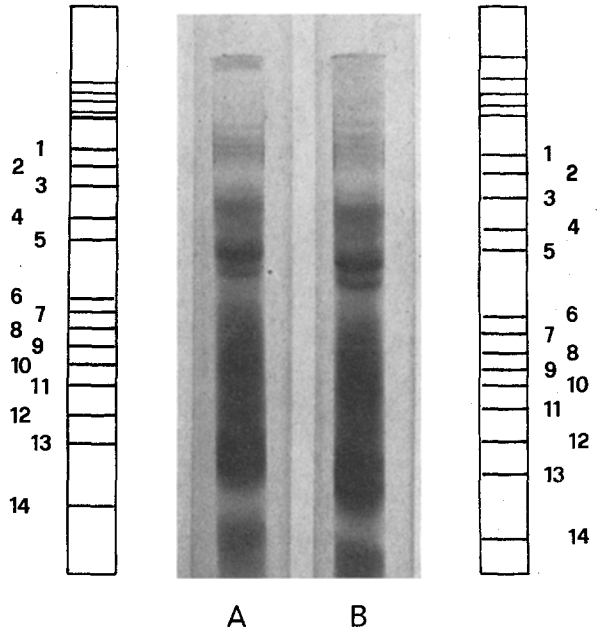
glycerol, 10^{-3} *M* N-ethyl-maleimide), using a magnetic stirrer at 10 °C for 20 min (1 part extraction volume to 4 parts of original tissue weight). DNA was extracted from the insoluble residue with 5% $HClO_4$ at 70 °C for 15 min and determined by the method of BURTON[8]. Total NHP amounts were determined in the GRONOW and GRIFFITHS' extract by the method of LOWRY[9]. Protein samples were separated by disc-electrophoresis on 3 × 60 mm gels with the system of SHAPIRO et al.[10], as modified by ELGIN and BONNER[11]. Methodological details have been published elsewhere[12].

NHP/DNA ratios in the developing and adult liver and brain of the rat

Organ	Age	Experiments			Mean
		1	2	3	
Brain	5 d	2.4	2.4	1.8	2.2
	10 d	3.2	2.7	2.5	2.8
	20 d	3.1	2.6	2.4	2.7
	adult	2.6	3.4	2.4	2.8
Liver	5 d	2.1	2.8	1.7	2.2
	10 d	2.4	2.6	2.2	2.4
	20 d	3.3	2.7	3.0	3.0
	adult	3.2	2.8	2.7	2.9



Disc-electrophoresis of GRONOW and GRIFFITHS' extract of total brain from a 5-day-old (A) and adult (B) rat. 20–50 µg proteins were separated for $1^1/_2$ h with $1^1/_2$ mA per gel. Gels were then stained with amido black.

Results and discussion. A reproducible pattern of 14 main bands for brain and 12 for liver nuclei was obtained from adult animals. Comparison of these patterns shows that the GRONOW and GRIFFITHS soluble proteins have a limited organ specificity, which we have described in another publication[12]. The electrophoresis of protein samples extracted from 5-, 10- and 20-day-old rats and adult animals all gave the same general pattern and number of bands (Figure). There is a slight increase of the NHP/DNA ratio for both liver and brain nuclei during ontogenesis, as can be seen from the Table. At the age of 5 days liver and brain nuclei both have a ratio of 2.2, whereas in adult rats the ratios for brain and liver nuclei were found to be 2.8 and 2.9 respectively.

This unchanged NHP pattern during ontogenesis is not compatible with the supposed function of NHP in transcription processes in brain nuclei. Although NHP are synthesized during the first 10 postnatal days, no new bands could be demonstrated by electrophoresis in that period and we could not find any quantitative changes in the densitometric profile of the gels. Our findings are consistent with the observations of BURDMAN[2] who did not find any differences between the NHP patterns of 1-day- and 8-day-old rat brains, but noticed an increase of total nuclear protein during that period. In rat liver, however, electrophoretic differences of NHP during ontogenesis[3] and during liver regeneration have been observed[13]. It is therefore possible that the changes in the nuclear proteins of brain nuclei during development are too small to be detected by present techniques.

Zusammenfassung. Die «non-histone» - Proteine (NHP) aus Hirn- und Leberzellkernen der Ratte wurden bei 5, 10 und 20 Tage alten und adulten Tieren extrahiert und das NHP/DNA-Verhältnis bestimmt. Die NHP aus den Gehirnzellkernen wurden disk-elektrophoretisch aufgetrennt. Leber- und Gehirnkerne zeigten eine geringe Zunahme des NHP/DNA - Quotienten während der ersten 20 postnatalen Tage. Es konnten jedoch für diese Altersstadien keine Veränderungen im Proteinmuster des Hirns gefunden werden.

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A Bipolar Electrode for Localized Directional Stimulation

During a recent study of the cerebral cortical consequences of stimulation of the pyramidal tract, it has been found that unless great care is taken, interference occurs due to inadvertant stimulation of the adjacent medial lemniscus[1]. This problem is especially difficult in experiments on smaller mammals such as rats, and a special bipolar electrode has therefore been devised to overcome as far as possible problems of stimulus spread.

Materials and methods. A diagram of the tip of the electrode to be described is shown in Figure 1. The method of construction was as follows: a hollow dental needle of external diameter 400 µm was used and through this was

threaded a length of insulated copper or steel wire of 100 µm diameter. A length of this wire was dipped in insulating varnish (Voltalac) and then drawn back into the needle. The assembly was left overnight at 120 °C to harden the varnish, thus permanently fixing the wire within the needle.

Electrical contact with the external portion of the electrode was effected directly onto the needle. The internal wire was gently scraped free of insulation where it protruded from the needle, and was then fixed permanently onto the needle exterior by means of either insulating varnish, or a similar fixative material such as Araldite®. An electrical connection was then made onto the exposed part of the centre wire. It was necessary that this connection be firmly fixed in relation to the body of the electrode, since movement of the wire soon led to breakage.

Having completed the basic construction of the electrode, the needle was clamped, lowered into a sufficient volume of insulating varnish to cover that part of the electrode which was to be inserted into the brain, and then slowly withdrawn. The electrode assembly was again heated at 120 °C overnight to harden the varnish.

The final stage in preparation consisted of cutting and filing the stimulating end of the electrode to an angle which was usually between 30° and 45° as shown in Figure 1. A very fine grained file and great care were needed to ensure that the filing process did not lead to the establishment of electrical contact between the inner and outer components of the electrode.

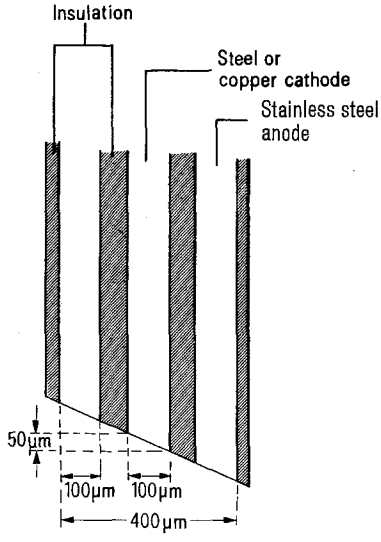

Fig. 1. Diagrammatic representation of the tip of the concentric bipolar electrode described in the text.

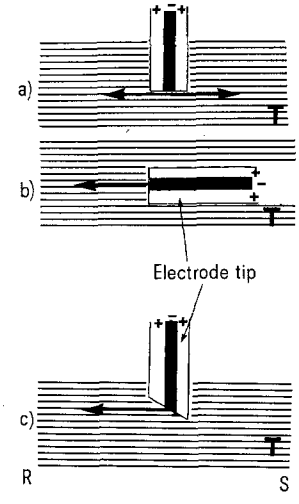

Fig. 2. a) A conventional concentric bipolar electrode is illustrated here. When inserted into a tract such as the pyramidal tract (T), fibres can be excited in an orthodromic and an antidromic direction. b) To excite fibres in either the orthodromic or antidromic directions, the electrode would have to be inserted longitudinally into the tract. c) The electrode described in the text has an angled stimulating tip, so that it can be inserted at right angles to a tract. When the centre wire is used as a cathode, fibres can only be activated in one direction (in this case impulses will only travel towards R, not S).

Results and discussion. Many of the previous investigations on the pyramidal tract have been carried out using bipolar electrodes, but these have been of a conventional form, usually consisting of 2 separated poles about 1 mm apart and placed on the surface of the exposed pyramid [2,3]. Such an arrangement cannot effect much restriction of stimulus spread. Furthermore, in those instances where a concentric bipolar electrode has been used [4], the electrode core is still able to activate fibres travelling in any direction away from the point of stimulation. In the case of the pyramidal tract, for example, a perpendicularly oriented electrode of this could activate fibres in an orthodromic and antidromic direction (Figure 2a). The electrode would have to be inserted parallel to the fibre tract in order to stimulate in only one direction (Figure 2b).

With the electrode described here only a small area of tissue, approximately 50 µm in diameter is exposed to the full stimulus, and the stimulus may be directed to a large extent rostrally or caudally in the fibre tract as illustrated in Figure 2c.

The efficacy of this electrode was confirmed when stimulating in the pyramidal tract of rats. The central wire was used as the cathode. Stimulation in a rostral direction allows the production of normal cortical α- and β-waves [1]. When using a train of six stimuli at 100 Hz and a stimulus strength of 20 V, no limb movement was seen. This indicates that the electrode was not activating descending fibres to the spinal cord. When the electrode faced caudally in the pyramidal tract, however, the stimulus parameters just described readily caused limb movement. It was also possible to differentially stimulate the closely adjacent pyramidial tract and medial lemniscus of the rat [1]. These observations illustrate the potential usefulness of this type of electrode in restricting stimulus spread in the central nervous system.

Résumé. Une électrode est décrite avec laquelle on peut stimuler une aire donnée du cerveau. On peut contrôler la direction du stimulus par cette électrode (Figure 2). Un diagramme de l'extremité de l'électrode est présenté à la Figure 1.

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