Stereotaxic Approach to Hypothalamic Nuclei of the Shiba Goat with Radiographic Monitoring

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ABSTRACT. Practical method was devised for precise approach to hypothalamic nuclei in the Shiba goat. A stereotaxic instrument and a brain atlas with stereotaxic coordinates were developed. For an accurate placement of probes into specific hypothalamic regions a radiographic method was employed in which radio-opaque material was injected into the lateral ventricle and the ventricular outline was depicted. A sagittal diagram showing the arrangement of hypothalamic nuclei in relation to the brain ventricular system was constructed from the transverse stereotaxic atlas. This diagram was revealed extremely useful in pinpointing the target on the radiographs of lateral view. Precision of this method was evaluated in female Shiba goats (n=4) by comparing radiographically estimated positions of hypothalamic nuclei with those histologically determined. Despite of cranial variability among individual animals these two parameters matched well each other in all the nuclei examined. Furthermore, chronic cannulae were implanted into different hypothalamic structures of one goat and the accuracy of their placement was confirmed histologically. Thus, it was revealed that the stereotaxy by aid of radiography herein described was accurate enough to apply to various neuroendocrinological studies in the Shiba goat.—KEY WORDS: brain cannulae, hypothalamus, radiography, Shiba goat, stereotaxic surgery.

The Shiba goat is a Japanese indigenous goat with relatively small body size, adult female weighing about 20 kg. Since the establishment of its closed colony [6, 7], experimental utilization of the Shiba goat has been steadily growing. In the field of reproductive physiology the Shiba goat is considered of particular importance as an experimental model for domestic ruminants, especially for cattle because of similarities between the two species in various reproductive aspects including endocrinological parameters [8, 10]. The Shiba goat is now also in demand at neuroendocrinological studies, and a method has therefore been devised for precise approach to the central nervous system of the Shiba goat.

Stereotaxic approach to the specific brain structures, e.g. hypothalamic nuclei, is a crucial measure for neurological studies where intracranial probes are to be placed accurately into the target region. Brain atlases are generally used in small laboratory animals such as rats and cats to obtain necessary stereotaxic coordinates. A few brain atlases have also been published for goats [13, 14, 15], but there has been only one atlas available for the hypothalamic area of the goat (Saanen breed) with stereotaxic coordinates [13]. This is, however, not sufficiently detailed and lacking some hypothalamic structures such as vascular organ of the lamina terminalis (OVLT) and arcuate nucleus, both of which are considered playing important roles in neuroendocrine regulation of reproduction. Furthermore, in species like goat the

accuracy of stereotaxic surgery is limited, if it is merely based on the brain atlas, due to large cranial variability among individual animals as compared with small laboratory animals. To circumvent this problem a radiographic technique has been successfully introduced to a stereotaxy for relatively large experimental animals such as dogs [4], sheep [3, 11] and rhesus monkeys [2].

The purpose of the present paper is to provide a practical method for precise approach to the hypothalamic nuclei of the Shiba goat. We have herein devised a radiographic stereotactic technique, in which radio-opaque material is injected into the lateral ventricle, to visualize the outline of the brain ventricular system as a guide to specific hypothalamic structure.

MATERIALS AND METHODS

Apparatus: The stereotaxic instrument for the Shiba goat is illustrated in Fig. 1. We modified the apparatus that is originally designed for cats and dogs (SN-2, Narishige, Tokyo, Japan) to fit for the goat head. Major modifications were as follows: The distance between the both sides of anterior-posterior (AP) bars was widened to be 200 mm. The hind edge of the base frame was removed to accommodate the abdominal portion of the goat. The ear bars and the head holder were newly designed for the Shiba goat so that the external auditory meati (horizontal zero; H 0) was positioned 30 mm above the upper ridge of the AP bars. The position of the skull in relation to the instrument is shown in Fig. 2. The head was supported with a pair of ear bars and the head holder. The ear bars were inserted via the ear adaptor into the external auditory meati, and clamped to the slots in the frame in position with their tips equidistant from each side of AP bars. Here the interaural line was defined as stereotaxic zero in the AP direction (AP 0). The front of the head was supported by the head holder that consisted of a metal plate and 4

![Diagram](image-url)
vertical rods clamped to it: i.e., pairs of eye bars and mouth bars. The plate position was adjustable forwards and backwards, and the rods were free to slide and swing. The horizontal ends of mouth bars were inserted at the sides of the mouth to rest against the maxilla. The lower extremities of eye bars were adjusted to be exactly 25 mm below the HO plane (this level was equal to the lower margin of the orbit) passing through the meeting point of the ear bars. This method allowed the basis encephali to be held horizontally, no matter how large the cranium was. This set-up of stereotaxic instrument enabled lateral radiographic access to the whole hypothalamic area as shown in Fig. 3 without being obstructed by frames.

**Fluoroventriculography:** The surgery for fluoroventriculography was conducted under anesthesia with intravenous (i.v.) infusion of ketamine HCl and xylazine HCl as previously described for the Shiba goat [9] through a 18 gauge jugular catheter (Japan Sharwood, Tokyo). When the animal reached a surgical plane of anesthesia, it was mounted in the stereotaxic instrument, a set of ear adaptor was inserted into the external auditory meati, and the head was fixed as mentioned above.

After parietal disinfection, about 5 cm median incision was made into the scalp and the calvarium was exposed. Then a small hole was drilled 25 mm rostral to AP O, and
4–5 mm lateral to the midline. A spinal needle (21 gauge, 70 mm long, Terumo, Tokyo) attached to a micromanipulator (SM-15, Narishige, Tokyo) was carefully lowered until the tip reached the lateral ventricle 20–25 mm below the dura. A 0.5 ml aliquot of cerebrospinal fluid (CSF) was withdrawn through the needle and a 0.5 ml of radio-opaque material, Iopamidol (Iopamiron 370, Schering, West Germany) [1] was infused slowly into the lateral ventricle. A lateral radiography was taken 30 sec later with conditions of 70 kVP and 5 mAs (10 mA, 0.5 sec). The film cassette was placed 14 cm behind the subject that was 84 cm from the X-ray source. The magnification factor was 1.15, and all the measures on radiography were thus compensated. The layout of the X-ray equipments and the stereotaxic instrument was always kept constant for accurate calculation.

Construction of a sagittal diagram of the hypothalamus: Precise approach to the hypothalamic area relies largely on the lateral radiography which designates the outline of brain ventricular system and some cranial structures. For this reason sagittal arrangement of several hypothalamic nuclei was examined. The sagittal projection was made as shown in Fig. 4 according to three dimensional coordinates on the brain atlas for the Shiba goat developed in our laboratory [in preparation for publication]. Information on the lateral width of ventricles was also given on the diagram, since ventricular width is reflected as the tone of radio-opaque material. This is another useful landmark for pinpointing a specific target.

Histological evaluation: Four adult female Shiba goats, weighing 18–35 kg, were subjected to fluoroscopy as described above. They were then intravenously injected with sodium pentobarbital (Nembutal, 40 mg/kg BW). Catheters were inserted into the common carotid arteries, and the animals were perfused with physiological saline followed by 10% formalin. The jugular veins were cut for drainage, and the perfusion was maintained for at least 1 hr. The animal was decapitated, the parietal bone was removed and the head was re-

![Fig. 4. Reconstruction of a sagittal projection of hypothalamic structures (left) based on the 3 dimensional coordinates on a transverse brain atlas for the Shiba goat (right), which is 30 mm anterior to the APO plane in this case. AC, anterior commissure; LS, lateral septum. For further explanation, see text.](image-url)
mounted in the stereotactic instrument. Reference needles (23 gauge) were inserted vertically into the 6 points: i.e., 15, 25 and 35 mm rostral to the AP O and 5 mm bi-lateral to the midline. Two horizontal reference needles were also inserted parallel to the sagittal plane.

The brain was post-fixed in 10% formalin solution for one week in situ, then removed from the cranium. The distances among reference needles were measured for assessment of the shrinking rate during fixation. The brain was trimmed, embedded in 10% gelatin in physiological saline and kept in 10% formalin saline solution containing 15% sucrose for 4 days. The material was frozen by dry ice, and serial sections of 50 μm thickness were cut into transverse plane which was parallel to the vertical reference needles. Sections every 200 μm were stained with cresyl violet. During the course of histological process, the rate of shrinkage of the brain tissue was revealed negligible (less than 1%).

The hypothalamic structures such as vascular organ of the lamina terminalis (OVLT), suprachiasmatic nucleus (SCH), paraventricular hypothalamic nucleus (PVH) and anterior commissure (AC) were identified microscopically in individual animals, and the outlines of these structures were traced on sheets of paper by a camera lucida. Their positions in relation to the third ventricle (3V) were determined by measuring the vertical distances from the optic recess and horizontal distances from the anterior margin of the interthalamic adhesion. The positions of these structures were superimposed on the lateral radiograph of each animal that had been enlarged 1.74 times on a film (Kodak FX 5060) to give a final magnification rate of 2.0 times of the original brain size. The arrangement of the hypothalamic landmarks thus histologically examined was compared with those radiographically estimated.

Placement of intracranial cannulae: The method was further evaluated from a practical point of view. One adult female Shiba goat weighing 26.0 kg was stereotaxically implanted with 4 chronic cannulae in the preoptic area (PO), PVH, median eminence (ME) and in the lateral ventricle. Each cannula consisted of outer (20 gauge) and inner (24 gauge) stainless steel tubings with different lengths depending on depth of the target.

Following intravenous injection of ketamine HCl (10 mg/kg) and xylazine HCl (0.02 mg/kg), the animal was intubated and anesthetic state was maintained with halothane inhalation (2–3% in oxygen). The stereotaxic approach to respective hypothalamic nuclei was carried out as follows. The tip of each cannula was oriented to the target region on the lateral radiograph refering to the sagittal diagram: the tip being placed just above the optic recess for PO, about 3 mm below the rostral edge of the interthalamic adhesion for PVH, and just above the infundibular recess for ME. Each of the cannulae was fixed to the calvarium with acrylic resin. The scalp was then sutured leaving the guide cannulae exposed. The animal was released from the stereotaxic instrument and given antibiotics for 3 days. The positions of intracranial cannulae were examined by the computed tomography (CT) of transverse and horizontal planes as well as by the lateral radiography. The CT scanning was carried out with CT-W400 (Hitachi Medico, Japan). Upon completion of physiological experiments the localization of cannulae tips were examined histologically as described above.

RESULTS

The sagittal arrangement of hypothalamic nuclei in the Shiba goat is illustrated in Fig. 5 with stereotaxic coordinates. The transverse stereotaxic planes of the brain as well
as reconstructed sagittal diagrams were examined in each of four animals. Individual variations in anterior-posterior coordinates were very small irrespective of variability in their cranial sizes.

Lateral radiographs have clearly depicted the outlines of cerebro-ventricular system and some landmarks such as the interventricular foramen, the optic recess and the infundibular recess which are useful to approach to the hypothalamic structures. The radiographically estimated arrangement of the hypothalamic nuclei in relation to the ventricular system was consistent with those which were determined histologically in all the four animals examined despite of variance in individual cranial sizes (Fig. 6).

The goat that had been implanted with 4 intracranial cannulae showed no symptom of encephalitis and maintained normal appetite as well as behavior throughout the 3 months post-operative experimental period. Positions of cannulae are shown in Fig. 7 and Fig. 8. The CT imaging has been proven very useful in monitoring the cannulae positions in transverse and horizontal planes where ordinary radiographical approach is difficult because of complicated bone structures. Histological examination revealed that the tip of each cannula was placed accurately in expected target region, and that development of gliosis was limited only to the vicinity of each cannula as shown in Fig. 7.
Fig. 6. Positions of some hypothalamic structures radiographically estimated based on the sagittal diagram (shaded areas) and those reconstructed from transverse histological sections (lines) in four individual goats. For abbreviations, see Fig. 5.
Fig. 7. Lateral radiography of the Shiba goat showing chronic cannulae placed into preoptic area (PO), paraventricular nucleus (PVH) and median eminence (ME) (A). Asterisks indicate tips of cannulae. Transverse sections of the brain showing the tracks of cannulae (T) and their tips (*) in PO (B) and ME (C). Abbreviations: ME, median eminence; SO, supraoptic hypothalamic nucleus; Further abbreviations, see Fig. 5.
DISCUSSION

In neurological studies the usage of large experimental animals such as monkeys, pigs, sheep and goats has certain advantage particularly when long-term recording of multiple physiological parameters are required. The number of available animals, however, is generally limited as compared with small laboratory animals. This consequently makes individual animals more valuable, and so it is crucial to adopt the most precise stereotaxic approach.

We have therefore designed a new instrument suitable for a stereotaxy of Shiba goats, made a brain atlas (in preparation for publication) and innovated a technique of stereotaxic surgery. Precision and accuracy of the method herein described were proven satisfactory.

The positions of hypothalamic structures in relation to the stereotaxic coordinates were remarkably constant despite of considerable cranial variations among individual animals. Tindal et al. [13] also noted surprisingly small variations in anterior-posterior coordinates among brains in Saanen goats. Variations in total brain size and/or in cranial cavity could be accounted for primarily by differences in the extent of the cerebral cortex. In spite of these remarkable consistency in stereotaxic coordinates of hypothalamic structures, Richard [12] and Tindal et al. [13] have pointed out that extreme care should be taken when inserting the ear bars to avoid damaging the cartilage of the ear, since, if damage is caused, a correct insertion of the bars be difficult and the head may become displaced by 2-3 mm in relation to the anterior-posterior coordinates. This means that absolute errors involved in stereotaxy in the goat would be larger than those encountered in small laboratory animals. However, we have shown in this paper that these problems could be circumvented with an assist of radiographical compensations, since the arrangement of the hypothalamic nuclei around the third ventricle is extremely consistent in the Shiba goat.

The method herein described has enabled various neurological approach to the hypothalamic region of the goat. For example, we are currently using this technique for intracranial microinjections of neuroactive substances to see the effects on the pituitary function, and for recording the multiunit activity of hypothalamic nuclei with chroni-
cally implanted electrodes (data not shown). The technique for fluoroventriculography has also provided a method for long-term frequent sampling of ventricular cerebrospinal fluid (CSF), by which we have already been able to characterize diurnal patterns of CSF melatonin concentrations in conscious goats [5].

By careful comparison between the brain atlases for the Saanen goat [13, 14] and for the Shiba goat we have found that arrangements of hypothalamic nuclei are virtually identical between the two breeds despite of large difference in their body sizes (22 vs. 50 kg on the average). This may imply that the present method, the radiographic stereotactic approach to the hypothalamic structures, is not only useful to the Shiba goat but also applicable to many other subspecies that are distributed throughout the world.

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REFERENCES

要約

脳室造影法を組み入れたシバヤギ用脳定位手術法の開発：森裕司・武内ゆかり・島田正浩・林絹治1）星野邦夫（東京農工大学家畜臨床繁殖学教室、1）東京都神経科学総合研究所解剖発生）——シバヤギは実験動物として、特に反芻動物のモデルとして種々の生理的実験に供試されている。これらの研究の進展にともない、中枢神経系、特に視床下部への神経内分泌学的アプローチの必要性が増大してきた。目的とする視床下部神経核に正確にアプローチするために、シバヤギ用脳定位固定装置を開発し、精度の高い標準脳定位地図を作成した。さらに脳室と視床下部神経核との相対的位置関係を矢状断面図として再構成し、脳室造影法を組み入れた脳定位手術法の基礎的手法を確立した。4頭のシバヤギを供試し脳室造影後に組織切片を作製し、いくつかの神経核について脳室との位置関係を解析したところ、その位置関係はほぼ一定で個体差は少ないことが判明し、脳定位手術の高い再現性が示唆された。さらに1頭のシバヤギを供試し、実際にこの手法を用いて視床下部の3部位および側脳室内にカニューラを慢性的に留置し、本手術の実用性について検討した。その結果、精度の高いアプローチが可能であり、さらにカニューラを長期間留置して動物の生理状態に重大な影響を与えることなく実験を行うことが判明した。