Morphometric Analysis of the Nucleus Accumbens Using the Mulligan Staining Method

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BACKGROUND: **There is a need to further anatomically describe the nucleus accumbens (NA), as there is a growing neurosurgical interest in this locus but a limited understanding of its structure. In this study, we evaluated quantitative NA parameters and spatial relationships with adjacent structures found in the telencephalon.**

METHODS: **A total of 155 NA specimens from coronal sections and 3 NA specimens from transverse sections were stained using the Mulligan technique as modified by Barnard et al. The distance from the NA to other structures was then measured.**

RESULTS: **The mean radius of the 155 NAs in the coronal sections was 6.23 ± 0.964 mm, averaging 8.99 ± 2.02 mm from midline (coordinate *x*), 27.09 ± 3.15 mm from the insula, 12.95 ± 3.21 mm from the outer border of the putamen, 10.52 ± 2.66 mm from the upper border of the caudate, and 8.84 ± 2.93 mm from the midline of the lateral ventricle. The mean distance from the NA center of gravity to the middle of the intercommissural line parallel to the midline (coordinate *y*) was 17.08 ±3.61 mm, and the mean vertical distance from the intercommissural line to the NA was 8.12 ± 1.265 mm.**

CONCLUSIONS: **We obtained the stereotactic coordinates of (*x*, *y*, *z*) = (8, 17, −8) for the NA. From this and other delineations of the described position of the NA, it is possible to contribute to stereotactic surgical atlases, improving neurosurgical interventions in this structure.**

INTRODUCTION

The nucleus accumbens (NA) is a part of the ventral striatum and is considered a gateway nucleus to the basal ganglia.[1] This nucleus occupies an area of the forebrain rostral to the anterior commissure,[2] below the anterior arm of the internal capsule, lateral to the vertical part of the diagonal band of Broca, and medial to the claustrum and piriform cortex.[2,3] Unlike most of the other brain nuclei, the NA's boundaries are not completely well defined, extending dorsolaterally to the ventral putamen and dorsomedially to the ventral caudate nucleus.

The anatomofunctional basis of the NA is related to limbic and prefrontal cortico-striatal-pallidal-thalamic circuits, which suggests that this nucleus is related to several diseases.[2,4-7] Neurosurgical interest in this nucleus increased after preliminary studies identified the NA as a potential target for neurosurgical interventions for obesity[3] and schizophrenia,[7] as well as for refractory cases of drug addiction,[8] although there is no consensus that this site is the best target for stimulation in these diseases.[8] Thus, studies of the anatomic structure and activity of the NA are needed.

The objective of the present anatomic study was to examine brains obtained from human cadavers to determine quantitative parameters of the NA and its spatial relationships with adjacent structures in the telencephalon.

METHODS

Data Acquisition

This experimental, analytical, descriptive study was performed in the Anatomy Department of the Biological Sciences Sector at the Federal University of Paraná in Brazil. Because the anatomic cuts

Key words
- Deep brain stimulation
- Nucleus accumbens
- Neuroanatomy

Abbreviations and Acronyms
DBS: Deep brain stimulation
GC: Gravity center
MRI: Magnetic resonance imaging
NA: Nucleus accumbens
OCD: Obsessive-compulsive disorder

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and brain slices of the Department of Anatomy are available for study and research purposes, the study was approved by the university's Research Ethics Committee.

Initially, a total of 164 NA specimens from coronal sections (with separated evaluations, owing to the possibility that 1 side might not be well stained or well preserved), and 8 NA specimens from transverse sections were isolated from human brains (the same observations were applied to NAs on either side) from cadaveric donations used for research purposes. We obtained 2 coronal sections from each brain from a total of 41 entire encephalons. For transverse cuts, we made 1 cut per encephalon, with a total of 4 encephalons used for these sections. Thus, we used a total of 45 encephalons. In addition, 4 transverse sections were obtained from 4 encephalons that were fixed in formalin solution and then cut using a professional 12-inch-long knife, yielding ~5-mm-thick brain slices.

Inclusion and Exclusion Criteria

For this study, the inclusion criteria were integrity of the brain before transverse cuts, integrity of the coronal and transversal cuts, presence of the NA in the obtained sample, and good differentiation of white and gray matter after using a staining method. The exclusion criteria were destruction of or defects in the anatomic specimens, absence of the NA in the cuts except in the transverse sections in the intercommissural line and parallel areas, and poor differentiation of white and gray matter after using a staining method.

Sample Data

A total of 151 NAs in coronal sections and 6 NAs in transverse sections that met the foregoing inclusion criteria were obtained (Figure 1). We excluded 13 NAs in coronal sections and 2 NAs in transverse sections owing to poor conservation of the anatomic specimens. These coronal cuts were grouped according to anatomic planes: A) coronal sections in the region of the anterior commissure (posterior to the optic chiasm), 21 NAs; B) coronal sections in the region of the optic nerve (close to the optic chiasm), 71 NAs; and C) coronal sections in the region of the gyrus rectus and the olfactory sulcus, 59 NAs.

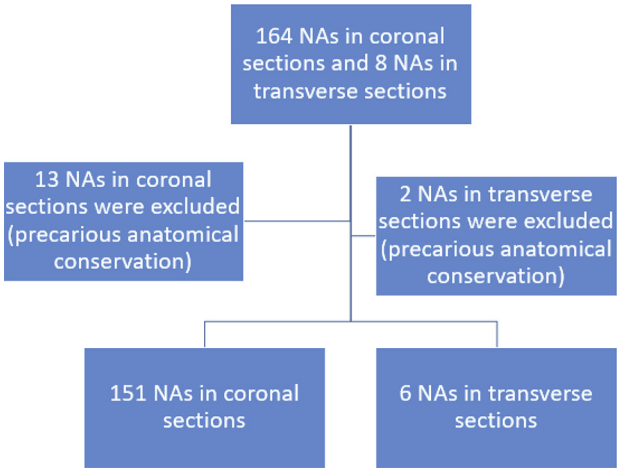

Figure 1. Process of selection of anatomic material for the study.

Starting with this selected anatomic collection, the sections were stained according to the Mulligan technique as modified by Barnard et al.[9,10]

Staining Technique

All samples were stained by the Mulligan technique as modified by Barnard et al. Before staining, it was necessary to submit the anatomic sections to washes so that the formalin solution deposited on the surface of the cuts would be maximally removed to allow for better staining impregnation.

After the washing step, the Mulligan histochemical technique as modified by Barnard et al., which highlighted the gray matter in blue to enhance the morphometric analysis. The staining occurred in well-defined stations, in which chemical and physical reactions occurred. According to this technique, the samples were submitted to 1) Mulligan solution, 2) washing in warm water, 3) washing in running water, 4) washing in 1% ferric chloride solution, 5) washing in 1% potassium ferrocyanide solution, and 6) washing in 10% formaldehyde and 2% hydrochloric acid solution. These components, the duration of incubation, and required temperature are detailed in Table 1.

Once the stations were completed, the staining process was followed by these additional steps: 1) Mulligan solution; 2) washing in warm water; 3) washing in 1% ferric chloride solution; 4) washing in running water; 5) washing in 1% potassium ferrocyanide solution; 6) washing in warm water; and finally, 7) washing in 10% formaldehyde solution.

Morphometric Data Acquisition

After the 7 steps of the staining process, photographic documentation of the recently stained anatomic cuts was performed using an 8-megapixel camera (Figures 2 and 3). This documentation was done as soon as possible, because the brain sections tend to darken to darker blue as time passes, which could compromise the precision of the morphometric analysis.

In the first step, the center of gravity of the NA in each cut was obtained. Considering that its shape in the coronal cuts most closely approximates a semicircle, the center of gravity may be thought of as the mathematical center of this nucleus. The following calculation was used:

$$GC = \frac{4r}{3\pi}$$

where GC is the gravity center, r is the radius, and $\pi \approx 3.1416$.

Once the NA's center of gravity was obtained, measurements from that point were taken at the shortest distance to the midline, the insula (at the lateral sulcus in the coronal sections), the external border of the putamen, the upper border of the caudate nucleus, the lower border of the gyrus rectus (when present in coronal sections), and the midline of the lateral ventricle. It was also possible to obtain approximate measurements of the diameter of each NA.

In addition, the stereotactic coordinates of the NA in relation to the midcommissural point were obtained (where $x, y, z = 0, 0, 0$,

Table 1. Description of the Coloring Process by the Mulligan Staining Technique Modified by Barnard et al

Station	Components	Temperature (°C)	Time (minutes)
Mulligan solution	1000 mL of water (l) + 1.25 g or 1.05 mL of hydrochloric acid (l) + 40 g or 37.73 mL of phenol (l) + 5 g of copper (II) sulfate (s)	60–65	2–3
Washing in warm water	Warm water	—	1
Iron(III) chloride 1% solution	1000 mL of water (l) + 10 g of iron(III) chloride (s)	—	3
Washing in running water	Running water	—	1
Potassium ferrocyanide 1% solution	1000 mL of water (l) + 10 g of potassium ferrocyanide (s)	—	<1
Formaldehyde 10% solution + hydrochloric acid 2%	1000 mL of water (l) + 100 mL of formaldehyde (l) + 20 mL of hydrochloric acid (l)	—	—

i.e., the middle of the intercommissural line between the anterior commissure and the posterior commissure).

ImageJ[11] software (available in the public domain from the National Institutes of Health) was used to measure the NA and its distance from reference points of neurosurgical interest (Figure 4), as well as for calibration.

Statistical Analysis

Numerical data were tabulated and submitted to simple statistical analysis (using standard deviation, mean and median calculations), adopting 95% confidence intervals, using Excel 2016 (Microsoft, Redmond Washington, USA) and R.[12]

RESULTS

Coronal Cuts

Morphometric analysis of the 155 NAs was obtained from the selected anatomic cuts, consisting of coronal cuts of 151 NAs (from 38 encephalons) and transverse cuts of 6 NAs (from 3 encephalons). As described previously, the NA, approximating a semicircle in a coronal section, had a mean radius of 6.231 ± 0.964 mm and a mean distance to the center of gravity of 2.644 ± 0.409 mm. In physics, the center of gravity, also known as the center of mass, is the unique point at which the weighted relative position of the distributed mass sums to 0.

The mean distances from the NA's center of gravity to nearby anatomic structures were as follows: 1) 8.99 ± 2.02 mm to midline (representing the stereotactic coordinate X); 2) 27.09 ± 3.15 mm to the insula; 3) 12.95 ± 3.21 mm to the external border of the putamen; 4) 10.52 ± 2.66 mm to the upper border of the caudate nucleus; 5) 15.84 mm ± 2.94 mm to the gyrus rectus when it was present in coronal sections (which occurred in 52 hemispheres); and 6) 8.84 ± 2.93 mm to the midline of the lateral ventricle. Owing to the proximity of the sections belonging to each of the 3-incidence cut groups, the data were also assessed collectively.

Transverse Cuts

Of the 6 NAs in transverse sections that met the inclusion criteria for this study, the NA center of gravity was located 8.99 mm from the midline. We consider the coronal sections a more reliable measure owing to the ease of delineating the NA in the coronal plane as opposed to the difficulty of determining boundaries in a transverse section.

Thus, when establishing the NA's center of gravity cross-sectionally, the mean distance of this center to the middle of the intercommissural line in a parallel plane to this line was 17.08 ± 3.61 mm. This parameter was used only for transverse cuts because it was of the most neurosurgical interest in this plane, corresponding to stereotactic coordinate y.

Using the hemispheres made by the transverse cuts in the sagittal plane, we were able to obtain the distance from the NA to the intercommissural plane in the vertical direction (corresponding to stereotactic coordinate z). This mean distance was 8.12 ± 1.26 mm inferiorly; therefore, $z = -8.12$ mm, because it was inferior to the stereotactic anterior commissure–posterior commissure line.

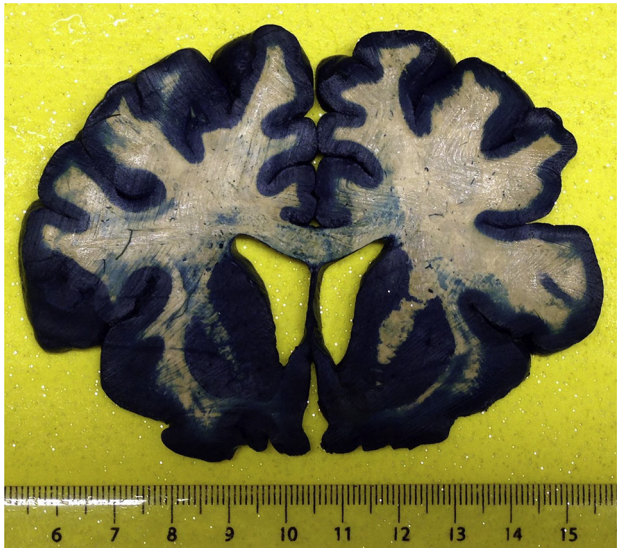

Figure 2. A coronal section stained by the Mulligan staining technique modified by Robert, Barnard, and Brown. Blue, gray matter; white, white matter. This coronal cut is in the region of the gyrus rectus and the olfactory sulcus.

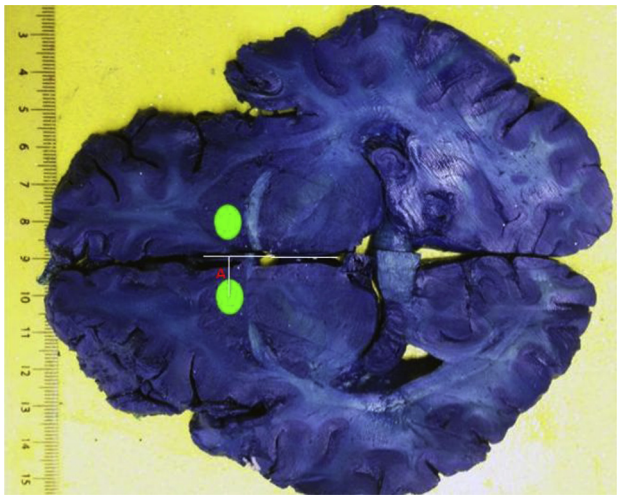

Figure 3. Transverse section stained with the Mulligan staining technique modified by Robert, Barnard and Brown. in green, approximate location of the nucleus accumbens (NA). The white lines are the ImageJ software measurement lines. (**A**) Distance from the gravity center of the NA to midline. In a transverse cut, in the sagittal plane we obtained the distance from the NA to the intercommissural plane in the vertical orientation (which corresponds to the stereotactic coordinate z). This mean value was 8.12 ± 1.26 mm; therefore, z = −8.12 mm, because it was inferior to the stereotactic plane (0, 0, 0).

DISCUSSION

Didactically, the NA is divided into 2 parts: a shell at the ventromedial margin of the nucleus (associated with the limbic system) and a heart (associated with the extrapyramidal motor system).[2] Therefore, it influences the limbic–motor interface and is involved in several cognitive, emotional, and psychomotor functions and their respective diseases, such as Alzheimer disease, depression, and obsessive-compulsive disorder (OCD).[13-16]

Although the main contributions of the NA are from GABAergic neurons, dopamine is the main neurotransmitter in this nucleus.[17] Indeed, the NA has been recognized as a critical center for reward and pleasurable experiences.[18] For this reason, the NA has also been associated with obesity, addiction to opioids and other drugs, and other psychiatric diseases; therefore, this nucleus has been listed as a potential target for neurosurgical intervention for disorders related with these conditions.[3,4,7,8]

Sturm et al.[17] reported the first use of deep brain stimulation (DBS) of the NA in humans for the treatment of OCD and anxiety disorder. Gao et al.[19] performed neurosurgical stereotactic ablation of the NA for the relief of psychological addiction to opiates with a minimally invasive technique; this was the first report of this intervention in humans. Over the last 8 years, the use of DBS applied to the NA has increased,[1] and DBS is currently used in carefully selected patients with refractory major depression, OCD, or Tourette syndrome.[18,20,21]

Previous studies on the NA were based purely on stereotactic coordinates or imaging tests, such as magnetic resonance imaging (MRI), without both anatomic and stereotactic information in the same study. It is important to note, however, that most typical atlases used in stereotactic neurosurgery, including Talairach and Tournoux (1957 and 1988)[22,23] and Schaltenbrand and Bailey (1959 and 1976),[24,25] were based on a small number of brains. Talairach and Tournoux's atlas of 1957 was based on only 1 encephalon. For Schaltenbrand and Bailey's atlas, 111 brains were used, but only 34 cuts from 7 brains were analyzed. The largest number of brains analyzed in a stereotactic atlas until now was 30 brains in the Afshar atlas (1978).[26] It is also worth noting that the intercommissural line was used as a stereotactic landmark after the publication of the Talairach and Tournoux atlas in 1957.[26]

A review of the literature on dedicated human anatomic studies identified no major morphometric studies of the NA. For this reason, our main goal in the present study was to determine, in the brains of human cadavers, quantitative parameters of the NA and their spatial relationships relating to more constant structures in the telencephalon to aid in correct localization of the NA. The present study is the first study with such characteristics.

Studies of human anatomic tissue have advantages over other animal or imaging techniques. Neto et al.[2] reported that the NA is poorly defined by MRI (T1-weighted sequences) compared with anatomic techniques, because the NA does not exhibit a distinctive signal intensity in the MRI images.

In contrast, anatomic slices require formaldehyde fixation, which can lead to minor tissue dehydration and change the dimensions in sample analysis compared with in vivo brains.

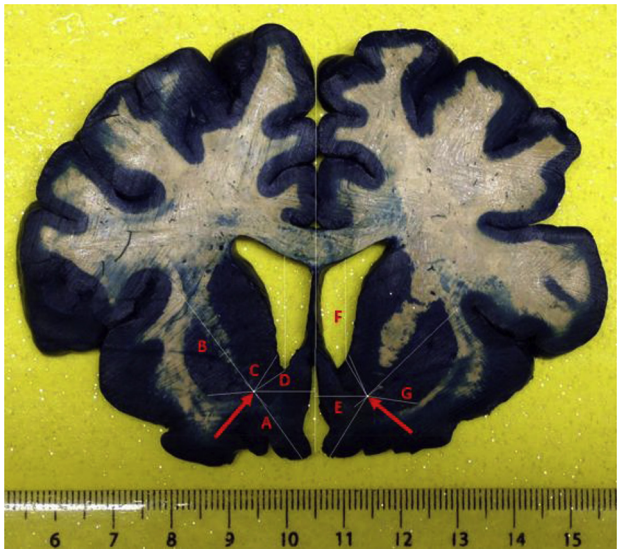

Figure 4. A coronal section stained with the Mulligan staining technique modified by Robert, Barnard and Brown. Blue, gray matter; white, white matter. This coronal cut is in the region of the gyrus rectus and the olfactory sulcus. The white lines are the ImageJ software measurement lines. The *arrow* indicates the gravity center of the nucleus accumbens (NA), calculated by the formula. Other measurement lines extend from the NA gravity center. (**A**) Gravity center to the gyrus rectus. (**B**) Gravity center to the insula. (**C**) Gravity center to the upper border of the caudate nucleus. (**D**) Gravity center to the midline of the lateral ventricle. (**E**) Gravity center to the midline (representing the stereotactic coordinate *x*). (**F**) Midline of the lateral ventricle. (**G**) Gravity center to the external border of the putamen.

In cases where the NA is a stereotactic target,[17,18,21,27] the most commonly used reference structures are the midline, the plane between the anterior and the posterior commissures, and the anterior margin of the anterior commissure. It is well known that the difficulties in delineating the NA are related to several factors, especially due to the fusion of different neuronal systems in the ventral striatum, which represents the separation of the NA from the putamen and the caudate nucleus.[4] This difficulty in establishing a precise delineation of this nucleus (because it is not possible to visualize its entire perimeter, but only an "estimate") supports the use of the center of gravity as a parameter of greater reliability for analysis. The inability to perfectly visualize the whole perimeter may be an inherent disadvantage in all studies of this structure.

The measures corresponding to the stereotactic coordinates x, y, and z are of paramount importance for the new stereotactic surgeries whose target has been the NA. In this respect, it is possible to compare the current results with those reported by Mavridis et al.,[28] including $y = 2$ mm anterior, $x = 8$ mm lateral, and $z = 4$ mm inferior, with 32 anatomic slices and 26 MRI images. However, Mavridis et al. considered the anterior border of the anterior commissure in the midline as a stereotactic reference point [$(x, y, z) = (0, 0, 0)$], whereas in our study the middle of the intercommissural line (anterior commissure—posterior commissure) was used as a reference in surgical practice.[23] Moreover, according to the methodology used by Mavridis et al.,[28] it was not possible to observe where the measurements were made in the NA. This is certainly important because in the case of millimeters, a few units are already representative. In our methodology, we chose to obtain measurements from the center of gravity of the NA to standardize the measurements of interest. A comparison of other parameters evaluated in our study with data published in the literature is not currently possible, because there is no such information. This study is valuable in its anatomic and surgical aspects, because the distances measured between structures or points to the NA allow coordinates in MRI to be obtained for the evaluation of the NA as a stereotaxic target.[2] Therefore, this study may become a descriptive study that can support others, as well as allowing for future comparisons.

CONCLUSIONS

In this study, we obtained the stereotactic coordinates of the NA as, on average (x, y, z), 8 mm lateral, 17 mm anterior, and 8 mm behind the middle of the intercommissural line connecting the anterior commissure to the posterior commissure. Moreover, the morphometric delimitations of the NA and their respective relationships to other points considered more constant in the brain allow for direct application in the anatomic determination of stereotactic coordinates in surgeries targeting the NA.

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