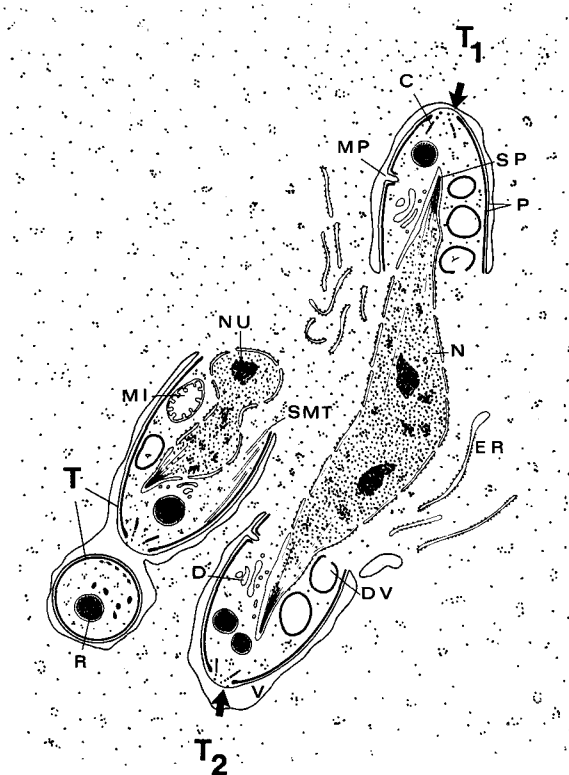


*akimovae*⁸ kann bei *Eimeria tenella* eine Kernteilung einsetzen, bevor die vorangegangene abgeschlossen ist. Ein genauerer Bericht über Wachstum und Kernteilungsvorgänge im Schizonten folgt an anderer Stelle.



Schematische Darstellung von 4 Tochterzellen (= Merozoiten) in der Randzone des Schizonten. T₁, T₂ zu einem Drittel differenzierte, synchron heranwachsende Tochterzellen (T) über den Spindelpolen (SP) eines Schizontenkerns (N); C, Conoid; DV, dickwandiger Vesikel; ER, endoplasmatisches Reticulum; MI, Mitochondrium; MP, Mikropore; NU, Nukleolus; P, Pellicula; R, Rhoptriesanlage; SMT, subpelliculärer Mikrotubulus; V, Vakuole in Verbindung mit der parastophoren Vakuole.

Die Bildung von Tochterzellen ist mit der letzten Teilung der Schizontenkerne in der Mutterzelle gekoppelt und unterscheidet sich nicht wesentlich von den früheren Kernteilungen. In einer neueren Arbeit wird die Tochterzellbildung dargestellt⁹. Über jedem Kernspindelpol entsteht die Anlage einer Tochterzelle. Sie hat ein Conoid und einen inneren Membrankomplex, der nach aussen von einer Cytoplasmamembran kappenartig begrenzt wird. Zu diesem frühen Zeitpunkt der Entwicklung lassen sich auch 4 Tochterzellanlagen an zwei noch nicht vollständig getrennten Kernen beobachten. Die Pelliculamembranen der Tochterzellanlagen vergrössern sich synchron während der fortschreitenden Teilung des Schizontenkerns und umschliessen dabei einen Spindelpol mit den benachbarten Centriolen und ein Dictyosom (Figur).

Die neuen Ergebnisse zeigen feinstrukturelle Übereinstimmungen im Vermehrungsablauf bei *Eimeria tenella* und den Toxoplasmatea (BIOCCA 1968) und sprechen für eine enge verwandtschaftliche Zusammengehörigkeit.

Summary. Fine structural aspects of development in 2nd and 3rd generation schizonts were studied with experimentally infected chickens. Two developmental steps were observed in schizogony: multiplication of nuclei and succeeding merozoite formation associated with the last division of each nucleus in the schizont. Dividing nuclei had eccentric intranuclear spindles and centrioles near their poles.

GISELA HOPPE

Institut für Virologie und Seuchenhygiene der Medizinischen Hochschule Hannover, Karl-Wiechert-Allee 9, D-3 Hannover-Kleeefeld (Bundesrepublik Deutschland, BRD), 31. Mai 1974.

⁸ G. L. KELLEY and D. M. HAMMOND, *J. Parasit.* 59, 1071 (1973).

⁹ G. HOPPE, *Protistologica* 10, 185 (1974).

Labelling of TCA-Soluble Fraction and Protein of the Rat Visual Cortex After Learning a Pattern Discrimination Task

The possibility of training to perform a visual vertical-horizontal discrimination monocularly has been demonstrated by BUREŠOVÁ and NADEL¹. Intact visual cortex contralateral to the intact eye was shown to be essential for acquisition and retention of the new experience. No transfer to the opposite hemicortex takes place. This paradigm was used in the present experiments in an attempt to find out whether this kind of learning would cause any difference in the labelling of protein in the visual hemicortex of rat.

Materials and methods. Experiments were performed on the male hooded rats weighing 180–200 g. The horizontal-vertical discrimination training procedure was essentially that already published¹. Briefly, visual input was restricted by covering one eye with an opaque plastic occluder. The rats were trained to escape or avoid shocks during 5 sec by entering an unlocked door. Immediately after reaching a criterion of 12 correct in 13 consecutive responses, the occluder was removed.

After 30 min of rest (period of maximal chemical response²), 0.5 ml saline containing about 16 μ Ci L-(U-¹⁴C)-leucine or about 13 μ Ci L-(1-¹⁴C)-leucine was injected s.c. After definite time intervals, the animals were sacrificed by decapitation in a cold room at 2–4 °C. The samples of both visual hemicortices were dissected with the aid of a celluloid stencil³ and frozen in liquid nitrogen. Tissue samples were then homogenized in the ice-chilled 6% trichloroacetic acid (TCA) containing 2 mg/ml ¹²C-leucine. TCA-precipitates were dissolved in 1 N NaOH after treatment by the procedure⁴. In aliquots,

¹ O. BUREŠOVÁ and L. NADEL, *Physiol. Behav.* 5, 849 (1970).

² J. KŘIVÁNEK, in *Neurobiological Basis of Memory Formation* (Ed. H. MATTHIES; VEB Verlag Volk und Gesundheit, Berlin 1974), p. 595.

³ E. L. BENNETT, M. C. DIAMOND, D. KRECH and M. R. ROSENZWEIG, *Science* 146, 610 (1964).

⁴ P. SIEKEVITZ, *J. biol. Chem.* 195, 549 (1952).

Radioactivity of protein and TCA soluble fraction in the visual cortex of rats after monocular acquisition of a pattern discrimination task

Amino acid used	Time after ¹⁴ C-leucine injection (min)	Conditions of experiment	Specific activity of protein (cpm/mg)	Specific activity of TCA soluble fraction (cpm/mg protein)	Relative specific activity of protein (protein/TCA _{sol}) × 100	
L-(U- ¹⁴ C) leucine	7	Trained rats	Naive cortex	42.3	101.3	35.0
			Trained cortex	42.3	100.1	35.8
			Change (%)	±0	-1.2	+2.3
			Change ± SEM	±0 ± 0.23	-1.2 ± 1.85	+0.8 ± 0.72
			P (n = 9)	N.S.	N.S.	N.S.
	11	Trained rats	Naive cortex	60.4	131.3	44.9
			Trained cortex	60.9	128.7	46.9
			Change (%)	+0.8	-2.0	+4.1
			Change ± SEM	+0.5 ± 0.6	-2.6 ± 6.2	+2.0 ± 1.1
			P (n = 11)	N.S.	N.S.	N.S.
15	Trained rats	Naive cortex	141.0	180.9	76.3	
		Trained cortex	145.8	169.9	78.0	
		Change (%)	+3.4	-6.1	+10.1	
		Change ± SEM	+4.8 ± 2.03	-11.0 ± 2.02	+7.7 ± 1.19	
		P (n = 25)	<0.05	<0.003	<0.003	
Control rats	Naive cortex	Naive cortex	128.0	175.5	75.6	
		Trained cortex	124.0	167.7	77.6	
		Change (%)	-3.1	-4.4	+2.6	
		Change ± SEM	-4.0 ± 1.72	-7.8 ± 1.77	+2.0 ± 1.76	
		P (n = 11)	<0.05	<0.003	N.S.	
L-(1- ¹⁴ C) leucine	15	Trained rats	Naive cortex	147.0	124.7	113.2
			Trained cortex	153.3	118.9	125.5
			Change (%)	+4.3	-4.7	+10.9
			Change ± SEM	+6.3 ± 1.27	-5.8 ± 1.85	+12.3 ± 2.53
			P (n = 6)	<0.01	<0.05	<0.01

protein content⁵ and radioactivity⁶ were determined. In the TCA supernatant fluids after extraction with ether, radioactivity was determined⁷ and related to the protein content. All preparation and analytical work was done blind, i.e. without knowing which eye had been occluded.

Results and discussion. 15 min after ¹⁴C-leucine administration slight but statistically significant elevation of protein labelling (+3.4%, $p < 0.05$) in the visual cortex contralateral to the intact eye (Trained cortex in the Table) as compared to the opposite (naive cortex in the Table) was found. In contrast, radioactivity in the TCA-soluble fraction declined by 6.1% ($p < 0.003$). Using the leucine labelled on the carboxyl carbon only, the same results were obtained: 4.3% increase in the protein specific activity and 4.7% drop of TCA-soluble radioactivity ($p < 0.01$ and < 0.05 resp.). Therefore most likely, alteration in TCA-soluble fraction does reflect that of leucine itself⁸. No proper method is available to determine directly radioactivity of the precursor in the pool from which it is used for incorporation into protein. Since in our experiments consistent trend of changes in the precursor radioactivity in the soluble fraction to that bound to protein is reversed for all time intervals studied (7-60 min, Table and ²), it seems likely that change in the protein labelling is not merely a reflection of that in the precursor radioactivity, but may rather indicate enhanced incorporation rate. Two groups of rats (control rats in the Table) were treated essentially in the same way as the experimental animals (same light input, mean dosage electrical stimulation, mean time of treatment) except that stimulus cards had been removed (no training). In their visual hemicortices contralateral to the open eye ('trained' cortex in the Table) 4.4% decrease of TCA-soluble labelling ($p < 0.003$) was found, while radioactivity of protein reflects only decrease of precursor in the soluble fraction.

Although an intact visual cortex is a prerequisite for acquisition and retention of this kind of discrimination task¹, it does not necessarily mean this is the place where memory trace is formed and/or stored. Also the nature of the protein labelling changes cannot be characterized more precisely as yet. The results do demonstrate, however, that a visual discrimination training is followed by redistribution of protein radioactivity between control and trained visual hemicortices, while labelling of the soluble fraction, probably attributable to the differential light input only, appears to be unaffected. Since both, control and experimental parts, of the tissue are taken from the same animal, small changes can be revealed and also the complex of the side effects like frustration, hormonal influences, locomotor activity etc. can be eliminated. A similar approach has been used previously in a few studies concerning changes in RNA⁹ and acetylcholinesterase^{10,11} during 'handedness' and RNA during imprinting with 'split-brain' chicken¹².

A number of papers have accumulated demonstrating changes in the labelling of protein or RNA in the visual

⁵ O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. Y. RANDALL, *J. Biol. Chem.* 193, 265 (1951).

⁶ N. P. MADSEN, *Analyt. Biochem.* 29, 542 (1969).

⁷ G. BRAY, *Analyt. Biochem.* 7, 279 (1960).

⁸ G. BANKER and C. W. COTMAN, *Arch. Biochem. Biophys.* 142, 565 (1971).

⁹ H. HYDÉN and P. W. LANGE, *Proc. natn. Acad. Sci., USA* 53, 946, (1965).

¹⁰ N. G. ALEKSIDZE and M. N. BALAVADZE, *Dokl. Akad. Nauk, SSSR* 198, 1455 (1971).

¹¹ J. KRIVÁNEK and O. BUREŠOVÁ, *Experientia* 28, 291 (1972).

¹² G. HORN, A. L. D. HORN, P. P. G. BATESON and S. P. R. ROSE, *Nature, Lond.* 229, 131 (1971).

cortex during learning or light stimulation¹³⁻²¹. Most of them differ, however, in many respects from the paradigm explored in the present study (early life period, total dark versus strong light stimulation, very often control and expt. groups treated separately, correction of the

¹³ H. HYDÉN, P. W. LANGE, L. J. MIHAILOVIĆ and B. PETROVIĆ-MINIĆ, *Brain Res.* 65, 215 (1974).

¹⁴ W. POHLE and H. MATTHIES, *Pharmac. Biochem. Behav.* 2, 573 (1974).

¹⁵ S. C. BONDY and F. L. MARGOLIS, *Expl Neurol.* 27, 344 (1970).

¹⁶ A. J. DEWAR and A. K. WINTERBURN, *J. neurol. Sci.* 20, 279 (1973).

¹⁷ J. CLIFFORD, J. GAITO and T. MASAYUKI, *Nature New Biol.* 234, 90 (1971).

¹⁸ P. METZGER, M. CUENOD, A. GRYNBAUM and H. WAELSH, *Life Sci.* 5, 1115 (1966).

¹⁹ B. RENSCH and H. RAHMAN, *Pflügers Arch.* 290, 158 (1966).

²⁰ S. P. R. ROSE, *Nature, Lond.* 215, 253 (1967).

²¹ G. P. TALWAR, S. P. CHOPRA, B. K. GOEL and B. D'MONTE, *J. Neurochem.* 13, 109 (1966).

²² S. P. R. ROSE, *Brain Res.* 38, 171 (1972).

²³ S. C. BONDY and B. S. MORELOS, *Expl. Neurol.* 31, 200 (1971).

²⁴ S. C. BONDY and J. L. PURDY, *Brain Res.* 76, 542 (1974).

²⁵ The author express his appreciation to Dr. JAN BUREŠ and Dr. OLGA BUREŠOVÁ for discussion and advice, to Mrs. L. JERÁBKOVÁ for training the rats and to Mr. V. ŠILLING for skillful technical assistance.

precursor changes made almost exclusively at one fixed time interval measuring total TCA soluble radioactivity even when tritiated amino acid used). Nonetheless, differences in the pool of some amino acids²², blood flow²³ and blood-brain barrier permeability²⁴ between light restricted and stimulated visual cortex revealed from these kinds of experiments might contribute to the changes in TCA-soluble activity detected in our experiments.

Zusammenfassung. Ratten lernten mit einem Auge eine horizontal-vertikale Diskrimination. In der optischen Hirnrinde, kontralateral zum offenen Auge, konnte bereits 15 min nach Applikation von ¹⁴C-Leucin eine kleine, aber statistisch signifikante Erhöhung der Proteinmarkierung sowie eine Verminderung der Radioaktivität in der Trichloressigsäure-löslichen Fraktion gefunden werden.

J. KRIVÁNEK²⁵

Institute of Physiology, Czechoslovak Academy of Sciences, Budějovická 1083, CS-142 20 Praha 4 - Krč (Czechoslovakia), 9 September 1974.

Pattern in the Hunger Signal of Hornet Larvae (*Vespa orientalis*)

In previous publications the nature of the hunger signals produced by larvae of the oriental hornet (*Vespa orientalis*) was reported¹⁻³. It was found that the pattern of contractions of the larvae when producing spontaneously the hunger signals is rhythmic and the average between consecutive contractions is 3 to 4 sec. In the present experiment, the pattern of contractions induced by a mechanical stimulus was studied.

Materials and methods. 3 hornet queen larvae were kept in their optimal natural conditions in separate combs to prevent the transfer of signals from one to the other. The experiment continued for 8 days: during the first 6 days the larvae were completely starved and during the latter 2 days they were fed. On each day a series of mechanical stimuli were presented to the larvae. Initially a light knock was given to the back of the comb by a vibrator. Consequently a series of bodily contractions was observed and recorded by a pick-up transducer (B & K) attached to a tape recorder (Philips) and to a polygraph (Grass). When a series of contractions was followed by a relatively long interval (5-10 min) containing no contractions, the stimulus was repeated and the results again recorded.

This cycle of stimulus and recorded response was continued for a one-half-hour period on each day for each larva.

Results. In Figure 1 three typical series of signals induced by stimuli are presented. In the top trace the series is relatively short and contains a total of 12 contractions. The series is preceded by a period of resting which was followed by the stimulus. Another period of rest follows the series. The interval between the first and second contraction is about 2 sec, whereas the interval between the last 2 signals is about 4-5 sec. In the other two traces there is a similar lengthening of the intervals between contractions as the series continued, although the number of contractions and the absolute length of the series differ (Figure 1).

32 series of contractions, each of which contained at least 30 signals, were analyzed. If a relationship can be

¹ L. SHAUDINSCHKY and J. ISHAY, *J. acoust. Soc. Am.* 14, 1290 (1968).

² J. ISHAY and E. M. LANDAU, *Nature, Lond.* 237, 5353, 286 (1972).

³ J. ISHAY, A. MOTRO, S. GITTER and M. B. BROWN, *Animal Behav.* 22, 741 (1974).

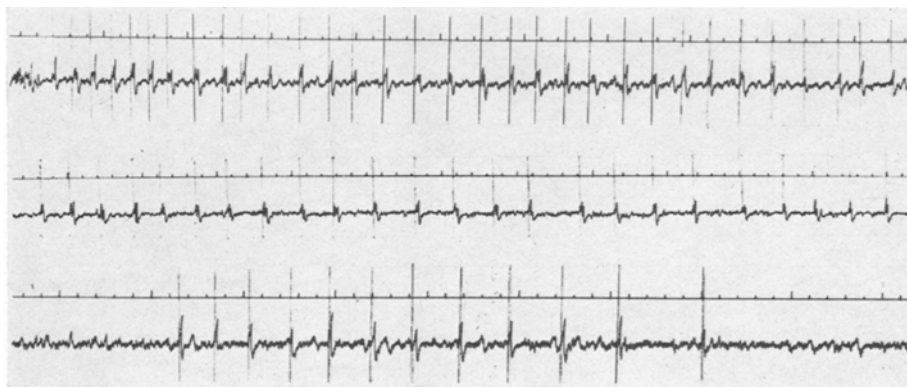


Fig. 1. The polygraph tracing of the recordings of 3 typical series of contractions. The upper line in each tracing is a timer, each interval represents 1 sec. The vertical lines are drawn to designate the start of the contractions.