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**Título artículo / Títol article:** Critical evaluation of the anatomical location of the Barrington nucleus: relevance for deep brain stimulation surgery of pedunclopontine tegmental nucleus

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**Revista:** Neuroscience, 2013, Volume 247

**Versión / Versió:** Post-print

**Cita bibliográfica / Cita bibliogràfica (ISO 690):** BLANCO, Lisette, et al. Critical evaluation of the anatomical location of the Barrington nucleus: relevance for deep brain stimulation surgery of pedunclopontine tegmental nucleus. *Neuroscience*, 2013, vol. 247, p. 351-363.

**url Repositori UJI:** <http://hdl.handle.net/10234/89110>

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## Accepted Manuscript

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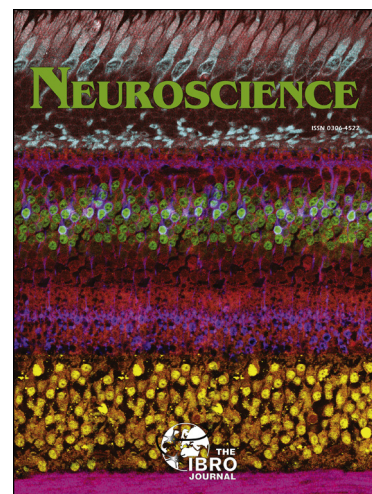
PII: S0306-4522(13)00480-6  
DOI: <http://dx.doi.org/10.1016/j.neuroscience.2013.05.047>  
Reference: NSC 14641

To appear in: *Neuroscience*

Accepted Date: 23 May 2013

Please cite this article as: L. Blanco, J.E. Yuste, M.C-d. Sauvage, A. Gómez, E. Fernández-Villalba, I. Avilés-Olmos, P. Limousin, L. Zrinzo, M.T. Herrero, Critical Evaluation of the anatomical location of the Barrington nucleus: relevance for deep brain stimulation surgery of pedunclopontine tegmental nucleus, *Neuroscience* (2013), doi: <http://dx.doi.org/10.1016/j.neuroscience.2013.05.047>

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# Critical Evaluation of the anatomical location of the Barrington nucleus: relevance for deep brain stimulation surgery of pedunclopontine tegmental nucleus

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**Abbreviations:** Bar, Barrington nucleus; CRF, corticotropin release factor; DBS, deep brain stimulation; LC, locus coeruleus; LDTg, laterodorsal tegmental nucleus; PD, Parkinson disease; PPN, pedunclopontine nucleus; TH, tyrosine hydroxylase; VACHT, vesicular acetylcholine transporter.

**Keywords:** Barrington nucleus, pedunclopontine nucleus, locus coeruleus, laterodorsal tegmental nucleus, micturition centre

**Running Title:** Critical Evaluation of the Anatomical Location of the Barrington Nucleus

**ABSTRACT**

Deep brain stimulation (DBS) has become the standard surgical procedure for advanced Parkinson's disease (PD). Recently, the pedunculopontine tegmental nucleus (PPN) has emerged as a potential target for DBS in patients whose quality of life is compromised by freezing of gait and falls. To date, only a few groups have published their long-term clinical experience with PPN stimulation. Bearing in mind that the Barrington (Bar) nucleus and some adjacent nuclei (also known as the micturition centre) are close to the PPN and may be affected by DBS, the aim of the present study was to review the anatomical location of this structure in human and other species. To this end, the Bar nucleus area was analyzed in mouse, monkey and human tissues, paying particular attention to the anatomical position in humans, where it has been largely overlooked. Results confirm that anatomical location renders the Bar nucleus susceptible to influence by the PPN DBS lead or to diffusion of electrical current. This may have an undesirable impact on the quality of life of patients.

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There has been growing enthusiasm for the use of surgery as therapy for advanced Parkinson's disease (PD) patients, due primarily to the limits of a more efficient pharmacological treatment in some patients and following a better understanding of the pathophysiology of the motor circuits of the basal ganglia involved in the disease (Obeso et al., 2000). In this context, deep brain stimulation (DBS) for PD was introduced, targeting different structures depending on patient symptoms (Benazzouz and Hallett, 2000). Clear improvement is obtained by stimulation of the subthalamic nucleus (STN) and the globus pallidus pars interna (GPi) in patients with dyskinesias, motor fluctuations, tremor and rigidity (Guridi et al., 2000; Nilsson et al., 2008). However, gait disorders refractory to dopamine remain a clinical challenge as they may not improve and may even deteriorate following DBS at these targets.

The pedunculopontine nucleus (PPN) forms part of the mesencephalic locomotor region and is implicated in the gait disturbance that characterizes parkinsonian syndromes (Pahapill and Lozano, 2000). In animal models, PPN lesions have been reported to impair attention, executive function, working memory and learning (Keating et al. 2002; Keating and Winn, 2002; Inglis et al., 2000, 2001; Inglis and Winn 1995; Kozak et al., 2005; Garcia-Rill, 1991). High frequency PPN DBS in a non-human primate model had similar effects. The macaque was then rendered parkinsonian with intravenous MPTP. Low frequency DBS (5 to 10 Hz) improved motor activity (Jenkinson et al., 2005; Jenkinson et al., 2004; Jenkinson et al., 2006).

To date, only a few groups have published their long-term clinical experience with PPN stimulation (Stefani et al., 2007; Capozzo et al., 2009; Ferraye et al., 2010; Moro et al., 2010). Information is scarce concerning the collateral damage to structures surrounding the PPN that may be affected during or after surgery given the tightly packed structure of the human brainstem and the variation in electrode placement in relation to the PPN.

Paraesthesia and oscillopsia have been reported during PPN stimulation (Ferraye et al., 2009). It is therefore necessary to assess the possible risks to structures located in the trajectory of the surgical target, or which may be affected by the expanded stimulus in order to minimize side effects due to affection of others brainstem structures.

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In the present work, we evaluate one of the crucial structures neighbouring the PPN, the so-called Barrington (Bar) nucleus, which controls the micturition reflex. Micturition is the process by which the bladder is emptied. It consists in the contraction of the bladder and the simultaneous relaxation of the posterior urethra. If this coordination of contraction and relaxation is altered, micturition disorders may appear (Barrington, 1914). A spino-bulbo-spinal pathway passing through two coordination centres, the periaqueductal gray matter and the pontine micturition centre, located in the caudal brainstem mediates the micturition reflex. This pathway is in turn modulated by higher centres of the cerebral cortex involved in the voluntary control of micturition (Groat and Yoshimura, 2010). In addition, four specific areas of the mammalian neural system are important for the control of micturition and continence: I) the ganglion cells of the bladder wall and the sympathetic (autonomic) and dorsal (sensory) root chains; II) the motor neurons and the sensory interneurons of the spinal cord; III) the caudal brainstem (pontine micturition centre); and IV) the cortical and subcortical areas (Blok, 2002; Sugaya et al., 2005).

The “micturition reflex centre” was described by Barrington in the cat and is located in a small region of the dorsolateral pontine tegmentum (Barrington, 1925). Since their description, many electrophysiological studies have investigated the physiology and function of the “pontine micturition centre” and micturition reflex (Nathan, 1952; Kuru, 1965; Satoh et al., 1978). Importantly, bilateral lesions of the dorsolateral pons, immediately ventral to the medial edge of the superior cerebellar peduncle, result in a permanent inability to empty the urinary bladder (Barrington, 1925). Furthermore, electrical stimulation of the dorsolateral pontine tegmentum, in the region of the *locus coeruleus* (LC), evokes contraction of the urinary bladder (Barrington, 1925), which gives evidence for the specific role of these nuclei in the micturition reflex. The pontine micturition centre or Bar nucleus directly excites the motor neurons of the sacral spinal cord that innervate the bladder during micturition (Blok, 2002) and inhibits the motor neurons that innervate the sphincter of the external urethra. On the other hand, the pontine continence centre activates the sphincter of the external urethra during continence. The midbrain periaqueductal gray matter receives bladder-filling information (Blok, 2002).

The micturition centre that Barrington described in the cat corresponds to a group of cells lateral to the laterodorsal tegmental (LDTg) nucleus in the pontine tegmentum of the mouse (VanderHorst and Ulfhake, 2006) and rat (Swanson, 1998).

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According to these descriptions, the Bar nucleus has been well defined in rodents, and even in non-human primates; indeed, it is included in common atlases (Paxinos and Watson, 1997; Paxinos et al., 1999; Paxinos and Franklin, 2001). However, the area occupied by Bar nucleus is not delineated in the commonly used human brain atlases (**Fig. 1**) (Schaltenbrand and Wahren, 1977; Paxinos and Huang, 1995) and consequently may be overlooked in some surgical procedures.

In clinical practice, the location of the PPN on standard clinical imaging can only be inferred from the visualized surrounding structures on MRI (Zrinzo et al., 2008). The PPN lies in the lateral pontine and mesencephalic tegmental reticular zones, straddling the pontomesencephalic junction, with its long axis roughly parallel to that of the fourth ventricle floor. The rostral pole lies at mid-inferior collicular level and the nucleus extends caudally to reach the rostral pons. In the pontine tegmentum, the PPN lies medial to the curved lemniscal tracts and lateral to the superior cerebellar peduncle en route to its decussation (Paxinos and Huang, 1995).

Both trans-ventricular and extra-ventricular approaches to the PPN have been described (Aviles-Olmos et al., 2011; Khan et al., 2010). However, since both surgical trajectories commence from the ipsilateral frontal region, the deep aspect of the DBS lead may come to lie in close proximity to the “micturition reflex centre” and its connections. Indeed, micturition problems following PPN DBS have recently been reported (Aviles-Olmos et al., 2011). An alternative surgical approach involves stereotactic brain magnetic resonance imaging (MRI) and contrast ventriculography to define the bi-commissural line and the fourth ventricle (Piallat et al., 2009). Stereotactic lead implantation carries a small but definite risk of bleeding (Zrinzo, 2012). This may have serious consequences in the brainstem leading some authors to recommend unilateral stimulation since the PPN has bilateral connections.

A review of the anatomical localization of the Bar nucleus is warranted. In the present study, we analyze the location of the Bar nucleus in the mouse and monkey and propose the exact location of this structure in the human brainstem.

## EXPERIMENTAL PROCEDURES

### Human and Animal Tissue

All studies were performed in accordance with the Ethical Committee of the University of Murcia and the Brain Bank of the *Hospital Virgen de la Arrixaca* and the Guidelines of the European Convention for the protection of Vertebrate Animals used for

1 Experimental and other scientific purposes of the European Communities Council  
2 Directive (2010/63/ECC) and the Helsinki Declaration.

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4 Five adult C57BL/6J mice were used in the present study. Mice were group-  
5 housed under controlled photoperiod (12 h day-night cycles), at constant room  
6 temperature (22 °C) and had access to food and water *ad libitum*. Animals were  
7 sacrificed by an overdose of Ketamine anaesthesia (50 mg/kg) and perfused with  
8 phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in 0.1M  
9 PBS. The brains were removed and postfixed in 4% PFA solution for 24h.

10  
11 The monkey brain tissue used was obtained from two intact adults male  
12 macaques (*Macaca fascicularis*), which have been studied in the Primate Unit of the  
13 University of Murcia. The monkeys were sacrificed with a lethal pentobarbital injection  
14 after ketamine anaesthesia. The brains were removed and fixed for 4 days in 4% PFA  
15 dissolved in 0.1 M PBS.

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17 The human brainstem was obtained from the Brain Bank of the *Hospital Virgen*  
18 *de la Arrixaca* (Murcia, Spain). The brain samples were maintained in a fixative  
19 solution containing 10% PFA in PBS during 10 days before its processing.

### 20 21 22 23 24 25 26 27 28 29 30 31 **Specific staining**

32 All brains were post fixed for 24 h and immersed in 30% sucrose in PBS for 4 days.  
33 Serial sections (20 µm coronal sections for mouse and and 40 µm transverse sections for  
34 human and monkey, starting downward from SNpc) were then cut on a freezing  
35 microtome (Microm HM 400).

36 The free floating sections of the brainstem were stained with an antibodies against  
37 tyrosine hydroxylase (TH) (Sheep polyclonal, 1:1000; Chemicon, Temecula Inc., CA) in  
38 order to observe the noradrenergic neurons from the LC, for vesicular acetylcholine  
39 transporter (VAcHT) (Rabbit monoclonal, 1:1000; Sigma Aldrich, Saint Louis, MO) in  
40 order to stain the cholinergic cells from the laterodorsal tegmental nucleus (LDTG) and  
41 for corticotropin release factor (CRF) (Rabbit polyclonal, 1:250; Peninsula Laboratories,  
42 LLC, San Carlos, CA) in order to stain neurons from the Bar (See Table 1 for antibody  
43 details).

44 For TH and CRF immunohistochemistry, endogenous peroxidase activity was  
45 inhibited with 0.3% H<sub>2</sub>O<sub>2</sub> and non-specific Fc binding sites were blocked with 10% horse  
46 serum. Sections were incubated for 48 hours (at 4 °C, constant shaking) with primary  
47 antibody diluted in PBS containing 1% horse serum, 0.5% Triton X-100, and 0.1%



1 sodium azide. Sections were incubated for 4 hours in secondary antibody diluted in  
2 antibody solution. Binding of antibody was detected with avidin-biotin peroxidase ABC  
3 kit (Vectastain, Vector Labs) and 3,3'-diaminobenzidine (DAB; Peroxidase Substrate Kit;  
4 Vector Laboratories). Sections were mounted on gelatin-coated slides and dehydrated in  
5 graded ethanol series and xylene before being coverslipped. For human TH and CRF co-  
6 immunohistochemistry, the same section of human brain tissue was firstly stained for TH  
7 and revealed with DAB Peroxidase Substrate Kit without nickel chloride solution and  
8 secondly it was stained for CRF and revealed with DAB Peroxidase Substrate Kit without  
9 nickel chloride solution to obtain a gray-black stain. CRF immunohistochemistry was  
10 only performed in human sections since the human brain was the main object of this  
11 research. Thionine histochemistry was also performed to visualize the outline of the  
12 nuclei in the area.

21 The cholinergic neurons were stained by histoenzimology in human and monkey  
22 brainstem detecting the NADPH-diaphorase activity of the nitric oxide synthase (NOS)  
23 (Table 1). The detection of NADPH-diaphorase activity was carried out according to a  
24 previously published protocol (Hunot et al., 1996). Free floating sections (40  $\mu\text{m}$ ) were  
25 washed three times in 0.1 M PBS (pH=7.4) and then incubated 24 hours at 37 °C in  
26 0.1M phosphate buffer (pH=7.4) containing 0.9 mg/ml nitroblue tetrazolium, 2 mg/ml  
27  $\beta$ -NADPH and 12% (v/v) dimethylsulfoxide. The reaction was stopped by placing the  
28 sections in 0.1M PBS solution. Sections were mounted on gelatine-coated slides, dried  
29 and coverslipped with Eukitt (O. Kindler, Freiburg, Germany).

38 The architectonic borders of the sections traced according to the thionine stain  
39 using a camera lucida. Following this, the immunoreacted sections were matched to the  
40 drawings and the immunopositive cell bodies marked. To be considered  
41 immunopositive, the cell bodies had to exhibit clearly stained dendrites/axons. These  
42 drawings were scaled down, scanned, and redrawn using the Image J drawing program.  
43 High power digital photomicrographs were captured using a Zeiss Axioskop and the  
44 Axiovision software. No pixilation adjustments or manipulation of the captured images  
45 were undertaken, except for the adjustment of contrast, brightness, and levels using  
46 Adobe Photoshop 7.

54 The images were captured with a high-resolution digital camera (AxioCam HRC,  
55 Karl Zeiss) connected to a conventional optical microscope (Zeiss AxioPlan 2).

## RESULTS

1 The most common atlases used for DBS indicate that the PPN is located in the caudal  
2 mesencephalic tegmentum, extending from the caudal border of the red nucleus to the  
3 parabrachial nucleus [for extended review on PPN anatomy sees Alam et al., 2011]. The  
4 PPN is bordered medially by fibres of the superior cerebellar peduncle and its  
5 decussation and laterally by the medial lemniscus (**Fig. 1**). Most importantly for this  
6 study, the most caudal part of the PPN pole is adjacent to neurons of the LC (Pahapill  
7 and Lozano, 2000). In fact, the LC and other structures, like the LDTg, can be clearly  
8 observed medial, posterior and caudal to the PPN (**Fig. 2**). The brain atlases of different  
9 species normally indicate the Bar nucleus in this particular area, adjacent to the LC and  
10 the LDTg, as we will discuss in following sections. However, atlases for human brain  
11 do not mention this structure at all (**Fig. 2**).

### Bar nucleus in rodent

22 Conventional rodent brain atlases locate the Bar nucleus medially to the LC and  
23 laterally to the LDTg, adjacent to the PPN (**Fig. 3**). In a series of coronal sections, it is  
24 possible to visualize this area starting from Bregma -5.34 mm to Bregma -5.68 mm in  
25 the mouse (Paxinos and Franklin, 2001).

26 In rodents, the Bar nucleus has been anatomically defined as a small group of  
27 neurons located bilaterally in the pontine tegmentum, ventromedial to the rostral pole of  
28 the LC (Cano et al., 2000). In our study, in order to identify the Bar nucleus in mouse  
29 brain sections, we used the anatomical references of the LC and the LDTg with two  
30 specific markers. The neurons of the LC can be clearly stained with TH  
31 immunohistochemistry while the LDTg is clearly evidenced by VACHT  
32 immunostaining. In addition, we also use a structural staining with thionine in order to  
33 facilitate the localization of surrounding structures and nuclei (as groups of neurons).

34 Thionine staining revealed the presence of different groups of cell bodies with  
35 several morphologies in three areas laterally to the IV ventricle: an area with small and  
36 rounded cell bodies, the LDTg, followed by an area of ovoid cells, which corresponds to  
37 the Bar nucleus and a clearly distinguishable area of large neurons with an intense  
38 thionine staining, the LC (**Fig. 4B-C**). TH immunohistochemistry confirmed the  
39 presence of the noradrenergic neurons of the LC separated medially from the IV  
40 ventricle (**Fig. 4 G-H**). In addition, VACHT immunohistochemistry revealed the  
41 presence of cholinergic neurons in the caudal brainstem, which correspond to the LDTg

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(**Fig. 4 D-E**). LDTg appears as an elongated structure, which is extended in laterodorsal position closely opposed to the IV ventricle. Rostrally, the lateral concavity of the LDTg is oriented to the midline; caudally the concavity is oriented to the lateral position. Between the LDTg and LC, there is an area of transition where the VAcHt<sup>+</sup> cholinergic neurons from the LDTg are intermingled with the TH<sup>+</sup> noradrenergic cell bodies of the LC (Luppi et al., 1995), this area corresponds with the putative location of the Bar nucleus. Since CRF is the major neurotransmitter in Bar nucleus, the neurons of this area can be specifically stained with antibodies against CRF (Valentino et al., 2011). In mouse sections, we can observe some CRF<sup>+</sup> fibers and neurons, limited laterally by the neurons of the LC and medially by the LDTg (**Fig. 4**).

### Non-human primates

Commonly used brain monkey atlases indicate that the PPN, consisting of *pars compacta* and *dissipata*, is similar to that of humans (**Fig. 5**; at Bregma -18.90 mm). The Bar nucleus is also indicated in the same atlas but located 4 mm posterior to the PPN (**Fig. 5**; at Bregma -22.95 mm). As with rodents, the Bar nucleus is limited laterally by the LC and medially by the LDTg. In our study we selected two levels of sections adjacent to -22.95 mm where the Bar nucleus could be clearly detected according to Paxinos et al. (1999) (**Fig. 5**). The structures of anatomical reference for Bar nucleus, the LDTg and LC, were easily detected in monkey brain at sections from -22.55 mm to -23.85 mm from the Bregma. Immunohistochemistry for TH revealed a clear positive staining of noradrenergic neurons of the LC, and NADPH-diaphorase activity revealed evident neurons of the LDTg nucleus (**Fig. 6**). However, very few NADPH-diaphorase positive neurons were found in the caudal levels (**Fig. 6**).

### Human Tissue

As with non-human primates described above, the atlas of the human brainstem shows a similar distribution of the LC and the LDTg at the pontine levels (corresponding with Obex +28 and Obex +26 mm); however, the Bar nucleus is not indicated (**Fig. 2**). In our study, we chose sections from these two levels in order to identify the neurons of the Bar nucleus with CRF specific staining. In the first level (Obex +28), the three areas of interest may be identified: the LDTg, LC and Bar with thionine, TH and NADPH-diaphorase staining, as with monkey brain tissue. Immunohistochemistry confirmed that there were a discrete number of TH<sup>+</sup> noradrenergic cells of the LC at this level (**Fig. 7 D-E**). Simultaneously, a large number of medium sized cells were seen in the dorsal

border of the putative Bar nucleus area. These cells were labelled with histoenzymology for NADPH-diaphorase activity and were seen to correspond to cholinergic cells from the LDTg. The transition area between LDTg and LC presented abundant cells with brown pigmentation due to neuromelanin accumulated in noradrenergic cells from the LC. These neurons could clearly be distinguished from the dark blue precipitate of the NADPH-diaphorase reaction product in cholinergic cells from the LDTg (**Fig. 7 F-G**). Furthermore, CRF<sup>+</sup> cell bodies can be clearly observed between the LC and LDTg corresponding to the neurons in the micturition centre or Bar nucleus (**Fig. 7 H-I**). At the second level (Obex +26), the LC can be clearly observed but very few neurons of the LDTg can be seen (**Fig. 7**).

Thionine staining revealed a large number of pigmented cell bodies corresponding to noradrenergic cells from the LC, intermingled with ovoid cells belonging to Bar nucleus (**Fig. 7 K-L**). A robust labelling of TH<sup>+</sup> neurons could be seen in the LC area (**Fig. 7 M-N**). Magnification revealed that a large number of processes (apparently dendrites, but probably also axons) were found outside the nuclear core of the LC (**Fig. 7 M-N**). It was possible to see a few NADPH<sup>+</sup> cholinergic cells, close to the most caudal pole of LDTg (**Fig. 7 O-P**). At this level, some CRF<sup>+</sup> neurons could still be seen but less apparent than in the previous level (**Fig. 7 Q-R**). Double staining of adjacent sections of the first level (Obex +28) with TH and CRF revealed CRF<sup>+</sup> cell bodies at the border of the LC where TH<sup>+</sup> neurons and fibres intermingled (**Fig. 7 S-U**), confirming the presence of the neurons of the micturition reflex centre. Therefore, the Bar nucleus in human is bounded on its lateral side by the LC and on its medial side by the LDTg. Superiorly, the Bar nucleus CRF<sup>+</sup> neurons extend posteriorly and laterally towards the LC, which is adjacent to the most inferior pole of the PPN.

## DISCUSSION

In the present study, the anatomical location of several pontine nuclei (LDTg, Bar and LC) related with the micturition reflex is reviewed in different species, including a description of the Bar nucleus in humans (until now lacking). Further work should take into consideration the anatomical proximity between the caudal PPN and brainstem structures implicated in the control of micturition or their connections since electrical spread from the relatively large diameter of currently available DBS leads may affect nearby structures in an unpredictable manner in the compact human brainstem.

1 Neurosurgeons with an interest in the potential of the PPN as a DBS target in PD  
2 (Jenkinson et al., 2005; Stefani et al., 2007; Alam et al., 2011) should be aware of the  
3 location and important functions of these nuclei.  
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5 Functional imaging studies localising the PMC in humans are limited to axial  
6 sections at 4mm intervals (Blok et al., 1997). Comparison of images through the lower  
7 extent of a well-placed PPN lead and the PMC would suggest that parts of the PMC  
8 may be as little as 4 mm away. With standard stimulation parameters it has been  
9 suggested that the outer boundary of the volume of influence of the electrical field may  
10 be estimated to be at a radius of 2–5 mm from the electrode contact (Volkman et al.,  
11 2006). Thus, it is plausible that PPN DBS may influence the pontine micturition centre,  
12 either directly or via descending connections. This has led some authors to propose that  
13 DBS of the PMC could be a promising treatment of voiding disturbances caused by  
14 lesions in suprapontine voiding centres such as can be seen in multiple sclerosis and PD  
15 (Andersen, 1985; Giannantoni et al., 1998).  
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18 The LDTg is a group of cholinergic cells lying ventromedial to the LC in all  
19 species studied, in agreement with previous publications (Wang and Morales, 2009).  
20 These cells seem to play an important role in the regulation of the micturition reflex  
21 (Noto et al., 1991). In humans, the LDTg (LDTGv) extends ventral to the  
22 central/periaqueductal gray matter, posterior and medial to the PPN (Paxinos et al.,  
23 1990).  
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25 Together with the LDTg, the LC appears in close proximity to the caudal PPN in  
26 all the species studied. The LC has a close morphophysiological relation to the Bar  
27 nucleus and is also involved in the micturition reflex and other sympathetic functions.  
28 In PD, the degenerative process affects the LC in a similar manner to the PPN and  
29 substantia nigra *pars compacta* (Delaville et al., 2011). Ventriculography and atlas  
30 coordinates were used to target the LC in 3 patients with the aim of treating spasticity  
31 and epilepsy (Feinstein et al., 1989). However, the report makes no mention of the  
32 effects off stimulation on micturition and does not provide anatomical or radiological  
33 images confirming the locus of stimulation.  
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36 Proximal colon distension increases Fos expression in the LC and in the Bar  
37 nucleus, which suggest that LC and Bar nucleus are also involved in the bowel  
38 movements. These Fos-IR cells from the Bar nucleus formed a characteristic cluster  
39 surrounded by dense TH<sup>+</sup> cells from the LC (Wang et al., 2009). Using retrograde tract  
40 tracing from the spinal cord and the LC, others authors have provide evidence that  
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1 neurons project to both regions, suggesting that this nucleus can coordinate sacral  
2 parasympathetic activity with brain noradrenergic signal (Pavcovich et al., 1998).  
3 Divergent projections to the spinal parasympathetic and brain noradrenergic nucleus,  
4 make this system a putative substrate for the co-morbidity of pelvic visceral symptoms  
5 in DBS patients (Pavcovich et al., 1998).  
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9 Our study used CRF<sup>+</sup> labelling to review the anatomical location of the so-called  
10 Bar nucleus in a range of species (mouse, non-human primates and human). The Bar  
11 nucleus was found to be present in humans with a similar location to that seen in  
12 monkeys and rodents. Interestingly, CRF<sup>+</sup> neurons revealed the specific cells in the  
13 micturition centre in human brain sections (**Fig. 8**). Human brain atlases do not refer to  
14 the Bar nucleus. However, according to Olszewski and Baxter (1954), the caudal part of  
15 the oral pontine reticular nucleus (PnO), limited laterally by the LC and *sublocus*  
16 *coeruleus* (subLC) may envelop the Bar nucleus in humans. Ventral to the LC, in the  
17 dorsal part of subLC, there are strands of smaller, more diffusely arranged and non  
18 pigmented cells, which constitute what can be called *pars alpha* of the LC. Part of this  
19 region was included in the LC, as depicted by Olszewski and Baxter (1954). However,  
20 these so-called *pars alpha* neurons of the LC located bilaterally in the dorsolateral pons  
21 are probably involved in micturition control and may be the pontine micturition centre  
22 coincident with the Bar nucleus in the human brain.  
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34 In rodents our study shows that the Bar nucleus is limited rostrally by the LC,  
35 LDTg and the mesencephalic trigeminal tract and caudally by the LC and medial  
36 longitudinal fasciculus. The region is concordant with the medial cell group in the  
37 laterodorsal pontine tegmentum and its long axis roughly goes parallel to the midline. In  
38 human tissue, however, the Bar nucleus is limited on its lateral side by the LC and on its  
39 medial side by the LDTg. Rostrally, the Bar nucleus extends dorsally and laterally  
40 towards the LC, while caudally is located ventral to the LC reaching the medial  
41 longitudinal fasciculus. In accordance with previously published descriptions in  
42 humans, the putative micturition centre belongs to the pontine tegmental nuclei that are  
43 close to the midline, where they form a series of rod-shaped cell clusters, distributed in  
44 the medial and lateral regions of the periventricular grey matter of the pons (Huang et  
45 al., 1992).  
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56 In addition to controlling the micturition reflex, the Bar nucleus also appears to  
57 be involved in more diverse functions than previously appreciated. For example, it  
58 receives neural inputs from numerous supraspinal regions implicated in fluid  
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homeostasis, blood pressure regulation, the neuroendocrine function, and sexual behaviour, suggesting that it may function as an integrative centre involved in the coordination of forebrain activity with parasympathetically-controlled visceral functions (Cano et al., 2000). Interestingly, study of the topographical distribution of apelinergic neurons, which contain apelin, a neurohumoral modulator of the cardiovascular system involved in the fluid homeostasis, revealed numerous immunoreactive nerve fibres in the Bar nucleus (Reaux et al., 2002).

Divergent innervations of LC and sacral spinal cord from a subset of the Bar nucleus neurons, have recently been reported and proposed to provide a substrate for integration of sacral parasympathetic activity with central noradrenergic function (Valentino et al., 1996). The Bar nucleus also seems to be related to mechanisms underlying the response to stress, since changes in c-Fos and corticotropin-releasing hormone gene expression have been reported in this nucleus after acute and chronic stress in rats (Houshyar et al., 2003).

PD often results in problems with micturition (Chaudhuri and Schapira, 2009). Nevertheless, the patient in question did not experience any symptomatic disturbance of micturition until implantation of the PPN DBS lead. Therefore, PPN DBS surgery may have precipitated a symptomatic disturbance in a patient with reduced physiological reserve (Aviles-Olmos et al., 2012). PPN DBS is performed to address gait disturbance resistant to dopaminergic medication. Since this is often a late occurrence in the progression of PD (Macht et al., 2007), this observation is of particular relevance to the patient group in question (Aviles-Olmos et al., 2012). The question arises whether micturition disturbance is a part of a parkinsonian syndrome or merely signs and symptoms related to ageing. Some studies had reported frequent urinary disturbances (70%) in PD, which improve during treatment with increased dose of levodopa (Krygowska-Wajs et al., 2002; Blackett et al., 2009) but the limited number of reported patients that have undergone DBS of the PPN region, the wide variation in patient selection criteria and anatomical location of DBS leads in relation to the PPN proper may explain why symptomatic disturbance has not been more commonly reported.

Taking into account the importance of the Bar nucleus as the core of the pontine micturition centre and its several important functions (as well as its relation with LDTg and the LC in the micturition reflex), the presence of these nuclei in the human brain should be highlighted. Future research should detail further the functional neuroanatomy of the region. Information about the location of the Bar nucleus in the

1 human brainstem, as well as others nuclei related with the micturition, should be taken  
2 into account when planning DBS procedures in order to minimize adverse events.  
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## 5 **CONCLUSIONS**

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7 In summary, the present study indicates that anatomical location makes the Bar nucleus  
8 susceptible to being involved by the PPN DBS lead or to being affected by diffusion of  
9 electrical current. This can have an undesirable impact on the quality of life of patients  
10 and could also interfere with the results of experimental studies that attempt to explain  
11 the mechanisms underlying the beneficial effects of DBS as a surgical alternative to  
12 Parkinsonian patients. Taken together, our results support the importance of deepening  
13 accurate stereotaxic localization as well as anatomical and functional relationships of  
14 the Bar nucleus with other brainstem nuclei.  
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## 23 **COMPETING INTERESTS**

24 The authors have declared that no competing interests exist.  
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## 29 **ACKNOWLEDGEMENTS**

30 This work was supported by a Fellowship from the *Fundación Séneca* to LB  
31 (09765/IV2/08) and to MTH (09629/EE2/08) and by grants from the Spanish Ministry  
32 of Science and Innovation (FIS PI10 02827; SAF-2004/07656 C02-02), *Fundación*  
33 *Séneca* (FS/05662/PI/07; FS/15329/PI/10) and CIBERNED (*Centro de Investigación*  
34 *Biomédica en Red sobre Enfermedades Neurodegenerativas*). The Unit of Functional  
35 Neurosurgery, UCL Institute of Neurology, Queen Square, London is supported by the  
36 Parkinson's Appeal for Deep Brain Stimulation, the Edmond J. Safra Philanthropic  
37 Foundation and the Monument Trust. The authors would also like to thank Mr. P.  
38 Thomas for comments and language suggestions on this manuscript.  
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## FIGURE LEGENDS

**Figure 1: Anatomical location of the PPN in two of the most common atlases used in the DBS surgery in humans.** (A) Location of the PPN nucleus in coronal sections, 15.5 mm (top pictures) and 16.5 mm (bottom pictures) posterior to the mid-commisure point, according to Schaltenbrand and Wahren (1977). The PPN, also named nucleus *tegmenti pedunculopontinus* (Tg.pdp) is indicated with a red circle. (B) Location of the PPN *pars compacta* (PPNC) and PPN *pars dissipata* (PPND) and major surrounding structures along the axis of the brainstem 34 mm (image on the left) and 35 mm (image on the right) rostral to the obex according to Paxinos and Huang (1995). The PPN area is indicated by the red coloured area.

**Figure 2: Targeting the PPN nucleus in DBS involves the rostrocaudal axis of the brainstem.** (A) Images from the Paxinos and Huang atlas show sections from the rostral portion of the brainstem, at the level of the PPN (indicated by the red area), to the caudal portion of the brainstem reaching the *locus coeruleus* (LC) (indicated by the green area). Importantly, the Bar nucleus does not appear represented in the atlas images. (B) Schematic representation of the brainstem with the four levels of coronal sections represented in A. Sections 1, 2, 3 and 4 correspond to Obex +36, Obex +34, Obex +28 and Obex +26 respectively. (C) Schematic diagram of the sections of the four levels indicated in B along the rostrocaudal axis of the brainstem. Note that the PPN area is anatomically very close to the putative location of the Bar nucleus, the area also known as the pontine micturition centre.

**Figure 3: Anatomical location of the PPN and Bar nucleus in the mouse.** Diagram on the left shows a coronal section -4.84 mm from Bregma (Figure 71 according to Paxinos and Franklin, 2001) where the PPN can be identified (PPN is indicated with the red coloured area). Diagram on the right shows a coronal section -5.40 mm from Bregma (Figure 76 according to Paxinos and Franklin, 2001) where the LC (green coloured area) and the Bar nucleus (blue coloured area) can be identified.

**Figure 4: Histological analysis of the Bar nucleus and surrounding structures in relation with the PPN in mouse brain.** (A) Schematic diagrams from the two sections showed in figure 3, showing the Bar and PPN areas. A magnified section at the level of the Bar nucleus is also shown. The red square indicates the areas studied histologically. (B, C) Photomicrographs of 20- $\mu$ m-thick coronal sections at the level of caudal mouse brainstem stained with thionine. LC, Bar LDTg can be distinguished and are indicated with a broken line area. (D, E) Immunohistochemistry of vesicular acetylcholine transporter (VAChT) in mouse sections stains specifically neuronal bodies of the LDTg nucleus (broken line). LDTg is shown magnified in F where neuronal bodies can be clearly distinguished. (G, H) Immunohistochemical staining of tyrosine hydroxylase (TH) for noradrenergic neurons shows clearly the LC. (I, J) Immunohistochemistry for corticotropin release factor (CRF) specifically stains the Bar nucleus as indicated by the broken line. Magnification of the Bar area is indicated in K. Scale bar for B, D, G and I = 0.5 mm; scale bar for C, E, H and J = 200  $\mu$ m; scale bar for F and K = 25  $\mu$ m.

**Figure 5: Anatomical location of the PPN, LC and Bar in the macaque brain.** Location of the PPN *pars compacta* (PPNC) and PPN *pars dissipata* (PPND) and major surrounding structures along the axis of the brainstem -18.90 mm (image on the left) and -22.95 mm (image on the right) from the Bregma in the macaque according to

Paxinos et al., 1999. The PPN area is indicated by the red coloured area while LC is indicated in green and Bar nucleus in blue.

**Figure 6: Histological analysis of the monkey midbrain region.** (A, H) Schematic representation of two coronal sections of the monkey brainstem at the level of the Bar nucleus. The rostral (A) and caudal part (H) of the Bar nucleus are indicated close to the LDTg and LC in both diagrams. (B, C, I, J) Photomicrographs of 40- $\mu$ m-thick cross sections at the level of Bar nucleus stained by thionin. Insert in B and I are magnified in C and J, respectively. The boundaries of LC, and LDTg can be distinguished and are indicated by the broken line areas. (D, E, K, L) Immunohistochemistry for TH clearly showing the LC in macaque brain. Insert in D and K are magnified in E and L. LC is indicated with a broken line area. (F, G, M, N) NADPH – diaphorase staining for cholinergic neurons clearly shows the LDTg nucleus. Insert in F and M is magnified in G and N. LDTg is indicated by the broken line area Scale Bar in M = 0.5 mm; scale bar in N = 50  $\mu$ m.

**Figure 7: Histological analysis of the Bar nucleus in the human midbrain region.** (A, J) Schematic coronal sections of the human brainstem, previously showed in figure 2, show the rostral (A) and caudal (J) part of the Bar nucleus. (B, C, K, L) Photomicrographs of 40- $\mu$ m-thick cross sections at the level of human Bar nucleus stained by thionin. The neurons of the LC, LDTg and Bar can be observed and are indicated by broken line areas. Insert in B and K are magnified in C and L respectively. LDTg and Bar are more evident in the rostral part than the caudal part. (D, E, M, N) Immunohistochemistry for TH shows clearly the LC. Insert from D and M are magnified in E and N respectively. LC is limited by the broken line area. (F, G, O, P) NADPH-diaphorase staining of cholinergic neurons of the LDTg nucleus. Insert from F and O are magnified in G and P. LDTg can be clearly seen in rostral part but not in the caudal part. (H, I, Q, R) Immunohistochemical staining of corticotropin release factor (CRF) in the midbrain sections shows the neurons of the Bar nucleus. (S, T, U) Double immunohistochemistry for TH (brown) and CRF (dark blue) in the human brain stem. Insert from H and Q are magnified in I and R. Bar nucleus is clearly evidenced by CRF in rostral but also in caudal part. Arrowheads indicate TH positive neurons (brown) from the LC. Arrows indicate the CRF positive neurons (dark blue) characteristic from Bar nucleus. Scale bar in Q = 2 mm; scale bar in R = 300  $\mu$ m; scale bar in U = 35  $\mu$ m. Note that some pigmented neurons of the LC, containing neuromelanin, seen in brown color in some of the displayed images, should not be identified as a staining.

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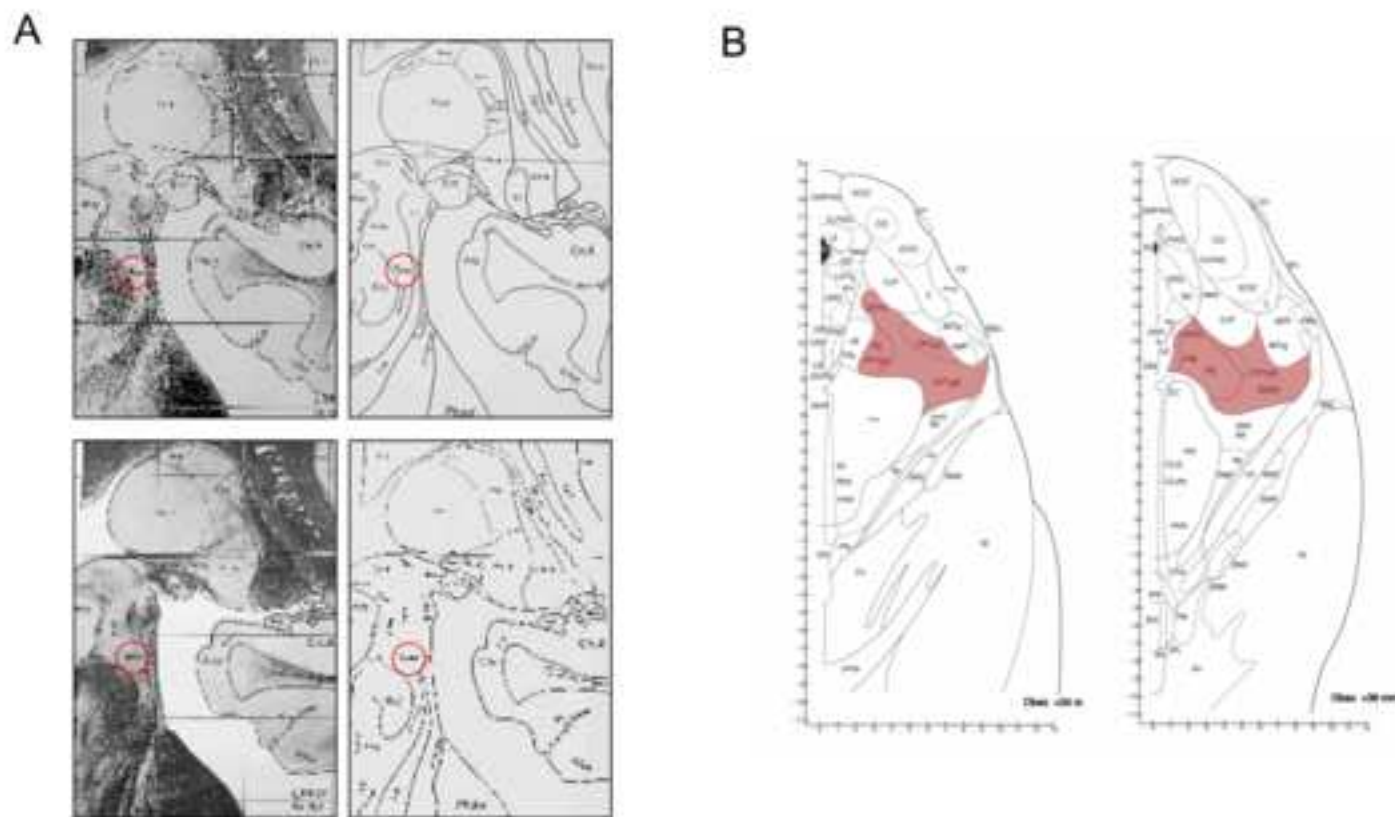
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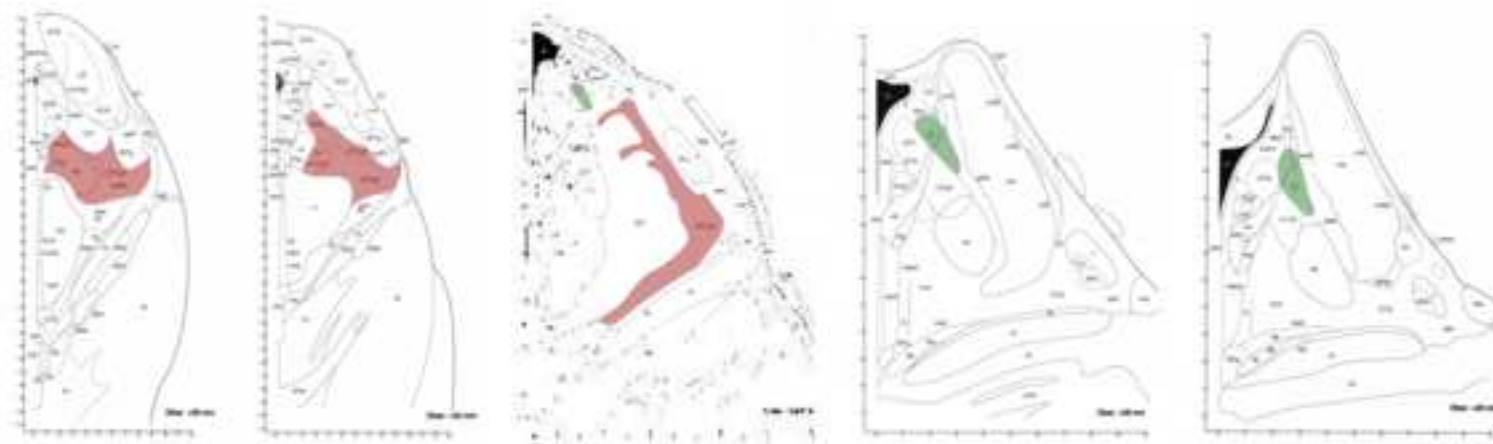


**HIGHLIGHTS**

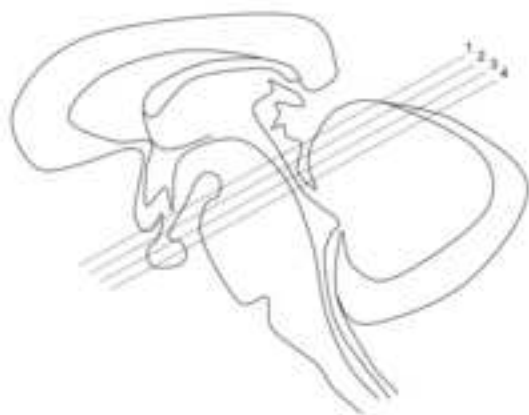
1. The Bar nucleus (Bar) is not indicated in the atlas of the human brainstem.
2. CRF<sup>+</sup> neurons in the Bar were observed in mouse and in human sections.
3. Double staining shown CRF<sup>+</sup> cell bodies from Bar mixed with TH<sup>+</sup> neurons from LC.
4. Human's Bar is bounded laterally by the LC and, medially by the LDTg.
5. Human's Bar extends towards the LC, adjacent to the caudal pole of the PPN.



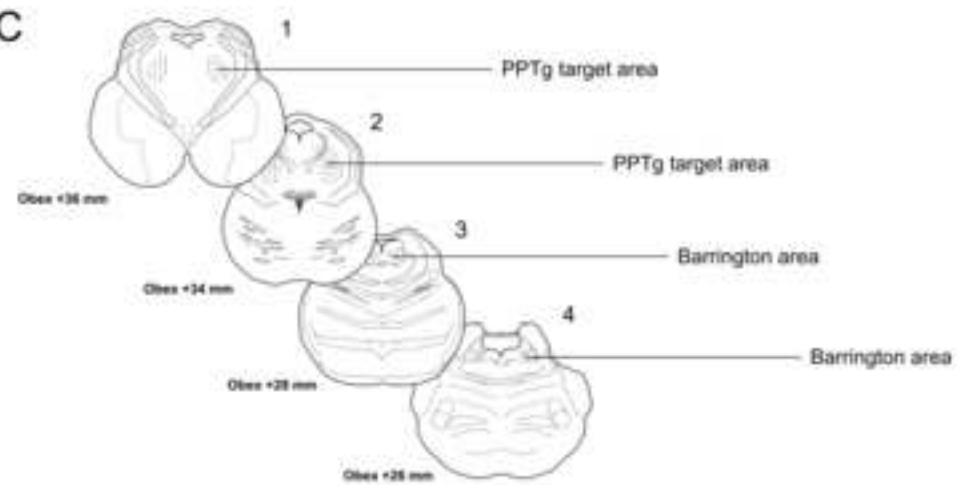
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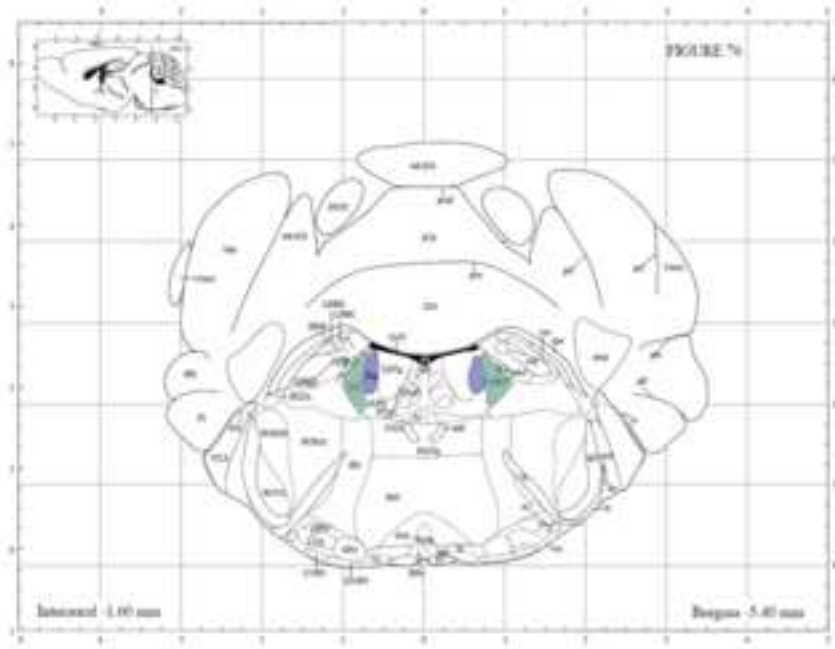


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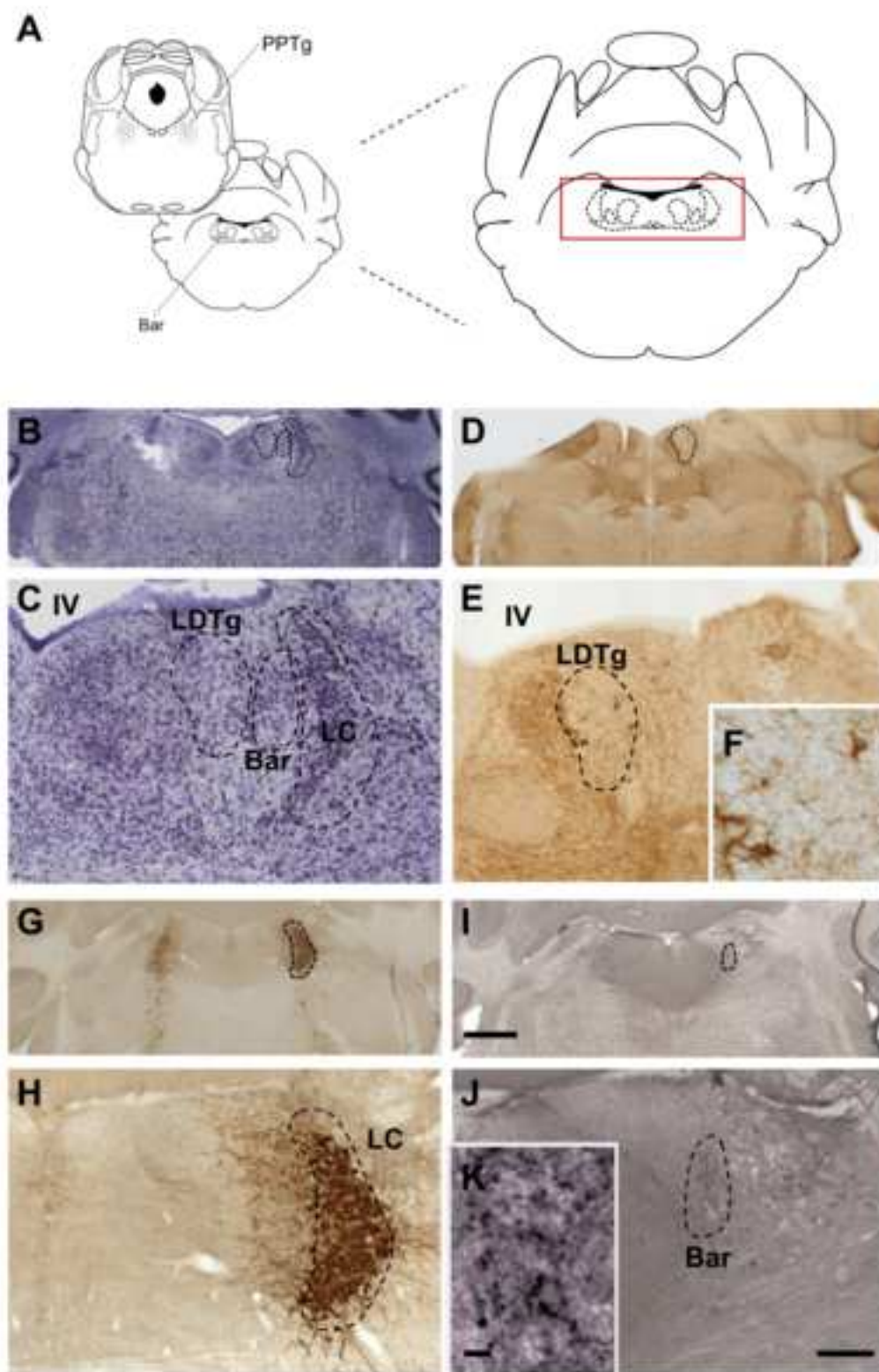


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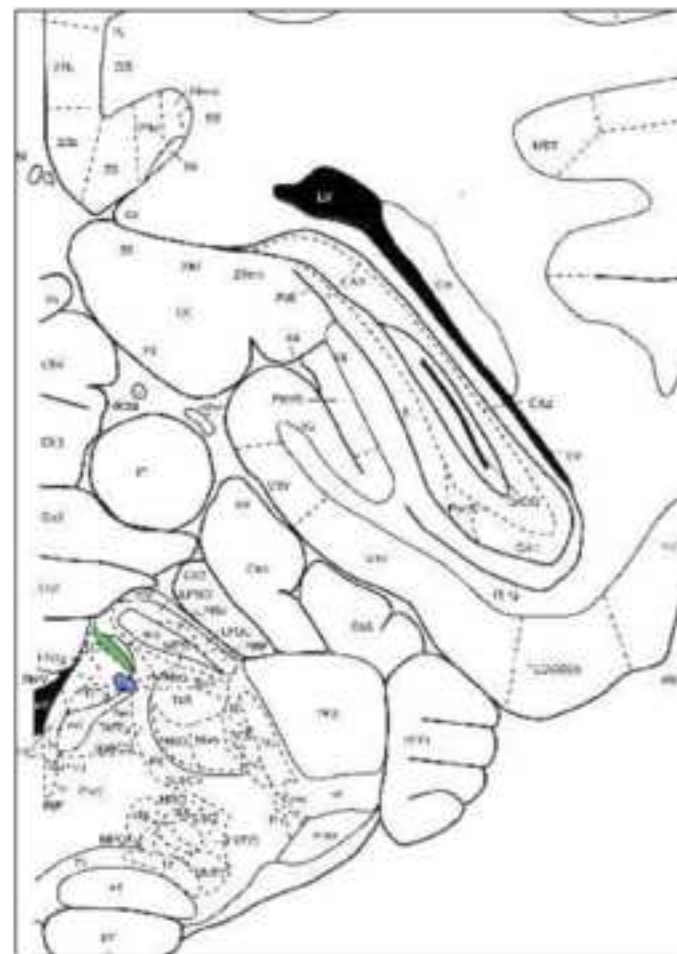
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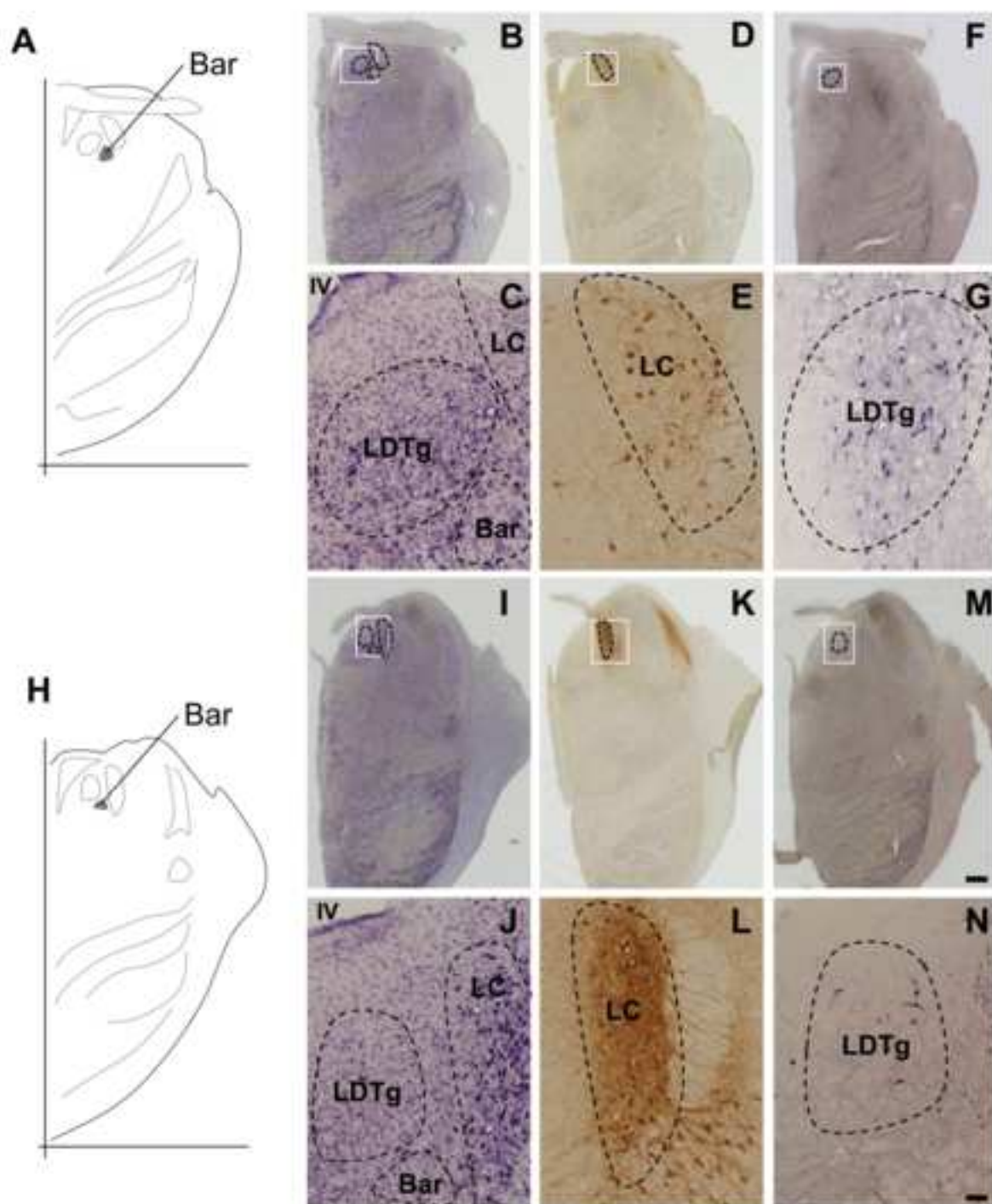
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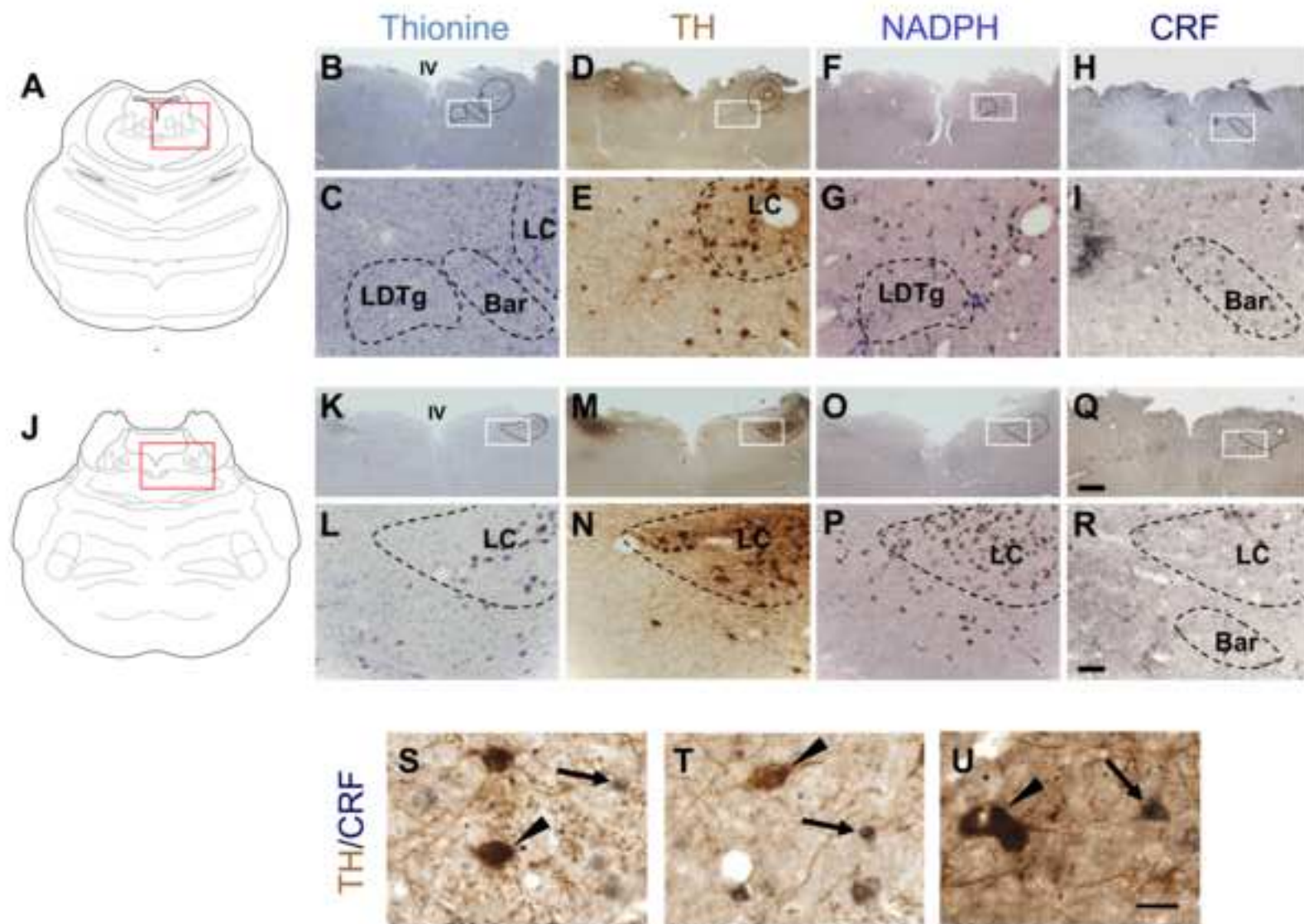
Bregma -18.90 mm



Interaural -01-05 mm

Bregma -22.95 mm







**Table 1.** Summary of different morphological staining techniques (and primary antibodies employed to identify cholinergic, noradrenergic and corticotrophin-positive neurons) performed in the study.

Species	Histology	Histoenzymology	Immunohistochemistry		
	Thionin staining	NADPH-diaphorase	Anti-TH NA neurons (LC)	Anti-VAcH ChAT neurons (LDTg)	Anti-CRF neurons in Bar nucleus
Mouse	X		X	X	X
Non-human primates	X	X	X		
Human	X	X	X		X