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RABBIT STEREOTAXIC TECHNIQUES

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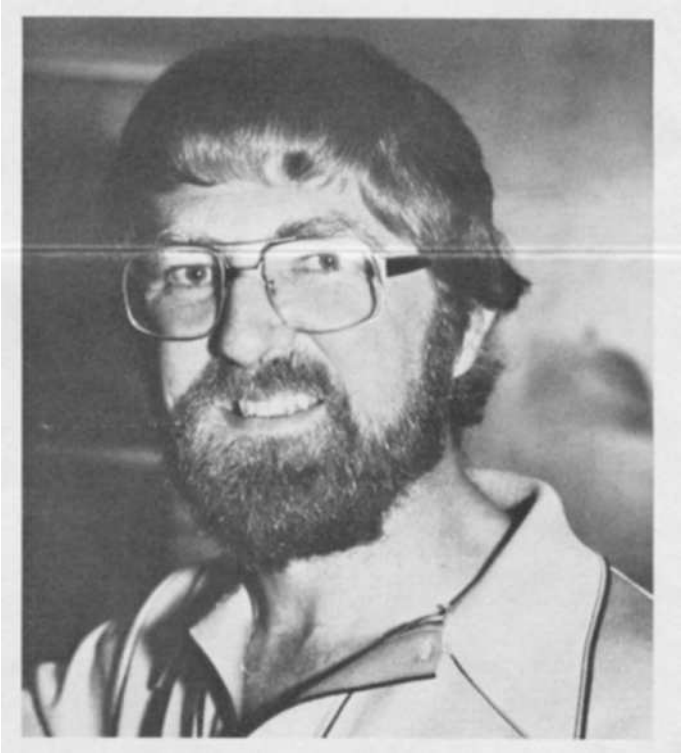
This article is the first of two which will appear in the Carrier, the second will be on cat restraint techniques.

THE USE OF THE RABBIT STEREOTAXIC HEADHOLDER

The rabbit is a very useful laboratory animal for various behavioral and neurophysiological studies. Indeed the similarities between the rabbit and human eye have made it a prime subject for eye research (e.g. Prince, 1967). In psychology, one of the rabbit's main uses has been in classical conditioning studies of the eyelid or nictitating membrane response following Gormezamo's elegant delineation of this preparation in 1962 (Gormezamo, Schneiderman, Deaux & Fuentes, 1962). However, despite a large behavioral literature on rabbit classical conditioning, the brain of the rabbit has been much less extensively studied than that of the cat. This situation is likely due, at least in part, to the easy availability of cats during early periods of neurophysiological research, which led to a perpetuation of cat neurophysiological models and to the relative ease with which the cat can be anesthesized with common barbiturates.

The current trend, however, seems to be toward the increased use of the rabbit in certain neurobehavioral and neurophysiological studies. Gormezamo's elegant behavioral paradigm with its vast accumulation of data seems to be one impetus for this trend. Other reasons include (1) theincreased availability of the rabbit, (2) the genetic background can be closely controlled by obtaining a standard strain such as the New Zealand white, (3) the low cost of the rabbit, and (4) perhaps the ultimate edibility of the animal if only behavioral studies are performed. One major problem in the use of the rabbit in neuropsychology is the difficulty of obtaining good stereotaxic placement of brain electrodes. Unlike the cat, with its optimally placed external auditory meatus which allows solid placement of ear bars for stereotaxic holders, the rabbit's ears are constructed such that there is almost no possibility for the use of stereotaxic ear bars. The most widely used and convenient rabbit stereotaxic holder is the Kopf machine, the current model shown in Figure 1. This device holds the animal by means of a tooth bar, nose

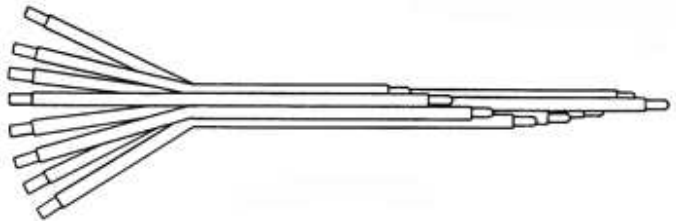


clamp, and two zygoma clamps. Due to the variability of clamp placement, with no accurate external landmarks or holding points, each subject must be individually aligned in the holder and stereotaxic frame prior to electrode implantation. In addition, the rabbit is somewhat harder to anesthetize than the cat. The processes of anesthesia and stereotaxic adjustment of the rabbit will be discussed here.

Anesthesia

Sodiumpentobarbital. Perhaps the most common rabbit anesthetic agent is sodium pentobarbital (Nembutal). It is easily administered intravenously (I.V.) through the medial or marginal ear vein via a 23 gauge butterfly infusion set (e.g. Minicath-23 infusion set, Deseret Pharmaceutical Cat. #2312). Initially, the tube and needle should be filled with normal saline, inserted into the vein and taped in place prior to anesthetic injection. The vein cannulation is most easily accomplished

with the animal in a restraining box, and the vein can be more easily visualized by shaving or wetting the back of the ear, then tapping the vein gently to produce dilation. Often the usual method of aspiration to determine if the needle is in the vein does not result in blood being drawn back into the tube or syringe; probably due to the vein wall blocking the needle lumen. It is thus best to inject very small amounts of saline once the needle is thought to be in the vein lumen. If a bleb does not appear, more saline can be injected to be certain a good placement has been made.



CIRCULAR ARRAY

Shown above is one of the many arrays fabricated by Rhodes Medical for those researchers who need specialized electrodes for a particular study. Arrays are available with wire and contacts ranging from 0.25mm diameter to as small as 0.lmm diameter.. Numerous contacts and configurations are available.
To enable us to quote price and delivery, please submit the number of contacts in the array, spacing geometry, contact diameter, contact exposure and electrode length. A new electrode catalog is available on request.

NEW PRODUCTS
RAT SPINAL #980

David Kopf Instruments will introduce a new rat spinal at the Society for Neuroscience meeting in Anaheim, November 7-9.

The modular concept and optional extras make this the most flexible rat spinal unit available. The model 980 is ideal for intracellular procedures. The standard spinal unit consists of a 1.9 x 30.4 x 40.6 cm base, two round adjustable A-P bars, a new type vertebrae clamp and hip spikes. Optional extras include two standard calibrated 18.7 mm A-P bars. These will allow the use of the 1460 or 1760 SB carriers on either side of the spinal frame; all of our standard accessories can be used with the new spinal unit. Other options available are retractors, dual prong clamps and a rat head holder.

If a complete stereotaxic frame as well as a spinal unit is desired, a 40.6 x 55.9 cm base plate can be supplied that will accommodate our standard 900 Small Animal Stereotaxic Frame.

1244 RABBIT ALIGNMENT TOOL

The ability to rapidly load a rabbit into our 1240 head holder has been enhanced by the addition of a new David Kopf Instruments skull alignment device. This tool combines the features of the planilabe, designed by Crawford, I. L., Kennedy, J. L, & Lipton, J. M., for bregma-lambda alignment with the side to side skull orientation.

The 1244 attaches to the electrode carrier in the same manner as any of the electrode holders.

An adjustment is provided in the A-P plane to allow for differences in the distance between the bregmalambda points. The lambda point is 1.5 mm lower than the bregma point. In addition to this, reference points crossing laterally are provided. These are spaced 5 mm either side of the bregma coronal point.

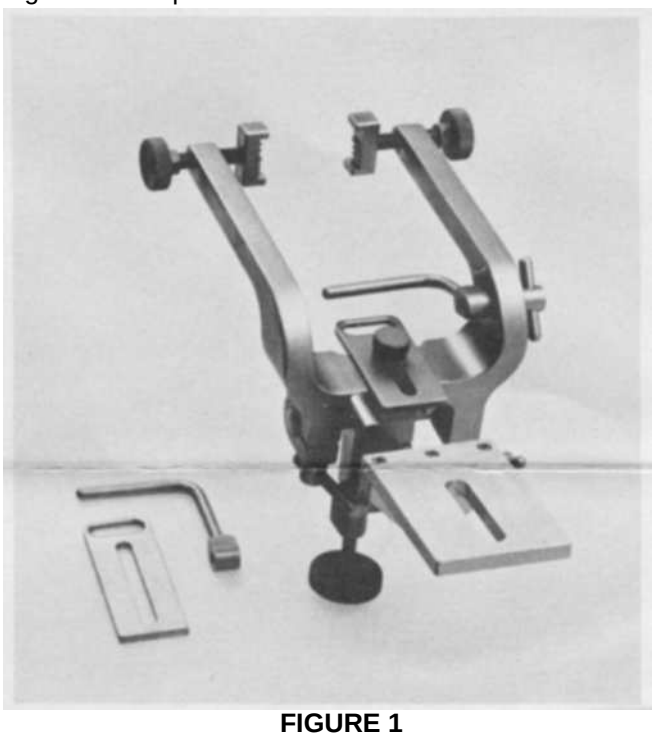


FIGURE 1

Continued from Page 1

The nembutal can then be injected from a second syringe followed by more saline to flush the anesthetic into the vein.

For most 1.9-2.5 kg. animals, 50 mg. of nembutal given over 30-40 sec. and flushed in with saline is sufficient to produce anesthesia. The animal will become limp almost immediately after the initial injection, but continue to become more deeply anesthetized for several minutes. If the initial dose is insufficient to produce surgical anesthesia in about 5 minutes, 1-2 cc (5-10 mg) additional can be given. Care must be taken however, as the rabbit has a small safety margin with pentobarbital and can be lost with the addition of very small amounts of anesthesia above that necessary to do surgery. The absence of a moderate leg flexion to hard toe pinch or eye blink to corneal touch is a danger sign, and the animal will stop breathing if a bit too much anesthesia is given.

Breathing can be maintained under such circumstances by grasping the animal's chest just behind the front legs with the experimenter's thumb and forefinger, and squeezing. Each sqeeze will usually produce reflex breathing which can often save the subject. Once a suitable anesthetic level is reached and surgery begun, it is necessary to give small additional doses of anesthesia every 30-45 minutes to maintain surgical levels, as the drug is short acting in the rabbit. For this reason, it is advisable to leave the cannula taped in the vein for the duration of surgery, flushing occasionally with saline to prevent clogging. Recovery from pentobarbital is uneventful, and the animal is usually mobile within an hour after the last dose. Interperitoneal pentobarbital injection is not recommended due to the extreme variability in effectiveness

of the anesthetic when given by this route.

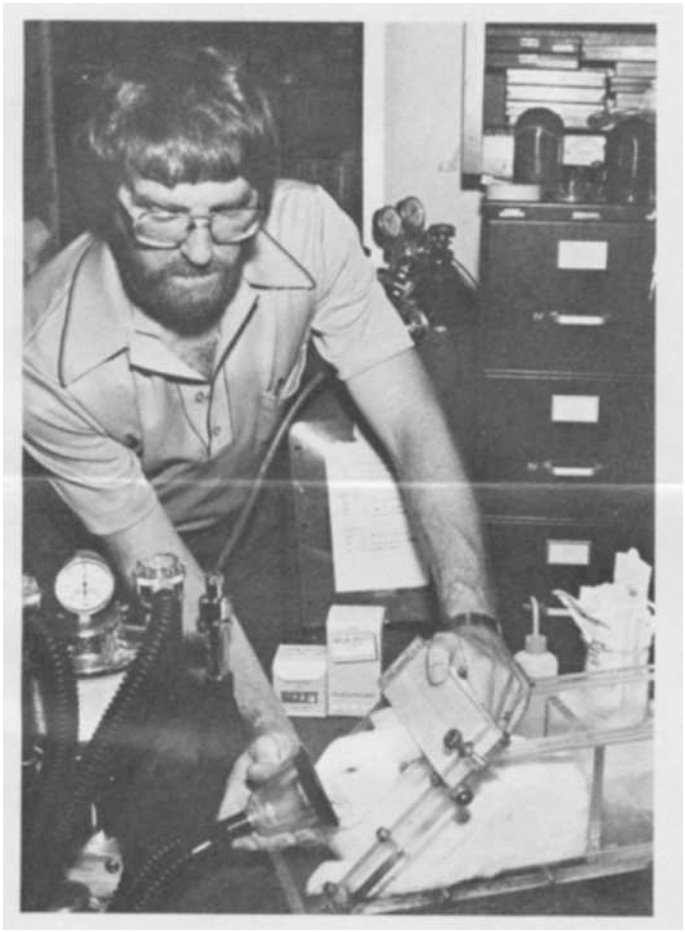


FIGURE 2

Alpha Chlorolose. If a more excitable nervous system is desired for such projects as brain mapping, etc., alpha chlorolose can be used in the rabbit. Gormezamo (personal communication) has recently found that a dose of 85-90 mg./kg. body weight of chlorolose dissolved in propylene glycol and injected LV. as above, produces good surgical anesthesia. For animals below 2 kg., the lower dose is indicated, while above 2 kg. the 90 mg/kg. is often required. As with pentobarital, the rabbit has a small safety margin for alpha chlorolose and care must be taken to avoid overdose. The induction time for alpha chlorolose is about 20-30 minutes after LV. injection, and the first signs of induction include a pronounced horizontal nystagmus. As with nembutal, the cannula should be left in place and flushed before and after anesthetic administration. To prepare the anesthesia, it is best to mix a 5'% solution by putting 1.0 gram of chlorolose powder into 20 cc of propylene glycol heated to 40°C. The powder is not sufficiently soluble in cool glycol and even at 40°C, stirring for about 30 minutes is necessary to dissolve the powder. If the glycol is heated to over 40°C, some of the alpha chlorolose will be changed to beta chlorolose, a less effective anesthetic. Once in solution, the mixture can be held at body temperature for administration and will keep in a water bath for several hours.

Following induction, chlorolose anesthesia is prolonged, one dose sufficient for 2-3 hours. Small supplementary doses can be given through the ear vein cannula. Recovery is risky although we have let several

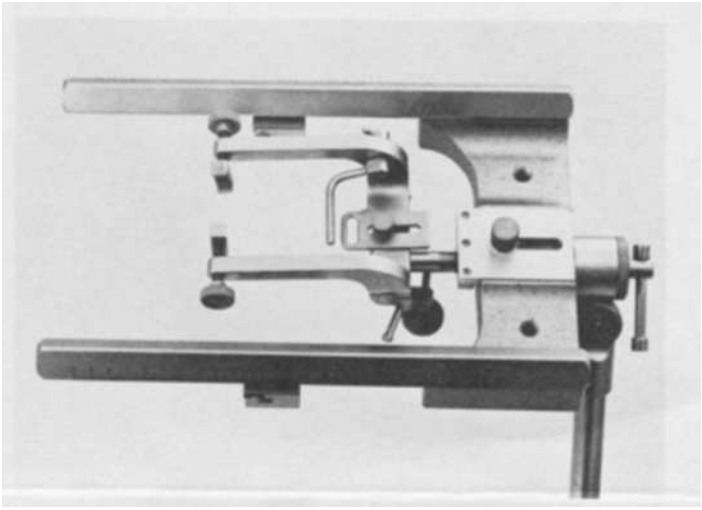


FIGURE 3

animals recover with no apparent ill effects such as kidney damage. However, chlorolose is not recommended for procedures in which recovery is necessary.

When an even more active brain is necessary and no recovery is desired, chlorolose and urethane can be combined. A dose of 40 mg./kg. chlorolose and .75 grams/kg. urethane LV. may be used. The chlorolose is mixed as above, and the urethane mixed in distilled water. The animal's response will vary somewhat to this combination, but the effect is long lasting and produces an active nervous system. Kidney damage and edema of the lungs are side effects of the urethane, thus recovery is not to be expected.

Halothane. Probably the most easily used rabbit anesthesia in terms of safety is the gas, halothane. (Ayerest Laboratories, Inc., New York, 10017, Fluothane brand.) As with many inhalation anesthetics Fluothane should be used in well ventilated rooms or with closed delivery systems, due to possible deleterious effects with long exposure in humans.

While extremely safe in terms of the animal, the gas anesthesia requires expensive equipment and a special vaporizer for its use. This equipment is commercially available from most medical instrument supply houses (e.g. Victor Instruments, Inc., P.O. Box 17900, Irvine, California 92713). Anesthesia induction can be carried out with the rabbit restrained in a box or wrapped in a towel by placing a small animal nose cone over the nose and mouth as seen in Figure 2. With a 5% halothane concentration delivered in oxygen, induction takes 10-15 minutes. Recovery to the stage of struggling, after this brief induction time is about 3-5 minutes, so the subject must quickly be placed in the surgical apparatus and put on anesthesia again after initial induction. The concentration can then be reduced to 1.5-2% and will provide anesthesia for many hours. We have had almost no fatalities with this gas, and the animal does not have respiratory distress such as that often seen with pentobarbital.

One of the major problems of using a gas anesthetic for the rabbit when stereotaxic surgery is to be performed is in delivery of the gas while the animal is in the stereotaxic frame. Unlike the cat which is easily intubated, and can thus be respirated through an endotrachial tube while in a stereotaxic holder, the rabbit

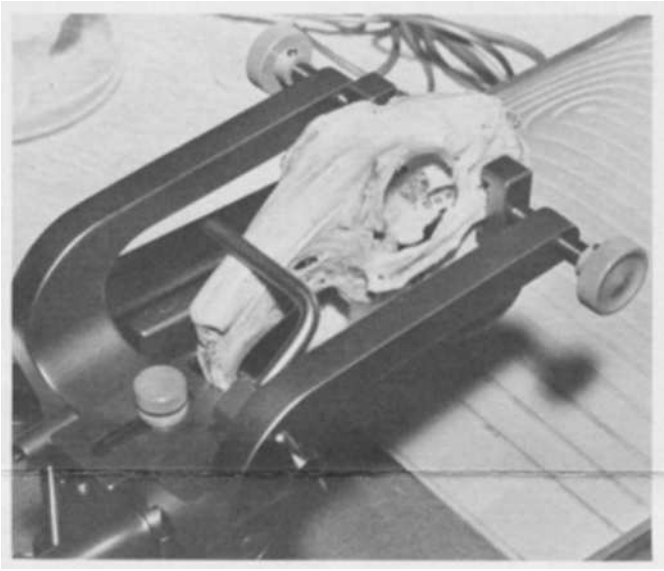


FIGURE 4

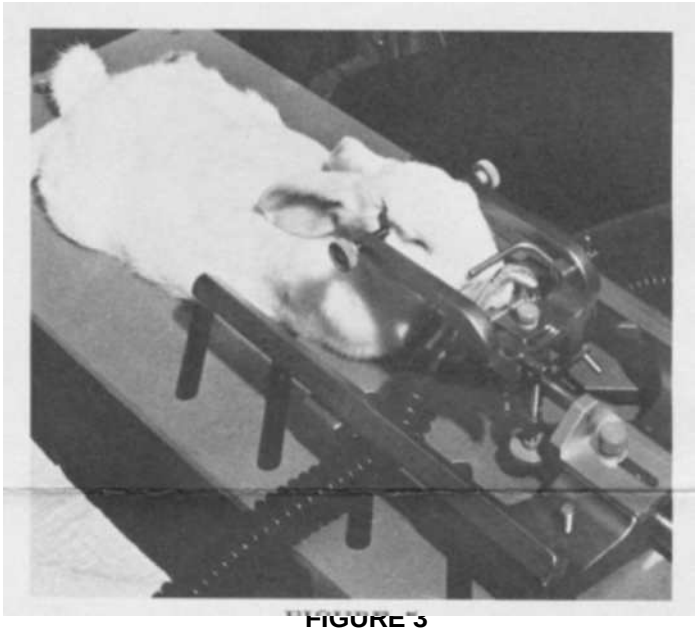


FIGURE 3

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.

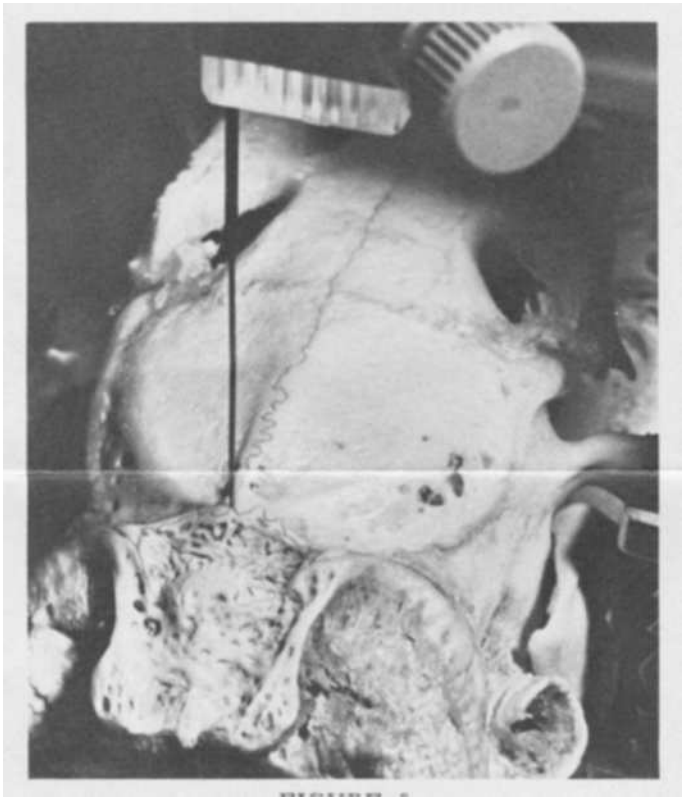


FIGURE 6

The problem of respiratory distress while putting animals anesthetized with halothane into the clamps does not occur. Obviously, if the animal begins to struggle while the clamps are being tightened, it should be released and more anesthesia administered. Once the animal is in place and the clamps on the head, the screws should be tightened until firm when turned, both screws being advanced about equally. Care should be taken not to tighten the clamps too tightly as this will break the temporal zygomatic process and perhaps the underlying mandibular condyles. It is usually necessary to retighten the clamps a turn or two after 30-45 minutes as the tissue fluids are forced from the tissues under the pressure of the clamps. Figure 5 shows a rabbit correctly placed in the stereotaxic instrument under halothane anesthesia. Note the gas mask adaptor in place.

Once the animal's head is firmly secured in the headpiece, the top of the head should be shaved and a 5 cm incision made in the midline starting between the eyes and extending back to the point where the skull slopes sharply down. Hemostats clamped midway along the skin edges open the wound for easy visualization and scraping of the tissue away from the skull. The periosteum should be separated from the bone until most of the top of the skull is clear. In smaller rabbits, the zygoma clamps may interfere with obtaining sufficient lateral clearing of the skull and in this case, the head may have to be repositioned with the clamps set somewhat lower.

Once bared, the skull usually has several areas of bleeding which can be stopped with bone wax or silver nitrate cautery. The skin and muscle edges also may need some mild cautery to stop oozing of blood although often a small covering of cotton or gelfoam will suffice. The bleeding must be completely stopped in order that the skull landmarks can be pinpointed and if electrodes are to be chronically implanted, so that the dental cement will adhere to the bone.

Due to the fact that the positioning of the head is variable in the headholder during initial placement, the head !Trust be leveled within the stereotaxic frame using skull bony landmarks. Most current rabbit atlases (for a free list of atlases, write to David Kopf Instruments) are constructed with the skull positioned such that the two main skull landmarks, bregma and lambda, are set with lambda below bregma. In order to visualize these marks, the skull should be cleaned with normal saline, and if a bleached bone is required, with a dilute hydrogen peroxide solution. The cleaning usually leaves the skull sutures clearly visible as red or brown lines across the field. Bregma, the crossing of the longitudinal and coronal sutures, is often difficult to place precisely due to the failure of the two halves of the coronal suture to meet at the midline. In this case, a felt tip pen should be used to make a mark midway between the two halves of the coronal sutures for future reference. Lambda, the crossing of the occipital and longitudinal sutures, is also sometimes hard to accurately place for similar reasons and because it is on the steep down slope at the back of the skull. A mark at the best guess for lambda is also indicated in such cases. Once the two suture landmarks have been defined, the head must be positioned level from side-to-side and with lambda 1.5 mm lower than bregma. A blunt needle is best used for this purpose as a sharp needle is more apt to penetrate the skull and not be noticed. The needle is put in an electrode carrier and moved to touch bregma. Care should be taken not to force the needle onto the skull too -tightly when lowering the carrier as this can result in several tenths of a millimeter error. With the needle tip resting on bregma, the dorsoventral reading is taken from the carrier. The electrode carrier is then raised and moved to lambda (it is shown in this position in Figure 6) where a second reading is taken. Care must be taken to accurately position the needle for the lambda reading since the slope of the skull can cause the needle to slip down as it is lowered, causing large errors. If the bregma-lambda difference is 1.5 mm, the front-back plane is correct. If not, the head holder can be tipped up or down. Figure 1 shows the Kopf adaptor with a screw adjustment for tipping up or down. This feature can be added to most older adaptors.

With practice, the experimenter will be able to adjust the head to within =.1 mm by eye. The experimenter should also check the position of the longitudinal suture as the electrode is moved from front to back. If the head is not straight, the simple expedient of screwing in one zygoma clamp while releasing the other will not straighten the head, it will simply slide the head over. To adjust the alignment in this plane, the clamps must be loosened and the skin pulled through under one or the other clamps, or the clamp rotated slightly to force the nose one way or the other. In some cases, the animal will have to be repositioned completely. Obviously, the saggital plane is the hardest to straighten since there is no adjustment in the head holder which allows the holder to swing from side to side. Thus, if this adjustment is necessary, it should be made before further attempts to level the bregma-lambda axis.

Once the skull has been positioned correctly in the saggital plane with the bregma-lambda difference correctly set, it is very important to make sure the side-toside or horizontal plane is level. For this the electrode should be taken 5 mm to

each side of bregma and vertical readings taken. If the side-to-side variation is over 0.1 mm, the holder should be rotated about its central axis to assure a level horizontal placement. This adjustment is crucial for deep electrodes, as a horizontal error compounds with depth.

These corrections for head placement can be somewhat time-consuming, especially for the inexperienced investigator. Obviously, they are crucial in achieving accurate electrode placement, and therefore, must be done with care. In a recent article, Crawford, Kennedy and Lipton, (1977), have described a simple device for helping place the head in the correct bregmalambda attitude. Called a planilabe, it is a metal plate with one fixed and one moveable tip which extends 1.5 mm below the fixed tip. When placed in an electrode carrier, the fixed tip is placed on bregma and the moveable one moved to lambda. Since the two tips are in the correct dorsal-ventral plane, if both touch the skull landmarks, the skull is positioned correctly. If not, the head bolder can simply be moved up or down as necessary. If in addition, two other tips were placed 5 mm on either side of the fixed bregma tip, the skull could also be placed in the correct saggital and horizontal planes in one operation. The planilabe is an excellent device and its use would undoubtedly reduce stereotaxic error considerably.

With the skull in the correct stereotaxic position, the experimenter can then put the desired electrode, cannula, etc., in the electrode carrier and proceed to zero the device at the bregma landmark. For this, the device to be introduced into the brain is lowered to touch bregma and the three coordinates read from the carrier. Appropriate corrections are then made to bring the device to the skull position desired and holes drilled for placement. Stereotaxic implantation and skull attachment can then be done as usual.

With proper care in adjusting the head in the head holder, good, reliable brain placements can be achieved in the rabbit despite the lack of the secure mounting anatomy the cat possesses. We would caution the beginner to take extra care in placing the head in the holder and leveling the skull in all planes, as the procedure must be done with accuracy to achieve good brain placements. However, with the introduction of such refinements as gas anesthesia, the planilabe and further adjustments on the head holder itself, the routine placement of devices accurately in the rabbit brain is readily achieved.

It has been our experience that the rabbit is quite resistant to infections from surgery. It is usually not necessary to observe strict asceptic measures when doing implants, although good, clean technique is necessary. We have also found that rather than using suture for wound closing following chronic implants, lesions, etc., stainless steel woundclips (9 mm size) work very well for closing. The clips fall out when the wound is healed, and the animal makes no attempt to dislodge them during recovery. Thus, removal of the clips in long term chronic animals is not necessary.

ACKNOWLEDGMENTS

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FIGURES

FIGURE 1. The Kopf rabbit adaptor for stereotaxic surgery. Note the long nose clamp and tooth bar for use with smaller animals and the screw adjustment for up and down positioning of the instrument.

FIGURE 2. Method for inducing anesthesia in a rabbit with halothane. The mask covers both the nose and mouth and delivers a 5'% halothane 95% oxygen induction mixture.

FIGURE 3. Proper placement of the rabbit adaptor in the stereotaxic frame is shown in this view.

FIGURE 4. The rabbit skull is shown correctly placed in the adaptor. Note the position of the zygoma clamps on the temporal process of the zygomatic arch. With smaller animals, the nose clamp will strike the skull further forward as the tooth bar is moved back in order to achieve correct zygoma clamp placement.

FIGURE 5. A rabbit under halothane anesthesia correctly mounted in the adaptor. The zygoma clamps are positioned such that the ears are held slightly back, but not sufficiently high to preclude free access to the back of the skull. The anesthesia is delivered through the gas delivery mask developed for the rabbit adaptor (see text).

FIGURE 6. A view of the rabbit skull with a needle electrode positioned on the lambda bony landmark. Note the bregma landmark at the coronal and longitudinal suture crossing just behind the orbits, and the slight offset of the coronal sutures at the midline.

THE MIGHTY RUTH

Shown Above

For those attending the annual meeting of the Society for Neuroscience, Sunday, November 6, the Mighty Ruth will be available for your listening and viewing pleasure. Starting time 1 p.m.

Kopf's Old Time Music Company proudly presents a gigantic Ruth & Sohn Concert Organ direct from the Oktoberfest in Munich, Germany.

Built in 1908 in the Black Forest village of Waldkirch which was at that time the organ manufacturing capital of Europe, this crowd pleaser is a superb example of the finest in German craftsmanship. Its ornate wooden front features 7 hand-carved figures which are animated to keep time to the music.

And what wondrous music! This 96 note keyless, book-operated concert organ plays more than 500 wooden pipes along with a glockenspiel, drums and cymbal. The sweet melodious tone of the organ allows it to play overtures as well as grand opera. More than 900 meters of classical German music including marches provide many hours of listening pleasure.

This gem of the Black Forest has had only two previous owners who each used it in conjunction with rides at fairs; from 1908-1942 the Rohweder family in what is now East Germany, and from 1942-1976 Willy Gast in Munich. Mr. Gast hid the organ in the Black Forest during World War II to protect it from possible destruction and has played it only at the Oktoberfest since 1948.

It is not likely you will ever again see or hear anything quite like this. Out of approximately 50 large organs manufactured in that era, there are probably fewer than 6 in existence today. At 8-1/2 feet high x 16 feet long, this Ruth, model 38 a, is the largest organ of its type in the U.S.

Although many parts were so well made that they have never needed repair, this magnificent music machine has been lovingly restored, where necessary, to play as it did in 1908 when its beautiful tone was first heard echoing across the valley of Waldkirch thrilling the hearts of all who heard it.