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An exploring chemitrode device for direct chemical stimulation of the brain

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MORGANE, P. J., J. D. BRONZINO, AND W. C. STERN. *An exploring chemitrode device for direct chemical stimulation of the brain.* J. Appl. Physiol. 32(1): 138–142. 1971.—This paper presents a detailed plan for constructing an exploring chemitrode device that can be lowered through the brain in 1-mm stages in the behaving unanesthetized cat. Various advantages of this type of chemitrode system over the older "fixed" models are emphasized. By use of such a device we are able to explore as many as 10 points in the brain of a single animal, including stimulating multiple loci in the brain bilaterally or longitudinally along a neural fiber system. This type of system for chemostimulation thus allows more systematic mapping or charting of neurobehavioral systems in the brain. Exploring the brain point by point means that stratified behavioral circuits can be "dissected" out of a conglomerate heterogeneous bundle containing several functionally different populations of neurons all bound together and not spatially laminated. Chemostimulation begins above a critical region, extends into the region, and then explores below the region. Additionally, this exploring cannula can be used to "back-up" so that the same loci can be restimulated at a later date. The chemitrode described in this paper thus represents a versatile device for chemically mapping neurobehavioral circuits in the brain.

brain stimulation; exploring cannula system; chemical mapping of the brain; chemospecificity of neural systems

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CHEMICAL STIMULATION of the brain has been accomplished in the past by injection of chemical agents into the direct cranial circulation (especially the carotid arterial system), by intraventricular or intracisternal injection, and by placing of chemical agents in liquid or solid form directly into the brain substance. Especially in the last decade, interest in studying the effects of various chemical agents on specific structures of the brain has encouraged the development of special cannula devices often referred to as chemodes or chemitrodes. This latter combines electrodes with the system so that electrical or chemical stimulation can be carried out at the tips. Use of these systems enables introduction of chemicals precisely and reproducibly into predetermined target areas deep within the brain.

The advantages of chemical stimulation as compared to electrical stimulation of the brain have been discussed in several previous papers (2, 6, 9, 13, 15, 18) and will be only briefly summarized here. Chemical stimulation produces orthodromic stimulation, whereas both orthodromic and antidromic stimulation is produced by electrical stimulation of neurons. Thus, it is thought that chemical agents act on synaptic membranes and not on the nerve fibers themselves, whether myelinated or nonmyelinated (16). There is recent evidence indicating that chemical and electrical stimulation at the same locus excites different neuronal populations (2), thus accounting for the different effects produced by these two methods of stimulation. Based on their "chemical code" (17) it has been possible to chart or map neural systems by directly stimulating along their course and observing behavioral events and concomitant electrographic activity. Another important advantage of the chemical stimulation method is that the induced behavioral changes and associated electrographic indicators can be followed over an extended time course not possible with the more stimulus-bound effects of electrical stimulation.

The relative advantages of crystalline versus liquid chemical stimulation have been debated recently (6, 9, 18) and need not be gone into here in any detail. It is sufficient to note that the slowly dissolving crystal induces a more localized stimulation and several studies indicate that powdered chemical agents inserted into the brain spread little more than a radius of 1 mm in a period of up to 40 min (7, 13, 19, 20). On the other hand, it is more difficult to quantitate the amount of chemical agent delivered to the brain when using agents in crystalline form. As to critical nature of dose, however, Delgado (4) observed that, beyond a certain level, a 10-fold increase in dose does not increase certain evoked effects. We have repeatedly found that it is possible to deliver without particular difficulty 1 μg of agent representing a single crystal of chemical agent, i.e., carbachol, into the brain.

With regard to cannula devices in general, most workers stimulating the brain with chemicals have used fixed cannula systems so that only one locus could be stimulated in a single animal. With this type of device it is certainly not surprising that failure to replicate results using movable devices has occurred (11, 14). With fixed types of systems it is extremely easy to miss a critical neurobehavioral system which might lie even a single millimeter above or below such a locus. We feel that a fixed device builds in distinct disadvantages in trying to chemically "dissect" neurobehavioral systems within neural circuits having meandering trajectories and containing stratified neuron populations. Hence, the chemitrode system described below represents a considerable advance over other devices that have been previously developed (3, 7, 10, 12, 19, 23) in that, not one, but several specific sites in the same brain can be stimulated by lowering the cannula system through the brain millimeter by millimeter in the behaving unrestrained animal connected by means of a slip-ring system for chronic EEG recording. For long-range charting of the course of behavioral systems in the brain, use of these devices has proven to be a great time-saving development as they decrease the total number of animals used and allow for collection of more data from the same animal.

The chemitrode described in this paper represents a modification and further development of the device originally designed in the laboratory of Hernández-Péon and used by the present senior author in that laboratory in the early 1960's. In a previous paper (11) we showed only a simple sketch of a similar single chemitrode model but without description as to its design, con-

struction, and special capabilities. Hence, we now describe our present model in sufficient detail so that they may be constructed in other laboratories.

MATERIALS AND METHODS

The purpose of this report is to describe the construction, implantation, uses, and advantages of exploring cannula systems. With a machine shop available, these devices can be built from the various directions and figures presented herein. All measurements given in Figs. 1–4 are mixed between the metric and English systems since stereotaxic measurements are standardly presented

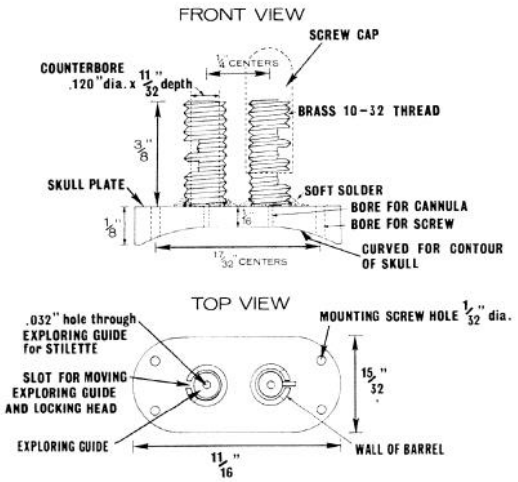

FIG. 1. Front and top views of a double-barrel chemitrode device showing the two barrels mounted atop skull plate (*front view*) and details as to fittings of exploring guide and stylet inside the barrels (*top view*).

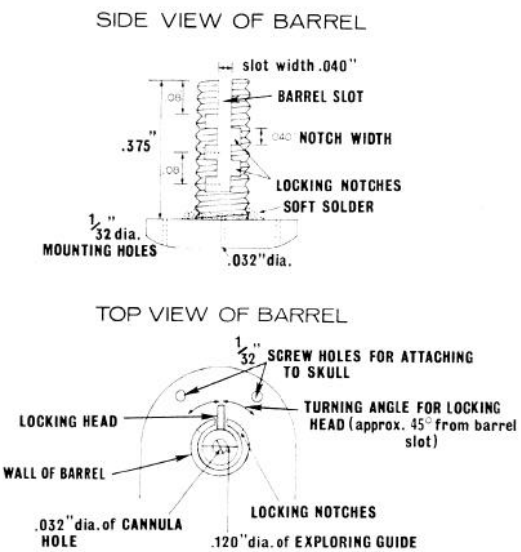

FIG. 2. Side and top views of chemitrode barrel showing details and measurements. Note locking notches into which locking head fits. When screw cap is tightened down against locking head (with locking head in locking notch) movement of exploring guide and cannula is prevented. When locking head is turned about 45° out of one locking notch it is then moved down with exploring guide and cannula and then turned about 45° to opposite side into the next locking notch. Thus, cannula tip is lowered exactly 1 mm and locked into place for stimulation.

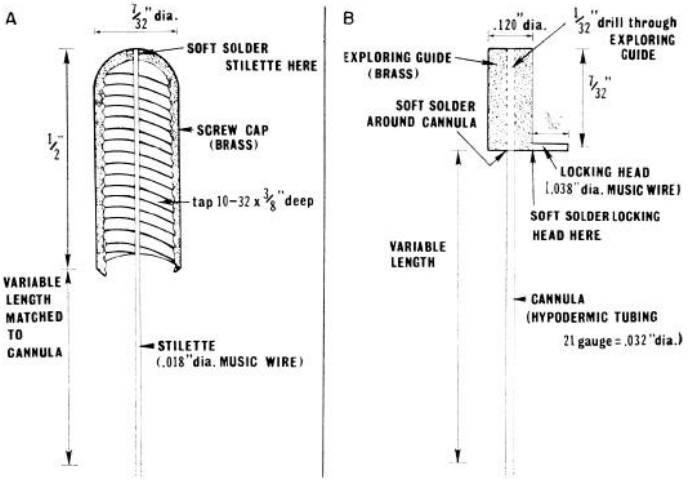

FIG. 3. *A:* tamping stylet measurements. Stylet is soft-soldered into screw cap. *B:* details of exploring guide and locking head. Note that cannula is pushed through brass exploring guide and soft-soldered therein. Locking head, which forms a critical part of functioning system, is soft-soldered to base of the exploring guide.

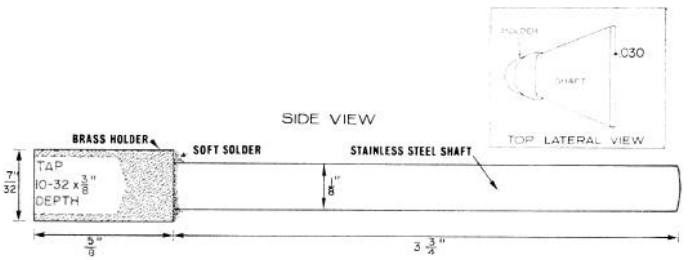

FIG. 4. Stereotaxic driving device for moving chemitrode on stereotaxic instrument. Brass holder screws onto one of threaded barrels of chemitrode device. Once chemitrode has been screwed and cemented into place driving device is unscrewed and removed.

in millimeters, whereas machinists' tools are standardized in the English system. Fractions are given in terms of 1/8 of an inch where applicable since machinists' tools are calibrated in such fractions, i.e., 8's, 16's, 32's, 64's, etc. Otherwise, decimal calculations are given. The drawings represent our artist's quantitative sketches drawn from drafting plans designed by our machine shop.

The animals are prepared for stereotaxic surgery in a routine manner used in our laboratory. They are anesthetized with Nembutal, inserted into the stereotaxic machine, and their heads shaved. The area is cleaned with Merthiolate and pHisoHex as routine operative procedure. The chemitrode device itself is dipped in 70% alcohol before insertion and animals are given antibiotics for 3 days following surgery.

The basic components of our exploring chemitrode unit are illustrated in Figures 1–4. The devices may be designed as either single or double chemitrode systems depending on the particular investigator's needs. Brass stock is used for the skull plate (Fig. 1), chemitrode barrels (Figs. 1 and 2), screw cap (Fig. 3*A*), and exploring guide (Fig. 3*B*). Figure 1 shows the dimensions of the double-barrel chemitrode, the outer portion of both barrels being threaded the entire length of the barrel (3/8 inch) using a 10-32 die. The interior hole of 0.032-inch diameter is drilled through each exploring chemitrode guide (Fig. 1, top view; Fig. 3*B*). The wall of each barrel is notched on alternate sides as illustrated in Fig. 2 to provide five 1-mm increments for movement of the exploring guide and cannula. Alternate notching of the wall of the

barrel lends support for the locking head at each stage. This enables the cannula, which is inserted through the barrel, to be locked into any one of the five slots or notches. This is accomplished by turning the locking head and exploring guide (Fig. 3*B*) about 45°, moving it down to the next locking notch (Fig. 2), and turning the locking head 45° into the notch.

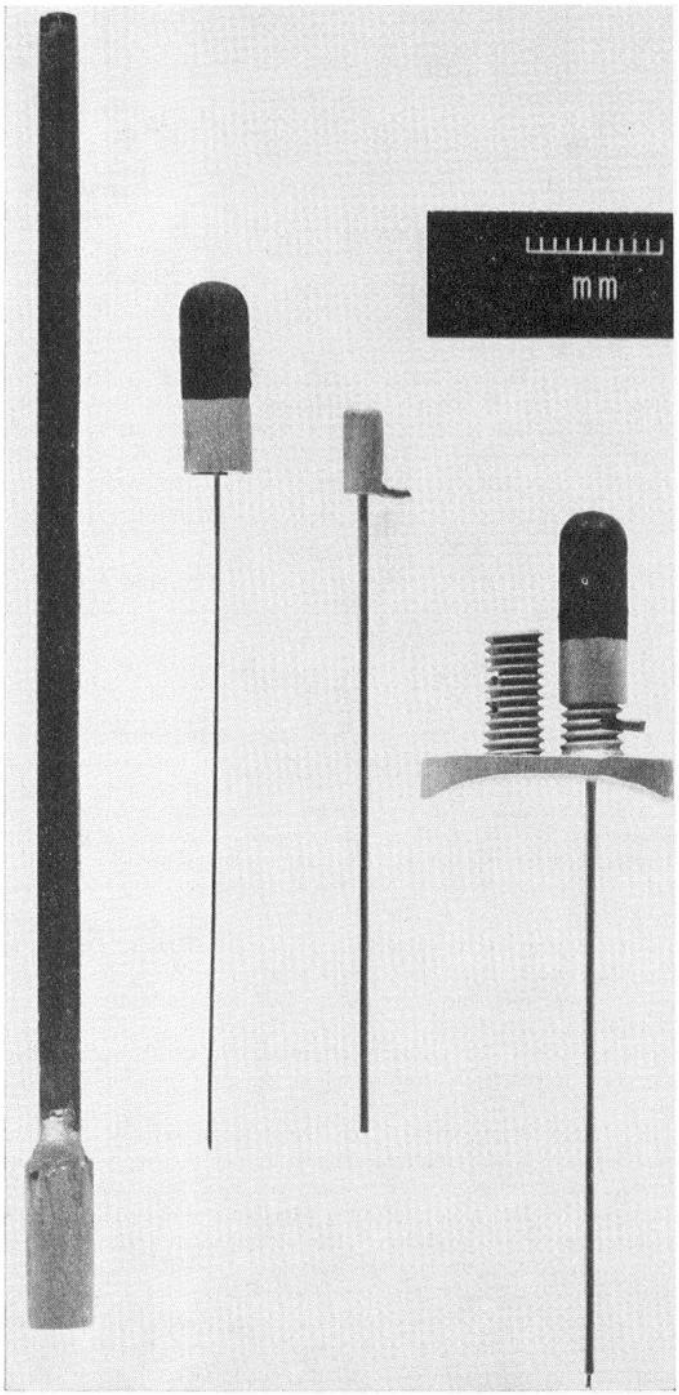

FIG. 5. Photograph of stereotaxic driving device, tamping stylet, exploring guide with locking head and cannula, and skull plate showing one threaded barrel and two notches therein and a second barrel onto which screw cap has been placed. This screw cap will be tightened down against locking head shown immediately below it. In this photograph, we have allowed tip of stylet to protrude approximately 1 mm beyond tip of cannula simply for purposes of illustration. In our experiments we cut stylet exactly flush with tip of cannula so that as cannula moves down, millimeter by millimeter, stylet in each new position remains flush with tip of cannula since screw cap moves down against locking head.

The chemitrode barrels are soldered to the skull plate (Figs. 1 and 2) which is contoured for the curvature of the skull. We have found it best to also secure the skull plate onto the skull with four screws (Fig. 1, mounting screw holes). This, of course, firmly anchors the device which is additionally stabilized by the dental cement built up around the skull plate and bases of the barrels. The tamping stylet or plunger is illustrated in Fig. 3*A* and is made of music wire which is soft-soldered into the head of the screw cap. The interior of the screw cap is threaded $\frac{3}{8}$ inch deep, using a 10-32 tap. The stylet is of variable length depending on the length of the cannula which, in turn, is cut depending upon the structures to be reached in the depths of the brain. The stylet is cut flush with the cannula tip and moves down with the cannula when the screw cap is tightened down against the locking head at each stage. Thus, the stylet always is flush with the cannula tip to ensure release of the crystal into the brain. The exploring guide and cannula system (Fig. 3*B*) is made of several components including a 21-gauge hypodermic needle (cannula) which is inserted through and soldered to the exploring guide. The stainless steel locking head is soldered to the base of the exploring guide (Fig. 3*B*).

All of the fittings on the chemitrode device should be "worked" in advance of implantation to make certain that they are functional and move with a reasonable degree of freedom. If they are too tight, there is a great difficulty in moving the exploring guide up and down once it is implanted in the animal. A stereotaxic driving device (Fig. 4) is used during implantation and has been designed to fit the stereotaxic carrier holder and screws onto one barrel of the chemitrode device. Figure 5 is a photograph of a double chemitrode assembly and driving device.

It is essential that some general points be kept in mind with respect to construction of the chemitrode system. The locking notches in the barrel (Figs. 1, 2, and 5) should be spaced 1 mm apart so as to guarantee a movement of the cannula only a single millimeter at each stage. Attempts to build devices moving less than 1 mm would reduce the width of the protruding barrel wall which supports the locking head under each notch. This would then be more likely to break off when the locking head is tightened against it as the screw cap is tightened down. In the double chemitrode device the spacing between the two holes through which the cannula barrels pass through the skull plate (Fig. 1, $\frac{1}{4}$-inch centers) below the barrels is limited by the width of the screw caps that must screw down on each barrel when the stylet is introduced (Fig. 1). We are able to vary this distance slightly but a minimal distance between the cannulas is usually about 4 mm. The only way to decrease this interval is to make a device with smaller diameter barrels (thus, having smaller screw caps) but this can also be a disadvantage since these barrels may bend easier if the cat rubs against the cage or other objects.

The locking head forms a key part of the exploring assembly and it is to be emphasized that this must be welded onto the base of the exploring guide with some precision and strength. If the locking head breaks off the exploring guide, the device is then not usable since it cannot "explore" and lock into place at each stimulation point. In this same regard, the stylet cap should not be screwed down too tightly against the locking head or it may break off this latter. The locking notches must be machined deeply enough in the barrel wall so that the locking head will not easily move out of place when the screw cap is turned to remove the stylet prior to exploration. The locking heads themselves should be so constructed as to fit quite snugly into the locking notches for an additional safeguard against the locking head being turned out of the notch when the screw cap is removed. The exploring guide into which the cannula is attached must also not be too loose within the barrel or else it is possible that a "rocking" of the

exploring assembly may occur resulting in pendular movement of the cannula tip in the brain.

As a final point, it might be of interest to note that it is possible to expand the range of exploration in these chemitrode devices by building the barrel housing up higher with more locking notches. However, in cat preparations, we have found the size discussed in this paper (5-mm exploring capacity) to be satisfactory so that not too long a structure projects above the cement cap. In our experience, devices, such as described in this paper, when implanted may remain perfectly well in place for many months or even years. Histological analysis of cannula tracks weeks or months after implantation show the usual glial sleeve along the tracks but only in rare instances have hemorrhages or abscesses occurred. In fact, the histological picture is similar to that with long-term implanted wire electrodes, i.e., minimal long-term neural reaction along the path of the cannula barrels. In the usual situation when systematic mapping of neurobehavioral systems is being carried out, it is likely that each point in the brain may be stimulated on the downward trajectory of the cannula system and a second time on an upward trajectory. If chemical stimulation is carried out once a day or once every other day, this means that basically one chemitrode system can be completely worked through in a period of 10–20 days. Using devices of this type, we have thus been able to systematically map out neurobehavioral systems in chronically recorded freely moving animals and, in addition, secure many "control" behavioral points above and below specific neuronal trajectories. These assemblies, therefore, have many advantages in mapping out multiple points in the brain of a single animal and in elucidating the functional elements within long polysynaptic fiber systems such as the medial forebrain bundle. In implantation of the exploring device, the first stimulation point should be established above the area suspected of a given function and then "explored" down through the system under study and into loci below the critical functional area. To confirm the stratification of systems, the cannula device can then be "backed-up" again millimeter by millimeter so that the same loci may be restimulated as the cannula is raised.

DISCUSSION

It is generally assumed that chemical stimulation allows a functional "dissection" within a given heterogeneous neural system since the same brain locus may be stimulated with different presumed neurotransmitter or neuromodulator agents to produce, at the same site, different behavioral repertoires (11). Therefore, a given neural pathway or system which might contain cholinergic, serotonergic, noradrenergic, dopaminergic pathways could have these specific elements activated by application of microamounts of these respective chemicals into the conglomerate system, thus "dissecting out" each single functional system within the pathway. Of course, the behavioral and electrographic effects may well be due to stimulation of locally sensitive cholinergic, adrenergic, etc. neuron pools (5) but the exact mechanism of action in this type of stimulation is not known. It is possible, for example, that depolarizing blockade of a neural system may be produced by high concentration of drugs injected into a neuronal pool. Thus, instead of activating a system we might well be producing a type of "chemical ablation."

With the present double-barreled exploring cannula system, depending on how it is oriented and precalibrated, it is also possible to stimulate the same longitudinally oriented fiber bundle at different anteroposterior sites along its path. This orientation becomes extremely valuable, for example, when one wishes to produce chemical blockade of neurobehavioral and electrographic effects evoked by chemical stimulation through the other cannula (22). Use of chemitrode devices oriented anteroposteriorly also helps to determine the polarity of a neurobehavioral system since one can determine whether a behavioral effect produced by chemical stimulation can be selectively blocked either "upstream" or "downstream" from the other cannula by placing a blocking agent or local anesthetic in the second cannula. Using a double chemitrode, we can stimulate 10 loci in a single animal and can also arrange the system across the brain to stimulate the two sides of the brain. Thus, the device described in this paper has a great degree of flexibility and can be used in a wide variety of ways to explore the brain chemically.

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