Deep Brain Surgery in Animal Research

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The Brain is different from most other organs. Every location in it has a different function for the animal's behavior or bodily control. So much so that mapping the brain as to chemistry, connectivity and function is a major part of what neuroscience is about. Neuroscientists study functional localization in the brain partly so that we can know where in the brain a particular symptom set arose. To study localization, it is necessary to be able to reproducibly place probes of various kinds (lesion electrode, recording electrode, microdialysis probe, stimulating electrode, injection needle, withdrawal cannula, and more) in very specific locations in the brain. In primates or animals such as cats or dogs, such placement would have to be guided by an image of the particular brain under study, since individual differences are very large. Such surgery is done in humans for medical reasons.

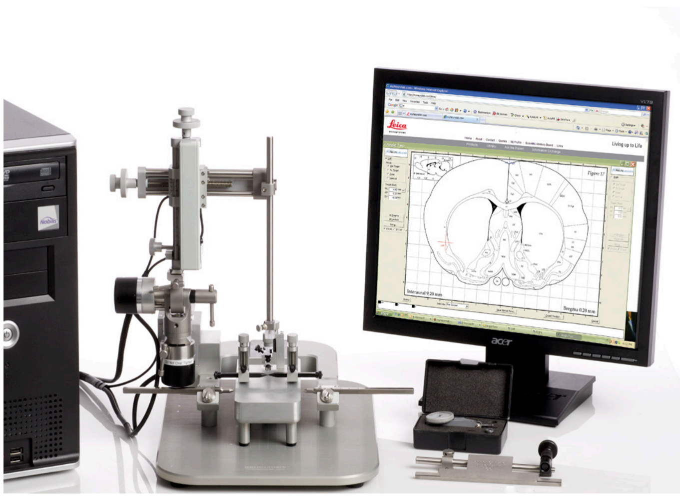

Fig. 1: The Leica Angle Two Stereotaxic Instrument

In rodents of a given species and age range, however, there are only very small individual differences in brain size and shape. Thus, it is possible to make a 3D map of the brain with coordinate overlay giving dimensions of how to reach any part of the brain (a Stereotaxic Atlas). A stereotaxic instrument is then used to move a probe from a selected external zero point to a specified coordinate location in the brain.

A new small animal stereotaxic instrument (Leica Angle Two) significantly improves accuracy over conventional vernier scale stereotaxic instruments, and enables the operator to reach any target site in brain from any chosen angle accurately and consistently.

A stereotaxic instrument is composed of a species-specific head holder mounted on a base plate and used to orient an anesthetized animals head in a defined position, and a 3D manipulator with movement axes aligned with the head holder. The manipulator is used to move a probe tip to a selected target coordinate in the brain of that animal relative to a chosen zero point. The zero point is usually skull landmarks Bregma or Lambda, which are visible lines on the skull where bone plates have grown together. These points overlie the brain at consistent positions relative to brain structures in rodents (less reliably in primates or most other mammals). They are at a crossover of the midline suture, and the anterior (Bregma) and posterior (Lambda) coronal sutures across the skull perpendicular to the midline. Conventional stereotaxic instruments have three vernier scales to align to measure from Bregma to the target.

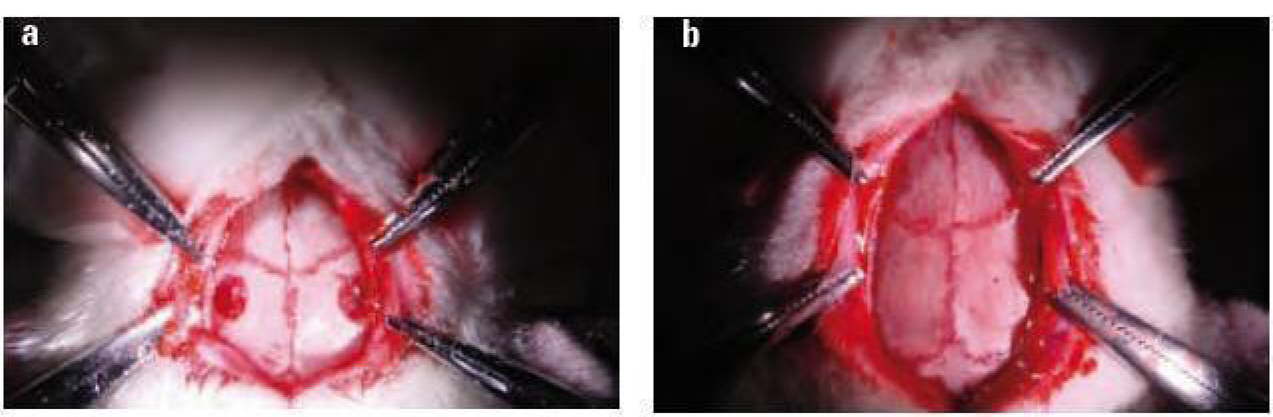

Fig. 3: a-b Intersections of Bregma and Lambda

The vernier scale instruments have the flexibility to tilt or rotate the manipulator to a different approach angle, but doing so usually requires a pilot study to see where the tips are reaching to, and adjusting the target coordinates. As a result, stereotaxic surgery is much easier to do, and takes much less time with less risk of error, and fewer animals, if the probe always approaches in a straight vertical position with the head in the “skull flat” position.

However, this results in confounding the path of approach with the action at target. For example, if making injections into hypothalamus from a glass pipette, a little injectate will leak out on the way down. Then, when a bolus is forced out, some will run up the path of least resistance, the already opened needle track. Lastly, when the pipette is pulled out, it leaves a vacuum behind and more injectate is sucked up into the needle track. So overall, the injection is not just in hypothalamus, but all along the needle path through brain. Which leaves the question when results are seen later, are they caused by the injection at the target site, or the injected material somewhere above the target? A similar analysis applies to lesion or recording studies, and tissue damaged by passage of the electrode.

The Angle Two manipulator features linear encoders on the 3 linear axes, and rotary encoders on tilt and rotation movements, 5 instrumented axes in all. These connect to a computer, into which a target point in conventional atlas coordinates relative to Bregma and assuming “skull flat” (Bregma and Lambda at the same vertical coordinate) is entered either by typing, or by scrolling an onscreen atlas of coronal sections of the brain, and clicking on the desired target point in the atlas pictures.

The animal is then anesthetized and installed in the head holder, the skull landmarks exposed, and the manipulator tilted and/or rotated to any chosen complex angle. The rotary encoders supply the computer with exact information about the angle of approach of the probe. The operator only needs to touch the probe tip to Bregma and click to show the computer the location of Bregma in the tilted and rotated coordinates. The computer has the math algorithms to display how far to move on the 3 linear axes to move the tip to the target point, given the tilt and rotation.

With the new Angle Two, every surgery can be done from a different approach angle, without need for pilot studies or manual math problem solving. In fact, it is faster and more efficient than the vernier models, with less risk of error. If the same result is achieved over several different approach paths, then it is the action at target, not something along the approach path, that caused the result. Interpretation of results is better served by varying the angle of approach.

The three displayed coordinate numbers count down to zero as the target coordinates are approached. The atlas display on screen shows where the probe tip is above or in brain as the operator moves it toward the target. The user can see what structures are being traversed as the probe moves toward the target.

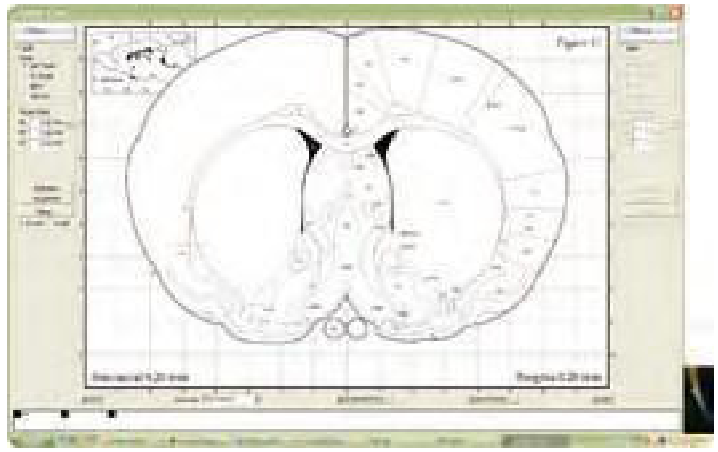

Fig. 2: Angle Two screen

The Angle Two includes the Virtual Skull Flat™ feature. Adjusting the animal’s head to skull flat is normally a trial and error and time consuming process. Very small errors in head tilt give large errors in position reached, especially if the probe is being lowered to a position deep in brain. Touching the probe to Lambda, and clicking a button on screen, after showing the computer where Bregma is located, enables the computer to calculate the degree of head tilt, and how that alters the target position. The path to the target is then recalculated given the head tilt, and a mathematically correct target position presented. It is no longer necessary to achieve actual skull flat adjustment.

The Angle Two allows better science, a higher yield of accurate placements, and faster operating speed.

We are pleased that the Angle Two stereotaxic instrument and accompanying accessories will be on display at the upcoming Society for Neuroscience symposium in Chicago, IL. October 17-21, 2009. Please visit us at booth 729.