Neuropeptides 4: 331-341, 1984

BEHAVIOURAL ACTIONS OF VASOACTIVE INTESTINAL PEPTIDE (VIP)

G.A. Cottrell, H.D. Veldhuis, W.H. Rostene[1] and E.R. de Kloet, Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands and [1]INSERM U.55, Hôpital St. Antoine, 184 rue du Fg. St. Antoine, 75654 Paris Cedex 12, France.

ABSTRACT

The effect of vasoactive intestinal peptide (VIP) was studied on fear-motivated behaviours, exploration of a novel environment and on novelty and ACTH-induced grooming. VIP was administered via a plastic cannula into the lateral ventricle. Retention of a step-through passive avoidance task was inhibited by 10 and 30 ng VIP injected 1 hour before the retention test. Extinction of pole-jumping active avoidance behaviour was facilitated by 10 and 100 ng VIP. Mild effects were observed in an open field test on exploration and grooming activity. In conclusion, VIP produces inhibitory effects on fear-motivated behaviours.

INTRODUCTION

Vasoactive intestinal peptide (VIP), a highly basic 28 amino acid peptide, originally isolated from hog intestine (1) has a discrete localization in the central nervous system as shown by immunocytochemistry and radioimmunoassay. Highest concentrations of the peptide have been found in the cerebral cortex, hippocampus, hypothalamus and amygdala (2-6). Hypothalamic VIP has been implicated in neuroendocrine control of pituitary function, in particular prolactin release (7-11). VIP has been suggested to regulate brain energy metabolism through its glycogenolytic effects (12). The vasodilatory effects of VIP (13,14) may be explained by localization of the peptide in cerebrovascular nerves. Recent evidence suggests that VIP participates in the control of hippocampal functions. VIP is present in hippocampal interneurons that project locally (2,6,15,16). Specific binding sites for VIP have been demonstrated in hippocampus (17,18) and electrophysiological studies showed excitatory effects of the peptide on hippocampal and cortical neurons (19,20). VIP modulates *in vitro* the number of serotonin receptor (5 HT_1) receptors in subregions of the hippocampus as shown on membranes (21) and by quantitative autoradiography (22). VIP level (23), $5HT_1$ receptors (24,25) and 5HT synthesis rate (26) in hippocampus are under control of corticosterone, the rat's naturally occurring glucocorticoid, which has hippocampal neurons as an important target in the brain (27,28).

Since the interaction between 5HT and corticosterone in the hippocampus is thought to be involved in control of neural mechanisms underlying stress-responsiveness, learning and memory processes (28-30), it is possible that

VIP also participates in the control of these functions. We tested this hypothesis by studying the effects of VIP on extinction of fear-motivated tasks, exploration of a novel environment, and on novelty- and ACTH-induced grooming.

MATERIALS AND METHODS

Animals

Male Wistar rats were used in all experiments. They were individually housed in cages placed in a room with a 14 hr light: 10 hr dark cycle (lights on at 0500 hr), with free access to food and tap water. All experiments were performed in the morning.

Surgery

Rats weighing 140 - 160 g were implanted for intracerebroventricular (icv) injection with a polyethylene cannula in one of the lateral ventricles under Hypnorm anesthesia (31). They were allowed a minimum of 1 week recovery. The correct positioning of the cannula was checked by dissection of the brain.

Treatments

Native porcine VIP (supplied by Prof. V. Mutt, Stockholm, Sweden) and ACTH-(1-24) (Organon International BV, Oss, The Netherlands) were dissolved in 0.9% saline. Injections were given icv in a volume of 1 µl except in the grooming experiment where 3 µl were used. Control animals received the same volume of vehicle (saline).

Behavioural procedures

Pole-jump active avoidance.

Extinction of an active avoidance behaviour was studied in a pole-jumping situation as described previously (32,33). The conditioned stimulus (CS) was the light of a 40 W electric bulb; the unconditioned stimulus (US) was an electric shock of 0.20 mA delivered through the grid floor of the test chambre 5 sec after CS onset. The CS remained on during presentation of the US for a maximum of 20 sec. Rats were conditioned to avoid the US by jumping onto a pole (diameter 1.5 cm) located in the centre of the test box (30 x 30 x 40 cm). Ten trials per day, with an intertrial interval of 60 sec (range = 40 - 80 sec) were given for 4 consecutive days. Extinction sessions, of 10 non-reinforced trials, were given on Day 5. Those animals which made 8 - 10 avoidance responses on the first extinction session were used for further experimentation. The rats received VIP or saline treatment immediately after the end of the first extinction session and two more extinction sessions were run 2 and 4 hours later.

Passive avoidance behaviour

Animals were trained on a one-trial, step-through passive avoidance task (34). The experimental apparatus consisted of an illuminated platform attached to a large, dark compartment. Rats were habituated to the dark compartment for 2 min, then placed onto the platform and their step-through latency (STL)

into the dark compartment was recorded. Rats normally enter the dark compartment within 15 sec. On the following day, after three more trials, unavoidable scrambled footshock (0.4 mA, 2 sec) was delivered through the grid floor of the dark compartment. Ten sec after this single learning trial, rats were returned to their home cage. The retention test was given 24 hours later: the rat was placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 300 sec. Number of approaches to the door before actual entry was also recorded. Treatment with VIP or saline was given one hour prior to the retention test (pre-retention treatment) or immediately after the learning trial (post-learning treatment).

Open-field behaviour.

A circular open-field (radius 40 cm, height 31 cm), having a floor divided into oblong blocks and a 8 cm radius circle in the centre, was used. It was illuminated by a 60 W electric bulb, 40 cm above the floor. Each rat was placed into the centre of the open-field and given a 5 min test session. The number of floor units crossed (ambulation, divided into outer and inner), rearing (free or supported by the wall), the number and duration of grooming bouts and defecation were recorded (35). VIP or saline was injected icv 1 hr before the start of the session.

Grooming.

Grooming behaviour was recorded as described previously (36). Rats were placed individually into novel plastic boxes (24 x 12.5 ± 14 cm) in a low noise room and a 50 min observation period was started 15 min later. The occurrence of grooming (including face and body grooming, scratching and licking) or not grooming was recorded every 15 sec. In the first experiment, the effect of VIP on novelty-induced grooming over a 50 min period was observed. Rats were injected with 0, 10, 100 or 300 ng VIP icv at 15 min prior to testing. In the second experiment, the effect of VIP on ACTH-induced grooming was investigated. A standard dose - 300 ng/ 3 µl - of ACTH that reliably induces grooming(36), was given. A double injection method was used: saline-saline, saline-VIP, saline-ACTH or VIP-ACTH, with a 10 min inter-injection interval. The first injection occurred 20 min and the second injection 10 min prior to the test.

Statistical analysis.

Analysis of variance was used to analyse the pole-jump active avoidance behaviour, open-field behaviour and grooming behaviour. Passive avoidance was analyzed by Kruskal-Wallis analysis of variance.

RESULTS

The extinction of a pole-jump active avoidance task was significantly facilitated by 10 and 100 ng VIP at both 2 and 4 hours ($F = 58.784$ (2,13), $p < 0.001$ and $F = 59.265$ (2,13), $p < 0.001$) (Fig. 1). In the passive avoidance situation, 10 and 30 ng VIP injected 1 hr before the retention test significantly attenuated the retention response at 24 hours (K-W = 9.888, $p < 0.05$). VIP injected immediately after the learning session had no significant effect on retention at 24 hours, although a decrease was seen after 1 and 10 ng (Table I). There was no significant difference in number of ap-

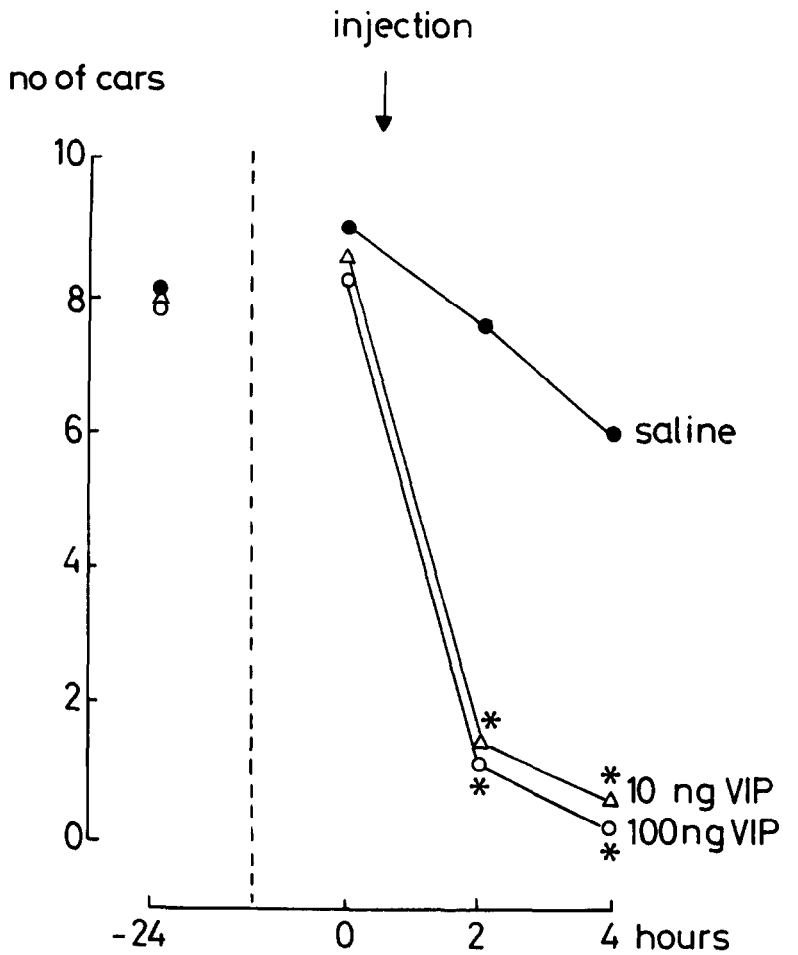

Fig. 1. Effect of VIP on the rate of extinction of pole-jumping active avoidance behaviour. Groups are depicted as follows: ● —— ● saline, △ —— △ 10 ng VIP, and ○ —— ○ 100 ng VIP. Number of rats per group was 5 for the 0 and 100 ng, and 6 for the 10 ng.
* $p < 0.001$ vs. saline.

proaches following either type of injection (Table I). VIP had no effect on ambulation in the open-field, 10 ng decreased free rearing and 100 ng decreased number of grooming bouts (Table II). However, in the grooming test, VIP had no significant effect on novelty-induced grooming measured over a 50 min period ($F = 2.475$ (3,16)) (Table III) or on ACTH-induced grooming (Table IV).

TABLE I

Effect of VIP on one-trial, step-through passive avoidance learning responses

pre-retention treatment	n	STL[2]	no. of approaches[3]
saline	16	137.5	5.6 ± 1.0
1 ng VIP[1]	12	136.5	4.4 ± 0.8
10 ng VIP	12	30.0+	3.6 ± 0.8
30 ng VIP	7	43.0+	2.3 ± 0.6
100 ng VIP	18	249.0	5.2 ± 1.1
post-training treatment			
saline	9	160.0	7.4 ± 1.7
1 ng VIP	8	91.5	5.1 ± 1.8
10 ng VIP	8	72.5	4.1 ± 0.8
100 ng VIP	8	128.5	4.4 ± 0.8

Statistical differences are expressed as follows:
For STL Mann Whitney U-test + $p < 0.05$.
For no. of approaches: Analysis of variance, $F = 1.381$ (4,60) and $F = 1.636$ (3,29(respectively.
[1] dose per rat icv (1 µl).
[2] median step-through latency in seconds.
[3] mean number of approaches.

DISCUSSION

The present experiments show that VIP facilitated extinction of pole-jumping active avoidance and attenuated retention of a passive avoidance response. VIP suppressed retrieval processes rather than consolidation of the aversive footshock experience, since only the pre-retention injection was effective. Mild effects were observed in an open field test; one dose of VIP (10 ng) decreased free rearing and another dose (100 ng) decreased the number of grooming bouts, while novelty- and ACTH-induced grooming were not affected. Thus, it appears that VIP exerts an inhibitory action on the expression of fear-motivated behaviour.

The inhibitory effect of VIP on passive avoidance behaviour disappears, when the dose is increased to 100 ng icv. Such a U-shaped effect of peptides on behaviour is a common observation. It may be that VIP embodies smaller sequences with a distinct and specific action on brain function and that is revealed at different dose levels. In addition, VIP has neuroendocrine effects (7-11) and altered release of pituitary hormones may have consequences for behavioral performance of the animals.

Interestingly, adrenal corticoids exert inhibitory effects on conditioned behaviour that are similar to the ones observed in the present study; a

TABLE II

Effect of VIP on open field behaviour in rats

treatment	amb. wall*	amb. centre	rearing wall	rearing free	grooming bouts	time grooming	defecation
saline	96.2 ± 7.6	14.2 ± 4.2	16.2 ± 1.8	10.5 ± 1.9	3.3 ± 0.5	20.9 ± 6.5	4.5 ± 0.9
1 ng VIP[1]	109.3 ± 6.2	15.5 ± 4.7	15.2 ± 1.3	9.3 ± 1.1	4.2 ± 0.4	26.9 ± 8.6	5.8 ± 0.7
10 ng VIP	105.5 ± 6.7	15.3 ± 3.1	18.7 ± 2.2	5.0 ± 1.3+	3.5 ± 1.0	21.6 ± 7.3	5.3 ± 0.7
100 ng VIP	101.5 ± 7.4	14.8 ± 2.9	16.8 ± 2.4	10.5 ± 1.6	1.3 ± 0.4‡	7.6 ± 4.0	5.5 ± 0.7

[1] dose per rat icv (1 µl).
* mean ± S.E.M. is given, n = 6.
+ $p < 0.05$: 10 ng vs. 0, 1 and 100 ng.
‡ $p < 0.05$: 100 ng vs. 0 and 1 ng.

TABLE III

Effect of VIP on novelty-induced grooming

treatment	n	Amt. of Grooming[2]
saline	5	58.2 ± 10.8
10 ng VIP[1]	5	35.2 ± 6.3
100 ng VIP	5	33.4 ± 2.9
300 ng VIP	5	39.8 ± 6.6

[1] dose per rat icv.
[2] mean of total positive 15th sec grooming scores are given ± S.E.M.

TABLE IV

Effect of VIP on ACTH-induced grooming

treatment	n	Amt. of Grooming[3]
saline-saline	9	33.3 ± 8.0
saline-VIP[1]	9	41.6 ± 8.2
saline-ACTH[2]	9	152.7 ± 9.3
VIP-ACTH	9	153.0 ± 14.6

[1] 100 ng VIP per rat icv (3 µl).
[2] 300 ng ACTH per rat icv (3 µl).
[3] mean of total positive 15th sec grooming scores are given ± S.E.M.

finding which has been explained as elimination of behaviour, that is of no more relevance. Corticoids given to intact rats or mice facilitated extinction of a pole-jumping avoidance response and suppressed passive avoidance retention after subcutaneously administration of rather high doses as well as after infusion or implantation in the hippocampus (37-40). Adrenalectomy facilitated extinction of food-rewarded (41) and attenuated extinction of fear-motivated behaviour (42) and these behaviours were normalized after corticosterone replacement (41,42). The behavioural effect of corticosterone was remarkably specific for the steroid, which corresponds to the binding specificity of the corticosterone receptors with principal localization in hippocampal neurons (43,44). Interaction of corticosterone and 5HT is thought to be involved in modulation of stress responsiveness and extinction of fear motivated behaviours (28-30). 5HT neurons project from the midbrain raphe nuclei to the hippocampus (30,45,46). Corticosterone increases 5HT synthesis rate (26) and 5HT level (29) and reduces the number of $5HT_1$ receptors in hippocampal terminal regions and in the raphe area (24,25). For full expression of corticosterone effects on certain behaviours an intact raphe-hippocampal 5HT system appeared necessary (47). Some of the interneurons in the

hippocampus contain VIP (2,6,15,16). VIP immunostaining is observed in bipolar basket non-pyramidal cells in stratum oriens and radiatum, and in multipolar neurons in stratum laucunosum moleculare (15,16). Pyramidal cells and the granular neurons of the dentate gyrus, which contain corticosterone receptors, do not contain VIP immunoreactivity. Adrenalectomy results in reduced hippocampal VIP level, that is normalized by corticosterone replacement (23).

VIP increases in vitro the number of $5HT_1$ receptors in the dorsal subiculum as judged by autoradiographical analysis (22) and the effect of VIP on $5HT_1$ receptors in the hippocampus can be modulated by corticosterone (48).

One possible physiological implication of the above-mentioned neurochemical effects of VIP could be that the peptide, the 5HT system and corticosterone interact in the hippocampus in the regulation of behaviour. Indeed, VIP and 5HT appear to have similar behavioural effects in relation to the sleep-waking cycle, which may be mediated by the hippocampus (49). It was reported by these authors that VIP overcomes the insomnia produced by PCPA, a 5HT synthesis blocker. In this connection it was reported that corticosterone treatment of adrenalectomized rats influences the diurnal pattern of both locomotor activity and paradoxical sleep (50). As was shown in this study VIP facilitated extinction of active and passive avoidance behaviour. It is conceivable that VIP also participates in the control of a neural mechanism underlying behaviour, that is also affected by corticosterone and 5HT.

ACKNOWLEDGEMENT

The authors are grateful to Dr. B. Spruyt for helpful criticism concerning the grooming experiment and they wish to thank J. Brakkee for his technical assistance.

REFERENCES

1. Said, S.I. and Mutt, V. (1970). Polypeptide with broad biological activity: Isolation from small intestine. Science 169: 1217-1218.
2. Lorén, I., Emson, P.C., Fahrenkrug, J., Björklund, A., Alumets, J., Håkanson, R. and Sundler, F. (1979). Distribution of vasoactive intestinal polypeptide in the rat and mouse brain. Neuroscience 4: 1953-1976.
3. Sims, K.B., Hoffman, D.L., Said, S.I. and Zimmerman, E.A. (1980). Vasoactive intestinal polypeptide (VIP) in mouse and rat brain: An immunocytochemical study. Brain Research 186: 165-183.
4. Fuxe, K., Hökfelt, T., Said, S.I. and Mutt, V. (1977). Vasoactive intestinal polypeptide and the nervous system: Immunohistochemical evidence for localization in central and peripheral neurons, particularly intracortical neurons of the cerebral cortex. Neuroscience Letters 5: 241-246.
5. Besson, J., Rotsztejn, W., Laburthe, M., Epelbaum, J., Beaudet, A., Kordon, C. and Rosselin, G. (1979). Vasoactive intestinal peptide (VIP): Brain distribution, subcellular localization and effect of deafferentation of the hypothalamus in male rats. Brain Research 165: 79-85.
6. Rostene, W.H., Leranth, C.S., Maletti, M., Mezey, E., Besson, J., Eiden,

L.E., Rosselin, G. and Palkovits, M. (1982). Distribution of vasoactive intestinal peptide (VIP) following various brain transections in the rat by radioimmunoassay and electronmicroscopic immunocytochemistry. Neuropeptides 2: 337-350.
7. Kato, Y., Iwasaki, Y., Iwasaki, J., Abe, H., Yanaihara, N. and Imura, H. (1978). Prolactin release by vasoactive intestinal polypeptide in rats. Endocrinology 103: 554-558.
8. Ruberg, M., Rotsztejn, W.H., Arancibia, S., Besson, J. and Enjarbert, A. (1978). Stimulation of prolactin release by vasoactive intestinal peptide (VIP). European Journal of Pharmacology 51: 319-320.
9. Shaar, C.J., Clemens, J.A. and Dininger, N.B. (1979). Effect of vasoactive intestinal polypeptide on prolactin release in vitro. Life Sciences 25: 2071-2074.
10. Vijayan, E., Samson, W.K., Said, S.I. and McCann, S.M. (1979). Vasoactive intestinal peptide: Evidende for a hypothalamic site of action to release growth hormone, luteinizing hormone, and prolactin in conscious ovariectomized rats. Endocrinology 104: 53-57.
11. Rotsztejn, W.H., Benoist, L., Besson, J., Beraud, G., Bluet-Pajot, M.T., Kordon, C., Rosselin, G. and Duval, J. (1980). Effect of vasoactive intestinal peptide (VIP) on the relase of adenohypophyseal hormones from purified cells obtained by unit gravity sedimentation. Neuroendocrinology 31: 282-286.
12. Magistretti, P.J., Morrison, J.H., Shoemaker, W.J., Sapin, V. and Bloom, F.E. (1981). Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: A possible regulatory mechanism for the local control of energy metabolism. Proceedings of the National Academy of Sciences of the United States of America 78: 6535-6539.
13. Larsson, L.I., Edvinsson, L., Fahrenkrug, J., Kakanson, R., Owman, C., Schaffalitzky de Muckadell, O.B. and Sundler, F. (1976). Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves. Brain Research 113: 400-404.
14. McCulloch, J. and Edvinsson, L. (1980). Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide. American Journal of Physiology 238: 4449-4456.
15. Köhler, C. (1982). Distribution and morphology of vasoactive intestinal polypeptide-like immunoreactive neurons in regio superior of the rat hippocampal formation. Neuroscience Letters 33: 265-270.
16. Léránth, Cs. and Frotscher, M. (1983). Commissural afferents to the rat hippocampus terminate on vasoactive intestinal polypeptide-like immunoreactive non-pyramidal neurons. An EM immunocytochemical degeneration study. Brain Research 276: 357-361.
17. Taylor, D.P. and Pert, C.B. (1979). Vasoactive intestinal polypeptide: Specific binding to rat brain membranes. Proceedings of the National Academy of Sciences of the United States of America 76: 660-664.
18. Besson, J., Dussaillant, M., Marie, J.C., Rostene, W. and Rosselin, G. (1984). In vitro autoradiographic localization of vasoactive intestinal peptide (VIP) binding sites in the rat central nervous system. Peptides in press.
19. Dodd, J., Kelly, J.S. and Said, S.I. (1979). Excitation of CA_1 neurons of the rat hippocampus by the octacosapeptide vasoactive intestinal polypeptide (VIP). British Journal of Pharmacology 66: 125P.
20. Phillis, J.W., Kirkpatrick, J.R. and Said, S.I. (1978). Vasoactive intestinal polypeptide excitation of central neurons. Canadian Journal of Physiology and Pharmacology 56: 337-340.

21. Rostene, W.H., Fischette, C.T., Rainbow, T.C. and McEwen, B.S. (1983). Modulation by vasoactive intestinal peptide of serotonin$_1$ receptors in the dorsal hippocampus of the rat brain: An autoradiographic study. Neuroscience Letters 37: 143-148.
23. Rotsztejn, W.H., Besson, J., Briaud, B., Gagnant, L., Rosselin, G. and Kordon, C. (1980). Effect of steroid on vasoactive intestinal peptide in discrete brain regions and peripheral tissues. Neuroendocrinology 31: 287-291.
24. Biegon, A., Rainbow, T.C. and McEwen, B.S. (1984). Corticosterone modulation of neurotransmitter receptors in rat hippocampus: a quantitative autoradiographic study (in press).
25. De Kloet, E.R. and Sybesma, H. Short-term effect of corticosterone on 5HT$_1$ receptors in hippocampus and raphe area (in preparation).
26. De Kloet, E.R., Kovács, G.L., Szabo, G., Telegdy, G., Bohus, B. and Versteeg, D.H.G. (1982). Decreased serotonin-turnover in the dorsal hippocampus of rat brain shortly after adrenalectomy: selective normalization after corticosterone substitution. Brain Research 239: 659-663.
27. McEwen, B.S., Weiss, J.M. and Schwartz, L.S. (1969). Uptake of corticosterone in rat brain and its concentration by certain limbic structures. Brain Research 16: 227-241.
28. De Kloet, E.R. and Veldhuis, H.D. (1984). Adrenocortical hormone action on the brain. In: Lajtha, A. (ed.) Handbook of Neurochemistry, vol. 8. Pergamon Press, New York, in press.
29. Kovács, G.L., Telegdy, G. and Lissak, K. (1976). 5-Hydroxytryptamine and the mediation of pituitary-adrenocortical hormones in the extinction of active avoidance behavior. Psychoneuroendocrinology 1: 219-230.
30. Azmitia, E.C. (1978). The serotonin producing neurons of the midbrain median and dorsal raphe nuclei. In: Iversen, L.L., Iversen, S.D. and Snyder , S.H. (eds.) Handbook of Psychopharmacology, vol. 9. Plenum, New York, pp. 233-314.
31. De Wied, D. (1976). Behavioral effects of intraventricularly administered vasopressin and vasopressin fragments. Life Sciences 19: 685-690.
32. De Wied, D. (1966). Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. Proceedings of the Society for Experimental Biology and Medicine 122: 28-32.
33. Van Wimersma Greidanus, Tj.B. and De Wied, D. (1971). Effects of systemic and intracerebral administration of two opposite acting ACTH-related peptides on extinction of conditioned avoidance behavior. Neuroendocrinology 7: 291-301.
34. Ader, R., Weijnen, J.A.W.M. and Moleman, P. (1972). Retention of a passive avoidance response as a function of the intensity and duration of electric shock. Psychonomic Science 26: 125-128.
35. Weijnen, J.A.W.M. and Slangen, J.L. (1970). Effects of ACTH-analogues on extinction of conditioned behaviour. Progress in Brain Research 32: 221-235.
36. Gispen, W.H., Wiegant, V.M., Greven, H.M. and De Wied, D. (1975). The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: Structure-activity studies. Life Sciences 17: 645-652.
37. Van Wimersma Greidanus, Tj.B. (1970). Effect of steroids on extinction of an avoidance response in rats. A structure-activity relationship study. Brain Research 32: 185-191.
38. Bohus, B. (1973). Pituitary-adrenal influences on avoidance and approach behaviour of the rat. Progress in Brain Research 39: 407-420.

39. Bohus, B. (1975). The hippocampus and the pituitary-adrenal system hormones. In: Isaacson, R.L. and Pribram, K.H. (eds) The hippocampus. Plenum Press, New York, pp. 323-353.
40. Micheau, J., Destrade, C. and Soumireu-Mourat, S. (1982). Post-trial injection of corticosterone in dorsal hippocampus of the BALB-C mouse facilitates extinction of appetitive operant condition in the Skinner box. C.R. Academy of Sciences Paris 294: 1109-1112.
41. Micco, D.J., McEwen, B.S. and Shein, W. (1979). Modulation of behavioral inhibition in appetitive extinction following manipulation of adrenal steroids in rats: implications for involvement of the hippocampus. Journal of Comparative Physiology and Psychology 93: 323-329.
42. Bohus, B. and De Kloet, E.R. (1981). Adrenal steroids and extinction behavior: antagonism by progesterone, deoxycorticosterone and dexamethasone of a specific effect of corticosterone. Life Sciences 28: 433-440.
43. Gerlach, J.L. and McEwen, B.S. (1972). Rat brain binds adrenal steroid hormone: radioautography of hippocampus with corticosterone. Science 175: 1135-1136.
44. Warembourg, M.Y. (1975). Radioautographic study of the rat brain after injection of 1.2 ^{3}H-corticosterone. Brain Research 89: 61-70.
45. Jacobs, B.L., Wise, W.D. and Taylor, K.M. (1974). Differential behavioral and neurochemical effects following lesions of the dorsal and median raphe nuclei in rats. Brain Research 79: 353-361.
46. Steinbusch, H.W.M. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat cell bodies and terminals. Neuroscience 6: 557-618.
47. De Kloet, E.R., Nyakas, Cs., Koppenaal, D., Burger, K.J. and Veldhuis, H.D. Corticosterone and exploratory behavior of adrenalectomized rats: Effect 5.6-dihydroxytryptramine lesion of fornix-cingulum serotonergic input in hippocampus (in preparation).
48. Rostene, W.H., Fischette, C.T., Dussaillant, M. and McEwen, B.S. (1984). Adrenal steroid modulation of vasoactive intestinal peptide effect on binding sites in the reat brain shown by in vitro quantitative autoradiography. Neuroendocrinology, in press.
49. Riou, F., Cespuglio, R. and Jouvet, M. (1982). Endogenous peptides and sleep in the rat: The hypnogenic properties of vasoactive intestinal polypeptide. Neuropeptides 2: 265-277.
50. Micco, D.J. Jr., Meyer, J.S. and McEwen, B.S. (1980). Effect of corticosterone replacement on the temporal patterning of activity and sleep in adrenalectomized rats. Brain Research 200: 206-212.

Accepted 17/5/84