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The extended application of *The Rat Brain in Stereotaxic Coordinates* in rats of various body weight

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A R T I C L E I N F O

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A B S T R A C T

Background: Stereotaxic procedures in rats are frequently used in neurological research. The commonly used rat brain stereotaxic atlases are*The Rat Brain in Stereotaxic Coordinates,* the second most cited book in science, and *Stereotaxic Atlas of Rat Brain* (in Chinese) which based on Wistar rats of 290 g and SD rats of 225 g, respectively. However, rats of different weight are frequently used, while the stereotaxic coordinates of their brain regions show obvious differences with the rats on which the atlases were based.
New method: The craniometric parameters were measured as accurately as possible, then curve fitting equations obtained from the parameters were used to deduce the relative coordinates in rats of different weight for the first time.
Results: The results revealed that if the coordinates of target brain region were defined as AP(a), ML(b), DV(c) in "standard" rats, the corresponding coordinates AP(a'),ML(b'),DV(c') for rats weighing*m*(g) were described as follows:

For Wistar rats using *The Rat Brain in Stereotaxic Coordinates*:

$$a' = \frac{0.138\times\ln(m)+0.177}{0.138\times\ln(290)+0.177}\times a$$

$$b' = \frac{0.055\times\ln(m)+0.515}{0.055\times\ln(290)+0.515}\times b$$

$$c' = \frac{0.054\times\ln(m)+0.706}{0.054\times\ln(290)+0.706}\times c - 0.00012\times(m\text{-}290)$$

and for SD rats using *Stereotaxic Atlas of Rat Brain*(in Chinese):

$$a' = \frac{0.127\times\ln(m)+0.176}{0.127\times\ln(225)+0.176}\times a$$

$$b' = \frac{0.049\times\ln(m)+0.556}{0.049\times\ln(225)+0.556}\times b$$

$$c' = \frac{0.077\times\ln(m)+0.604}{0.077\times\ln(225)+0.604}\times c - 0.00010\times(m\text{-}225)$$

Unit of a, b, c, a', b', c' are centimeter, unit of *m* is gram.

Comparison with existing method: *The Rat Brain in Stereotaxic Coordinates* is applicable for rats weighing about 290 g, while our equations extended the use of atlas to rats weighing 90–400 g.
Conclusions: In this manner, the application of the rat brain atlases could be extended and the results would be of great help in stereotaxic procedures.

1. Introduction

Stereotaxic procedures are frequently used to eliminate, enhance or disturb the function of certain regions of the brain as part of neurological research, pharmacological evaluation or central nervous system (CNS) disease related experiments. As rodents are the most widely used animals for neuroscience research, using stereotaxic atlases of rat brains to determine the coordinates of certain brain regions for stereotaxic procedures is of paramount importance. The two commonly used rat brain stereotaxic atlases based on flat skull position are *The Rat Brain in*

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Stereotaxic Coordinates (Wistar rats) and *Stereotaxic Atlas of Rat Brain* (in Chinese, Sprague Dawley rats). The former is the second most cited book in science (Elsevier, 2016). Both of these resources provide invaluable information when analyzing the structure and function of certain brain regions.

A lot of tedious and meaningless work was needed if atlases were prepared with rats of different body weight, which is also a waste of time and money. So the atlases used rats of certain body weight. *The Rat Brain in Stereotaxic Coordinates* used Wistar rats weighing 290 g (Paxinos and Watson, 2008) and *Stereotaxic Atlas of Rat Brain* (in Chinese) used SD rats weighing 225 g (Bao and Su, 1991). However, rats of different gender, strain and weight are often used in experiments. The differences between the experimental subjects and the animals on which the stereotaxic atlases were based inevitably lead to errors.

To examine the influence of rat gander, strain and weight, Paxinos et al. studied the stereotaxic position of certain regions of rat brain. The results showed that there were only tiny errors when using rats of different genders and strains. However, if the rats were of different weights, there were giant deviation (Paxinos et al., 1985). In order to examine the use of stereotaxic atlas for juvenile (180 g) or mature (436 g) rats, Paxinos et al. measured the craniometric parameters of the skull of juvenile and mature Wistar rats and compared them with those of “standard” rats (290 g). The results showed that there were substantial deviations, especially for the AP (anteroposterior) distance between the interaural line and bregma (0.77 cm in the juvenile and 0.97 cm in the mature rats), and therefore concluded that the atlas is not suitable for juvenile or mature rats. In spite of this, it was hinted that the deviation between the juvenile or mature rats with “standard” rats could be speculated, such that misplacement of the probes could be reduced through modulating stereotaxic coordinates. There is evidence to show that the bottom plane of cerebrum is identical with transverse plane of interaural line, and the posterior plane of cerebrum is close to the coronal plane of interaural line form Fig. 1A (obtained from *The Rat Brain in Stereotaxic Coordinates*). Furthermore, MRI imaging also shows that the brain fits well with the cranial cavity in rats (Fig. 1B), giving further reason why correcting stereotaxic coordinates based on landmarks on the skull is reasonable. Furthermore, although the rat brain develops rapidly after birth and enormous changes occur, after postnatal day 7(PND-7), the brain structures are similar to those of adult rat brains (Ramachandra and Subramanian, 2011), thus it could be speculated that the stereotaxic coordinates of certain brain region also change proportionately with the skull parameters.

In *the Rat Brain in Stereotaxic Coordinates*, the weight/age variation in craniometrics, primarily in terms of (anteroposterior) landmark deviation, were discussed in rats weighing 180 g and 436 g. However, similar work for rats with a range of body weights has not been carried out. In addition, while stereotaxic coordinates are composed of AP, DV (dorsoventral) and ML(mediolateral), professor Paxinos ignored the DV and ML coordinates as they believed that DV variation with weight/age is trivial (we don’t know why they also ignored ML, maybe they believed that the existing data already showed the incompatibility between non-standard rats and the atlas). In our study, variations in DV and ML cranial dimensions are also considered, using the depth and the width of the cranial cavity to represent variations in DV and ML. Accordingly, these parameters of the cranial cavity of rats with different weight were calculated as accurately as possible to obtain the curve equations. These equations were used to calculate the deviations between “corrected” coordinates of particular brain regions in rats of various weights and “uncorrected” coordinates in the “standard” rats. These deviations were analyzed and then used to establish a new set of coordinates specific to the non-standard rat. The implication of these new formulas are more accurate localization can be achieved in nonstandard weight rats, eventually extend the application of stereotaxic atlases in rats of different body weight.

In our study, the youngest rats selected were 1 week old because their brains are similar to adult rats. Besides, the most frequently used adult rats were under 10 weeks (about 400 g body weight). So the animals selected in our study were between 1 week to 10 weeks, besides, a group of 34 weeks rats were also added to observe that whether there were significant differences between the mature animals and adult animals in craniometric parameters.

2. Materials and methods

2.1. Animals

Forty eight male Wistar and forty eight male Sprague-Dawley (SD) rats were purchased from Vital Rivers (Beijing, China), aged from 1 week to 10 weeks, and 34 weeks. The rats were given food and water *ad libitum* and raised on a 12 h to 12 h light/dark cycle in a room temperature of 24 ± 1°C.They were housed in groups of four per cage. All experiments were performed in accordance with the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Peking Union Medical College and Chinese Academy of Medical Sciences. Animals were divided into 10 groups according to their week ages (including 1-10weeks), and the rats whose ages were 34 weeks were divided into two groups, including light (34 L) and heavy body weight (34 H).

2.2. Magnetic resonance imaging (MRI)

Rats in each group were anaesthetized with 3% isoflurane (RWD, China) in 95% oxygen/5% carbon dioxide for examination. MRI experiment was conducted on a 7 T PharmaScan small-animal MR scanner (300 MHz, Bruker Biospin, Germany). The MRI protocol was a T2_TurboRARE, with TR/TE = 4300/35 ms, 4 averages, 40 × 40 field-of-view, size 256 × 256, and 0.3 ~ 0.5 mm thickness. The planar resolution was 0.15 × 0.15 mm. The brain slice images were collected for 6 consecutive sections with identical section height. Each section was analyzed using Image-Pro Plus 6.0 software. The volume of cerebrum (white parts within cranial cavity) was calculated by adding all brain slices together. The cranial cavity volume (the parts underneath skull) was measured by adding all cavity slice areas together.

Cerebrum volume/cranial cavity(%) = The volume of cerebrum/ the volume of cranial cavity × 100

2.3. Data measurements

After body weights were recorded, rats were anaesthetized with chloral hydrate (400 mg/kg; i.p.), perfusion-fixed with 4% paraformaldehyde in PBS, then the cadaveric heads were removed by cervical dislocation. After the skins and muscles were peeled off, the data of skulls were measured by vernier caliper (Santo, Shanghai, China) in a flat skull position (*i.e.* the height of lambda and bregma skull points were in the same horizontal lane). All the needed craniometric data were illustrated in Fig. 1C. For different groups, the incisor bar was lowered under the interaural line for distance 5 (vertical height from interaural line to incisor bar, I-IB, Table 1); this allowed for a flat skull position on Fig. 1C. A total of 5 craniometric parameters were gauged in each rat, as shown in Fig. 1C, including distance 1 (horizontal length from lambda to interaural line, L-I_h), distance 2 (horizontal length from bregma to interaural line, B-I), distance 3 (horizontal length from the rear-end of orbital bone to the sagittal sutures, Width), distance 4 (vertical height from lambda to interaural line, L-I) and distance 5. In accordance with the atlases, the lambda was defined as the midpoint of the curve of best fit along the lambdoid suture, while the bregma was defined as the point of intersection of the sagittal suture with the curve of best fit along the coronal suture, as the redefined reference points are more reliable than the "true" points. If the two sides of coronal suture met the sagittal suture with different points, the bregma point was defined as the midpoint of the two points (Paxinos and Watson, 2008).