Cranial and Cerebral-Ventricular Landmarks for Accurate Stereotaxic Approach to Hypothalamic Nuclei in the Goat Brain

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ABSTRACT. This paper describes a new stereotaxic coordinate system for the goat brain based on cranial landmarks. An osseous triangle (a-b-c) formed by the point of junction of the crista galli with the caudal ventral part of the frontal sinus septum (a), the external occipital protuberance (b), and the midsagittal projection of the external acoustic meatus (c), was measured using lateral radiographs and ventriculographs and showed a constant mathematical relation. The rostral angle was 20.3 ± 1.0 (mean $\pm$ SD) degree in 23 goats studied regardless of their cranial size which varied considerably from one animal to another. The hypotenuse length (a-b distance) was found to be a good predictor of the rostral nuchal position of the anterior commissure (AC) and the infundibular recess of the third ventricle (INF), by which the individual variance of the stereotaxic coordinates for a given hypothalamic structure could be compensated. The anterior-posterior distances from the external acoustic meatus was highly correlated with the a-b distance for AC ($r=0.88$) and INF ($r=0.90$). Using these cranial landmarks and the method outlined in this paper, uncertainty in coordinate values for AC and INF in the goat brain was reduced considerably in comparison to deviation observed when the ordinary Horsley-Clarke axis (Reid's plane) was employed.—**KEY WORDS:** goat, radiography, stereotaxy, ventriculography.

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Domestic ruminants (sheep and goat) have been widely employed in neuroscientific research such as studies on neuroendocrine control of the maternal, feeding and sexual behavior. Currently available stereotaxic maps of the goat brain [16] have been based on the Horsley-Clarke plane as the horizontal axis, but they have not paid any attention to the relationship between the skull and the brain which varies from one animal to another. It is, therefore, still difficult to perform brain surgeries with sufficient accuracy. Actually, it has been reported in monkey [12] and cat [4] that the coordinate system using ventricular landmarks based on the intercommissural line between the anterior and posterior commissure would be more adequate in terms of precision and reproducibility of stereotaxic approach.

Precise positioning of the hypothalamic nuclei has been achieved in the Shiba goat by using the lateral radioventriculography [10]. However, from practical points of view, it would be more beneficial if we could improve precision of stereotaxic approach without employing radioventriculography because the techniques require certain skill and injection of radiopaque material into the cerebral ventricular system may have adverse effects on the brain function.

We therefore tried to develop a routine method to calculate accurately the location of subcortical structures using cranial or ventricular landmarks. In this paper information about cranial landmarks obtained from lateral radiographs of the goat head was analyzed in combination with lateral cerebral ventriculographs. This allowed us to

establish a new stereotaxic coordinate system based on cranial landmarks easily observable in lateral radiographs. The system improved the effectiveness of calculating exact positions of brain structures to locate any experimental device.

MATERIALS AND METHODS

Animals: A colony of the miniature “Shiba” goat, which is an indigenous Japanese variety of *Capra hircus*, has been established for experimental uses [7]. It is a white colored, horned and relatively small goat, with body weight averaging 25 and 20 kg for male and female, respectively.

Radiographic examination was carried out in 3 females and 5 males of varying body size and age to obtain information about the skull modification associated with the development of horns. In addition, 15 female goats were used to study the topographical relation between the cranial reference points and ventricular landmarks.

Stereotaxic instrument and radiographic equipment: The stereotaxic instrument was described in detail elsewhere [10]. Briefly, the goat head was supported with a pair of ear bars and the anterior head holder. Both of them were designed so that the external acoustic meatus (horizontal axis) was located 3 cm above the upper ridge of the anterior-posterior (AP) bars. The ear bars were inserted, via an ear adapter holder, into the external acoustic meatus and placed in the frame at an equidistant position from each of the AP bars. The front of the head was supported by the head holders which consisted of a horizontal metal plate with 4 vertical rods clamped to it. Two of the vertical rods worked as eye bars and the other two as mouth bars.

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The instrument was equipped with two micro-manipulators adapted to the AP bars, which permitted accurate placement of any device into the brain from different angles of approach.

A portable X-ray machine (model TP20, TANKA) was located at a fixed distance of 70 cm from the midsagittal plane of the stereotaxic instrument. This arrangement ensured that all radiographs were taken from a constant height and distance thereby producing images of the same magnification ($\times 1.16$).

Lateral radiographs and lateral ventriculographs: The goats were lightly anesthetized and kept prone while lateral radiographs of the head were taken. Then each animal was placed in the stereotaxic instrument to obtain similar lateral radiographic images.

Radio-ventriculography was performed according to the previous description for the Shiba goat [10]. Briefly, the goat was given intravenous administration of anesthetics (ketamine HCl and xylazine HCl), and mounted in the stereotaxic instrument. The position of the penetration site of the ventriculographic cannula could be determined with sufficient accuracy according to the rostral nuchal coordinates regarding to the external acoustic meatus. After median incision of the scalp, the external surface of the cranium roof was exposed and a hole was drilled about 5 mm lateral to the midsagittal suture and 25 mm rostral to the IA line. With the aid of the micro-manipulator a spinal needle (21 gauge, 70 mm long; Terumo, Tokyo) was inserted through the hole until the tip reached the lateral ventricle, approximately 25 mm below the dura mater. Slowly 0.5 ml of cerebrospinal fluid was withdrawn and the equal volume of radio-opaque material, Iopamidol (Iopamiron 370, Schering, Germany) was injected. A lateral ventriculograph was taken 30 sec later. All the radiographs and ventriculographs were taken on condition of 70 kVp and 5 mAs (10 mA, 0.5 sec).

Cranial landmarks: In lateral radiographs 3 osseous points appeared easily visible and relatively consistent in their location (Fig. 1). The rostral point (a) corresponds to the junction of the crista galli with the lowest part (caudal ventral part) of the frontal sinus septum. The nuchal point (b) is the external occipital protuberance, and the central ventral point (c) represents the midsagittal projection of the external acoustic meatus. The distance and the angle (rostral and caudal) of the triangle formed by the referred points were measured in all of the individuals examined.

Ventricular landmarks: Cerebral ventriculographs were taken from 15 does on the stereotaxic instrument, and the rostro-caudal and dorso-ventral location of the following 3 ventricular points were measured (Fig. 4). The anterior commissure (rostral ventral aspect of the interventricular foramen), the infundibular recess of the third ventricle, and the most ventral aspect of the olfactory recess of the lateral ventricle were chosen as the most evident areas outlined in the lateral ventriculograph.

RESULTS

Cranial Landmarks: Figure 1 showed the osseous triangle formed by the junction of the crista galli with the caudal ventral part of the frontal sinus septum (a), the external occipital protuberance (b), and the midsagittal projection of the external acoustic meatus (c).

The triangle formed by these 3 osseous landmarks showed a constant relationship in its shape and size irrespective of sex and variation in the body size as shown in Table 1. The rostral angle was particularly constant,

Fig. 1. Drawing of the goat cranial cavity showing the location of the ventricular system (black area), the interthalamic adhesion (white area) and the skull landmarks observed on lateral radiographs. The stereotaxic axes (X_0 and Z_0) and the tangents of the brain (dorsal and rostral) were established using the osseous triangle formed by the point of fusion of the crista galli with the lower part of the frontal sinus (a), external occipital protuberance (b) and the central projection of the lumen of the external acoustic meatus (c).

Table 1. Craniometric parameters of the Shiba goat

Goat number	Sex	Weight (kg)	a-b line (mm)	b-c line (mm)	a-c line (mm)	Angle I (rostral)	Angle II (caudal)
26	Female	26.0	88.0	34.0	62.0	19.0	35.0
16	Female	20.0	89.0	37.0	63.0	20.0	35.0
14	Female	13.0	81.5	32.0	60.0	19.0	37.0
46	Male	13.0	80.0	34.0	62.0	21.0	40.0
42	Male	18.0	83.0	35.0	64.0	21.0	40.0
7	Male	19.0	87.0	36.5	65.0	22.0	41.0
51	Male	13.0	83.0	34.0	62.0	20.0	40.0
53	Male	16.0	86.0	37.0	65.0	20.0	40.0
Minimum		13.0	80.0	32.0	60.0	19.0	35.0
Maximum		26.0	89.0	37.0	65.0	22.0	41.0
Mean		17.2	84.7	34.9	62.9	20.2	38.5
SD		4.5	3.3	1.8	1.7	1.0	2.3
CV.%		26.2	3.9	5.2	2.7	4.9	6.0

a) Point of fusion of the crista galli and the lower part of the frontal sinus.

b) External occipital protuberance.

c) Midsagittal projection of the external acoustic meatus.

SD: Standard deviation.

CV: Coefficient of variation.

showing 20.3 ± 1.0 (mean $\pm$ SD) degrees. Therefore, it represents, together with the external acoustic meatus, the most accurate radiological guides to establish the stereotaxic coordinates.

Ventricular landmarks: The ventriculography clearly outlined the ventricular system and adjacent diencephalic areas. The most evident points visualized were the anterior commissure (rostral ventral aspect of the interventricular foramen), and the infundibular recess of the third ventricle (Fig. 2). The posterior commissure was not visible in the majority of the cases.

The distance between the anterior commissure and the infundibular recess showed little variation in the 15 does studied. It was 12.2 ± 0.6 mm (mean $\pm$ SD) despite individual variation of the skull length (a-b line) which ranged from 74–91 mm. Figure 3 graphically showed a notable correlation ($r=0.89$) between the length of the a-b line (x) and the rostral-nuchal position of the anterior commissure (y) with the equation of $y = -7.08 + 0.42x$. Similarly, the a-b line length (x) highly correlated ($r=0.90$) with the position of the infundibular recess of the third ventricle (y) and the equation was $y = -5.37 + 0.37x$.

Stereotaxic coordinates: Since there was a consistent topographical correlation between the osseous triangle and the ventricular system, new stereotaxic coordinates for goat brain were established as follows using cranial landmarks as reference guides.

The horizontal axis (X_0) was defined as the plane that passed through the external acoustic meatus with an angle of 10° below the a-c line (Fig. 1). This X_0 was practically parallel to the floor of diencephalon and to the principal telencephalon axis, but not parallel to the mesencephalon axis. It cut the pituitary gland approximately in the middle of its dorsal-ventral dimension, and more rostrally it passed at a tangent to the ventral part of the olfactory bulb.

The vertical axis (Z_0 or IA line) was established as the

Fig. 2. Goat sagittal ventriculograph showing the location of the lateral and third ventricles in relation to the a-b line. The radio opaque material was injected into the lateral ventricle through an intracerebroventricular cannula.

plane that passed through the external acoustic meatus and intersected the X_0 plane at 90° . The rostro-caudal location of the structures was referred to as the distance from Z_0 . Every vertical plane parallel to Z_0 thus cut the hypotenuse of osseous triangle (a-b line) forming a caudal angle of 80° . Figure 4 illustrates individual locations of the anterior commissure and the infundibular recess in this stereotaxic coordinate system in 15 female goats.

The third axis (Y_0 or sagittal plane) corresponded to the midsagittal ventricular plane perpendicular to X_0 and Z_0 . It was virtually identical to the interhemispheric sulcus.

Fig. 3. Upper: The correlation ($r=0.88$) between the rostral-caudal location of the anterior commissure (rostral ventral aspect of the interventricular foramen), measured in mm from IA line, and the length of the a-b line. Lower: The correlation ($r=0.90$) between the rostral-caudal location of the infundibular recess of the third ventricle, measured in mm from IA line, and the length of the a-b line.

Fig. 4. Stereotaxic coordinates for three ventricular points, namely the most ventral aspect of the olfactory recess, the anterior commissure, and the infundibular recess in 15 goats.

DISCUSSION

As a demand for more sophisticated anatomical and functional studies of the brain develops, greater precision in locating specific brain structures is required. At present, a critical need exists in studies with experimental animals for precise placement of electrodes, microcatheters or other devices into specific subcortical anatomical structures.

The most widely used way to place the head in a stereotaxic frame is based on the method using cranial landmarks described by Horsley and Clarke [5], in which the horizontal plane passes through the center of the external acoustic meatuses (mid-auricular points) and through the lowest part of the orbital ridge (infra orbital point). This stereotaxic approach has been used for establishing brain atlases of most mammalian species utilized in the neuroscience.

Unreliability of this system, however, has been documented in several species including rat [14], rabbit [17], cat [4] and monkey [13]. Corrections of the stereotaxic coordinates based on the position of the bregma have been satisfactory in rats [11] and newer brain maps based on this system were actually available [8, 15]. On the other hand, osseous landmarks, such as the posterior tip of the sphenoid bone in rhesus monkey [1] and the top margin of the optic foramen in marmoset [9] have been used as effective predictors for positioning the hypothalamic structures.

A new ventricular landmark coordinate system, based on the intercommissural line (anterior and posterior commissure), appeared to be more adequate for use in monkeys [12] and cats [4]. Ventricular coordinates proposed for cats [6] and monkeys [3] have greatly improved accuracy in locating subcortical structures.

These parameters, however, were not necessarily adequate for use in goats, since, for example, the posterior commissure was not well-visualized by lateral ventriculographs, resulting in that the widely-used intercommissural line could not be a reliable reference-line for this species. In addition, the silhouette of the sella turcica

was covered by the zygomatic arch on lateral radiographs. Therefore, in this study, a system using coordinates based on cranial landmarks and intracerebral landmarks, was applied to develop a more reliable method for stereotaxic approach.

Our statistical study revealed that no correction factor could be deduced from body weight, sex, or age. Moreover, the position of the bregma point (interfrontal-interparietal join) was variable due to the development of horns. The parietal-interparietal suture and the lambda point were not easily observable because of the area rugosa covering them in adult goats. These facts make it difficult to establish any stereotaxic system based on distances from either the bregma or the lambda point.

Ventriculographs have been shown to provide good guides for accurate placement of microinjection cannulae or other devices into the hypothalamic nuclei [10]. Present measurements confirmed this observation and the stereotaxic coordinates established by using ventricular landmarks (ventricular coordinates) may therefore represent the most exact method to construct brain topographic maps as Percheron proposed in his study on monkeys [13].

In the goat the osseous triangle formed by the frontal septum, the external occipital protuberance and the projection of the external acoustic meatus, was found to be an easily accessible and clear reference guide to establish the stereotaxic coordinates for locating telencephalic and diencephalic anatomical structures. The landmarks of the goat skull examined in the present study were closely correlated with those of the ventricular system. Moreover, the a-b distance accurately reflected the rostral nuchal position of the anterior commissure. Therefore, the horizontal (X_0) and rostral nuchal (Z_0) axes were indirectly referred to ventricular landmarks.

In conclusion, by using the lateral radiograph of the head, the a-b line was easily measured and thus the position of other points of the osseous triangle, as well as the location of the anterior commissure and infundibular recess, can be estimated with sufficient accuracy without any sophisticated equipment. Our findings are in accordance with previous stereotaxic studies carried out in other species [2, 4, 12]. In each species individual variations of the brain in relation to the skull should be examined in detail to establish the most accurate stereotaxic coordinate system by using cranial or ventricular landmarks.

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