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A MASK FOR RABBIT STEREOTAXIC GAS ANESTHESIA

Michael M. Patterson, Ph.D. College of Osteopathic Medicine Ohio University, Athens, Ohio 45701

and

I. Gormezano
Department of Psychology
University of Iowa, Iowa City, Iowa 52240

Drs. Patterson and Gornrezarto are currently professors at their respective institutions. The article on which this issue of the Carrier is based was written following a summer research leave during which the authors were Associate Visiting Professor and Visiting Professor respectively in the laboratory of Dr. Richard F. Thompson at the University of California, Irvine. The mask described here was developed there, and the original article was published in the Psychonomtic Society jour-nal, Behavior Research Methods and Instrumenta-tion (Patterson and Gormezano, 1978). 1i is adapted and reprinted here with the kind permission of the Psychonomic Society.

A mask suitable for delivering gas anesthesia to a rabbit in a stereotaxic headholder is described. The mask allows simple delivery of any volatile anesthesia to the rabbit for long periods during stereotaxic surgery, providing an ideal means for rabbit anesthesia when recovery in important.

The rabbit is a useful animal for many kinds of behavioral and neurophysiological studies (e.g., Gormezano, Schneiderman, Deaux, & Fuentes, 1962; Cegavske, Thompson, Patterson, & Gormezano, 1976; Patterson, 1977; Skelton, Mauk & Thompson, 1988; Thompson, 1986). Indeed, over the past several years, the rabbit has become a very widely used subject for studies on the neurophysiology of learning, especially those using the classical conditioning paradigm developed by Gormezano and his colleagues (Gormezano, et. al., 1962). Thompson and his associates have performed many studies on the association between various brain areas and classical conditioning, (e.g., Skelton, Mauk and Thompson, 1988), and many other investigators have be gun using the preparation for similar studies (e.g., Kao & Powell, 1988; Moore, Desmond & Berthier, 1982).

While providing a behaviorally cooperative subject, the use of the animal in stereotaxic surgery for lesions or brain implants is considerably more difficult than is the cat. While the cat has well placed skull features for stereotaxic mounting, the rabbit must be held with somewhat less accurate and cumbersome means (Patterson, 1977). In addition, the cat can easily be intubated with noninjurious endotracheal tubes, allowing for delivery of gas anesthesia during stereotaxic surgery, whereas the rabbit is very difficult, if not impossible, to intubate under normal conditions. Thus, most rabbit stereotaxic stir gery is performed under an injectable anesthesia such as Nembutal (sodiurm pentobarbital), which is often not well tolerated (Patterson, 1977), and recovery time may be substantial. The ability to utilize an easily controlled, delivered, and well tolerated gas anesthesia such as halothane or penthrane during stereotaxic surgery ,vitlr the rabbit Would make such surgery simpler and less risky. The mask described here was developed to allow gas delivery to the rabbit in a Kopf stereotaxic head holder and has proved to (live good results over several hours of surgery followed by complete, nontraumatic recovery from anesthesia.

CONSTRUCTION

The mask itself is shown in Fig tire 1. It consists of three Pieces of Plexiglas tubing, a Plexiglas plate, and a rubber face adapter. The large tube forms the body of the mask and is cut from a tube 50 mm (about 2 in.) O.D., 42 mm (about 1-1/4 in.) I.D., and about 50 mm long. This tube is cut at a 45-deg. angle, starting from 3 mm from the front and slanting down to the back. Starting at (Continued on page 2, col. 2)

Editor's Column

The issues of animal rights continue to generate controversy and debate. It seems that almost every day we hear move about the activities of groups

which are attempting to stop the use of animals in research and education, as well as in drug testing and other areas. Of cour se, we must vain it that in some areas, the call for humane treatment of animals has made a positive impact on the way in which research is conducted and animals are treat- ed. I owever, in many cases, it is obvious that the animal activists are not interested in humane treat ment, as we would see it, but in stopping all research use of animals. This is inimical to the pursuit of science in the biological realm.

It amazes me when I see statements like that in the Winter 1989 issue of the APA Division 28 newsletter. Here, Linda Dykstra, Division president, pointed out that during the recent crisis at Cornell University over Dr. Okamoto's cat research, not one letter was received from the scientific community in support of the research! As scientists, we must change that situation and rally to the support of our colleagues when they are threatened by irrational and militant animal rights activists. The knowledge that one has support from ones peers is very helpful in such a situation, while conversely, the feeling that one is alone could be devastating. Support those who are threatened by writing to them, by writing to their institutional officials and to their politicians. Also, support those groups Which are springing up to help in the area of animals in research. One such group is iiFAR-incurably ill For Animal Research. This group is starting to put together a very effective campaign to show the benefits of animal research. For more information on iiFAR, write to Steve Carroll, iiFAR, P.O. Box 1873, Bridgeview, IL 60455. Let them know you appreciate their efforts in education and in support of good, humane research.

Michael M. Patterson. Ph.D.
Science Editor
College of Osteopathic Medicin
Ohio University
Athens, OH 45701

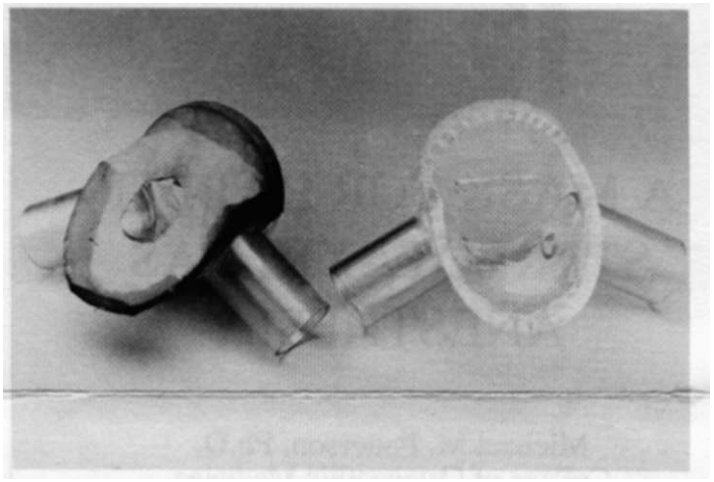

Figure 1. The rabbit anesthesia mask complete (left) and without the rubber membrane attached. Note the asymmetry of the sides in relation to the front slot, and the side tubes and suturing holes visible on the incomplete model.

the 3-mm point from the front, the sides are then cut down about 20 mm on the right (when viewed from the front) and about 12 mm on the left, leaving a 3-mm rim on the top of the mask. Holes 21 mm (7/8 in.) in diameter and centered 15 mm back and 10 mm below the horizontal midline are drilled into the body tube. Side tubes 21 mm (7/8 in.) long are sealed into the holes with Plexiglas solvent (e.g., ethylene dichloride). The side tubes slant down to allow attachment of flexible anesthesia tubes under the stereotaxic frame bars. A round 3-mm thick plate with a diameter that of the body tube is sealed to the front of the tube. The plate has a milled slot 25 mm long and 3 mm wide centered side to side with the top of the slot on the horizontal midline. the slot is just large enough to pass the Kopf tooth bar. A flexible rubber membrane is sewn to the mask trough 1-mm holes drilled around the open sides. The sewing is done using a 0 or 1 silk suture swaged to a quarter curved needle. Since the rubber must be airtight around the mask, the suture is brought back through each hole twice to provide a continuous loop over the rubber. We did not find a glue that would adhere well enough to joint the vinyl rubber to the Plexiglas. However, other rubbers or other glues may be used to make the sewing unnecessary. In fact, the rubber that is used to make the rubber bags on most bas anesthesia machines makes an excellent membrane for the mask and is easily obtained from many medical suppliers. This

(Continued on page 3, Col. I)

rubber resists the gas anesthesias, and is easily glued to the Plexiglas with common contact cement, unlike the vinyl rubber. This rubber is an excellent alternative to the vinyl rubber. A hole about 12 mm in diameter is cut in the rubber to allow the animal's nose and mouth to enter the mask, but provide a stug fit.

OPERATION

The mask is fitted to the Kopf rabbit adapter as shown in Figure ?. The slot fits tightly over the. tooth bar and the space in the bar is filled with clay to provide a seal. The asymmetric side cuts in the main tube allow the nose clamp on the right to come down to Be animal's nose while provid-ing a higher support on the left. To position the animal, the mask is pushed as far back onto the bar as possible. Initial anesthesia is given by a regular small animal face cone while the animal is in a restraining box or wrapped in a towel. If halolhane is used, a 5% concentration in oxygen produces sufficient anesthesia in about 15 min. The animal can then be placed on the tooth bar, the mask slid up over the nose and mouth and gas flow started through the mask. It is easiest to have two sets of hoses for the anesthesia machine, one attached to the cone and one to the mask, switching hoses at the machine rather than from cone to mask. Attempting to place the hoses on the mask after the animal is in place is difficult and may take longer than the time available before the animal becomes too light. It is very important when adjusting the animal in the mask to make sure the Mouth is well into the chamber and open, with the tongue out This can be accomplished by pushing a set of hemostats inside the rubber and spreading the mouth while lightly grasping the tongue. If the mouth is closed, the animal May slowly become hypoxic since, with the nose clamp in place, breathing is largely through the mouth. If the animal suddenly expires some time after being placed in the apparatus. slow suffocation leading to vomiting and aspiration is to be Suspected, probably due to the mouth not being sufficiently open. Care should also be taken that a good respiration volume is maintained and that no leaks around the mask are present. IN sure that the clay around the slot i the tooth bar is scaled firmly, as even a small leak can cause Sufficient loss of anesthesia. Once the animal is secured in the mask and the nose clamp is down, the zygoma chimps may be adjusted into position and the gas mixture changed to maintenance levels (1%- 1 1 /2% for halothane).

When used properly, the mask maintains excellent anesthesia levels for many hours. Problems are usually due to improper mask position, allowing leakage or blocking proper ventilation of the animal. Used properly, the mask allows rabbits to be easily maintained on gas anesthesia during stereotaxic surgery over long periods with practically no ancathesia connected fatalities.

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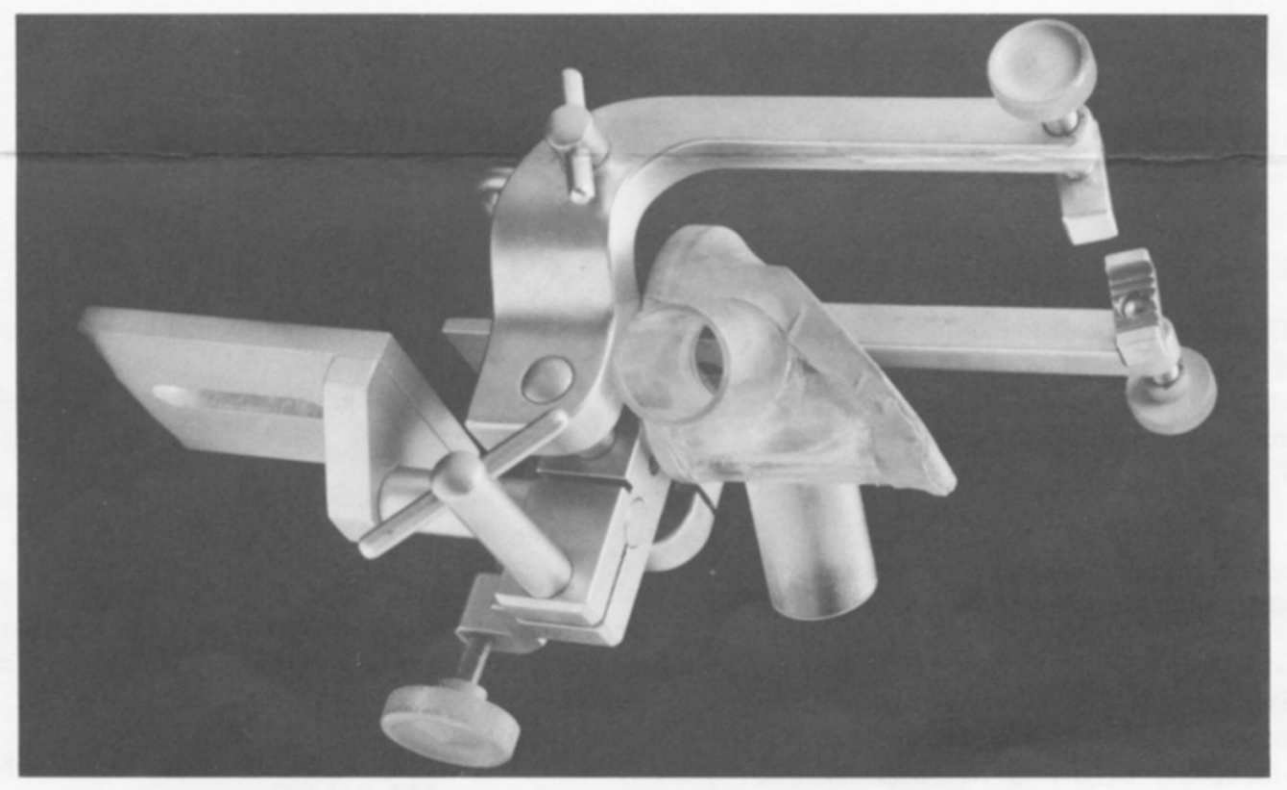

1 Figure 2. The mask mounted on the Kopf Rabbit Adapter

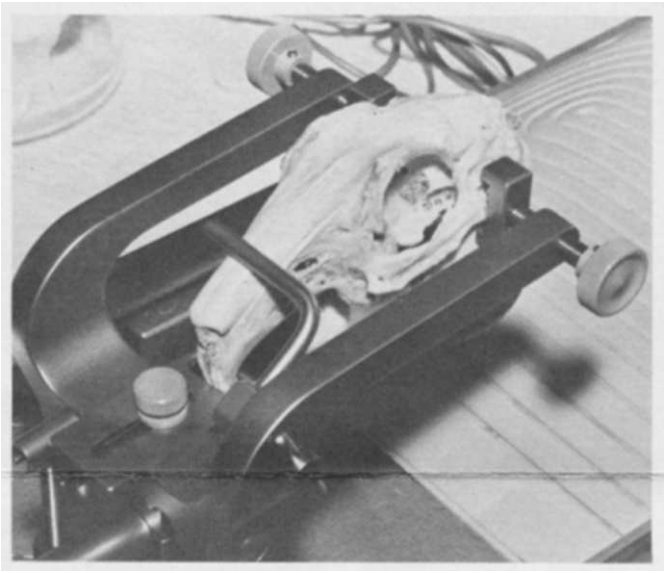

FIGURE 4

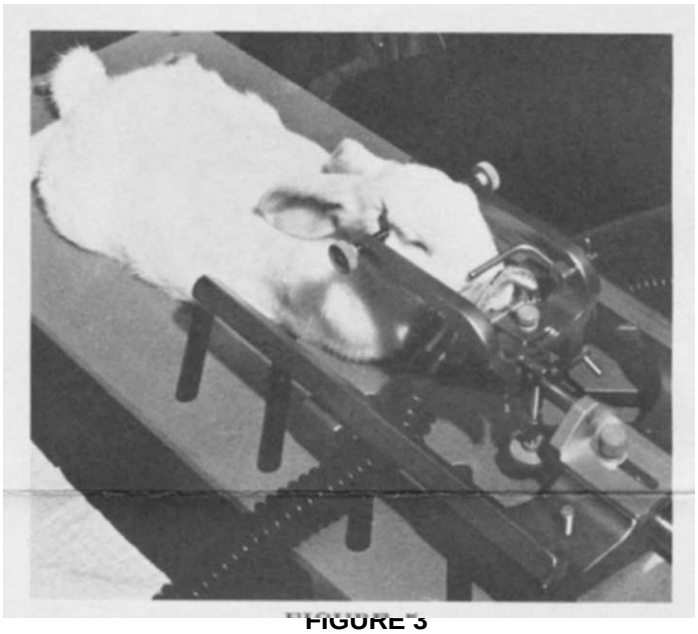

FIGURE 3

cannot be intubated without clipping the teeth almost completely off and then only with great difficulty. Gormezamo and I have recently devised a special delivery mask which fits over the Kopf tooth bar and allows the gas to be administered to the rabbit during surgery. Basically a plexiglass cylinder with inflow and outflow tubes and a rubber membrane through which the animal's nose and mouth fit, the mask slides over the tooth bar prior to the placement of the rabbit in the holder. In this way, surgical gas anesthesia can easily be given to the rabbit for long periods. Details of the mask are available from the author and will soon be published.

Of the several basic rabbit anesthesia methods described here, sodium pentobarbital will be the drug of choice for most laboratories due to its low cost and ease of delivery, and the fact that no equipment other than needle and syringe are needed for its use. It has been our experience that some variability will be seen between strains in tolerance to pentobarbital and that some dose adjustment will be necessary before a low loss rate is achieved. However, once the experimenter has had some experience with his particular strain, he should find almost no loss due to anesthesia if it is given IN. and sufficient care is taken to monitor the animal's level during induction. However, if funds are available, the gas anesthesia is safer and requires less care during surgery than pentobarbital, provided a good delivery system is used. Other hints necessary for good rabbit gas anesthesia delivery during surgery are given below. As noted, chlorolose should be used only in cases where recovery is not necessary and an excitable brain is desired, while urethane precludes recovery. More information can be obtained from William Lumb's book, Small Animal Anesthesia (Lea & Febiger, 1963).

Placing the Rabbit in the Headholder.
Once a suitable level of anesthesia is induced, the rabbit must be placed in the stereotaxic instrument. The headholder should be mounted in the stereotaxic frame as shown in Figure 3. For subjects below about 1.5 kg, the long tooth bar and nose clamp (see Figure 1) should be used.

For initial placement in the headholder, the animal should be grasped by the back of the head with one hand while the experimenter opens the lower jaw with the other. The upper teeth can then be put over the tooth bar. Care should be taken not to get the tongue caught in the bar as this restricts free breathing. The nose clamp should then be brought down over the nose and tightened lightly. Once the teeth are in place, positioning of the zygoma clamps is begun. The temporal process of the rabbits zygomatic arch extends about twothirds of the way from the eye to the ear at a level just below the eye. The clamps should be positioned vertically over these processes, as shown by the rabbit skull in the headmount in Figure 4. It is necessary at this point to turn the clamp screws in about equally until the clamps are almost touching the animal's head to see whether the clamps will contact the correct area. If the head is too far anterior or posterior, the tooth bar can be repositioned further forward or backward in the frame. Once the correct anterior-posterior position is reached, the zygoma clamps can be tightened lightly on the head.

The head should then be checked visually to see that it is about level from side to side and that it is straight in the holder. If a gross side-to-side tilt is noted, the clamps can be loosened and the head straightened. If any deviation of alignment from front to back is seen, the skin on the side too far back can be slipped forward under the zygoma clamp to make the nose slide to the opposite side. If this does not correct a less than perfect alignment, reset the clamps. When it appears that the head is straight in both dimensions (front-to-back and side-to-side), the clamps should be tightened until they are snug.

If the animal is under pentobarbital anesthesia, this is the most crucial part of the operation since the clamp pressure frequently causes the animal to stop breathing. Typically, a slightly too heavily anesthetized animal will exhale and remain in forced exhalation until the heart stops if the chest is not squeezed. Often one or two reflexly produced inhallations will be enough to restart normal breathing. If prolonged respiratory difficulty is seen, a leather thong or light wire tied around the chest with a reasonable pressure will cause continued respiration and save the animal. If breathing cannot be restarted within about 20 seconds, the clamps should be released and the animal allowed to rest for a few minutes before another attempt is made to put it into the headpiece.

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