due to taping. This left a total of 14 rats, or seven pairs, for comparison.

The Table shows that five of six rats with bilateral amygdaloid lesions displayed a greater plasma corticosterone response to the stress of immobilization than their respective paired controls with cortical lesions. There was a reversal in only one case, experiment 4. A unilateral lesion of the amygdala (experiment 9) produced no appreciable difference in plasma corticosterone level. On being taped for immobilization, the amygdaloid lesion rats were in general more emotionally reactive and harder to handle than the controls.

Examination of effects of electrode placement in individual amygdaloid sub-nuclei showed that, when there was substantial bilateral damage to the central nucleus, but no bilateral damage to any other amygdaloid subnucleus, as in experiments 1, 5, and 7, the plasma corticosterone level was distinctly more elevated in the rat with bilateral amygdaloid lesions. The most clear-cut symmetrical lesions of the central nucleus were obtained in experiment 1 (Figure), where the rat with amygdaloid lesions showed an increase in plasma corticosterone following stress that was 16.75 µg/100 ml of plasma greater than the increase for the control.

These three experiments, 1, 5, and 7, showed on the average a difference of 12.83 µg of corticosterone per 100 ml of plasma in favor of the animals with amygdaloid lesions, compared to an average of 1.25 µg in favor of the rats with amygdaloid lesions in the remaining four experiments, 3, 4, 6, and 9. In experiment 4, where as noted above, a reversal occurred, there was substantial bilateral damage to the central nucleus, but there was in addition bilateral damage to the basal nucleus. This was the only instance in this series where this occurred.

These individual comparisons of histologically-determined damage in individual amygdaloid sub-nuclei with the plasma corticosterone response to emotional stress of the rat therefore suggest, in particular for experiments 1, 5, and 7, that bilateral lesions confined to the central nucleus of the amygdala result in an increased plasma corticosterone response to emotional stress in the laboratory rat. In view of the small number of animals and of the topographical scattering of the lesions among various amygdaloid sub-nuclei (Table) statistical methods cannot be justifiably applied to this series[8]. These observations suggest that under normal conditions the central nucleus in the rat amygdala may exert an inhibitory effect upon ACTH release.

Our results, taken together with previous work[2,3] suggest the possible existence of separate inhibitory and facilitatory mechanisms for the pituitary-adrenal response to stress at the amygdaloid level in the rat. Such inhibitory and facilitatory mechanisms have been found at the hypothalamic level in the dog[9] and at the mid-brain level in the rat[10].

Zusammenfassung. Im Vergleich zu Kontrolltieren mit zweiseitigen Rindenläsionen führt beidseitige Zerstörung des Nucleus centralis im Mandelkernkomplex der Ratte zu erhöhter Plasma-Corticosteron-Produktion nach kurzfristigem Stress durch entsprechende Immobilisierung.

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[8] An overall comparison of plasma corticosterone response between experimental and control rats was nevertheless made. This showed an average plasma corticosterone level of 28.46 µg per 100 ml of plasma for the seven experimental rats, compared to 22.25 µg for the seven paired controls, a difference of 6.21 µg. This difference was not statistically significant. However, since this sample, for reasons given above, does not lend itself to a meaningful statistical analysis, this negative result does not invalidate the findings. For the purposes of this study, effects of lesion placements in individual sub-nuclei are more relevant, but this work should be repeated on a larger series of animals to allow for the application of statistical methods.

[9] T. Suzuki, E. B. Romanoff, W. P. Koella, and C. K. Levy, Amer. J. Physiol. *198*, 1312 (1960).

[10] M. A. Slusher and V. Critchlow, Proc. Soc. exp. Biol. Med. *101*, 497 (1959).

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PRO EXPERIMENTIS

Fine Tipped Metal Microelectrodes with Glass Insulation

It is profitable to use very fine tipped metal microelectrodes, when leading off extracellularly action potentials from single nerve cells in the brain, because the finer the electrode tip, the greater is the recorded potential from a nearby cell in respect to those from units farther away.

With the electropolishing technique, it is possible to make very fine tipped microelectrodes of tungsten[1] or steel[2], which are then insulated with lacquer. It is, however, very difficult to obtain a thin, smooth lacquer layer when the electrodes have a long shaft of less than 20 µ in diameter. Moreover, the mechanical strength of the lacquer layer is unsatisfactory, and when coagulating with high frequency alternating current, the loss over the insulation (or through minute cracks?) is great. A glass insulating layer would be much more satisfactory. Indeed, Johnson and Manhof[3] drew a coat of glass around a platinum wire, but the tips of their electrodes were not thinner than the wire used and blunt. I therefore developed a method for drawing a thin layer of pyrex glass, under microscopical inspection, around an electropolished platinum wire and so obtained tip sizes of controlled, very small dimensions.

A length of platinum wire (10 or 20 µ diameter), a little longer than the desired shaft length, is soldered with silver solder to a short length of thicker copper wire to facilitate the handling (Figure 1a).

Polishing is done in the same way as with tungsten[1]: the utmost tip of the platinum wire is dipped in saturated sodium or potassium nitrite solution, and an alternating

[1] D. H. Hubel, Science *125*, 549 (1957).

[2] J. D. Green, Nature *182*, 962 (1958).

[3] M. W. Johnson and L. J. Manhoff, Science *113*, 812 (1951).

current is passed through the electrolyte between the Pt electrode and a carbon rod. The potential difference has to be 1 to 1.5 V. The immersed tip of the platinum wire is dissolved (the process is slower than with tungsten) until in the end the contact with the nitrite solution is suddenly broken, resulting in a very fine tapered point. The tapering of the point depends on the surface tension of the electrolyte. The point becomes short and pencil-like at high surface tension, but, when surface tension lowering agents are added, it tapers more gradually.

After electropolishing, the electrode is rinsed in water, alcohol, and dried.

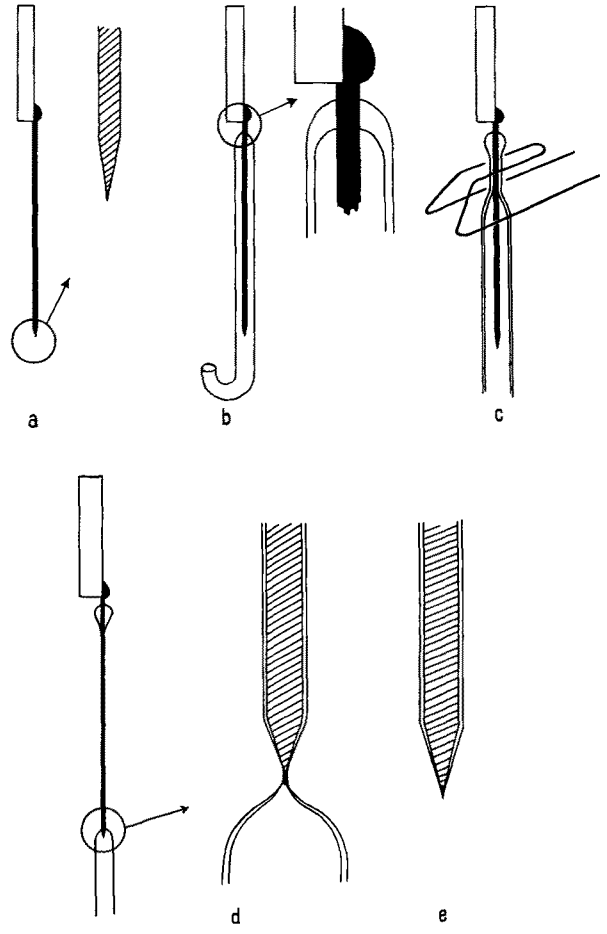

Fig. 1. (a) Platinum wire with electropolished point, soldered to a thicker copperwire, (b) a pyrex capillary with a hook on one end is fixed, (c) by heating the element a thin film of glass is drawn around the platinum, (d) when a tip of suitable size is not yet fused to the glass, the rest of the capillary is broken off, (e) in the vicinity of the tip the glass layer is very thin.

Thin walled pyrex tube of about 3 mm diameter is heated over a gas flame and drawn to capillaries of less than $^1/_2$ mm diameter. Under a dissecting microscope, a short length of the capillary is slid over the platinum wire and fixed to the metal at the upper end by melting with a microburner. At the other end, the capillary is bent to a hook (Figure 1b).

In order to obtain a thin and tight insulating layer, the glass is now heated and drawn out over the electrode in the following way: The electrode is clamped in a holder, which can be moved up and down with a rack and pinion (Figure 2d), and a small weight (Figure 2c) is hooked on. An electric heating filament (Figure 2a) (made of 0.3 mm nichrome wire) is put around the upper end of the electrode and heated, under visual control with a dissecting microscope, to a bright red. The heating current is drawn from a 6.3 V transformer (Figure 2e), which is fed from the mains over a variac (Figure 2f) by which the heating current can be regulated. When the pyrex glass softens, the capillary is drawn out by the weight, the glass forming a thin layer around the electrode (Figure 1c). By raising the electrode with the rack and pinion support, one can draw a thin layer of glass over the whole length of the electrode. In the vicinity of the tip, the heating current is diminished to slow down the process so that one is able to stop heating just when a tip of suitable size is not yet bonded to the glass (Figure 1d). A sharp pull at the hook of the capillary breaks the glass just around the tip, where it forms a very thin layer. The tip region is now briefly heated to ensure a good and smooth bond between the glass and the platinum. The result (Figure 1e) is an electrode with a very fine tip and a smooth and sturdy but thin insulation.

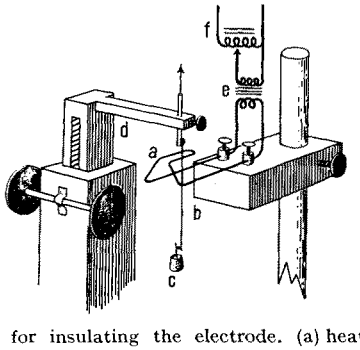

Fig. 2. Set-up for insulating the electrode. (a) heating filament, (b) electrode, (c) weight, (d) rack and pinion support, (e) heating transformer 6.3 V, (f) Variac.

Zusammenfassung. Es wird eine Methode beschrieben, die es ermöglicht, glasisolierte Platinelektroden mit beliebig feiner Spitze herzustellen.

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Zoological Laboratory of the University of Groningen (Netherlands), April 25, 1961.

Technique d'inclusion et d'ultramicrotomie, destinée à l'étude du développement des organelles dans une cellule isolée[1]

Introduction. L'étude, par coupes ultra-minces, des modifications structurelles qui se déroulent dans les cellules au cours de leur développement, pose certains problèmes de localisation. On peut comparer ces problèmes à ceux que rencontre, en embryologie, l'étude de la genèse d'organes et de systèmes d'organes. Dans l'un et l'autre cas, une démonstration satisfaisante du cours du développement implique deux nécessités: d'une part un contrôle précis du stade évolutif au moment de la fixation, d'autre part la connaissance exacte de l'orientation de l'objet et du plan de coupe. Le nombre élevé d'objets qui

[1] Travail effectué dans le cadre d'une recherche faite en collaboration avec les Instituts d'Histologie et de Biophysique, grâce à une subvention de «Fritz Hoffmann-La Roche-Stiftung zur Förderung wissenschaftlicher Arbeitsgemeinschaften in der Schweiz».