



UvA-DARE (Digital Academic Repository)

A roadmap towards standardized neuroimaging approaches for human thalamic nuclei

Segobin, S.; Haast, R.A.M.; Kumar, V.J.; Lella, A.; Alkemade, A.; Bach Cuadra, M.; Barbeau, E.J.; Felician, O.; Pergola, G.; Pitel, A.-L.; Saranathan, M.; Tourdias, T.; Hornberger, M.

DOI

[10.1038/s41583-024-00867-1](https://doi.org/10.1038/s41583-024-00867-1)

Publication date

2024

Document Version

Final published version

Published in

Nature Reviews Neuroscience

License

Article 25fa Dutch Copyright Act (<https://www.openaccess.nl/en/policies/open-access-in-dutch-copyright-law-taverne-amendment>)

[Link to publication](#)

Citation for published version (APA):

Segobin, S., Haast, R. A. M., Kumar, V. J., Lella, A., Alkemade, A., Bach Cuadra, M., Barbeau, E. J., Felician, O., Pergola, G., Pitel, A.-L., Saranathan, M., Tourdias, T., & Hornberger, M. (2024). A roadmap towards standardized neuroimaging approaches for human thalamic nuclei. *Nature Reviews Neuroscience*, 25(12), 792-808. <https://doi.org/10.1038/s41583-024-00867-1>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

A roadmap towards standardized neuroimaging approaches for human thalamic nuclei

Shailendra Segobin¹✉, Roy A. M. Haast^{2,3}, Vinod Jangir Kumar⁴, Annalisa Lella⁵, Anneke Alkemade⁶, Meritxell Bach Cuadra^{7,8}, Emmanuel J. Barbeau⁹, Olivier Felician¹⁰, Giulio Pergola^{5,11,12}, Anne-Lise Pitel¹³, Manojkumar Saranathan¹⁴, Thomas Tourdias^{15,16} & Michael Hornberger¹⁷✉

Abstract

The thalamus has a key role in mediating cortical–subcortical interactions but is often neglected in neuroimaging studies, which mostly focus on changes in cortical structure and activity. One of the main reasons for the thalamus being overlooked is that the delineation of individual thalamic nuclei via neuroimaging remains controversial. Indeed, neuroimaging atlases vary substantially regarding which thalamic nuclei are included and how their delineations were established. Here, we review current and emerging methods for thalamic nuclei segmentation in neuroimaging data and consider the limitations of existing techniques in terms of their research and clinical applicability. We address these challenges by proposing a roadmap to improve thalamic nuclei segmentation in human neuroimaging and, in turn, harmonize research approaches and advance clinical applications. We believe that a collective effort is required to achieve this. We hope that this will ultimately lead to the thalamic nuclei being regarded as key brain regions in their own right and not (as often currently assumed) as simply a gateway between cortical and subcortical regions.

Sections

Introduction

Neuroimaging of human thalamic nuclei

Implications of segmentation challenges

Towards better thalamic nuclei segmentation

Conclusions

Introduction

The thalamus is known to play a key role in the interactions between the cortex and subcortical brain regions that underlie a range of brain functions, including motor, sensory, cognitive, behavioural and affective functions¹. It is made up of several nuclei that have unique characteristics in terms of their connectivity, functionality and specialization when compared to other cortical and subcortical brain areas and that are known to be important in health and disease¹.

Many recent neuroscientific studies have focused much effort on the evaluation of human brain regions with the aim of unravelling distinct roles for their subdivisions. This is the case not only for the thalamus but also the basal ganglia, the cerebellum and several cortical regions that were previously considered functional units and are now thought of as composite structures, which will be discussed below.

However, the concept of segmentation is particularly important for the thalamus for several reasons that relate to its anatomy and function. First, a crucial characteristic of the principal thalamic nuclei is that they are not connected with each other – with the exception of indirect connections mediated via the thalamic reticular nucleus – and are instead tightly connected to specific projection areas². This connectivity pattern is unlike that of the basal ganglia, which form a circuit by themselves and have common neurochemical properties. It is also unlike the cerebellum, in which the nuclei and cortex are functionally arrayed to coordinate complex operations. The connectivity of the thalamic nuclei suggests that they subservise multiple functions rather than being a cluster of interconnected nuclei underlying the same function, meaning that a clear delineation of thalamic nuclei will help us distinguish those functions.

A second unique feature of the thalamus is its burst and relay firing modes, which – due to the exclusive connectivity that it has with the cortex – enable the thalamus to rapidly switch functional activity between entire cortical networks². This feature is enhanced by the notable synaptic weight of glutamatergic thalamocortical synapses through which the thalamus can drive cortical neurons (unlike most other subcortical afferents, which instead employ neuromodulators)³. Thus, the mechanism by which thalamic nuclei modulate cortical activity is an important, if not unique, feature of these regions.

Finally, thalamic nuclei are highly specialized when compared to those in other brain regions: the lateral geniculate nuclei alone are made up of six subdivisions, while higher-order thalamic nuclei (such as the mediodorsal nuclei and the pulvinar) exhibit multiple cytoarchitectural structures across their different portions².

These unique thalamic features explain why the thalamus is challenging to study, why thalamic research requires substantial granularity and why the thalamus is of such clinical importance. Still, the delineation of thalamic nuclei remains controversial. Approximately 40 thalamic nuclei have been defined by histology in the human brain⁴ but current thalamic segmentation approaches only allow a subset of these to be reliably delineated in neuroimaging (Box 1). This limits our understanding of the thalamus and its function in health and disease, making comparisons of findings across studies (whether among human populations or between human and animal models) challenging. There is currently no consensus in the field as to which thalamic nuclei can be reliably imaged or how to exploit emerging techniques that can potentially allow more robust delineation of thalamic nuclei in humans.

To address and resolve the shortcomings of neuroimaging-based delineation and segmentation of human thalamic nuclei, we have formed a consortium, the Thalamus Nuclei Neuroimaging Group (**TANGO**). Herein, we briefly review the current challenges and opportunities in the segmentation of human thalamic nuclei via neuroimaging and illustrate

how research and clinical applications are currently limited by prevailing thalamic nuclei neuroimaging methods. Finally, we propose a Roadmap for the future of thalamic nuclei imaging that will allow for advances in our understanding of the role of this important brain structure.

Neuroimaging of human thalamic nuclei

Approaches to delineate human thalamic nuclei via the acquisition and segmentation of MRI data have had varying success over recent decades. The relaxation times and/or proton densities of different thalamic nuclei are very similar to each other when data is acquired at 3 T or 7 T field strengths^{5,6} (Fig. 1a). Even at very high field strengths, such as 9.4 T, thalamic nuclei delineation remains challenging, making their distinction via conventional T1-weighted (T1w) imaging and T2*-weighted imaging difficult⁷. A different approach uses specific acquisition sequences, such as white-matter-nulled (WMn) imaging to improve the delineation of thalamic nuclei⁵. Nulling the white matter improves the intra-thalamic contrast as well as overall thalamic contrast by removing the signal from the white-matter lamellae that surround the nuclei as well as those outside the thalamus, resulting in better delineation of the thalamic nuclei as well as the lateral borders of the thalamus. WMn acquisition allows better thalamic nuclei delineation even at 3 T and 7 T (Fig. 1b) but restrictions in spatial resolution still make the imaged voxels prone to partial volume effects and associated blurring⁶. Unsurprisingly, therefore, thalamic nuclei delineations from MRI data can yield low accuracy – especially for smaller thalamic nuclei – hindering the reliability of human thalamic neuroimaging findings.

Despite these limitations, MRI remains the preferred in vivo non-invasive method for imaging thalamic nuclei in humans. Recent developments in acquisition (Box 2 and Table 1) and segmentation show promise in bridging the gaps in contrast and resolution that exist between in vivo MRI data and data gathered from post-mortem and/or histological studies, which remain the gold standard⁸. The following sections give a brief overview of current and emerging approaches for the segmentation of human thalamic nuclei in MRI data and discuss their challenges and opportunities.

Structural MRI-based segmentation

Current available thalamic segmentation approaches for structural MRI can be broadly divided into those based on the co-registration of structural MRI data to histological atlases (Box 1), those involving the direct labelling of structural MRI data, and those in which there is a combination of histology with structural MRI data.

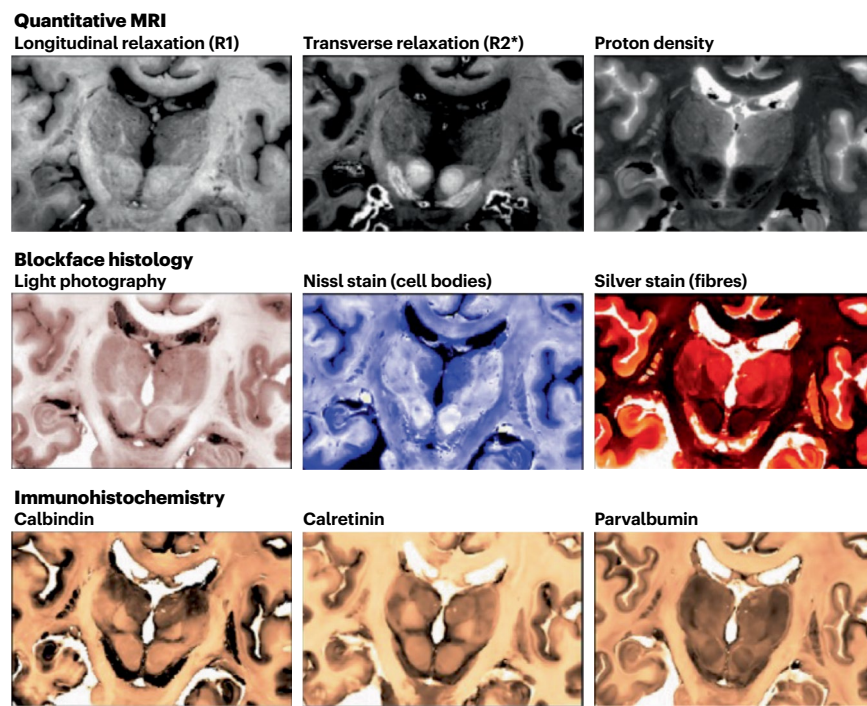
To date, most histology-based MRI segmentations of the thalamus are created using the Morel stereotactic human thalamic atlas^{4,8,9} (Fig. 2) and the Schaltenbrand atlas^{10–12}. Both atlases are in common usage but the accuracy of their spatial correspondence to the thalamus of a specific person largely depends on the accuracy with which they can be deformed to match their anatomy through a process of image registration (a complex mathematical operation that non-linearly warps the atlas to the specific person). Individual thalamic nuclei segmentations can show significant variability between people for structural MRI, diffusion MRI and functional MRI (fMRI)¹³ (Fig. 2). This means that probabilistic maps (maps in which a voxel is indexed as being part of a particular region when it exceeds a given statistical threshold, thus capturing the inter-person variability of the thalamic nuclear boundaries) across the different modalities often provide mere approximations of the positions and sizes of the nuclei, particularly the smaller ones (Fig. 2).

Volumetric quantification of thalamic nuclei using these histological atlases has contributed to many studies such as those analysing

Box 1 | Histological approaches for the delineation of thalamic nuclei

Post-mortem histological approaches are considered the ‘gold standard’ for assessing anatomical boundaries within the thalamus. The spatial resolution that can be achieved using microscopy is much greater than that possible using currently available MRI methods. However, histology-based atlases can be used in several different ways to help parcellate brain structures in MRI data.

Histology-based atlases of the human brain can serve as a reference that provides general information on the relative positions and sizes of individual structures and this can guide manual delineations of MRI data. However, the success of this labour-intensive approach is highly dependent on the visibility of the thalamic nuclei in the MRI images, which is often limited. One solution to this problem could be to register detailed histology-based information to MRI space. An example of such registration is shown in the figure, in which quantitative MRI images of a single post-mortem specimen acquired at a field strength of 7 T and with an isotropic resolution of 0.4 mm with different contrasts (longitudinal relaxation (R1), transverse relaxation (R2*) and proton density) are shown in the first row. The thalamic nuclei are more visible in the middle row of the figure, in which different histology stains of the post-mortem tissue are shown. Finally, the bottom row shows immunohistochemical staining with antibodies against parvalbumin, calretinin or calbindin for the same specimen. However, the co-registration of the histology-based information with the MRI data and subsequent validation of the underlying anatomy remains challenging. Alternatively, comparisons can be made between other atlases available in MRI space³⁰. Interestingly, some of the co-registration efforts even incorporate probabilistic information, in which statistical methods are used to estimate the likelihood of a certain image alignment. Instead of determining a single ‘best’ alignment, probabilistic approaches consider multiple possible alignments and assign probabilities to each based on image features and similarity metrics. This allows for a more robust and accurate co-registration, especially when



dealing with noisy or ambiguous image data. By incorporating uncertainty into the alignment process, probabilistic methods can help to avoid errors and improve the overall quality of the registered images²⁹.

Despite the benefits of combining histological information with MRI data, it remains difficult to assess the level of inter-person variations in the shape and volume of thalamic nuclei. Understanding these variations is crucial for classifying ‘normal brain anatomy’, disease detection and surgical planning. To resolve the challenges associated with the translation between histological and in vivo approaches, several methods have been developed to collect MRI data and histological information from the same specimens^{11,37,38,148–152}. The data sets created through these efforts can serve as a basis for the validation of existing co-registered atlases as well as future thalamus mapping efforts. Figure is adapted from ref. 38, CCBY 4.0.

the brain hubs involved in cognitive tasks in healthy controls^{44,15} or the involvement of thalamic nuclei in large-scale functional or nuclei-specific networks^{16,17}. The popularity of this approach is related to the embedding of these atlases into commonly used neuroimaging analysis pipelines. However, although these thalamic atlases are highly accessible to a large user base, they are not always well suited to the specific population scanned or the nuclei studied, and registration quality (in terms of goodness of fit between source and target data and the specific regions where the registrations fail) is not always reported. For example, although current registration algorithms show acceptable performance for young healthy brains, the accuracy of these methods decreases when applied to brains affected by age-related processes, pathophysiology or

surgical interventions, limiting their generalizability^{18,19}. Furthermore, the histological atlases were usually created from a single specimen (or an extremely limited number of specimens) that might not capture the inter-person variability of thalamic shapes and volumes. Finally, the tissues processed for histological analyses have substantial distortions, which need to be corrected via co-registration. The effects of these challenges are further exacerbated when moving from the higher resolution of histological data (in microns) to the lower resolution MRI space (typically 1 mm isotropic). In addition to these problems, there has been an evolution of the nomenclature for the human thalamus used in these histological atlases since the 1970s²⁰. This evolution can be attributed to several factors, including access to larger data sets (and hence reduced

inter-person variability) and technological advances in microscopic imaging, macroscopic imaging and co-registration algorithms. However, the inconsistent terminologies used across time hinder accurate mapping between the two modalities as the different naming conventions across studies and atlases can lead to misalignment and errors in identifying the loci of thalamic nuclei and, ultimately, make it difficult to establish reliable and comparable data analysis. Thus, although histology-based atlases are regarded as the gold standard for thalamic neuroimaging, they still cannot be easily generalized to MRI space.

A different segmentation approach involves directly labelling the structural MRI data by assessing several voxel-based image features (for example, (1) voxel intensities that are numerically similar and that tend to lie in close vicinity to each other can be grouped together, (2) gradients of voxel intensities defining potential boundaries or (3) anatomical landmarks that are distinct in their contrast among structures), which is more resilient to inter-person anatomical variations^{21–23}. Such MRI-based segmentation techniques seem to provide higher specificity for individual anatomies than segmentations driven solely by histological atlases. However, the underlying issue – that conventional structural MRI has limited intra-thalamic contrast – detracts from this argument. Specifically, the homogeneity of the relaxation times of different thalamic nuclei in data obtained using standard T1w imaging sequences hinders structural MRI data segmentation methods from providing reliable nuclei delineations. Moreover, the limited spatial resolution of T1w-MRI sequences limits the number of nuclei that can be segmented in vivo, even for more high-resolution MRI approaches²⁴. Thus, in theory, structural MRI thalamic segmentation methods might provide better segmentation but, in practice, they are often limited by the inaccuracy of structural MRI data.

A solution to overcome the issues associated with both of these methods is to combine them. Multimodal methods guide the structural MRI labelling process by using prior information from a single or multiple predefined atlases (either those based on histology or those based on manually defined labels)²⁵. The resulting probabilistic atlases allow for variability in shape and volume. For example, Bayesian inference techniques were used to overlay a mesh-based representation of an in-house-developed post-mortem thalamic atlas onto in vivo MRI data²⁶. This method has found wide usage since its inception, due to its implementation within the *FreeSurfer software suite*²⁷. Another combined approach, multi-atlas label fusion, leverages anatomical variability across individuals to improve thalamic segmentation within individuals²⁸ and has been implemented in the thalamus-optimized multi-atlas segmentation (*THOMAS*) atlas^{29,30}. However, the inherent contrast and resolution issues of standard T1w sequences still limit the number of segmented nuclei that can be identified via these methods compared to those identified via histology. To improve the delineation of nuclei boundaries, it has been proposed that images with optimized contrasts, such as those provided by white matter signal nulling^{5,31,32} or quantitative T1 mapping, should be used^{33,34}. Acquisition of quantitative parametric maps allows the generation of multiple intrinsically aligned MRI contrasts (that is, when the images are all reconstructed from the same raw data, they are inherently aligned for each and every voxel across all the images), and can provide additional contrast levels as the degree of difference in signal intensities among different tissues increases to reveal more details of the internal structure of the thalamus^{35,36}. For example, T1w maps can be used to generate both conventional and WMn data, providing two image contrasts that are perfectly registered as they are generated from a single parametric map. Histological preparations can provide even higher levels of anatomical detail, allowing visualization of individual thalamic structures that cannot be discerned on MRI. The co-registration of these

preparations with structural MRI data from the same individuals can facilitate co-registration of the histology results to MRI standard space^{37,38}.

Diffusion-based segmentation

Beyond anatomical landmarks and intensity differences, segmentation can also be achieved using diffusion-weighted MR imaging (DWI). DWI is based on measuring the microscopic motion of water molecules, which are restricted by the underlying microstructural anatomy of the brain. From these measurements, one can segment thalamic nuclei based on their microstructural properties or their structural connectivity with respect to their cortical projections.

Thalamic nuclei segmentation from DWI mostly involves unsupervised data-driven techniques that group data based on local diffusion properties (within the thalamus) or long-range connections through fibre tracking. As such, these techniques can be broadly categorized into three groups. The first group of techniques are based on the different local diffusion properties of the various nuclei such as the full diffusion tensor^{39–42}, the principal diffusion directions^{43–46} or the distribution of diffusion orientations^{47,48}. The second group of techniques measures the global diffusion-based connectivity between the different nuclei and the rest of the brain and is thus based on structural connectivity. These techniques can be tractography methods, which estimate the long-distance projections of each nucleus to the cortex^{49,50}, or super-resolution track density-based methods^{51,52}. Finally, the third group of techniques use a hybrid approach, combining local and global diffusion properties^{53,54}.

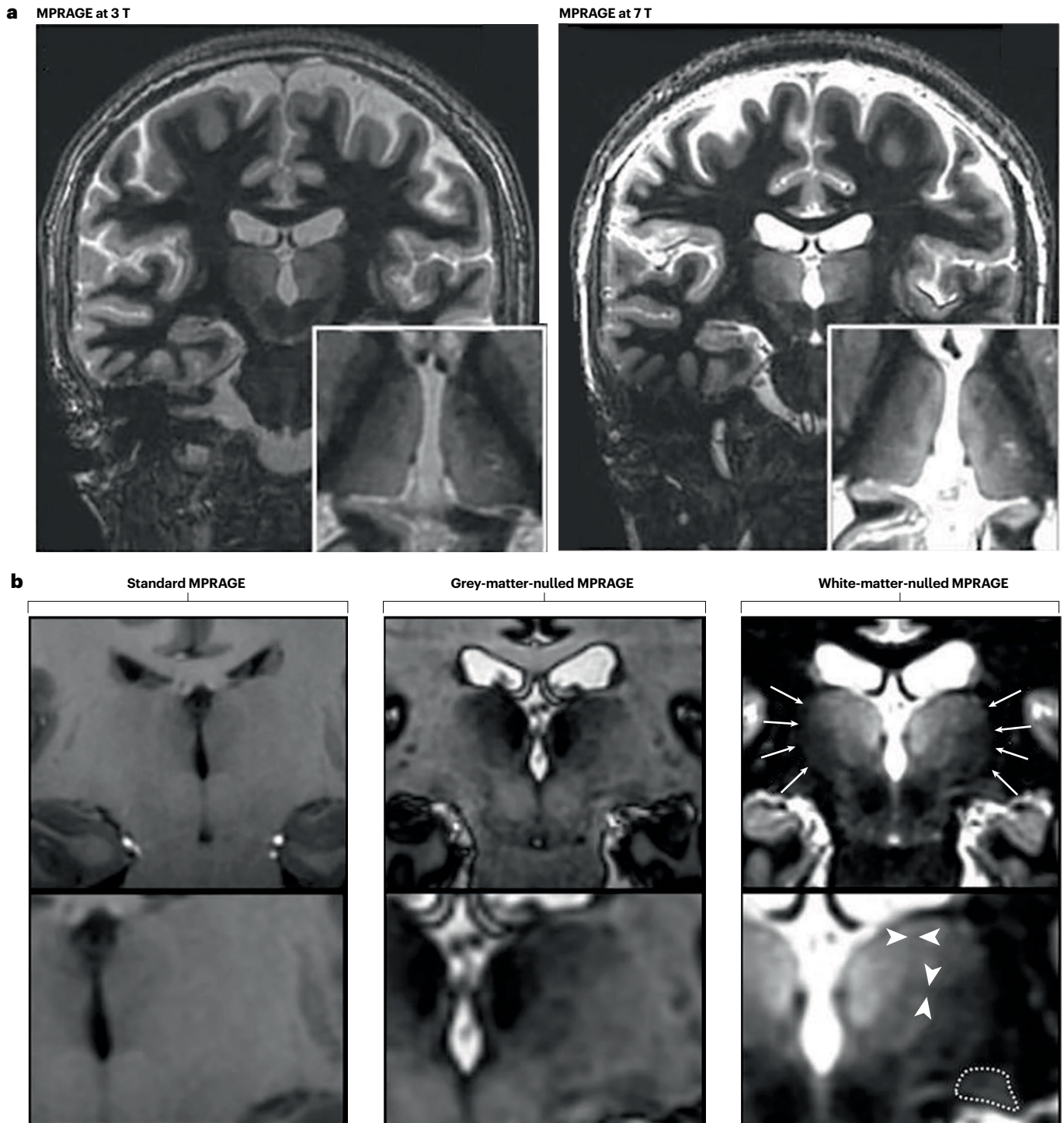
Although in vivo diffusion-based MRI segmentation allows for a tailored person-specific non-invasive analysis of the thalamus, it tends to produce inconsistent mappings of nuclei, largely due to the researcher's subjective choice of the number of nuclei or clusters. Unlike other MRI acquisition sequences (such as T1w imaging, T2-weighted imaging or susceptibility-based imaging), DWI is not currently capable of exploiting the potential signal enhancements achievable at ultra-high field strengths such as 7 T (ref. 55).

Despite providing insight into microstructural thalamic changes and the relationship of thalamic nuclei to cortical regions, DWI has an overall relatively low spatial resolution (typically 2–3 mm isotropic) and some complex fibre crossing configurations might be difficult to image^{56,57}. It is thus currently unclear to what extent corticothalamic and thalamocortical projections form bundles that are consistent enough to be captured by DWI, particularly within the cortex. DWI is also more susceptible to image distortion due to factors like eddy currents and susceptibility artefacts, compared to the anatomical approaches discussed above. As a result, only a few nuclei, often the larger ones, can be precisely identified via DWI (Fig. 2) and these techniques offer only a coarse anatomical view for now⁴⁷. In the future, track density-based techniques^{52,58}, which focus on fibre density estimation instead of directionality, might overcome some of these limitations as they can reach a histological level of nuclei identification. However, these techniques require high-quality data and rely on more advanced acquisition schemes such as multi-shell DWI. Finally, the accuracy of clinical DWI data remains to be evaluated, particularly since global structural connectivity analyses are constrained by brain lesions, which can compromise tractography results⁵⁹.

fMRI-based segmentation

The final major approach to delineating thalamic nuclei is based on the use of resting-state fMRI (rs-fMRI) to either examine the functional relationships of cortical areas with the thalamus^{16,60} or to segregate

Roadmap



thalamic subdivisions by conducting an independent component analysis (ICA) of thalamocortical connectivity maps^{61–64}. Both approaches primarily focus on segmenting the thalamus based on correlations in the resting-state activity of the thalamus and cortex.

In the first of these approaches, temporally coherent spontaneous fluctuations of the blood oxygen level-dependent (BOLD) signal are

assessed using a ‘winner-takes-it-all’ approach⁶⁵: each thalamic voxel is labelled based on its strongest correlation with a specific cortical region, also termed a ‘seed’, that ‘wins’ over other possible correlations. It thus becomes feasible to group similarly labelled voxels, resulting in the formation of thalamic maps that reveal the spatial arrangement of thalamic voxels based on their functional connectivity to different

Fig. 1 | Visualization of thalamic nuclei with differing field strengths and MRI pulse sequences, highlighting current segmentation challenges.

a, White-matter-nulled magnetization-prepared rapid gradient echo (MPRAGE) images acquired at 3 T and 7 T field strengths. When the white-matter signal is suppressed, delineation of several nuclei becomes easier, as indicated in the insets. While the gain in resolution at 7 T looks modest when compared to data at 3 T for the same sequence, the acquisition time is highly reduced at this field strength, thus minimizing motion artefacts, while the signal-to-noise ratio is increased²³. **b**, Images of the thalamus obtained at 1-mm³ resolution at 3 T using three different MPRAGE acquisition sequences: standard MPRAGE (left), grey-matter-nulled (middle) and white-matter-nulled (right). The poor contrast of

the standard MPRAGE 3 T image is because the tissue within thalamic nuclei have similar relaxation times, offering poor distinction of the boundaries between the nuclei and even between the nuclei and the white-matter tissues that surround the thalamus. The grey-matter-nulled sequence shows somewhat better delineation of thalamic nuclei but boundary definitions between the nuclei remain challenging. However, the white-matter-nulled sequence shows clear delineation of the thalamus from the surrounding white matter (indicated by the white arrows) as well as the boundaries between thalamic nuclei (white arrowheads in the higher magnification inset). There is also better delineation of the lateral geniculate nucleus (white dashed line)²⁵. Part **a** is reprinted with permission from ref. 31, Wiley. Part **b** is adapted with permission from ref. 5, Elsevier.

cortical regions^{16,65–67}. However, the functional thalamic subdivisions that are identified using this approach do not correspond to the anatomical thalamic subdivisions identified in histological or probabilistic structural MRI atlases¹³ (Fig. 2). This discrepancy raises intriguing questions: are the traditionally defined anatomical boundaries less pertinent when considering the dynamic functional roles of the thalamus? Alternatively, could the methods used to derive functional subdivisions be capturing a different dimension of thalamic organization, one not strictly tied to its cytoarchitectural structure? Another major drawback of this approach is that the cortical regions assessed are commonly defined based on their cytoarchitecture and/or structural anatomy, which may not correspond to the functional domains of the brain. To address this limitation, some approaches instead depict the highest correlations between each thalamic voxel and resting-state functional networks¹⁶. Thus, it remains unclear whether the histologically defined templates reflect the functional properties of corticothalamic connectivity because most thalamic nuclei exhibit dense internal connectivity and might potentially be divided into more distinct functional areas or smaller subdomains⁶⁸.

The ICA-based method, which focuses on the identification of similar BOLD signal patterns within the thalamus rather than associations with predefined cortical areas, provides an alternative approach to thalamic parcellation⁶⁹. When comparing the functional segmentation achieved via the region or seed-based analysis and the ICA approach, whole-brain analyses resulted in comparable group-level thalamic subdivisions^{70,71}.

A quantitative assessment of the spatial overlaps between fMRI-based segmentation and a structurally defined thalamic atlas revealed significant differences for both methods between functional imaging results and post-mortem histology⁶² (but see ref. 68). Indeed, the structural and functional thalamocortical connectivity maps both vary considerably, suggesting that the underlying brain communication architecture and its functional use during the resting state is complex and requires further examination. rs-fMRI thalamus parcellation methods also do not take into account the dynamics of the fMRI signal, which exhibits heterogeneous timing properties across individual thalamic nuclei, resulting in highly structured thalamocortical network dynamics⁷². Ultra-high field fMRI might be needed to identify these temporal dynamics and to provide sufficient granularity to capture the detailed interactions within and between individual thalamic nuclei. Finally, changes for each specific thalamic nucleus cannot be currently observed using these techniques.

Emerging methods

Although most of the thalamic nuclei segmentation methods developed to date use a single MRI acquisition technique (T1w, diffusion

MRI or rs-fMRI), emerging methods exploit the use of multiple MRI contrasts, leveraging the complementary benefits of each MRI acquisition technique. For example, incorporating DWI information into the T1w-MRI-based segmentation has been proposed to better delineate boundaries⁷³. Similarly, there have been approaches to incorporate T1w, T2-weighted and WMn magnetization-prepared rapid gradient echo (MPRAGE) data into diffusion-based scalar maps (such as those based on mean diffusivity, fractional anisotropy or fibre orientation) to create synthetic images that are all resampled (that is, their spatial resolution or orientation has been altered so that the dimensions and coordinate system of the different image types are matched) and registered to the T1w-MRI data⁷⁴.

In terms of data-driven methods, deep learning-based methods are nowadays ubiquitous in any field, including MRI segmentation. The first deep learning approach for thalamic nuclei segmentation used a type of neural network known as a residual U-net, which was trained using manually segmented 3 T and 7 T WMn MPRAGE data sets⁷⁵. A similar network was trained using jointly acquired WMn and standard MPRAGE data and used for segmentation of 7 T T1w data⁷⁶. Another approach used a cascaded synthesis-segmentation scheme to synthesize WMn MPRAGE from standard T1w data (thus providing improved thalamic contrast) before segmentation⁷⁶. Finally, a 3D U-net-based architecture that uses T1, fractional anisotropy and Knutsson edge maps (the latter two of these data sets being calculated from diffusion tensor imaging (DTI) data) was used to segment thalamic nuclei⁷⁷. However, although these deep learning-based approaches show promise, challenges remain, including the need for large data sets and/or annotations for training and the well-known lack of generalizability to out-of-domain cases such as data obtained using different field strengths, different pathological conditions or sequence settings used by scanner manufacturers. These are areas of active research in the general machine learning community.

The combination of information obtained using different MRI acquisition sequences can be difficult given the vastly different spatial resolutions of T1w-MRI, DTI and rs-fMRI as well as the discrepancies between structural and functional boundaries. However, improvements in technology, including ultra-high-field imaging and multi-band echo-planar imaging⁷⁸, will likely harmonize these methods in the near future.

Implications of segmentation challenges

The difficulties in using MRI to segment thalamic nuclei currently limit its clinical and research applications. Indeed, many of the segmentation methods described above have been mainly developed in healthy, young adult volunteers. They face additional challenges when transposed to developmental or ageing cohorts or to specific

Box 2 | Acquisition approaches for thalamic nuclei

How well thalamic nuclei can be segmented via MRI is affected by the acquisition sequences that are chosen (that is, the set of radiofrequency pulses and magnetic field gradients applied to generate an image). Different sequences emphasize different tissue properties and careful selection of a sequence can substantially impact the visibility and delineation of thalamic nuclei. MRI can be classified into three broad categories: anatomical and/or structural MRI, diffusion MRI (dMRI) and functional imaging. Anatomical imaging commonly involves T1-weighted (T1w) imaging, T2-weighted imaging and susceptibility-weighted imaging. dMRI measures the average diffusion of water molecules, which indirectly probes the structure of the biological tissue at a scale that is much smaller than the resolution of conventional MRI (typically 2–3 mm). Functional MRI (fMRI) measures the change in signal produced by deoxygenated haemoglobin — the so-called blood oxygen level-dependent effect — and can be employed in cognitive and/or behavioural tasks or as resting-state fMRI.

The sensitivity of T1 relaxation times to myelin content and the presence of differing myelin content across thalamic nuclei have been exploited to enable thalamic nuclei segmentation via T1w-MRI⁴³. Diffusion-based methods, which explore microstructure at a local scale (by examining the anisotropy behaviour of the diffusion tensor, for example) or a global scale (by using tractography to assess the structural connectivity between different cortical regions and the thalamus, for example), have also been used for the segmentation of thalamic nuclei or (more coarsely but more reliably) subdivisions of clustered thalamic nuclei⁴⁹. Similarly, cortical connectivity-based parcellation has been performed using resting-state fMRI data⁶⁷. Despite the capacity of T1w-MRI to determine the myelin content of nuclei and its high spatial resolution (1 mm isotropic), this method has generally been suboptimal for delineating intra-thalamic and internal capsule boundaries. Both dMRI and fMRI are acquired using the echo-planar imaging sequence, which is limited in spatial resolution and subject to distortion (especially in areas of increased magnetic susceptibility such as the temporal lobes and areas bordering the sinuses).

The field strengths of MRI scanners can also make a notable difference in thalamic nuclei acquisition and subsequent segmentation approaches. Most research and clinical MRI scanners have a field strength of 1.5 T or 3 T, with 7 T MRI scanners only

being found in highly specialized research centres. 7 T MRI can afford a significantly higher signal-to-noise ratio (approximately twice that of 3 T MRI) but this comes at the cost of increased tissue heating (with a specific absorption rate that is four times that of 3 T MRI) and dielectric artefacts, which increase inhomogeneity. Furthermore, 7 T MRI is more susceptible to variations in the main magnetic field, B₀, at air–tissue interfaces, leading to artefacts in the image and higher signal loss. These artefacts arise when the small magnetic fields created by tissues interact with B₀ and lead to fluctuations in the latter. The higher the magnetic field, the more susceptible are the tissues to cause such fluctuations. Nevertheless, 7 T MRI has been used for many specific applications, including susceptibility-weighted imaging and quantitative susceptibility mapping¹⁵³, in which its increased susceptibility can be leveraged. Such applications enhance thalamic nuclei segmentation¹⁵⁴ based on manual delineation such as the identification of motor thalamic nuclei for deep brain stimulation. Another advantage of 7 T MRI over 3 T MRI is that it allows higher acceleration factors (a measure of how much faster the scan is compared to a conventional scan) in parallel imaging¹⁵⁵ (an imaging technique used to speed up MRI scans by using less data points within an array of receiver coils), although these are accompanied by higher geometry (g)-factors (a measure of image quality loss associated with parallel imaging). Thus, when combined with its higher signal-to-noise ratio, 7 T MRI enables substantial reductions in scan times or improved spatial resolution (0.8 mm isotropic or better) with comparable scan times, when compared to 3 T MRI. To overcome shading artefacts (dark shades that appear on an MRI image due to an uneven signal intensity across the image and are often caused by non-uniformity in the radiofrequency field during image acquisition), a variant of magnetization-prepared rapid gradient echo (MPRAGE) that uses two inversion preparation segments (magnetization-prepared 2 rapid acquisition gradient echoes; MP2RAGE)¹⁵⁶ has been introduced. In this approach, the final T1w image is a ratio image, resulting in cancellation of shading artefacts. The same acquisition sequence can also be used to generate T1 maps, from which newer contrasts, such as white-matter-nulled MPRAGE³⁴, can be synthesized. Lastly, variants of T1w-MRI, such as white-matter-nulled MPRAGE⁵, have been demonstrated to provide optimal intra-thalamic contrast at 7 T, with clear delineation of the lateral borders of the thalamus.

pathological conditions. Even across healthy ageing, there are substantial structural changes within the thalamus and neighbouring regions that might affect segmentation⁷⁹. Furthermore, it is unclear how pathological changes to the thalamus, such as thalamic infarcts due to stroke (Fig. 3a), neurodegeneration (Fig. 3b) and alcohol use disorders (Fig. 3c), affect its segmentation. In the sections below, we illustrate some situations for which having information at the thalamic nucleus level could be useful but for which current methodological shortcomings limit reliable outcomes.

Thalamic imaging during and across the life course

Development. It is likely that neurodevelopmental conditions affect the thalamus but the extent to which this is the case and which nuclei

are involved remain poorly understood because we do not have a good understanding of the changes in the thalamus during healthy development and because of the associated segmentation challenges^{80,81}. Most thalamic developmental studies use manual segmentation of the thalamus and its nuclei⁸² given that current segmentation algorithms were not developed for infants or children. Approaches to standardize the segmentation of the thalamus in children have thus far been unsuccessful⁸³. In particular, it is difficult to determine which age to use for the standardization as thalamic volumetrics differ significantly between infants and teenagers⁸⁴. Age range-specific standardization protocols⁸⁵ are therefore required but do not currently exist. A further complication is the fact that MRI scans from children are known to vary in contrast intensity across ages, which creates a challenge

for segmentation algorithms⁸⁶. Finally, paediatric scans are often of comparatively lower resolution than adult scans, increasing the partial volume effects that challenge segmentation approaches when standard structural acquisition sequences are applied⁸⁷. Thus, developing better thalamic segmentation for scans of children is crucial to improve both developmental studies and our understanding of paediatric conditions involving the thalamus.

Ageing. Previous meta-analyses have shown that overall thalamic volume decreases with age⁸⁸. However, the exact relationship between this overall volume decrease and changes in specific thalamic nuclei is yet to be determined. Several studies have measured the differential effects of ageing on the volumes of individual nuclei with atlas-based parcellation based on WMn MPRAGE^{29,89} or standard T1w acquisition sequences⁹⁰. While some studies indicated that thalamic nuclei involved in cognitive, visual, and auditory or vestibular functions presented atrophy at higher rates than those associated with motor and somatosensory functions⁸⁹, others demonstrated a somewhat inverse pattern, with higher atrophy

in nuclei involved in motor functions and in the pulvinar⁹⁰. Such contradictory results highlight, once again, the need for age-adjusted thalamic segmentation approaches. Thus, it will be important to clarify which current thalamic segmentation approaches are most appropriate for ageing cohorts and for future developments in segmentation algorithms to take ageing into account.

Thalamic lesions in clinical populations

Intra-thalamic alterations. Although isolated thalamic stroke lesions could represent an ideal means to study the role of the thalamus and of specific thalamic nuclei in cognitive functions^{91–93}, the use of neuroimaging to determine which nuclei are injured after a vascular lesion faces several issues. First, morphological changes in the thalamus induced by a stroke challenge thalamic segmentation as the lesions can shift the thalamic region that is used as a reference in the corresponding atlas. To our knowledge, none of the existing thalamic segmentation approaches takes this into account, even though it clearly affects the localization of the lesion and its corresponding

Table 1 | Summary of acquisition methodologies and processing software for thalamic nuclei segmentation

Neuroimaging class	Acquisition sequence	Spatial resolution	Contrast property	Processing methods	Atlas	Software
Structural MRI at 3T	MPRAGE and variants 3D multi-echo Gradient echo	0.8–1mm ³	Myelin content Iron content	Bayesian probabilistic atlas	Probabilistic atlas of human thalamic nuclei ²⁶	FreeSurfer
				Multi-atlas segmentation	THOMAS ²⁹	THOMAS
				Manual segmentation	Modified segmentation procedure ¹⁴²	HIPS-THOMAS
				Quantitative susceptibility mapping	Multimodal platform for deep brain stimulation surgery ¹⁴³	Lead-DBS
Structural MRI at 7T	MP2RAGE 3D multi-echo Gradient echo	0.5–0.7mm ³	Myelin content Iron content	Bayesian probabilistic atlas Multi-atlas segmentation Manual segmentation Quantitative susceptibility mapping	THOMAS ²⁹	THOMAS
Diffusion MRI	Echo-planar imaging	2.0–2.4mm ³	Orientation of diffusion tensor Orientation of diffusion tensor plus cortical connectivity	Local: k-means clustering of principal eigenvector or ODF coefficients	Diffusion MRI-based thalamic atlas ⁴⁷	Zenodo
				Global: connectivity-based parcellation (tractography)	Thalamic connectivity maps ⁴⁹	FMRIB Software Library
Functional MRI	Echo-planar imaging	2–3mm ³	BOLD plus cortical connectivity	Connectivity-based parcellation Instantaneous connectivity parcellation	Human Brainnetome Atlas ¹⁴⁴	Human Brainnetome Atlas
Multiple contrasts	MPRAGE Multimodal MP2RAGE EPI Proton density	Various	Mixed	Bayesian probabilistic atlas	Joint segmentation from T1 and diffusion tensor imaging ⁷³	FreeSurfer
				Deep learning synthesis	Convolutional neural network-based thalamic segmentation ⁷⁶	CNN-THOMAS
				Polynomial synthesis	Thalamic segmentation by polynomial intensity transformation ¹⁴²	HIPS-THOMAS
					Multi-contrast human brain atlas ¹³⁷	Human Brain Atlas

BOLD, blood oxygen level-dependent; EPI, echo-planar imaging; HIPS, histogram-based polynomial synthesis; Lead-DBS, lead-deep brain stimulation – a toolbox for facilitating deep brain stimulation electrode reconstruction and computer simulation based on postoperative MRI and CT imaging; MPRAGE, magnetization-prepared rapid gradient echo imaging; MP2RAGE, magnetization-prepared 2 rapid acquisition gradient echoes; ODF, orientation distribution function; THOMAS, thalamus-optimized multi-atlas segmentation.

a Axial view

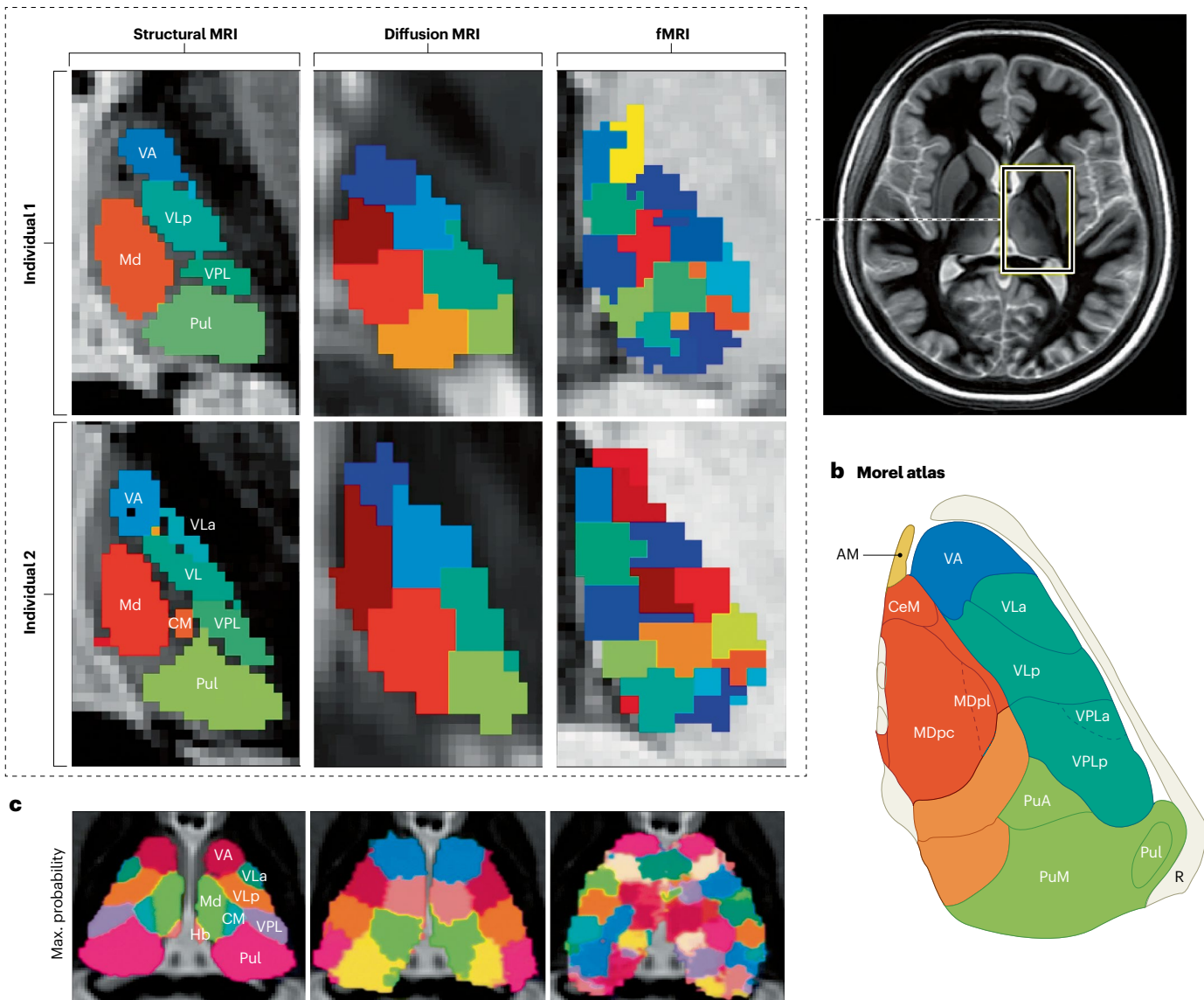


Fig. 2 | Variability in shapes and volumes of thalamic parcellations across individuals and imaging methods. The figure illustrates the variability in the shapes and volumes of thalamic nuclei that have been observed across individuals and through the use of distinct MRI data acquisition and segmentation techniques. **a**, Similarities and differences in the segregation of thalamic nuclei in data obtained from structural, diffusion or functional MRI (fMRI) in two individuals. The structural MRI data were segmented using thalamus-optimized multi-atlas segmentation²⁹, the diffusion MRI data were segmented using the orientation distribution function method⁴⁶ and the fMRI data were segmented via functional parcellation using time courses of instantaneous connectivity⁴⁵. **b**, A schematic illustration of a section from the Morel histology-based atlas is also shown for comparison. **c**, Probabilistic maps that account for the shapes and volumes of the thalamic nuclei across

individuals. Max. probability refers to the highest likelihood that the indexed nucleus will be within that voxel. These maps also reflect the high variability in parcellations output based on image acquisition and segmentation methods used. AM, anterior medial nucleus; CeM, central medial nucleus; CM, centre median nucleus; Hb, habenular nucleus; Md, mediodorsal nucleus; MDpc, mediodorsal nucleus parvocellular part; MDpl, mediodorsal paralamellar; PuA, anterior pulvinar; PuL, inferior pulvinar; PuM, medial pulvinar; R, reticular; VA, ventral anterior nucleus; VLa, ventral lateral nucleus; VLa, ventral lateral anterior nucleus; VLP, ventral lateral posterior nucleus; VPLa, ventral posterior lateral nucleus anterior part; VPLp, ventral posterior lateral nucleus posterior part. Parts **a** and **c** are adapted from ref. 13, Springer Nature Limited. Part **b** is adapted from ref. 47, Springer Nature Limited.

symptomatology. One proposed solution to this problem is to use the contralateral thalamus as a reference⁹⁴. However, the commonly voiced (yet poorly documented) intra-person morphological

variability between the two thalami (in terms of shape and location or size of thalamic nuclei), means that the utility of this approach remains undemonstrated. Second, the precise location of the nuclei,

along with their vascularization pattern⁹⁵, can have large inter-person variability, which can make group lesion mapping studies very challenging. Third, the mammillothalamic tract runs through the thalamus towards the anterior thalamic nuclei and could therefore be a useful anatomical landmark; however, vascular lesions of the thalamus may damage this small tract⁹⁶ (although this has not been consistently examined⁹⁷).

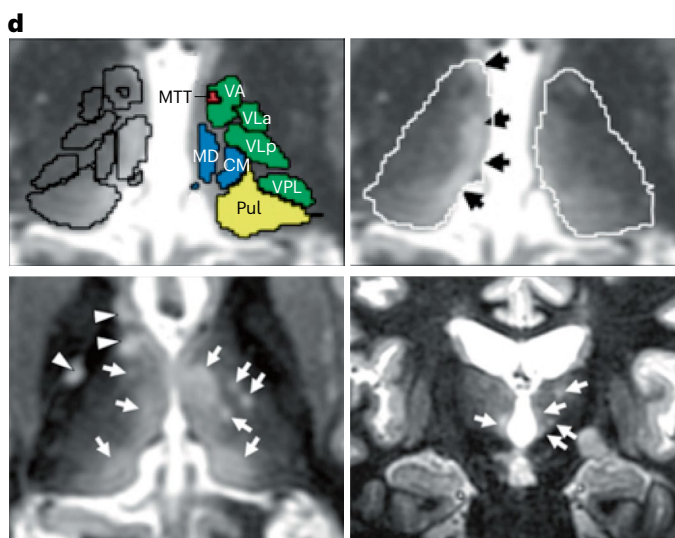
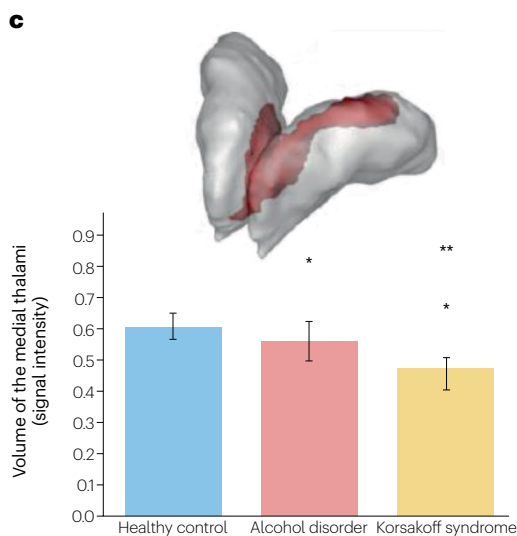
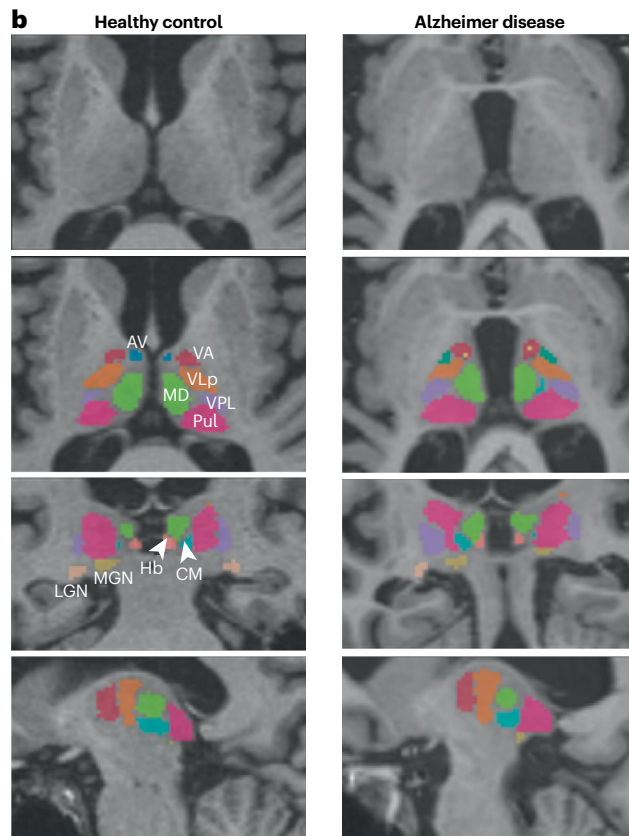
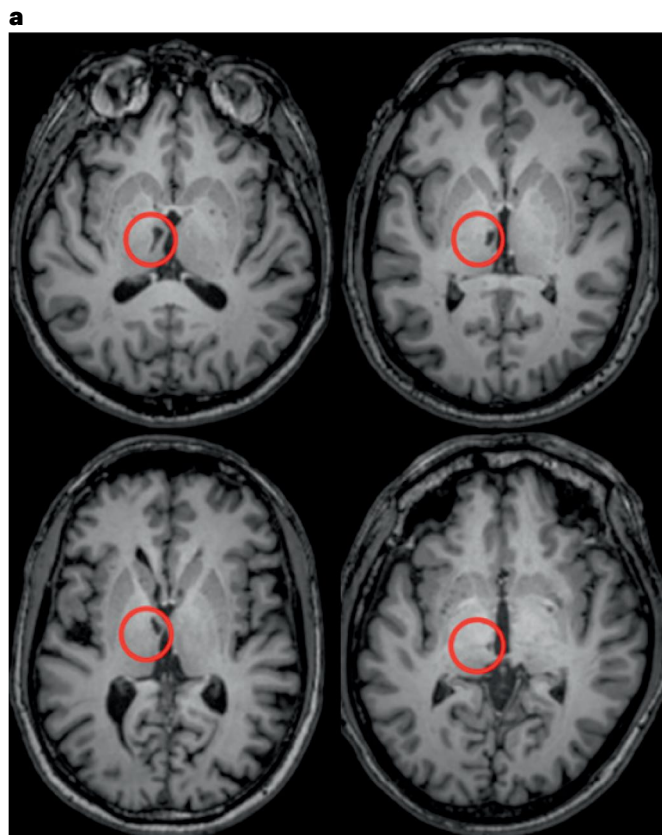
Conditions that result in severe thalamic atrophy can also bias currently available thalamic segmentation methods. At the moment, it is not clear how to deal with such scenarios, and researchers and clinicians often have to pick their segmentation approaches and neuroimaging atlases without objective performance measures. This increases the risk that tools will be selected based on their capacity to replicate previous results, with the inherent dangers of circular reasoning and confirmation bias. One potential way to reduce this bias is to use existing neuropathological findings as a 'ground truth' to guide the researcher or clinician in their thalamic nuclei segmentation approach. The measures are taken *ex vivo* and the tissues are physically quantified, hence diverging from using the 'same toolbox' for the 'same population' *in vivo*, where physical measurements are effectively impossible. For example, in Korsakoff syndrome, which most frequently occurs in patients with alcohol use disorder, post-mortem studies systematically report mediodorsal and anterior nuclei being affected⁹⁸. A study using a DTI-based thalamic segmentation replicated these neuropathological findings *in vivo* in Korsakoff syndrome⁹⁹, providing confidence in the reliability of the chosen method, but whether other approaches could also replicate the findings was not tested. A similar reasoning can be applied in Alzheimer disease research, where neurofibrillary tangles are found in the anterior thalamus early in the disease course according to neuropathological data¹⁰⁰. *In vivo*, atlas-based segmentation studies have replicated this finding^{26,101,102}. However, in contrast to the neuropathological data, these studies have also shown mediodorsal and medial geniculate thalamic nuclei changes.

Even thalamic microscopic abnormalities, such as inflammatory changes, can affect the thalamic nuclei differentially. For example, the thalamus is particularly vulnerable to the pathophysiological mechanisms related to multiple sclerosis¹⁰³, and inflammation changes due to multiple sclerosis can affect thalamus integrity. However, it is currently not clear whether inflammation changes the tissue properties of the thalamic nuclei (and, therefore, their radiological signatures), and there have been some expected difficulties in clustering-based segmentation approaches. There might also be some focal inflammatory lesions within the thalamus of individuals with multiple sclerosis¹⁰⁴ and the impact of these on the accuracy of the anatomical-based segmentations is undefined (as discussed above for ischaemic lesions). Microstructural changes have also been revealed by DWI within the thalamus in psychiatric disorders, such as early psychosis and chronic schizophrenia, revealing both widespread diffusion-related alterations across the whole thalamus in early psychosis and heterogeneous anomalies particularly in the mediodorsal and posterior thalamus¹⁰⁵. Overall, the effect of macrostructural and microstructural changes on thalamic nuclei is still far from clear, as is the relationship between the underlying pathophysiology and the segmentation.

Extra-thalamic modifications. The interruption of thalamocortical projections via anterograde or retrograde degeneration¹⁰⁶ can also affect thalamic segmentation. For example, white matter lesions in

multiple sclerosis can affect not only thalamic nuclei but also their cortical connections¹⁰⁷, thereby impacting thalamic segmentation methods that use structural or functional thalamocortical connectivity. These methods might therefore be unreliable in conditions disrupting white matter integrity, such as multiple sclerosis and other neurodegenerative conditions. This challenge is also likely to apply to conditions associated with focal lesions that interrupt thalamic projections such as stroke. The thalamus can indeed be indirectly damaged by secondary degeneration resulting from infarcts involving the more direct (mammillothalamic tract) or even indirect (cortical) projections from lesion sites^{108,109}. For many years, such remote effects have been captured *in vivo* for the whole thalamus¹¹⁰, but it is only recently that the remote effects of stroke on specific thalamic nuclei have begun to be explored¹⁰⁸. It is also interesting to note that the disconnection of specific thalamic projections has been associated with focal iron accumulation in the affected nuclei, which is apparent in susceptibility-weighted imaging MRI and quantitative susceptibility mapping, and is expected to complicate some anatomical-based segmentation methods. Iron levels can also increase diffusely within the thalamus in several neurodegenerative conditions, resulting in a similar challenge for anatomical-based segmentations^{108,111}. How this affects thalamic segmentation approaches remains largely unknown despite its clear relevance for symptom management and treatment of patients.

Ventricular enlargement is a common landmark of ageing and can be exacerbated by pathophysiological changes affecting the cerebrospinal fluid or adjacent tissues. Enlarged lateral or third ventricles can potentially affect the shape of the thalamus and, hence, hinder accurate thalamic segmentation processes²⁰. Such ventricular enlargement could impact the medial and dorsal thalamic nuclei disproportionately due to their proximity to the ventricles. For example, a higher rate of atrophy in the posterior and medial thalamic nucleus groups that are in direct contact with the cerebrospinal fluid has been reported in multiple sclerosis, while there is a relative sparing of the more lateral groups¹¹². This could also explain the 'ependymal-in' gradient of thalamic damage related to meningeal inflammation that has been reported, which has a greater impact on thalamic nuclei in contact with the third ventricle¹¹³. However, the possible contribution of any errors when measuring nuclei adjacent to the ventricles (due to their changing morphology) to these findings is unknown. Similarly, global atrophy in Alzheimer disease significantly enlarges the lateral ventricles, which can prevent reliable thalamic nuclei segmentation and delineation¹¹⁴. Interestingly, for prodromal Alzheimer disease or cohorts at genetic risk of Alzheimer disease, the findings seem to be more consistent^{101,102}, suggesting that, indeed, ventricular enlargement in Alzheimer disease can impact on thalamic segmentation. The lateral ventricles are also enlarged in individuals with normal pressure hydrocephalus, which causes a swelling of the ventricular system¹¹⁵. Ventricular enlargement also ranks among the most replicated findings for schizophrenia^{116,117}. At the same time, there is burgeoning literature showing thalamic nuclei changes in people with schizophrenia^{118,119} and sometimes in their unaffected first-degree relatives^{120,121}, suggesting that there may be a genetic component to thalamic changes. However, due to the ventricular enlargement (which may increase with disease progression), a consensus is still lacking as to how to separate thalamic changes from ventricular changes in schizophrenia^{122–125}. Overall, this brief overview shows that changes to adjacent ventricles can impact thalamic shape and bias clinical inferences drawn from imaging of thalamic nuclei.



Future thalamic nuclei targets

Most thalamic imaging studies are currently conducted on the subset of nuclei that are the easiest to delineate via neuroimaging atlases. This means that there are many thalamic nuclei currently ‘missing’ or neglected that might have a relevance in health and disease. For example, the adhesio interthalamica is not part of any neuroimaging atlases, even though it connects thalamic hemispheres in approximately 70–80% of people and has a potential compensatory role after

lesions^{126,127}. Along the same lines, the midline thalamic nuclei, including the reuniens and rhomboid nuclei, are extensively investigated in animal studies for their contribution to memory¹²⁸ but inter-species homologies are still undefined for human studies. Furthermore, the central lateral nucleus is a very thin band-like nucleus at the interface between medial and lateral nuclei that acts as a central node within fronto-striatal networks. It has been identified as a promising target for deep brain stimulation (DBS) to improve executive function and quality

Fig. 3 | Alterations to thalamic nuclei across healthy ageing and disease.

a, A series of axial T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) scans of thalamic strokes in the left hemisphere that affect the mediodorsal nucleus (red circles indicate the lesion location)^{96,146}. These images indicate the variability of lesion location across patients and show that the lesions also potentially affect neighbouring nuclei. **b**, Thalamic atrophy in Alzheimer disease, as shown in T1-weighted scans¹⁰¹. The left column of images shows the thalamus of a healthy older person with different nuclei highlighted. The right column of images shows thalamic changes in a person with Alzheimer disease. Note how the overall brain atrophy but also the atrophy of specific thalamic nuclei can affect delineation of the thalamic nuclei. **c**, Changes in the medial thalamus in control individuals, individuals with alcohol disorder and individuals with Korsakoff syndrome. Both alcohol disorder and Korsakoff syndrome result in changes in the volume of the medial thalamus; however, the level of severity is larger in Korsakoff syndrome. This is shown in the 3D rendering of the thalamus of a healthy control individual, with the red overlay depicting the corresponding cluster of voxels for which volume in the Korsakoff syndrome group is significantly smaller than that in the alcohol disorder group (based on a voxel-based morphometry full-factorial analysis (ANCOVA equivalent))¹⁴⁷. The graph shows the comparison of grey matter volumes in this cluster of voxels for control versus alcohol disorder and Korsakoff syndrome groups (measured by

calculating the mean grey matter signal intensity within the red cluster, with Tukey post hoc comparisons). *, significant with respect to healthy controls; **, significant with respect to the alcohol disorder group; both Tukey, $P < 0.05$. **d**, Thalamic changes in multiple sclerosis as shown in white-matter-nulled MPRAGE scans at 7 T (ref. 104). The top row shows the delineation of thalamic nuclei in an individual with multiple sclerosis, with black arrows indicating multiple sclerosis lesions that appear as bands along the medial surface of the thalamus and project within the pulvinar (Pul), mediodorsal nucleus (MD) and ventral anterior nucleus (VA), based on overlap with the segmentation. The right column shows the scan of the thalamus in one individual with multiple sclerosis (axial and coronal slice), showing that white-matter-nulled MPRAGE can highlight several thalamic multiple sclerosis lesions that are either ovoid (indicated by white arrowheads) or more diffuse along the surface of the ventricle (indicated by white arrows). AV, anterior ventral nucleus; CM, centromedian nucleus; Hb, habenular nucleus; LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus; MTT, mamillothalamic tract; VL_a, ventral lateral anterior nucleus; VL_p, ventral lateral posterior nucleus; VPL, ventral posterior lateral nucleus. Part **a** is adapted with permission from ref. 96, Wolters Kluwer. Part **b** is adapted with permission from ref. 101, IOS Press. Part **c** is adapted with permission from ref. 147, Wolters Kluwer. Part **d** is adapted with permission from ref. 104, SAGE Publications.

of life after traumatic brain injury and even consciousness in coma¹²⁹. Favourable treatment outcomes with DBS of other thalamic nuclei have been demonstrated for several neurological conditions, including tremor¹³⁰ and epilepsy¹³¹. However, the success of DBS greatly varies across patients, which is related to the major challenges in the direct visualization and/or precise delineation of the respective thalamic nuclei in each patient using currently available methods. Similarly, there are currently no atlas-based segmentations of the anterodorsal nucleus despite neuropathology showing that it is the earliest and most specifically affected thalamic nucleus in Alzheimer disease^{100,132}. For now, all Alzheimer disease neuroimaging studies include the anterodorsal nucleus within the larger anterior group, which probably results in an underestimation of the specificity of thalamic nuclei changes in this condition. In the addiction field, segmentation of the ventromedial nuclei that are part of the thalamo-insular circuit would be extremely relevant, since they are considered to play a key role in interoception and craving¹³³. Finally, in schizophrenia, the thalamus reticular nucleus has received increasing interest¹³⁴ since animal models have shown that this nucleus is particularly vulnerable to disease development¹³⁵. Therefore, there is a strong need to develop techniques for investigating the thalamus reticular nucleus in human brain imaging^{136,137}.

From these few examples, it should become clear that we are currently only investigating ‘the tip of the iceberg’ of thalamic nuclei in humans, giving us an incomplete or even biased view of the thalamic contribution to the healthy brain and of its clinically relevant changes.

Towards better thalamic nuclei segmentation

As we have discussed, current thalamic nuclei segmentation approaches limit our ability to study the true importance of the thalamus in health and disease. If we want to gain a better understanding of the specific roles of thalamic nuclei and their contributions to cognitive development, healthy ageing, disease progression and symptomatology, more precise, robust and standardized neuroimaging approaches are needed. Fortunately, recent developments in approaches for MRI acquisition and segmentation promise to provide better insights into the thalamus in the future. It is therefore now timely to propose a Roadmap towards better thalamic nuclei

neuroimaging and a more standardized way to approach thalamic nuclei identification. Based on our brief review of the current segmentation techniques and their research and clinical implications, we propose that the community should take the following steps, potentially in this order of priority.

First, we recommend setting up a multidisciplinary group – consisting of expertise in thalamic histology, microscopy and neuroimaging – in order to establish thalamic ‘ground truths’ such as the number of thalamic nuclei, their location and morphology, nomenclature, and intra-person and inter-person nuclei variations. Here, we take inspiration from the [Hippocampal Subfields Group](#), which has led the way in the development of consensus protocols for segmenting the hippocampus and its subfields¹³⁸. Importantly, the Hippocampal Subfield Group build their work on the joint effort by the European Alzheimer’s Disease Consortium and Alzheimer’s Disease Neuroimaging Initiative to bring together international experts to agree on hippocampal boundaries and segmentation protocols in a bid to homogenize manual segmentation of MR-imaged hippocampus, resulting in the creation of the [Harmonized Hippocampal Protocol](#). The Hippocampal Subfields Group has been highly successful in building on this European Alzheimer’s Disease Consortium–Alzheimer’s Disease Neuroimaging Initiative effort to delve into localizing and agreeing on the key landmarks for parcellating the hippocampal subfields. Still, key challenges remain¹³⁹ such as the need to (1) compare different histological delineations of the medial temporal lobe region, (2) harmonize the identification of the hippocampal subregions in different settings, and (3) establish harmonized in vivo MRI segmentation protocols in clinical settings. We believe that similar challenges remain for the thalamus, which is far less explored than the hippocampus but even more important for a plethora of brain functions and clinical syndromes. The goal of the proposed thalamic multidisciplinary group would be not only to close the translation knowledge gap between thalamic histology and neuroimaging but also to agree on and clearly identify the existence of differences and similarities between histological data sets, to agree on solutions to resolve the differences¹³⁹, and to work together towards a harmonized in vivo MRI segmentation protocol. Indeed, it is our belief that

Glossary

Bayesian inference

An analysis technique that involves the use of probabilities to infer a hypothesized outcome.

Blood oxygen level-dependent (BOLD) signal

BOLD variations occur due to changes in deoxyhaemoglobin (which is paramagnetic) that are in turn caused by local changes in blood flow due to neuronal activity.

Diffusion MRI

Refers to imaging the microscopic motion of water molecules. When magnetic field gradients are applied on either side of the refocusing pulse in a spin-echo experiment, motion will result in reduction of the refocused MRI signal, which can be quantified and related to the diffusion coefficient.

Diffusion tensor imaging

(DTI). In diffusion MRI, by applying gradients in specific directions, information on preferential direction of motion can be inferred. One such method involves representing it as a tensor with the ellipsoid representing the three principal directions.

Echo-planar imaging

An ultra-fast variant of gradient or spin-echo MRI, in which multiple (or even all) k-space lines are acquired within a single repetition time. Echo-planar imaging is used when very fast acquisitions are required. Its high temporal resolution allows imaging of rapid physiological processes with decreased motion artefacts.

Fractional anisotropy

A scalar measurement from DTI that reflects the microstructural integrity of white matter tracts.

Functional MRI

(fMRI). A means for depicting brain activity by measuring regional BOLD variations in the brain.

Image registration

The mathematical operation that warps one image, called the source, to a target image. Registration can be rigid body, when the source and target are from the same brain, or non-linear, when matching a source to a template.

Mesh-based representation

A method that generally involves partitioning an image into tiny polygons in a process called tessellation. This term is used in the field of computer vision to describe the representation of 3D objects.

Nulling

The process of eliminating the magnetic signal coming from a particular tissue.

Partial volume effects

Effects that occur when a voxel contains signals from two or more tissues. The resultant signal is therefore an average of the signal arising from these tissues. Qualitatively, the image appears blurred and is quantitatively biased. Partial volume effects are particularly prominent in small regions or along borders of regions.

Proton density

The concentration of protons in each voxel, indicated by the voxel intensity.

Quantitative susceptibility mapping

Uses both magnitude and phase as a combined process to highlight the presence of compounds that could be diamagnetic (for example, calcification), paramagnetic (for example, deoxyhaemoglobin, due to fewer red blood cells causing anaemia) or ferromagnetic (for example, high iron content, making it behave as a magnet on its own).

Relaxation

The spin from hydrogen atoms aligns with the scanner's main magnetic field (B_0) in the longitudinal plane. When a radiofrequency pulse is applied, the spins absorb energy and their magnetization flips from the longitudinal into the transverse plane. When removed, the spins undergo relaxation to align again with the main B_0 magnetization.

Relaxation times

The time taken by the spins from the hydrogen atoms to lose their energy. Transverse and longitudinal relaxation times are labelled T1 and T2, respectively.

Resting-state fMRI

(rs-fMRI). Refers to measuring the fluctuations occurring in the brain when not subject to a specific task. Studies can be hypothesis driven, directly looking at the synchronicity between regions in either static (time invariant) or dynamic (observing switching or transitions) models, at a whole-brain level, through independent component analysis or graph theory methods.

Susceptibility

Each hydrogen atom has a local magnetic field associated to it. The human body also contains other compounds (for example, calcium) that have magnetic properties, hence distorting the local magnetic field. This leads to a change in the phase of local tissue and hence to a change in the signal measured.

Susceptibility-weighted imaging

Shows the presence (through magnitude only) of tissue susceptibility by weighting the resulting MRI images.

T1-weighted (T1w) imaging

Imaging in which the contrast between tissues is due to the differences in their T1 relaxation times. The longer the T1 relaxation time, the darker the signal (for example, cerebrospinal fluid has much longer T1 relaxation times than white or grey matter and is dark in T1w-MRI).

T2-weighted imaging

Imaging in which the contrast between tissues is due to the differences in their T2 relaxation times.

T2*-weighted imaging

Imaging in which the contrast between tissues is due to the differences in their T2* relaxation times. This method takes into account magnetic field inhomogeneities in addition to T2 relaxation and is therefore faster than T2.

Tractography

Modelling of the pathway of white matter tracts using scalar and vector measurements from diffusion tensor imaging.

inconsistencies will persist as long as problems are tackled individually and not as a grouped effort. The latter will ensure that research will advance along an agreed line of thought, allowing biases and inconsistencies to be identified and addressed more easily. Moreover, since identifying and consolidating the ground truth is one of the missions of the TANGO group, we will be using a formal consensus approach¹⁴⁰ towards boundary definitions. One potential solution we have identified is to have several experts in the cytoarchitecture of the human thalamus segment high-resolution post-mortem data working separately. The parcellations will then be compared qualitatively

(by rating the segmentations among the experts) as well as quantitatively (by measuring superimposition, dice index, test-retest and intraclass correlation coefficients). Where agreements among experts cannot be reached, a probabilistic approach can be considered for certain complex delineations. Nevertheless, there will remain a challenge for high-resolution segmentation data acquired in vivo as these are bound to be hampered by motion artefacts that directly affect the precision with which a particular nucleus can be located.

Second, we recommend establishing a harmonized nomenclature for the specific thalamic nuclei that can be identified by neuroimaging

in humans. We propose setting up a consensus group to define the nuclei that can currently be reliably imaged within healthy and diseased cohorts. The group would further propose a list of ‘target’ thalamic nuclei that are not currently available in neuroimaging atlases but should be added to make an important difference to future research across fields. This nuclei target list would inform future research to advance the field, including the development of specific MRI acquisition sequences for harder-to-image nuclei. These sequences would be tested in post-mortem data sets first before validating them in vivo in healthy populations and in those with disease. We also suggest that what would ultimately become a ‘standard’ high-resolution image of the whole thalamus should be acquired for all research protocols dealing with thalamic nuclei imaging. The idea would be to use these images to build a common platform across imaging centres worldwide, from one acknowledged reference image, to which parcellations of thalamic nuclei can be iteratively and incrementally added using conventional and artificial intelligence techniques to ultimately produce a robust atlas. More specifically, as imaging data bases become larger and larger, the iterative addition of intricate thalamic nuclei to the thalamic atlas can be managed via machine learning and deep learning algorithms.

Defining the probable location of small nuclei that are beyond the reach of advanced MRI methodology represents value in itself. It creates awareness that the signal derived from larger neighbouring structures is likely to represent a mixed signal arising from the larger visible structure and the smaller neighbouring structure. This will bring further nuance to the interpretation of BOLD activation patterns. Information on the location of a nucleus can also help drive MRI optimization of structural scanning, which will hopefully lead to the identification of the structures that are currently beyond the reach of MRI techniques.

Third, we suggest that recommendations for ‘best practice’ thalamic nuclei MRI acquisition sequences and protocols should be outlined for general neuroimaging, imaging of specific nuclei or imaging of specific study populations. Establishing these MRI acquisition guidelines will improve thalamic nuclei imaging overall and allow approaches towards human thalamic MRI acquisition to be standardized, which would enable the establishment of large-scale, open-source, gold-standard data sets for human thalamic nuclei. For example, research centres should report the type of quality control that has been conducted on the data that they have acquired, highlighting any differences observed during those quality checks, including notable artefacts due to movements, susceptibility, inter-person variability and differences observed when the same sequences are used but scanner manufacturers differ. Such checks should not result in simple binary decisions to accept or reject data but should contain a grading of the data quality so that variability of data quality can be considered within the delineation of thalamic nuclei. Such data-driven approaches can be complemented by expert-based approaches: for example, thalamic neuroanatomy experts can identify agreements and disparities in the delineation of thalamic nuclei in different studies, and can use this information to establish agreed landmarks for their delimitation.

Fourth, best-practice thalamic nuclei segmentation algorithms and/or toolboxes should be created for general use, the imaging of specific nuclei or the imaging of specific study populations. This will clarify which algorithms and/or toolboxes are the most appropriate for different age groups, pathologies or interventions (such as DBS). This step will improve the data and research outputs for specific thalamic nuclei and, in turn, improve meta-analytic approaches to determine the roles of specific thalamic nuclei across different populations. It is also

clear that the structural and/or functional alterations of thalamic nuclei that are associated with particular tasks or conditions will depend on the imaging modality under which the nuclei are analysed. For example, fMRI studies focus on functional dynamics and might therefore prioritize parcellation based on connectivity patterns rather than strictly histological boundaries. Thus, adopting a rigid parcellation strategy based solely on histology may overlook the interplay between structure and function, which varies across different states and conditions. Given these considerations, it seems sensible, when developing best-practice guidelines for nuclei segmentation, to adopt a flexible, multi-level approach that accommodates the inherent limitations and diverse methodologies of current imaging technologies and goes beyond simple structural–functional associations.

Fifth, we recommend gathering large, open-source, gold-standard data sets for thalamic nuclei. Obtaining such data sets – for example, by ensuring that all imaging centres include in their acquisition sequences one common MRI sequence – will foster research on thalamic nuclei by providing a benchmark data set for comparison and/or validation or for the development of new segmentation approaches. This operation will also establish quality assurances when sharing data sets obtained using different approaches and MRI strengths among imaging centres.

Finally, we recommend that guidelines should be published for the development and validation of new acquisition sequences or segmentation methods for thalamic nuclei. We recommend convening as an international consensus group, using the Delphi approach¹⁴¹, to establish these guidelines and promote cutting-edge thalamic nuclei research developments.

Conclusions

We are convinced that the Roadmap steps that we have outlined above will allow not only a better understanding of thalamic structure and function in normal and pathological conditions but also a better understanding of the importance of a functional thalamus for brain function and of how we can modulate thalamic function to treat neurological and psychiatric disorders. It is time to establish thalamic nuclei as separate brain structures in any neuroimaging investigation and to acknowledge that they are differentially involved in separate brain processes. We call on any reader who is interested in being involved in this process to contact the TANGO consortium for further information.

Published online: 17 October 2024

References

1. Usrey, W. M. & Sherman, S. M. In: *The Cerebral Cortex and Thalamus* (eds Usrey, W. M. & Sherman, S. M.) 3–10 (Oxford Univ. Press, 2023).
An updated and comprehensive volume on thalamic nuclei and their contributions to cortical mechanisms.
2. Jones, E. G. *The Thalamus* (Cambridge Univ. Press, 2007).
3. Halassa, M. M. & Sherman, S. M. Thalamocortical circuit motifs: a general framework. *Neuron* **103**, 762–770 (2019).
4. Krauth, A. et al. A mean three-dimensional atlas of the human thalamus: generation from multiple histological data. *Neuroimage* **49**, 2053–2062 (2010).
5. Tourdias, T., Saranathan, M., Levesque, I. R., Su, J. & Rutt, B. K. Visualization of intra-thalamic nuclei with optimized white-matter-nulled MPRAGE at 7T. *Neuroimage* **84**, 534–545 (2014).
6. Segobin, S. & Pitel, A. L. The specificity of thalamic alterations in Korsakoff’s syndrome: implications for the study of amnesia. *Neurosci. Biobehav. Rev.* **130**, 292–300 (2021).
7. Kumar, V. J., Scheffler, K., Hagberg, G. E. & Grodd, W. Quantitative susceptibility mapping of the basal ganglia and thalamus at 9.4 Tesla. *Front. Neuroanat.* **15**, 725731 (2021).
8. Morel, A., Magnin, M. & Jeanmonod, D. Multiarchitectonic and stereotactic atlas of the human thalamus. *J. Comp. Neurol.* **387**, 588–630 (1997).
Most probabilistic atlases that incorporate histological data are derived from this histological atlas.

9. Niemann, K., Mennicken, V. R., Jeanmonod, D. & Morel, A. The Morel stereotactic atlas of the human thalamus: atlas-to-MR registration of internally consistent canonical model. *Neuroimage* **12**, 601–616 (2000).
10. Schaltenbrand, G. & Wahren, W. *Atlas for Stereotaxy of the Human Brain* (Thieme, 1977).
11. Chakravarty, M. M., Bertrand, G., Hodge, C. P., Sadikot, A. F. & Collins, D. L. The creation of a brain atlas for image guided neurosurgery using serial histological data. *Neuroimage* **30**, 359–376 (2006).
12. Sadikot, A. et al. Creation of computerized 3D MRI-integrated atlases of the human basal ganglia and thalamus. *Front. Syst. Neurosci.* **5**, 71 (2011).
13. Iglehart, C., Monti, M., Cain, J., Tourdias, T. & Saranathan, M. A systematic comparison of structural-, structural connectivity-, and functional connectivity-based thalamus parcellation techniques. *Brain Struct. Funct.* **225**, 1631–1642 (2020).
14. Hwang, K., Shine, J. M., Cole, M. W. & Sorenson, E. Thalamocortical contributions to cognitive task activity. *eLife* **11**, e81282 (2022).
15. Antonucci, L. A. et al. Flexible and specific contributions of thalamic subdivisions to human cognition. *Neurosci. Biobehav. Rev.* **124**, 35–53 (2021).
16. Kumar, V. J., Beckmann, C. F., Scheffler, K. & Grodd, W. Relay and higher-order thalamic nuclei show an intertwined functional association with cortical-networks. *Commun. Biol.* **5**, 1187 (2022).
17. Wen, H. et al. Pulvinar response profiles and connectivity patterns to object domains. *J. Neurosci.* **43**, 812–826 (2023).
18. Dadar, M., Fonov, V. S. & Collins, D. L. A comparison of publicly available linear MRI stereotaxic registration techniques. *Neuroimage* **174**, 191–200 (2018).
19. Klein, A. et al. Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. *Neuroimage* **46**, 786–802 (2009).
20. Mai, J. K. & Majtanik, M. Toward a common terminology for the thalamus. *Front. Neuroanat.* **12**, 114 (2019).
A paper that underlines the importance of a common nomenclature and discusses how it can be achieved.
21. Deoni, S. C. L., Josseau, M. J. C., Rutt, B. K. & Peters, T. M. Visualization of thalamic nuclei on high resolution, multi-averaged T1 and T2 maps acquired at 1.5T. *Hum. Brain Mapp.* **25**, 353–359 (2005).
22. Deoni, S. C. L., Rutt, B. K., Parrent, A. G. & Peters, T. M. Segmentation of thalamic nuclei using a modified k-means clustering algorithm and high-resolution quantitative magnetic resonance imaging at 1.5T. *Neuroimage* **34**, 117–126 (2007).
23. Traynor, C. R., Barker, G. J., Crum, W. R., Williams, S. C. R. & Richardson, M. P. Segmentation of the thalamus in MRI based on T1 and T2. *Neuroimage* **56**, 939–950 (2011).
24. Mulder, M. J., Keuken, M. C., Bazin, P.-L., Alkemade, A. & Forstmann, B. U. Size and shape matter: the impact of voxel geometry on the identification of small nuclei. *PLoS One* **14**, e0215382 (2019).
25. Iglesias, J. E. & Sabuncu, M. R. Multi-atlas segmentation of biomedical images: a survey. *Med. Image Anal.* **24**, 205–219 (2015).
26. Iglesias, J. E. et al. A probabilistic atlas of the human thalamic nuclei combining ex vivo MRI and histology. *Neuroimage* **183**, 314–326 (2018).
27. Fischl, B. FreeSurfer. *Neuroimage* **62**, 774–781 (2012).
28. Wang, H. et al. Multi-atlas segmentation with joint label fusion. *IEEE Trans. Pattern Anal. Mach. Intell.* **35**, 611–623 (2013).
29. Su, J. H. et al. Thalamus Optimized Multi Atlas Segmentation (THOMAS): fast, fully automated segmentation of thalamic nuclei from structural MRI. *Neuroimage* **194**, 272–282 (2019).
30. Saranathan, M., Iglehart, C., Monti, M., Tourdias, T. & Rutt, B. In vivo high-resolution structural MRI-based atlas of human thalamic nuclei. *Sci. Data* **8**, 275 (2021).
31. Saranathan, M., Tourdias, T., Bayram, E., Ghanouni, P. & Rutt, B. K. Optimization of white-matter-nulled magnetization prepared rapid gradient echo (MP-RAGE) imaging. *Magn. Reson. Med.* **73**, 1786–1794 (2015).
32. Sudhyadhom, A., Haq, I. U., Foote, K. D., Okun, M. S. & Bova, F. J. A high resolution and high contrast MRI for differentiation of subcortical structures for DBS targeting: the Fast Gray Matter Acquisition T1 Inversion Recovery (FGATIR). *Neuroimage* **47**, T44–T52 (2009).
33. Brun, G. et al. Automatic segmentation of deep grey nuclei using a high-resolution 7 T magnetic resonance imaging atlas-Quantification of T1 values in healthy volunteers. *Eur. J. Neurosci.* **55**, 438–460 (2022).
34. Datta, R. et al. Fast automatic segmentation of thalamic nuclei from MP2RAGE acquisition at 7 Tesla. *Magn. Reson. Med.* **85**, 2781–2790 (2021).
35. Weiskopf, N. et al. Quantitative multi-parameter mapping of R1, PD*, MT, and R2* at 3 T: a multi-center validation. *Front. Neurosci.* **7**, 95 (2013).
36. Caan, M. W. A. et al. MP2RAGEME: T1, T2*, and QSM mapping in one sequence at 7 tesla. *Hum. Brain Mapp.* **40**, 1786–1798 (2019).
37. Alkemade, A. et al. 7 Tesla MRI followed by histological 3D reconstructions in whole-brain specimens. *Front. Neuroanat.* **14**, 536838 (2020).
38. Alkemade, A. et al. A unified 3D map of microscopic architecture and MRI of the human brain. *Sci. Adv.* **8**, eabj7892 (2022).
39. Duan, Y., Li, X. & Xi, Y. Thalamus segmentation from diffusion tensor magnetic resonance imaging. *Int. J. Biomed. Imaging* **2007**, 90216 (2007).
40. Jonasson, L. et al. A level set method for segmentation of the thalamus and its nuclei in DT-MRI. *Signal. Process.* **87**, 309–321 (2007).
41. Rittner, L., Lotufo, R. A., Campbell, J. & Pike, G. B. In 2010 IEEE International Symposium on Biomedical Imaging: From Nano to Macro 1173–1176 (2010).
42. Wiegell, M. R., Tuch, D. S., Larsson, H. B. W. & Wedeen, V. J. Automatic segmentation of thalamic nuclei from diffusion tensor magnetic resonance imaging. *Neuroimage* **19**, 391–401 (2003).
43. Kumar, V., Mang, S. & Grodd, W. Direct diffusion-based parcellation of the human thalamus. *Brain Struct. Funct.* **220**, 1619–1635 (2015).
44. Mang, S. C., Busza, A., Reiterer, S., Grodd, W. & Klöse, A. U. Thalamus segmentation based on the local diffusion direction: a group study. *Magn. Reson. Med.* **67**, 118–126 (2012).
45. Ziyani, U., Tuch, D. & Westin, C.-F. Segmentation of thalamic nuclei from DTI using spectral clustering. *Med. Image Comput. Comput. Interv.* **9**, 807–814 (2006).
46. Ziyani, U. & Westin, C.-F. Joint segmentation of thalamic nuclei from a population of diffusion tensor MR images. *Med. Image Comput. Comput. Interv.* **11**, 279–286 (2008).
47. Najdenovska, E. et al. In-vivo probabilistic atlas of human thalamic nuclei based on diffusion-weighted magnetic resonance imaging. *Sci. Data* **5**, 180270 (2018).
48. Battistella, G. et al. Robust thalamic nuclei segmentation method based on local diffusion magnetic resonance properties. *Brain Struct. Funct.* **222**, 2203–2216 (2017).
49. Behrens, T. E. J. et al. Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat. Neurosci.* **6**, 750–757 (2003).
50. O’Muircheartaigh, J. et al. Clustering probabilistic tractograms using independent component analysis applied to the thalamus. *Neuroimage* **54**, 2020–2032 (2011).
51. Calamante, F. et al. Super-resolution track-density imaging of thalamic substructures: comparison with high-resolution anatomical magnetic resonance imaging at 7.0T. *Hum. Brain Mapp.* **34**, 2538–2548 (2013).
52. Basile, G. A. et al. In vivo super-resolution track-density imaging for thalamic nuclei identification. *Cereb. Cortex* **31**, 5613–5636 (2021).
53. Stough, J. V. et al. Automatic method for thalamus parcellation using multi-modal feature classification. *Med. Image Comput. Comput. Interv.* **17**, 169–176 (2014).
54. Semedo, C. et al. In *Medical Image Computing and Computer Assisted Intervention – MICCAI 2018* (eds Frangi, A. F., Schnabel, J. A., Davatzikos, C., Alberola-López, C. & Fichtinger, G.) 383–391 (Springer International Publishing, 2018).
55. Huang, S. Y. et al. Connectome 2.0: developing the next-generation ultra-high gradient strength human MRI scanner for bridging studies of the micro-, meso- and macro-connectome. *Neuroimage* **243**, 118530 (2021).
56. Behrens, T. E. J., Berg, H. J., Jbabdi, S., Rushworth, M. F. S. & Woolrich, M. W. Probabilistic diffusion tractography with multiple fibre orientations: what can we gain? *Neuroimage* **34**, 144–155 (2007).
57. Johansen-Berg, H. et al. Functional-anatomical validation and individual variation of diffusion tractography-based segmentation of the human thalamus. *Cereb. Cortex* **15**, 31–39 (2005).
58. Basile, G. A. et al. In vivo probabilistic atlas of white matter tracts of the human subthalamic area combining track density imaging and optimized diffusion tractography. *Brain Struct. Funct.* **227**, 2647–2665 (2022).
59. Maier-Hein, K. H. et al. The challenge of mapping the human connectome based on diffusion tractography. *Nat. Commun.* **8**, 1349 (2017).
60. Shine, J. M., Lewis, L. D., Garrett, D. D. & Hwang, K. The impact of the human thalamus on brain-wide information processing. *Nat. Rev. Neurosci.* **24**, 416–430 (2023).
Highlights the role of the thalamus in a large number of human brain functional signatures.
61. Mezer, A., Yovel, Y., Pasternak, O., Gorfine, T. & Assaf, Y. Cluster analysis of resting-state fMRI time series. *Neuroimage* **45**, 1117–1125 (2009).
62. O’Muircheartaigh, J., Keller, S. S., Barker, G. J. & Richardson, M. P. White matter connectivity of the thalamus delineates the functional architecture of competing thalamocortical systems. *Cereb. Cortex* **25**, 4477–4489 (2015).
63. Toulmin, H. et al. Specialization and integration of functional thalamocortical connectivity in the human infant. *Proc. Natl Acad. Sci. USA* **112**, 6485–6490 (2015).
64. Yuan, R. et al. Functional topography of the thalamocortical system in human. *Brain Struct. Funct.* **221**, 1971–1984 (2016).
65. Zhang, D. et al. Intrinsic functional relations between human cerebral cortex and thalamus. *J. Neurophysiol.* **100**, 1740–1748 (2008).
66. Zhang, D., Snyder, A. Z., Shimony, J. S., Fox, M. D. & Raichle, M. E. Noninvasive functional and structural connectivity mapping of the human thalamocortical system. *Cereb. Cortex* **20**, 1187–1194 (2010).
67. Ji, B. et al. Dynamic thalamus parcellation from resting-state fMRI data. *Hum. Brain Mapp.* **37**, 954–967 (2016).
68. Kumar, V. J., van Oort, E., Scheffler, K., Beckmann, C. F. & Grodd, W. Functional anatomy of the human thalamus at rest. *Neuroimage* **147**, 678–691 (2017).
Compares and contrasts parcellations of the thalamus obtained from structural versus functional connectivity data and shows that there is no one-to-one mapping between them.
69. Kim, D., Park, B. & Park, H. Functional connectivity-based identification of subdivisions of the basal ganglia and thalamus using multilevel independent component analysis of resting state fMRI. *Hum. Brain Mapp.* **34**, 1371–1385 (2013).
70. Hale, J. R. et al. Comparison of functional thalamic segmentation from seed-based analysis and ICA. *Neuroimage* **114**, 448–465 (2015).
71. Zhang, S. & Li, C.-S. R. Functional connectivity parcellation of the human thalamus by independent component analysis. *Brain Connect.* **7**, 602–616 (2017).
72. Setzer, B. et al. A temporal sequence of thalamic activity unfolds at transitions in behavioral arousal state. *Nat. Commun.* **13**, 5442 (2022).

73. Tregidgo, H. F. J. et al. Accurate Bayesian segmentation of thalamic nuclei using diffusion MRI and an improved histological atlas. *Neuroimage* **274**, 120129 (2023).
An optimized segmentation procedure using T1w MRI and DWI data that is available within the new FreeSurfer pipeline.
74. Yan, C. et al. Segmenting thalamic nuclei from manifold projections of multi-contrast MRI. *Proceedings of SPIE* <https://doi.org/10.48550/arXiv.2301.06114> (2023).
75. Majdi, M. S. et al. Automated thalamic nuclei segmentation using multi-planar cascaded convolutional neural networks. *Magn. Reson. Imaging* **73**, 45–54 (2020).
76. Umapathy, L., Keerthivasan, M. B., Zahr, N. M., Bilgin, A. & Saranathan, M. Convolutional neural network based frameworks for fast automatic segmentation of thalamic nuclei from native and synthesized contrast structural MRI. *Neuroinformatics* **20**, 651–664 (2022).
Describes the use of deep-learning procedures to enhance thalamic nuclei segmentation procedures, arguably the method of the future.
77. Shao, M. et al. Evaluating the impact of MR image harmonization on thalamus deep network segmentation. *Proc. SPIE Int. Soc. Opt. Eng.* **12032**, 120320H (2022).
78. Setsompop, K., Feinberg, D. A. & Polimeni, J. R. Rapid brain MRI acquisition techniques at ultra-high fields. *NMR Biomed.* **29**, 1198–1221 (2016).
79. Williams, B., Nguyen, D., Vidal, J. P. & Saranathan, M. Thalamic nuclei segmentation from T1-weighted MRI: unifying and benchmarking state-of-the-art methods. *Imaging Neurosci.* **2**, 1–16 (2024).
80. Jaimes, C. et al. Probabilistic tractography-based thalamic parcellation in healthy newborns and newborns with congenital heart disease. *J. Magn. Reson. Imaging* **47**, 1626–1637 (2018).
81. Jakab, A. et al. Mental development is associated with cortical connectivity of the ventral and nonspecific thalamus of preterm newborns. *Brain Behav.* **10**, e01786 (2020).
82. Lidauer, K. et al. Subcortical and hippocampal brain segmentation in 5-year-old children: validation of FSL-FIRST and FreeSurfer against manual segmentation. *Eur. J. Neurosci.* **56**, 4619–4641 (2022).
83. Hashempour, N. et al. A novel approach for manual segmentation of the amygdala and hippocampus in neonate MRI. *Front. Neurosci.* **13**, 1025 (2019).
84. Tutunji, R. et al. Thalamic volume and dimensions on MRI in the pediatric population: normative values and correlations: (a cross sectional study). *Eur. J. Radiol.* **109**, 27–32 (2018).
85. Turesky, T. K., Vanderauwera, J. & Gaab, N. Imaging the rapidly developing brain: current challenges for MRI studies in the first five years of life. *Dev. Cogn. Neurosci.* **47**, 100893 (2021).
86. Gousias, I. S. et al. Magnetic resonance imaging of the newborn brain: manual segmentation of labelled atlases in term-born and preterm infants. *Neuroimage* **62**, 1499–1509 (2012).
87. Morey, R. A. et al. A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *Neuroimage* **45**, 855–866 (2009).
88. Pomponio, R. et al. Harmonization of large MRI datasets for the analysis of brain imaging patterns throughout the lifespan. *Neuroimage* **208**, 116450 (2020).
89. Choi, E. Y. et al. Thalamic nuclei atrophy at high and heterogeneous rates during cognitively unimpaired human aging. *Neuroimage* **262**, 119584 (2022).
90. Pfefferbaum, A., Sullivan, E. V., Zahr, N. M., Pohl, K. M. & Saranathan, M. Multi-atlas thalamic nuclei segmentation on standard T1-weighted MRI with application to normal aging. *Hum. Brain Mapp.* **44**, 612–628 (2023).
91. Schmähmann, J. D. Vascular syndromes of the thalamus. *Stroke* **34**, 2264–2278 (2003).
92. Carlesimo, G. A., Lombardi, M. G. & Caltagirone, C. Vascular thalamic amnesia: a reappraisal. *Neuropsychologia* **49**, 777–789 (2011).
93. Golden, E. C., Graff-Radford, J., Jones, D. T. & Benarroch, E. E. Mediodorsal nucleus and its multiple cognitive functions. *Neurology* **87**, 2161–2168 (2016).
94. Pergola, G. et al. Quantitative assessment of chronic thalamic stroke. *AJNR Am. J. Neuroradiol.* **34**, E51–E55 (2013).
95. Percheron, G. The anatomy of the arterial supply of the human thalamus and its use for the interpretation of the thalamic vascular pathology. *Z. Neurol.* **205**, 1–13 (1973).
96. Danet, L. et al. Thalamic amnesia after infarct: the role of the mammillothalamic tract and mediodorsal nucleus. *Neurology* **85**, 2107–2115 (2015).
97. Hwang, K., Shine, J. M., Bruss, J., Tranel, D. & Boes, A. Neuropsychological evidence of multi-domain network hubs in the human thalamus. *eLife* **10**, e69480 (2021).
98. Harding, A., Halliday, G., Caine, D. & Kriil, J. Degeneration of anterior thalamic nuclei differentiates alcoholics with amnesia. *Brain* **123**, 141–154 (2000).
99. Segobin, S. et al. Dissociating thalamic alterations in alcohol use disorder defines specificity of Korsakoff's syndrome. *Brain* **142**, 1458–1470 (2019).
100. Braak, H. & Braak, E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239–259 (1991).
101. Bernstein, A. S., Rapcsak, S. Z., Hornberger, M. & Saranathan, M.; Alzheimer's Disease Neuroimaging Initiative. Structural changes in thalamic nuclei across prodromal and clinical Alzheimer's disease. *J. Alzheimers Dis.* **82**, 361–371 (2021).
102. Forno, G. et al. Thalamic nuclei changes in early and late onset Alzheimer's disease. *Curr. Res. Neurobiol.* **4**, 100084 (2023).
103. Azevedo, C. J. et al. Thalamic atrophy in multiple sclerosis: a magnetic resonance imaging marker of neurodegeneration throughout disease. *Ann. Neurol.* **83**, 223–234 (2018).
104. Planche, V. et al. White-matter-nulled MPRAGE at 7T reveals thalamic lesions and atrophy of specific thalamic nuclei in multiple sclerosis. *Mult. Scler.* **26**, 987–992 (2020).
105. Alemán-Gómez, Y. et al. Multimodal magnetic resonance imaging depicts widespread and subregion specific anomalies in the thalamus of early-psychosis and chronic schizophrenia patients. *Schizophr. Bull.* **49**, 196–207 (2023).
106. Henry, R. G. et al. Connecting white matter injury and thalamic atrophy in clinically isolated syndromes. *J. Neurol. Sci.* **282**, 61–66 (2009).
107. Ontaneda, D. et al. Deep grey matter injury in multiple sclerosis: a NAIMS consensus statement. *Brain* **144**, 1974–1984 (2021).
108. Kuchcinski, G. et al. Thalamic alterations remote to infarct appear as focal iron accumulation and impact clinical outcome. *Brain* **140**, 1932–1946 (2017).
109. Linck, P. A. et al. Neurodegeneration of the substantia nigra after ipsilateral infarct: MRI R2* mapping and relationship to clinical outcome. *Radiology* **291**, 438–448 (2019).
110. Tamura, A. et al. Thalamic atrophy following cerebral infarction in the territory of the middle cerebral artery. *Stroke* **22**, 615–618 (1991).
111. Moon, Y., Han, S.-H. & Moon, W.-J. Patterns of brain iron accumulation in vascular dementia and Alzheimer's dementia using quantitative susceptibility mapping imaging. *J. Alzheimers Dis.* **51**, 737–745 (2016).
112. Blyau, S. et al. Differential vulnerability of thalamic nuclei in multiple sclerosis. *Mult. Scler. J.* **29**, 295–300 (2023).
113. Magliozzi, R. et al. "Ependymal-in" gradient of thalamic damage in progressive multiple sclerosis. *Ann. Neurol.* **92**, 670–685 (2022).
114. Lee, J.-S., Heo, D.-Y., Choi, K.-H. & Kim, H.-J. Impact of the ventricle size on alzheimer's disease progression: a retrospective longitudinal study. *Dement. Neurocogn. Disord.* **23**, 95–106 (2024).
115. Oliveira, L. M., Nitrini, R. & Román, G. C. Normal-pressure hydrocephalus: a critical review. *Dement. Neuropsychol.* **13**, 133–143 (2019).
116. Johnstone, E., Frith, C. D., Crow, T. J., Husband, J. & Kreel, L. Cerebral ventricular size and cognitive impairment in chronic schizophrenia. *Lancet* **308**, 924–926 (1976).
117. Van Erp, T. G. M. et al. Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. *Mol. Psychiatry* **21**, 547–553 (2016).
118. Pergola, G., Selvaggi, P., Trizio, S., Bertolino, A. & Blasi, G. The role of the thalamus in schizophrenia from a neuroimaging perspective. *Neurosci. Biobehav. Rev.* **54**, 57–75 (2015).
119. Pergola, G. et al. Grey matter volume patterns in thalamic nuclei are associated with familial risk for schizophrenia. *Schizophr. Res.* **180**, 13–20 (2017).
120. Honea, R. A. et al. Is gray matter volume an intermediate phenotype for schizophrenia? A voxel-based morphometry study of patients with schizophrenia and their healthy siblings. *Biol. Psychiatry* **63**, 465–474 (2008).
121. Cooper, D., Barker, V., Radua, J., Fusar-Poli, P. & Lawrie, S. M. Multimodal voxel-based meta-analysis of structural and functional magnetic resonance imaging studies in those at elevated genetic risk of developing schizophrenia. *Psychiatry Res. Neuroimaging* **221**, 69–77 (2014).
122. Akudjedu, T. N. et al. Progression of neuroanatomical abnormalities after first-episode of psychosis: a 3-year longitudinal sMRI study. *J. Psychiatr. Res.* **130**, 137–151 (2020).
123. Cobia, D. J., Smith, M. J., Wang, L. & Csernansky, J. G. Longitudinal progression of frontal and temporal lobe changes in schizophrenia. *Schizophr. Res.* **139**, 1–6 (2012).
124. Gaser, C., Nenadic, I., Buchsbaum, B. R., Hazlett, E. A. & Buchsbaum, M. S. Ventricular enlargement in schizophrenia related to volume reduction of the thalamus, striatum, and superior temporal cortex. *Am. J. Psychiatry* **161**, 154–156 (2004).
125. Guo, J. Y. et al. Longitudinal regional brain volume loss in schizophrenia: relationship to antipsychotic medication and change in social function. *Schizophr. Res.* **168**, 297–304 (2015).
126. Borghesi, A., Piracha, A. & Sani, S. Prevalence and anatomical characteristics of the human massa intermedia. *Brain Struct. Funct.* **226**, 471–480 (2021).
127. Trzesniak, C. et al. Adhesion interthalamic alterations in schizophrenia spectrum disorders: a systematic review and meta-analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **35**, 877–886 (2011).
128. Cassel, J.-C. et al. The reuniens and rhomboid nuclei of the thalamus: a crossroads for cognition-relevant information processing? *Neurosci. Biobehav. Rev.* **126**, 338–360 (2021).
129. Schiff, N. D. et al. Thalamic deep brain stimulation in traumatic brain injury: a phase 1, randomized feasibility study. *Nat. Med.* **29**, 3162–3174 (2023).
130. Wong, J. K. et al. Deep brain stimulation in essential tremor: targets, technology, and a comprehensive review of clinical outcomes. *Expert. Rev. Neurother.* **20**, 319–331 (2020).
131. Middlebrooks, E. H., He, X., Grewal, S. S. & Keller, S. S. Neuroimaging and thalamic connectomics in epilepsy neuromodulation. *Epilepsy Res.* **182**, 106916 (2022).
132. Aggleton, J. P., Pralus, A., Nelson, A. J. D. & Hornberger, M. Thalamic pathology and memory loss in early Alzheimer's disease: moving the focus from the medial temporal lobe to Papez circuit. *Brain* **139**, 1877–1890 (2016).
133. Craig, A. D. How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* **3**, 655–666 (2002).
134. Steullet, P. et al. The thalamic reticular nucleus in schizophrenia and bipolar disorder: role of parvalbumin-expressing neuron networks and oxidative stress. *Mol. Psychiatry* **23**, 2057–2065 (2018).
135. El Khoueiry, C. et al. Developmental oxidative stress leads to T-type Ca²⁺ channel hypofunction in thalamic reticular nucleus of mouse models pertinent to schizophrenia. *Mol. Psychiatry* **27**, 2042–2051 (2022).
136. Viviano, J. D. & Schneider, K. A. Interhemispheric interactions of the human thalamic reticular nucleus. *J. Neurosci.* **35**, 2026–2032 (2015).

137. Schira, M. M. et al. HumanBrainAtlas: an in vivo MRI dataset for detailed segmentations. *Brain Struct. Funct.* **228**, 1849–1863 (2023).
138. Boccardi, M. et al. Delphi definition of the EADC-ADNI harmonized protocol for hippocampal segmentation on magnetic resonance. *Alzheimers Dement.* **11**, 126–138 (2015).
139. Baumeister, H. et al. Comparison of histological delineation of the entorhinal, perirhinal, ectohippocampal, and parahippocampal cortices by different neuroanatomy laboratories. *Alzheimers Dement* **19**, e076135 (2023).
140. Carter, P. et al. A demonstration of using formal consensus methods within guideline development; a case study. *BMC Med. Res. Methodol.* **21**, 73 (2021).
141. Nasa, P., Jain, R. & Juneja, D. Delphi methodology in healthcare research: how to decide its appropriateness. *World J. Methodol.* **11**, 116–129 (2021).
142. Vidal, J. P. et al. Robust thalamic nuclei segmentation from T1-weighted MRI using polynomial intensity transformation. *Brain Struct. Funct.* **229**, 1087–1101 (2024).
143. Oxenford, S. et al. Lead-OR: a multimodal platform for deep brain stimulation surgery. *eLife* **11**, e72929 (2022).
144. Fan, L. et al. The human brainnetome atlas: a new brain atlas based on connectonal architecture. *Cereb. Cortex* **26**, 3508–3526 (2016).
145. van Oort, E. S. B. et al. Functional parcellation using time courses of instantaneous connectivity. *Neuroimage* **170**, 31–40 (2018).
146. Danet, L. et al. Medial thalamic stroke and its impact on familiarity and recollection. *eLife* **6**, e28141 (2017).
147. Pitel, A.-L. et al. Macrostructural abnormalities in Korsakoff syndrome compared with uncomplicated alcoholism. *Neurology* **78**, 1330–1333 (2012).
148. Alegro, M. et al. In: *IEEE Computer Society Conference on Computer Vision and Pattern Recognition Workshops* 634–642 (IEEE Computer Society, 2016).
149. Alho, A. T. D. L. et al. Magnetic resonance diffusion tensor imaging for the pedunculo-pontine nucleus: proof of concept and histological correlation. *Brain Struct. Funct.* **222**, 2547–2558 (2017).
150. Alho, E. J. L. et al. High thickness histological sections as alternative to study the three-dimensional microscopic human sub-cortical neuroanatomy. *Brain Struct. Funct.* **223**, 1121–1132 (2018).
151. Mollink, J. et al. Evaluating fibre orientation dispersion in white matter: comparison of diffusion MRI, histology and polarized light imaging. *Neuroimage* **157**, 561–574 (2017).
152. Sitek, K. R. et al. Mapping the human subcortical auditory system using histology, postmortem MRI and in vivo MRI at 7T. *eLife* **8**, e48932 (2019).
153. Jorge, J. et al. Improved susceptibility-weighted imaging for high contrast and resolution thalamic nuclei mapping at 7T. *Magn. Reson. Med.* **84**, 1218–1234 (2020).
154. Abosch, A., Yacoub, E., Uğurbil, K. & Harel, N. An assessment of current brain targets for deep brain stimulation surgery with susceptibility-weighted imaging at 7 tesla. *Neurosurgery* **67**, 1745–1756 (2010).
155. Dешмане, А., Gulani, V., Griswold, M. A. & Seiberlich, N. Parallel MR imaging. *J. Magn. Reson. Imaging* **36**, 55–72 (2012).
156. Marques, J. P. et al. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. *Neuroimage* **49**, 1271–1281 (2010).

Acknowledgements

The Thalamus Nuclei Neuroimaging Group (TANGO) consortium can be followed and reached via our website. S.S. was supported by the French National Institute for Health and Medical Research (INSERM), Label Excellence de la Région Normandie, the French National Agency for Research (ANR), the Fondation pour la Recherche Médicale (FRM; ING20140129160). R.A.M.H. was supported by H2020 Marie Skłodowska Curie Actions. Grant/Award Number: 101061988. V.J.K. was supported by the Deutsche Forschungsgemeinschaft, DFG SCHE 658/17. G.P. and

A.L. were supported by RIPARTI – “assegni di Ricerca per riPARTire con le Imprese” initiative, APULIA REGION (POC PUGLIA FESR-FSE 2014 / 2020), Project Code 79ed97ad. G.P. was supported by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), “MNESYS, A multiscale integrated approach to the study of the nervous system in health and disease” (PE0000006) – (DN. 1553 11.10.2022). T.T. was supported by University of Bordeaux’s IdEx “Investments for the Future” RRI programme “IMPACT” (IMaging for Precision medicine within A Collaborative Translational programme), and IHU Precision & Global Vascular Brain Health Institute, ANR-23-IAHU-000, which received financial support from the France 2030 programme. M.B.C. is funded by CIBM Center for Biomedical Imaging, a Swiss research centre of excellence founded and supported by CHUV, UNIL, EPFL, UNIGE and HUG, and also by Swiss National Science Foundation grants 205321-157040. A.-L.P. was supported by the INSERM, Label Excellence de la Région Normandie, the ANR, the FRM, the French University Institute. A.A. was supported by ZonMW Open competition Grant 09120012110015 JPND/ZonMW (grant 73305113). M.S. was supported by National Institutes of Health NIBIB R01 E032674. M.H. was supported by the National Institute for Health Research and the Medical Research Council.

Author contributions

All authors researched data for the article, contributed substantially to discussion of the content, and reviewed and/or edited the manuscript before submission. M.H. and S.S. wrote the article.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Neuroscience* thanks Rosanna Olsen and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Related links

FMRI Software Library: <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases>

FreeSurfer software suite: <https://surfer.nmr.mgh.harvard.edu/>

Harmonized Hippocampal Protocol: <http://www.hippocampal-protocol.net/SOPs/index.php>

Hippocampal Subfields Group: <https://hippocampalsubfields.com/>

Human Brain Atlas: <https://hba.neura.edu.au/methods/>

Human Brainnetome Atlas: <https://atlas.brainnetome.org/>

Lead-DBS: <https://www.lead-dbs.org/>

TANGO: <https://thalamicsegmentation.github.io/>

THOMAS: <https://github.com/thalamicseg>

Zenodo: <https://doi.org/10.5281/zenodo.1253021>

© Springer Nature Limited 2024

¹Normandie University, UNICAEN, PSL Université Paris, EPHE, INSERM, U1077, CHU de Caen, Cyceron, Neuropsychologie et Imagerie de la Mémoire Humaine, Caen, France. ²Aix-Marseille University, CRMBM CNRS UMR 7339, Marseille, France. ³APHM, La Timone Hospital, CEMEREM, Marseille, France. ⁴Max Planck Institute for Biological Cybernetics, Tuebingen, Germany. ⁵Department of Translational Biomedicine and Neuroscience (DiBrain), University of Bari Aldo Moro, Bari, Italy. ⁶Integrative Model-based Cognitive Neuroscience Unit, Department of Psychology, University of Amsterdam, Amsterdam, The Netherlands. ⁷CIBM Center for Biomedical Imaging, Lausanne, Switzerland. ⁸Radiology Department, Lausanne University and University Hospital, Lausanne, Switzerland. ⁹Centre de recherche Cerveau et Cognition (Cerco), UMR5549, CNRS – Université de Toulouse, Toulouse, France. ¹⁰Aix Marseille Université, INSERM INS UMR 1106, APHM, Marseille, France. ¹¹Lieber Institute for Brain Development, Johns Hopkins Medical Campus, Baltimore, MD, USA. ¹²Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, USA. ¹³Normandie University, UNICAEN, INSERM, U1237, PHIND “Physiopathology and Imaging of Neurological Disorders”, NeuroPresage Team, Cyceron, Caen, France. ¹⁴Department of Radiology, UMass Chan Medical School, Worcester, MA, USA. ¹⁵Neuroimagerie diagnostique et thérapeutique, CHU de Bordeaux, Bordeaux, France. ¹⁶Neurocentre Magendie, University of Bordeaux, INSERM U1215, Bordeaux, France. ¹⁷Norwich Medical School, University of East Anglia, Norwich, UK.