Precision Stereotaxic Procedure for the Mouse *(Mus musculus):* Method and Instrumentation

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Abstract:
A precision stereotaxic procedure for mouse brain research is described accompanied by a new design in mouse stereotaxic head holder and a new device used to guarantee accurate alignment of the skull in the stereotaxic device. This method and instrumentation when applied in forthcoming research will contribute to the development of investigations of structure/function relationships in mouse brain.
Keywords: Stereotaxic, Brain Surgery, Mouse

Article:
Experimentation on the brain of the laboratory mouse poses serious problems of accuracy simply because the mouse brain, although very complex in organization, is quite small, rarely exceeding a volume of 0.5 cc. A number of researchers have found the mouse to be a useful animal for stereotaxic surgery, but careful studies have encountered problems of accuracy (e.g., [1]). Slotnick [4] reported that lesions aimed at the amygdala of CF-1 mice were sufficiently variable in accuracy and bilateral symmetry that subjects had to be separated into groups on the basis of actual lesion placements. The behavioural effects of the lesions differed significantly among the groups, demonstrating that these errors can indeed be important.

A recent study in our laboratory has identified several sources of error in stereotaxic surgery with mice [5]. First, the spatial inter-relations of the Bregma and Lambda points, interaural zero, and brain structures differ significantly among several strains of laboratory mice. Second, errors in placing the subjects in the stereotaxic instrument can contribute to within-strain variability, Finally, within-strain variability in the structure of the skull itself, including structures of Lambda, Bregma and the auditory meatus, can also inflate variability: All of these errors can be reduced by improving the methods of stereotaxic surgery.

Accordingly, we have developed new apparatus and procedures which give excellent results in stereotaxic electrode placements, These changes can he summarized briefly as follows: (1) the basic head holder designed by Slotnick [3] has been improved by adding a cross piece to the palate bar to give better rigidity, and by making the orientation in space of the head holder itself adjustable in three dimensions; (2) a precision alignment device fitting into the stereotaxic instrument allows the skull of each subject to be adjusted to a uniform orientation in three dimensions before spatial measurements are made; (3) a binocular microscope is used to insure greater accuracy in performing all operations; and (4) a very rigid stereotaxic instrument with fine, 10 graduations is used (Kopf professional model).

This new method has been employed successfully in a study of strain-specific stereotaxic coordinates [2].

The head holder (see Fig. 1) designed for this study is similar in basic construction to that of Slotnick [3]. The device is attached to a bar which is fastened securely into the ear bar fittings of the stereotaxic frame. The primary modification to the Slotnick design is one which permits much more accurate and reliable orientation of the skull relative to the true planes of the stereotaxic instrument. Incorporated into the design of the head holder are 3 adjustments, one rotating on a constant DV plane allowing accurate alignment of midline with true AP (midline adjust), one rotating on a constant AP plane allowing accurate alignment of bilateral homotopic skull points in the DV plane (horizontal adjust), and one rotating about a constant ML plane permitting accurate determination of the defined Bregma above Lamda position (vertical adjust). To facilitate accurate alignment of the skull with respect to the true cardinal planes of the instrument, a special alignment device (see Fig. 1) was constructed to precision specifications in such a way that, when attached to the Kopf stereotaxic frame via the tower and adapted electrode carrier, the three reference planes of the stereotaxic instrument can be compared directly to the orientation of the skull, and appropriate alignment corrections can be made using the adjustments available in the head holder. The alignment device was constructed in such a way that when viewed from the side, a line joining the pin points subtends an angle of 6 degrees (anterior pin high) relative to the true horizontal plane of the instrument (the mean Bregma above Lambda elevation across several strains, [5]). The pair of pins which are parallel to the stereotaxic AP plane are 4 mm apart, the mean Lambda-Bregma distance across several strains [5]. The two pins which are parallel to the stereotaxic ML plane lie equidistant from the two AP pins, their tips placed so as to follow, the 6° AP elevation.

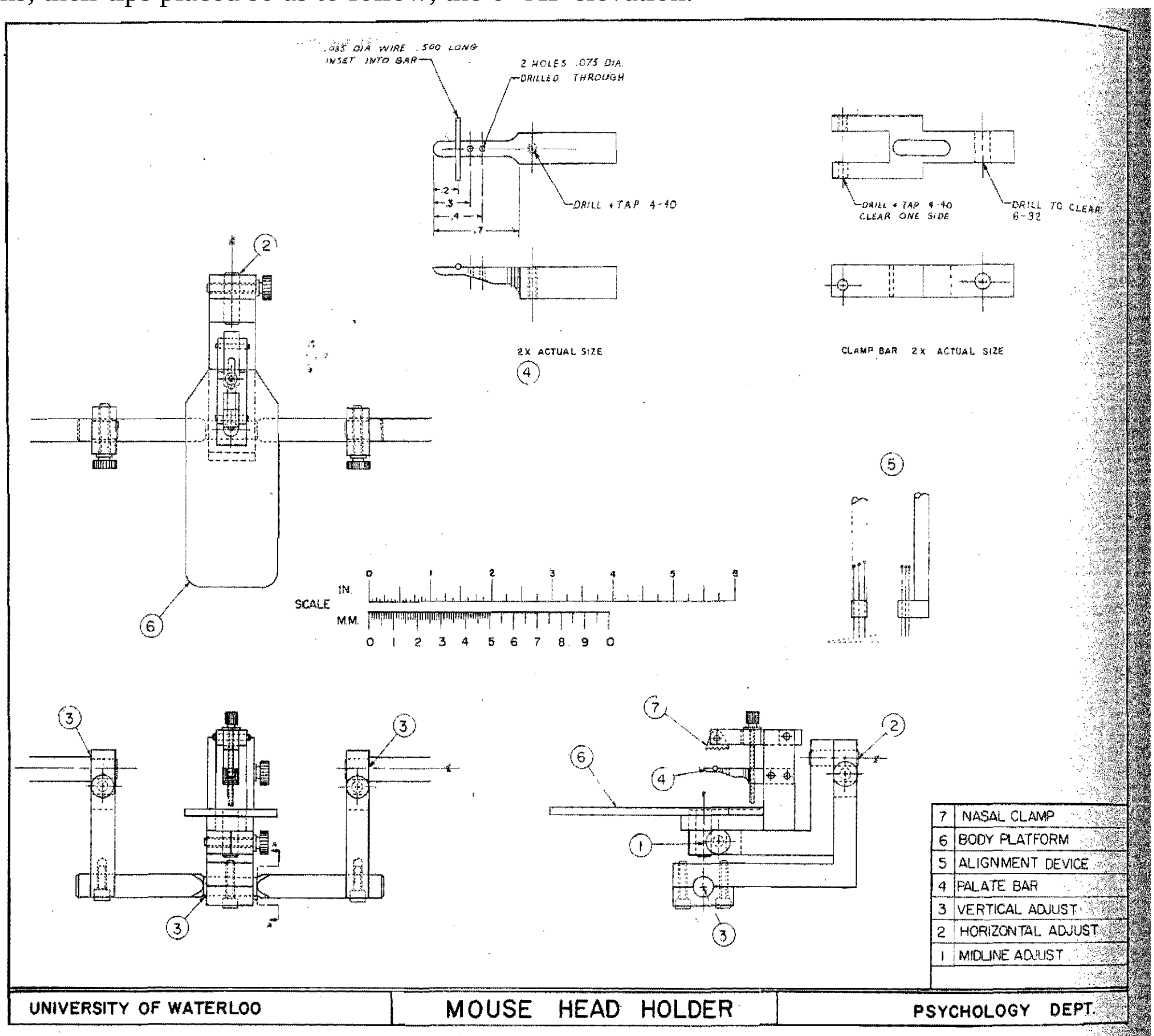
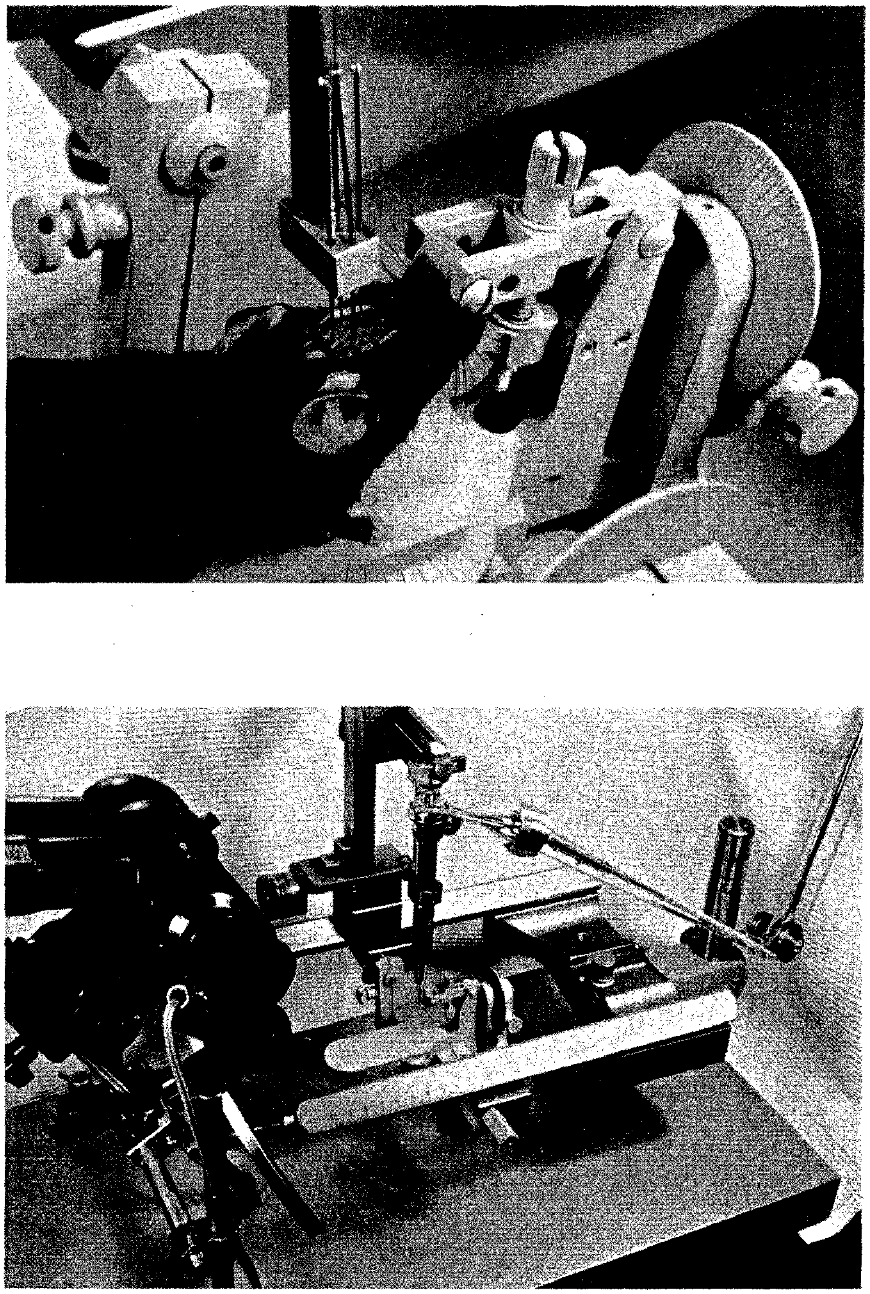


FIG. 1. Detail – Mouse stereotaxic head holder and alignment device.

Accurate location of trephine holes and the resultant opportunity to use extremely small burrs is accomplished by employing a modified electrode carrier and drill assembly as seen in Fig. 2. Drilling is conducted under stereoscopic observation which provides visibility of such a quality that holes can be drilled with no danger of superficial neurostructural damage or interruption of blood supply. This has proven to be a valuable asset in other, chronic preparations.

After appropriate surgical preparation, the subject is placed in the head holder by inserting the palate bar between the tongue and palate, such that the incisors' fit into the forwardmost of the two small holes (see Fig. 1), the cross-bar resting just posterior to the most posterior upper dentation. The Plexiglas nasal clamp is then lowered and tightened on the nasal bones resulting in a rigidly held preparation free from the encumbrances of protruding ear bars and tooth bar.

After making a midline incision and retracting the scalp, the alignment device is lowered to the skull of the animal such that the anterior pin rests over the Bregma intersection (Fig, 2). Viewing the preparation and alignment pins through the stereoscope, the surgeon can clearly view any error in skull orientation with respect to the true instrument planes and adjust the skull orientation accordingly. The headholder has been designed in such a way that the three adjustment planes are co-axial, with the intersection at the Bregma point. This feature permits the surgeon to make alignment corrections to the preparation without necessitating removal of the alignment pins from their skull land marks since movement of the skull in any plane leaves Bregma relatively stationary. With practice this procedure takes little time and results in consistent orientation of the skull of each subject in the instrument. The use of a moderate power (30-40 ×) stereoscope is essential to the procedure.



a

b

FIG. 2. (a) C57BL/6J mouse secured in the head holder. The alignment device is in place in preparation for the skull alignment procedure. (b) Stereotaxic apparatus including drill assembly, stereoscope, and head holder.

Having been properly aligned ,in the instrument the animal is now ready for the stereotaxic procedure. The alignment device is removed, and another electrode carrier, adapted to hold a small electric drill, is mounted in its place (see Fig. 2). Under stereoscopic observation, the drill tip is zeroed to Bregma and then, using the adjustments on the stereotaxic instrument, desired skull target points are located and drilled. Because of the accuracy of trephine hole placement, extremely small burrs can he employed for this procedure. Following creation of trephine holes, the drill carrier is removed, and replaced by a standard carrier holding an electrode or other intracranial device. The stereotaxic procedure then proceeds in the normal manner.

DISCUSSION

The reliability or consistency of this technique's accuracy has been demonstrated in a recent study [2] in which this methodology was employed to demonstrate genotypic differences in brain spatial organization. The need for a steady reference point from which to observe these differences necessitated development of a stereotaxic methodology with highly consistent accuracy. In this study, standard deviations of electrode, placement relative to the position of the Bregma intersection ranged from 0.096 mm to 0.248 mm with a mean of 0.155 mm. These small errors in placement compare favourably with the demands placed on a stereotaxic technique which is designed to position intracranial devices in target structures within the small brain of this species. Further, observed variability in electrode position may in part be attributed to minor within-strain differences in such factors as the location of Bregma in relation to underlying brain. Individual differences such as these set a lower limit on the measure of variability in electrode position which is truly irreducable when using reference points based on skull characteristics [5].

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