Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex, strain and weight

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Craniometric and stereotaxic data from rats of different sex, strain and weight were compared. It was found that stereotaxic atlases can be used with rats of different sex and strain provided that the weights of the rats conform to those used in the reference atlas. If rats of different weights are used, greater accuracy can be achieved if bregma is used as the reference point for work with rostral structures and the interaural line for work with caudal structures.

Misplacement of probes in stereotaxic surgery frequently frustrates experimental objectives. Although variations in the placement of probes may be attributable to experimenter error or the inaccuracy of the atlas used, it is often suspected that they are due to the incompatibility of the sex, strain or weight of the experimental subjects with those on which the reference atlas was based. It is this latter group of variables (sex, strain or weight) that are the subject of this study.

Stereotaxic atlases are usually based on a group of rats of a specified sex, weight and strain. For this reason, the experimenter has the onus of determining empirically the stereotaxic position of each structure of interest when the subjects used are of different sex, strain or weight from those on which the atlas was constructed. In the present study we report that, with atlases based on the flat-skull position, only marginal errors occur with use of rats of different sex and strain. Significant errors occur, however, with use of rats of different weight.

The subjects were 10 female Wistar rats of 282 ± 19 g weight, 10 hooded (Long–Evans) rats of 290 ± 20 g weight, 10 Sprague–Dawley rats of 299 ± 13 g weight, 10 juvenile Wistar rats of 180 ± 8 g weight and 10 mature Wistar rats of 436 ± 16 g weight. Craniometric and stereotaxic data from these rats were compared...
with those given for 130 Wistar rats of $290 \pm 16$ g weight on which the recently published atlas, *The Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 1982), is based.

The rats were anesthetized with sodium pentobarbital and placed in a Kopf small-animal stereotaxic instrument. The incisor bar was adjusted until the height of lambda and bregma skull points were equal (i.e. they lay in the same horizontal plane). For the different groups this flat-skull position was achieved by lowering the incisor below the horizontal zero the distances shown in the last column of Table I. Because the point of intersection of the lambdoid and sagittal sutures is variable, we defined lambda as the midpoint of the curve of best fit along the lambdoid suture (Fig. 1). This redefined reference point is considerably more reliable than the “true” lambda (see SDs in Table I). We also defined bregma as the point of intersection of the sagittal suture with the curve of best fit along the coronal suture. When the two sides of the coronal suture met the sagittal suture at different points, bregma usually fell midway between the two junctions. Table I presents the mean body weight and mean skull measurements for the different groups.

To establish the stereotaxic position of structures, reference needle tracks were made in the coronal plane. Needles were inserted at the anterior–posterior (AP) level of bregma as well as at 11.7 and $-1.3$ mm from the interaural line. The last two

![Fig. 1. Dorsal and sagittal views of the skull of a 290 g male Wistar rat.](image)
Table 1
CRANIOMETRIC AND STEREOTAXIC DATA (MEANS ± S.D.) FOR RATS OF DIFFERENT SEX, STRAIN AND WEIGHT ac, anterior commissure; Acb, accumbens nucleus; AP, anterior–posterior; B, bregma; DV, dorsal–ventral; 7n, facial nerve; I, interaural line; L, lambda; W, Wistar.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean weight (g)*</th>
<th>AP I–B (mm)</th>
<th>AP I–L (mm)</th>
<th>DV I–B (mm)</th>
<th>AP 1–Acb (mm)**</th>
<th>AP B ac (mm)**</th>
<th>AP 1–7n (mm)**</th>
<th>DV 1–incisor bar (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Atlas' W</td>
<td>290</td>
<td>9.1 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>10.0 ± 0.2</td>
<td>11.7</td>
<td>0.0</td>
<td>-1.3</td>
<td>-3.3 ± 0.4</td>
</tr>
<tr>
<td>Female W</td>
<td>282</td>
<td>9.3 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>10.0 ± 0.1</td>
<td>11.6</td>
<td>0.1</td>
<td>-1.2</td>
<td>-3.2 ± 0.5</td>
</tr>
<tr>
<td>Hooded</td>
<td>290</td>
<td>9.4 ± 0.4</td>
<td>0.3 ± 0.6</td>
<td>9.8 ± 0.2</td>
<td>11.9</td>
<td>0.0</td>
<td>-1.2</td>
<td>-3.9 ± 0.6</td>
</tr>
<tr>
<td>Sprague</td>
<td>299</td>
<td>9.0 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>10.1 ± 0.1</td>
<td>11.7</td>
<td>0.1</td>
<td>-1.2</td>
<td>-3.9 ± 0.5</td>
</tr>
<tr>
<td>Juvenile W</td>
<td>180</td>
<td>7.7 ± 0.4</td>
<td>-0.4 ± 0.3</td>
<td>9.9 ± 0.2</td>
<td>10.2</td>
<td>-0.1</td>
<td>-1.6</td>
<td>-2.0 ± 0.4</td>
</tr>
<tr>
<td>Mature W</td>
<td>436</td>
<td>9.7 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>10.7 ± 0.4</td>
<td>12.4</td>
<td>-0.1</td>
<td>-0.8</td>
<td>-2.7 ± 0.3</td>
</tr>
</tbody>
</table>

* S.D.s. ≤ 20 g.
** S.D.s. ≤ 0.4 mm.
positions are given, respectively, as the levels of the frontal pole of the accumbens nucleus (Acb) and of the descending limb of the 7th nerve (7n) in the atlas of Paxinos and Watson (1982).

After surgery, the rats were decapitated. In order to preserve the spatial relation between skull landmarks and brain structures, brains were frozen in the skull and the skull bones were then prized off the frozen brains. The fresh (unfixed) brains were cut at 40 μm parallel to the coronal plane. Sections containing the electrode tracks were stained with cresyl violet according to the procedure used by Paxinos and Watson (1982).

Table I shows craniometric data and the location of key brain structures for rats of different sex, strain and weight. It is clear that with atlases based on the flat-skull position no substantial stereotaxic error will occur when rats of different sex and strain are chosen provided the rats are of the same weight on which the atlas is based. Thus, for rats of different sex and strain but of the same weight the AP distance between the interaural line and bregma falls within 9.0 and 9.4 mm. The AP distance between the interaural line and lambda falls within 0.3 and 0.7 mm. The dorsoventral distance between the interaural line and the surface of the skull at bregma is nearly constant (9.8–10.1 mm) as is the AP distance of the Acb (11.7–11.9 mm) and the 7n (−1.3 to −1.2 mm) from the interaural line. The dorsoventral position of structures relative to reference points was tested in two rats of each of the groups of different sex and strain but of similar weight by making one horizontal needle insertion perpendicular to the coronal plane at 3.0 mm dorsal to the interaural line. In each case, the electrode track was found within ±0.1 mm of the expected position. Similarly, needles aimed at the basolateral amygdala to investigate the mediolateral variability of structures (in these same rats) reached the target area. It is evident, therefore, that atlases based on the flat-skull position can be used with rats of different sex and strain provided that the weights of the rats are similar to those used in the atlases.

On the other hand, skull measurements for juvenile (180 g) and mature (436 g) Wistar rats differ substantially from those of the other groups. The AP distance between the interaural line and bregma is 7.7 mm in the juvenile and 9.7 mm in the mature rats. (The means of all other groups fall between 9.0–9.4 mm.) Lambda is 0.4 mm posterior to the interaural line in juvenile rats and 0.6 mm anterior to this line in the mature rats. (The means of all other groups fall between 0.3–0.7 mm anterior to the interaural line.) Unexpectedly, the dorsoventral distance between the interaural line and bregma for the juvenile rats (9.9 mm) was almost the same as that of the rats of the atlas weight (10.0 mm). In the mature rats, the vertical distance between the interaural line and bregma was 10.7 mm.

A strikingly stable relation was found between bregma and the anterior commissure (ac) in all groups of rats used in this study (including the juvenile and mature groups). Bregma was always found to be above the most forward crossing fibers of the ac (±0.1 mm). This is the point at which the posterior limbs of the ac appear. Interestingly, a similar relation between bregma and the ac is found in mice (Slotnick and Leonard, 1975). These data confirm the observation of Whishaw et al. (1977) that bregma is more stable than the interaural line for positioning of
electrodes in brain structures close to, or anterior to, bregma. However, data from
insertion of needles aimed at the level where the facial nerve leaves the facial genu
show that the interaural reference point is more stable than bregma for localization
of such posterior structures. Therefore, if juvenile or mature rats are used, greater
accuracy can be achieved if bregma is used as the reference point for work with
rostral structures and the interaural line for work with caudal structures. A further
improvement in accuracy can be obtained by taking into account the actual location
of the Acb and 7n (Figs. 9 and 35 of the atlas of Paxinos and Watson). In agreement
with Slotnick and Brown (1980) we noticed that structures were more accurately
located when more than one reference system was used to give target coordinates. In
particular, it was found that a simple averaging of coordinates given by the
interaural and bregma reference systems was more than adequate when locating
structures in the brains of rats within the size range for which the atlas was
constructed.

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