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# Anatomical evidence for functional diversity in the mesencephalic locomotor region of primates

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ABSTRACT

The mesencephalic locomotor region (MLR) is a highly preserved brainstem structure in vertebrates. The MLR performs a crucial role in locomotion but also controls various other functions such as sleep, attention, and even emotion. The MLR comprises the pedunculopontine (PPN) and cuneiform nuclei (CuN) but their specific roles are still unknown in primates. Here, we sought to characterise the inputs and outputs of the PPN and CuN to and from the basal ganglia, thalamus, amygdala and cortex, with a specific interest in identifying functional anatomical territories. For this purpose, we used tract-tracing techniques in monkeys and diffusion weighted imaging-based tractography in humans to understand structural connectivity. We found that MLR connections are broadly similar between monkeys and humans. The PPN projects to the sensorimotor, associative and limbic territories of the basal ganglia nuclei, the centre median-parafascicular thalamic nuclei and the central nucleus of the amygdala. The PPN receives motor cortical inputs and less abundant connections from the associative and limbic cortices. In monkeys, we found a stronger connection between the anterior PPN and motor cortex suggesting a topographical organisation of this specific projection. The CuN projected to similar cerebral structures to the PPN in both species. However, these projections were much stronger towards the limbic territories of the basal ganglia and thalamus, to the basal forebrain (extended amygdala) and the central nucleus of the amygdala, suggesting that the CuN is not primarily a motor structure. Our findings highlight the fact that the PPN integrates sensorimotor, cognitive and emotional information whereas the CuN participates in a more restricted network integrating predominantly emotional information.

## 1. Introduction

The pedunculopontine nucleus (PPN) is located within the mesencephalic reticular formation of the upper brainstem. Together with the cuneiform nucleus (CuN), they have been named “the mesencephalic locomotor region” (MLR) because early studies showed that electrical stimulation of the MLR produces locomotion in various animal species (Garcia-Rill et al., 2014). Because of its anatomical location and the possibility to generate locomotor pattern, the MLR is considered to be a key generator of gait in the brain. However, the specific roles of the PPN and the CuN remain unclear, even if in decerebrate cat, stimulation of the CuN elicits locomotor patterns whereas stimulation of the PPN is able to change muscle tone (Takakusaki et al., 2003). Because the PPN and the CuN have no clear cut boundaries and are composed of clusters of various neuronal types (GABAergic, glutamatergic, cholinergic) (Mena-Segovia et al., 2009; Wang and Morales, 2009), their precise anatomical identification leads to divergence between authors and increases the confusion between these two nuclei.

*Abbreviations:* IV, trochlear nucleus; AC, anterior commissure; AM, amygdala; Ant, anterior; Assoc, associative territory; aud, auditory cortex; BC, brachium conjunctivum; BDA, biotine dextran amine; BM, nucleus basalis of Meynert; Ce, central nucleus of the amygdala; CM, centre median; cs, central sulcus; CuN, cuneiform nucleus; DBC, decussation of the brachium conjunctivum; DBS, deep brain stimulation, Dor: dorsal; FR, retroflex fascicle; DWI, diffusion-weighted imaging; GPi, internal pallidum; GPe, external pallidum; ia, anterior insula; IC, inferior colliculus; ins, insula; lat, lateral; Lim, limbic territory; MLF, medial longitudinal tract; MLR, mesencephalic locomotor region; NADPH, nicotinamide adenine dinucleotide phosphate; OT, optical tract; PAG, periaqueductal gray; PF, parafascicular nucleus; Post, posterior; PPN, pedunculopontine nucleus; Pu, putamen; SM, sensori-motor territory; SMA, supplementary motor area; SN, substantia nigra; STN, subthalamic nucleus; Sup, superior; temp, temporal cortex; VTA, ventral tegmental area.

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Recently, deep brain stimulation (DBS) of the PPN area has been attempted in patients with Parkinson disease to improve gait disorders (Ferraye et al., 2009; Mazzone et al., 2005; Moro et al., 2010). Even if expected alleviation of gait remains disappointing, clinical and electrophysiological results strongly suggest that the MLR controls locomotion also in humans (Lau et al., 2015; Piallat et al., 2009; Tattersall et al., 2014). However, the precise implantation target within the MLR remains undefined, some suggests that DBS of the CuN should give better results than PPN DBS (Moro et al., 2010).

Besides locomotion, the MLR is known to control many other functions in the brain. As a part of the ascending reticular activating system, the PPN regulates sleep and arousal in rat (Datta, 2002; Steriade et al., 1990). Lesioning the rat PPN also affects attentional resources (Ainge et al., 2006; Okada et al., 2009), learning, reward and reinforcement processes (Inglis et al., 2001; Kozak et al., 2004; Steiniger and Kretschmer, 2004). PPN manipulations in rat are able to alter self-administration of nicotine and cocaine (Corrigall et al., 2002). In the macaque, PPN neurons send positive reward-related signals to nigral neurons where dopaminergic neurons are known to encode motivational values (Hong and Hikosaka, 2014; Okada and Kobayashi, 2013). In PD patients, DBS of the PPN has been shown to modulate non-motor functions. Indeed, PPN DBS could significantly improve sleep (Arnulf et al., 2010), executive functions and working memory (Stefani et al., 2013). These results emphasise the complex and integrative role of the PPN (Lau et al., 2015). The role of the CuN is much less known but this nucleus seems implicated in locomotion if related to aversive reactions, and in the perception of nociception in rodents (Allen et al., 1996). This result highlights the fact that the CuN is not just devoted to generating locomotion – it also integrates contextual information.

Analysis of the detailed connectivity of the MLR should help us understand the specific role that the PPN and CuN could play in motor, cognitive and emotional functions. Tract tracing studies in monkey have revealed that the PPN receives afferents from the motor cortices (Matsumura et al., 2000), from the output structures of the basal ganglia (internal pallidum (GPi) and substantia nigra (SN)) and from the subthalamic nucleus (STN) (Lavoie and Parent 1994a,b; Shink et al., 1997). The PPN ascending pathway projects to non-specific nuclei of the thalamus, in particular to the centre-median-parafascicular nuclei (CM-PF) (Parent et al., 1988; Steriade et al., 1988). The PPN descending outputs project to the ponto-bulbar reticulospinal formation (Rolland et al., 2011). Even if the connections of the CuN are less known, it has been demonstrated in monkey that the CuN only receives projections from the SN (Rolland et al., 2011) and projects back to various thalamic nuclei (Lavoie and Parent, 1994a), to dopaminergic neurons of the mesencephalon (Hong and Hikosaka, 2014), and to the reticulospinal formation (Rolland et al., 2011). Anatomical connectivity has also been explored *in vivo* in primates using diffusion weighted imaging (DWI). This technique is the only non-invasive method allowing access to white matter structural connectivity (Le Bihan et al., 1986) through tractography algorithms (Mori and van Zijl, 2002). Results of DWI-based analyses provide evidence for strong connections between the PPN and the cortex, pallidum, STN, thalamus and spinal cord in macaque and human (Aravamuthan et al., 2007; 2009; Muthusamy et al., 2007).

Altogether, these anatomical results provide insights into the complex connectivity of the MLR and its close relationship with basal ganglia. However, the delineation of the PPN remains controversial, the CuN connectivity remains poorly studied, and the connectivity of these two specific nuclei has not been determined in relation to the anatomo-functional subdivisions of different brain structures. This partial anatomical knowledge of the MLR limits our understanding of the specific role of the PPN and the CuN. The aim of our study was to examine the PPN and the CuN inputs and outputs focusing on how projection patterns relate to the cortical, basal-ganglia, amygdala and thalamic anatomo-functional territories that process sensorimotor, cognitive and emotional information. For this purpose, we used tract-tracing experiments in monkeys and DWI-based tractography in humans.

## 2. Material and methods

### 2.1. Monkeys and volunteers

All experiments were carried out in strict accordance with the European Community Council Directive of 2010 (2010/63/UE) for care and use of laboratory animals. The authorisation for conducting our experiments was approved by the local Committee on the Ethics of Animal Experiments. The animals were kept under standard conditions (12-h light/dark cycle [light on at 20 h], 23 °C and 50% humidity). We used five adult monkeys weighing between 2 and 5 kg (four *Macaca fascicularis*, MI53, MI58, MI82, MIW7 and one *Cercopithecus aethiops*, CA8). Both species are Old World monkeys, with comparable body size and brain development.

Data from 30 healthy volunteers (12 males, range 22 to 35 years) were included in this study, provided by the Human Connectome Project (HCP) (Van Essen et al., 2013). HCP experiments were performed in accordance with relevant guidelines and regulations and the experimental protocol was approved by the Institutional Review Board.

### 2.2. Tracer injections and analysis

All the procedures used have already been described in detail (Jan et al., 2000; Rolland et al., 2011). For all stereotaxic injections, we used biotin dextran amine (BDA, Sigma, St-Louis, MO) as an anterograde and retrograde tracer (10% in 0.01 M phosphate buffer saline). We made unilateral (CA8, MI58, MIW7) or bilateral (MI53) injections in the MLR at -3.5 to 4.5 mm from the Posterior Commissure (PC), 3 to 4 mm from the midline, and -6 to 7 mm below PC. We also performed one injection in the infralimbic cortex (MI82) at 12 mm from the frontal pole, 1 mm from the midline and at a depth of 8 mm from the dura.

Thirteen days after injections, animals were sacrificed, brains removed and cut on a freezing microtome into 50 µm coronal sections perpendicular to the Anterior Commissure (AC)-PC line. The BDA tracer was revealed on regularly spaced sections (500 µm) as previously described (Rolland et al., 2011). A second series of sections were first processed to precisely localise the BDA injections, and then processed with nicotinamide adenine dinucleotide phosphate diaphorase histochemistry (NADPH) to identify PPN cholinergic neurons (Hirsch et al., 1987). Representative sections distributed regularly over the whole extent of the regions studied were selected.

NADPH+ neurons were used to delimit the boundaries of the PPN, following criteria previously used (Rolland et al., 2011). For the delineation of the CuN, we used anatomical landmarks: medially, the periaqueductal grey matter, dorsally the colliculi, laterally the lateral lemniscus, and ventrally the PPN. Maps of the retrograde BDA-labelled cell bodies and anterograde labelled terminals (characterised by thin, varicose and bifurcating fibres) were drawn using a computer assisted image analysis (Mercator, ExploraNova, La Rochelle, France). Contralateral labelling was not considered. All the data obtained from the left side were flipped to the right side to simplify comparisons.

Brain structure functional territories were delineated from previous studies: in the STN (Karachi et al., 2009), in the SN (François et al., 1994), in the GPi and GPe (François et al., 2004), in the posterior intralaminar thalamic nuclei (centre median (CM), parafascicular nucleus (PF)) (Galvan and Smith, 2011). The ventral tegmental area (VTA), considered as a part of the limbic system, was delineated following criteria already described (François et al., 1999). An overview of the PPN and the CuN where the tracer was injected and their basal ganglia and thalamic target regions in monkey is summarized (Fig. 1A).