

périphérique étant un flash (L). Sur la troisième ligne, un choc électrique est appliqué à une patte postérieure (PPD), et on note que sur la racine ventrale ipsilatérale à la stimulation (RVL7d), la décharge bulbo-spinale est précédée d'une composante à courte latence qui vraisemblablement représente le réflexe de flexion: lors de l'interaction (NCd + PPD), ce dernier n'est pas affecté à cette intensité du stimulus.

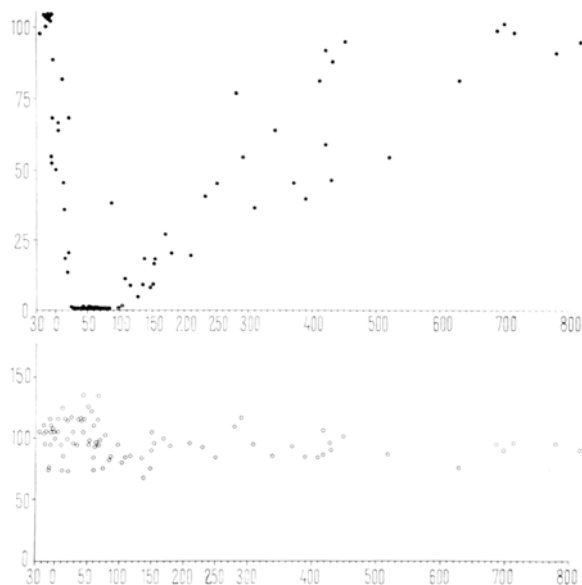


Fig. 2. Evolution de l'effet dépressur en fonction de l'intervalle séparant les 2 stimuli. En abscisse, on a porté en msec l'intervalle compris entre le début de la stimulation centrale (qui correspond au temps 0) et la stimulation périphérique, ici un choc électrique appliqué à la patte postérieure gauche. En ordonnée, on a mesuré l'amplitude de la réponse tardive recueillie sur la racine ventrale gauche du septième segment lombaire (courbe du haut) et celle du réflexe ipsilatéral de flexion (courbe du bas). Dans les deux cas on a donné la valeur 100 à l'amplitude moyenne des réponses non conditionnées.

Les courbes obtenues en combinant à intervalles divers le stimulus caudé et le stimulus périphérique montrent l'existence d'un tel effet dépressur pour des intervalles compris entre 0 et 400 msec, avec un maximum entre 20 et 80 msec (Figure 2).

Bien que certaines caractéristiques du phénomène, en particulier sa fragilité, inclinent à penser qu'il s'agit d'une inhibition, il n'est pas actuellement possible de décider de sa localisation ni de sa nature précise. Cette action du noyau caudé est à rapprocher de celle exercée par la même structure sur les systèmes ascendants non primaires⁴ qui sont, au même titre que les voies descendantes réticulo-spinales, considérablement facilitées par l'action de l'anesthésique utilisé; le blocage de la voie ascendante vers le cortex moteur par la stimulation du noyau caudé produit de son côté une suppression de la décharge pyramidale réverbérée d'origine sensorielle⁵. Cette action descendante de la stimulation striatale n'exclut pas d'autre part un effet sur le réflexe lui-même, qui se note probablement dans d'autres conditions expérimentales⁶.

Summary. In cats under chloralose anaesthesia, the efferent spinal discharges of extrapyramidal – presumably reticulo-spinal – origin, which can be elicited by various types of sensory stimuli, can easily be suppressed by short trains of electric shocks applied to the head of the caudate nucleus.

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⁴ G. KRAUTHAMER et D. ALBE-FESSARD, *J. Neurophysiol.* 28, 100 (1965).

⁵ P. BUSER, H. ENCABO et P. BORENSTEIN, *C. r. hebdom. Séanc. Acad. Sci., Paris* 253, 538 (1961).

⁶ J. P. SEGUNDO, E. F. MIGLIARO et J. A. ROIG, *J. Neurophysiol.* 27, 391 (1958).

Localization of Histone Fractions by Radioautography

It is now known that macromolecules, including intact proteins, can penetrate into living cells of several types of tissue^{1,2}, although the mechanism is not understood³. In the present investigation, this property was used to determine the degree of penetration of different fractions of histone into the roots of the broad bean, *Vicia faba*. The bean root was selected for these studies because of the reported ability of plant roots to incorporate intact, basic protein molecules larger than histones^{4,5}, and also because of its favorable cytological characteristics. The plan of the experiment was to add tritium-labeled *Vicia* root histone fractions to *Vicia* roots, and then to observe the distribution by radioautography.

Methods. To prepare tritium-labeled histone fractions, freshly excised root tips of *Vicia faba* were incubated in aerated sterile water containing 2–6 mc/l H³-arginine and H³-lysine for 3 h. Nuclei were isolated from the roots by slight modifications of the method of KÜHL⁶ and histone fractions were isolated from the nuclei by a differential acid extraction method⁷. Details of these isolations and

the characterization of the fractions by amino acid analysis and by acrylamide gel electrophoresis are to be described in another report; however the main characteristics of the histones used here are summarized in the Table.

The radioactive proteins were dissolved at a concentration of 1 mg/ml and *Vicia* seedlings were placed in these

¹ L. LEDOUX, in *Progress in Nucleic Acid Research and Molecular Biology* (Ed. J. N. DAVIDSON; Academic Press, New York 1965), vol. 4, p. 258.

² C. M. FELDHER and C. V. HARDING, *Protoplasmatologia* v/2, 3 (1964).

³ H. J.-P. RYSER and R. HANCOCK, *Science* 150, 501 (1965).

⁴ W. A. JENSEN and A. D. McLAREN, *Expl Cell Res.* 19, 414 (1960).

⁵ A. D. McLAREN, W. A. JENSEN and L. JACOBSON, *Pl. Physiol.*, Lancaster 35, 549 (1960).

⁶ L. KÜHL, *Z. Naturf.* 19b, 525 (1964).

⁷ K. MURRAY, *J. molec. Biol.* 15, 409 (1966).

solutions for periods up to 3 h. Aliquots of solution and root tips were removed periodically, dried on planchettes and counted on a Nuclear-Chicago gas flow counter. At the end of 3 h, the root tips were cut off and fixed in 10% neutral formalin. Sections were cut 4 μ thick, deparaffinized, and dipped in NTB-2 emulsion. Radioautographs were developed according to standard procedures.

Results. Radioactivity gradually disappeared from the medium, with a 50% decrease occurring over a 3 h period. Simultaneously the root tips became radioactive. The radioautographs showed that most of the label was adsorbed to the cell walls, but, for each protein fraction, there was also some label within the cells. The quantity of intracellular label varied considerably between roots

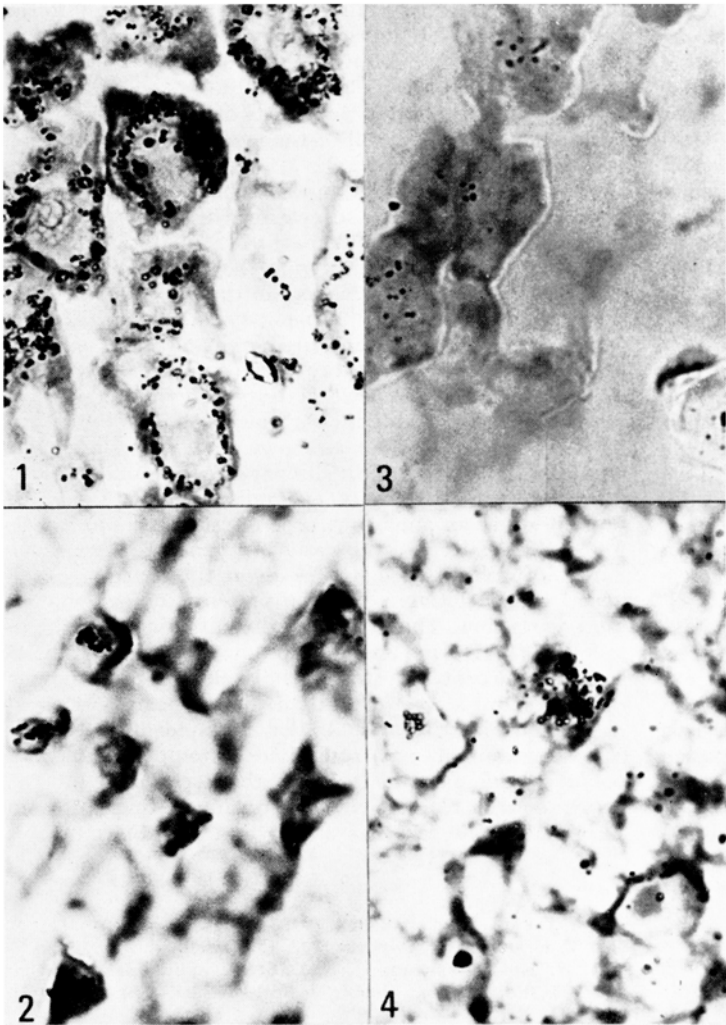
and was highest in the peripheral cells. Fraction I, the most lysine-rich histone, entered the cytoplasm only (Figure 1), but both fractions II and III were found primarily in the nucleus, with fraction II localized in the nucleolus (Figure 2). The total histone was in both cytoplasm and nucleus (Figure 4).

Discussion. Histones have been shown to be toxic^{2,8} and may inhibit root growth when used for a prolonged period of time⁹. However in a short period of time, as used here, no obvious evidence of cell damage was seen. The results are in general agreement with 3 recent studies, which however used different histones and different cell types. BUKRINSKAYA et al.¹⁰ demonstrated C¹⁴-chick embryo histone to be over the nucleus and especially around the nucleolus in Hep-2 cells and BLAZSEK and GYERGYAY¹¹, using fluorescent-labeled calf thymus histone with Ehrlich ascites tumor cells, reported fluorescence in the nuclei of some cells and in the nucleolus. RYSER and HANCOCK³, using far lower concentrations of

Characteristics of histone fractions

Fraction No.	pH of isolation	Basic-acidic amino acids	Lys/arg ratio	% of total histone	cpm/mg protein
I	2.8-1.8	1.9	7.8	25	137,000
II	1.8-1.4	1.5	2.5	30	41,000
III	1.4-0.6	1.4	1.5	45	18,000

⁸ C. W. KISCHER and L. S. HNILICA, *Experientia* 22, 1 (1966).
⁹ A. D. McLAREN and O. E. BRADFUTE, *Physiologia Pl.* 19, 1094 (1966).
¹⁰ A. G. BUKRINSKAYA, O. BURDUCEA, A. K. GITELMAN and T. A. ASSADULLAEV, *Expl Cell Res.* 42, 484 (1966).
¹¹ V. A. BLAZSEK and F. GYERGYAY, *Experientia* 22, 380 (1966).



Radioautographs with focus on silver grains. All $\times 100$. (1) Root treated with histone fraction I; (2) root treated with histone fraction II; (3) root treated with histone fraction III; (4) root treated with total histone.

histones, reported entry of histones into sarcoma-180 cells (but not into nuclei) as estimated by fluorescence microscopy. The use of hydrolyzed histone¹¹ or free amino acids¹⁰ gave a different picture with label primarily in the cytoplasm.

The reason for a different localization of the lysine-rich fraction I than the 2 arginine-rich histones is not clear without further work. It is possible that over a longer period of time, fraction I might also have entered the nucleus. On the other hand, there might be a permeability barrier of the nuclear membrane to fraction I, but not to fractions II and III. This would agree with the idea that lysine-rich histone is synthesized in the nucleus¹² but arginine-rich histones are synthesized in the cytoplasm and must then enter the nucleus¹³.

The fact that histones, both native and foreign, are able to penetrate various cell types, even to the nucleus, should be useful in further studies of histone functions *in vivo*, as well as in studies of permeability. It might be possible, not only to localize various histone fractions more specifically, but also to use added histone fractions to alter the normal histone complement of a cell¹⁴.

Zusammenfassung. Wurzeln von *Vicia faba* nehmen ³H-markierte Histon-Bruchstücke auf. Mikroautoradiographien lassen auf eine unterschiedliche intrazelluläre Lokalisation der Histon-Bruchstücke schliessen.

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¹² B. R. REID and R. D. COLE, *Proc. natn. Acad. Sci.* 51, 1044 (1964).
¹³ D. BLOCH and S. BRACK, *J. Cell Biol.* 22, 327 (1964).

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Nystagmus and the Activity of Visual Cortex Cells

In the study on sleep with low voltage fast EEG activity and made with an unanaesthetized cat sleeping in a dark cage¹, it emerged that the highest discharge rate of visual cortex cells tended to commence after saccadic bursts of rapid eye movements. It was thought that during that stage of sleep, a common brain stem factor had affected both the eye muscles and the visual cortex. Regarding the corollarity of peripheral oculomotor activity and the visual cortex discharge which occurred in sleeping cats without light stimulation of the retina, a decision was made further to study this corollarity in alert monkeys by changing the input to the central oculomotor system, i.e. by using labyrinthine stimulation.

Methods. In the present work, the single unit activity of the area striata was compared with the postcaloric nystagmus pattern when light stimulation of the retina was avoided. Major attention was paid to the possible grouping of neuronal discharges in area striata and to the relationship of this grouping to the nystagmus rhythm. The experiments were carried out on monkeys (*Macaca mulatta*). During the recording session, the monkey sat in a primate chair with the head bent backward at an angle of 60° and restrained according to the method reported elsewhere². The eyes were covered by opaque contact occluders after application of the local anaesthetic. The stimuli consisted of 1–2 sec of irrigation of the left external ear canal with 2 cm³ of water at 20–23°C, thereby provoking horizontal nystagmus with the fast phase to the right. The phasic activity of the right lateral rectus muscle corresponded, in this case, to the rapid phase of postcaloric horizontal nystagmus.

Results. Thirty-two units from striate cortex on the left side were recorded during the horizontal postcaloric nystagmus, together with the EMG of the right lateral rectus eye muscle. Occasionally the discharge rate of most visual units showed a change in relation to the nystagmic rhythm. Two main types of relation were found between visual cortex cell discharge and nystagmus, both comprising about 40% of the total material: (1) the unit

discharges tended to occur in a general relationship to the fast phases of nystagmus. The highest frequency of unit activity corresponded to the ocular EMG or alternatively to the periods immediately before or after the EMG burst, including the initial and final parts of the slow phase and the momentary standstill of the eyes during the turning points of nystagmus (Figure 1). (2) The unit discharges occurred mainly during the slow phase of nystagmus, often in regular series and ceasing with the fast phases (Figure 2). In the remaining part of the material, the unit grouping deviated so much from the nystagmus rhythm that there was a continuous shifting in the temporal relation between these 2 phenomena. Sometimes the relation between visual unit discharge and the lateral eye muscle activity was of the same type, regardless of whether the ocular muscle activity contributed to nystagmus or non-nystagmic 'spontaneous' saccadic eye movements. Earlier¹ it was shown that during sleep the highest rate of visual unit discharge was found to occur after the small bursts of eye muscle activity. In the present study an analogous type of relationship was also found, but only in some cases. In general, there is no doubt in both of these studies about the contribution of visual cortex activity, but the present findings showed more variations. The reason for this difference may be as follows: (1) During sleep with low voltage fast EEG activity, the central system of the oculomotor phenomena may be more stereotyped than during postcaloric nystagmus in an alert animal. For example, in the latter case, the animal may sometimes attempt to make a voluntary eye movement during the nystagmus. (2) The visual cortex reaction to labyrinthine stimulation in 'encéphale isolé' cats has a long and variable latency³.

¹ P. VALLEALA, *Archs ital. Biol.* 105, 1 (1967).

² E. V. EVARTS, *J. Neurophysiol.* 27, 152 (1964).

³ H. H. KORNHUBER and J. S. DAFONSECA, in *The Oculomotor System* (Ed. BENDER; Hoeber Medical Division, New York 1964), p. 556.