References and Notes

1. V. C. Scheffer, *Seals, Sea Lions, and Walruses: A Review of the Pinnipedia.* (Stanford Univ. Press, Stanford, Calif., 1958), pp. 14–15.
2. T. C. Poulter, *Science* **139**, 753 (1963).
3. W. N. Kellogg, R. Kohler, H. N. Morris, *ibid.* **117**, 239 (1953); J. C. Lilly and A. M. Miller, *ibid.* **133**, 1689 (1961); W. E. Evans and J. H. Prescott, *Zoologica* **47**, 121 (1962); W. E. Schevill, R. H. Backus, J. B. Hersey, in *The Sea*, M. S. Hill, Ed. (Interscience, New York, 1962), vol. 1, *Physical Oceanography*, chap. 14, pp. 556–57; W. E. Schevill and W. A. Watkins, "Whale and porpoise voices." A phonograph record (Woods Hole Oceanographic Institution, Woods Hole, Mass., 1962).

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6 May 1963

Influence of Methodology on Electroencephalographic Sleep and Arousal: Studies with Reserpine and Etryptamine in Rabbits

Abstract. *In studies with reserpine and with etryptamine, quite opposite electroencephalographic effects were obtained when either of these two drugs was administered to rabbits prior to surgical preparation for EEG recording as opposed to the drug being given after the rabbits had been fully prepared for EEG recording. With the one method, EEG rhythms indicative of sleep were obtained, while with the other method, EEG rhythms indicative of central nervous system stimulation were obtained, although with both methods, the amount of drug, duration of drug in animal, and type of acute preparation were identical for both sets of findings. The presurgical injection procedure produced EEG results more in accordance with behavioral and biochemical findings, even though the conventional procedure is to administer the experimental drug after the animal has been prepared for EEG recording.*

In most electroencephalographic (EEG) drug studies involving an acute animal preparation, the animal does not receive the experimental drug until after it has been fully prepared for EEG recording. While the technical details vary from laboratory to laboratory, this type of preparation generally involves a temporary period of general anesthesia as well as a prolonged period of local anesthesia, the implantation of several recording electrodes either to the surface of the brain or directly within the brain tissue, and, in many experiments, artificial respiration with its consequent tracheotomy and curarization. Each of these procedures produces its own physiological consequences and, in this sense, all acute animal experiments of this type are unphysiologic. Within limits, these procedures do not vitiate the experimental results, since the acute preparation is, itself, a major condition of the experiment. This assumes, however, that the procedures utilized in the preparation of the animal for EEG recording exert a constant influence throughout the experiment and also that they do not interact in some unique way with the pharmacological actions of the drug under investigation.

Our laboratory has found in studies with reserpine and with etryptamine that quite opposite EEG effects were obtained in rabbits when the experimental drug was administered to the animal prior to the preparation for EEG recording as opposed to the drug being given after the animal had been fully prepared for EEG recording, even though with both methods the amount of drug, duration of drug in animal, and type of acute preparation were identical for both sets of findings. With either reserpine or etryptamine, with one method EEG rhythms indicative of sleep were obtained, while with the other method, EEG rhythms indicative of central nervous system stimulation were obtained. These differing EEG effects of sleep and arousal generally lead to opposite kinds of interpretation of drug action.

We have studied etryptamine (alpha-ethyltryptamine), which is a weak inhibitor of the enzyme system monoamine oxidase. While its mechanism of action is not fully understood, it exhibits central nervous system stimulatory properties, an action shared by other and stronger inhibitors of monoamine oxidase. Our laboratory undertook an EEG, behavioral, and neurochemical analysis of the action of this chemical agent (*1*). New Zealand male albino rabbits were tracheotomized under ether and local pontocaine anesthesia, curarized and artifically respired. Five coaxial electrodes were implanted directly into brain tissue through trephined holes in the exposed skull. A control EEG recording was taken to assess the initial responsiveness of the animal, 5.0 mg/kg of etryptamine was then administered by femoral vein, and the rabbits were sacrificed at intervals varying from 15 to 360 minutes after injection for determination of brain serotonin. Electroencephalographic recordings were taken throughout the drug period. Activation of the EEG was usually apparent by 30 to 40 minutes after the administration of etryptamine in those animals which were studied for the longer time periods. The stimulatory effects, as evidenced by sustained EEG alerting, persisted unabated until the sacrifice of the animal. While brain serotonin levels were not found to be significantly elevated as compared to the usual findings for the stronger inhibitors of monoamine oxidase, it was apparent from the increasing and decreasing levels across time that this brain amine could not be associated with any EEG activation beyond about 3 hours of drug time. The rabbits studied for 6 hours, however, were still displaying EEG arousal at the time of sacrifice.

Because of the biochemical findings, a second set of animals was studied with the same surgical procedures and electrode implantation but under a method whereby the drug was administered prior to the preparation of the animal for EEG recording. In this second group, 5.0 mg/kg of etryptamine was given by marginal ear vein and the animals were placed back in their boxes until a period 2 hours prior to the time the animals were to be sacrificed for serotonin determinations. At 2 hours before sacrifice, the animals were then prepared for EEG recording in the same manner as described above for the postsurgical injection method. The surgical procedures required approximately 30 minutes, thus giving 90 minutes of EEG recording prior to sacrifice. With this second method of drug study, it was determined that the drug-induced EEG alerting usually subsided by 3 to 4 hours after injection, a duration bearing closer correspondence with the return of brain serotonin to control levels.

Figure 1, *A* and *B*, presents EEG tracings from each of two animals studied with the differing presurgical and postsurgical procedures for administration of etryptamine. Each recording was taken just before the sacrifice of the animals for serotonin determinations. As can be seen from Fig. 1, tracings *A* and *B* depict quite opposite EEG states. In Fig. 1*A*, the EEG rhythms are characteristic of the

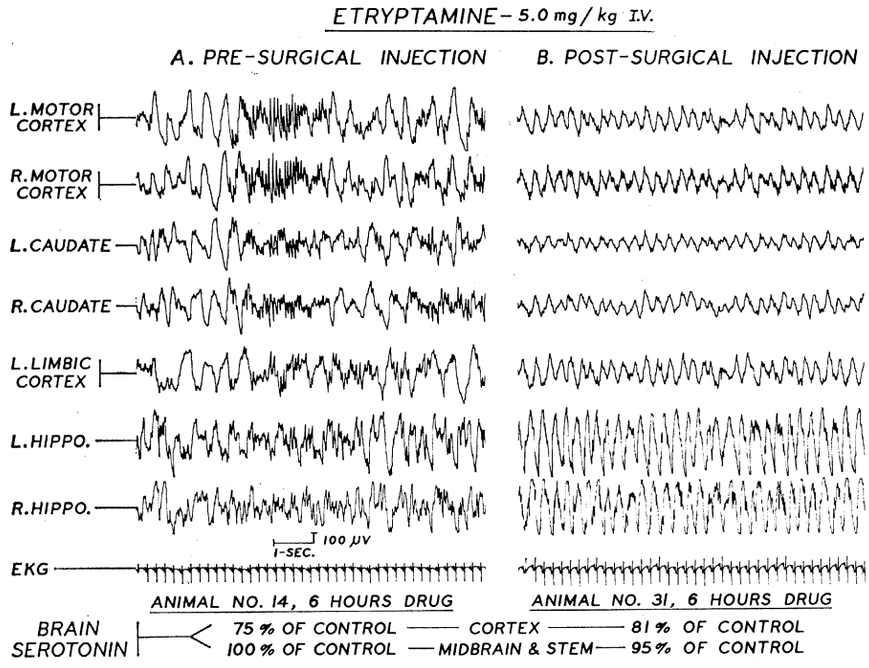

Fig. 1. Difference in EEG activity from presurgically and postsurgically injected rabbits 6 hours after injection.

normal "sleep" pattern for the rabbit. Since the presurgically injected animals studied for only 3 hours presented EEG patterns of arousal, this finding of sleep rhythms suggests that the stimulatory effects of etryptamine have subsided prior to the EEG study period for the 6-hour animals. In Fig. 1*B*, the EEG rhythms are characteristic of the normal "arousal" pattern for the rabbit. In the absence of the presurgical injection data, this would lead one to conclude that the central nervous system stimulatory action of etryptamine was still in effect at the sixth hour of drug study. Both animals were selected for presentation from a group of ten animals on the basis of the close similarity of brain serotonin levels for the two animals at the time of sacrifice.

We have also studied reserpine, a well-known tranquilizing agent having as one of its pharmacological actions the depletion of brain norepinephrine and brain serotonin. A curious but easily reproduced reserpine effect is the appearance of EEG alerting at a time when the animal is behaviorally sedated (*2*, *3*).

Our laboratory had occasion some months ago to study the cholinoyltic action of chlorpromazine in the reserpinized preparation. The type of experiment necessitated the giving of reserpine several hours before the animals were prepared for EEG recording in order to achieve a satisfactory level of amine depletion. In each instance, the animals were found to be comparatively insensitive to peripheral stimulation such as pain or touch when the EEG recordings were taken 5 hours after the reserpine pretreatment. This finding of diminished responsiveness in conjunction with the appearance of EEG rhythms indicative of sleep was in accord with the behavior of the animals at the time they were prepared for EEG recording but it was not in accord with the type of EEG result usually encountered with reserpine when it is administered after the animal has been prepared for EEG recording. After a delay of 30 to 60 minutes, reserpine produces a continuous pattern of EEG arousal lasting throughout the day (*2*).

At this juncture, the full significance of the etryptamine findings as to time of injection was finally appreciated. As a consequence, two sets of animals were studied; one group received 1.0 mg of reserpine per kilogram intravenously *after* they had been prepared for EEG recording, while the second group received the same dosage intravenously *before* they had been prepared for EEG recording. The details as to animal preparation followed the procedures described above for etryptamine, with the presurgically injected animals being returned to their boxes after drug administration until the appropriate time for EEG preparation 2 hours prior to sacrifice.

Figure 2, *A* and *B*, presents EEG tracings from each of two animals studied by means of the differing presurgical and postsurgical methods for administration of reserpine. Each recording was taken just prior to the

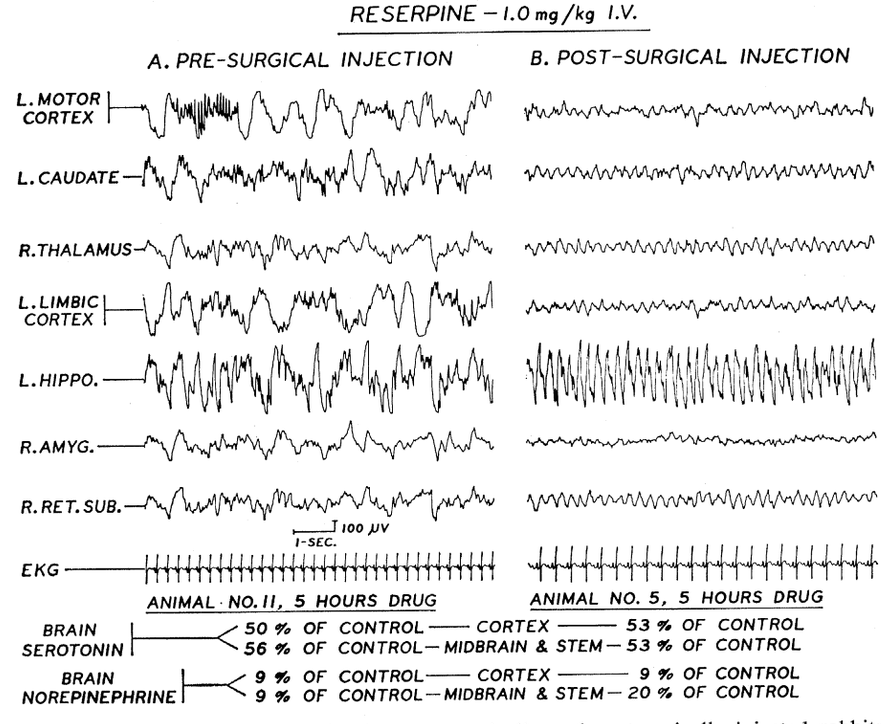

Fig. 2. Difference in EEG activity from presurgically and postsurgically injected rabbits 5 hours after injection.

sacrifice of the animals, the final selection of animals for presentation from a group of ten animals being based on the close similarity of brain amine levels for the two animals at the time of sacrifice. The EEG tracings of Fig. 2*B* present the familiar EEG arousal pattern in rabbits reported by Rinaldi and Himwich (*2*) for reserpine. Similar patterns of EEG arousal were obtained for every animal studied by means of postsurgical reserpine administration after a minimum drug duration of approximately 1 hour. The EEG tracing of Fig. 2*A* reflects the enhancement of the resting rhythms of the brain, which is the expected finding based on the calming action of reserpine. Again, as with etryptamine, the treatment of these two animals was identical except for the administration of drug either before or after surgical preparation for EEG recording.

An obvious conclusion from the results presented is that under some circumstances the type of EEG effect obtained is a direct function of whether the experimental drug has been given before or after the animal has been prepared for EEG recording. The effects reported take on added significance in view of the extensive use made of the rabbit in pharmacological and neurophysiological research. While the mechanism responsible for this difference in effects is unknown, it may relate, in the two drug studies described, to the length of time the animals are in a state of "acute" preparation. In most instances, the phenomenon encountered in these studies would not negate the conclusions drawn from acute preparations but it would seem advisable, in light of these results with reserpine and etryptamine, to utilize presurgical injection procedures if the type of problem requires the use of an acute preparation for long periods of time with postsurgical injection procedures. The presurgical injection method also seems advisable when postsurgical injection methods yield EEG findings at odds with either behavioral or biochemical findings.

Regarding the mechanisms involved in this phenomenon, it is well known that EEG patterns of sleep and arousal are altered under conditions which produce a depression of cortical function such as in the case of traumatic brain injury. This results, in part, from a disturbance in the corticofugal regulatory influences on brain-stem mechanisms (*4*). The effects reported in this paper, however, are of a different kind, since numerous control experiments over a period of several years show that the cortical responsiveness of the rabbit is not materially impaired by the surgical procedures employed in these studies. Electroencephalographic alerting can always be elicited by peripheral stimulation within 10 to 15 minutes after the completion of the electrode implantation. Secondly, in rabbits that had received reserpine for a duration of only 1 hour, EEG activation was apparent by about 45 minutes after either the presurgical or the postsurgical injection procedure, even though the presurgically injected animals had undergone electrode implantation not over 25 minutes prior to the appearance of the reserpine-induced arousal pattern. Consequently, the difference in EEG effects associated with the differing injection procedures cannot be ascribed simply to depression of cortical function resulting from the various procedures involved in electrode implantation.

Whether the anomalous results encountered with reserpine and etryptamine in rabbits are due to a nonspecific deterioration of the "acute" preparation or to some special aspect of the drugs employed is not clear. Studies in progress at this laboratory indicate that the same degree of alteration in brain levels of norepinephrine and serotonin is produced by reserpine in conjunction with either injection procedure, and consequently the phenomenon is probably unrelated to an altered release of brain amines in the case of this pharmacological agent. In any event, both the reserpine and etryptamine studies demonstrate that an alteration in method of drug administration can produce profoundly differing results in terms of EEG sleep and arousal patterns in rabbits.

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References

1. W. G. Steiner, G. R. Pscheidt, E. Costa, H. E. Himwich, *Psychopharmacologia*, in press.
2. F. Rinaldi and H. E. Himwich, *Ann. N.Y. Acad. Sci.* **61**, 27 (1955).
3. H. Gangloff and M. Monnier, *Helv. Physiol. Pharmacol. Acta* **15**, 83–104 (1957).
4. W. R. Adey, J. P. Segundo, R. B. Livingston, *J. Neurophysiol.* **20**, 1 (1957).

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Visual Reinforcement in Siamese Fighting Fish

Abstract. *Male Siamese fighting fish* (Betta splendens) *were conditioned to emit an instrumental response to obtain the visual image of another male of the species. The relative positive reinforcing effects of three visual stimuli capable of eliciting aggressive display were compared.*

Stimuli which evoke unlearned aggressive behavior can act as positive reinforcers for instrumental responses (*1*). The visual image of a mature fighting cock was found to act as a positive reinforcer for a key-pecking response in another fighting cock. The present study is an extension of this line of investigation, in which the relative reinforcing properties of several visual stimuli evoking aggressive display in *Betta splendens* (Siamese fighting fish) are examined. The purpose of this experiment was to establish the positive reinforcing effects of the visual image of one male of the species for the instrumental behavior of another. In addition, the reinforcing properties of three visual stimuli in maintaining the same response were compared.

Four male fish (*B. splendens*), from about 6 to 10 cm long, purchased from a local aquarium supply store, were the experimental subjects. The fish were maintained on a diet of six to 10 tubifex worms, once a day, throughout the course of the experiment. Each fish was housed in a 2-liter pyrex beaker filled with conditioned tap water maintained at 26° to 29°C, when not in the experimental test chamber. The test tank was about 30 by 20 by 25 cm, with transparent Lucite walls (Fig. 1). A Lucite ring about 12½ cm in diameter with a circular aperture of about 7½ cm was suspended in the tank from a translucent Lucite top. A beam of light was focused across the aperture of the ring, falling on a photoelectric cell (Clairex Cl-404) imbedded in the plastic of the opposite side of the ring. A response was recorded when the fish swam through the ring, breaking the beam of light, then continuing through, allowing the light to again fall upon the cell (*2*). The back wall of the tank was used for presentation of visual stimuli.

Three stimuli were sequentially examined for reinforcing properties. In the first procedure a mirror was pre-