Anesthesia and Paralysis in Experimental Animals: Report of a Workshop held in Bethesda, Maryland, October 27, 1984

Organized by the Visual Sciences B Study Section, Division of Research Grants, National Institutes of Health

Sponsored by the National Eye Institute and the Division of Research Grants, National Institutes of Health

Notice from the editors of *Visual Neuroscience*

The following report, which represents the conclusions of a workshop on anesthesia and paralysis in experimental animals, is being published in *Visual Neuroscience* at the request of the organizers of the workshop. The Editors believe that the report is important and may serve as a useful guide for the conduct of experiments on anesthetized, paralyzed animals—a common situation in the field of visual neuroscience. However, the Editors remind the reader that the proceedings are *not* the official policy either of the journal or of any organization, and are not published as having any official sanction. Any inquiries about the material in the report should be addressed to the authors.

Background

Experiments to investigate the responses of neurons in the visual pathway usually require that images on the retina be stationary, or that their movements be controlled. Most investigators achieve this by immobilizing the animal with curariform muscle relaxants, thereby paralyzing both the extracocular muscles and all other voluntary muscles. Under these conditions, pain or distress in the experimental animal is difficult to evaluate.

Responding to concerns expressed by Study Sections, the National Advisory Eye Council issued a policy statement on the use of paralyzed mammals in research supported by the National Eye Institute; the text of this statement is reproduced in Appendix I. Adequate guidelines for controlling and monitoring the condition of a paralyzed experimental animal do not presently exist. Therefore, Visual Sciences B Study Section of the Division of Research Grants of the National Institutes of Health, which evaluates many research projects involving paralyzed experimental animals, initiated a workshop on this topic. The workshop, jointly sponsored by the Division of Research Grants and the National Eye Institute, was held in Bethesda, Maryland in October 1984. This report summarizes the recommendations of the workshop.

In this document, we address two sets of issues. In the first part we consider the difficulties encountered when general anesthesia accompanies systemic paralysis, and offer some recommendations for ensuring the adequacy of a particular anesthetic regimen. We also consider briefly some experimental methods that do not require systemic paralysis. In the second part we consider the more difficult situation in which a systemically-paralyzed animal is to be studied without anesthesia. This case requires and receives more specific recommendations.

Experiments on anesthetized, paralyzed animals

Experiments on paralyzed animals are normally performed while the animal is anesthetized. Because paralysis prevents the experimenter from using common indices of anesthetic depth, it is important to ensure that the procedures employed are adequate.
Evaluation of anesthetic state

Anesthetics are commonly thought to have three relatively distinct effects: analgesia, loss of awareness, and amnesia. Of these, maximal analgesia is of course the most desirable, but there is no reason to assume that analgesia alone is sufficient to remove all potential sources of distress in an experimental animal. Loss of awareness offers the most complete guarantee of freedom from distress, but equating loss of behavioral responsiveness with loss of awareness may not always be warranted in experimental animals. Human observers use an animal’s behavioral responses to stimulation to assess its “awareness”, “pain” and “distress”. The interpretation of these responses involves an anthropomorphic judgment, and may vary from one individual to another. It is therefore desirable that, where possible, objective criteria be used to make these judgments. It might seem appropriate to use criteria like those used to assess anesthetic effects in human patients, but these depend heavily on postanesthetic recollection to establish awareness and sensitivity to pain, and amnesia is a chief criterion of clinical effectiveness. It is neither possible nor reasonable to apply this criterion to animal subjects.

How, then, can the level of awareness and analgesia be assessed acutely in an animal subject? An indication of a level of awareness may be obtained for subjects capable of voluntary movement, but systemic paralysis makes this assessment difficult. In the unparalyzed animal, it may be firmly stated that the absence of phasic motor or autonomic responses to nociceptive input (e.g., withdrawal reflexes, heart rate and/or blood pressure changes, EEG arousal, tearing) can be taken to indicate relatively deep (“surgical”) levels of anesthesia. Since such levels may well be considerably deeper than necessary to produce the desired analgesia and lack of awareness, the presence of autonomic reflexes need not indicate inadequate anesthesia. It is, for example, acknowledged that a human patient can be clinically unaware of and unable to appreciate pain, while still retaining the ability to respond to certain kinds of stimuli (e.g., commands and certain classes of noxious input). Nevertheless, the presence of vigorous and organized withdrawal responses of more than reflex character to noxious stimulation indicates an inadequate level of anesthesia.

During systemic paralysis, of course, withdrawal reflexes are unobservable, and other signs must be sought. Muscle relaxants do not abolish cardiovascular reflexes, and substantial cardiovascular or other autonomic responses (e.g., salivation and lacrimation), when elicited by noxious stimuli, provide a clear indication of distress. Because common anesthetic drugs can modify autonomic responses, the absence of these indicators cannot, however, be taken as a reliable guide that all is well. Pupil dilation and lacrimation normally indicate distress, but in experiments on the visual system, where the pupil is normally dilated and contact lenses are worn, such signs are usually not observable.

The simple two-electrode electroencephalogram (EEG) may provide an indication of the depth of anesthesia. In human patients the form of the electrical activity changes progressively with depth of anesthesia. Its dominant frequency generally moves from around 10–12 Hz in wakefulness to around 3–4 Hz when anesthesia is deep, though certain anesthetics, both alone and in combination with atropine, may produce abnormal patterns and alter the relationship between EEG and behavioral state. Moreover, interpretation of the EEG in the absence of a running spectral analysis is difficult; even with this analysis available, interpretation is rarely unambiguous, and the simple EEG does not by itself form a sufficiently reliable indicator of anesthetic depth.

A potentially more useful and accurate electroencephalic indicator of anesthetic depth is the shift from posterior-dominant to anterior-dominant activity that can be discerned in multielectrode EEG recordings in humans, macaque monkeys and dogs (e.g., Tinker et al., 1977). This shift appears to mark a transition between awareness and unawareness that occurs at lower anesthetic levels than those required for “surgical” anesthesia. It may thus indicate a point of more interest and relevance to experimental studies involving animals on whom no further surgery is to be performed. This observation is, however, preliminary, and its generality across species and anesthetics has not yet been established.

In view of the difficulties inherent in the evaluation of anesthetic state in paralyzed animals, it is important to establish that the anesthetic regimen to be employed is adequate in a trial preparation identical to the experimental one in all respects save systemic paralysis. This trial should be of the same duration as an experimental preparation, and should involve all manipulations that might cause distress. This “trial” method is important regardless of the type of anesthetic to be used.

With inhalational anesthetics, the intraspecies variability is very low, and the minimum effective alveolar concentration established for a particular agent can be used to obtain consistent levels of anesthesia. It is important to note that appropriate methods for measuring the alveolar concentration of an inhalational agent must be employed, rather than merely establishing the concentration of the agent in the inspired gas mixture. When another form of anesthetic agent (e.g., intravenous or intramuscular) is to be used, this “trial” method is important because of the greater variability in response to be expected. For some commonly used intravenous agents the basic parameters needed to estimate plasma concentrations are known, and it is possible to establish infusion regimens that maintain steady and reasonably predictable levels of anesthesia.

Another suitable method for evaluating anesthetic level is periodically to withhold the muscle relaxant to
test the animal’s reflex responses. Supplementary anesthesia is required if evidence of organized movements of more than reflex character appears. Use of a modern muscle relaxant whose action is of short persistence makes this procedure quite practical.

Because of the difficulty of assessing anesthetic state in the paralyzed preparation, surgical procedures performed on anesthetized animals after the induction of paralysis should be confined to the minimum consistent with the needs of the experiment.

Choice of anesthetic agent

The use of an anesthetized preparation gives rise to another concern: that the anesthetic itself acts directly on the neurons under study, or otherwise alters their environment, so that normal neuronal behavior cannot be observed. This objection is a serious one, since anesthetics have substantial effects upon the sensitivity of visual neurons, and may also alter their receptive field properties. The difficulties caused by anesthesia can be minimized by careful choice of anesthetic, and careful monitoring of its depth. Recent advances in anesthetic practice have rendered obsolete many methods that remain in common use in animal experiments, and investigators are encouraged to consult veterinary anesthesiologists for advice on anesthetic methods appropriate to their experimental situation. Appendix II provides information on obtaining professional advice on anesthetic technique and practice.

Alternatives to systemic paralysis

Eye immobilization

It should be recalled that the goal of paralysis in experiments on the visual system is to prevent movement of the eyes. Systemic paralysis need not accompany ocular immobilization, and two methods for immobilizing the eyes of unparalyzed animals are known. Section of the cranial nerves that innervate the extraocular muscles may be used to produce reasonable stability of the eye. The surgery involved is, however, complex. Alternatively, direct injection of the extraocular muscles with a long-lasting neuromuscular blocking agent may prove to be an expedient method for immobilizing the eyes for some kinds of experiment. In either of these cases, systemic paralysis is unnecessary and the problems of anesthetic monitoring are correspondingly reduced.

Awake, behaving preparations

Effective short-term control of eye position may also be obtained in alert animals trained to fixate visual targets. This method has many attractions: one knows whether or not the animal is alert and comfortable, the technique is economical of animals, and it may provide the only satisfactory method of studying the physiology of certain types of visual function. However, its application is restricted to species that can be readily trained to fixate and whose eye movements can be monitored accurately. Moreover, it is ill suited to studies that require detailed histological reconstruction of electrode tracks, and may not yield the exact stabilization of the eyes required for the study of very small receptive fields. Because of the trained animal’s fixation behavior, it may also be difficult to study receptive fields in the central fovea.

Experiments on awake, paralyzed animals

Some experiments depend upon the animal being awake. For example, the cells under study may be thought to have some special role (e.g., recognizing some significant object) that could not be demonstrated were the animal unconscious. In most cases an experiment of this type would be better undertaken on an awake, behaving animal than a paralyzed one. We cannot however, be certain that there exist no situations in which the use of awake, paralyzed experimental subjects is appropriate. Moreover, the National Advisory Eye Council’s policy statement (Appendix I) permits investigators to request special consideration by the Council for projects of this kind. Specific guidelines for this difficult case are therefore required, and our recommendations follow. No significant omission from the following list would be acceptable.

Experimental procedures

- Any necessary surgical procedures should be performed under general surgical anesthesia and prior to the induction of paralysis. If such procedures might have painful consequences during the subsequent period of paralysis, local anesthesia should be used in a manner shown by the experimenter to be effective over comparable periods after surgery in alert, freely moving animals of the same species. Endotracheal intubation and other preparatory procedures should be accomplished under general anesthesia, as should the induction of paralysis.

- Fixation of the skull in a stereotaxic plane requires suitable implanted devices previously fitted under surgical anesthesia. These are known to be well tolerated and neither painful nor stressful in awake, unparalyzed animals. Local anesthesia of “pressure points” from a stereotaxic frame is inadequate.

- Other devices attached to the animal should be placed to provide maximum comfort. The pharynx and larynx should be treated with topical anesthetic. The tracheal cannula should be coated with a lubricant containing a local anesthetic agent, and must be immobilized so
that it cannot be moved inadvertently after general anesthesia has subsided. Intravenous catheters must likewise be immobilized.

- The animal should be placed in one of its natural resting positions. Minor adjustments in posture form time to time may be helpful in preventing pooling of blood in great veins.

- Salivation should be controlled with a suitable drug, or some other innocuous means employed to prevent the accumulation of secretions in the throat.

- The duration of paralysis must be dictated by the time over which the physiological condition of the animal, and hence its comfort and well-being, can readily be maintained. For most species this will not exceed 4–6 hours. Some form of intravenous supplementation may be needed, as will cannulation of the bladder in certain species (e.g., tree shrews) in the event that emptying does not occur spontaneously.

- If the animal is to recover from the paralysis and reestablish its respiration, this must be accomplished while the animal is under general anesthesia. The skilled use of chemical antidotes may ease this process of recovery so that anesthesia may be brief and light in level.

- No procedure should be undertaken until it has been shown, when the animal is fully alert, paralyzed and capable of expressing its reactions, that an identical procedure elicits no sign of discomfort or distress. This specifically includes, but is not limited to, insertion or manipulation of recording or other devices, and electrical, chemical or other stimulation.

Monitoring of the animal’s condition

- In an otherwise comfortable situation, the principal source of distress that occurs in paralyzed humans (and therefore presumably animals) is the sense of respiratory distress that accompanies elevation of arterial $P_{CO_2}$. Therefore end-tidal $P_{CO_2}$ must be continuously monitored with a reliable, calibrated instrument, and its concentration be kept significantly below the 4.7% level at which respiratory distress may begin. It should be appreciated that $CO_2$ monitors designed for use in humans typically do not accurately measure end-tidal $CO_2$ in smaller animals because excessive gas mixing occurs in the sampling apparatus. Also, relatively short periods of artificial ventilation are often accompanied by changes in blood chemistry and pulmonary function that alter the normal relationship between end-tidal $P_{CO_2}$ and arterial $P_{CO_2}$. Regular direct measurement of arterial blood gas concentration is therefore an indispensable component of this monitoring, and monitoring with a respiratory gas analyzer alone is inadequate.

- The heart rate must be monitored. Preferably, a rate meter should sound an alarm if heart rate rises above or falls below its natural range for conditions of relaxed rest. Indeed, once the immobilized animal has recovered from the general anesthesia, the heart rate may provide the experimenter with a ready index of the animal’s state, and an elevation of rate due to a relatively innocuous stimulus provides some assurance that the animal is both alert and not experiencing a significant degree of stress.

- Body temperature must be monitored and strictly maintained within the limits normal for resting animals of that species. In most instances there will be a tendency for a very gradual fall in body temperature because of immobilization, and provision must be made for keeping the animal warm. Devices used for this purpose must be incapable of overheating that either causes distressingly high temperatures or produces uncomfortable heating of the skin.

- Provision must be made for the rapid and effective delivery of an anesthetic agent in the event that any indication of pain or distress occurs and its cause cannot be immediately identified and rectified.

- In cases in which an animal is to be repeatedly subjected to experiment under these conditions, signs of specific aversion to the experimental setting should be taken as evidence that the precautions employed are inadequate. In these circumstances, experiments must be discontinued until appropriate procedural changes are made.

- All of the foregoing precautions and procedures apply equally to the encephalé isolé preparation, remembering that although somatosensation from the body is interrupted, massive visceral input is still intact, as is sensation from the head.

- The midpontine pretrigeminal preparation may reasonably be assumed to produce effective analgesia, but the uncertain level of awareness of this preparation makes it prudent to apply as many of the criteria listed above as is reasonable.
Appendix I: Text of National Advisory Eye Council Policy Statement

Research on paralyzed mammals

The National Advisory Eye Council gives special attention to grant applications on which concerns have been raised about the intended use of research animals. In general, the NIH policy statement on “Responsibility for Care and Use of Animals” (NIH Guide to Grants and Contracts, November 10, 1978) provides guidance on the humane care and use of vertebrate animals under NIH awards.

There are, however, certain experimental procedures for which the National Advisory Eye Council feels it important to provide more specific guidance to the vision research community. In particular, the Council has decided not to recommend approval of any research or training projects proposing to use paralyzed, awake mammals. The Council encourages the use, when feasible, of existing alternative methods for studying awake animals and the development of other techniques and approaches. In stating its position on this issue, the Council recognizes that important vision research problems may exist for which no acceptable experimental approach is currently available. Since a Council recommendation for approval is required before an award can be made by the National Eye Institute, investigators may, in truly exceptional cases, request special consideration by the Council.

Appendix II: Sources of Anesthesiological Information

Investigators may obtain information about matters of veterinary anesthetic practice and the names of veterinary anesthesiologists in their area who are willing to consult on these matters, through

Dr. Robert Paddleford, Executive Secretary
American College of Veterinary Anesthesiology
Department of Urban Practice
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It would also be appropriate to consult modern reference works such as Lumb and Jones (1984) or Covino et al. (1985) for general recommendations and information.

References


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