Pharmacology Biochemistry & Behavior, Vol. 15, pp. 705–709, 1981. Printed in the U.S.A.

Dopamine Induced Changes in L-Fucose Incorporation into Proteins of Rat Hippocampus and Corpus Striatum During Postnatal Development[1]

B. LÖSSNER, R. JORK AND H. MATTHIES

Institute of Pharmacology and Toxicology, Medical Academy Magdeburg, 301 Magdeburg (G.D.R.)

Received 1 June 1981

LÖSSNER, B., R. JORK AND H. MATTHIES. *Dopamine induced changes in L-fucose incorporation into proteins of rat hippocampus and corpus striatum during postnatal development.* PHARMAC. BIOCHEM. BEHAV. **15**(5) 705–709, 1981.—In 60 day old (adult) male Wistar rats dopamine caused a dose-dependent increase of L-fucose incorporation into total proteins of both hippocampus and corpus striatum slices up to +47.8±6.0% (n=6) and +53.2±8.5% (n=20), respectively, when compared to corresponding controls. Under these conditions the dopamine concentration leading to a maximum stimulation of fucose incorporation was 5×10^{-4} M in hippocampus and 1×10^{-3} M in corpus striatum. In the latter tissue the range of dopamine concentrations causing a significant elevation in incorporation rates was larger than in hippocampal tissue. In the corpus striatum of 9 day old rats dopamine was ineffective, but by 30 days the transmitter stimulated fucose incorporation rate reached the maximum observed for any age studied. This developmental pattern seems to be related to the ontogenesis of dopamine receptor sites or dopamine sensitive adenylate cyclase formation in this brain structure. In the hippocampus the postnatal development of dopamine induced augmentation of glycoprotein synthesis showed a longer latency, but the maximum effect was also seen in 30 day old animals. These results support our assumption that at the end of the postnatal differentiation period the glycoprotein synthesis in brain tissue may be controlled (at least to some extent) by the state of dopaminergic receptors and/or of dopamine sensitive adenylate cyclase.

Rat Hippocampus Corpus striatum Dopamine Glycoprotein synthesis Postnatal development L-fucose

NUMEROUS biochemical investigations indicate changes in the incorporation of precursors into proteins and glycoproteins of rat hippocampus related to training experiments [6, 9, 10, 16, 18, 19, 20, 24, 27, 31]. There is evidence for an increased incorporation of L-fucose into hippocampal glycoproteins during the acquisition and consolidation of a foot-shock motivated brightness discrimination in rats [20, 24, 27]. Similar alterations in glycoprotein formation of rat hippocampus are elicited by the action of dopaminergic agonists (dopamine and apomorphine), both *in vivo*[13] and *in vitro* [12,14]. Since, in rats, the intrahippocampal application of apomorphine prior to, or immediately after training improves the retention of this brightness discrimination task [7], a link between the consolidation of a long-term memory trace and the activation of dopamine sensitive structures as well as of glycoprotein synthesis within rat hippocampus might be assumed.

With the exception of some recent findings [1, 8, 28] the existence of a dopaminergic transmitter system within the rat hippocampus is still an open question. However, in slices of this brain region dopamine stimulates the accumulation of cyclic AMP [15]. Both the dopamine induced stimulation of glycoprotein synthesis and the dopamine stimulated accumulation of cAMP is blocked by haloperidol (dopamine receptor antagonist) [15]. This indicates the presence of dopamine receptor sites and of dopamine sensitive adenylate cyclase in rat hippocampus. Moreover, exposure of hippocampal slices to dibutyryl-cyclic AMP (db-cAMP) enhances the incorporation of L-fucose [15].

Based on these findings it is assumed that these two responses of rat hippocampus to dopamine, i.e., accumulation of cAMP and augmentation of glycoprotein synthesis, are causally related. It is important to determine whether the dopamine stimulated glycoprotein synthesis in rat hippocampus [12, 13, 14, 15] is the result of dopamine-dopamine receptor-interaction. For this purpose the dopamine effects on L-fucose incorporation were contrasted between hippocampus and corpus striatum (a brain region known to be rich in dopaminergic synapses and receptor sites [3]). In addition, to answer the question as to whether this transmitter induced augmentation of glycoprotein synthesis is related to the existence and density of dopamine receptors, the ontogenetic development of this dopamine action was tested in slices of corpus striatum and hippocampus.

[1]This work was supported by the Ministry of Science and Technology of the G.D.R.

METHOD

Male Wistar rats from our own breeding stock aged 9, 14, 20, 25, 30, 40 and 60 days (adult) were used. After dissecting from the brain both hippocampus and corpus striatum [25], 0.5 mm thick slices were prepared. The hippocampus was sliced perpendicularly to its longitudinal axis [17,29]. For each tissue, alternate slices were used for control and experimental groups. After preincubation for 15 min in 0.8 ml incubation mixture (134 mM NaCl, 5 mM KCl, 16 mM $NaHCO_3$, 0.75 mM $CaCl_2$, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$ and 10 mM glucose; pH 7.35) at 37°C, test substances were added in a volume of 0.1 ml. Ten min later 25 μCi L-(1-^{3}H)-fucose (specific activity: 1 Ci=37 GBq per mmole; The Radiochemical Centre, Amersham, Great Britain), in a volume of 0.1 ml of incubation medium was added to control and experimental slices. The final concentration of labelled L-fucose was 1×10^{-3} M [4]. The medium was aerated throughout the experiment with carbogen (95% O_2/5% CO_2).

In order to stop any further incorporation after the 1 hour incubation period, the slices were repeatedly washed with ice-cold incubation medium and then frozen on solid CO_2. The slices were homogenized in 0.3 ml of 0.1 N NaOH. In order to determine total incorporated radioactivity of proteins a 0.1 ml aliquot of the homogenate was treated with 0.5 ml ice-cold 12% trichloracetic acid (TCA; w/v), and the precipitated proteins were pelleted by centrifugation (10,000 G × 10 min). The pellet was washed twice with 6% TCA, water and ethanol. Afterwards the proteins were solubilized in 0.3 ml of a mixture of 0.66% sodium dodecylsulfate (SDS), 10% mercaptoethanol, 0.03% dithiothreitol and 0.02 M K_2CO_3 (final concentration) by heating at 90°C for 10 min. Aliquots of solubilized proteins were treated with Hyamine hydroxide, subsequently dissolved in a dioxane containing scintillator [17] and counted in a scintillation spectrometer (Intertechnique, Plaisir, France) for determination of radioactivity. The protein content of the solubilized fraction was determined using the amido black technique [26] with bovine serum albumin as standard. To calculate the incorporation rate (IR) (nmoles L-fucose being incorporated per mg protein per hour), the specific radioactivity of total proteins (d.p.m. × mg protein^{-1}) was corrected by the amount of radioactive L-fucose in the incubation medium using the following equation:

$$IR = \frac{\text{d.p.m.} \times \text{mg protein}^{-1} \times \text{hr}^{-1}}{\text{d.p.m.} \times \text{nmoles L-fucose}^{-1}\text{ (medium)}}$$

This calculation is based on the assumption that within a few minutes the specific radioactivity of the intracellular space (d.p.m. × nmoles L-fucose^{-1}) will be identical with that of the incubation medium [4], i.e., 1×10^{-3} M.

The age dependent developmental pattern of the basal rate of protein and glycoprotein synthesis in hippocampal slices, was studied simultaneously using L-(U-^{14}C)-leucine (specific radioactivity: 185 mCi=6.8 GBq/mmole, purchased from UVVR, Prague, CSSR) and L-(1-^{3}H)-fucose as previously described [11].

RESULTS

In adult rats the effects of dopamine on L-fucose incorporation into glycoproteins were studied in slices of corpus striatum and compared to that seen in hippocampus. As depicted in Fig. 1, dopamine caused a dose-dependent increase in fucose incorporation into total proteins of both hippocampus and corpus striatum. Corpus striatum appeared to be less sensitive to dopamine than hippocampus. Maximum stimulation of glycoprotein synthesis by dopamine occured at 5×10^{-4} M in hippocampus but at 1×10^{-3} M in corpus striatum. However, in hippocampus the range of dopamine concentrations resulting in a significant increase of L-fucose incorporation was 5×10^{-4} to 1×10^{-3} M, while in striatum a significant effect was obtained with 5×10^{-4} M up to 7.5×10^{-3} M.

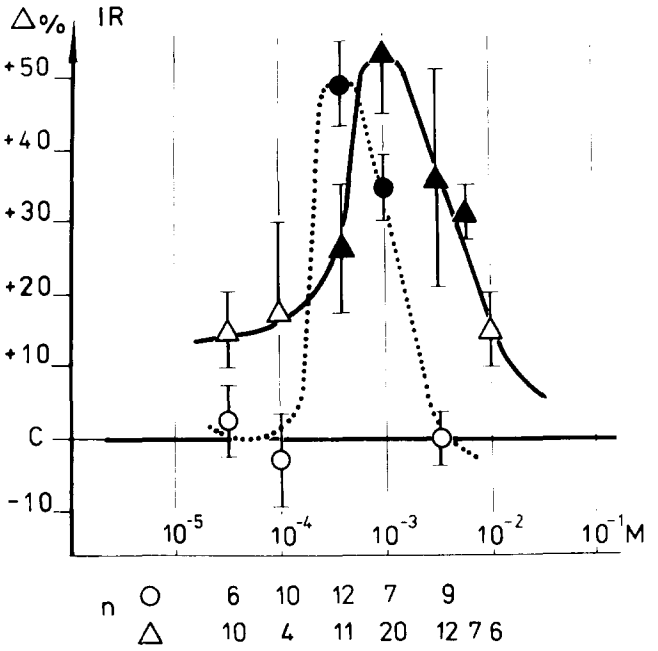

FIG. 1. Mean values (±SEM) of the percentage deviation of L-fucose incorporation rate (IR; nmoles/mg protein/hr) from control levels (C) in hippocampal (○···○) and striatal slices (△–△) of adult rats in the presence of dopamine (5×10^{-4} M). The IR-values of controls were 0.41 nmoles/mg protein/hr in hippocampus and 0.30 nmole/mg protein/hr in corpus striatum. The filled circles and triangles denote statistically significant values ($p<0.05$) according to Wilcoxon's matched pairs signed rank test [30].

Figure 2 shows the developmental pattern of incorporation rate of hippocampal and striatal slices. These data indicate a decrease in glycoprotein synthesis in both hippocampus and corpus striatum between the 9th and 30th day after birth. In comparison to adult rats the IR-values of 9 day old animals are five-to sixfold higher in hippocampus and only twofold higher in corpus striatum, indicating a faster decline in glycoprotein synthesis rate during the postnatal period in the hippocampus compared to the striatum. The IR-values in hippocampus as well as in striatum reached the adult level between 25th and 30th day (Fig. 2). However, even in adult rats the IR-values of hippocampal slices were above those of the corpus striatum.

The effect of dopamine on the *in vitro* incorporation of L-fucose into total glycoproteins of striatum during the postnatal period is demonstrated in Fig. 3. Dopamine is ineffective on slices from 9 day old rats. Bewteen 9 and 30 days of age there is an increase in dopamine induced stimulation of fucose incorporation in striatal slices. Maximal dopamine

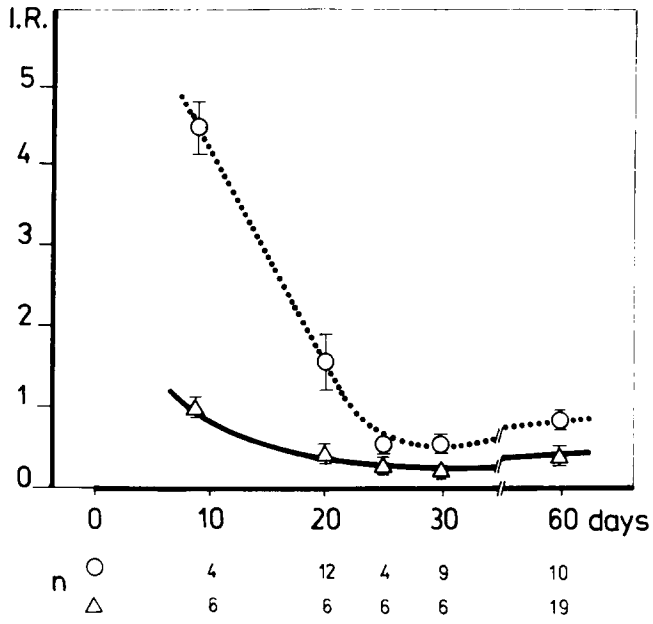

FIG. 2. Mean values ±SEM of incorporation rate (IR) of L-fucose in rat hippocampal (○···○) and striatal slices (△−△) during postnatal period. If not otherwise indicated the number of animals per group is equal to 6.

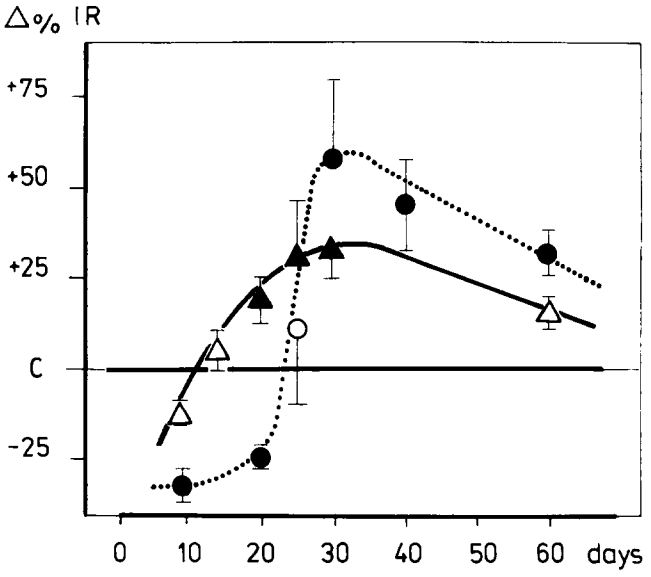

FIG. 3. Mean values of the percentage deviation of the IR-value in rat hippocampal (○···○) and striatal slices (△−△) from control level (C) in the presence of 5×10^{-4} M dopamine during the postnatal period.

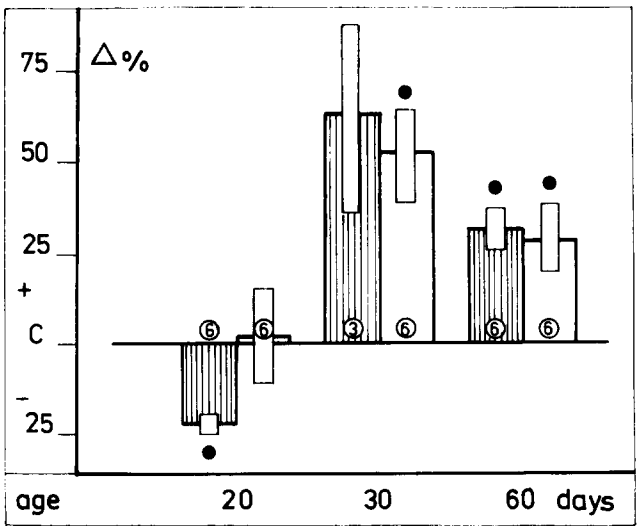

FIG. 4. The effect of dopamine (5×10^{-4} M: hatched columns) and of db-cAMP (2.5×10^{-3} M; white columns) on the incorporation of L-fucose into total proteins from hippocampal slices of 20, 30 and 60 days old rats. Data are presented as means ±SEM of percentage deviation of IR values from control levels (see Fig. 2).

stimulation was observed at 30 days and subsequently the dopamine induced stimulation declined slightly.

In contrast, the postnatal development of dopamine action on fucose incorporation into hippocampal slices shows a pattern that is different from that observed in corpus striatum. From 9 to 20 days after birth, dopamine caused a significant decrease in glycoprotein synthesis. Between 20 and 30 days of age the dopamine effect on fucose incorporation shows a transition from inhibition to stimulation (Fig. 3). As in the striatum, maximum stimulation by dopamine is observed at 30 days and with increasing age the stimulation also declines. Thus, the ontogenetic development of dopamine stimulated fucose incorporation in hippocampus seemed to have a longer latency than in striatum.

In addition, the postnatal development of the action of cAMP on hippocampal glycoprotein synthesis was studied *in vitro*. The effects of 2.5×10^{-3} M db-cAMP on fucose incorporation into total proteins were tested in hippocampal slices of 20, 30 and 60 day old rats. The results demonstrate (see Fig. 4) that the developmental pattern of the effect of db-cAMP closely corresponds to that of dopamine.

DISCUSSION

Previous investigations in our laboratory demonstrated a statistically significant augmentation of L-fucose incorporation into hippocampal proteins of adult rats under the influence of dopaminergic agonists, both *in vivo* [13] and *in vitro* [12, 14, 15]. These effects seemed to be mediated by cAMP. This supposition is based on our findings of a dopamine induced accumulation of cAMP in hippocampal slices. In addition, the *in vitro* administration of db-cAMP results in an enhanced incorporation of L-fucose into total proteins of hippocampal slices [15]. Both of these effects were blocked by haloperidol, a dopaminergic antagonist, indicating the existence of dopamine sensitive structures within the rat hippocampal formation [15].

However, previous reports provide conflicting evidence concerning the existence of a dopaminergic transmitter system or dopamine sensitive structures within this rat brain

TABLE 1

POSTNATAL DEVELOPMENT OF BASAL INCORPORATION RATES OF L-(^{14}C-U)-LEUCINE AND L-(1-^{3}H)-FUCOSE IN RAT HIPPOCAMPAL SLICES

Age (days)	IR-values expressed as nmoles of precursors incorporated per mg protein per hr (^{14}C)-leucine Mean SEM	(^{3}H)-fucose Mean SEM	Ratio	n
9	13.71 ± 0.92	4.49 ± 0.32	3.05	3
20	2.48 ± 0.32	1.55 ± 0.20	1.60	6
25	0.66 ± 0.09	0.51 ± 0.06	1.29	3
30	0.64 ± 0.08	0.55 ± 0.05	1.16	6
60	0.63 ± 0.07	0.83 ± 0.09	0.76	10

region [1, 3, 8, 12, 13, 23, 28]. In adult rat corpus striatum, (a brain structure known to be rich in dopaminergic synapses), dopamine stimulates the incorporation of L-fucose into total proteins, also. But, the corpus striatum appears to be less sensitive than the hippocampus, because the dopamine concentration needed to induce the maximum stimulation of fucose incorporation is higher (see Fig. 1). This finding is unexpected if one assumes a relationship between the extent of dopamine induced augmentation of glycoprotein synthesis and the density of dopamine receptor sites. The latter is higher in corpus striatum than in hippocampus [3]. However, the slope of the dose-response curves up to the maximum effective dopamine concentration is much steeper in hippocampal tissue than in striatal slices (see Fig. 1). This indicates that in the striatum, dopamine is still active in enhancing glycoprotein synthesis in a concentration range that is ineffective in the hippocampus. The question as to whether this finding is the result of a higher efficacy of dopamine on striatal glycoprotein synthesis *in vivo* must be answered by future investigations. Nevertheless, the differences in the slopes of dose-response curves could be the result of either a different behavior of striatal and hippocampal dopamine receptors within these two brain structures.

Furthermore, the similar time course of postnatal development of dopamine stimulated fucose incorporation into total proteins of corpus striatum (see Fig. 3) and the increase in both the specific ^{3}H-haloperidol binding [23] and the dopamine sensitive adenylate cyclase [2] support our conclusion that these effects of dopamine on glycoprotein synthesis may be mediated by a dopamine receptor-adenylate cyclase system. The dopamine concentration (5×10^{-4} M) causing a statistically significant increase in fucose incorporation in adult rat hippocampus, elicits in both 9 and 20 day old animals a significant suppression of glycoprotein synthesis (about 70 to 75% of that of control level). Between 20 and 30 days after birth a transition in the dopamine effect on fucose incorporation from suppression to maximum stimulation takes place. Moreover, as in striatal slices the extent of dopamine stimulated fucose incorporation into hippocampal proteins is smaller in adult rats when compared to 30 day old animals (see Fig. 3). Since the developmental pattern for dopamine induced augmentation of glycoprotein formation in rat hippocampus demonstrates some differences when compared to that of corpus striatum (Fig. 3), there might be regional variation in the time course of postnatal development of dopamine receptors. This supposition should be tested by future studies of the ontogenesis of specific ligand-binding to dopamine receptor sites in these two regions. However, the results described in this paper may indicate that the dopamine stimulated glycoprotein synthesis [13,14] is a general phenomenon in dopaminergic innervated and/or sensitive structures of the rat brain.

According to our previously published data [15,21] it might be assumed that the dopamine induced elevation of the incorporation of L-fucose into hippocampal proteins is mediated by cAMP. The age dependency of the db-cAMP effects on the *in vitro* fucose incorporation into hippocampus is comparable to that of dopamine (see Fig. 4). One could deduce from these findings that both the dopamine receptors and the intracellular mechanisms involved in the enhanced glycoprotein formation mature during the postnatal period. Dibutyryl-cAMP was ineffective in stimulating the fucose incorporation into proteins of hippocampal slices in rats younger than 20 days. This finding might indicate that the cAMP-system of this brain structure becomes important in controlling and regulating glycoprotein synthesis during ontogenetic development. During the first stages of brain maturation, the observed high rate of glycoprotein synthesis (see Fig. 2) is mainly dependent on the rapid synthesis of polypeptide chains (see Table 1 and [5]) which could function as acceptors for glycosylation. During this early period, glycoproteins would serve to permanently connect functional activated synapses without additional influences. As aminergic systems mediating emotional or motivational influences mature during postnatal development, and as the early intense protein synthesis decreases, the dopaminergic influence begins to control glycoprotein synthesis. Thus, this dopaminergic system gains in importance in the formation of permanently activated synaptic connections underlying the consolidation of the memory trace. This supposition should be evaluated by further investigations.

ACKNOWLEDGEMENTS

The skillful technical assistance of Mrs. Ilona Linke and Mrs. Angelika Reichel is gratefully acknowledged. We wish to thank Dr. V. Aloyo (Institute of Molecular Biology, Utrecht, The Netherlands) for revision of the English of the manuscript.

REFERENCES

1. Bischoff, S., B. Scatton and J. Korf. Biochemical evidence for a transmitter role of dopamine in the rat hippocampus. *Brain Res.* **165:** 161–165, 1979.
2. Coyle, J. T. and P. Camphiaro. Ontogenesis of dopaminergic-cholinergic interaction in the rat striatum: a neurochemical study. *J. Neurochem.* **27:** 673–678, 1976.
3. Creese, I., D. R. Burt and S. H. Snyder. Biochemical actions of neuroleptic drugs: focus on the dopamine receptor. In: *Handbook of Psychopharmacology, Vol. 10, Neuroleptics and Schizophrenia*, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1978, pp. 37–89.
4. Dunlop, D. S., W. van Elden and A. Lajtha. Measurement of rates of protein synthesis in rat brain slices. *J. Neurochem.* **22:** 812–830, 1974.

5. Dunlop, D. S., W. van Elden and A. Lajtha. Developmental effects on protein synthesis rates in regions of the CNS in vivo and in vitro. *J. Neurochem.* **39:** 939–945, 1978.
6. Dunn, A. J. The chemistry of learning and the formation of memory. In: *Molecular and Functional Neurobiology*, edited by W. H. Gispen. Amsterdam: Elsevier Scientific, 1976, pp. 347–387.
7. Grecksch, G. and H. Matthies. Role of dopaminergic mechanisms in the rat hippocampus for consolidation in a brightness discrimination. *Psychopharmacology*, in press.
8. Howlett, D. R., Helen Morris and S. R. Nahorsky. Anomalous properties of (^{3}H)-spiperone binding sites in various areas of the rat limbic system. *Molec. Pharmac.* **15:** 506–514, 1979.
9. Hyden, H. and P. W. Lange. Protein changes in nerve cells related to learning and conditioning. In: *The Neurosciences. Second Study Program*, edited by F. O. Schmitt. New York: Rockefeller University Press, 1970, pp. 278–289.
10. Hyden, H., P. W. Lange and C. L. Perrin. Protein pattern alterations in hippocampal and cortical cells as a function of training in rats. *Brain Res.* **119:** 427–437, 1977.
11. Jork, R., B. Lössner and H. Matthies. The influences of cholinergic transmitter substances on the incorporation of (^{14}C)-leucine and (^{3}H)-fucose into the total proteins of hippocampus in vivo and in vitro. *Pharmac. Biochem. Behav.* **11:** 243–245, 1979.
12. Jork, R., B. Lössner and H. Matthies. Dopamine and macromolecule synthesis in rat hippocampus. *Pharmac. Biochem. Behav.* **11:** 247–249, 1979.
13. Jork, R., G. Grecksch, M. Jirka, B. Lössner and H. Matthies. Apomorphine and glycoprotein synthesis in rat hippocampus. *Pharmac. Biochem. Behav.* **12:** 317–318, 1980.
14. Jork, R., B. Lössner and H. Matthies. The influence of dopamine in the incorporation of different sugars into total proteins of hippocampal slices. *Pharmac. Biochem. Behav.* **13:** 303–304, 1980.
15. Jork, R., B. Lössner and H. Matthies. Dopamine and glycoprotein synthesis in rat hippocampus. In: *Proceedings of the 6th International Neurobiological Symposium on Learning and Memory*. Magdeburg, 1980. IBRO Monograph Series, edited by C. Ajmone-Marsan. New York: Raven Press, in press.
16. Levitan, I. B., G. Ramirez and W. W. Mushynski. Amino acid incorporation in the brains of rats trained to use the nonpreferred paw in retrieving food. *Brain Res.* **47:** 147–156, 1972.
17. Lössner, B., R. Jork and H. Matthies. Einbau von (^{3}H)-Leuzin in die Gesamtproteine verschiedener Sommerscher Sektoren des Hippokampus der Ratte in vivo und in vitro. *Acta Biol. med. germ.* **36:** 1221–1229, 1977.
18. Lössner, B., R. Jork, G. Grecksch, W. Wetzel and H. Matthies. The incorporation of leucine into proteins of different rat brain structures during acquisition and consolidation of a "brightness discrimination." In: *Biological Aspects of Learning, Memory Formation and Ontogeny of the CNS*, edited by H. Matthies, M. Krug and N. Popov. Berlin: Akademie-Verlag, 1979, pp. 125–130.
19. Lössner, B., R. Jork, M. Krug and H. Matthies. Protein synthesis in rat hippocampus during training and stimulation experiments. In: *Proceedings of the 6th International Neurobiological Symposium on Learning and Memory*. Magdeburg, 1980. IBRO Monograph Series, edited by C. Ajmone-Marsan. New York: Raven Press, in press.
20. Matthies, H., W. Pohle, N. Popov, B. Lössner, H.-L. Rüthrich, R. Jork and T. Ott. Biochemical mechanisms correlated to learning and memory formation. Facts and hypothesis. *Acta physiol. hung.* **48:** 335–355, 1976.
21. Matthies, H. Learning and memory. In: *Advances in Pharmacology and Therapeutics, Vol. 5, Neuropharmacology*, edited by C. Dumont, Oxford: Pergamon Press, 1978, pp. 117–135.
22. Meyerhof, J. L., G. J. Kant and R. H. Lenox. Increase in dopamine in cerebral cortex and other regions of rat brain after microwave fixation: possible diffusion artefact. *Brain Res.* **152:** 161–169, 1978.
23. Pardo, J. V., I. Creese, D. R. Burt and S. H. Snyder. Ontogenesis of dopamine receptors binding in the corpus striatum of the rat. *Brain Res.* **125:** 376–382, 1977.
24. Pohle, W., H.-L. Rüthrich, N. Popov and H. Matthies. Fucose incorporation into rat hippocampus structures after acquisition of a brightness discrimination. A histoautoradiographic study. *Acta Biol. med. ger.* **38:** 53–63, 1979.
25. Popov, N., W. Pohle, B. Lössner, S. Schulzeck, S. Schmidt, T. Ott and H. Matthies. Regional distribution of RNA and protein radioactivity in the rat brain after intraventricular application of labelled precursors. *Acta Biol. med. germ.* **31:** 51–62, 1973.
26. Popov, N., M. Schmitt, S. Schulzeck and H. Matthies. Eine störungsfreie Mikromethode zur Bestimmung des Proteingehaltes in Gewebehomogenaten. *Acta Biol. med. germ.* **34:** 1441–1446, 1975.
27. Popov, N., S. Schulzeck, W. Pohle and H. Matthies. Changes in the incorporation of (^{3}H)-fucose into rat hippocampus after acquisition of a brightness discrimination reaction. An electrophoretic study. *Neuroscience* **5:** 161–167, 1980.
28. Scatton, B., H. Simpson, M. le Moal and S. Bischoff. Origin of dopaminergic innervation of the rat hippocampal formation. *Neurosci. Lett.* **18:** 125–132. 1980.
29. Thiemann, W., M. Krug, W. Pohle und H.-L. Rüthrich. Ein einfaches Gerät zum Schneiden von frischen Hirnteilen. *Acta Biol. med. germ.* **34:** 527–529, 1975.
30. Weber, E. *Grundlagen der biologischen Statistik für Naturwissenschaftler und Mediziner*, 7 auflage. Jena: Fischer Verlag, 1972.
31. Yanigihara, T. and H. Hyden. Protein synthesis in various regions of rat hippocampus during learning. *Expl Neurol.* **31:** 151–164, 1971.