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The Application of Stereotaxic Procedures to Reptiles

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Introduction

In the past ten years the number of neuroscience studies utilizing reptiles has increased dramatically. While some excellent studies have been done on turtles and snakes the majority have used lizards. Two lizard stereotaxic atlases have appeared in print: An atlas for the large lizard Iguana iguana (Distal 1976) and one for the small Anolis carolinensis (Greenberg, 1982). Although these atlases are based on standard stereotaxic principles there are substantial modifications of the typical approaches used with mammals.

Equipment

Both atlases are designed for use with standard stereotaxic machines. Distal used a Kopf cat-monkey stereotaxic while Greenberg used a Kopf small animal (rat) stereotaxic. The major modification in equipment is in the ear bar system. Since lizards do not have a boney auditory canal the head cannot be held rigidly with ear bars. Instead, the lizards' heads were held steady by some combination of a "bite plate," nose-holder, and tipped ear bars. In this arrangement the upper jaw rests on a plate that holds the head horizontal and prevents vertical movement. To increase stability Distal used a bar across the border of the frontonasal bones to press the upper jaw into the thin layer of styrofoam covering the bite plate. Greenberg lowered a rubber strap over the animal's snout and pressed the jaw into the dental elastic impression material with which he covered the plate. Greenberg further stabilized the head by pressing blunt ear-bars (freshly tipped with dental cement) against the sides of the lizard's head.

Co-ordinate System

It is in the reptile co-ordinate system where the greatest divergence from the mammalian pattern is encountered. Since there is no ear bar zero, external landmarks must be used for zero reference points. There are, however, no sutures, boney landmarks, or scale patterns that are constant from animal to animal. The only zero reference point readily available is the parietal eye. The parietal or third eye (a rudimentary eye in lizards thought to function in connection with the endocrine system) is located on the midline at the top of the skull. It is a small point and provides a convenient place to begin any co-ordinate system. The exact stereotaxic co-ordinate systems derived from this parietal eye zero point are substantially different however in the two atlases. To best understand why this is so it is expedient to examine the two systems separately.

A. *Iguanas*

The brain of *Iguana iguana* lies in the skull at a 50° angle. (See Figure 1.) This is typical of most lizards where angles between 40° and 60° are commonly seen. Because of this brain-skull angle Distal's system utilizes a horizontal brain axis that is 50° up from the horizontal plane of the stereotaxic instrument. That is, in order to drive an electrode through the length of the brain, from forebrain to spinal cord, the electrode must be driven in at an angle of 50° relative to the bite plate. (See Figure 1.) Since the anterior-posterior (AP) co-ordinates in the atlas relate to standard cross sections of the brain it means that Distal's AP numbers refer to millimeters down a tract that runs 50° from true horizontal. In the typical mammalian stereotaxic system the electrode is lowered into the brain at an angle of 90° from the longitudinal axis of the brain. The extent of this lowering is given by the dorsal ventral (DV) co-ordinate. The anterior-posterior (AP) co-ordinate is set by moving the electrode carrier at right angles to this DV co-ordinate. In Distal's system this is reversed: The electrode is advanced into the brain along the longitudinal axis, the extent of advancement being given by the AP coordinate. If the electrode carrier is moved at right angles to this AP co-ordinate then the dorsal ventral pith of the electrode through the brain is changed. A practical disadvantage of this can be seen from the following example illustrated in Figure 1 and Figure 2. Suppose an investigator wants to implant an electrode into nucleus sphericus (a telencephalic nucleus) of the Iguana. Using Distal's atlas one notes that nucleus sphericus has an AP co-ordinate of 8mm. Since the brain begins 6mm down the AP line the electrode must be advanced a total of 14mm from the top of the skull. If the electrode path is begun at the parietal eye, however, it will pass dorsal to nucleus sphericus. The DV coordinate given in the atlas for nucleus sphericus is 4mm. That is, the proper electrode path is 4mm "below" (parallel to) the tract that begins at the parietal eye. There are two approaches one can take to solve this

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Editor's Column

IAlthough it is hard to believe, sitting here in the vicious heat and humidity of Southeast Ohio in early September, it is almost time for the new academic year to begin. By the time you receive this issue of the *Carrier*, it will be well into the fall season and most of us will be settled into the new routine of the academic year. In addition, many of us will be getting ready to make the annual trek to the Neuroscience meetings, this year in Boston.

As I stated in the last issue of the *Carrier*, it is our intent to publish articles of general interest to the neuroscience community, not only on issues relating directly to stereotaxic methods and equipment, but to the broad range of neuroscience. In this issue, Robert Tarr has provided an article giving an overview of the problems and techniques encountered in working with iguanid lizards. I again issue the invitation for any reader who wishes to provide an article for the *Carrier* to contact me about it at the address or phone number given below. I will also be at the Kopf Booth at various times during the Neuroscience meetings, so please feel free to stop to chat about ideas, suggestions, or comments that you have for the *Carrier*.

Another feature which will be a regular part of this column is questions and answers or comments submitted by readers. One question I have been asked is the following: "During normal surgical procedures, the standard electrode carriers often get dirty. Besides the normal washing, should any other care be given to the moving parts of the carriers, such as the slides or swivels?" The Kopf engineers answer: "The electrode" carriers, like other stereotaxic equipment, should be cleaned in a mild soap solution, rinsed carefully and dried (Zephrin may also be used for cleaning). Moving parts should be lubricated with a light oil. Be sure to store in a dust-free area."

"In addition, the carriers should not be autoclaved or exposed to temperatures above 180°F. although they may safely be gas sterilized. Given this sort of care these precision parts will last, with original accuracy, for many years."

Please feel free to contact me at the address or phone number given below with your ideas for articles or with questions, comments or suggestions, or stop by the Kopf Booth at the Neuroscience meetings. I'll look forward to seeing you there.

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Stereotaxic Procedures *(Continued)*

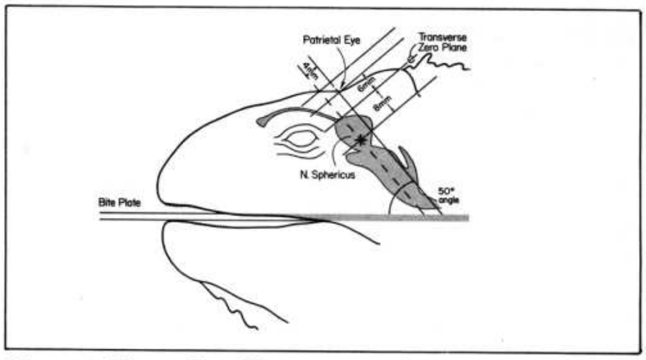

Figure 1. Illustration of *Iguana iguana* brain in situ. The brain rests in the head at a 50° angle relative to true horizontal. In order to implant an electrode in nucleus sphericus the electrode must be moved down a longitudinal line (which runs 4mm ventral to the parietal eye) for a distance of 14mm.

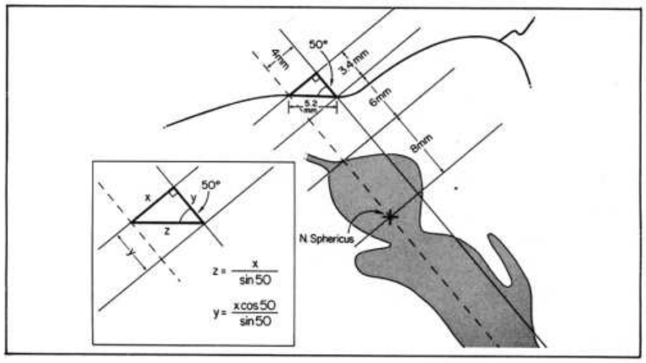

Figure 2. In order to obtain the desired DV coordinate ("X" or 4mm in the case of nucleus sphericus) the electrode carrier must be moved anterior from the parietal eye by an amount equal to $\frac{X}{\sin 50°}$. In the case of nucleus sphericus this equals 5.2mm anterior to the parietal eye. This maneuver adds 3.4mm to the final AP co-ordinate.

problem: utilization of a specialized stereotaxic adaptor or a trigonometric conversion of the coordinates. By using the Kopf 1261 AP slide attachment (permitting movement perpendicular to an angled electrode carrier) the required movement can be accomplished directly. If, however, the standard electrode carrier is utilized then what one must do (since the lizard's head is to be kept level) is move the electrode carrier some distance anterior to the parietal eye, then advance the electrode to hit the target. The distances involved, however, are not the atlas values. Figure 2 illustrates that any DV coordinate (in this case 4mm) should actually be obtained by moving anterior from the parietal eye by the amount of the co-ordinate divided by sin 50°. In this case the investigator should move the carrier forward 5.2mm then advance the electrode an additional 3.4mm (or 17.4mm total) down the AP path to hit nucleus sphericus.

B. *Anolis*

The brain of Anolis carolinensis is positioned in the skull in the normal mammalian horizontal fashion. Thus Greenberg's co-ordinate system is identical to the standard stereotaxic approach. For example, to hit nucleus rotundus (a prominent thalamic nucleus) one would look in the atlas for the AP co-ordinate (1.2mm posterior to the parietal eye in this case) and move the electrode carrier back to this spot. Next, referring to the atlas for the lateral co-ordinate (in this case 0.25mm) one would move the carrier 0.25mm lateral to the midline. Lastly, the electrode is lowered to the prescribed DV co-ordinate (2.25mm) below the surface of the skull.

Accuracy

Both authors assessed the accuracy of their atlases. Distal compared x-ray data, head and body measurements and electrode positions in 17 animals. He concluded that the co-ordinates are accurate within Imm2. Greenberg compared 15 Anolis, all with snout-vent lengths between 63 and 65mm and found less than 1.0mm variation in the location of specific landmarks such as the anterior commissure.

As both authors point out several factors limit accuracy in reptile stereotaxic work. First, the brain grows more slowly than the surrounding tissue. The reptile brain tends to rest comparatively loosely in the cranial cavity and can move as a result of changes in body position. This mobility also means that as an electrode enters the cranial cavity it can move the brain a considerable distance before the pia is penetrated. It should be appreciated that these factors plus the relatively small size of the reptile brain can introduce considerable variation in actual stereotaxic practice. Figure 3 illustrates the scattering of points observed in a study on Fence Lizards (Tarr, 1982). The Fence Lizard (Sceloporus occidentalis) is a small iguanid lizard with a brain-skull angle like Iguana iguana. The variability shown in Figure 3 is compiled from 60 animals with snout vent length between 7.0 and 8.0mm all of which had electrode implants advanced to the same co-ordinates (0.5mm posterior, 1.0mm lateral and 2.0mm ventral to the parietal eye). Similar studies (unpublished) on the large Tupinambis tequixin, the Desert Iguana (Dipsosaurusdorsalis) and A noliscarolin-ensis show a similar scattering of points. In all the above species except Anolis carolinensis the brain is set in the skull at an angle. The scattering was observed in all species, however, whether electrode angle was set at 90° or 50° to horizontal. Naturally the smaller the species of lizards the more severe the consequences of

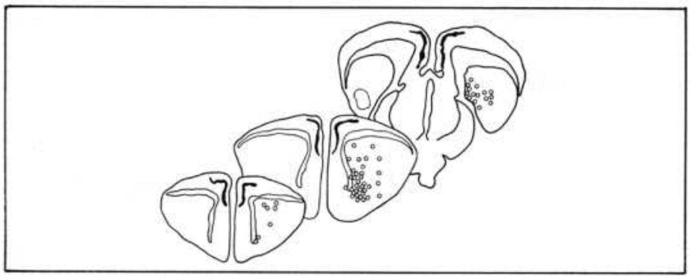

Figure 3. Cross section through the telencephalon (cut at 25%, 50% and 75% through the forebrain) of the Fence Lizard, *Sceloporus occidentalis* showing the scattering of points resulting from the placement of electrodes using one stereotaxic co-ordinate.

Product Changes

New Rat Spinal Unit

A new version of the 980 Rat Spinal Unit will soon be available.

This instrument will feature a new slotted base plate that will be large enough to accommodate the Model 900 Small Animal Stereotaxic. The new unit will include: Seven #982 adjustable base mounts with post and clamp, one pair #985 hip spikes, one #986 vertebrae clamp, one pair #987 V-notch spikes and one pair of #988 retractors. This new Rat Spinal Unit will be made available at no increase in cost to our customers.

All standard #980 accessories are compatible.

Improved 705 Plug-In Coil

We now have in stock a new type 705 Plug-In Coil that will withstand higher temperatures than the older coil. All orders are now being filled with these coil brackets.

701 Patch Clamp Bracket

The patch clamp micro-electrode accessory attaches to the slide block assembly of the vertical pipette puller. It has two vertically adjustable rods, with 30mm of travel, both rods have swing-away brackets. The patch clamp bracket allows one to make rapid, repeatable first and second pipette pulls. Easily attachable to all 700 series Kopf vertical pipette pullers.

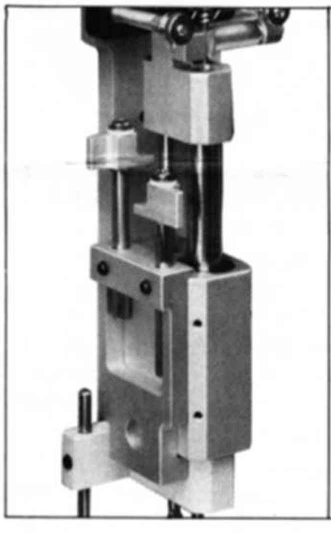

Figure 4. 701 Patch Clamp Bracket attached to 700D.

Figure 5. 701 Patch Clamp Bracket.

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any variability. In a small animal like the Fence Lizard (Figure 3) the scattering forms a sphere with a radius of about 1mm which results in electrode placements in functionally separate regions. This uncertainty can be used to advantage, however, in experiments where the experimenter is best blinded relative to exact electrode location until histologic examination is complete. For example, in behavioral studies where the design involves determining which neural structures control a given behavior then the behavioral data is collected prior to knowing the lesion or stimulation site. Animals with electrodes outside the target area function then as a control group and give a measure of the effects of nonspecific ablation or stimulation. Obviously, where the design calls for careful accuracy in electrode location the kinetic reptile brain will rule out a stereotaxic approach.

Thus, although stereotaxic procedures are inherently limited in their application to reptiles, the reptile brain maps in the literature give the interested neuroscientist ample background to begin experimenting with a wide variety of non-mammalian species.

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