sponse was recorded by an intracellular micropipette in 23 α motoneurones identified by antidromic stimulation of the central cut end of L7 ventral root. The stimulation frequency of the LGS nerves was kept within values capable of evoking a steady frequency of discharge, which generally ranged from 7 to 12 c/s. Then the stimulation frequency was suddenly raised in order to inhibit the motoneurone response. Threshold intracellular stimuli, previously established when the membrane potential was at resting values, were then applied through the same micropipette[4] in order to test the motoneurone excitability. Then the stimulation frequency was suddenly lowered to the previous values.

During the reflex inhibition induced by repetitive orthodromic stimulation, no hyperpolarization of the postsynaptic membrane was observed (Figure, A) and the threshold intracellular stimuli were able to evoke a normal response (Figure, A, B, C). When the orthodromic stimulation frequency was decreased to the initial values, the same initial stimulus-response ratio was immediately resumed (Figure, A, B, C).

The lack of the hyperpolarization and the normal excitability of the postsynaptic membrane seem to rule out the possibility that any postsynaptic inhibition is involved in this kind of reflex depression. The peculiarities of the reflex discharge reappearance, which occurs when the stimulation frequency is lowered, rule out the hypothesis of the 'receptor desensitization' of the postsynaptic membrane[5].

These experiments support the hypothesis that the reflex discharge suppression observed in the spinal motoneurones during the repetitive orthodromic stimulation is due to a presynaptic mechanism.

*Riassunto.* La stimolazione elettrica delle fibre afferenti del Gruppo I è in grado di provocare nel singolo motoneurone spinale una depressione della scarica riflessa che è funzione della frequenza di stimolazione. I risultati della presente ricerca sembrano dimostrare che tale depressione riflessa è da attribuirsi ad un meccanismo inibitorio presinaptico.

M. DECANDIA and L. PROVINI

*Istituto di Fisiologia Umana dell'Università di Milano (Italy), September 11, 1965.*

[4] T. ARAKI and T. OTANI, J. Neurophysiol. *18*, 472 (1955).
[5] S. THESLEFF, J. Physiol. (London), *148*, 659 (1959).

## Morphogenetic Field Properties of the Forebrain Area of the Neural Plate in an Anuran

*Background.* The early amphibian neural plate is known to be, to a large extent, already *determined* for later regional development, along both the anteroposterior and the medio-lateral axes[1]. Thus isolation, transplantation, or reversal of many portions of the early plate fail to prevent their development according to their prospective significance. The presumptive forebrain region, for example, forms in isolation only those structures characteristic of the forebrain[2]. Within this area, however, developmental *plasticity* is found, since isolated subregions form other forebrain structures in addition to those for which they were specifically fated[3]. Quantitative differences between the differentiation of the forebrain area *in situ* and *in vitro* also demonstrate that such alterations in the developmental pathways of cells can still take place at the early neural plate stage[2]. Finally, the fact that the experimental 'activation' of neural differentiation from competent ectodermal cells typically results in an isolated but complete forebrain is a further indication of the creation first of a *field* of 'forebrain' cells, in which only subsequently does a heterogeneous spatial pattern develop[4]. Since most of the experimental analysis of forebrain morphogenesis has been carried out in urodeles, the author's previously cited study of the development of neural plate fragments of the African clawed toad, *Xenopus laevis laevis* (*Daudin*), offered an opportunity to examine this question in an anuran form.

*Experimental.* The transparency of the skin in this species made it possible to recognize eye formation in the living explanted tissue by means of the darkly pigmented *tapetal* outer layer. When examined histologically the tapetum was found to be associated with a fragment of retina, continuous with a neural vesicle or mass. (Retina alone was not encountered under the present experimental conditions, which included the presence of large amounts of mesodermal cells inside a jacket of ectoderm). In almost 100 preparations of varying size and origin, but all lacking any tissue from the presumptive forebrain area, there were no cases of eye formation. Eye structures were found in 7 out of 8 explants of the entire estimated forebrain area of the early neurula, in 2 out of 4 explants of only the transverse neural fold (presumptive telencephalon) and in all 4 explants of the anterior lateral fold only (future diencephalon roof). When the anterior most region of the neural *plate* was tested (presumptive eye and ventral forebrain) an eye developed in 8 out of 17 cases. With slightly more caudal plate (mostly diencephalon floor) 4 out of 10 did so. In addition, eye formation occurred in all seven explants made with anterior neural fold tissue excised at the stage of closed neural folds (i.e. *late* neurula), a region which forms only telencephalon in

[1] H. EYAL-GILADI, Arch. Biol. (Liège) *65*, 180 (1954). – C. VON WOELLWARTH, Roux Arch. Entw. Mech. *145*, 582 (1952). – J. GALLERA, Roux Arch. Entw. Mech. *145*, 143 (1951). – M. A. CORNER, J. comp. Neurol. *123*, 243 (1964). (For overall surveys of differentiation tendencies in the early plate.) J. C. VAN DE KAMER, Thesis, University of Utrecht (1949). – A. STEFANELLI, Quart. Rev. Biol. *26*, 17 (1951). – C. O. JACOBSON, Zoöl. Bidr., University of Uppsala *36*, 73 (1964). (For specific cellular and nuclear development in particular regions of the brain.)
[2] E. C. BOTERENBROOD, Thesis, University of Utrecht (1962). The total volumes of telencephalon, diencephalon, and eye were measured and compared. Simple spatial patterns were often obtained even in completely disaggregated-reaggregated cell masses.
[3] P. D. NIEUWKOOP et al., Arch. Néerl. Zoöl. *13*, Suppl. 1, 167 (1958); J. Anim. Morph. Physiol. *11*, 21 (1964). – J. C. VAN DE KAMER[1].
[4] P. D. NIEUWKOOP, Develop. Biol. *7*, 255 (1963) for the most recent and precise treatment of this question. This is moreover one of the few experiments where all the future forebrain cells were with certainty exposed to essentially identical conditions during the 'activation'. An anuran form (*Rana pip.*) was studied in addition to a urodele (*Amblystoma punct.*).

normal development[5]. Plasticity of the forebrain region was also evident in the donors of the explanted neural tissue, as normally proportioned paired eyes were found in all seven *Xenopus* larvae examined histologically after earlier removal of large portions of the anterior neural plate. Except for two explants in which a pair of eyes developed, eye formation in vitro always resulted in a single, compact structure.

*Discussion.* It is clear from the above results that in an anuran as well as in urodeles the forebrain area of the future central nervous system behaves initially like a 'morphogenetic field'. The final developmental instructions, although limited to forebrain types, are not yet fixed in the individual cells. Although the kinetics of 'crystallization' into the definitive pattern are unknown, field properties are in evidence at least through the end of neurulation. This is not necessarily to say, however, that the presumptive forebrain area is uniform ('equipotential') at the time of testing. Recent quantitative experiments have in fact established, by measuring the *differentiation tendencies* of different areas, that regional differences exist at least as early as the open neural plate stage[6]. Apparently the postulated initial *equi*potentiality of the field is quickly lost. The way in which this occurs is not known, but it is noteworthy that a qualitatively normal structural pattern develops even in tissue activated in vitro[4]. This implies that slight differences in the 'micro-environment' at different points within the cell mass are sufficient to select different developmental pathways. Such differences could in turn consist partly of gradients resulting from metabolic or secretory activity[7].

The determination of the forebrain structural pattern in situ is strongly influenced by regional factors which are non-existent under in vitro conditions. The best established of these is an *eye-depressing* influence from the underlying medial mesoderm[8]. This effect could also explain why no eye developed in a percentage of cases in the *Xenopus* forebrain material reported here[9]. The relatively small size of the neural fragments used was possibly also a contributing factor since it is known that eye formation frequently fails to occur if the mass of forebrain cells is small[4,6]. Taking in perspective the known facts about the histogenesis of the eye, it becomes clear that the developmental fate of individual eye cells is specified in the following step-wise sequence. During 'primary induction' all potentialities ('genes'?) become blocked for cellular differentiation other than to one of the *forebrain* types. The potentialities become further restricted in the future eye-forming region by a secondary process of *segregation* during neurulation, so that ultimately only cell types of the eye can develop. Eye morphogenesis begins shortly afterwards by cellular migration and finally, at about the optic cup stage, the definitive development of each cell is determined according to its location within the 'eye field'[10].

*Résumé.* Une analyse du développement des yeux faite sur de petits fragments de l'ébauche neurale («neural plate») a montré que la région du futur prosencéphale se comporte comme un *champ* morphogénétique. Bien que cette région ne soit plus «*équi*potentielle» même dans la neurula jeune, les propriétés du champ persistent pendant toute la neurulation.

M. A. Corner

*Central Institute for Brain Research, Amsterdam (The Netherlands), September 6, 1965.*

[5] M. A. Corner, J. exp. Zool. *153*, 301 (1963) and [1] for an approximate fate map of the major brain divisions in the *Xenopus* neurula. – C. O. Jacobson, J. Embr. exp. Morph. *7*, 1 (1959). – C. von Woellwarth, Roux Arch. Entw. Mech. *152*, 602 (1960). (For precise maps of the forebrain area in the early neurulae of *Amblystoma mex.* and *Triturus alp.* respectively.)

[6] P. D. Nieuwkoop, J. Anim. Morph. Physiol. *11*, 21 (1964) using *Triturus alp.*; also from unpublished experiments of the author with *Amblystoma mex.*: the presumptive eye region of the early neurula formed almost exclusively eye in vitro while relatively less eye and more neural material developed from the other forebrain areas.

[7] W. F. Loomis, in *Biological Structure and Function* (Academic Press, New York 1961), p. 509, suggests a differential gene activation according to the $pCO_2$ built up at each point in the tissue, while S. M. Rose, Biol. Rev. Cambr. Philos. Soc. *32*, 351 (1957) has proposed that a hierarchy of specific self-inhibitory cell products initiates the spatial pattern. Boterenbrood[2] has suggested that telencephalic differentiation is favoured in cells located towards the *surface* of the neural mass, which in itself is consistent with either of the mechanisms mentioned above.

[8] See *Discussion* in Nieuwkoop et al.[3], this factor may in fact be identical with the 'transforming principle', which blocks all forebrain differentiation tendencies in the more caudal neural regions. It appears also to favour diencephalic differentiation within the forebrain area at the expense of telencephalic.

[9] M. A. Corner, unpublished (*Amblystoma mex.*): the inclusion even of ventral mesoderm in an explant led to the formation of extensive mesenchyme and absence of eye formation in presumptive eye tissue. A neural structure formed in its place.

[10] V. Lopashov and O. E. Stroeva, in *Advances in Morphogenesis* (Academic Press, New York 1961), p. 331: differentiation can still be guided into either tapetum or retina, by properly choosing the environmental conditions. The experiments of L. S. Stone, J. exp. Zool. *145*, 85 (1960) and of G. Székely, Acta biol. hungar. *5*, 157 (1954) have demonstrated, moreover, that at about this time individual retinal cells become specified to make synaptic connections at appropriate points in the optic tectum.

## Relationships Between Cerebral Transit Time of Non-Diffusible Indicators and Cerebral Blood Flow. A Comparative Study with Krypton$^{85}$ and Radioalbumin

Methods available at present have not yet yielded reliable measurements of the parameters of the cerebral circulation suitable for clinical use. In recent years a consistent effort has been directed towards estimation of indices of cerebral blood flow (CBF) by externally recording thepassage through cerebral vessels of a bolus of a $\gamma$-emitting, non-diffusable tracer, such as radiohyppurane or radioalbumin[1,2].

The reliability of such methods is based essentially on two assumptions: (1) the recorded curve of radioactivity versus time secures reliable information about the mean transit time ($\bar{t}$) of the blood circulating in the explored region of the head; (2) the cerebral blood volume is a constant. Under these conditions, $\bar{t}$ would behave as a linear index of CBF: $1/\bar{t} = F/V$, where F is the blood flow and V the blood volume in the explored region[3].

[1] W. H. Oldendorf, J. nucl. Med. *3*, 382 (1962).

[2] C. Fazio, C. Fieschi, and A. Agnoli, Neurology *13*, 561 (1963).

[3] M. Zierler, in *Dynamic Clinical Studies with Radioisotopes* (Ed., R. Kniseley, Atomic Energy Commission, 1964), p. 55.