann Arbor, Michigan: Ann Arbor Scientific Publ., 1976, pp. 635–643.
22. Wimersma Greidanus, Tj.B. van and D. de Wied. Effects of systemic and intracerebral administration of two opposite acting ACTH-related peptides on extinction of conditioned avoidance behavior. *Neuroendocrinology* **7:** 291–301, 1971.
23. Wimersma Greidanus, Tj.B. van and D. de Wied. The physiology of the neurohypophysial system and its relation to memory processes. In: *Biochemical Correlates of Brain Function*, edited by A. N. Davison. London: Academic Press, Inc., 1976, in press.
24. Wimersma Greidanus, Tj.B. van, B. Bohus and D. de Wied. Effects of peptides hormones on behavior. In: *Progress in Endocrinology*, Proc. 4th Int. Congr. Endocr., Int. Congr. Series no. 273, edited by R. Scow. Amsterdam: Excerpta Medica, 1973, pp. 197–201.
25. Wimersma Greidanus, Tj.B. van, B. Bohus and D. de Wied. Differential localization of the influence of lysine vasopressin and of ACTH 4-10 on avoidance behavior: A study in rats bearing lesions in the parafascicular nuclei. *Neuroendocrinology* **14:** 280–288, 1974.
26. Wimersma Greidanus, Tj.B. van, J. Dogterom and D. de Wied. Intraventricular administration of anti-vasopressin serum inhibits memory in rats. *Life Sci.* **16:** 637–644, 1975.
27. Wimersma Greidanus, Tj.B. van, B. Bohus and D. de Wied. The role of vasopressin in memory processes. In: *Hormones, Homeostasis and the Brain, Progress in Brain Research*, Volume 42, edited by W. H. Gispen, Tj.B. van Wimersma Greidanus, B. Bohus and D. de Wied. Amsterdam: Elsevier, 1975, pp. 133–141.
28. Wimersma Greidanus, Tj.B. van, B. Bohus and D. de Wied. CNS sites of action of ACTH, MSH and vasopressin, in relation to avoidance behavior. In: *Anatomical Neuroendocrinology*, Proc. Conference on Neurobiology of CNS-hormone interactions, Chapel Hill (N.C.) 14–16 May, 1974, edited by W. E. Stumpf and L. D. Grand. Basel: Karger, A. G., 1975, pp. 284–289.
29. Wittkowski, W. Elektronenmikroskopische Studien zur intraventrikulären Neurosekretion in den Recessus infundibularis der Maus. *Z. Zellforsch.* **92:** 207–216, 1968.