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MEASUREMENTS OF TURTLE HEAD AND SEMICIRCULAR CANAL ORIENTATION USING A MODIFIED SMALL ANIMAL STEREOTAXIC DEVICE

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INTRODUCTION

The vestibular system detects and encodes changes in angular and linear accelerations of the head. This information is then used in a broad repertoire of vertebrate motor behaviors essential for survival. We are trying to understand some of the underlying mechanisms of vestibular function by studying components of the vestibulocollic reflex (VCR), one of the most basic mechanisms by which adjustment of head position is achieved. The function of the VCR is to stabilize head and cranial sense organs in space and is elicited by perturbations of the head. These perturbations stimulate angular and linear labyrinthine receptors and result in compensatory counter-rotations of the neck. Mammals have been typical subjects for such studies but in our work we chose turtles because this animal offers a number of theoretical and practical advantages for analyzing vestibular function in general and the VCR in particular. One advantage is that since turtles have a rigid shell and no back muscles, the vestibular control of head position must be achieved entirely by neck movements. Therefore the turtle might be considered a natural 'isolated head-neck' preparation.

A stimulus evoking the VCR is angular acceleration of the head and this stimulus is detected by a total of six semicircular canals: one horizontal and two vertical canals on each side of the head. The commonly held view regarding the spatial orientation of these canals is that the three ipsilateral canal planes are mutually perpendicular and this is perfectly mirrored by the contralat-eral canals. Closer examination of mammalian species has shown significant deviations from the 'ideal' arrangement suggesting that stimulation of one canal will result in some stimulation of another and the total pattern of activity set up by head rotation will depend on the angular relations between canals. As a consequence, it is necessary to know the planar relations between semicircular canals so that we may understand the labyrinthine output caused by head movement of any spatial form. What follows is a brief summary of a study we conducted to determine canal orientation in awake, resting turtles (Brichta et al., 1988).

TURTLE HEADHOLDER

To allow a case by case comparison of canal orientation it is necessary to hold the turtle head in a consistent and reproducible manner. Since turtle heads are significantly different from those mammals commonly used in animal stereotaxic devices, a modification in approach was required. In their turtle stereotaxic atlas Powers and Reiner (1980) provide a simple and effective solution in the form of a specially designed headholder. Dispensing with the normal rat mouthbar and ear-bars, they mounted the headholder on a Kopf small animal stereotaxic device (DKI 900) using cat earbars. The turtle headholder consists of two brass blocks with holes to accept the cat earbars. The blocks are connected by means of a precisely

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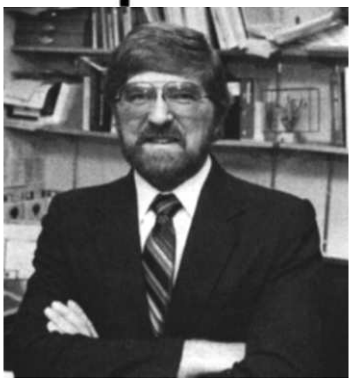

Editor's Column

Spring is coming to the Midwest (and, I suppose, to other parts of the country as well.) There are so many wonderful things in the world and spring seems to make them even better. Un-fortunatly, there are also many terrible things which we must continually strive to do what we can to correct. If only we can all be a little more understanding of one another, that will help.

I wanted to draw your attention to the April, 1994 issue of *Lab Animal.* This journal is a monthly publication which is sent to many of us who work with animals. In this issue, there are several articles which are of real interest to the research community. The first is by Frankie Trull and is entitled "The Research Movement". In her article, she reviews the development of the research movement, what it has meant to human progress, and how the Foundation for Biomedi-cal Research came to be. In a second article, (Public Relations and Animal Research) Gregory Mass of iiFAR gives a thoughtful discussion of public opinion and how we as scientist can better portray our issues. He also gives the history of iiFAR. In a third article, Jayne Mackta discusses various issues surrounding the communication to students of the benefits of animal research (Teaching Students the Benefits of Animal Research), while in a fourth article, Adrian Morrison discusses other ways to improve peoples perceptions of research (Improving the Image of Biomedical Research).

These articles are well worth reading and keeping for future reference. I would strongly encourage everyone to get copies of these articles for your files.
I hope you all have a great summer. We will be back in the fall with another issue of the *Carrier*. Our thanks to Alan Brichta for the very nice article in this issue.

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positioned mouthbar. The upper jaw of a turtle rests on the mouthbar and a nosebar clamps the snout to prevent movement especially about the interaural axis (pitching of the head) (Figure la). For our purposes the rat mouthbar is reintro-duced to provide a standard external reference point (Figurelb). Powers and Reiner emphasized two dimensions of the turtle headholder. 1) The distance between the center of the earbars and an imaginary plane continuous with the caudal face of the turtle mouthbar was 12.60 mm; 2) the distance between the interaural line and an imaginary plane continuous with the upper surface of the mouthbar was 3.86 mm. These precise dimensions not only ensure the turtle brain is oriented as it was in the atlas but also, as we determined, holds the horizontal canal plane parallel to the stereotaxic horizontal.

MEASUREMENTS

We used the modified small animal stereotaxic apparatus described above for both dry skull and live animal measurements. We collected two sets of data. One set of measurements were obtained from dry skulls and provided data on canal orientation. The second set of data, from both dry skulls and live animals, produced reference marks that would be used to determine head and predicted canal orientation in radiographic analyses of awake, resting turtles.

CANAL ORIENTATION

We placed the turtle skull into the stereotaxic apparatus and using a dorsal approach, we carefully exposed the canals of the bony labyrinth by drilling away the overlying bone. Using the method of Blanks et al. (1972) and the aid of a stereomicroscope we recorded the three-dimensional coordinates of up to 30 points along the fundus of each canal lumen using the tip of a fine needle held in a three-axis micromanipula-tor. Each canal data point was therefore expressed in x, y, and z coordinates using stereotaxic zero as the origin. Stereotaxic origin was defined as the midpoint of the interaural line when the skull was centered in the ear bars. In this coordinate system the x-axis is oriented earth-horizontally and aligned midsagittally with positive in the anterior direction. The y-axis is oriented earth-horizontally and aligned along the interaural line with positive in the animal's left direction. The z-axis is oriented vertically, positive up (Figure 2). The data

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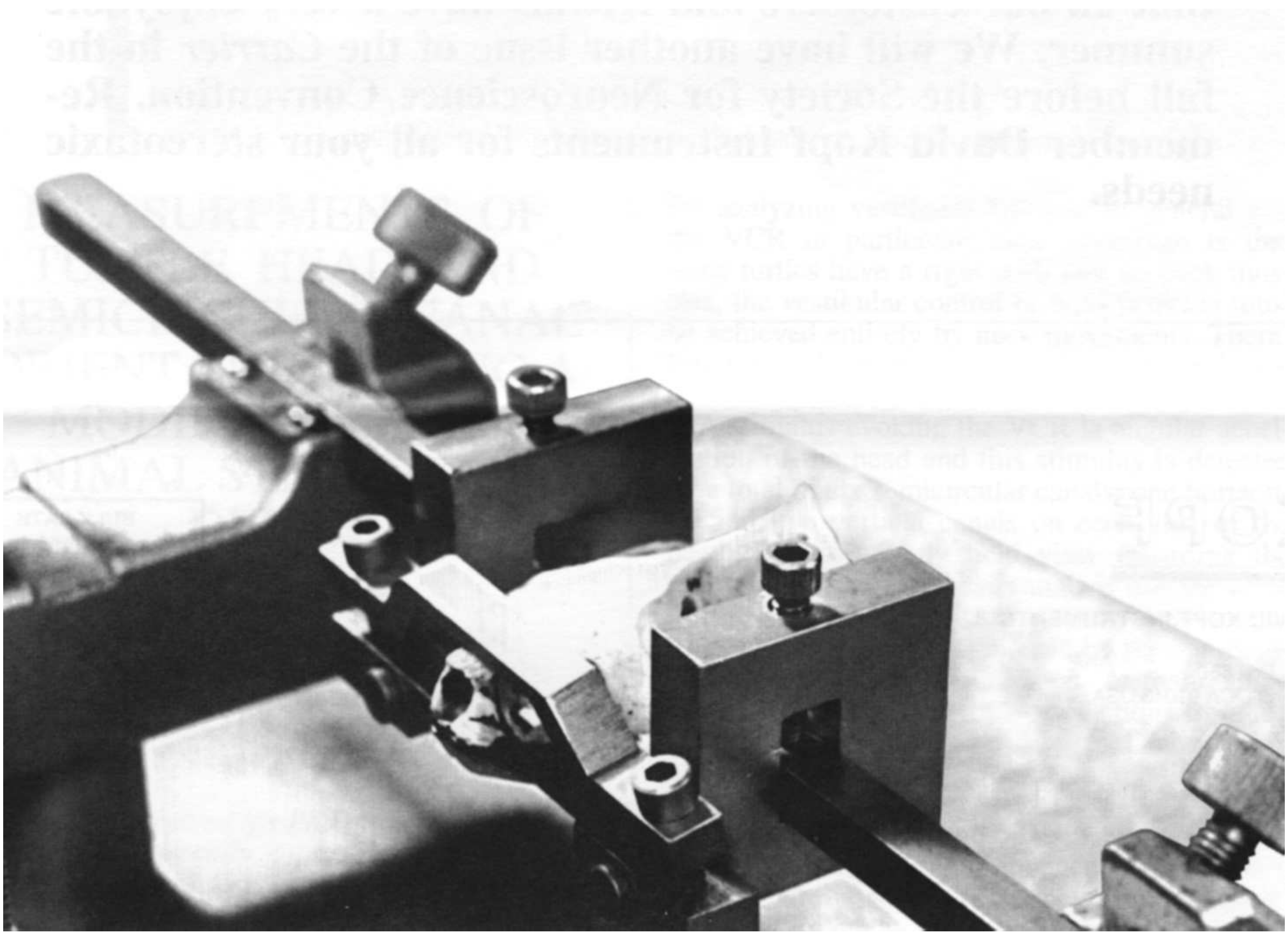

Figure la. Shows in detail the Powers and Reiner turtle headholder designed for a small animal stereo-taxic device.

points were then used to calculate planar equations of best fit for each canal. These equations, in turn, were used to calculate the angles between the various canals and between canal and stereotaxic planes.

REFERENCE POINT MEASUREMENTS

On dry skulls we recorded six points outside the bony labyrinth: five points on the skull surface (points anterior, posterior, left, right, relative to a center point directly above stereotaxic origin) and one external reference point on the overlying rat mouthbar. These reference points were used in two ways. First, the measuring needle was always referred back to the center point of the skull surface and to the external reference point before and after each canal measurement. This enabled us to correct for any drift in measurements or accidental displacement of the skull or needle. Second, the knowledge of skull surface point coordinates allowed us to relate the canal planes to these landmarks in our subsequent radiographic analyses. To determine canal orientation in live animals we needed to establish the same five reference points on the skull surface of live animals in a manner suitable for radiographic monitoring. We therefore measured, marked, and secured five lead implants in each of three turtles. The procedure was as follows. The animals were lightly anesthetized (Brevital 10 mg/ kg), intubated with polyethylene tubing, and then maintained at surgical plane with a mixture of approximately 80% Nitrogen and 20% Oxygen and 2-3% halothane. The external eardrum and overlying skin were resected, the head placed into the headholder and earbars positioned firmly

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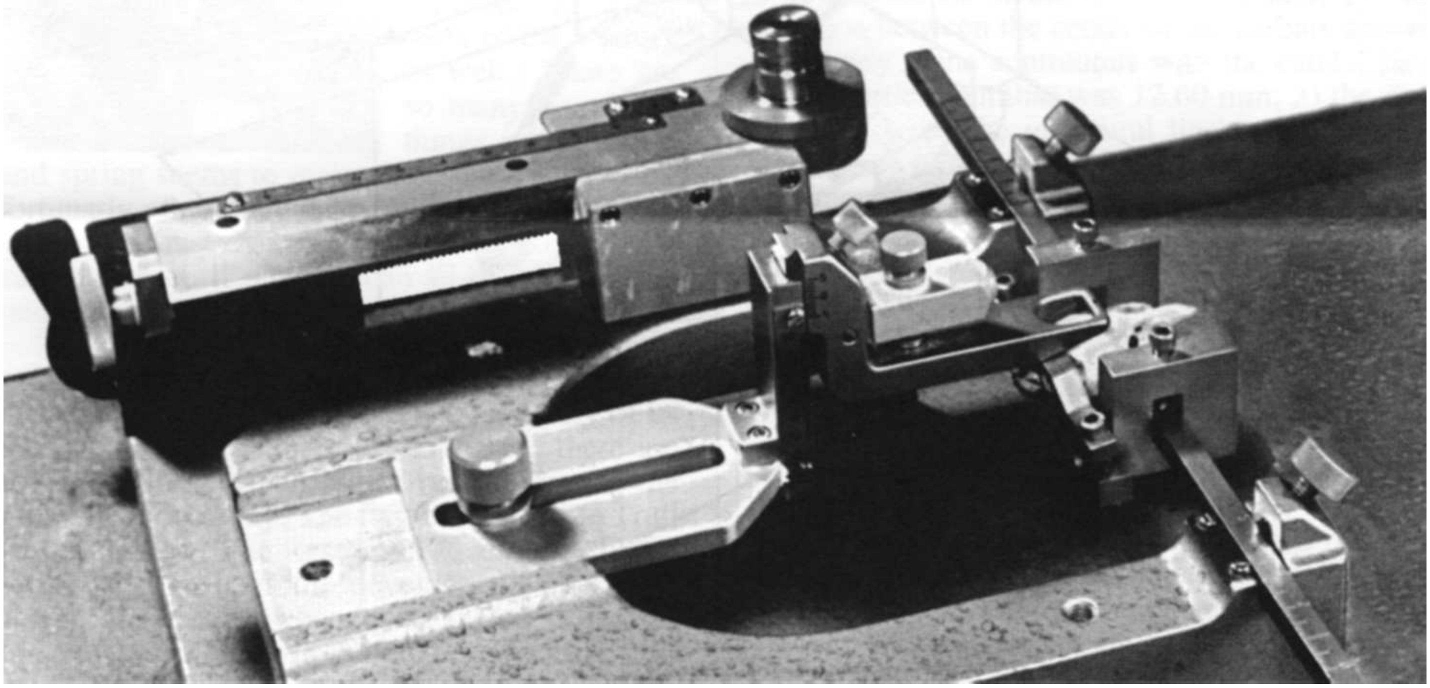

Figure 1b. Arrangement of turtle head holder with rat mouthbar in place. A mark on the rat mouthbar served as an external reference point for all canal measurements. By returning to this point and another reference point on the skull surface, before and after each series of canal measurements, we could detect drift or accidental movement of the needle or skull.

within the tympanic cavity. The animals were then mounted into the stereotaxic apparatus. Under a stereomicroscope we exposed the skull surface by reflecting the overlying skin and jaw adductor musculature, and we marked the five reference points using the tip of the probe dipped in waterproof ink and positioned stereotaxic ally via the three-axis micromanipulator. With a hand held drill we excavated, at each reference point, a depression deep enough to accept a lead marker (1 mm in diameter, 0.5 mm thick). Markers were secured in the skull with dental cement. The animals were then sutured, removed from the head-holder, and allowed to recover.

After 1-2 weeks, we used radiographs and fluoroscopy to record head position. Radiographs generate a high resolution but static measure of resting position. In contrast fluoroscopy yields a dynamic image but at lower resolutions. In combination the two techniques allowed us to monitor and measure resting head position and any variations in resting position. A fourth animal was used as a control to asses any effects of surgery (which had necessitated damage to the tympanum). We observed no difference in resting head position betwen control and experimental animals.

CONCLUDING REMARKS

From the bony labyrinth data we determined that turtle ipsilateral canals are not mutually orthogonal nor are complementary canals coplanar, although they approach this idealized condition more closely than do the canals of other vertebrates for which quantitative data exists. One significant departure from the perfectly orthogonal configuration is that both vertical (anterior and posterior) canals are slightly rotated towards the frontal plane. As a result we speculate that the

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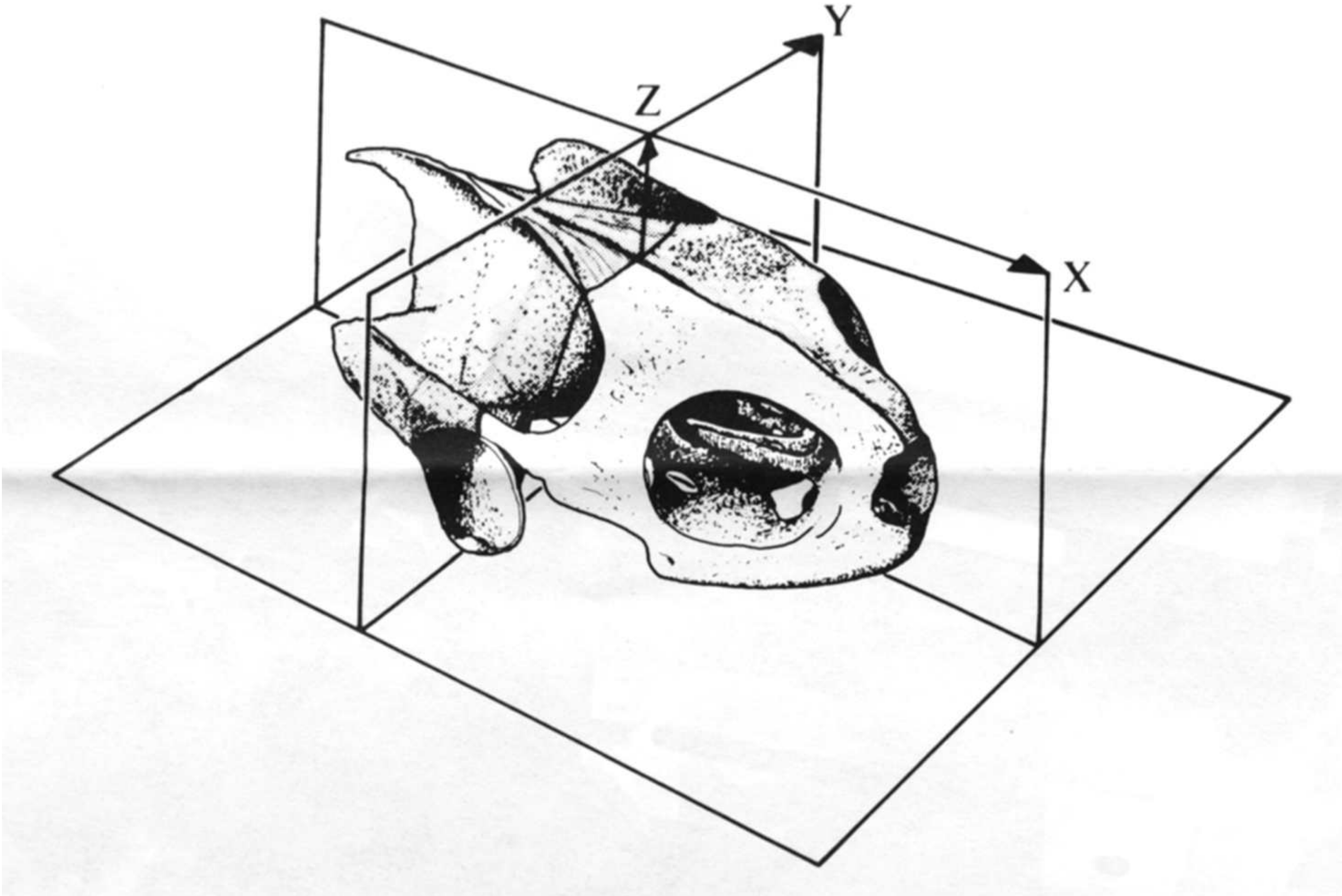

Figure 2. Diagram of stereotaxic axes superimposed on a turtle skull.

is the most obvious alteration in these large pyramidal turtle may be somewhat more sensitive to head roll than head rotations in other planes. We also found the turtle skull's flat dorsal surface is parallel to the horizontal canal and therefore provides a reliable external estimate of horizontal canal orientation. Radiographic analyses of the awake, resting turtles indicate that like other vertebrates they tend to keep the horizontal semicircular canal within a few degrees of earth horizontal.

As a postscript to the work described above we have recently completed a series of physiological studies on the response properties of ves-tibular primary afferents using in vivo and in vitro preparations of the turtle (Brichta and Golberg, in preparation). It has been reassuring though perhaps not surprising that physiological predictions of canal orientation are in close agreement with those calculated from stereotaxic measurements.

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