A Suitable Preparation for Pharmacological Analysis of EEG "Activation".* (24967)

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Electroencephalograms characterized by low voltage and high frequency (so-called EEG "activation") can be elicited by stimulation of brain stem reticular formation(1-5). This response often is accompanied by behavioral manifestations of wakefulness. In contrast, a persistent "deactivated" pattern consisting of high voltage, low frequency cortical electrical activity (and behavior which is characteristic of sleep) may be observed following brain stem lesions which destroy portions of mid-brain reticular formation, but which spare the leminiscal fillets; conversely interruption of the primary sensory pathways at mid-brain level without damage to the more medially placed reticular formation does not significantly modify alerting responses and EEG "activation"(4). Observations of EEG effects of diverse pharmacological agents have indicated that mid-brain reticular formation is an important site of drug action and have suggested that multineuronal, polysynaptic character of this area may play an important role in its response to drugs(6-17). Some studies have provided evidence of cholinergic mediation in the "reticular activating system"(12-14). Others demonstrated EEG "activation" following intravenous administration of adrenergic agents, and have suggested that the "reticular activating system" or a component within it is adrenergic(15-17). The anatomical location and functional relation between cholinergic and adrenergic components of the "reticular activating system" have not been clarified. The available data bearing on this problem contain discrepancies which may be due in part to species variation (some investigators using cats, other rabbits). In addition, there has been wide variation in type of preparation used to provide a "deactivated" EEG pattern as baseline for drug-induced "activation" studies. A consistent, reliable and uniform baseline is essential for analysis of unmistakable activation effects. Rothballer(17) stated the problem succinctly: "Whether or not any EEG changes could be detected after intravenous adrenaline depends primarily upon the background of activity, and to a much lesser extent on the dosage. Working against a background of "arousal," it was quite difficult to detect any effect at all." Our experience has substantiated this view, and brought to our attention certain disadvantages of some preparations commonly used. In the unanesthetized curarized cat, artificial respiration is necessary, often leading to disturbances in EEG pattern; external stimulation (noise, light, handling) must be reduced to extremely low levels, and even then a reliable "deactivated" EEG background is difficult to reproduce consistently. These difficulties can be overcome with the "cerveau-isolé"(20), but like other preparations in which the cranium is widely opened, cerebral edema generally develops. Moreover, the "cerveau-isolé" suffers from a tendency towards progressive cardiovascular deterioration, and lacks an intact brain stem. The cat with midbrain reticular coagulation(17) exhibits a good "deactivated" EEG pattern as well as reliable cardiovascular status, but here again the brain stem is not intact and coagulative lesions may eliminate important sites of drug-induced effects. Moreover, residual effects of anesthesia in acute preparations may be difficult to exclude. A "deactivated" pattern follows intra-cranial destruction of trigeminal nerves in the "encephale isolé" cat. Electrolytic transection of mid-brain at the rostro-pontine level (rostral to the trigeminal roots) produces a similar result(18-19). It is generally believed that physiological "activation" of EEG is dependent upon the play of impulses from collateral fibers feeding into the reticular formation. Our objective has been to produce a sufficient degree of somatic de-afferentation in an unanesthetized preparation with intact brain stem

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so as to elicit a consistently high degree of EEG "deactivation" without the aid of extraneous pharmacological agents. Such a preparation should also exhibit good cardiovascular status and should not require artificial respiration.

Methods. A preparation which meets the above desiderata can be accomplished by extra-cranial transection of the roots of maxillary and mandibular branches of trigeminal nerves followed by low transection of cervical spinal cord and sensory roots of cervical spinal nerves. 1. *Extra-cranial transection of trigeminal roots.* Cats anesthetized with pentobarbital sodium (35 mg/kg) are placed in the inverted position in a stereotaxic holder. The lower jaw is opened maximally to expose the roof of mouth. An incision 1.5-2.0 cm long is made 2 mm lateral to the edge of the pterygoid process of the sphenoid and the perpendicular plate of the palatine. Adequate exposure is achieved by careful separation and removal of the external and internal pterygoid muscles, and the maxillary and mandibular roots of trigeminal nerves are sectioned bilaterally at their points of emergence from the foramina ovale and rotundum. The fossae created by removal of pterygoid muscles are packed with sterile gelatin sponge and the incision sutured. In large cats (4-5 kg), cord transection can be done immediately following partial "trigeminalectomy," but in smaller cats this is done after 12 to 24 hour rest period. 2. *Low transection of cervical spinal cord and cervical sensory roots.* The surgical technic for cord transection is essentially similar to that described by Bremer(20), but with the following elaboration. The dorso-lateral surface of the atlas and the axis, and spinous processes and dorsal vertebral arches of C_3 to C_7 are exposed. The atlantal foramina and the intervertebral foramina between axis and atlas are located bilaterally and the dorsal roots of C_1 and C_2 cautiously sectioned to avoid bleeding from accompanying blood vessels. Dorsal arches and spinous processes of remaining cervical vertebrae are removed and their cut ends packed with sterile bone wax. With a dural hook, the dura at level of C_7 is then elevated and a longitudinal incision made rostrally to C_2. The dorsal roots of C_3 to C_8 are gently elevated and sectioned bilaterally after high frequency coagulation of dorsal radicular arteries that accompany these roots. The dorsal spinal artery is then coagulated just rostral to the point chosen for cord transection. The cord is then severed between last cervical and first thoracic vertebrae and the section is further explored by gentle suction to make sure that all connecting strands of cord tissue are separated. Sterile gelatin sponge is placed between sectioned ends of the cord, the dura sutured and the incision closed.

Results. This preparation recovers readily from spinal shock, and may be maintained 2 to 3 months if suitable precautions are observed and adequate nursing care provided. This care includes antibiotic administration when necessary, maintenance of reasonably uniform and physiological environmental temperature, bladder catheterization and feeding by stomach tube. Such preparations maintain spontaneous diaphragmatic respiration (24-30/min.) and blood pressures of 85-100 mm Hg. Although some degree of motor innervation to muscles of neck and forelimbs remains intact, the animal generally remains quiescent. Sensory inflow is limited to special and visceral afferents of cranial nerves, as well as somatic afferents to orbit and upper eyelid *via* the ophthalmic branch of trigeminal nerve. The eyes are able to follow an object passing through the visual field and react with pupillary changes to emotional stimuli.

All observations reported below have been repeated a number of times in several different preparations. With this preparation, it has been possible to carry out all of following procedures in the unanesthetized non-curarized cat. (a) To insert an indwelling polyethylene catheter *via* the right subclavian artery so that its tip lies at the bifurcation of innominate artery. With this technic intracarotid administration of drugs has been carried out without cannulation of these arteries. Bilateral and simultaneous delivery of drugs to both sides of brain has been accomplished and direct drug-induced effects on EEG observed without appreciable latency. (b) To place the animal in a stereo-taxic instrument without eliciting motor activity or EEG dis-

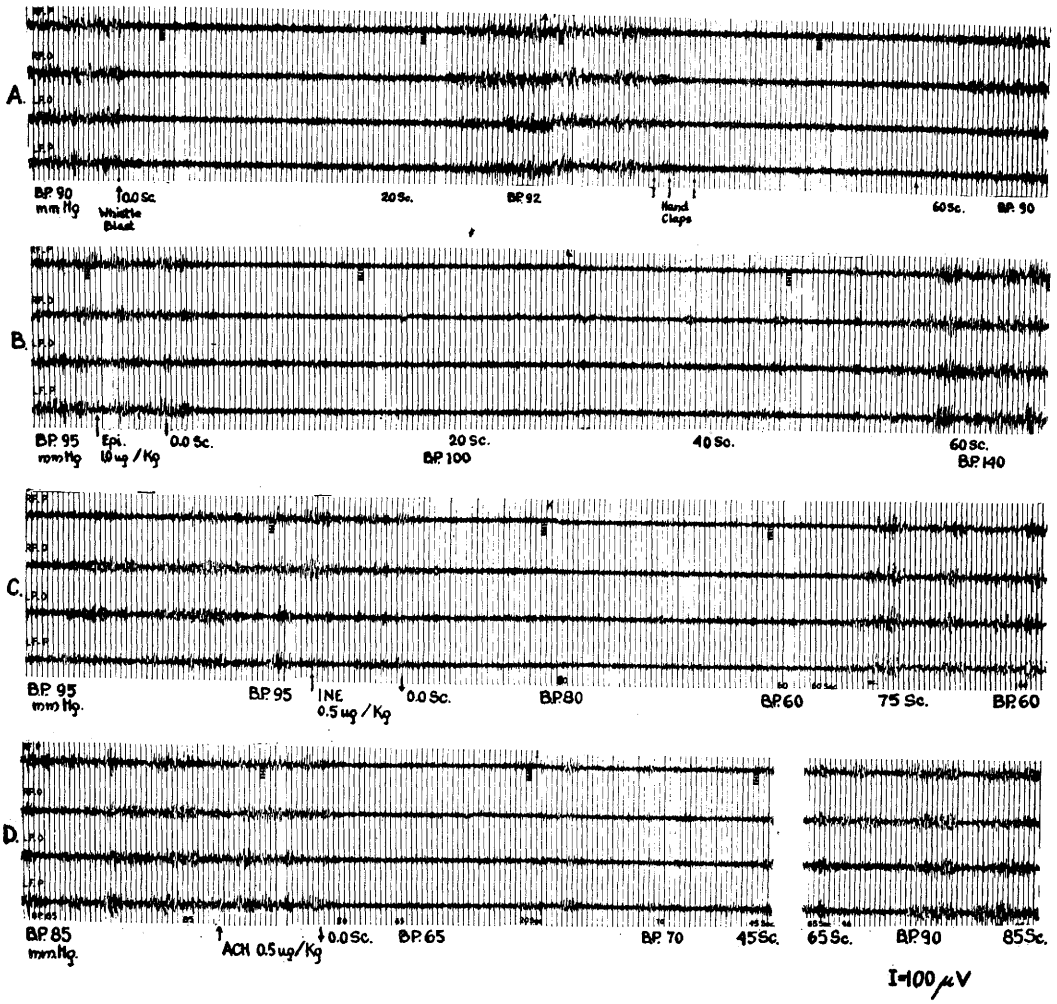

FIG. 1. "Activation" effects of auditory stimuli and of adrenergic and cholinergic agents administered by intra-innominate route in a de-afferented, unanesthetized, non-curarized cat with intact brain stem. All doses expressed as weight of free base. A: Audiogenic activation (whistle blast and hand claps). Prompt onset in both instances with abrupt reversion to the pre-activated pattern after 15-20 sec. B: Intra-innominate epinephrine (1 μg/kg). Injection bracketed by arrows. Immediate activation preceding pressor response (latency = approx. 3 sec.), and lasting approximately 57 sec. with abrupt reversion to control pattern while the pressor response is maximal (140 mm Hg). C: Intra-innominate isopropyl norepinephrine (0.5 μg/kg). Injection bracketed by arrows. Immediate activation preceding peak depressor response and lasting about 75 sec., with abrupt reversion to pre-existing pattern when the B.P. is still at 60 mm Hg. D: Intra-innominate acetylcholine (0.5 μg/kg). Injection bracketed by arrows. Immediate activation before peak depressor response (65 mm Hg), with a gradual return to pre-activated pattern.

turbances. (c) To expose the cranium and place screw electrodes through the skull with tips touching the dura for recording electrocorticograms. (d) To trephine the skull and insert tips of coagulating electrodes into the brain stem for production of electrolytic lesions. In such preparations, an indwelling arterial catheter has been maintained for several days so that the effects of a number of agents, even long-acting compounds, could be observed in the same animal. The importance of durable chronic preparations which can be studied after ample time for recovery from destructive lesions of the central nervous system has been stressed recently by Batsel *et al.* (21). Furthermore, the initially intact mid-

brain stem makes it possible for one side to act as a reliable control for drug-induced EEG effects, following mid-brain lesions of the contralateral side.

This preparation has provided a particularly useful tool for study of drug-induced "activation" of EEG, inasmuch as a highly deactivated EEG can readily be produced in absence of brain stem lesions and extraneous drug influences. In addition, cerebral edema is lacking and cortical excitability is good. After a few days of adaptation to its new situation, a Rothballer(17) type "D" pattern (persistent spindles in all leads) regularly appears when the animal is placed in a quiet, dimly lighted environment, even in the stereotaxic instrument. Low-voltage, fast frequency responses are usually observed during feeding. Marked and immediate EEG "activation" can be accomplished easily by suitable auditory (or visual) stimulation (Fig. 1A), but prompt reversion to a "deactivated" pattern follows removal of the stimulus. Moreover, this preparation manifests a consistently high degree of sensitivity to direct (intra-arterial) "activation" by both cholinergic and adrenergic agents (Fig. 1B, C, D).

Preliminary results with this preparation indicate that transitory drug-induced EEG "activation" immediate in onset, and with complete temporal independence of blood pressure, occurs at relatively low dose levels (0.25-1 μg/kg) following intra-innominate administration of epinephrine, norepinephrine, isopropyl norepinephrine and acetylcholine.

Summary. (1) A method is described for obtaining a suitably de-afferented preparation with intact brain stem in the unanesthetized cat whose resting EEG pattern is well "deactivated" and ideally suited to pharmacological and neurosurgical analysis of adrenergic and cholinergic components of the "reticular activating system," free from extraneous pharmacological influences. (2) Preliminary observations with both adrenergic and cholinergic agents suggest that complete temporal independence can be shown between onset and termination of action of "activating" agents introduced directly by intra-innominate administration and vascular effects of these agents as reflected in blood pressure alterations. (3) Advantages of this preparation over those commonly used for drug-induced EEG "activation" are reported. (4) This method currently is being used for further anatomical exploration of adrenergic and cholinergic components of pathways leading to diffuse "activation" of the electro-corticogram, and for studying the influence of psycho-tropic drugs on these systems.

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