Research Article

Combined T1-weighted MRI and diffusion MRI tractography of paraventricular, locus coeruleus, and dorsal vagal complex connectivity in brainstem-hypothalamic nuclei

Nikos Makris[1,2,3,4,5,6,7†*], Poliana Hartung Toppa[6†], Richard J. Rushmore[1,5,6†], Kayley Haggerty[6], George Papadimitriou[6], Stuart Tobet[7,8], Yogesh Rathi[1,6], Marek Kubicki[1,6,7,9] Edward Yeterian[6,10], Agustin Castañeyra-Perdomo[3], Jill M. Goldstein[2,7,11]

[1]Psychiatry Neuroimaging Laboratory, Department of Psychiatry, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States

[2]Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, United States

[3]Anatomy and Physiology Area, Department of Basic Medical Sciences, Faculty of Health Sciences, University of La Laguna, San Cristobal de La Laguna 38000, Tenerife, Spain

[4]Department of Cognitive, Social and Organizational Psychology, Faculty of Health Sciences, University of La Laguna, University Institute of Neuroscience, San Cristobal de La Laguna 38000, Tenerife, Spain

[5]Department of Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts 02118, United States

[6]Center for Morphometric Analysis, Department of Psychiatry and Neurology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02129 United States

[7]Innovation Center on Sex Differences in Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114, United States

[8]Department of Biomedical Sciences, School of Biomedical Engineering, Colorado State University, Fort Collins, Colorado 80523, United States

Abstract

Background: Current multimodal neuroimaging plays a critical role in studying clinical conditions such as cardiovascular disease, major depression, and other disorders related to chronic stress. These conditions involve the brainstem-hypothalamic network, specifically the locus coeruleus (LC), dorsal vagal complex (DVC), and paraventricular nucleus (PVN) of the hypothalamus, collectively referred to as the "DVC-LC-PVN circuitry." This circuitry is strongly associated with the norepinephrine (NE) and epinephrine (E) neurotransmitter systems, which are implicated in the regulation of key autonomic functions, such as cardiovascular and respiratory control, stress response, and cognitive and emotional behaviors. **Objectives:** To develop a methodology for delineating the DVC-LC-PVN circuitry in the human brain using multimodal neuroimaging. **Methods:** We combined structural T1-weighted morphometric magnetic resonance imaging (MRI) and diffusion MRI-based tractography to map the DVC-LC-PVN circuitry in the human brain. This methodology was applied to a pilot sample of brain datasets from five healthy adult subjects obtained from the publicly available Human Connectome Project repository and to one post-mortem human dataset. **Results:** The DVC-LC-PVN circuitry was delineated *in vivo* in five human subjects and one ultra-high resolution post-mortem dataset, allowing for refined anatomical observations. **Conclusion:** NE and E neurotransmitter systems engender substantial interest in both basic and clinical neuroscience due to their roles in the regulation of key autonomic functions, such as cardiovascular and respiratory control, stress responses, and cognitive and emotional behaviors. As demonstrated in this study, multimodal neuroimaging techniques provide a valuable approach for mapping small brainstem and hypothalamic structures and complex circuitries such as the DVC-LC-PVN circuitry.

Keywords: Diffusion magnetic resonance imaging, Dorsal vagal complex, Locus coeruleus, Major depressive disorder, Paraventricular nucleus of the hypothalamus

***Corresponding author**
Nikos Makris
(nikos@cma.mgh.harvard.edu)

Received: 24 July 2024; Revision received: 7 September 2024; Accepted: 8 October 2024; Published: 22 November 2024

How to cite this article: Makris N, Toppa PH, Rushmore RJ, et al. Combined T1-weighted MRI and diffusion MRI tractography of paraventricular, locus coeruleus, and dorsal vagal complex connectivity in brainstem-hypothalamic nuclei. *J Biol Methods*. 2024;11(4):e99010036. DOI: 10.14440/jbm.2024.0043

[9]Department of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, United States
[10]Department of Psychology, Colby College, Waterville, Maine 04901 United States
[11]Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114, United States
[†]The authors contributed equally to this paper

1. INTRODUCTION

The locus coeruleus (LC), dorsal vagal complex (DVC), and paraventricular nucleus (PVN) of the hypothalamus are among the most important structures in the noradrenergic (norepinephrine [NE]) and adrenergic (epinephrine [E]) brain circuitries. Collectively known as the "DVC-LC-PVN circuitry," this network is a key component of the brainstem-hypothalamus circuitry and constitutes a fundamental and critically important biological apparatus for the survival and healthy functioning of mammals. From lower invertebrates to vertebrates, the organization and development of the body, nervous system, and behavior have grown in complexity; a general rule is that increased complexity is associated with more prominent morphological and structural representation in the rostral part of the brain [1]. During human ontogenesis, the brainstem and hypothalamus appear between the 4th and 6th week of gestation, respectively, arising from the rhombencephalon and diencephalon – two visually recognizable enlargements at the rostral end of the neural tube [2]. Comparatively, the differences in size between the brainstem and diencephalon in macrosomatic mammals and primates have increased to a lesser degree than those in the neocortex. This trend parallels variations in the brain's functional performance and behavioral phenomenology. An important structural feature that the brainstem and hypothalamus share to a great extent across species is their chemical neuroanatomy [3,4]. The brainstem is a principal hub of cell groups that utilize catecholamine neurotransmitters, including NE, E, and dopamine. In particular, NE and E are synthesized in neurons located in the pons and medulla [2-4]. Since the 1960s, the NE and E transmitter-specified populations and their structural connectivity have been extensively studied in experimental animals and, more recently, in humans. This research has led to a significant integration of traditional neuroanatomy and chemical neuroanatomy. Recently, the advent of neuroimaging has further propelled this integration, creating novel research and clinical opportunities due to the non-invasive and *in vivo* nature of these techniques. The NE and E systems in the brainstem are organized by specific cells of origin and axonal fibers that follow topographically specific trajectories toward their terminations [3,4]. However, the complex anatomy of the brainstem and its spatial limitations present significant challenges for clinical structural neuroimaging investigations of fine-grained brainstem anatomy.

Herein, we report a novel methodology for investigating the brainstem–hypothalamic structural circuitry involving regions within the brainstem and hypothalamus, namely the DVC, including the nucleus of the solitary tract (NTS), and the dorsal motor nucleus of the vagus (DMN), the LC, and the PVN of the hypothalamus. We refer to this circuitry as the DVC-LC-PVN circuitry. This multimodal imaging approach combines structural T1-weighted magnetic resonance imaging (MRI) morphometric with diffusion MRI (dMRI) tractographic analyses in humans. The method was tested for reliability and subsequently applied to five healthy human datasets from the publicly available Human Connectome Project (HCP) repository [5]. Moreover, we determined the finer topography and trajectory of the individual fiber tracts constituting the DVC-LC-PVN circuitry in one ultra-high-resolution post-mortem dataset [6]. The MRI-based and anatomically curated delineation of central catecholamine neuronal systems, particularly the NE/E systems, is expected to be highly beneficial in both basic and clinical neuroscience. This is especially relevant given the critical role of NE- and E-related circuitry in disorders associated with acute and chronic stresses, such as cardiovascular disease, major depressive disorder (MDD), and post-traumatic stress disorder (PTSD), among many others.

2. METHODS

2.1. Rationale

The reasoning behind the approach to determining the circuitry of DVC (including NTS and DMN), LC, and PVN circuitry is grounded in the conceptual framework of "MRI-based brain volumetrics." This morphometric approach has been developed at the Center for Morphometric Analysis at Massachusetts General Hospital (MGH)/HMS and the A. A. Martinos Center for Biomedical Imaging since the 1990s [7,8]. This framework gave rise to the Harvard Oxford Atlas, distributed in FSL in the early 2000s, and served as a testbed for validating the most current automated methods for brain morphometry, particularly FreeSurfer. An important concept in this morphometric approach is the consideration of neuroanatomical individuality, acknowledging the biological structural variability of each individual's brain and generating reliable methods for analysis. Thus, the definition of brain regions of interest (ROIs) is based on landmarks that can be reliably identified by certain neuroimaging modalities. Using T1-weighted images, visible features such as fissures, sulci, protuberances or bulges, and gray-white matter contrasts are identifiable [8,9]. Morphometric procedures are driven by a thorough knowledge of neuroanatomy, which is particularly relevant for structures with complex neuroanatomical

compositions, such as the brainstem and hypothalamus [7,8]. The methodology used in the present study consisted of two consecutive steps, as illustrated in detail in Figure 1.

In the first step, we defined and segmented the relevant structural ROIs in the brainstem and hypothalamus from the T1-weighted dataset, focusing on the DVC, LC, and PVN. The DVC comprises the NTS and the DMN, anatomically located in the dorsal-medial sector of the posterior upper medulla (B1p-dm and the adjacent B1p-vm), adjacent to the area postrema (AP) in the vicinity of the obex (Figures 1A and C). The LC is within the dorsal-medial sector of the posterior upper pons (P1p-dm and the adjacent P1p-vpl) (Figure 1A-C), while the PVN is situated in the anterior and tuberal regions of the hypothalamus. The morphometric method used for analyzing the anterior and tuberal regions of the human hypothalamus has been previously detailed [7]. Further, the segmentation method for the human brainstem was also priorly reported [10] and has been applied in several studies by our group [10]. Segmentation was performed using the "neurosegmentation module" of the publicly available 3D Slicer software platform (www.slicer.org).

In the second step, the DVC, LC, and PVN ROIs were sampled and used as "seeds" for tractography. This step involved dMRI-based tractography to delineate the structural connectivity of the DVC-LC-PVN. The virtual fiber tracts

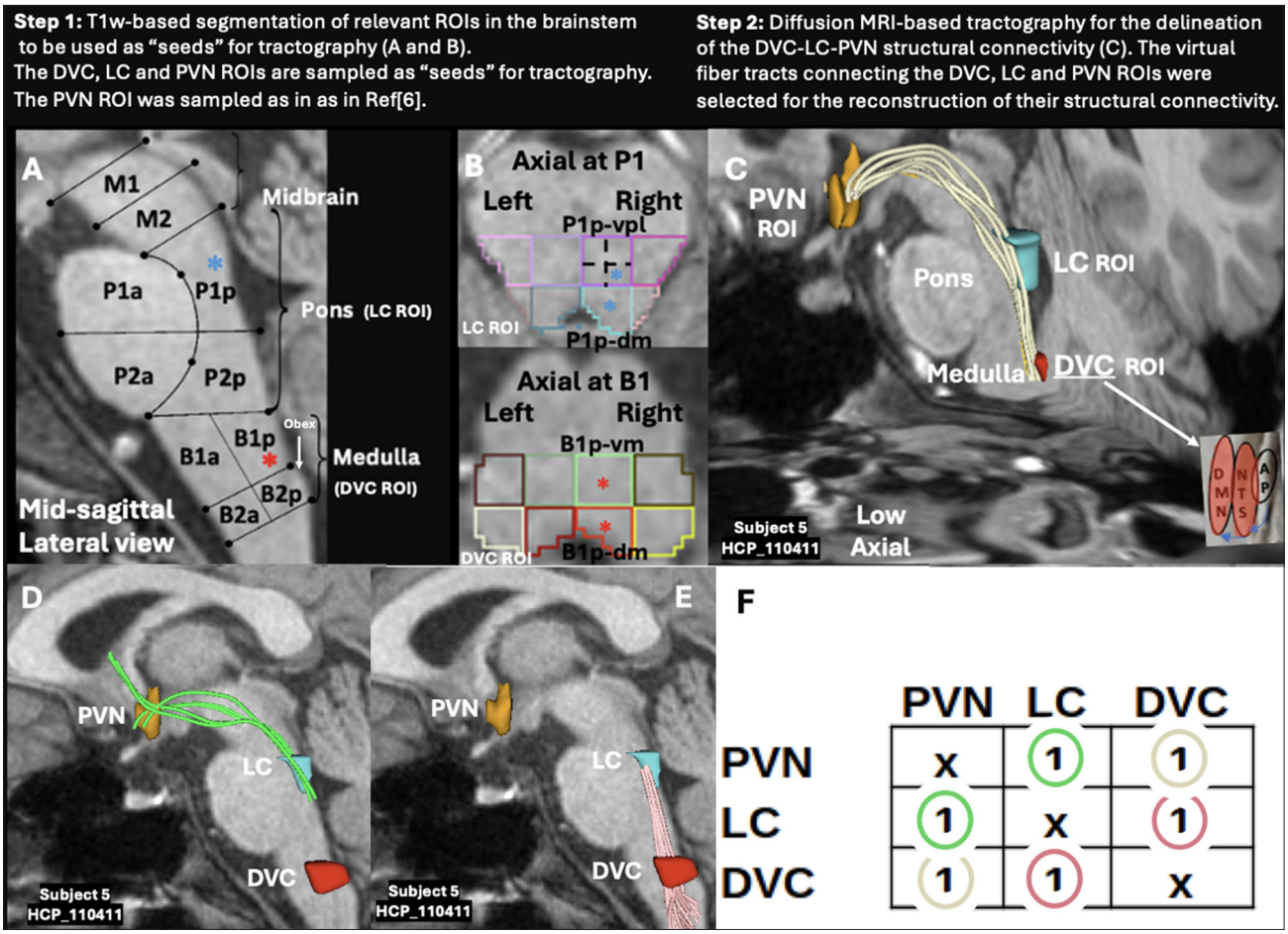

Figure 1. Method for structural MRI-based delineation of the DVC-LC-PVN circuitry as shown in Step 1 (A and B) and Step 2 (C) above and explained in detail in the main text. The diffusion MRI (dMRI)-based tractographic data shown in (C), (D), and (E) are from Subject 5 (HCP_110411). Panels (A) and (B) show morphometric parcellation performed on T1-weighted images for the entire brainstem. Using this procedure, we subdivided the brainstem into the midbrain (M1 [upper midbrain] and M2 [lower midbrain]), anterior and posterior pons (P1a and P1p [upper pons]; P2a and P2p [lower pons]), and anterior and posterior medulla (B1a and B1p [upper medulla]; B2a and B2p [lower medulla]). The DVC region of interest (ROI) is highlighted in red in panels (C), (D), and (E) and is located in the caudal-medial sector of the posterior upper medulla (B1p-dm and B1p-vm ROIs, indicated by a red asterisk in panels [A] and [B]). The LC ROI, shown in blue in panels (C), (D), and (E), is situated within the caudal-medial sector of the posterior upper pons (P1p-dm and P1p-vpl ROIs, indicated by a blue asterisk in panels [A] and [B]). Please note that the DVC consists of the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMN), and is adjacent to the area postrema (AP), shown in panel (C) (magnified as indicated by the arrow), and the vicinity of the obex, indicated by the arrow in panel (A). Along with the LC and the PVN of the hypothalamus (PVN), these structures form the key components of the brainstem–hypothalamic circuitry under investigation. Panel (F) illustrates the pairwise structural connectivity matrix of the DVC-LC-PVN circuitry.

DVC: Dorsal vagal complex, LC: Locus coeruleus, MRI: Magnetic resonance imaging, PVN: Paraventricular nucleus, ROI: Region of interest.

connecting the DVC, LC, and PVN "seed" ROIs were selected for the reconstruction of their structural connectivity using the Slicer dMRI module of the 3D Slicer software platform (www.slicer.org), as illustrated in Figure 1C.

2.2. Exploratory analysis of DVC and LC telencephalic connectivity

Although this study focused on the brainstem–hypothalamic DVC-LC-PVN circuitry, we extended our tractographic analysis to explore the structural connectivity of the LC and DVC with telencephalic regions. As a result, we excluded the PVN from our hypothalamic ROI. Given the exploratory purpose of this analysis and its anatomically extensive nature, we referred to this fiber system as the "extended DVC-corticolimbic fiber system" or DVC-CLFS.

2.2.1. Reliability assessment

Before proceeding to the second step, including the tractographic reconstruction of fiber tracts, we performed morphometric segmentation in all five subjects and computed intra-rater and inter-rater reliability measurements using the Dice coefficient.

2.3. Subjects and information of neuroimaging protocols and dMRI tractographic analysis

The five datasets were part of the publicly available HCP repository [5], and their subject codes are as follows: 100307, 101107, 101915, 103111, and 110411. The subjects were classified as healthy controls. Datasets were acquired from the HCP, as described in a previous study [5] (https://www.humanconnectome.org). The ACPC-aligned T1w MRI images ($0.7 \times 0.7 \times 0.7$ mm^3 voxel size) from the HCP Young Adult dataset were used for structural MRI analysis. dMRI analysis was performed on the same subjects. The T1-weighted and dMRI protocols are as follows: T1-w: 3D MPRAGE, TR = 2400 ms, TE = 2.14 ms, TI = 1000 ms, Flip angle 8°, and voxel size 0.7 mm isotropic. dMRI: Spin-echo EPI, with TR = 5520 ms, TE = 89.5 ms, and flip angle of 78°. The refocusing flip angle was set at 160°. The multifactor was 3, with an echo spacing of 0.78 ms and a voxel size of 1.25 mm isotropic. The b-values used were 1000, 2000, and 3000 s/mm^2, each b-shell with 90 diffusion directions and 6 b = 0s. The whole brain tractography was performed using a multitensor unscented Kalman filter method [11,12] as implemented in 3D Slicer. This algorithm uses tractography to drive the local fiber model estimation, that is, model estimation (in this case, the multiple tensors) is done while tracing a "fiber" from seeding to termination.

2.4. DVC-LC-PVN circuitry in one ultra-high resolution post-mortem dataset

To contrast our findings in HCP publicly available datasets at commonly used resolutions in basic and clinical neuroscience, we analyzed one ultra-high resolution post-mortem MRI publicly available human brainstem and diencephalon human dataset. Post-mortem MR imaging was performed by Calabrese *et al*. [6], and the protocol is fully described in their publication [6]. In brief, Calabrese *et al*. [6], using a 7 Tesla MRI system, acquired a T2-weighted MRI anatomical dataset at 50 microns isotropic voxel resolution with a total acquisition time of 14 h. Furthermore, they acquired a dMRI dataset at 200 microns isotropic voxel resolution with a total acquisition time of 208 h. Importantly, in a previous anatomical study by our group, we parcellated morphometrically a T2-weighted MRI dataset from Calabrese *et al*. and delineated all of the principal nuclei of the human brainstem. These findings were published by Rushmore *et al*. and were used in the present study as a template for the selection of the DVC and LC ROIs [8]. The PVN ROI was parcellated on the post-mortem human hypothalamus based on a study by Makris *et al*. [7]. It should be noted that because of the ultra-high resolution of the post-mortem dataset, the DVC and PVN are highly representative of actual anatomical nuclei, as has been emphasized in our recent publication by Rushmore *et al*. [8]. Furthermore, dMRI data at 200 microns isotropic voxel resolution can be considered ground truth in the field of dMRI currently. Therefore, the DVC-LC-PVN circuitry delineation in the ultra-high resolution post-mortem dataset used herein can be conceived of as a ground truth comparator for the commonly used HCP datasets. The dMRI tractographic delineation of the DVC-LC-PVN structural connectivity was performed following the two-step method as described above.

3. RESULTS

We successfully delineated the neuroanatomy of brainstem–hypothalamic structural circuitry in five healthy human subjects, involving structures in the brainstem and hypothalamus, that is, the DVC (which includes the NTS and DMN) in the upper medulla, the LC in the upper pons, and the PVN in the anterior and tuberal hypothalamus (Figures 2-5). The specific DVC-LC-PVN circuitry was determined and contrasted with the more extensive DVC-LC structural connectivity with the cerebrum, or "extended DVC-corticolimbic fiber system" (DVC-CLFS), as illustrated in Figures 1-5. Furthermore, the individual observations in all five subjects are shown in Figures 1-5. The pair-wise

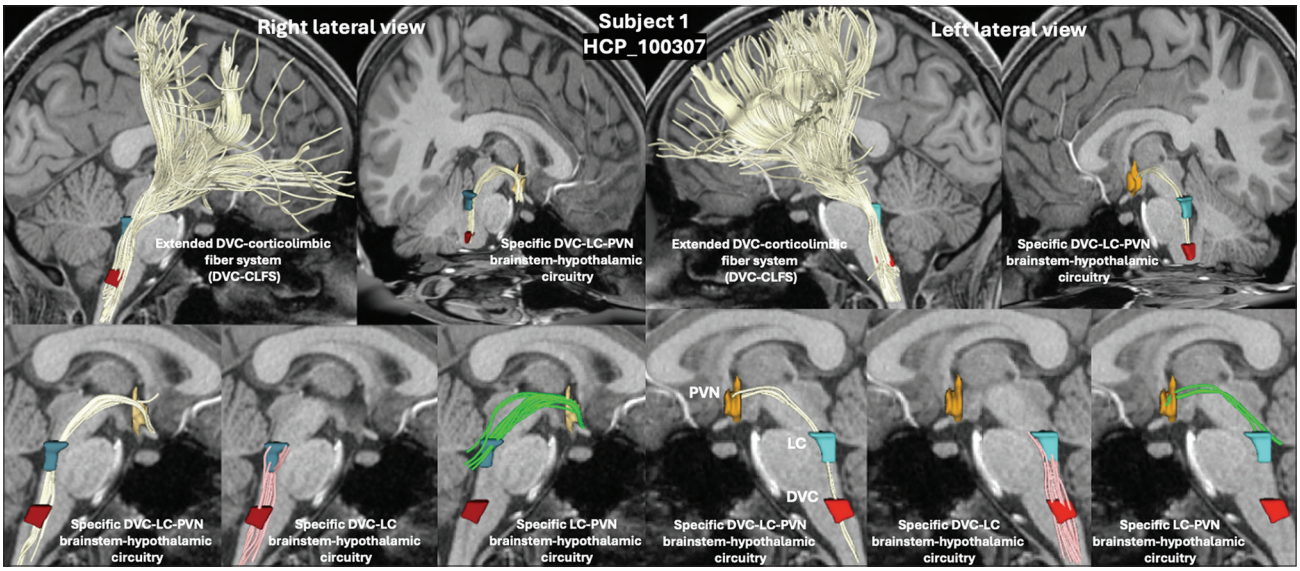

Figure 2. The complete results of diffusion magnetic resonance imaging tractographic analysis for Subject 1 (HCP_100307) are shown for the left and right sides of the brain. The structural connectivity specific to the DVC-LC-PVN circuitry is shown in detail in the six panels in the lower row of the figure. Furthermore, as explained in the rationale of the method, the "extended DVC-corticolimbic fiber system," or DVC-CLFS, is shown in the right and left hemispheres (The same applies to Figures 3-5 except subject number).
CLFS: Corticolimbic fiber system, DVC: Dorsal vagal complex, LC: Locus coeruleus, MRI: Magnetic resonance imaging, PVN: Paraventricular nucleus.

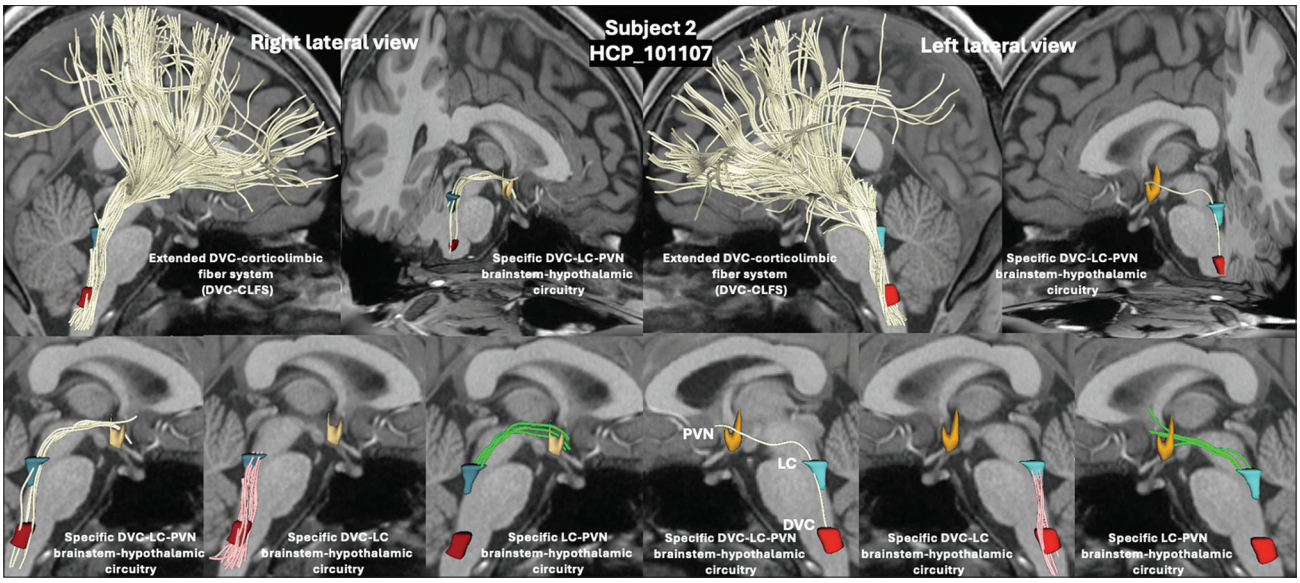

Figure 3. The complete results of diffusion magnetic resonance imaging tractographic analysis for Subject 2 (HCP_101107).
CLFS: Corticolimbic fiber system, DVC: Dorsal vagal complex, LC: Locus coeruleus, MRI: Magnetic resonance imaging, PVN: Paraventricular nucleus.

connections between the DVC, LC, PVN, and the DVC-CLFS were delineated bilaterally in all subjects. Inter-rater reliability of the ROIs in the upper medulla for DVC "seeding" and in the upper pons for LC "seeding" was excellent. The Dice coefficient was 0.99 for intra-rater reliability and 0.95 for intra-rater reliability.

Finally, we were able to delineate the DVC-LC-PVN circuitry in an ultra-high resolution post-mortem human dataset of the brainstem and diencephalon, as shown in Figure 6.

4. DISCUSSION

In this study, we utilized multimodal neuroimaging to delineate the structural brainstem–hypothalamic circuitry involving the DVC, LC, and PVN, that is, the DVC-LC-PVN circuitry, which is critically associated with the cellular origin and regulation of NE and E. Furthermore, we uniquely identified the distinct pair-wise connections between the DVC and LC, the LC and PVN, and the DVC and PVN. Moreover, we delineated the extended structural network of the DVC with the telencephalon, which is critical for

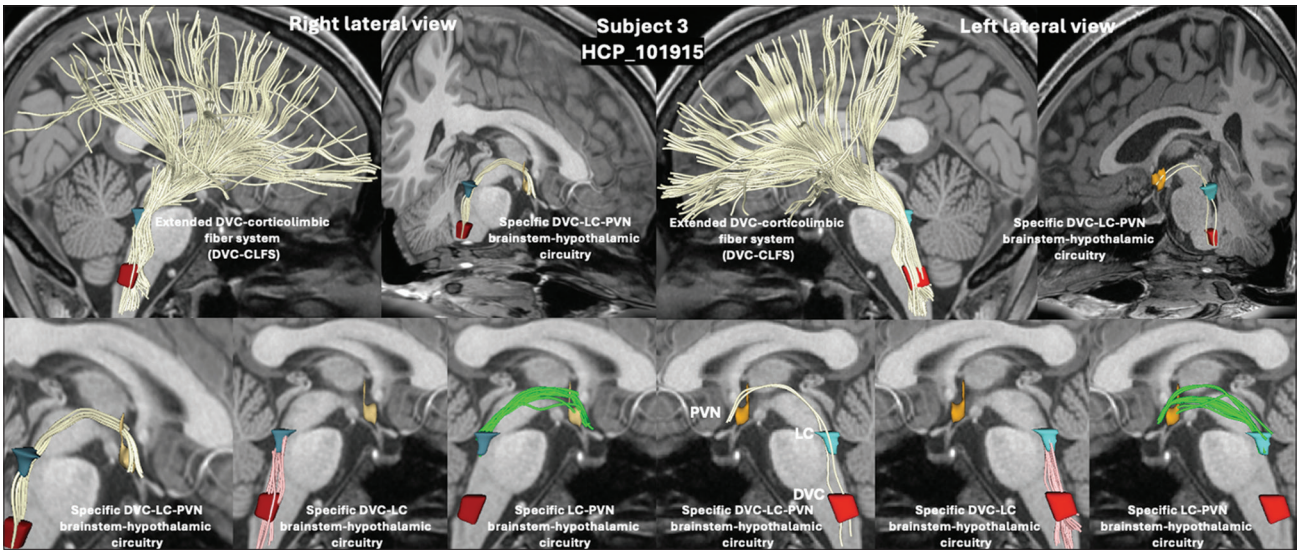

Figure 4. The complete results of diffusion magnetic resonance imaging tractographic analysis for Subject 3 (HCP_101915).
CLFS: Corticolimbic fiber system, DVC: Dorsal vagal complex, LC: Locus coeruleus, MRI: Magnetic resonance imaging, PVN: Paraventricular nucleus.

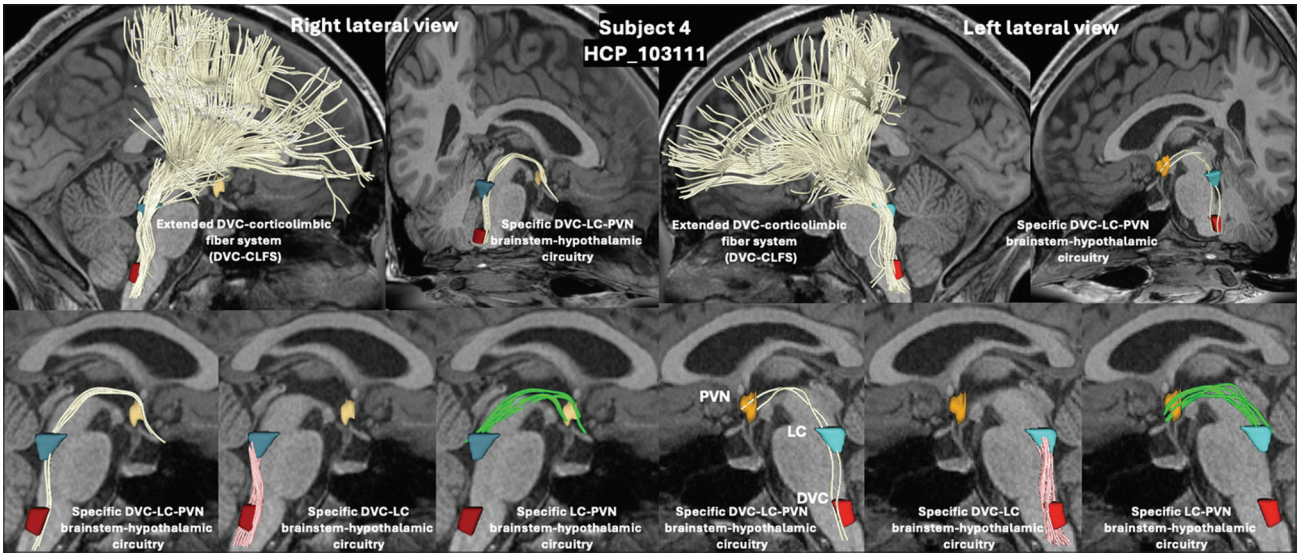

Figure 5. The complete results of diffusion magnetic resonance imaging tractographic analysis for Subject 4 (HCP_103111).
CLFS: Corticolimbic fiber system, DVC: Dorsal vagal complex, LC: Locus coeruleus, MRI: Magnetic resonance imaging, PVN: Paraventricular nucleus.

cognitive and affective behavioral investigations in basic and clinical neuroscience. Importantly, we demonstrated that when neuroimaging analyses were guided by thorough anatomical knowledge, structural T1-weighted MRI morphometry combined with dMRI tractography provided tremendous capability for the investigation of very complex and difficult circuitries, such as in the brainstem and hypothalamus. The *in vivo* and non-invasive nature of neuroimaging allows us to perform clinical investigations and assessments of high relevance in neuropsychiatric research. Significantly, we compared the DVC-LC-PVN circuitry results from the five HCP subjects to an ultra-high resolution dMRI dataset of the human brainstem and diencephalon. This comparison showed striking similarity between the two types of datasets in terms of origins, terminations, and trajectory of the DVC, LC, and PVN structural connectivity.

4.1. Neural systems organization and anatomy of NE and epinephrine systems involving the LC, DVC, and PVN

NE, E, and dopamine constitute the group of catecholamine neurotransmitters in the brain. The NE and E systems in the brainstem are organized by means of (i) specific cells of origin, (ii) fields or areas of termination, and (iii) specific fiber pathways of which their axons are a part. Thus, a complete and comprehensive characterization of these neural systems needs to address all these requisite structures. To achieve anatomical accuracy as well as neuroimaging precision and specificity, we combined T1-weighted MRI-based morphometry and dMRI-

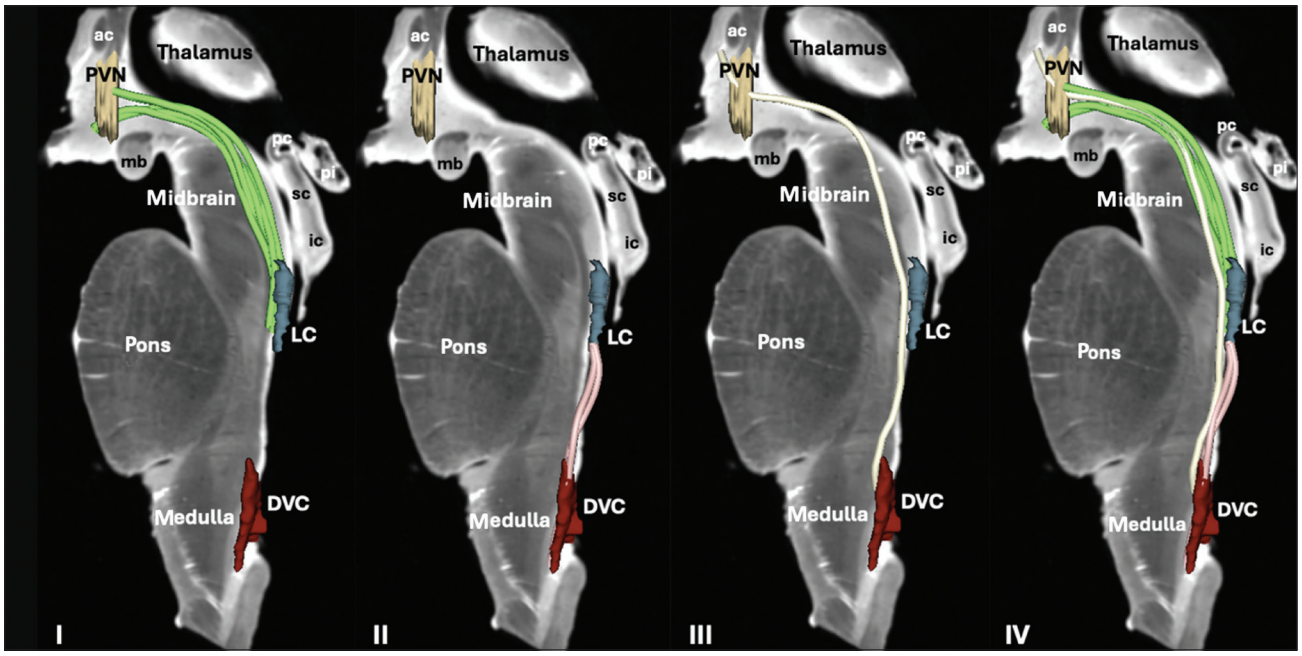

Figure 6. The results of the combined morphometric [6] and dMRI tractographic analysis of an ultra-high resolution human post-mortem brainstem and diencephalon dataset [12] illustrate the DVC-LC-PVN circuitry in four panels, I–IV. Specifically, panel I shows the connection (green) between the LC of the pons and the PVN of the hypothalamus. Panel II shows the connection (pink) between the DVC (NTS and DMN) of the medulla and the LC of the pons. Panel III shows the connection (white) between the DVC of the medulla and the PVN of the hypothalamus. Panel IV shows the set of the three pair-wise connections among the DVC, LC, and PVN.
ac: Anterior commissure, DMN: Dorsal motor nucleus of the vagus, DVC: Dorsal vagal complex, ic: Inferior colliculus, LC: Locus coeruleus, mb: Mammillary body, NTS: Nucleus of the solitary tract, pc: Posterior commissure, pi: Pineal gland, PVN: Paraventricular nucleus of the hypothalamus, sc: Superior colliculus.

based tractography. Classical neuroanatomy of structural connectivity of long fiber connections is principally grounded in experimental animal studies.

In the brainstem, the NE system surges in the pons and medulla. Specifically, the cells of origin of the NE system are located within the A6, A6cg, and Asc in the LC and adjacent regions, which are located in the dorsomedial part of the upper pons. In our T1 MRI-based brainstem morphometric parcellation methodology, this parcellation unit (PU or ROI) comprises P1p-dm and the adjacent P1p-vpl **(**Figure 1**)**. There are also important, non-cerulean cell groups of origin of the NE system, such as the A2 cell group in the dorsal upper medulla as well as the A1, A5, and A7 in the pontine lateral tegmentum. In this study, however, we focused only on the LC, given our prior experience with morphometric analysis of the brainstem using ultra-high resolution T2 MRI of *ex vivo* human data [8]. In the latter study, we were able to identify and segment the LC, NTS, and DMN; however, we were unable to identify and segment cell groups such as the A1, A2, A5, and A7. The connections of the NE system are extensive, including connections between the LC cells of origin within the cerebrum and spinal cord. The ascending and descending fibers of cerulean origin fasciculate within the large dorsal noradrenergic bundle [13,14] and the smaller dorsal periventricular bundle, which is part of the dorsal longitudinal fasciculus of Schutz [4,13,15-17]. The axonal fibers that form these origins are topographically gathered principally into the central tegmental tract (CTT) in the brainstem and continue within the medial forebrain bundle toward the diencephalon (including the hypothalamus) and the telencephalon [13,18,19]. Through these pathways, the LC is connected with several brain structures, including the PVN rostrally and the NTS and DMN caudally. By contrast, the non-cerulean origins give rise to fibers that assemble and course as part of the ventral and dorsal noradrenergic bundles [20] and the dorsal periventricular bundle. Further, propriobulbar connections [4] connect A1 and A2 cell groups with several nuclei, such as the NTS, DMN, and parabrachial nuclei [4,21,22]. The cells of origin of the E system are within the C1, C2, and C3 groups located in the upper medulla posterior to the inferior olivary nucleus. In our T1 MRI-based morphometric brainstem parcellation methodology, the PU (or ROI) corresponding to that region of the posterior upper medulla is represented by B1p-dm and the adjacent B1p-vm **(**Figure 1**)**. The connections of the E system ascend from the upper medulla and form part of the ventral noradrenergic pathway and, thus, of the CTT [13,18,19]. Through ascending fibers, these structures in the upper medulla connect with several brainstem and diencephalic structures, including the NTS and VMN in the medulla, the LC in the pons, and the PVN in the hypothalamus. Furthermore, through descending fibers, they establish connections with the spinal cord. Overall, the results of

the present study approximated remarkably what is known from fiber tract reconstruction in the experimental animal literature.

4.2. Functional and clinical implications

The functions of the DVC-LC-PVN circuitry are associated with a wide range of visceral processes, including cardiovascular control and respiration [23,24]. Furthermore, this circuitry is essential for the preservation of cognitive and affective functions, which is critically important for the aging brain [25]. In terms of chemical anatomy, it is closely related to the NE and E systems given the key positional location of the LC in the center of this circuitry. LC is the most important NE center of the brain and is connected with the PVN in the hypothalamus as well as the DVC and the adrenergic cells of origin in the upper medulla. The NE and E systems are key players in the two major systems that respond to stresses, that is, the sympathoadrenal medullary axis and the hypothalamic-pituitary-adrenal axis [23,24]. Based on experimental animal studies, it has been emphasized that the LC cells are activated by stressful stimuli, such as threats, eliciting cardiovascular responses such as increases in heart rate and blood pressure as encountered in the sympathetic "fight" behavioral response [4]. Importantly, through telencephalic connections, the LC and DVC connect with the amygdala, hippocampus, and key cortical inhibitory regions such as the anterior cingulate and medial and orbital prefrontal cortices. Given the inhibitory action of LC neurons on their targets, it has been proposed that LC has a "stress-dampening" action [4]. The notion that NE released by the LC has a protective effect on its target neurons during mentally and emotionally challenging or "alarming" situations emphasizes the relevance of the LC circuitry in the maintenance and preservation of our cognitive reserve, which is critical during aging [25]. Thus, the involvement of the DVC-LC-PVN circuitry with cognitive and emotional functioning is carried out by an array of brainstem-limbic-neocortical relationships that can be investigated with multimodal neuroimaging. Prolonged negative stress can produce persistent structural and physiological changes in the LC and DVC-LC-PVN circuitry, a condition that may lead to increased risk for cardiovascular and psychiatric disorders such as MDD and PTSD [26-28]. MDD and PTSD are also highly comorbid. Thus, understanding the role of the "DVC-LC-PVN" circuitry and associated physiology implicated in mood, anxiety, and cardiovascular disease may provide novel targets for treatments for comorbid disorders of the brain and heart.

4.3. Neuroendocrine pathways associated with cardiovascular disease involving the PVN, LC, and DVC (NTS and DMN)

The PVN, LC, and DVC, as well as three circumventricular organs, namely, the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), and the AP, are associated with arterial blood pressure regulation and cardiac function. As elaborated on in a recent report by our group [29], these structures participate in the secretion of vasopressin or antidiuretic hormone (ADH) through a pathway involving circulating angiotensin II (Ang II), which reaches the SFO and the OVLT. In turn, these organs stimulate the PVN through axonal connections to secrete ADH. Ang II also arrives at the AP via a separate pathway associated with the NTS, the DMN, the nucleus ambiguus, the rostral ventrolateral medulla, and the PVN, and affects arterial blood regulation and cardiac function. Ultimately, alterations in either of these two pathways may lead to cardiovascular disease. Given the intimate structural and functional relationships of the circumventricular organs with the hypothalamus and DVC, it is highly probable that the LC, NTS, and PVN may be impacted when the integrity of the blood–brain barrier is disrupted by peripheral inflammation [29].

4.4. Practical implications for clinical studies of DVC-LC-PVN circuitry using neuroimaging

Recently, the importance of neuroimaging in detecting neuroinflammatory and neurodegenerative brain alterations and thus assisting the practice of neuropathology has been highlighted [29]. Given that neuroinflammatory or neurodegenerative changes can be present in the DVC-LC-PVN circuitry in different pathological conditions, these could be investigated clinically using an array of neuroimaging techniques. Neuroinflammation can be assessed with currently available imaging techniques such as dMRI and positron emission tomography (PET) [30], whereas metabolic and neurodegenerative abnormalities may be detected by PET. Further, functional analyses can be conducted using functional MRI (fMRI) and fMRI connectivity techniques. The structural determination of the DVC-LC-PVN circuitry, as performed in the present study, can be integrated with other imaging modalities in a multimodal imaging context to provide a basis for the localization of the circuitry. This approach will empower basic and clinical research in a useful and effective way.

4.5. Limitations and future studies

It should be noted that the MRI-based approach presented here does not allow us to pinpoint specific fiber tracts with comparable accuracy as techniques used in experimental animals. Most of what we know regarding structural connections in the human brain is derived from other animals, such as rodents, cats, dogs, or non-human primates, given the invasive and destructive nature of the techniques used to obtain this information. Further, T1 MRI and dMRI have their weaknesses as well, especially those related to limited spatial resolution. Moreover, we should also keep in mind that dMRI can only inform us regarding the orientation and not the directionality of water molecular diffusion. Thus, what we

show here is an outline of the DVC, LC, and PVN structural connectivity, which can be further investigated by currently available multimodal structural neuroimaging in clinical settings. This is relevant, given the tremendous opportunity neuroimaging offers to study this critical brain circuitry in clinical conditions. Although the present study was strictly an anatomical investigation of structural connectivity, future studies using more sophisticated structural neuroimaging protocols with sizeable voxel sizes and other imaging modalities, such as fMRI and PET, will be of great use to advance diagnosis and monitoring of treatment targeting the DVC-LC-PVN circuitry in medical research and practice. Furthermore, the present investigation presented a novel structural connectivity finding that can serve as a basis for future functional neuroimaging and metabolic studies using PET imaging. These multimodal neuroimaging studies are expected to shed light on the complex relationships between structural and functional connectivity, a field of great relevance and challenge.

5. CONCLUSION

Using multimodal neuroimaging, we delineated the structural brainstem–hypothalamic circuitry involving the DVC, LC, and PVN in five healthy human brains, as well as in an ultra-high-resolution post-mortem dataset of the human brainstem and diencephalon. The DVC-LC-PVN circuitry is profoundly represented by the architectural connectivity of NE and E; it plays a crucial role in key autonomic functions, such as cardiovascular and respiratory control, stress response, and cognitive and emotional behaviors. In addition, using dMRI tractography, we were able to delineate, for the first time, the distinct connections between the DVC and LC, the LC and PVN, and the DVC and PVN. Given the important role of the NE system in cognitive and affective behaviors, we delineated the extended structural network connecting the DVC and LC with the telencephalon, which is vital for investigating cognitive and affective behaviors in both basic and clinical neuroscience. Importantly, multimodal structural neuroimaging combining T1-weighted MRI morphometry and dMRI tractography allows for anatomically curated delineation of complex circuitries, particularly in the brainstem and hypothalamus. The unique nature of neuroimaging, allowing for non-invasive investigations in the living human, is a critical factor for medical research in clinical conditions associated with the DVC-LC-PVN circuitry, such as cardiovascular disease, depression and anxiety disorders, and others associated with exposure to chronic negative stress.

ACKNOWLEDGMENTS

We would like to recognize Dr. Allan Johnson and Dr. Evan Calabrese for providing excellent post-mortem data to the neuroscience community, in particular the brainstem/diencephalon human dataset used in this study.

FUNDING

We would like to acknowledge the following grants for their support: R01MH112748 (MRK, NM, RJR), R01AG042512 (MRK, NM), K24MH116366, R01MH132610, R01MH125860 (NM), R21NS136960 (RJR, NM), and R01NS125307 (RJR, NM). In addition, work on this manuscript was supported by ORWH-NIMH U54 MH118919 (JMG & SAT, mPIs).

CONFLICT OF INTEREST

Jill M. Goldstein serves on the scientific advisory board of, and holds equity interest in, Cala Health (a neuromodulation company). Jill M. Goldstein's interests are managed by MGH and MassGeneral Brigham HealthCare in accordance with their conflict of interest policies. However, the work presented in this study was conducted before this relationship, and thus no conflict of interest exists. The other authors declare they have no competing interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Nikos Makris, Agustin Castañeyra-Perdomo, Jill M. Goldstein
Investigation: Nikos Makris, Poliana Hartung Toppa, Richard J. Rushmore, Kayley Haggerty, George Papadimitriou, Agustin Castañeyra-Perdomo, Jill M. Goldstein
Methodology: All authors
Writing – original draft: All authors
Writing – review & editing: All authors

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

Data were provided in part by the HCP, WU-Minn Consortium (Principal Investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657) funded by the 16 NIH Institutes and Centers that support the NIH Blueprint for Neuroscience Research, and by the McDonnell Center for Systems Neuroscience at Washington University. https://www.humanconnectome.org/study/hcp-young-adult/document/hcp-citations

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