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Electrode and Cannulae Implantation in the Brain by a Simple Percutaneous Method

In an investigation of the psychologically active motivational (reward and punishment) systems of the brain stimulated by electrical means, we have utilized "roving" electrodes implanted in the unanesthetized monkey's brain (*1–4*). The technique of implantation by hammering sleeve-shaped guides into the skull for these movable electrodes apparently has not been used before and simplifies the problem in chronic preparations (*2, 3, 5, 6*). The stereotaxic instrument (*7*) can be used to place the guides. During times when experiments are not being carried out on the animal, all electrodes and cannulae can be removed, leaving inconspicuous self-closing and self-healing skin lesions.

The method consists of implanting in the skull (but beneath the skin and outside the dura) a hollow tube (sleeve) which guides the electrode (or electrode array or cannula)through the skull in a definite direction into the brain. An electrode (*8*) is pushed through the skin and subcutaneous tissues, into the outer end of the sleeve in the skull, through the barrel, and thence into the brain.

The sleeves are made from stainless steel (type No. 316) hypodermic needle tubing (No. 20, 0.90 mm outside diameter, 0.57 mm inside diameter, in one case—a macaque implantation) as is shown in Fig. 1c.

In the spot desired for the implantation, a small indentation is made in the soft tissues and bone with a hardened steel spear-shaped tool (Fig. 1*a*), which is guided through a long tube-shaped rigid bearing in a director used in place of the electrode carrier in the stereotaxic instrument. The director has a cone-shaped lower end which is pressed into the skin; the spear is lightly pounded into the bone (for a distance of about ½ mm) and then is withdrawn. The sleeve is placed on the mandrel (as in Fig. 1*b*); the mandrel is inserted in the director; the mandrel and the sleeve are driven into the bone by light hammering on the outer end of the mandrel. After each one-half millimeter or so of the guide is driven into the bone, the mandrel is manually tugged lightly upwards; if it comes out of the sleeve easily, the lower end of the guide (Fig. 1*c'*) has passed the inner table of the skull (but not the dura).

After the sleeve is in place in the skull, the skin and the subcutaneous tissues are allowed to pull together over the upper end and to heal. The sleeves are placed in definite patterns in the skull by means of the stereotaxic instrument and allowed to protrude above the skull about 2 mm. The operator palpates these ends through the soft tissues and finds the opening in the sleeve with the spear's sharp tip. By pressing the cone end of the spear into the guide's outer end, the skin and subcutaneous tissues are pierced. The skin is held in place with a forceps, the spear is withdrawn, and a sharp needle is inserted far enough to puncture the dura. The needle is withdrawn, and the electrode or cannula is inserted into the sleeve and lowered into the brain. To measure the depth of penetration of electrode or cannula, a pointed scale is used to measure the distance from the outer end of the sleeve to the outer end of the inserted cylinder of the electrode or cannula. The length of the sleeve varies with the animal and the loci in the skull. For the top of the skull of a macaque of 6 kg (13.2 lb) weight, for example, suitable lengths are 3½ to 4½ mm; for the skull of a porpoise, 20 to 50 mm.

After 5 to 6 weeks, a thin plate of bone grows over the ends of guides which are flush with the skull's surface. This is easily drilled out with two beveled hypodermic needles—first with one smaller than and then with one the same size as the electrode. Five months after an implantation the bone has not grown over the outer ends of guides which protrude 1.0 to 2.0 mm above the periosteum.

From 20 to 60 zones with 1 to 2 mm resolution have been explored along each track, running from pial cortex through the brain to the base of the skull. Previously, with a stereotaxic button and roving electrodes (*4*), we explored about 500 zones in two monkeys, with no problems assignable to either intracranial bleeding or infection. Currently, two monkeys are being investigated, with sleeves in their skulls at interguide intervals of 2 mm (one with four and one with 20 sleeves, to date). Figure 2 shows an x-ray of the skull of the animal with 20 implanted sleeves (3½ to 4½ mm long) and one electrode in place.

The animal is restrained to avoid pulling out the electrodes or cannulae (*9*). Self-limited amounts of bleeding from penetration of veins does occur but does not cause detectable signs in an upright monkey nor in a floating porpoise with a closed calvarium. Using roving electrodes, we have not yet seen (in exploration of about 30 tracks, in four animals, over a period of 18 months) any

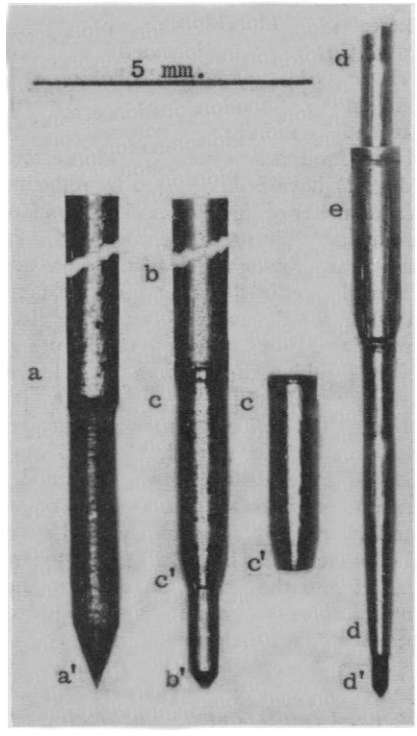

Fig. 1. Parts used in method of electrode implantation described in this report: *a,a'*, the lower end of the spear-shaped hardened steel tool (41 mm over-all length) used for starting the hole in the bone; *b,b'*, the lower part of the mandrel (41 mm over-all length), with a sleeve on the small cylindrical lower end (made of tungsten wire, 0.56 mm in diameter) (*b'*); *c,c'*, sleeves (one on mandrel and one by itself); *d,d'*, electrode; *e*, a sleeve guide on the electrode, showing a tight fit at the tapered inner end.

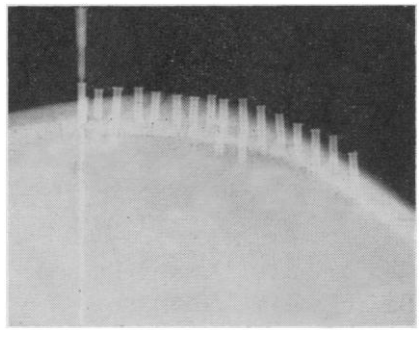

Fig. 2. X-ray photograph of monkey's skull (No. 230857, Horatio) containing 20 sleeves and one electrode; 16 sleeves are in the midplane and two are on each side, 10 mm lateral to the midplane. The latter four sleeves are displaced downward because of skull curvature, not because of deeper penetration. Careful inspection of stereo x-ray pairs shows that none of the sleeves penetrates more than a small fraction of a millimeter beneath the inside surface of the skull. Some angulation of those sleeves which were started on sloping parts of the skull can be seen; this "angulation error" has been reduced by modifications in the size of the sleeve and in the fit of the director on the sleeve (see text).

signs of tearing of an artery or signs of increased intracranial pressure from any cause. Infections are avoided by the liberal use of 70-percent alcohol on skin and on all of the aforementioned parts. Recently reductions in the hammering force required and in "angulation error" (Fig. 2) have been effected by reducing the diameter of the sleeve guide from No. 20 hypodermic needle tubing to No. 22 and by improving the fit of the director's channel on the sleeve's outside surface. The outside diameter of the roving electrodes and cannulae is reduced by use of No. 27 tubing in place of No. 24, decreasing their stiffness and, possibly, increasing the danger of arterial puncture.

Recently, sleeves made of No. 15 hypodermic needle tubing were manually hammered into the skulls of two restrained porpoises under only local anesthesia; electrodes in No. 18 needles were passed into the brain and used to find intracerebral motivational systems in experiments lasting up to 7 days.

JOHN C. LILLY

Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland

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12 December 1957

Basis of a Genetic Change Which Invariably Occurs in Certain Maize Heterozygotes

Contrary to the well-established Mendelian principle that alternative genetic elements in heterozygotes do not merge into, or otherwise regularly transmute, each other, the entire class of R^rrr seeds resulting from rr ♀ × R^rR^{st} ♂ matings are weakly pigmented, whereas standard R^rrr kernels within the same inbred strain are darkly mottled (*1*). The atypical phenotype recurs in orderly fashion in subsequent testcross generations, and thus is heritable. Corresponding change in the stippled character, if any, is slight, and hence is difficult to establish (*2*). Subsequent to these observations it was found that a similar, but not the same, change in pigment-producing potential occurs in R^r derived from R^rR^{mb} (marbled) heterozygotes also (*3*).

Theoretically, the basis of the alteration in R^r phenotype could be either cytoplasmic or chromosomal. It is the purpose of this report to present data which exclude the cytoplasm as the site of the hereditary change in question.

One might postulate that stippled (R^{st}) maize plants carry a pollen-transmissible plasmid or cytoplasmic genetic element (E) which is capable of shifting the R^rrr phenotype from the standard to the altered form. Thus R^rrr nuclei would give darkly mottled aleurone in standard cytoplasm but weakly colored aleurone in E cytoplasm.

It had previously been shown that the effect of stippled on the R^rrr aleurone phenotype, whatever its basis, did not appear at once after $R^{st}R^{st}$ ♀ × standard R^rR^r ♂ matings (*2*). That is to say, no change in R^r expression occurs in stippled cytoplasm immediately after fertilization. The possibility remained, however, that the postulated cytoplasmic element (E) becomes effective, in terms of altering the R^r phenotype, only after R^r and E have been present together during development of the sporophyte. An adequate test of the plasmid hypothesis required that allowance be made for this contingency.

Standard R^rR^r individuals were pollinated by $R^{st}r$ ♂♂. The R^rR^{st} and R^rr offspring were identified retroactively by the kernel phenotypes resulting from self-pollination. Each such R^rR^{st} and R^rr plant also was testcrossed on standard rr ♀♀. A control set of testcrosses was made by using, as the staminate parents on rr ♀ ♀, R^rr plants from standard rr ♀ × standard R^rR^r ♂ (that is, stippled was not in the ancestry). The R^rrr kernels resulting from the three kinds of testcrosses may be designated as follows, giving effect to the assumed plasmid (E). (i) $A = R^rrr$ (E) from rr ♀ × R^rR^{st} (E) ♂. (ii) $B = R^rrr$ (E) from rr ♀ × R^rr (E) ♂. (iii) $C = R^rrr$ from rr ♀ × R^rr (control) ♂.

The B and C ears were coded, and a random sample of 100 R^rrr kernels from each was scored for aleurone pigmentation. The scoring was done at 13 × magnification and involved determination of the proportion of seeds in each ear sample in which pigmentation in a predetermined area exceeded that of a particular kernel of intermediate grade selected as a reference specimen.

Expectation on the plasmid hypothesis is that the control kernels (C) will show the dark mottling characteristic for standard R^r in single dose, and that both the A and B kernels will be weakly pigmented. This is based on the proposition that, if stippled plants carry a pollen-borne plasmid capable of changing the R^r phenotype from the standard to the altered form, this cytoplasmic element will be transmitted with the sperm to the eggs, and thus to the ensuing sporophyte, by the r as well as the R^{st} pollen grains formed by $R^{st}r$ plants. The significant question, therefore, is whether the B and A testcross kernels conform in phenotype.

The experimental results showed that (i) the A and B kernels did not conform to each other in phenotype but, on the contrary, were widely unlike and (ii) the B kernels did not differ significantly in aleurone pigmentation from the controls (C). Thus, there is no evidence for a plasmid (E) accompanying the r gene in the r class of pollen formed by $R^{st}r$ plants.

All the R^rrr seeds on the nine A testcross ears were much more weakly colored than the reference kernel, a result in accord with earlier observations. Scoring of the 100-kernel samples from the 10 ears in the B group gave the following percentages of seeds darker than the specimen kernel: 76, 29, 74, 48, 65, 38, 58, 57, 46, and 50. The average is 54.1. The corresponding values for the ten control ears (C) were: 43, 48, 76, 31, 31, 43, 30, 47, 59, and 42. The average in this case is 45.0. Thus, the B kernels were somewhat more darkly pigmented, on the average, than the controls. The difference between the means, 9.1 ± 10.21 kernels, however, lies well within sampling limits.

The data, therefore, negate the hypothesis that the change in R^r pigment-producing potential arising in R^rR^{st} plants is attributable to a plasmid. The breeding facts, on the other hand, lend positive support to the conclusion not only that the phenomenon is chromosomal but also that it is the R^r region which is involved. Assortment of the capacity to promote heritable change in R^r action with R^{st}, but not r, gametes formed by $R^{st}r$ plants shows that the stippled allele, or a neighboring factor, induces the transallelic effect. Similarly, the regularity with which the change induced in the homologous chromosome subsequently follows R^r in inheritance demonstrates that it is the R^r allele, or a closely associated element, which is genetically altered in R^rR^{st} heterozygotes (*4*).

R. ALEXANDER BRINK

Department of Genetics, College of Agriculture, University of Wisconsin, Madison

References and Notes

1. The gene symbols used are as follows: R^r = self-colored aleurone, except in R^rrr endosperms, which are darkly mottled; R^{st} = stippled aleurone; R^{mb} = marbled aleurone; r = colorless aleurone.
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