Micromethods in Neuropharmacology: an Approach to the Study of Anesthetics

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SEVERAL micromethods are now available for the administration of chemical substances in the immediate extracellular environment of a given nerve cell. Thus far, these methods have been principally used to assess the pharmacological sensitivities of different neuron types to substances suspected of being neurohumoral transmitters and to study the actions of synergists and antagonists. In this paper we first describe these micromethods, particularly the iontophoretic technique as used in our laboratory (microelectrophoresis); we then review selected studies on the effects of anesthetics; finally, we suggest an approach—recently developed in our laboratory—for a more precise assessment of drug actions on central neurons, including the actions of anesthetics.

Micromethods

Common to all micromethods are provisions for unit recording and for drug administration through a micropipette. The drug can be administered by pressure, by diffusion (micro-tap technique) or by electrophoresis.

ADMINISTRATION BY PRESSURE. This method has been found useful with *in vitro* cell systems [23, 25] but has not proven practical for the pharmacological study of central neurons.[29] One problem with this method is the difficulty of precisely controlling the rate of flow. Also bothersome is the slowness of the onset and of the cessation of drug administration. Thirdly, volume effects resulting from the injection of the solution near a neuron may mask or distort the drug effect. Lastly, if the pipette tip is of sufficient diameter to allow the ejection of the solution by pressure, uncontrollable leakage between tests is likely to occur.

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MICRO-TAP. Leakage due to diffusion or bulk flow is made use of in an ingenious device called a "micro-tap." [9] It consists of two concentric micropipettes of which the outer contains a drug solution, while the inner serves as a recording microelectrode (fig. 1*E*). By means of a microdrive, the tip of the inner electrode may be moved into the tip of the outer, the two being so shaped that this forward movement results in the occlusion of the outer drug-containing micropipette. When the appropriate neuron has been found, a backward movement of the inner micropipette, relative to the outer, permits the escape of the drug from the tip, without disturbing control recording conditions. This technique has the advantage of permitting the administration of any water-soluble substance, irrespective of whether the substance ionizes in solution. On the other hand, it is limited in that it permits the testing of only one substance and because the rate of administration in any given situation can not be altered, being exclusively dependent upon the physical characteristics of that particular electrode tip. Additionally, the possibility of unwanted leakage cannot be fully ruled out despite the relatively large impedance (100 mohms or so) of the drug barrel in the tip-occluded position. Spontaneous efflux of acetylcholine (ACh) from 100 mohms micropipettes has been found to be of the order of 10^{-15} moles/sec.[32] and sufficient to excite very sensitive neurons, such as Renshaw cells.[13, 52] Nevertheless, the usefulness of the micro-tap technique, particularly with regard to testing the effects of tissue extracts, should not be overlooked.

MICROELECTROPHORESIS. Microelectrophoresis is the most practical micromethod of administering drugs that ionize well in aqueous solutions.

Principles of Microelectrophoresis. If a potential of positive polarity, relative to tissue

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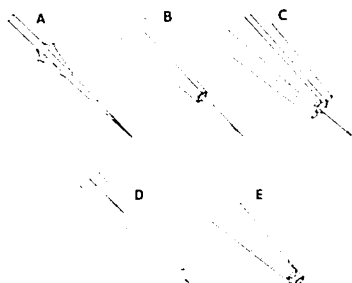

FIG. 1. Microelectrodes used for administering substances to neurons: *A*, 5-barrel electrode; *B*, concentric electrode; *C*, multibarrel concentric electrode; *D*, 2-barrel electrode; *E*, micro-tap.

fluid, is applied to the drug solution in a micropipette, cations will be ejected from the tip while anions will be ejected if the potential is made negative. If the drug is fully ionized, the ionic movement will be proportional to the current, and the rate of ejection for a univalent ion would theoretically be $n \times 10^{-14}$ gram equivalents/nanoamp./sec., where n is the transport number of the ion. For individual micropipettes, a simple proportionality between current flow and rate of release holds true in most cases, but an appreciable variation in rates of release between different micropipettes has been observed.[31, 32] For this reason, it is preferable to specify the current used to eject a given active ion rather than the theoretical concentration.

Unless the tip of the micropipette were infinitesimally small, some spontaneous outflow of drug ions could occur between tests and possibly contaminate the results. Such outflux can be minimized through the use of a "retaining" current,[18] that is via the application of a potential opposite in polarity to that required to eject active ions from the microelectrode tip. Obviously, since this results in the ejection of ions of opposite charge between tests, great care must be exercised to exclude the possibility that these ions also may affect the activity of the neuron under study.

The magnitude of the retaining current to be used relates, among other factors, to the internal diameter of the tip of the micropipette, which largely determines the electrode resistance. Hence, the larger the tip diameter, the lower the resistance, the greater the spontaneous outflux and the larger the retaining current required to counteract the diffusion of active ions. Since there are practical limits to the magnitude of the retaining current that can be used and to its efficacy, pipettes with tip diameter larger than 2 μ should be avoided.[32]

The theoretical concentration of the active ion at a given distance from the electrode tip—considering the latter as a "continuous point source"—can be calculated from the equation [8, 26]:

$$C = \frac{m}{4\pi Dr} \operatorname{erfc} \frac{r}{2(Dt)^{\frac{1}{2}}}$$

where C is the concentration at distance r, m is the rate of ejection, t is the time and D is the diffusion coefficient, assuming a uniform medium. A series of curves can then be plotted of the theoretical concentrations at various distances, knowing the rate of ejection and the period of administration.[14, 26] However, when drugs are administered within the central nervous system (CNS), the precise distance from the electrode tip to the recorded neuron and the diffusion coefficient can be only roughly estimated. Furthermore, the extracellular space cannot be considered as a uniform medium since physical barriers to diffusion, enzymatic degradation or binding may occur between ejection and receptive sites. Hence, although a range of concentrations can be estimated, the exact concentration required to activate neuronal receptors can not be accurately determined.

The Technique of Microelectrophoresis. This technique was first developed for the pharmacological study of the neuromuscular junction *in vitro*.[18, 36] In this case, a recording microelectrode can be inserted into the muscle fiber at the junctional region and one or more drug-containing micropipettes can be independently positioned near the end-plate under microscopic control. Similar techniques apply for studying the "micropharmacology" [19] of other isolated cell systems, such as the sympathetic ganglia [4] and the abdominal ganglion of the marine mollusk *Aplysia*.[50] This approach, however, is not feasible in the CNS, given the absence of microscopic control for

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the independent positioning of recording and drug-containing micropipettes. In this case, all micropipettes must be so arranged prior to their insertion in the CNS that their tips are located in close proximity with one another to assure that the drugs are actually applied at, or very near, the site of recording. Several configurations are possible (fig. 1) the most practical being the 5-barreled microelectrode described below.[12]

A 5-barreled microelectrode (fig. 1A) is constructed by fusing 5 pieces of 3 mm. diameter Pyrex tubing so that 4 of the pieces are radially arranged around the fifth, which is longer than the others. The fused tubings are then heated, drawn by hand to a blunt tip 3–4 mm. in diameter and subsequently redrawn using a vertical micropipette puller.[21] The resulting ultrafine tip ($< 0.5\ \mu$) is bumped with a glass rod under microscopic control to an overall diameter of 2–6 μ, making sure that all five barrels end in the same plane. The electrode is then boiled in distilled water under reduced pressure until all barrels are filled, and can be stored in this condition for a few days. Prior to use, the distilled water is removed from the shanks of the 5 barrels by aspiration through a fine plastic tubing and substituted with appropriate salt and drug solutions. The central barrel, used for recording, is filled with 4.8*M* NaCl; one outer barrel with 3*M* NaCl and the other three with freshly prepared aqueous solutions of drugs, usually at concentrations of about 1*M* (or near saturation if their solubility is poor) in order to achieve satisfactory conductance. When required, the *p*H of the solution is adjusted for optimal ionization but kept above 3.5 to minimize the possibility of hydrogen ion effects. The electrode is then centrifuged at 1,500 *g* for 30 minutes to increase the rate of drug movement into the tip. Following microscopic examination to determine the absence of air bubbles and of breakage, the d-c resistance of each barrel is measured and if too high for satisfactory recording (*i.e.*, > 10 mohms for the recording and > 500 mohms for the drug barrels), first a d.-c. current is passed through the high-resistance barrel, and if this proves unsuccessful, the tip is enlarged. However, electrodes with overall tip diameter greater than 8 μ are rejected because of the increased likelihood of uncontrolled leakage and because they do not appear to record the activity of small neurons—thus increasing the bias of the sample towards the larger cells.

The electrode is firmly attached to a micromanipulator mounted rigidly on a stereotaxic frame. Silver-silver chloride wires make the electrical contacts between the central recording barrel and a cathode follower,[1] and between the outer barrels and the source of the electrophoretic current.

Since the electrode resistance may change considerably during the passage of current, it is essential that the source of the electrophoretic current be as constant as possible. The electrophoretic unit used in our laboratory (see Appendix) was developed by R. Cox

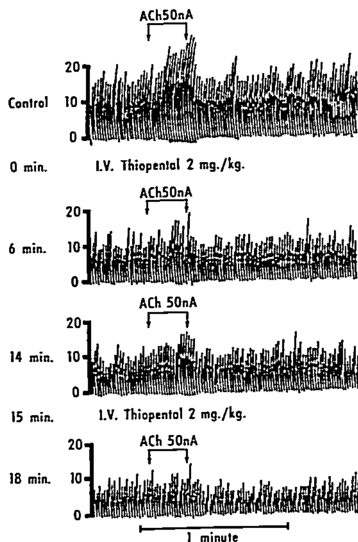

FIG. 2. Polygraph records of the effect of thiopental on the acetylcholine responses of an interneuron in the cat spinal cord. Control record, 2 minutes prior to thiopental administration. Two subsequent records, 6 minutes and 14 minutes, respectively, after the intravenous administration of thiopental (2 mg./kg.). Bottom record, 3 minutes after a second intravenous administration of thiopental (2 mg./kg.). Scale is in impulses/sec.

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and A. P. Oliver utilizing the property of photocells to yield a current which is relatively independent of the load and has an output impedance equivalent to 10,000 Mohms. Additionally, it automatically passes through the 3*M* NaCl barrel of the electrode, a neutralizing current which is opposite in polarity to the algebraic summation of the currents in the other channels. Therefore, since there is essentially no potential difference between the tip of the microelectrode as a whole and ground, possible electrotonic effects of the electrophoretic current are minimized. Moreover, the neutralization channel can also be used for the application of anodal or cathodal current when testing of the electrical excitability of the neuron is desired.

Extracellular action potentials recorded by the central barrel of the electrode are displayed on an oscilloscope and photographed when required or stored on magnetic tape. In addition, they are fed into a voltage-gaiting device to separate them from base-line noise, audio-monitored and converted into pulses of fixed amplitude and duration which are displayed on the second beam of the oscilloscope for comparison with the original signal and fed into a counter set to recycle at a preselect interval, usually once a second. The output of the counter is then displayed on one channel of an ink-writer to produce a continuous record of the neuron's discharge rate (figs. 2 and 3), while other channels of the ink-writer monitor electrophoretic currents and any other physical or biological variable of interest.

Studies on Anesthesia

Micromethods have thus far been principally used to study the responsiveness to suspected transmitters of individual neurons in many CNS regions [6, 12, 22, 27, 28, 40, 42] and have led to the recognition of ACh [13, 17] and of norepinephrine (NE) [38, 39, 41] as central synaptic transmitters. In only a few studies were attempts made to compare results under different conditions of anesthesia but before reviewing these observations and proposing a new approach, it seems profitable to briefly examine the possible neuronal sites of anesthetic action. For the purpose of this analysis, these sites will be examined separately, bearing in mind, however, that complex neuronal effects may follow the administration, and be responsible for the action, of any one anesthetic substance.

Possible Sites and Modes of Action. Most studies have been conducted on motoneurons or simpler *in vitro* systems and there is as yet no evidence that the information derived from these studies is applicable to other

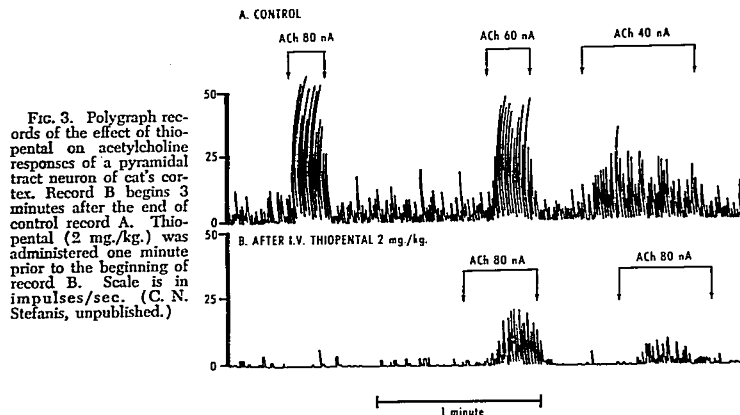

Fig. 3. Polygraph records of the effect of thiopental on acetylcholine responses of a pyramidal tract neuron of cat's cortex. Record B begins 3 minutes after the end of control record A. Thiopental (2 mg./kg.) was administered one minute prior to the beginning of record B. Scale is in impulses/sec. (C. N. Stefanis, unpublished.)

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nerve cell systems. Nevertheless, the following results have been obtained.

Blockade of Axonal Conduction. Both volatile and non-volatile anesthetics have been shown to be capable of blocking impulse conduction along nerve fibers.[7, 45, 46] Blockade of conduction, however, may reflect a common toxic effect of all anesthetics at high concentrations, unrelated—or only partially related—to their central anesthetic action at therapeutic levels. Suggestive of this possibility is the observation of Larrabee and Posternak[33] that pentobarbital, ether and chloroform reduce the postsynaptic response of cervical sympathetic ganglia at concentrations several times lower than those required to effect nerve conduction. Similarly, normal conduction of afferent fibers in a monosynaptic spinal reflex pathway was shown to occur at depths of ether or barbiturate narcosis at which the postsynaptic response was markedly reduced.[34, 48]

Alteration of Transmitter Release. A second possibility is that anesthetics may affect all, or only certain, presynaptic terminals altering transmitter release. There is no evidence to suggest that anesthetics may increase the release of an inhibitory transmitter but there is some evidence for a decreased release of excitatory transmitter/s. A number of nonvolatile central depressants have been found by Matthews and Quilliam[35] to reduce ACh release at the neuromuscular junction and in the superior cervical ganglion, while Paton and Speden[37] found volatile anesthetics to block transmitter release from nerve networks of the guinea-pig intestine. Although not directly relatable to these findings, the observation of Crossland and Merrick[10] that ACh increases in the CNS during general anesthesia is well worth remembering.

Alteration of Neuronal Excitability. This could be brought about by changes in the resting potential, in membrane properties or in the sensitivity of post-synaptic receptors.

(1) *Resting potential:* Changes in resting potential affect the probability of impulse generation. In frog skeletal muscle, however, Thesleff[51] found that several nonvolatile anesthetic agents affect membrane excitability independently of changes in the resting membrane potential. Similarly, no appreciable change in the resting membrane potential of motoneurons has been observed with barbiturates, ether and urethane.[34, 44, 47, 49]

(2) *Membrane properties:* Subtle alteration by anesthetics of some properties of the cell membrane is suggested by studies showing the excitability of the membrane to be reduced by anesthetics. In motoneurons, Sasaki and Otani[44] found barbiturate anesthesia to cause a marked increase in the threshold to slowly rising currents (accommodation) but not in the rheobase. Similarly, Shapovalov[47] observed a rise in the threshold for direct electrical stimulation of motoneurons after administration of barbiturates, ether and urethane, as well as a partial blockade of repetitive antidromic invasion. Somjen and Gill[49] also found that ether increased the depolarization required to initiate an orthodromic impulse in approximately 50 per cent of the motoneurons studied. On the other hand, Løyning *et al.*,[34] studying the effect of thiamylal in cats already anesthetized with a barbiturate, could not observe any change in threshold, accommodation or membrane resistance. A possible mechanism for the decreased excitability of motoneuron membrane by anesthetics may reside in alterations in Na conductance as suggested by Thesleff[51] for skeletal muscle and as shown by Hagiwara and Saito[24] in neurons of the marine mollusc *Onchidium*.

(3) *Sensitivity of postsynaptic receptors:* Another mechanism by which anesthetics could affect neuronal excitability would be by altering the property of post-synaptic receptors to respond to excitatory and/or inhibitory transmitters. We are not aware of any study reporting on the effects of anesthetics on inhibitory postsynaptic potentials (IPSP's) but several studies on motoneurons have shown the amplitude of excitatory postsynaptic potentials (EPSP's) to be reduced by anesthetics.[34, 47, 49] The observation that the amplitude of EPSP's may be reduced in some motoneurons in the absence of a decreased excitability of the membrane[34, 49] suggests that release of excitatory transmitters and/or sensitivity of post-synaptic receptors may be particularly sensitive to anesthetic agents. With present techniques it is not possible to directly measure transmitter release at the neuronal

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level in the CNS, but it is possible—by using microelectrophoresis—to test for the effects of anesthetics on receptor sensitivity. A few such studies have been carried out and are summarized below.

Studies by Microelectrophoresis. By and large these studies have shown anesthetics to decrease the responsiveness of cholinoceptive neurons to electrophoretically administered ACh.[5, 30, 43] Typical effects are illustrated in figures 2, 3 and 4. There is suggestive evidence, however, that different anesthetics may not affect ACh responses equally throughout the CNS. Biscoe and Krnjević,[3] for example, found responses of Renshaw cells to ACh and to ventral root stimulation to be unaffected by chloralose. On the other hand, chloralose and barbiturates were found by Bloom *et al.*[5] to decrease ACh responsiveness of caudate nucleus neurons and to grossly alter the probability of eliciting facilitatory or depressant responses to ACh. An intriguing observation in this study by Bloom *et al.* was the finding—illustrated in figure 4—that a small dose of a fast-acting barbiturate would suppress the facilitatory response to ACh of a caudate neuron but not the depressant response to ACh of a simultaneously recorded neighboring unit and that this differential effect could be duplicated by electrophoretically administered procaine. Caudate neurons responses to NE and dopamine also were reduced by anesthetics, though by larger doses than required to reduce ACh facilitatory responses.

Depression by barbiturates presumably partially account for the failure of Curtis *et al.*[17] to find, aside from responsiveness of Renshaw cells to ACh, other spinal cord neurons sensitive to electrophoretically administered ACh, NE and serotonin (5-HT). Our own studies[42, 52–54] have shown the presence of ACh, NE and 5-HT sensitive neurons in the lumbar segments of the spinal cord of cats decerebrated or lightly anesthetized with ether. Similar findings have now been reported by Engberg and Ryall[20] and by Biscoe and Curtis.[2]

In summary, studies by microelectrophoresis have shown that barbiturate anesthesia can reduce ACh excitation of neurons and probably also NE and 5-HT responses, suggesting that receptor sensitivity in the CNS may be decreased by anesthetics, as demonstrated by Thesleff[51] at the neuromuscular junction. Figures 2 and 3, however, show that the rate of spontaneous discharge of the neuron was often

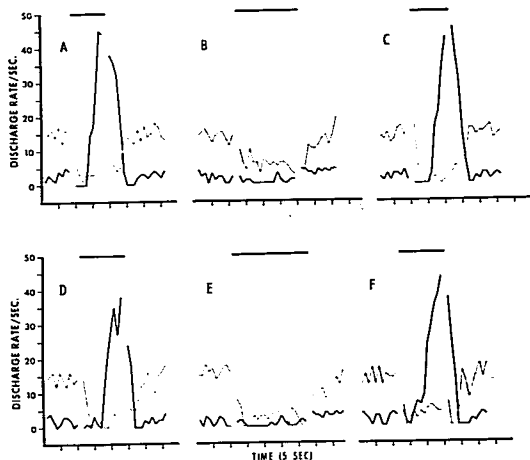

FIG. 4. Effect of hexobarbital on acetylcholine responses of neurons in the caudate nucleus of the cat. Plots from filmed records of two caudate nucleus neurons recorded simultaneously. The electrophoretic administration of acetylcholine by 20 nA of current is indicated by horizontal bars. Upper records: A, control; B, after the intravenous administration of hexobarbital (1.5 mg./kg.); C, 10 minutes later. Lower records: D, 15 minutes after C; E, during the electrophoretic administration of procaine; F, 4 minutes later. (Bloom *et al.*[5])

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decreased even by a small dose of the anesthetic. While this finding would be expected if the anesthetic depressed receptors' sensitivity to excitatory transmitters, the observations that can be made with microelectrophoresis when recording extracellular unit activity do not permit exclusion of the possibility that the reduced responsiveness may have resulted —at least in part—from decreased excitatory impingement upon these neurons or from changes in membrane properties. Intracellular recording and extracellular drug administration would be required to distinguish between level of facilitation, receptor sensitivity and membrane properties.

Theoretically, this could be achieved simply by arranging 2 micropipettes concentrically as shown in figure 1B. However, with this arrangement—extensively used by Curtis and collaborators [11, 14-17]—uncontrollable leakage of the drug from the outer, drug-containing, micropipette may grossly distort the results in that all obtainable drug effects may have occurred by the time a cell is found and control recordings are made. Additionally, since there is only a single extracellular pipette, the electrophoretic current cannot be neutralized nor can tests be made to distinguish between drug effects and electrotonic effects of current. Lastly, even if these difficulties could be surmounted, the usefulness of this instrument in parmacological studies is limited in that the effect of only one drug can be tested on a given neuron. For these reasons, the following microelectrode was developed by A. P. Oliver in our laboratory.

Multibarrel Concentric Electrode. The multibarrel concentric electrode (fig. 1C) is essentially a refinement of the 5-barreled glass micropipette. Four 3 mm. diameter Pyrex tubings are fused radially around a large glass micropipette, then partially drawn by hand and then finally redrawn with a vertical micropipette puller. This last stage is critical in that a judicious use of heat and pull is required to give the electrode a shape such that once the electrode tip is broken off to an overall diameter of 8–10 μ, most of it is taken up by the opening of the center barrel, the openings of the others still being less than 2 μ—as in conventional 5-barreled electrodes—thus allowing control of diffusion with a retaining current. Once this electrode is filled with appropriate drug and salt solutions in the outer barrels, a very fine single micropipette with tip diameter $<0.5\ \mu$, suitable for intracellular recordings, is threaded through the center barrel with a microdrive till its tip protrudes 20–60 μ and then held in this position during insertion into the CNS. Electrophoretic drug administration via 3 of the 4 outer pipettes and current neutralization via the 3M NaCl-filled fourth outer barrel is carried out as described in a previous section.

Since it provides for intracellular recording, the multibarrel concentric electrode makes it possible to study drug effects on the membrane resting potential as well as synaptic and action potentials and to determine changes in membrane excitability and conductance. Because several extracellular pipettes are available, several pharmacological agents can be applied to study receptor sensitivity or for recording and stimulating extracellularly to assess possible drug-induced changes in conduction of presynaptic fibers. Hence, this instrument has the potential of making the study of the precise mechanism of action of anesthetics on central neurons a feasible undertaking.

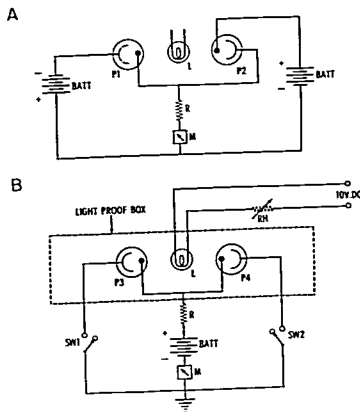

FIG. 5. *A.* Schematic showing the principle of the electrophoresis circuit. *B.* Schematic of the circuit for the matching of phototubes.

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Appendix—A Constant-Current Electrophoresis Unit

Vacuum phototubes wired as in figure 5A will pass a nearly constant current in the millimicroampere range which can be varied by changing the illumination intensity. The output current will be largely independent of inserted series resistance (R) and if the tubes are arranged in opposite polarity their currents will sum algebraically.

The circuit for activating one drug channel and achieving current null at the electrode tip is illustrated in figure 6. P1 and P2 (#922 phohotubes) are spaced six inches apart in a light-proof box (bottom of figure 7). A no. 47 pilot light (L1) is mounted between them on an adjustable bracket which allows variations in the ratio of illumination on the tubes. When SW1 is closed upward, L1 is lighted and negative current will flow through R1 and the drug channel. P2, which is opposite in polarity, will simultaneously pass a positive current through R3 into the 3M NaCl barrel of the microelectrode. The intensity of illumination, which governs the current output, is determined by the position of SW2 and the rheostats which are in series with L1 (bottom of figure 7). The output currents are made equal by proper positioning of L1, and a net current of zero at V and W indicates this position. The current steps are calibrated by monitoring the currents at V and W with a microammeter while changing the setting of RH1, 2 and 3, respectively, for each position of SW2. Dummy resistances of 22 megohms, interposed between V, W and ground (Z), are used to calibrate the circuit. P3 and P4 connected to the same electrodes cause reversal of current flow when L1 is extinguished and L2 is illuminated. Only three series rheostats (RH1, 2 and 3) are shown, but in practice one is used for each required current step.

Additional sets of phototubes are added in parallel for each microelectrode and in our application all additional outputs of the R3, R4 types terminate at the common point W (led to the 3M NaCl barrel), providing an automatic current null. B2 and B4 may be common to all additional P2 and P4 tubes, but individual B1's, B3's and R7's are necessary to monitor current for the independent electrodes. R8 which is common to all circuits may be monitored for the sum of the currents. During the experiments the R7's and R8 are monitored on a Grass polygraph equipped with DC pre-amplifiers. R1 and R2 are resistances used to match the output characteristics of the phototubes.

P5 and P6 are mounted in separate light-proof compartments (not shown in figure 7). When either L3 or L4 is activated by SW4, a positive or negative current—set with RH7—may be passed through the common electrode. This circuit unbalances the null but has no effect on the output of the independent channels.

The pairs of phototubes must have similar characteristics. They are cleaned carefully, removing the label too, and tested in the circuit of figure 5B.

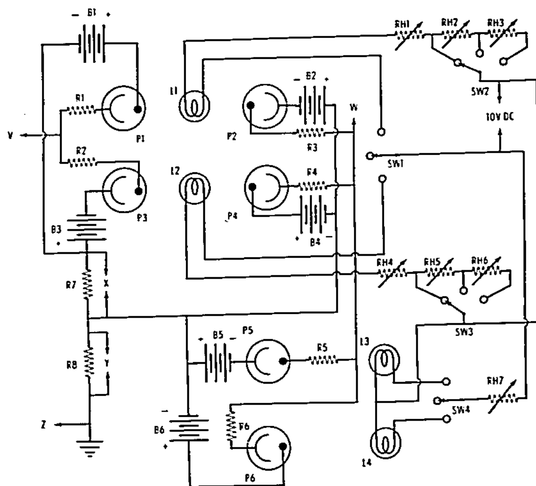

Fig. 6. Schematic of one electrophoresis channel and of the neutralizing circuit.

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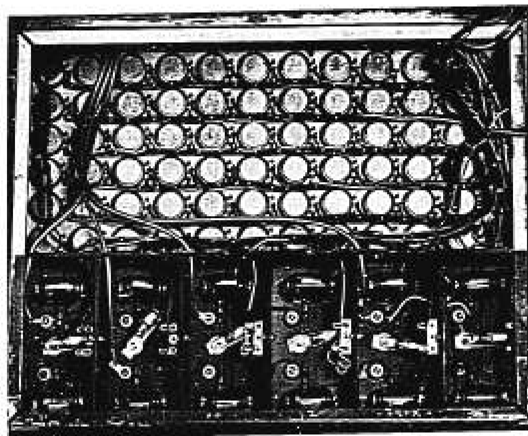

FIG. 7. Bottom view of the electrophoresis unit.

Tubes which pass more than 2 nanoamps (10^{-9} amp.) unilluminated, are rejected. The tubes are matched by selecting one with the highest output for a given illumination as a standard which is placed in P3 of figure 5B. The tubes to be tested are placed in P4 and a note made of their output as compared to P3 at varying intensities of illumination.

All output wires must be arranged to avoid capacitance between them or the chassis because the device is capable of charging relatively small capacitances. Storage batteries should not be used to power the pilot lights; we use a Con Avionics HT10-1.5. The phohotube batteries are 90 V. cells wired in series to yield 180 V.

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Discussion

Dr. Somjen: Would you comment on the difference between the results your group obtained and those of Drs. Curtis and Crawford, not yet published. I have the permission of Dr. Crawford to report the work. They tried DL-homocysteric acid and acetylcholine on cortical cells in the presence and absence of an anesthetic and found depression of the excitatory response to both. And if I recall correctly, in the caudate you found a difference, that is, acetylcholine excitation was diminished, but glutamate excitation was not. (Bloom, Costa, and Salmoiraghi: J. Pharmacol. 150: 244, 1965.) I wonder whether you consider this a difference between the two kinds of cells, or between glutamate and cysteine, or a technical difference.

Dr. Salmoiraghi: Your point is well taken; one should not lose sight of the possibility that glutamate and other excitant amino acids which we have been led to believe act unspecifically on mammalian neurons, may actually be synaptic transmitters. Dr. Krnjevic may wish to comment on this point. Whether the differences between our results in the caudate nucleus and those obtained by Crawford *et al.*, in the cortex are due to differences in the experimental conditions, in the amount or type of anesthetic administered or by differences between caudate and cortical neurons, I do not know; but certainly in decerebrate animals small doses of intravenously administered hexobarbital have a much more profound effect on facilitatory responses in caudate neurons to acetylcholine than on responses to glutamate or to norepinephrine.

Dr. Winters: You should not be too disturbed by your findings that norepinephrine and anesthetics produce the same effect on the caudate nucleus, since it has been shown by several workers. (Feldberg, W.: A Pharmacological Approach to the Brain from its Inner and Outer Surface, Evarts Graham Memorial Lectures, 1961, Edward Arnold Ltd., London.) We have also reported (Spooner, C. E. and Winters, W. D.: Experientia 21: 256, 1965) evidence for a direct action of monoamines on the chick central nervous system. That when noreipnephrine does get into the central nervous system, the effect it produces is a sleep-like, or anesthetic-like pattern.

Dr. Salmoiraghi: Yes, I'm aware of these experiments but I think that we ought to be clear on the kinds of questions we are asking. If the question is: what happens if we squirt norepinephrine into places where it is not normally present, one should not be surprised by any result. The question I ask is whether norepinephrine is indeed a central transmitter, and if so whether it produces excitation, inhibition or either excitation or inhibition, depending upon the properties of the postsynaptic membrane. What we now know is that not all neurons in the CNS are sensitive to norepinephrine. Moreover while certain cell types are invariably depressed by iontophoretically administered norepinephrine, others are invariably facilitated. The same duality in response also applies to acetylcholine. For example, if you apply norepinephrine and acetylcholine from two separate barrels of a multibarreled microelectrode to mitral cells in the olfactory bulb of the unanesthetized rabbit, you invariably find that when the cell is responsive to both substances, both depress the spontaneous activity of the neuron. Cortical pyramidal tract neurons, on the other hand, are excited by acetylcholine and depressed by norepinephrine, while Deiter's nucleus neurons in the brain stem are facilitated by both drugs. You can see, therefore, why I would hesitate to say that norepinephrine is a depressant, leaving aside the issue of whether or not it is a central transmitter. It is obvious that if it were a transmitter, the direction of its effect would have to be experimentally determined at each site.

Dr. Krnjević: It is relevant that Bradley, Dhawan and Wolstencroft (J. Physiol. 183: 658, 1966) found in the medulla, that cells sensitive to acetylcholine showed two kinds of response, some excitatory, some inhibitory. There was a pharmacological differentiation in that the acetylcholine effect on cells excited by acetylcholine, could be blocked by curare-like antagonists of acetylcholine, while the curare-like compounds had no effect on the inhibitory responses of the other type of cells. In the cortex, on the other hand, the inhibitory effects of acetylcholine were minor and difficult to detect. One other technical point that is probably worth mentioning; in addition to injecting non-ionized substances by pressure from a micropipette it is also possible to apply them by electroosmosis, a process of releasing fluid from the tip of an electrode by passing a small current through a solution having a low concentration of ions; it is a useful method for applying substances like certain anesthetics which are non-ionized. Dr. Galindo at McGill has recently shown that in this way you can apply halothane from a micropipette, effectively and repeatedly.

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