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A Simple Toothbar Adaptor for Neonatal Stereotaxic Surgery with Rat

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A common problem associated with neonatal stereotaxic surgery with rat is the stabilization of the cranium within the headholder-mainframe unit. First, neonatal rats (ca. 8-15 day) have poorly developed incisors. Thus, adult toothbars normally used with the Series 900 stereotaxic unit cannot be used for neonatal stereotaxic surgery. Second, the calvaria are poorly ossified in the neonate, particularly the frontal quadrant. The use of an adult nose clamp is therefore precluded to stabilize the neonates' upper incisor on specially constructed toothbars. Such factors have prompted many investigators to construct "whole-body" supports for neonatal stereotaxic surgery with rat to obtain some degree of stability in intended stereotaxic coordinates. Although "whole-body" supports at least partially circumvent problems associated with rigid cranial mounting of neonatal rats, such supports do not provide sufficient stability for reliable electrode or micropipette placements into deep brain structures. We describe herein a simple and reliable toothbar adaptor for use with a Series 900 stereotaxic unit. This adaptor provides an inexpensive and effective alternative to "whole-body" supports during stereotaxic surgery.

Guidelines for construction of the neonate toothbar were derived and modified from the stereotaxic atlas of Sherwood and Timiras (1970). That atlas offers several distinct advantages in investigations of the developing rat brain. First, coronal sections of the rat brain are provided at three developmental epochs (10 day, 21 day and 39 day), using both Nissl-stained material (Thionin) and myelin-stained material (Sudan Black B). Thus, both nuclei and fiber tracts are shown at various developmental periods. Second, variability of stereotaxic coordinates are provided as a function of age and specified stereotaxic alignment geometry.

The upper panel of Figure 1 shows construction details for the neonate toothbar adaptor that can be used with a Series 900 headholder. The adaptor

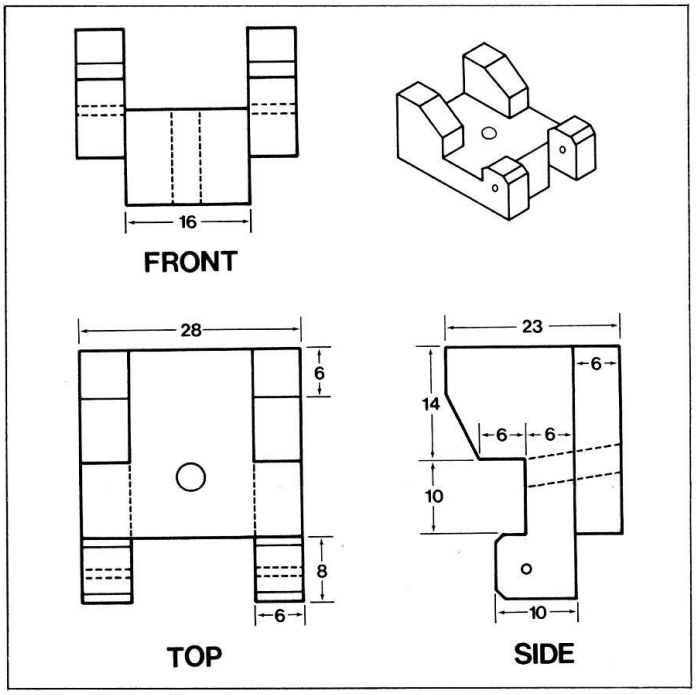

Figure 1—Upper Panel

is constructed entirely of lucite, with the exception of 21 gauge stainless steel tubing that serves as the upper incisor bar. The adaptor, as shown, can be used with neonates in the age range of 7-16 days. Older rats, within the age range of 18-40 days, can be stabilized with the standard adult toothbar. The lower panel of Figure 1 shows the adaptor mounted below the adult rat toothbar provided with the Series 900 unit. Toothbar elevation (for horizontal zero plane adjustment) can be

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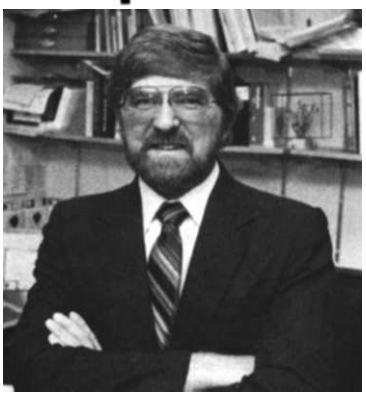

Editor's Column

It is time for another winter/ spring issue of the Kopf Carrier. We hope you enjoyed the last issue, the "tour" through the Kopf factory. It was a unique experience for me to go through the plant and to see how the equipment that has become the standard is made. I encourage anyone who is in the area of the Tujunga plant to make a point of stopping by to see the operation.

The new regulations put out recently by the DHE W on animal care and welfare contain several changes and additions which are designed to encourage better animal use in research. One of these is that not simply clean, but aseptic surgery must be used in all cases where any body cavity is opened and the animal is to survive surgery. This applies to all animals of the lagomorph or higher groups. While it may be argued that conscien-ciously applied clean surgical techniques are sufficient for implanting electrodes in rabbit deep brain structures, such surgery now falls within that necessitating aseptic conditions. If such conditions also necessitate the sterilization of the electrode carrier, either gas or sterilizing solutions should be used, not heat sterilization. The use of high temperatures will have adverse effects on the slides of the carriers, say the Kopf engineers, and may necessitate the return of the carrier to the factory for reconditioning. Just remember that the Kopf equipment is high precision and must be treated with care to maintain its unparalleled accuracy. If you have a question about the use or maintenance of your Kopf equipment, just call or write to the company for the answer.

As this is written, it is almost Christmas. The column is being written on an Apple He computer while we are driving through the farmlands of Illinois. Equipt with a portable power supply and a flat screen, the He is a very nice portable which sits on the lap with minimal inconvenience. In fact the biggest problem encountered thus far is the small amount of interference the computer is causing in the reception of a very weak station one of my sons is listening to on his Walkman personal radio. It seems he likes different sorts of music than do his parents, hence his dependence on the

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Walkman when in the car. The advances in personal computers (and in radios, as evidenced by the Walkman) have been dramatic since the first Apple computer was introduced about seven years ago. It seems likely that the next seven years will bring about even more radical advances in the field as personal computers become smaller and more powerful. We will soon think of the computer much as the electric motor was thought of in 1905, when it was said that the motor seemed like a good thing and perhaps every home should have one. Now, the average home in the US has over 50 electric motors. However, it will not take 80 years for that change to come with computers, but probably more on the order of 10.

We hope that all of the readers of the Carrier have had a good start on the new year. If you have any questions about Kopf equipment which you would like discussed in the Carrier, or would like to write an article for the publication, please contact the Editor at the address below.

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Toothbar Adaptor (continued)

adjusted by raising or lowering the adult toothbar and/or neonate adaptor as required. The reader is referred to the stereotaxic atlas of Sherwood and Timiras (1970) for further details of alignment geometry, and Glanzman & Lasiter (1981) for methods of stabilizing toothbar elevation as a function of skull size.

We have discussed elsewhere problems associated with barbiturate anesthesia in stereotaxic surgery (Lasiter & Garcia, 1984). Most notably, barbiturate anesthesia in rodents markedly depresses the myocardium, giving rise to hypoxia, hypercarbia and altered cardiovascular function. Such effects are greatly exacerbated in the neonatal rat. Because barbiturate anesthesia is associated with high mortality in neonatal rats, we routinely use methoxyflurane (Metofane™) as a general inhalation anesthetic agent. Methoxyflurane provides a wide margin of safety at surgical planes of anesthesia (level 3), promotes analgesia that persists for some time during postoperative recovery and does not markedly depress the myocardium or cardiovascular function. Details

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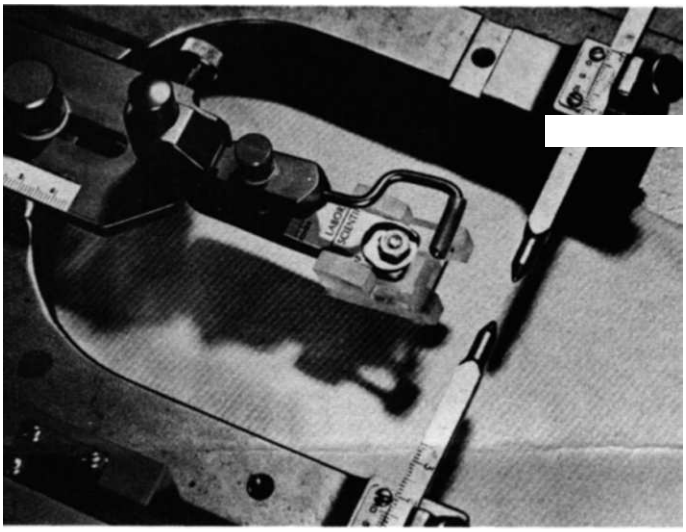

Figure 1 Lower Panel

concerning the operation of a methoxyflurane anesthesia system for stereotaxic surgery are described by Lasiter and Garcia (1984).

The upper panel of Figure 2 shows construction details for a methoxyflurane delivery system that is used with the neonate toothbar adaptor. As is evident, the nose cone assembly serves two functions: The nose cone a) stabilizes the neonates' upper incisor in the toothbar adaptor, and b) delivers vaporized methoxyflurane to maintain surgical planes of anesthesia. The adult nose clamp extends beyond the pivot point of the nose cone, which rests on the toothbar adaptor. Hence, as the adult nose clamp is tightened the nose cone exerts downward and rearward pressure on the neonates' frontal calvaria. This pressure stabilizes the neonates' upper incisor

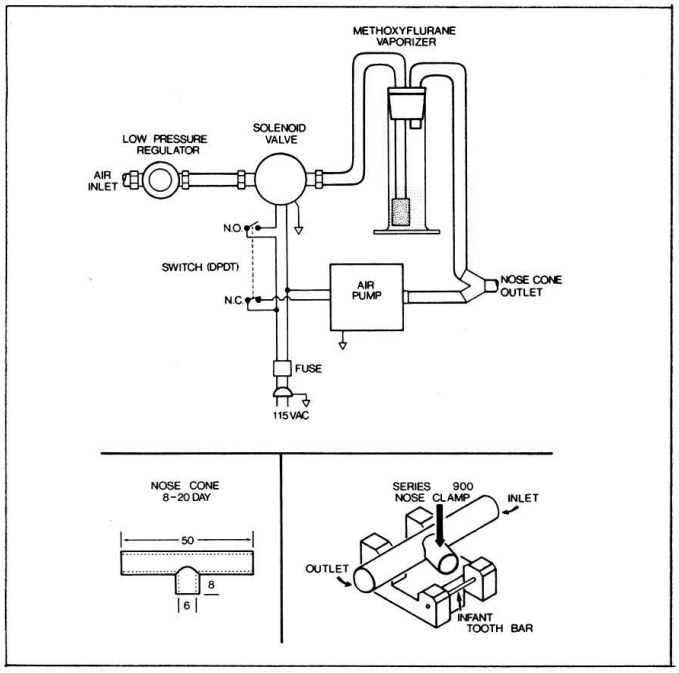

Figure 2 Upper Panel

on the toothbar adaptor without inducing excessive deformation of the frontal calvaria. It is important to note that a circular nose cone clamp is essential, regardless of whether or not methoxyflurane is used as an anesthetic agent, because the clamping load is distributed over a large surface area of the nares. As mentioned previously, the frontal calvaria are poorly ossified, and pressure exerted by the adult nose clamp can cause deformation and/or permanent damage to this region in the neonate.

The lower panel of Figure 2 shows a neonatal rat (10 day) mounted in a Series 900 unit using the toothbar and nose cone adaptors described herein. Tapered ear bars, which are normally used with blunt ear canal protectors, are used for 8-10 day old rats. During surgical procedures the animal is supported by a lucite block, measuring 7 cm (L) X 4 cm (W) X 4 cm (H), that receives warm circulating water (37-40°C). The warming block should be used in all surgical procedures lasting more than 15 minutes to minimize anesthesia-induced hypothermia.

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Figure 2 Lower Panel