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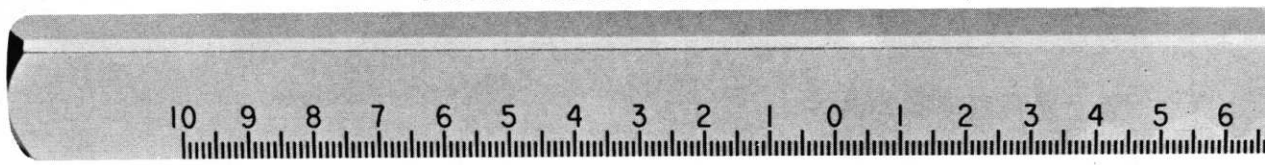

Using the Scouten Knife With Rabbits

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The destruction of fiber bundles in selected regions of the brain vis small knife cuts made with a knife assembly attached to the electrode carrier of a sterotaxic instrument has an obvious attraction to neuroscientists interested in functional changes resulting from brain manipulations. The development of the retractable wire knife blade makes it possible to lower a knife assembly, consisting of a piece of tubing containing a small diameter wire, to a preselected location in the brain and make a small cut by extending the wire a few millimeters and then raising or lowering the tube. This produces minimal damage to surrounding or overlying tissue, while cutting fibers that lie perpendicular to the blade. These kinds of knife cuts have been previously utilized in the rat (e.g. see Robertson, Laferriere & Mil-ner, 1986). The attractiveness of using this procedure in the rabbit originated from our concern that chemical or RF lesions could not be as easily accomplished in the rabbit as in rats due to the larger brain of the rabbit.

We have been studying the role that prefrontal structures, especially granular prefrontal cortex, play in classical (Pavlovian) conditioning of cardie as well a as other autonomic changes. We have used the rabbit preparation, developed by Gormazano (1966) and later Schniederman (1970), for his purpose. It appeared to us that tone possible way of assessing whether these structures were involved in associative cardiac changes, and their relationship lo other classically conditioned responses, would be to interrupt the descending fibers from these structures by making parasa-gittal knife cuts in the basal forebrain. After some initial experience with the Kopf Scouten knife assembly, we have come to view this instrument as especially good for this purpose. We have now used this knife not only for our original purpose of making parasagittal knife cuts to interrupt the sublenticular efferents from the amygdala and prefrontal cortex, but also to study the relationship between the mediodorsal nucleus (MD) in the thalamus and prefrontal cortex by making parasagittal cuts lateral to MD in the thalamus, which interrupt the efferents from MD to the prefrontal area.

We are using the Kopf 1204 stereotaxic frame, with a Kopf rabbit attachment to hold the animal's head in the instrument, and a 1260 electrode carrier modified with a mi-cropositioner for holding the Scouten knife assembly. The original knife assembly which we purchased was based on its use in the rat. However, the larger size of the rabbits head and its position in the 1204 frame did not allow enough "play" above the animal's head to properly place the knife blade over the animal's skull. Thus, a special knife blade assembly has been constructed by Kopf in which the blade assembly itself is approximately 1 centimeter shorter than the standard blade. This allows the proper placement of the carrier and the knife assembly above the animal's head. However, even with this shorter blade assembly there is little extra room for using the knife for this purpose in the rabbit. It is possible that other more recent stereotaxic frames may allow more distance between the animals head and the furthest extent of the microposilioner in the dorsal-ventral dimension.

The Scouten knife assembly can be mounted in any of the Kopf electrode carriers. The knife assembly itself contains a drive knob for extending and retracting the blade. The knife blade assembly, consisting of a cannula and the moveable wire blade, is inserted into a holder mounted on the electrode carrier and oriented in the proper direction by set screws. Once the blade is inserted in the assembly, proper extension of the knife blade itself to a preset dimension (varying from .05 to 3 mm) is accomplished by the drive knob. Set screws make it possible to extend the knife with the proper number of revolutions of the drive knob to its preset length. One revolution of the drive knob moves

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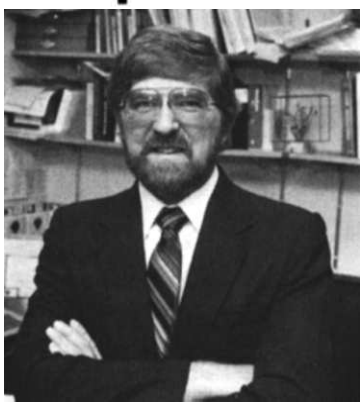

Editor's Column

The summer weather is in full swing here in southeastern Ohio. It has been hot and dry for so long that some of us are actually wishing for winter! The lawns are brown and in many communities there are watering bans in effect. The drought is becoming very severe and of great concern. As scientists, we need to be very concerned about the welfare of our animal subjects in this son of weather. The breakdown of air conditioning systems or the lapse of proper water supplies for even a short time can have very detrimental effects on the animals, so don't forget to check on your animals (both scientific subjects and pets) frequently during this sort of weather. It only takes a short time for an animal to succome to heat prostration.,

The response to the list of available back issues of the Carrier which was included in the last issue was fantastic. We had a real flood of requests for many of the available back issues and hope that everyone has received those that they requested by now. If you have not received any back issues that you requested, please let me know at the address below and I will get them to you as soon as possible. If you need a list of the available back issue, just let me know that also and I'll get it to you. Remember that Kopf Instruments also has available either from me or from the company, an annotated list of stercotaxic atlases which is free and will be sent to you at your request.

Please remember if your are sending in any of your stereotaxic equipment to the Kopf factory for repair or recalibration, that it must be clean (preferably sterilized) and accompanied by a letter that it is free of pathogens. If you were working on such equipment, I'm sure that you would want some assurance that it was at least clean.

I hope that your summer is productive and enjoyable. See you at Neurosciences in Toronto.

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the blade out by 1 mm.

We have used the 122 blade, modified as noted above for use with rabbits. After anesthetization with ketamine and chlorpromazine, the rabbit is placed in the stereotaxic frame and the head aligned using the Kopf rabbit adapter and zygoma clamps. A hole is drilled over the area of interest and the knife assembly is lowered to the proper depth in the brain after previously aligning the knife blade assembly in the proper orientation. This can be done in two ways, either by thumb screws, which hold the actual knife blade assembly in the holder, or by a thumb screw at the lop of the holder which allows for the rotation of the entire assembly. This knob is calibrated in 15 degree units and thus allows for proper calibration of orientation when bilateral cuts are necessary. In most of our experiments parasagittal knife cuts have been employed (although coronal cuts have also been made in some animals), so that the orientation of the knife blade when it is extended can be accomplished with reference to the midsagittal plane. We always extend the knife blade and then retract it just prior to lowering it into the brain to insure that the blade is still intact.

We puncture the dura above the area to be cut and lower the assembly with the knife blade retracted to a depth at the lowest level of the cut to be made. The blade is then extended. It is extremely important the the blade be extended slowly, and even more important that when the cut is made that the assembly be raised at an extremely slow rate. We use the DKI micromanipulator which was installed as a modification on the 1260 carrier. This allows the blade assembly to be raised at a rate of less than 1 millimeter per minute. We found by early manipulations with the knife assembly that if it was raised too rapidly uneven cuts resulted. We presume that this is caused by the fact that the knife blade bends with rapid raising of the assembly. In fact in some cases the blade actually broke off. Thus, through much experience with raising and lowering the as-"SCffibly, we have foondThat If it is raised at a rate of 1 mm per minute or less, good knife cuts usually are found on histological analysis. In some cases in which it was necessary that mylenated fiber tracks be cut (e.g. when going through the internal capsule), the blade has been retracted after the first cut and the assembly lowered to its original position and raised slowly a second time. This insures that all of the fibers are cut by making a second pass through the area. Our parasagittal knife cuts have all been made with a knife extension of 3 millimeters. We have determined thai it is impossible to make longer cuts. If the blade is extended more than 3 millimeters it is likely to break off during the time it is raised. In some cases in which we wanted to interrupt the sublcnticular as well as posterior internal capsule carrying downward fibers from the frontal cortex we have made two cuts on each side which overlap by 1 millimeter. The actual length of the cut was thus 4 millimeters in length. The overlap in ipsilateralcuts was to allow for possible errors in

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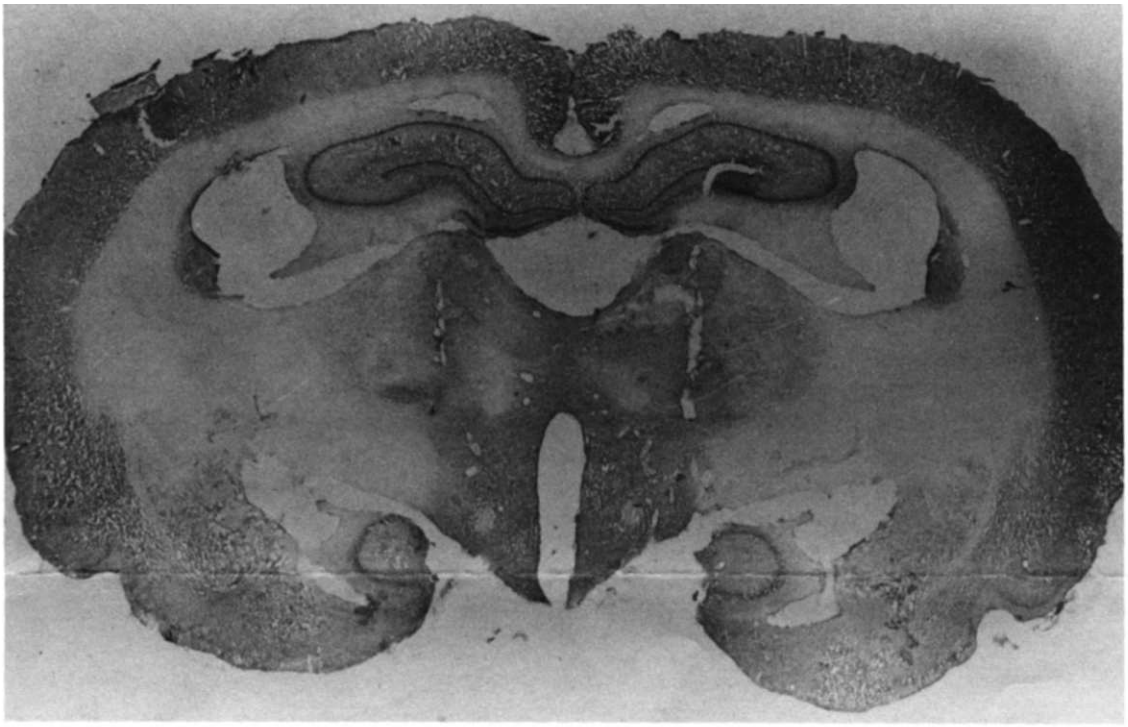

Figure 1. Photograph of section through the thalamus showing bilateral parasaggital knife cuts made just lateral to the mediodorsal (MD) nucleus (thiomin stain x 25).

head orientation or in placement of the assembly into the brain.

The blade itself is very fine, viz., .13 millimeters in diameter. The blade cannula that penetrates the brain is .3 millimeters in diameter. The small diameter of the blade and the fact that it can be adjusted in and out of the cannula puts some tension on the blade so that the knife tips break off from time to time as the blade is repeatedly extended and withdrawn. The blade supplied is 20 millimeters in length allowing extended use before the entire assembly needs replacement. However, we have found that rarely can we achieve (he extended length of 20 millimeters due to the frequent "catching" of the blade on the cannula as it is extended or retracted, or due to improper care of the blade.

It is imperative that the knife blade itself be properly cleaned after use. We believe that improper cleaning is the most frequent cause of breakage of the blade; the most frequent occurrence is not that the blade breaks off, but that it becomes impossible to extend the blade out of the cannula which holds it. This always occurs when the wire blade itself is not properly cleaned. We have found that in many cases proper cleaning consists simply of rinsing under tap water. However, it is then necessary that the blade be completely dried by shaking and blowing on it before it is retracted into the cannula. If blood or other tissue is on the blade after it is retracted it sticks to the cannula and the blade cannot then be extended. Similarly we have found that in some cases the blade stuck in the assembly because it was retracted while it was still wcl. Thus the best procedure is to clean it with ethanol which drys quickly.

In summary, we have made either parasagittal or coronal knife cuts in the preoptic area and hypothalamus and in the thalamus lateral to the mediodorsal and anterior nucleus in over 300 rabbits in our laboratory. An example of knife cuts lateral to MD in the thalamus is shown in Figure 1. Neuronal degeneration and glial proliferation in MD that resulted from this cut is illustrated in Figure 2 (on back page). Using the procedures which we have described it is possible routinely to get extremely precise cuts of the type illustrated in these figures.

REFERENCES

Gormezano, I.(1966). Classical conditioning. In J.B. Si-dowski (Ed.), *Experimental Methods and Instrumentation in Psychology*, New York: McGraw-Hill, 385-420.

Robertson, A., Laferriere, A., & **Milner, P.M**. (1986). The role of corico-cortical projections in self-stimulation of the prelimbic and sulcal prefrontal cortex in rats. *Behavioral Brain Research*., 21,129-142.

Schneidcrman, N. (1970). Determinants of heart rate classical conditioning. In S. Reymierse (Ed.), *Current Issues in Animal Learning,* Lincoln, NE, University of Nebraska Press.

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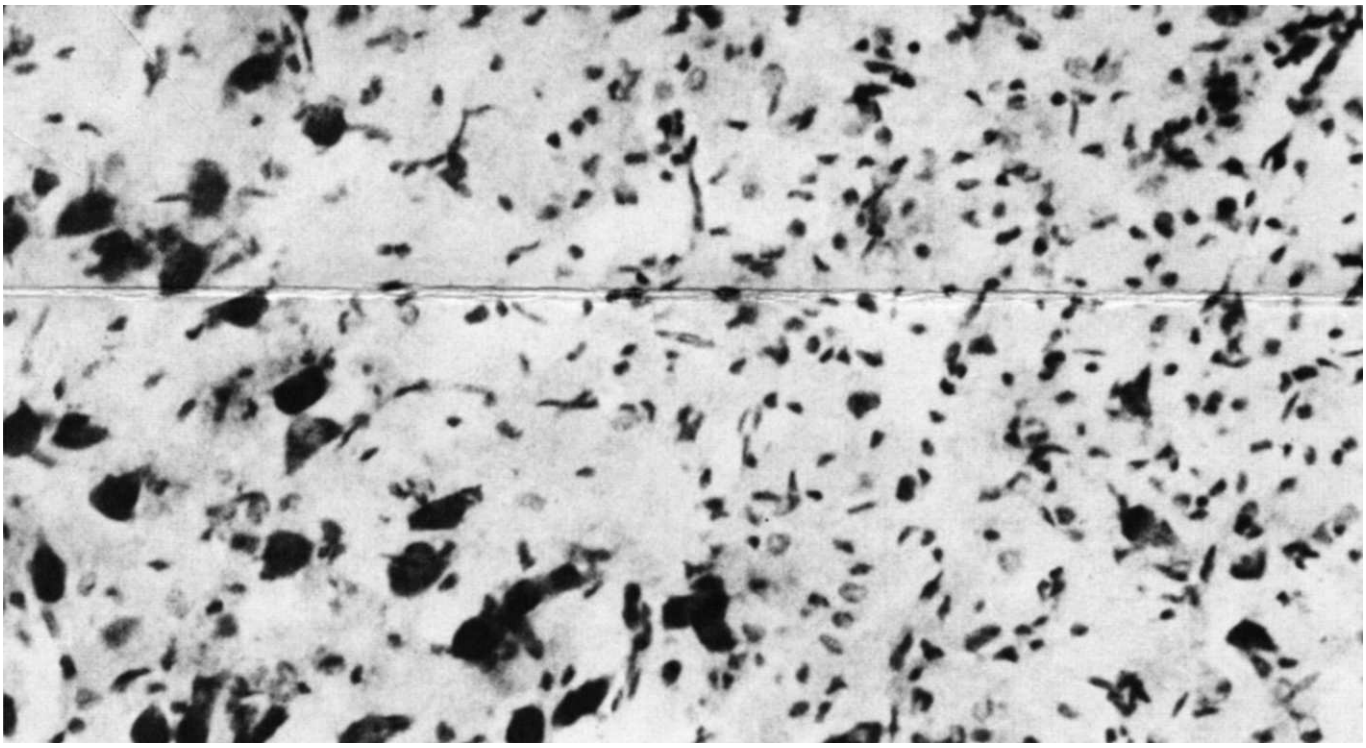

Figure 2. Higher magnification photomicrograph (x!25) of MD showing effects of severing MD effer-ents by knife cuts as illustrated in Figure 1. Note demarcation between neuron free area and glial proliferation (right) and undamaged area with normal neuronal cells (lower left). The upper right neuron free area is medial MD, while the lower left depicts the thalamic midline nuclei that project to the frontal areas in a medial direction through the cingulum and thus are unaffected by lateral knife cuts.