An anatomic atlas of the medulla oblongata of the adult goat

C. DEAN,1,2 L. K. GEIGER,1 B. M. SPRTEL,1 P. J. OHTAKE,1 AND H. V. FORSTER1

1Department of Physiology, Medical College of Wisconsin, Milwaukee 53226; and 2Department of Anesthesiology, Medical College of Wisconsin and Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin 53295

Dean, C., L. K. Geiger, B. M. Sprtel, P. J. Ohtake, and H. V. Forster. An anatomic atlas of the goat brain stem was developed for use in studies that analyze medullary neuronal groups, and factors that influence variability in the location of neuronal groups were determined. The medullas of 31 adult goats (weight, 17–88 kg) were fixed, harvested, frozen, serially sectioned, stained with 0.5% neutral red, and examined with a light microscope. Obex, the point at which the central canal opens into the fourth ventricle, was taken as the zero reference point from which the rostrocaudal and mediolateral coordinates of medullary neuronal groups were determined, whereas dorsoventral coordinates were calculated from the medullary surface. Histological variations with goat body weight were quantified, and linear regression analysis provided adjustment factors for weight in all three dimensions. Similar analysis of percentage of shrinkage on fixation and processing provided adjustment factors for precise coordinates of medullary neuronal groups. For accurate location of neuronal groups, body weight and histological procedure should be taken into account. The present study provided adjustment factors for body weight and standard histological processing to locate most major medullary neuronal groups in the adult goat.

histological atlas; retrotrapezoid nucleus; nucleus ambiguus; nucleus tractus solitarii

Glossary

AP Area postrema
CC Central canal
CN Cuneate nucleus
CON Cochlear nucleus
ECN External cuneate nucleus
FN Facial nucleus
FNl FN lateral division
FNm FN medial division
FTL Lateral tegmental field
G Nucleus gracilis
GVII Genu of the facial nerve
HP Nucleus praepositus hypoglossi
INT Nucleus intercalatus
IO Inferior olivary nuclei
LRN Lateral reticular nucleus internal division
NA Nucleus ambiguus
NAef External formation of NA
NTS Nucleus tractus solitarii
P Pyramidal tract
PGCL Nucleus paragigantocellularis reticularis lateralis
R Raphe nucleus
RB Restiform body
RG Nucleus gigantocellularis reticularis
RTN Retrotrapezoid nucleus
Sdm Dorsomedial subnucleus of the NTS
SI Intermediate subnucleus of the NTS
Sm Medial subnucleus of the NTS
SO Superior olivary nuclei
SSP Spinal trigeminal nucleus
SST Spinal trigeminal tract
Svl Ventrolateral subnucleus of the NTS
TB Trapezoid body
TS Tractus solitarius
V Vestibular nucleus
IV Fourth ventricle

GOATS ARE THE ANIMAL MODEL of choice for many studies, including some that examine the control of breathing (3, 7, 8, 12, 15). Although much research is focused on neural networks within the medulla oblongata, the locations of major neuronal groups in this region have not been clearly defined in previously published atlases of the goat brain stem (18, 19). To successfully complete studies on central neural control in the goat, there is a need for a histological atlas that details the location of major neuronal groups in the brain stem of this species. To this end, we have compiled an anatomic atlas of the goat medulla and examined factors that influence the stereotaxic coordinates of neuronal groups. This atlas does not contain details of the functions of medullary nuclei, as this topic has been discussed at length in appropriate publications (e.g., 1, 5, 6, 16). The intent of development of this atlas was to produce a histological aid for electrophysiologists to assist in the location of medullary neuronal groups by providing three-dimensional distances from a standard zero reference point and by including compensatory adjustments for weight variation of the goat and for tissue processing. Further refinement of electrode placement would require appropriate physiological criteria and challenges.

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VI Abducens nucleus
nVI Abducens nerve
X Dorsal motor nucleus of the vagus
nX Vagus nerve
XII Hypoglossal nucleus
nXII Hypoglossal nerve

MATERIALS AND METHODS

The protocol for this study was approved by the Animal Care and Use Committee at the Medical College of Wisconsin. Brain stems were studied from 31 adult goats of Alpine, Nubian, and La Manche varieties; the animals ranged in weight from 17 to 88 kg. Twenty-six of the goats were used in physiological studies pertaining to ventilatory control.

Goats were initially anesthetized with ketamine (Ketaset; 15 mg/kg im) and were then intubated; anesthesia was maintained with 1–2% halothane. In 9 of the 26 goats, in vivo measurements of ventral medullary cranial nerve landmarks were made. Each goat was placed on its left side, with the head rotated to a supine position. To expose the ventral surface of the medulla, the trachea was retracted laterally, through a midline incision, to view the foramen magnum. The basilar occipital bone was drilled, and the dura was removed. The abducens (nVI) and the hypoglossal (nXII) nerve rootlets were exposed to permit the lateral and rostrocaudal measurements between the VI nerves and XII nerves where they exit the medulla. These data were subsequently compared with the same measurements made after fixation, histochemical processing, and mounting (postprocessing) to determine the magnitude of brain stem shrinkage. The relationship between shrinkage vs. body weight was attained by linear regression analysis.

A catheter was inserted rostrad into a common carotid artery to perfuse the brain under full anesthesia. The goat was killed through injection of Beuthanasia (90 mg/kg pentobarbital sodium iv). PBS (0.1 M) was perfused through the arterial catheter until the exudate from the excised jugular veins was clear. Subsequently, 4–5 liters of 4% paraformaldehyde fixative in 0.1 M PBS was perfused over 30 min. The brain stems were removed and stored at 4°C in 10% sucrose in PBS until the brain sank (1–2 days), after which time the cryoprotectant sucrose gradient was increased to 20 and then 30%.

For histological analysis, frozen transverse sections (40 µm) through the medulla were cut on a freezing microtome (Reichert-Jung Cryocut 1800). Serial sections were placed on chrome alum-treated slides and were stained with 0.5% neutral red. Sections were analyzed by using light microscopy to identify major brain stem nuclei. The locations of the rostral poles of the dorsal motor vagal nucleus (X), hypoglossal nucleus (XII), nucleus ambiguus (NA), and the caudal pole of the facial nucleus (FN) were measured with respect to obex (the point at which the central canal opens into the 4th ventricle), the midline, and the dorsal or ventral surface. These data were subsequently compared with the same measurements made after fixation, histochemical processing, and mounting (postprocessing) to determine the magnitude of brain stem shrinkage. The relationship between shrinkage vs. body weight was attained by linear regression analysis.

RESULTS

Figures 1–3 are examples of transverse sections taken at 2-mm intervals throughout the medulla of a goat of the middle weight range (35 kg). The illustrations extend from 2 mm caudal to 12 mm rostral to obex. The obex was the rostrocaudal zero reference point used throughout this study. The midline was taken as zero reference in the mediolateral plane and for the atlas, and zero in the dorsoventral plane was taken as the most dorsal point of the dorsal surface of each section. In the text, the dorsoventral measurements were made relative to the surface point directly above or below the center of each nucleus. Descriptions of the locations of major medullary neuronal groups are detailed below. The coordinates given are averages taken from 10 goats in the middle weight range (30–38 kg). The numbers in parentheses (−2 to +12) refer to the level(s) of the sections illustrated in Figs. 1–3.
Dorsal motor vagal (X) nucleus. This nucleus extended from ~4 mm caudal to the obex to 5.5–6 mm rostral to obex. The caudal pole was located dorsal to the hypoglossal nucleus (XII) and ventromedial to the tractus solitarius (TS; −2.0); and the rostral pole was located lateral to the nucleus praepositus hypoglossi (HP; +4.0). Adjacent to the central canal at levels caudal to obex, the nucleus extended 0.5–1.8 mm lateral to the midline, and 1.5–1.9 mm from the dorsal surface (−2.0). More rostrally, the nucleus migrated...
lateral, and, at its rostral extent, was situated 2.6–3.0 mm from the midline and 1.9–2.3 mm from the dorsal surface.

Hypoglossal (XII) nucleus. This nucleus extended from −3 mm caudal to obex to 3.5 mm rostral to obex (−2.0 to +2.0). Caudal to obex, the nucleus was located ventrolateral to the central canal, extending 0.2–1.5 mm from the midline, and it was located 2.0–3.2 mm from the dorsal surface (−0.2). The XII nucleus lay ventral to the X nucleus at the caudal pole through 1.5–2 mm rostral to obex. At 2-mm rostral to obex, the XII nucleus was visible ventral to nucleus intercalatus (INT). The rostral pole of the XII nucleus was located ventral to the nucleus praepositus hypoglossi (HP), ventromedial to the X nucleus, and 0.8–1.1 mm from the floor of the fourth ventricle (IV), extending 1.6–2 mm from the midline (+2.0).

Nucleus tractus solitarii (NTS). The NTS lay longitudinally in the dorsomedial medulla, extending from the spinomedullary junction to the level of the caudal pole of the facial nucleus. In the adult goat, the caudal pole of the tractus solitarius (TS), at 3.5 mm caudal to obex, was located 0.5 mm lateral and 0.5 mm dorsal to the X nucleus. At this level, the TS lay 1.5–1.9 mm from...
the midline and ~1–1.5 mm from the dorsal surface (−2.0). At obex, the NTS bordered medially with the X nucleus and the area postrema (AP; +0.0), while dorsally it bordered with the nucleus gracilis (NG; +0.0). At the intermediate level, from obex to 2 mm rostral, the NTS bordered medially with the AP. The rostral pole of the TS ranged between 6 and 6.5 mm rostral to obex. At the rostral end, the TS migrated laterally to 4.2–5 mm from the midline and 2.5–3 mm from the dorsal surface (+6.0). The caudal two-thirds of the NTS can be divided into subnuclei, generally following the nomenclature of Loewy and Burton (9). Figure 4 illustrates the subnuclei of the TS at the level of the obex. The ventrolateral subnucleus (Svl) contained clusters of large neurons that lay ventrolateral to the solitary tract. Medially, the intermediate subnucleus (Si), that contained relatively smaller cells, lay lateral to the X nucleus. The medial subnucleus (Sm) curved around the medial aspect of the solitary tract, dorsal to the X nucleus. Not shown at this level is the commissural
subnucleus of the TS, which is located caudal to the obex where it spans the region overlying the central canal laterally toward the Sm.

NA. The NA extended from 5 to 6 mm caudal to obex to 3.75–4.0 mm rostral to obex (−0.2 to +2.0). The nucleus was located 3–3.5 mm from the ventral surface and extended 3–3.5 mm from the midline at the caudal portion of the nuclear column. NA was located dorsal to the lateral reticular nucleus (LRN) throughout its extent; however, more rostrally, the nucleus migrated ventrally. At its rostral pole, NA was located 1.9–2.4 mm from the ventral surface and extended 4.0–4.5 mm from the midline (+2.0). The rostral pole of the NA (NAef; +4.0) and adjacent reticular formation corresponds to the retrofacial nucleus (RFN) (16), which has been closely associated with the Bötzinger complex (15) and the rostral ventral respiratory group (rVRG) (1), as described below.

Bieger and Hopkins (2) noted that inconsistent use of terminology regarding the NA had arisen because it “lacks the structural and functional homogeneity implicit in the classical neuroanatomical concept of a nucleus.” Their study carefully examined the neuroviscerotopic organization of the NA, and researchers generally adhere to the terminology of Bieger and Hopkins. Of the two major divisions recognized, one was the NA of classic anatomy, which was composed of compact, semicompact, and loose formations serially arranged rostrocaudally. The second was the external formation (NAef) which lay ventrolaterally. The complexity of the ventrolateral region of the medulla is enhanced by the inclusion of the ventral respiratory group (VRG) (1, 4, 5, 15). The caudal division of VRG (cVRG) lies between the spinomedullary junction and the obex (otherwise termed nucleus retroambigualis). There are two views on the subdivisions of the serially arranged neurons of the VRG rostral to obex. The view held by Feldman (5) and Smith et al. (16) assigned the rVRG as that coextensive with the NA and adjacent reticular formation, and they described the most rostral subdivision as the Bötzinger complex, which is associated with the external formation defined by Bieger and Hopkins (2) and the RFN (16). In contrast, von Euler (4) and Bianchi et al. (1) defined an intermediate VRG (iVRG), which included the NA and para-ambigual regions, and a rostral subdivision (the rVRG) which corresponded to the Bötzinger complex. The Bötzinger complex was considered part of the VRG, rather than a separate entity, because there was a functional rather than anatomic separation from surrounding neurons with different functions. The NA and the para-ambigual region of the iVRG cannot be separated anatomically (4). The distinction between these two regions and the definition of the subdivisions of the VRG are primarily determined by physiological criteria. These physiological considerations have been determined in species other than the goat and were not established in the present study. Therefore, in this atlas, we have labeled the classical NA. This allows the implication, for example, that the Bötzinger complex is located ventrolateral to the NA at its rostral extent or that the cVRG is similarly adjacent to the NA at levels caudal to obex. However, for clarification of position, further refinement should be based on functional considerations.

FN. The FN extended from 5.0 to 6.0 mm rostral to obex to >12 mm rostral to obex (+6.0 to +12.0). The caudal pole of the FN was located 1.5–2.4 mm from the ventral surface and extended 4.2–4.9 mm from the midline (+6.0). Through the most caudal 2 mm, the FN appeared as a compact oval-shaped nucleus. More rostrally, the nucleus separated into a medial and a larger lateral portion (+8.0, +10.0). At ~11 mm rostral to obex, at the level of the superior olives (SO), the FN became a singular nucleus 0.5 mm ventral to the trapzoid bodies (TB), ~1.7–2.5 mm from the ventral surface, and extended 4–5.3 mm from the midline (+12.0).

RTN. The RTN was delineated by retrograde labeling of WGA-HRP from the region of the NA. Figure 5 illustrates the location of cells retrogradely labeled with WGA-HRP; these cells stained with a granular blue reaction product in the rostral ventrolateral medulla ipsilateral to the injection site shown in Fig. 6. At 0.3 mm rostral to obex, the center of the injection site (arrow) was slightly ventral to the NA, into which the reaction product extended. Although injections were made bilaterally, retrograde projections to the RTN from this region of the ventrolateral medulla are considered to be ipsilateral (16). The contralateral injection site was similarly located in the region of the NA but ~300 µm more rostral. Neurons of the RTN stained in a discrete group that was ventral and ventromedial to the medial division of the FN and dorsolateral to the pyramidal tract from 6 to 9 mm rostral to obex. At the more caudal level, the RTN was located 4.2–4.6 mm from the midline and 0.4–0.9 mm from the ventral medullary surface (Fig. 2; +6.0). Fewer cells stained in the more caudal sections, and they tended to be further
from the ventral surface (Fig. 5; +6.0) compared with the more rostral sections. At the rostral level, a greater number of retrotrapezoid cells extended in a compact group ventral to the FN, from 3.1 to 4.1 mm lateral to the midline and closer to the ventral surface of the medulla (150–650 µm; Fig. 5, +8.0). The retrotrapezoid cells were located lateral to the more diffusely scattered stained cells in the nucleus paragigantocellularis lateralis (PGCL; Fig. 5) and reticular cells that extended over the pyramid tract. A few stained cells were located in the postpyramidal region of the raphe (R) complex.

Histological variation with body weight. The variations with body weight of the rostrocaudal distance from obex of the rostral poles of the X nucleus, XII nucleus, TS, NA, and the caudal pole of the FN are illustrated in Fig. 7. There was a statistically significant histological variation with body weight in the distance from obex with increasing weight for the rostral poles of the X and XII nuclei and for the caudal pole of the FN (P < 0.022). There was a trend toward an increase in the distances of the rostral poles of the TS and NA with body weight, but this was not statistically significant (P = 0.17 and 0.11, respectively). Over a range of body weight from 20 to 70 kg, the goat medulla increased up to 1.5 mm in the rostrocaudal direction. The slopes and intercepts of the trend lines for the variation of the rostrocaudal measurements for each nucleus with body weight are shown in Table 1.

The dimensions of the goat medullas also exhibited variations with body weight in the mediolateral and dorsoventral planes. Across a 20- to 70-kg range in animal body weight, the medulla increased up to 0.9 mm in the mediolateral plane. The distance of the rostral poles of...
the X nucleus and NA, and the caudal pole of the FN from the midline increased significantly with body weight (Table 1). The distances of the rostral poles of the TS and the XII nucleus from the midline also showed a trend to increase with body weight, but these values were not statistically significant (P = 0.082 and 0.2, respectively). Across a 20- to 70-kg range in animal body weight, the medulla increased up to 0.6 mm with regard to dorsoventral measurements. The distances to the NA and FN showed statistically significant increases with body weight, yet the variation for the X nucleus, XII nucleus, and TS did not reach significance (Table 1).

Medulla shrinkage from tissue fixation and processing. In nine goats, measurements of the lateral distances between both VI cranial nerves, both XII nerves, and the rostrocaudal distance between the VI and XII cranial nerves were made before fixation and after processing. The change in distance was divided by the measurement before fixation to determine the percentage the tissue shrank after fixation and histological processing. The rostrocaudal and the lateral values of percent shrinkages were plotted against body weight (Fig. 8). The medulla of a 20-kg goat shrunk 15.2% in the rostrocaudal direction, and this value increased to 29.4% for a 70-kg goat. Linear regression analysis of the percentage of shrinkage vs. goat weight indicated that the shrinkage in the rostrocaudal plane increased significantly with increasing body weight (P = 0.03). The effect of the shrinkage is large. For example, for the X nucleus in a 20-kg goat, the rostrocaudal distance from obex is 5.52 mm and the corrected value is 6.51 mm. For a 70-kg goat, the respective distance is 6.82 mm, with a corrected value of 9.63 mm (Fig. 7, bottom). The shrinkage in the mediolateral plane was <13.8% for a 20-kg goat, increased to 22.3% for a 70-kg goat (Fig. 8), and was found not to be statistically significant (P = 0.33).

DISCUSSION

Goats are used extensively in physiological studies, including those that examine the control of breathing (3, 7, 8, 11, 15). This study presents a histological atlas of the adult goat brain stem, detailing the stereotaxic location of major medullary neuronal groups, including the X nucleus, the XII nucleus, the NTS, the NA, the FN, and the RTN. It is beyond the scope of this paper to provide detailed descriptions of the functional roles of these important nuclei, a subject covered in detail elsewhere (see Refs. 1, 4–6, 16 for reviews). Rather, the aim of this study was to provide target coordinates for use in studies involving brain stem nuclei of the goat and to determine whether body weight, fixation, and processing influence their variability.

For tissue which had been similarly fixed and processed, the location of medullary nuclei varied significantly with body weight. This variability is small. With an increase in body weight of 50 kg, the rostrocaudal distance of, for example, the caudal pole of the FN increased only 1.45 mm. Interestingly, the size of a medulla does not appear to vary in direct proportion to body weight between species. In a 2- to 3-kg cat, the hypoglossal nucleus extends 3.5 mm in the rostrocaudal direction; in comparison, this nucleus extended 6.5 mm in a 35-kg adult goat medulla. In comparing these species, with an 81% increase in body weight, the extent of the nucleus increased only 46%. The disparity in the increase in brain size vs. increase in body weight can also be highlighted within a species by comparing the neonatal vs. the adult goat medullas. In general, in vivo measurements taken of the medulla of a 2-kg neonatal goat in this laboratory (10) are only 40%
smaller compared with those of a 35-kg adult goat. Although the neonate medulla is not as small as might be anticipated, it is smaller than would be predicted by calculations provided in this manuscript. The predicted distance of the rostral pole of the XII nucleus from obex in a 5-kg goat would be 2.85 mm, whereas the actual distance in a 5-kg neonate goat is 2.4 mm. This emphasizes the need for anatomic atlases appropriate for the species and maturity of an animal.

Fixation and histological processing of the goat medulla influenced the dimensions of medullary landmarks, as determined from measurements of distances between ventral surface cranial nerve landmarks in vivo and after fixation and processing. Paraformaldehyde fixation and standard histological processing caused a significant shrinkage of the brain stem in the rostrocaudal plane, 19.5% for a 35-kg goat in the middle of the weight range. This shrinkage requires that target coordinates be adjusted by 1% for every 3.5-kg increase or decrease from a 35-kg goat, on which the present histological atlas was based. The variation in the mediolateral plane was less, with the brain stem shrinking 16.4% in the mediolateral direction for a goat that weighed 35 kg. To correct the target coordinates, this value should be adjusted by 1% for every 6-kg change in body weight. The reason for the discrepancy in fixation and/or processing shrinkage in the two planes is not apparent, but it could be due to the cytoarchitecture of the fiber tracts around the periphery of the medulla that may limit shrinkage in the mediolateral plane. Furthermore, shrinkage may be related to the columnar orientation in the rostrocaudal plane of the majority of the neuronal groups and central fiber tracts.

To effectively use the atlas that is presented in this manuscript for precise stereotaxic location of medullary nuclei in the adult goat medulla, the following three-step process should be followed. First, identify the coordinates of the target location from Figs. 1–3; second, correct the coordinates for variations in fixed tissue that result from differences in body weight; and finally, adjust the coordinates for shrinkage that occur between in vivo and fixed conditions. The following example predicts, in one dimension, the distance of the rostral pole of the XII nucleus from obex in a 5-kg goat. For a 50-kg goat by using the equation shown in Fig. 8, the calculated value of target distance for fixed tissue is multiplied by 1/1.0 – (FS/100), which, for this example, is 6.3 (1/1.0 – 0.236) and equals 2.3% mm. Therefore, for a 50-kg goat, the predicted distance from obex of the rostral pole of the X nucleus in vivo is 2.2 mm. This atlas can be used to obtain the coordinates for nuclei other than those listed in Table 1. For example, to obtain the coordinates for the rostral RTN, the three-step process outlined above would be followed for the rostral pole of the FN. These coordinates would be adjusted for the location of the RTN relative to the FN. In other words, with knowledge about the relationship between any nucleus and one of the five examples listed in Table 1, coordinates can be calculated for any medullary nucleus.

In conclusion, for precise stereotaxic location of medullary neuronal groups, the body weight as well as the fixation and histological procedure should be taken into account. The present study provides adjustment factors for body weight (20–70 kg) and paraformaldehyde fixation with standard histological procedures to locate most major medullary neuronal groups in the adult goat.

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Address for reprint requests and other correspondence: H. V. Forster, Dept. of Physiology, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226 (E-mail:bforster@post.its.mcw.edu).

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