UNIVERSITAT JAUME·I

Título artículo / Títol article:	Critical evaluation of the anatomical location of the Barrington nucleus: relevance for deep brain stimulation surgery of pedunculopontine tegmental nucleus
Autores / Autors	Herrero Ezquerro, María Trinidad ; Blanco, Lisette ; Yuste Jiménez, José Enrique ;Carrillo de Sauvage, María Ángeles ; Gómez, Aurora ; Fernández Villalba, Emiliano ;Avilés Olmos, Iciar ; Limousin, Patricia ; Zrinzo, Ludvic
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 pedunculopontine tegmental nucleus. *Neuroscience*, 2013, vol. 247, p. 351-363.
url Repositori UJI:	http://hdl.handle.net/10234/89110

Accepted Manuscript

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

Lisette Blanco, José Enrique Yuste, María Ángeles Carrillo-de Sauvage, Aurora Gómez, Emiliano Fernández-Villalba, Iciar Avilés-Olmos, Patricia Limousin, Ludvic Zrinzo, María Trinidad Herrero
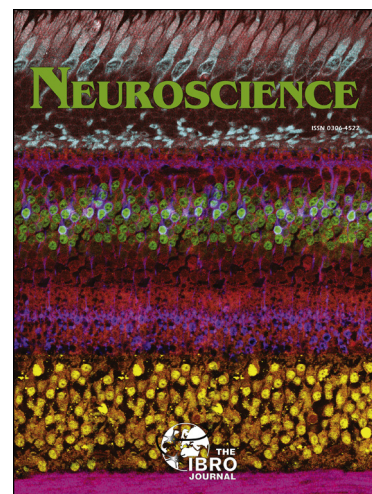
Please cite this article as: L. Blanco, J.E. Yuste, M.C-d. Sauvage, A. Gómez, E. Fernández-Villalba, I. Avilés-Olmos, P. Limousin, L. Zrinzo, M.T. Herrero, Critical Evaluation of the anatomical location of the Barrington nucleus: relevance for deep brain stimulation surgery of pedunculopontine tegmental nucleus, *Neuroscience* (2013), doi: http://dx.doi.org/10.1016/j.neuroscience.2013.05.047

Critical Evaluation of the anatomical location of the

Barrington nucleus: relevance for deep brain stimulation

surgery of pedunculopontine tegmental nucleus

Lisette Blanco[1,3], José Enrique Yuste[1,2], María Ángeles Carrillo-de Sauvage[1], Aurora Gómez[1], Emiliano Fernández-Villalba[1], Iciar Avilés-Olmos[4], Patricia Limousin[4], Ludvic Zrinzo[4], María Trinidad Herrero*[1,2]

[1]Clinical and Experimental Neuroscience, *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas* (CIBERNED), School of Medicine, University of Murcia, Campus de Espinardo, 30071, Murcia; [2]School of Health Sciences (Medicine). University Jaume I, Spain. [3]International Center of Neurological Restoration. Department of Experimental Neurophysiology. Ave. 25 #15805, 11300 Havana, Cuba, [4]Unit of Functional Neurosurgery, Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, University College London, Queen Square, London, WC1N 3BG, UK.

*Corresponding Author: María-Trinidad Herrero Ezquerro
Clinical and Experimental Neuroscience (NiCE- CIBERNED)
School of Health Sciences (Medicine)
University Jaume I
Av. de Vicent Sos Baynat, s/n 12071
Castelló de la Plana, SPAIN
mtherrer@um.es
ezquerro@uji.es

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

Keywords: Barrington nucleus, pedunculopontine 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.

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.

3

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 co-ordination 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).

4

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

5

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

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

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

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

Specific staining

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

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

For TH and CRF immunohistochemistry, endogenous peroxidase activity was inhibited with 0.3% H_2O_2 and non-specific Fc binding sites were blocked with 10% horse serum. Sections were incubated for 48 hours (at 4 ºC, constant shaking) with primary antibody diluted in PBS containing 1% horse serum, 0.5% Triton X-100, and 0.1%

6

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

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

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

The images were captured with a high-resolution digital camera (Axiocam HRc, Karl Zeiss) connected to a conventional optical microscope (Zeiss Axioplan 2).

7

RESULTS

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

Bar nucleus in rodent

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

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

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

8

(**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$^+$ cholinergic neurons from the LDTg are intermingled with the TH$^+$ 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$^+$ 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$^+$ 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^+ 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^+ 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 $NADPH^+$ cholinergic cells, close to the most caudal pole of LDTg (**Fig. 7 O-P**). At this level, some CRF^+ 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^+ cell bodies at the border of the LC where TH+ 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^+ 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.

10

Neurosurgeons with an interest in the potential of the PPN as a DBS target in PD (Jenkinson et al., 2005; Stefani et al., 2007; Alam et al., 2011) should be aware of the location and important functions of these nuclei.

Functional imaging studies localising the PMC in humans are limited to axial sections at 4mm intervals (Blok et al., 1997). Comparison of images through the lower extent of a well-placed PPN lead and the PMC would suggest that parts of the PMC may be as little as 4 mm away. With standard stimulation parameters it has been suggested that the outer boundary of the volume of influence of the electrical field may be estimated to be at a radius of 2–5 mm from the electrode contact (Volkmann et al., 2006). Thus, it is plausible that PPN DBS may influence the pontine micturition centre, either directly or via descending connections. This has led some authors to propose that DBS of the PMC could be a promising treatment of voiding disturbances caused by lesions in suprapontine voiding centres such as can be seen in multiple sclerosis and PD (Andersen, 1985; Giannantoni et al., 1998).

The LDTg is a group of cholinergic cells lying ventromedial to the LC in all species studied, in agreement with previous publications (Wang and Morales, 2009). These cells seem to play an important role in the regulation of the micturition reflex (Noto et al., 1991). In humans, the LDTg (LDTGv) extends ventral to the central/periaqueductal gray matter, posterior and medial to the PPN (Paxinos et al., 1990).

Together with the LDTg, the LC appears in close proximity to the caudal PPN in all the species studied. The LC has a close morphophysiological relation to the Bar nucleus and is also involved in the micturition reflex and other sympathetic functions. In PD, the degenerative process affects the LC in a similar manner to the PPN and substantia nigra *pars compacta* (Delaville et al., 2011). Ventriculography and atlas coordinates were used to target the LC in 3 patients with the aim of treating spasticity and epilepsy (Feinstein et al., 1989). However, the report makes no mention of the effects off stimulation on micturition and does not provide anatomical or radiological images confirming the locus of stimulation.

Proximal colon distension increases Fos expression in the LC and in the Bar nucleus, which suggest that LC and Bar nucleus are also involved in the bowel movements. These Fos-IR cells from the Bar nucleus formed a characteristic cluster surrounded by dense TH$^+$ cells from the LC (Wang et al., 2009). Using retrograde tract tracing from the spinal cord and the LC, others authors have provide evidence that

11

neurons project to both regions, suggesting that this nucleus can coordinate sacral parasympathetic activity with brain noradrenergic signal (Pavcovich et al., 1998). Divergent projections to the spinal parasympathetic and brain noradrenergic nucleus, make this system a putative substrate for the co-morbidity of pelvic visceral symptoms in DBS patients (Pavcovich et al., 1998).

Our study used CRF⁺ labelling to review the anatomical location of the so-called Bar nucleus in a range of species (mouse, non-human primates and human). The Bar nucleus was found to be present in humans with a similar location to that seen in monkeys and rodents. Interestingly, CRF⁺ neurons revealed the specific cells in the micturition centre in human brain sections (**Fig. 8**). Human brain atlases do not refer to the Bar nucleus. However, according to Olszewski and Baxter (1954), the caudal part of the oral pontine reticular nucleus (PnO), limited laterally by the LC and *sublocus coeruleus* (subLC) may envelop the Bar nucleus in humans. Ventral to the LC, in the dorsal part of subLC, there are strands of smaller, more diffusely arranged and non pigmented cells, which constitute what can be called *pars alpha* of the LC. Part of this region was included in the LC, as depicted by Olszewski and Baxter (1954). However, these so-called *pars alpha* neurons of the LC located bilaterally in the dorsolateral pons are probably involved in micturition control and may be the pontine micturition centre coincident with the Bar nucleus in the human brain.

In rodents our study shows that the Bar nucleus is limited rostrally by the LC, LDTg and the mesencephalic trigeminal tract and caudally by the LC and medial longitudinal fasciculus. The region is concordant with the medial cell group in the laterodorsal pontine tegmentum and its long axis roughly goes parallel to the midline. In human tissue, however, the Bar nucleus is limited on its lateral side by the LC and on its medial side by the LDTg. Rostrally, the Bar nucleus extends dorsally and laterally towards the LC, while caudally is located ventral to the LC reaching the medial longitudinal fasciculus. In accordance with previously published descriptions in humans, the putative micturition centre belongs to the pontine tegmental nuclei that are close to the midline, where they form a series of rod-shaped cell clusters, distributed in the medial and lateral regions of the periventricular grey matter of the pons (Huang et al., 1992).

In addition to controlling the micturition reflex, the Bar nucleus also appears to be involved in more diverse functions than previously appreciated. For example, it receives neural inputs from numerous supraspinal regions implicated in fluid

12

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

13

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

CONCLUSIONS

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

COMPETING INTERESTS

The authors have declared that no competing interests exist.

ACKNOWLEDGEMENTS

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.pdpo) 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-μm-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 hydroxilase (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 μm; scale bar for F and K = 25 μm.

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

15

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-μm-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. LTDg is indicated by the broken line area Scale Bar in M = 0.5 mm; scale bar in N = 50 μm.

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-μm-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 form **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 μm; scale bar in U = 35 μm. Note that some pigmented neurons of the LC, containing neuromelanin, seen in brawn color in some of the displayed images, should not be identified as a staining.

16

REFERENCES

Alam M, Schwabe K, Krauss JK (2011), The pedunculopontine nucleus area: critical evaluation of interspecies differences relevant for its use as a target for deep brain stimulation. Brain 134:11-23.

Andersen JT. Disturbances of bladder and urethral function in Parkinson's disease (1985), Int Urol Nephrol. 1985;17(1):35-41.

Aviles-Olmos I, Foltynie T, Panicker J, Cowie D, Limousin P, Hariz M, et al. (2011), Urinary incontinence following deep brain stimulation of the pedunculopontine nucleus. Acta Neurochir (Wien) 153(12):2357-2360.

Aviles-Olmos I, Foltynie T, Panicker J, Cowie D, Limousin P, Hariz M, Fowler C J, et al. (2012), Uncertainty, misunderstanding and the pedunculopontine nucleus. Acta neurochirurgica 154(5):839–841.

Barrington FJ (1914), The nervous mechanism of the micturation. Quart J. Exp. Physiol 8(1):33-55.

Barrington FJ (1925), The effect of lesion of the hind and mid-brain on micturation in the cat. Quart. J. Exp. Physiol 15:81-102.

Benazzouz A, Hallett M (2000), Mechanism of action of deep brain stimulation. Neurology 55(12) (Suppl. 6):13-27.

Blackett H, Walker R, Wood B (2009), Urinary dysfunction in Parkinson's disease: a review. Parkinsonism Relat Disord. 15(2):81-87.

Blok BF, Willemsen AT, Holstege G (1997), A PET study on brain control of micturition in humans. Brain: a journal of neurology 120(Pt 1):111–121.

Blok BF (2002), Central pathways controlling micturation and urinary continence. Urology 59 (Suppl 1):13-17.

Cano G, Card JP, Rinaman L, Sved AF (2000), Connections of Barrington's nucleus to the sympathetic nervous system in rats. J. Autonomic Nerv. Syst 79: 117–128.

Capozzo A, Florio T, Confalone G, Minchella D, Mazzone P, Scarnati E (2009), Low frequency stimulation of the pedunculopontine nucleus modulates electrical activity of subthalamic nucleus in the rat. J. Neural. Transmission 116:51-56.

Chaudhuri KR, Schapira AHV (2009), Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. Lancet neurology 8(5): 464–474.

17

Delaville C, De Deurwaerdère P, Benazzouz A (2011), Noradrenaline and Parkinson's disease. Front Syst. Neurosci 5:31. doi: 10.3389/fnsys.2011.00031

Feinstein B, Gleason CA, Libet B (1989), Stimulation of locus coeruleus in man. Preliminary trials for spasticity and epilepsy. Stereotact. Funct. Neurosurg 52(1):26-41.

Ferraye MU, Gerardin P, Debû B, Chabardès S, Fraix V, Seigneuret E, LeBas JF, Benabid AL, Tilikete C, Pollak P (2009), Pedunculopontine nucleus stimulation induces monocular oscilloscopia. J. Neurol. Neurosurg. Psychiatry 80(2): 228-231.

Ferraye MU, Debû B, Fraix V, Goetz L, Ardouin C, Yelnik J, Henry-Lagrange C, Seigneuret E, Piallat B, Krack P, LeBas JF, Benabid AL, Chabardès S, Pollak P (2010), Effects of pedunculopontine nucleus area stimulation on gait disorders in Parkinson's disease. Brain 133(Pt 1): 205-214.

Garcia-Rill E (1991) The pedunculopontine nucleus. Prog Neurobiol 36:363–389.

Giannantoni A, Scivoletto G, Di Stasi SM, Grasso MG, Vespasiani G, Castellano V. (1998), Urological dysfunctions and upper urinary tract involvement in multiple sclerosis patients. Neurourol Urodyn;17(2):89-98.

Groat WC, Yoshimura N (2010), Changes in afferent activity after spinal cord injury. Neurourol Urodyn 29(1):63-76.

Guridi J, Rodríguez-Oroz MC, Lozano A, Moro E, Albanese A, Nuttin B, Gybels J, Ramos E, Obeso JA (2000), Targeting the basal ganglia for deep brain stimulation in Parkinson's disease. Neurology 55(12) (Suppl. 6):21-27.

Huang F, Tork I, Halladay G, Paxinos G (1992), The Dorsal, Posterodorsal, and Ventral Tegmental Nuclei: A Cyto- and Chemoarchitectonic Study in the Human. The Journal of Comp. Neurol 318:117-137.

Houshyar H, Gomez F, Manalo S, Bhargava A, Dallman M (2003), Intermittent Morphine administration Induced dependence and is a chronic stressor in rats. Neuropsychopharmacology 28:1960–1972.

Hunot S, Boissiere F, Faucheux B, Brugg B, Mouatt-Prigent A, Agid Y, Hirsch EC (1996), Nitric Oxide Synthase and Neuronal Vulnerability in Parkinson's disease. Neuroscience 72(2):355-363.

Inglis WL, Winn P (1995), The pedunculopontine tegmental nucleus: where the striatum meets the reticular formation. Prog Neurobiol 47:1–29.

Inglis WL, Olmstead MC, Robbins TW (2000), Pedunculopontine tegmental nucleus lesions impair stimulus—reward learning in autoshaping and conditioned reinforcement paradigms. Behav Neurosci 114:285–294.

18

Inglis WL, Olmstead MC, Robbins TW (2001), Selective deficits in attentional performance on the five-choice serial reaction time task following pedunculopontine tegmental nucleus lesions. Behav Brain Res 123:117–131.

Jenkinson N, Nandi D, Miall RC, Stein JF, Aziz TZ (2004), Pedunculopontine nucleus stimulation improves akinesia in a Parkinsonian monkey. Neuroreport 15(17):2621- 2624

Jenkinson N, Nandi D, Aziz TZ, Stein JF (2005), Pedunculopontine nucleus: a new target for deep brain stimulation for akinesia. Neuroreport 16(17):1875–1876

Jenkinson N, Nandi D, Oram R, Stein JF, Aziz TZ (2006), Pedunculopontine nucleus electric stimulation alleviates akinesia independently of dopaminergic mechanisms. Neuroreport 17(6):639–641.

Jenkinson N, Nandi D, Muthusamy K, Ray NJ, Gregory R, Stein JF, Aziz TZ (2009), Anatomy, physiology, and pathophysiology of the pedunculopontine nucleus. Mov Disord 24(3):319–328.

Keating GL, Winn P (2002), Examination of the role of the pedunculopontine tegmental nucleus in radial maze tasks with or without a delay. Neuroscience 112:687– 696.

Keating GL, Walker SC, Winn P (2002), An examination of the effects of bilateral excitotoxic lesions of the pedunculopontine tegmental nucleus on responding to sucrose reward. Behav Brain Res 134:217–228.

Khan S, Javed S, Park N, Gill SS, Patel NK (2010), A magnetic resonance imaging-directed method for transventricular targeting of midline structures for deep brain stimulation using implantable guide tubes. Neurosurgery, 66(6 Suppl Operative), 234–237.

Krygowska-Wajs A, Weglarz W, Szczudlik ZD (2002), Micturition disturbances in Parkinson's disease. Clinical and urodynamic evaluation. Neurol Neurochir Pol. 36(1):25-32.

Kuru M (1965), Nervous control of micturation. Physiol. Rev 45:425-464.

Kozak R, Bowman EM, Latimer MP, Rostron CL, Winn P (2005), Excitotoxic lesions of the pedunculopontine tegmental nucleus in rats impair performance on a test of sustained attention. Exp Brain Res 162:257–264.

Luppi P, Aston-Jones G, Akaoka H, Chouvet G, Jouvet M (1995), Afferent projections to the rat locus coeruleus demonstrated by retrograde and anterograde

tracing with cholera-toxin B subunit and *Phaseolus vulgaris* leucoaglutinin. Neuroscience 65:119-160.

Macht M, Kaussner Y, Möller JC, Stiasny-Kolster K, Eggert KM, Krüger HP, Ellgring H (2007), Predictors of freezing in Parkinson's disease: a survey of 6,620 patients. Movement disorders: official journal of the Movement Disorder Society, 22(7):953–956.

Moro E, Hamani C, Poon YY, Al-Khairallah T, Dostrovsky J, Hutchison W, Lozano A (2010), Unilateral pedunculopontine stimulation improves falls in Parkinson's disease. Brain 133:215-224.

Nathan PW (1952), Micturation reflexes in man. J Neurol & Neurosurg Psychiat 15:148-149.

Nilsson MH, Jarnlo GB, Rehncrona S (2008), Functional balance performance in patients with Parkinson's disease after long-term treatment with subthalamic nucleus high-frequency stimulation. Parkinsonism & Rel Disord 14:291-297.

Noto H, Roppolo JR, Steers WD, De Groat WC (1991), Electrophysiological analysis of the ascending and descending components of the micturation reflex pathway in the rat. Brain Res 549(1):95-105.

Obeso JA, Benabid A, Koller W (2000), Deep Brain Stimulation for Parkinson's disease and tremor. Neurology 55(12) (Suppl. 6):1-6.

Olszewski J. and Baxter D. (1954). Cytoarchitecture of the Human Brainstem. Karger, NY.

Pahapill P, Lozano A (2000), The Pedunculopontine nucleus and Parkinson´s disease. Brain 123:1767-1783.

Pavcovich LA, Yang M, Miselis R, Valentino RJ (1998), Novel role for the pontine center, Barrington's nucleus: evidence for coordination of colonic and forebrain activity. Brain Res 784:355-361.

Paxinos G, Tork I, Halliday G, Mehler W. (1990) Human Homologs to Brainstem Nuclei identified in Other Animals as Revealed by Acetylcholinesterase Activity. In: The Human Nervous System. (Paxinos G, ed), pp 149-202. San Diego: Academic Press, Inc.

Paxinos G, Huang XF Atlas of the human brainstem. Academic Press, San Diego, 1995.

Paxinos G, Xu-Feng H, Toga A. The Rhesus Monkey Brain in Stereotaxic Coordinates Academic Press: San Diego, 1999.

20

Paxinos G, Franklin K. The Mouse Brain in Stereotaxic Coordinates. San Diego, Academic Press, 2001.

Piallat B, Chabardes S, Torres N, Fraix V, Goetz L, Seigneuret E, et al. (2009), Gait is associated with an increase in tonic firing of the sub-cuneiform nucleus neurons. Neuroscience 158:1201–1205.

Reaux A, Gallatz K, Palkovits M, LLorens-Cortes C (2002), Distribution of Apelin-Synthesizing neurons in the adult rat brain. Neuroscience 113(3):653-662.

Satoh K, Shimizu N, Tohyama M, Maeda T (1978), Localization of the micturation reflex center at dorsolateral pontine tegmentum of the rat. Neurosc. Lett 8:27-33.

Schaltenbrand G, Wahren W (1977), Atlas for Stereotaxy of the Human Brain. Second Edition.

Stefani A, Lozano A, Pepe A, Stanzione P, Galati S, Troperi D, Pierantozzi M, Brusa L, Scarnati E, Mazzone P (2007), Bilateral Deep Brain Stimulation of the pedunculopontine and subthalamic nuclei in severe Parkinson's disease. Brain 130(6):1596-1607.

Sugaya K, Hishijima S, Miyazato M, Ogawa Y (2005), Central nervous of micturation and urine storage. J Smooth Muscle Res 41(3):117-132.

Swanson LW (1998), Brain Maps: Structure of the Rat Brain: A Laboratory Guide with Printed and Electronic Templates for Data, Models, and Schematics, 2nd Revised Edition, pp vii, 267. Elsevier, Amsterdam; New York.

Valentino RJ, Chen S, Zhu Y, Aston-Jones G (1996), Evidence for divergent projections to the brain noradrenergic system and the spinal parasympathetic system from Barrington's nucleus. Brain Res 732:1-15.

Valentino RJ, Wood SK, Wein AJ, Zderic SA. (2011), The bladder-brain connection: putative role of corticotrophin-releasing factor. Nat Rev Urology 8: 19-28.

VanderHorst VG, Ulfhake B (2006), The organization of the brainstem and spinal cord of the mouse: relationships between monoaminergic, cholinergic, and spinal projection systems. J. Chem. Neuroanat 31(1):2-36.

Volkmann J, Moro E, Pahwa R (2006), Basic algorithms for the programming of deep brain stimulation in Parkinson's disease. Movement disorders: official journal of the Movement Disorder Society, 21 (Suppl 14): S284–9.

21

Wang HL, Morales M. (2009), Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. Eur. J. Neurosc 29:340-358.

Wang L, Martínez V, Larauche M, Taché Y (2009), Proximal colon distention induces Fos expresión in oxytocin-vasopressin-, CRF-and catecholamines-containing neurons in rat brain. Brain Res 1247:79-91.
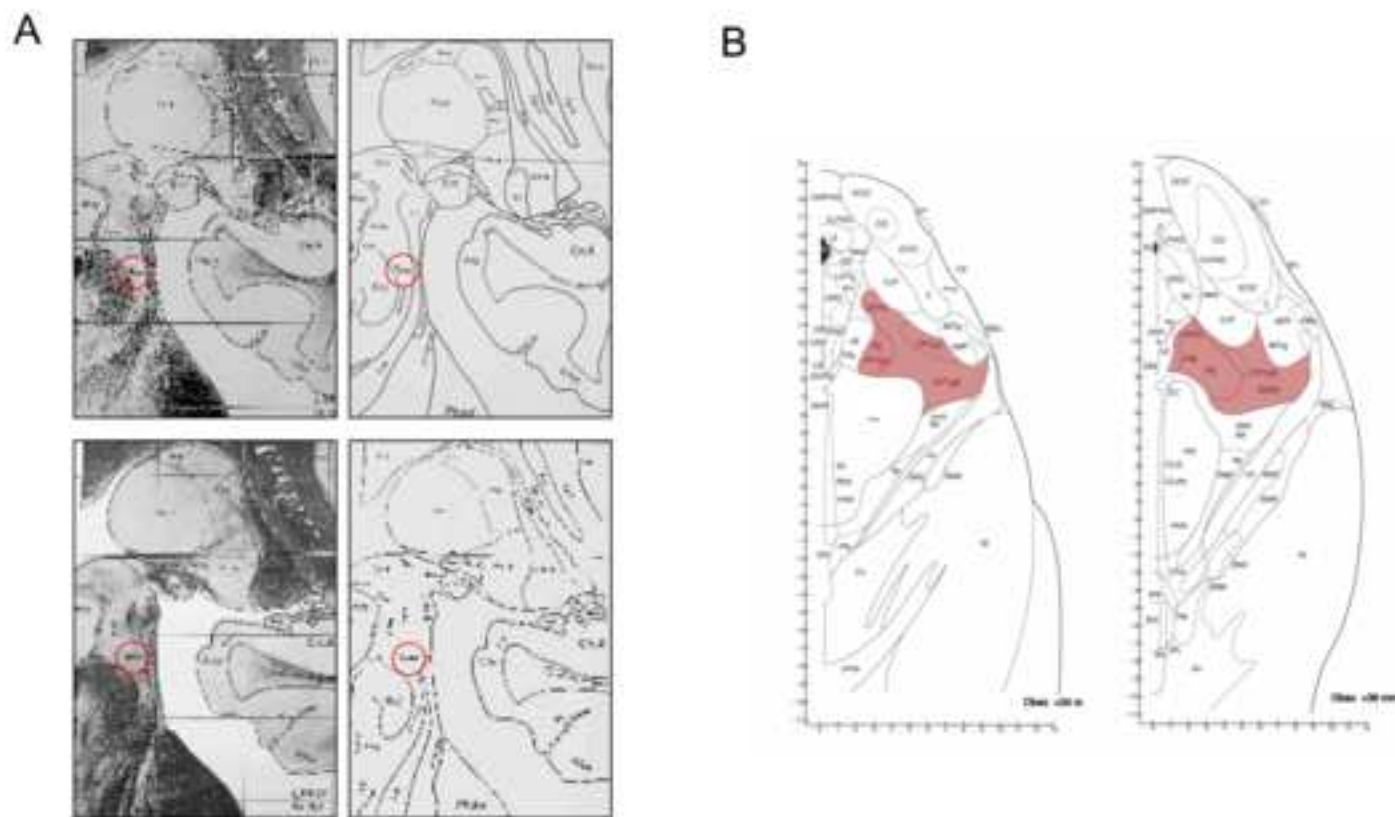
Zrinzo L, Zrinzo LV, Tisch S, Limousin PD, Yousry TA, Afshar F, Hariz MI. (2008), Stereotactic localization of the human pedunculopontine nucleus: atlas-based coordinates and validation of a magnetic resonance imaging protocol for direct localization. Brain 131:1588-1598.

Zrinzo L (2012), Pitfalls in precision stereotactic surgery. Surg Neurol Int. 3(Suppl 1): S53–S61.

HIGHLIGHTS

1. The Bar nucleus (Bar) is not indicated in the atlas of the human brainstem.

2. CRF$^+$ neurons in the Bar were observed in mouse and in human sections.

3. Double staining shown CRF$^+$ cell bodies from Bar mixed with TH$^+$ neurons from LC.

4. Human's Bar is bounded laterally by the LC and, medially by the LDTg.

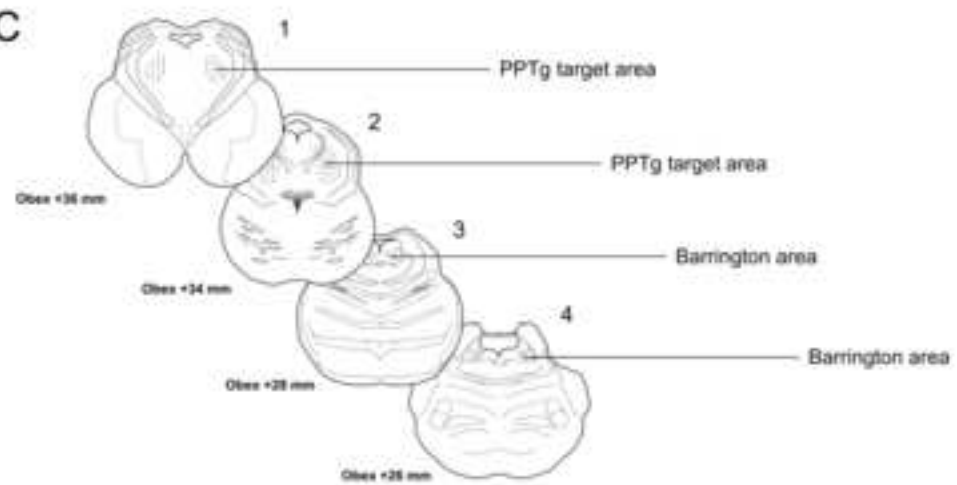5. Human's Bar extends towards the LC, adjacent to the caudal pole of the PPN.
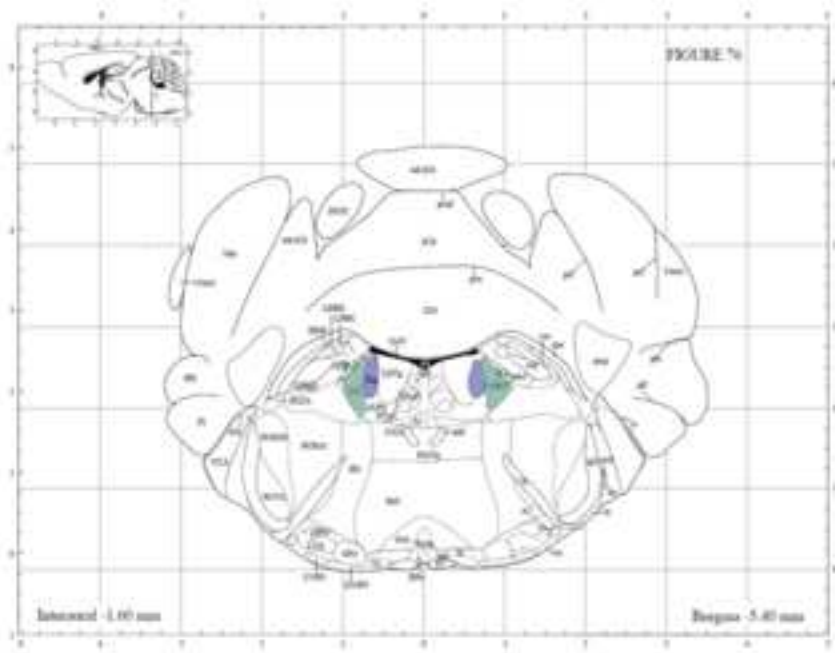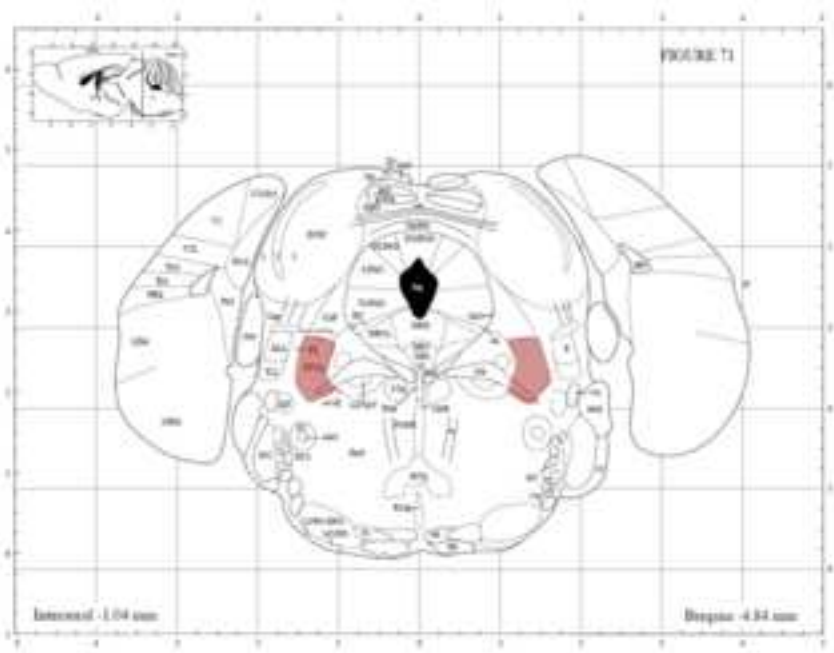
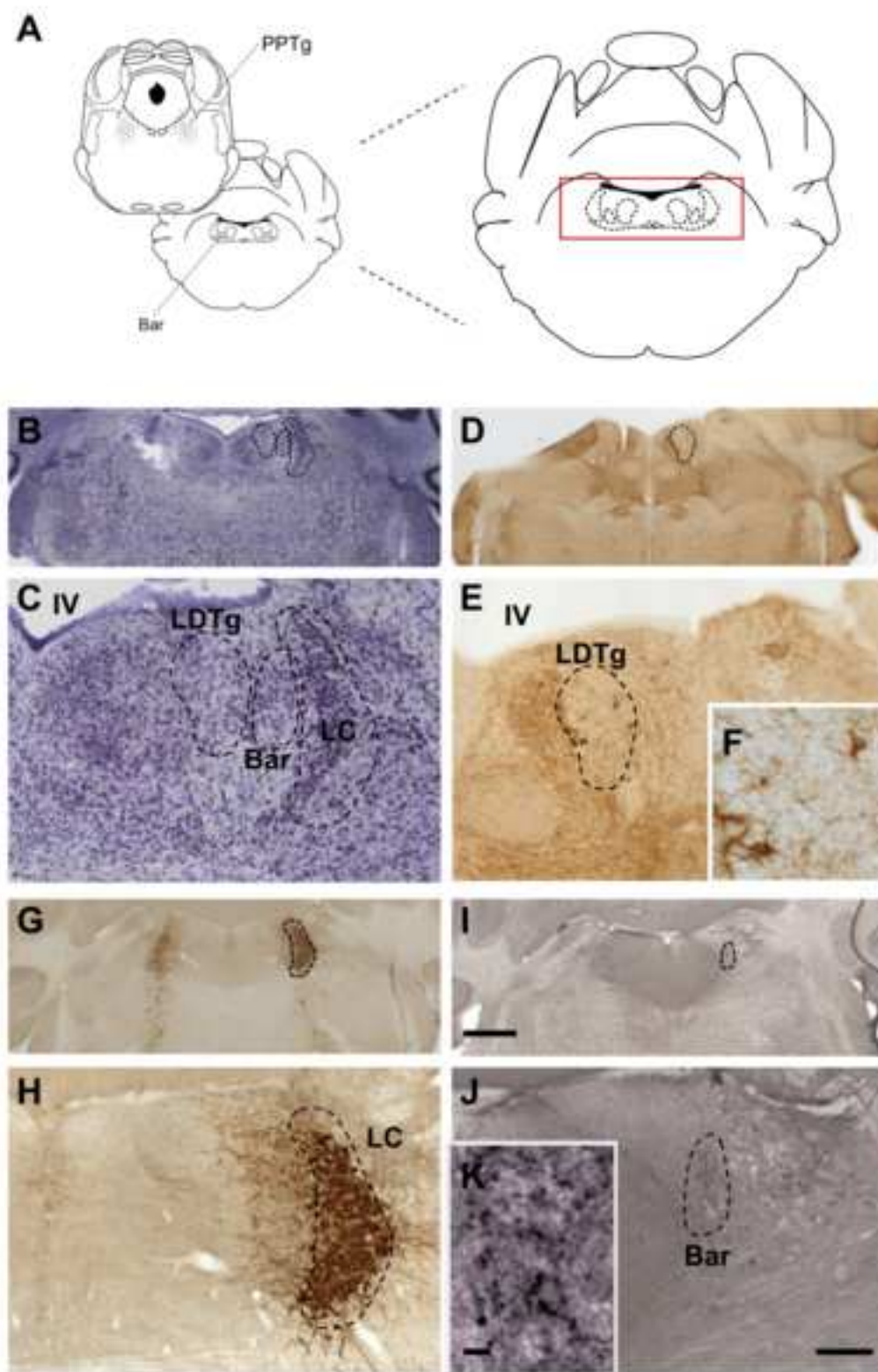**Figure 1**

Figure 2

A

B

C

1 — PPTg target area

2 — PPTg target area

3 — Barrington area

4 — Barrington area

Figure 3

Figure 4

Figure 5
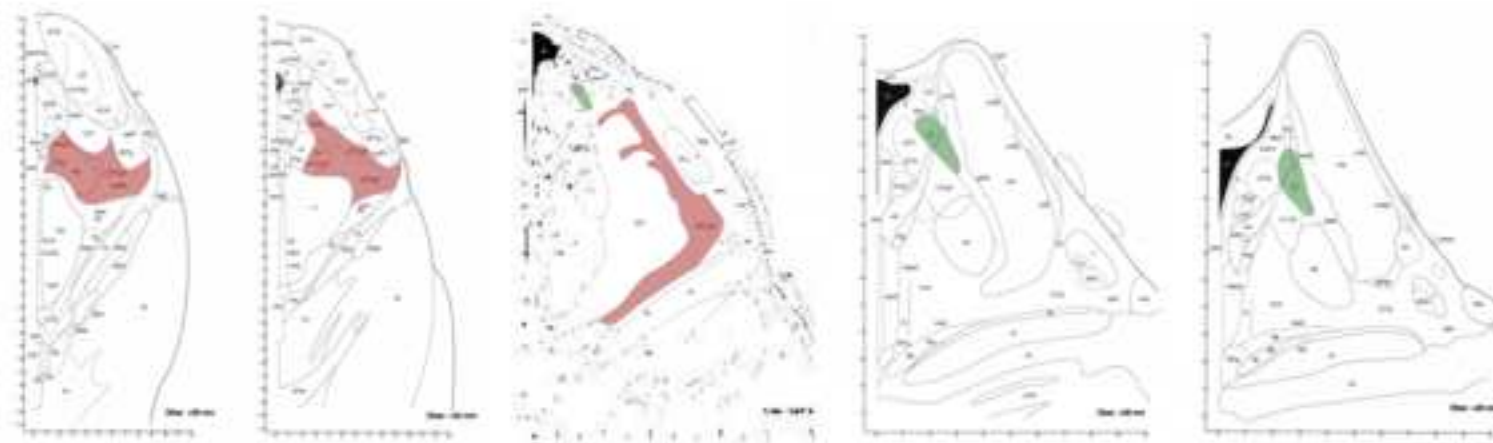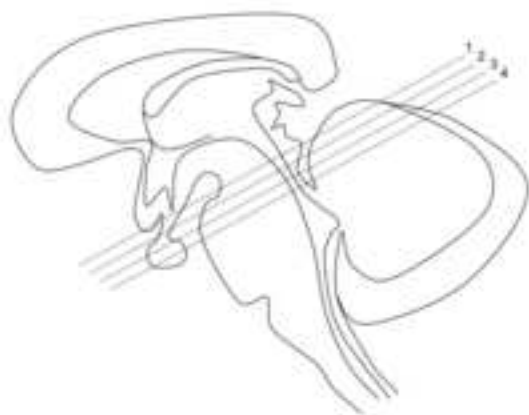
Interaural 03.00 mm   Bregma -18.90 mm
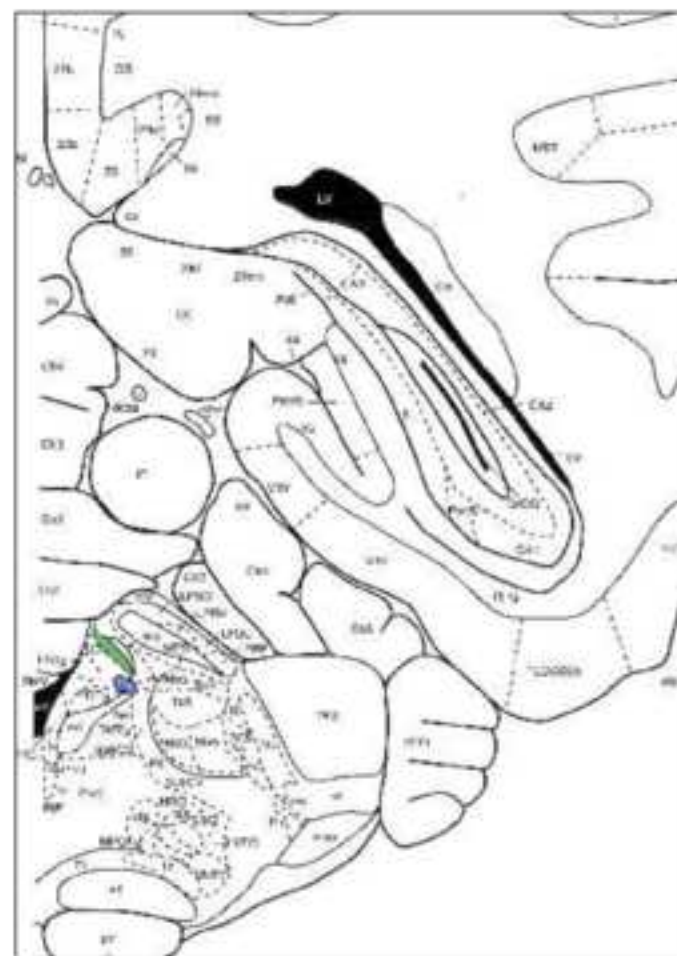
Interaural -01-05 mm   Bregma -22.95 mm

Figure 6

Figure 7
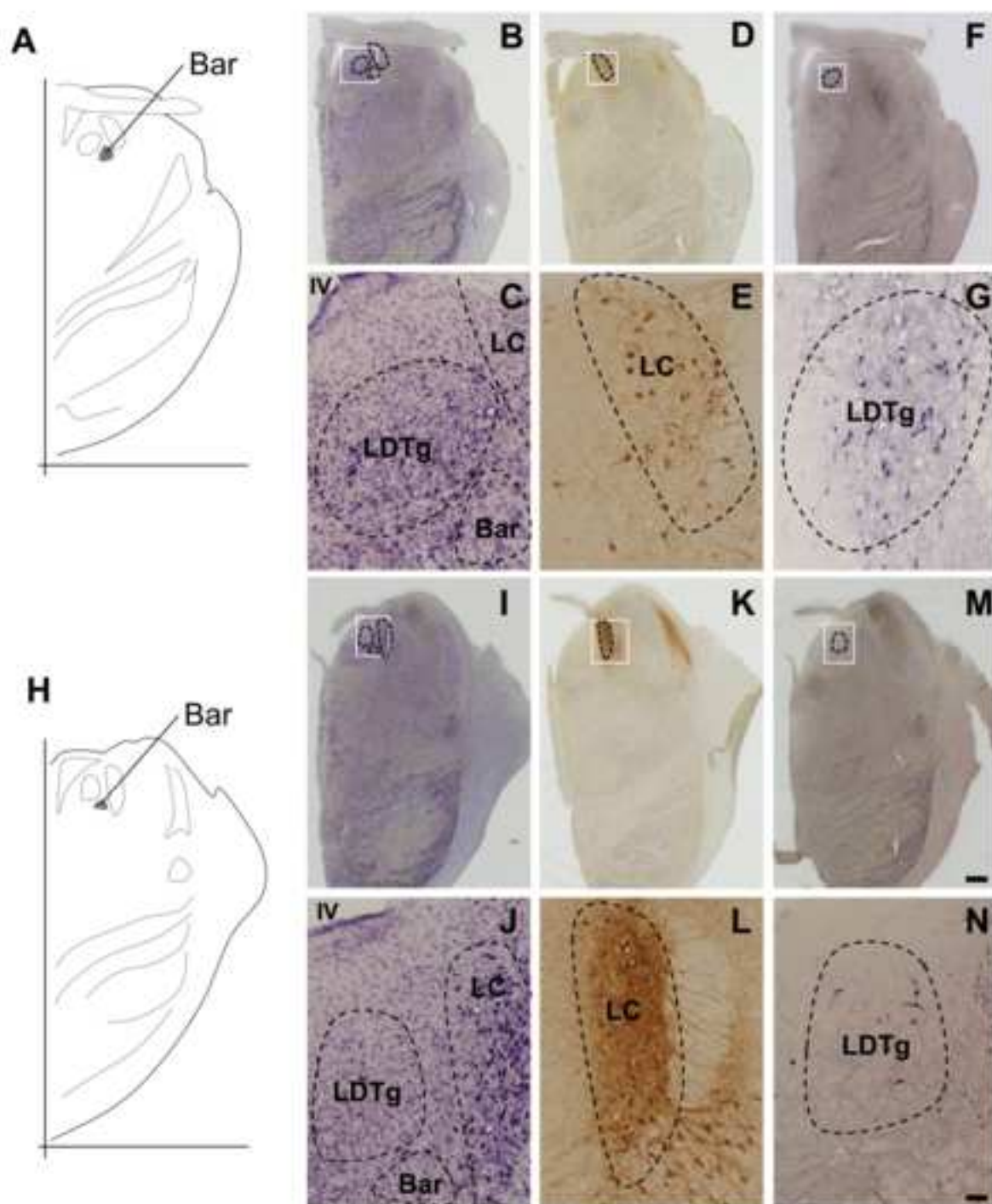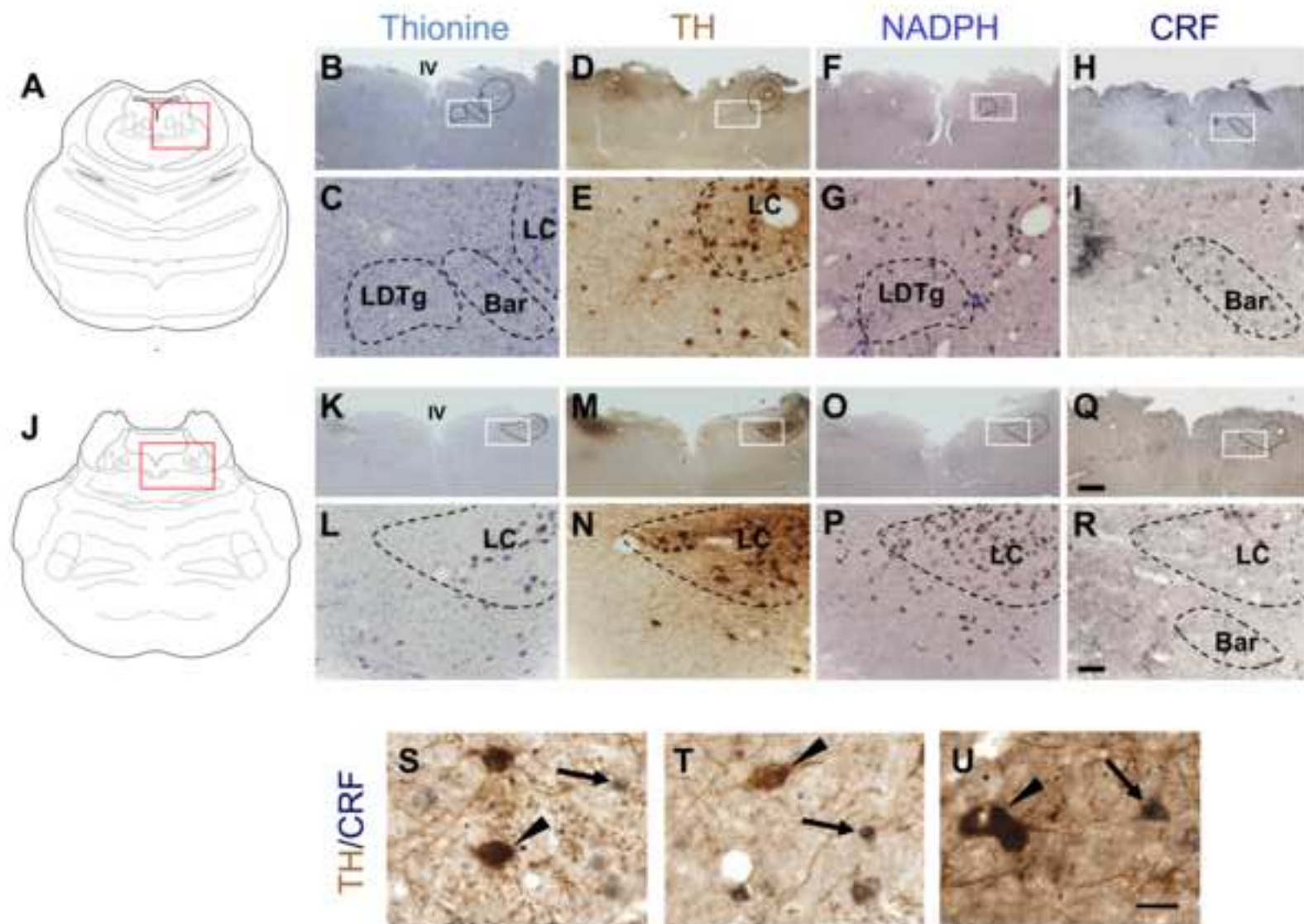
Table 1

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

Species	Histology	Histoenzymology	Immunohistochemistry		
	Thionin staining	NADPH-diaphorase	Anti-TH NA neurons (LC)	Anti-VAChT 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