Brussels, 19 May 2025

COST 027/25

DECISION

Subject: Memorandum of Understanding for the implementation of the COST Action “Advancing human brainstem MRI and its application in clinical research and practice” (ImagingBrainstemHealth) CA24118

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action Advancing human brainstem MRI and its application in clinical research and practice approved by the Committee of Senior Officials through written procedure on 19 May 2025.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA24118
ADVANCING HUMAN BRAINSTEM MRI AND ITS APPLICATION IN CLINICAL RESEARCH AND PRACTICE (ImagingBrainstemHealth)

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to investigate the brainstem's complex structure and its role in many diseases. Advances in high-resolution MRI now enable in vivo study, offering diagnostic and therapeutic potential, though challenges remain in standardization, training, and data collection to fully realize its clinical utility. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.

OVERVIEW

Summary
The brainstem, the phylogenetically oldest part of our brain, has traditionally been considered beyond the realm of standard brain imaging due to its small size and complex anatomical layout with a multitude of small structures. A particular vulnerability of brainstem structures characterizes the pre-symptomatic phase of many neurodegenerative conditions, rendering brainstem imaging an important tool for prodromal biomarkers. Moreover, dysfunctions in brainstem activity characterize a wide range of psychiatric or neurophysiological disorders but can currently not be considered for personalized treatments. Recent advances in magnetic resonance imaging (MRI) now render the human brainstem accessible also in standard clinical imaging setups. However, knowledge of and the ability to image the brainstem is still relatively sparse in clinical brain research. This disconnect between research developments and their clinical translation comes at a potentially enormous price, as assessing the brainstem in humans holds great promise for improved diagnoses and treatments of various pertinent health conditions in Europe. The Action will create a multidisciplinary network to facilitate and extend the use of brainstem MRI in clinical research and practice. It will a) establish a dialogue between methods development and clinical applications to define best practices suitable for clinical research and practice; b) train clinicians and young researchers to use advanced brainstem MRI; c) harmonize methods to establish big datasets across various clinical conditions; and d) establish the multidisciplinary research network necessary to support methods development for mapping currently uncharted areas of the brainstem and establishing new and reliable brainstem MRI biomarkers.

Areas of Expertise Relevant for the Action	**Keywords**
● Basic medicine: Neuroimaging and computational neuroscience ● Clinical medicine: Neurological disorders (e.g. Alzheimer's disease, Huntington's disease, Parkinson's disease) ● Basic medicine: Neuroanatomy and neurophysiology ● Clinical medicine: Psychiatric disorders	● Magnetic Resonance Imaging ● Brainstem Mapping ● Brainstem disorder ● Prodromal Neurodegeneration

Specific Objectives
To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination
● Establish the current state of the art of brainstem function in health and disease as well as current methodological standards in brainstem- specific anatomical atlases, imaging and analyses methods by a network of internationally leading experts.
● Build the multidisciplinary networks necessary to foster the development of new methods and insights for human brainstem MRI and facilitate their application in clinical research.
● Create harmonized research infrastructures and joint data repositories of brainstem imaging data

Capacity Building
● Build up a growing community of brainstem MRI experts
● Integrate a network of stakeholders, including health professionals, patient organizations, and policymakers

TECHNICAL ANNEX

1. S&T EXCELLENCE

1.1. SOUNDNESS OF THE CHALLENGE

1.1.1. DESCRIPTION OF THE STATE OF THE ART

Relevance of in vivo human brainstem imaging for alleviating health burdens in Europe: The brainstem is one of the phylogenetically oldest structures in the brain. Despite its small size, occupying only about 2% of total brain volume[1], it plays a vital role in controlling numerous central and peripheral functions. It is a primary relay centre or site of origin for a multitude of ascending and descending pathways and is densely packed with about 158 different grey matter structures and substructures[2], which project to the rest of the brain, spinal cord, and peripheral nervous system. These pathways and structures contribute importantly to the regulation of sensorimotor, cognitive, emotional, and autonomic functions, as well as pain processing, arousal, the sleep-wake cycle, or postural balance. **Due to its anatomical and functional complexity but also an often particular vulnerability, a selective dysfunction or degeneration of brainstem structures and pathways is implicated in a wide range of prevalent health conditions in Europe**. For instance, structures in the brainstem appear particularly vulnerable to protein pathologies in many neurodegenerative diseases and are often the first brain region affected, with dysfunctions likely contributing to early symptoms in the pre-symptomatic phase[3]. Indeed, an *in vivo* investigation of anatomical and functional changes of the human brainstem holds immense clinical potential for a) earlier prodromal diagnostics, b) new biomarkers of disease progression, c) new insights into disease mechanisms, and d) new targets for interventions. However, much of our current knowledge regarding the brainstem's involvement in pathology still comes from animal studies or *post mortem* data as the brainstem's position deep within the brain and the small size of individual component structures **require tailored approaches for *in vivo* investigations in humans**. Until recently, non-invasive neuroimaging of the pathways and small structures within the human brainstem was out of reach. However, recent developments in neuroimaging methodology, particularly in magnetic resonance imaging (MRI), now allow for submillimetre resolution and high-precision imaging[4] and open exciting opportunities to map the brainstem *in vivo*, becoming a potential game-changer in the diagnosis and management of several serious diseases as indicated in three examples below:

Early detection of neurodegenerative diseases: *Post mortem* data show that the noradrenergic locus coeruleus (LC) in the brainstem may be one of the epicenters for tau pathology in Alzheimer's disease (AD)[5]. Tau pathology in the LC is prevalent by the 3rd decade of life, about two decades before it is apparent in the cortex, and about four decades before clinical symptoms appear[5]. Animal data reveal that tau deposits in the LC render cells initially hyper-responsive and ultimately, as cell loss becomes more prevalent, under-responsive[6]. This may contribute to the development of symptoms such as sleep disruptions, agitation and anxiety in AD progression, which are challenging to manage and place a significant burden on both patients and their surroundings. Therefore, detailed and reliable assessments of **these early functional and anatomical changes in the LC** in living humans carries **enormous potential for monitoring the earliest prodromal diagnostics of brain changes in AD, and management of the disease**. Similarly, in Parkinson's disease (PD) and Lewy Body dementia (LBD), neurodegeneration of nigrosomes within substantia nigra (SN) precede the appearance of first motor symptoms by half a decade[7]. Recent advances in biologically-oriented MRI contrast mechanisms and high resolution imaging have allowed for developing anatomical sequences which visualize the LC and SN *in vivo* in humans[8]. However, a lack of standardisation as well as limited adoption in clinical research hinders an evaluation of these techniques as a **prodromal biomarker or much-needed precise marker of disease progression** to test disease modifying therapies in prodromal populations.

Personalised treatments in psychiatric conditions: Changes in the activity of the dopaminergic SN, ventral tegmental area (VTA), and noradrenergic LC, as well as the serotonergic dorsal raphe nucleus (RN), have been implicated in prevalent psychiatric conditions such as depression, post-traumatic stress disorder (PTSD), or attention-deficit/hyperactivity disorder (ADHD). For instance, animal studies suggest that a hyper-responsive LC through prolonged stress exposure might contribute to anxio-depressive disorders by fostering anxiety responses or aversive memories[9], while in ADHD, reduced dopaminergic control has been related to impulsive behaviour[10]. However, initial efficacy and long-term maintenance of treatment gains in pharmacological treatments targeting neuromodulation are often unsatisfactory, with overall response rates ranging between 30 and 70%[11]. Being able to assess

functional responses in neuromodulatory brainstem structures assumed to be over- or underactive could therefore provide important currently missing information for personalised psychopharmacological treatments as well as the monitoring of disease progression.

New therapeutic targets in chronic pain: Risk factors for developing chronic pain are currently still poorly understood. Recent animal and human brain imaging studies suggest that a dysregulation in an endogenous descending pain-modulation network including the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) in the brainstem might contribute to the pathological chronification of acute pain responses[12]. Cortical inputs to these brainstem sites display a connectivity pattern which is directly related to chronic pain sufferers' ongoing pain levels[13]. Moreover, interindividual differences in connectivity patterns can serve as a marker for treatment efficacy through non-invasive or pharmacological interventions[14]. Despite an understanding that the brainstem houses the core sites for regulating and inhibiting pain, it is only recently that MRI-based techniques have evolved which provide the spatial and temporal resolution necessary to resolve their involvement in human chronic pain states. Assessing interindividual differences in patterns of functional dysregulation in brainstem pain networks therefore holds great promise for informing more targeted therapeutic techniques in treating chronic pain and preventing pain chronification.

Timeliness of the challenge: Developments in neuroimaging methods now put the brainstem within reach in human research: Despite the potentially great relevance of *in vivo* brainstem imaging for a wide range of health conditions, the brainstem is still relatively neglected in human brain research. This is largely due to the fact that standard brain imaging methods are typically not suitable for investigating the small and heterogeneous structures of the brainstem. Positron emission tomography (PET) can visualize neurochemical properties but has insufficient spatial resolution and limited potential for functional or dense longitudinal assessments given its reliance on costly radioactive tracers. While providing important additional information, in particular for neuromodulatory structures, PET is thus insufficient for capturing atrophy or functional changes in particular in the smaller brainstem structures with volumes well below 100mm^3. In contrast, **recent developments in magnetic resonance imaging (MRI) techniques hold great promise as one of the core approaches for investigating the human brainstem *in vivo***. Specifically, the following recent developments now bring a more widespread clinical application of existing approaches in brainstem MRI within reach and allow for an increasing momentum in developing new brainstem MRI approaches: **a) Recent progress in ultra-high-field MRI** resulted in substantial increases of sensitivity and signal-to-noise ratio (SNR), enabling ultra-high-resolution imaging for both anatomical and functional MRI, which is key for small structures within the brainstem. At the same time, **b) new imaging sequences with higher SNR and flexible contrast mechanisms on conventional clinical field strengths** were enabled by recent progress in parallel imaging, image reconstruction and analysis. These innovations are strongly accelerated by the use of AI-based image analysis techniques, which have opened exciting opportunities to mitigate physiological artefacts and map brainstem anatomy quantitatively and with submillimetre resolution *in vivo*[15]. Moreover, **c) through a combination of *post mortem* and *in vivo* imaging methods**, contrast mechanisms in brainstem imaging can be more specifically tailored to the biological properties of specific brainstem structures to yield sufficient contrast to noise ratio (CNR) also at lower field strengths. Finally, **d) progress in quantitative MRI** transforms anatomical MRI from imaging modality to a quantitative assessment of subtle changes in brain microstructure, particularly valuable for longitudinal measures of disease progression in small brainstem nuclei. Despite these new methodological developments which suggest that MRI will be a core technique for human brainstem research, many challenges remain in establishing brainstem MRI as a reliable and informative approach in clinical applications.

1.1.2. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

The main aim of this Action is to establish a multidisciplinary and transnational network for advancing human brainstem MRI with a view of establishing and extending its use in clinical research and practice. The following challenges need to be met to reach this aim (cf. Figure 1):

Challenge 1: Need for harmonization and best practices: Promising imaging and analyses approaches for anatomical & functional brainstem MRI are developed and employed in different imaging centres across Europe and the World, but no best practice is established. a) **Delineation procedures and atlases** used to identify specific brainstem structures in anatomical or functional recordings are generated with diverse approaches (*post mortem* data or different sequences in *in vivo* data) and can **deviate substantially in shape and volume**[16]. b) **Functional and anatomical imaging sequences developed by different centres vary in key parameters** which often renders comparisons between studies difficult (e.g. are specific brainstem structures equally visible in different anatomical imaging sequences?, Which acquisition resolution is optimal given various sizes of brainstem structures?). Finally, as analyses guidelines are usually based on MRI for cortical areas with more SNR and larger structures, c) **no analyses standards exist** on, for example, required SNR in functional brainstem imaging and required CNR in anatomical brainstem assessments or required spatial precision in aligning assessments across time or individuals. Importantly, defining **best practices in mapping, imaging, and analyses methods** therefore also requires a **consensus on specific quality control criteria**.

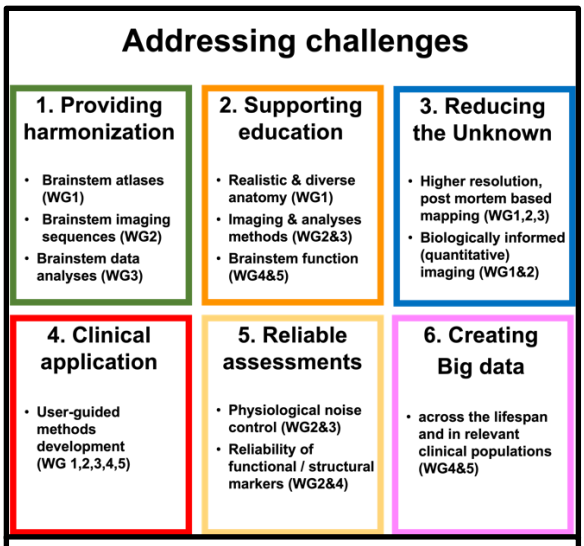

Figure 1: Overview challenges in human brainstem MRI and their link to specific work groups (WGs).

Challenge 2: Supporting education and knowledge sharing: Despite a suitable availability of MRI machines across Europe, brainstem MRI research is still dominated by a few and typically non-ITC (Inclusiveness Target Countries) in Europe (cf. Figure 2). At the same time, **research in non-ITC countries in Europe is internationally highly competitive** with about twice as many publications on brainstem MRI as compared to e.g. the US (0.41 vs. 0.2 publications per 100k inhabitants, source: pubmed). What is needed to advance brainstem MRI in Europe is a dedicated training of in particular YRIs (Young Researchers and Innovators) in ITCs with regard to a) **raising awareness** of the relevance of the brainstem in normal and pathological brain states, b) **best practices** for delineation, imaging, acquisition, and analyses methods in brainstem MRI, and c) introducing researchers to **realistic brainstem anatomy** based on a range of diverse *post mortem* and *in vivo* data and not schematic drawings of single *post mortem* brains in textbooks.

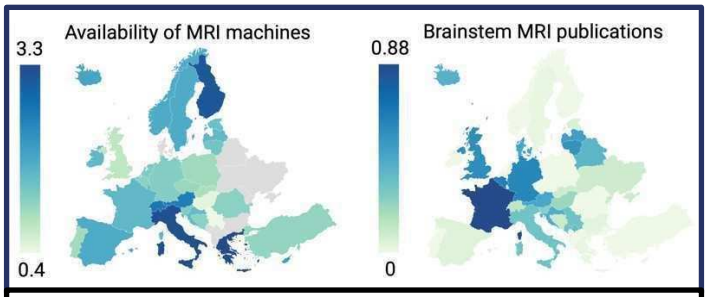

Figure 2: Left: Availability of MRI machines in 2020, grey: no data available, source: Eurostat. Right: Brainstem MRI publications, source: pubmed. Both: Data per 100k inhabitants.

Challenge 3: **Reducing the unknown across disciplines:** Brainstem MRI is still a pioneering field with many open questions and has reached a point where accelerating scientific progress is crucially dependent on a) consolidating and disseminating existing methodological knowledge, and b) setting up transnational research infrastructures for multidisciplinary exchange without which the necessary development of new methods and insights is not possible. Of the 158 known brainstem structures and substructures, only about 30-50 are currently accessible for in vivo research through 3D MRI atlases or probabilistic maps. Of the brainstem structures with available atlases and maps, only 5 appear to be investigated more intensively, that is, are addressed in more than 100 publications in human research (SN, PAG, VTA, LC and red nucleus (RN), source: pubmed). Moreover, available atlases and maps vary considerably in resolution and contained information on clinically altered anatomies as there is a gap between classical neuroanatomical delineations of brainstem structures, often performed on 2D histological sections from a few post-mortem brains, and in vivo MRI-based delineations, which have limited resolution but are in 3D and involve larger groups of participants, thereby capturing intersubject variability. Reducing the unknown means therefore a) **to reduce the uncharted areas** of the human brainstem anatomically with a particular focus of joining high precision *post mortem* assessments and

information on pathological alterations in *in vivo* data, b) **to increase data-driven knowledge** of normal and altered brainstem function and anatomy in humans.

Challenge 4: Integration of methods development and clinical application: Although suitable imaging protocols and analyses methods for brainstem MRI already exist, there is often a gap between advanced high-end ultra-high-field and ultra-high-resolution methods and clinically applicable protocols suitable for shorter imaging duration on lower field strengths.

Challenge 5: **Reliability of brainstem MRI markers:** If brainstem MRI is to be used as a diagnostic or therapeutic biomarker for detecting subtle changes within a single patient, anatomical and functional measurements need to be acquired with sufficient reliabilities, including adequate correction for physiological noise which in particular affects MRI recordings of the brainstem.

Challenge 6: **Building up Big Data:** Extensive datasets are needed to provide a reliable standard of brainstem anatomy and function in normal as well as various clinical populations across the lifespan. A comprehensive database for anatomical and functional *in vivo* brainstem MRI data in humans currently does not exist. This also delays the development of the next generation of AI-based analysis methods for detecting brainstem abnormalities which is reliant on big and various datasets, and needs to be addressed now in order to fully leverage the potential of emerging technologies[15].

1.2. PROGRESS BEYOND THE STATE OF THE ART

1.2.1. APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE OF THE ART

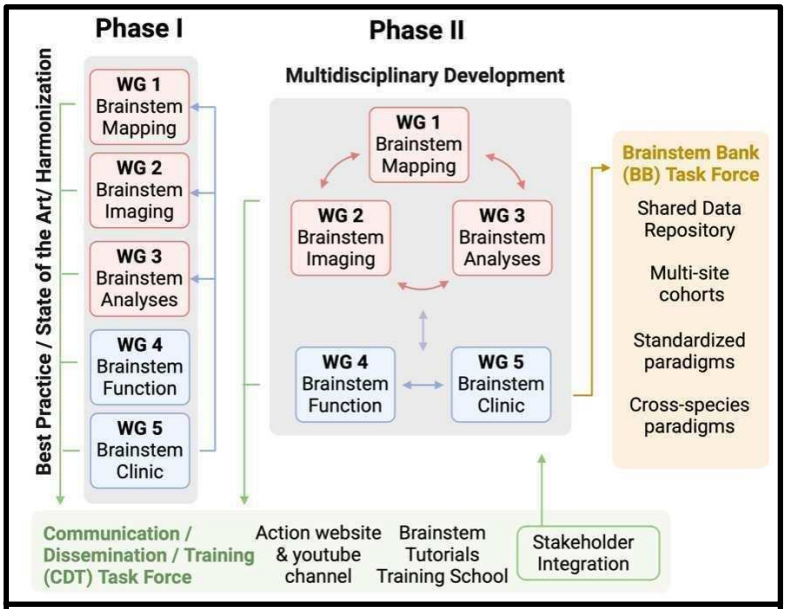

Figure 3: Overview of the Action network necessary to address the outlined challenges. Methodological Work Groups (WGs) in red and applied WGs in blue. Phase I will assess best practices also with a view on clinical applicability and identify gaps in knowledge and needs for methods development based on clinical demand. Phase II will work towards filling these gaps in multidisciplinary collaborations while including stakeholders (green) and creating lasting research infrastructures (brown).

Response to Challenge 1: Providing harmonization and best practice standards in brainstem MRI methods: Our multidisciplinary network will bring together leading European and international experts in brainstem mapping (Work Group, WG1), brainstem imaging (WG2) and brainstem MRI analyses (WG3). This will enable a) reviewing existing atlas resources, b) carrying out multisite comparisons of existing imaging approaches, and c) comparing different analyses approaches. Following a consensus on quality assessment criteria, the Action can therefore **determine reliabilities and best practices for brainstem MRI acquisition methods**, as well as support a better fit of imaging approaches with analyses and segmentation toolboxes. A particular focus in identifying best practices will be on **ensuring sufficient SNR and CNR with high resolutions** (which will typically be in the submillimeter range for functional ultra-high field imaging and millimeter range for lower field strengths, and millimeter or submillimeter range for anatomical imaging at ultra-high field or lower field strengths, depending on the biological specificity of anatomical imaging sequences)[4]. Moreover, recommendations will be made for **matching voxel resolutions with volumes and shapes of specific brainstem structures** to minimise partial voluming distortions.

Response to Challenge 2: Creating a 'one stop shop' for education in brainstem MRI: Together,

all work groups in the network will develop a comprehensive curriculum for multidisciplinary brainstem MRI training schools ('Brainstem Tutorials'). This includes the application of best practices in delineations, imaging and analyses, a comprehensive overview of current findings on brainstem function in normal and clinical populations, as well as information on realistic and diverse brainstem anatomy.

Response to Challenge 3: Joint exploration of the unknown in human brainstem anatomy and function: Current brainstem atlases and maps are in their majority based on standard anatomical sequences developed for the rest of the brain (e.g. T1w, T2w or diffusion imaging) which allow identification of a subset of brainstem structures visible in these sequences (e.g. PAG, SN, RN). Given the anatomical and neurobiological differences between the brainstem and the rest of the brain, in order to chart more currently invisible structures of the brainstem, it is necessary to a) optimize multivariate combinations of anatomical imaging sequences for identifying brainstem-specific neurobiological features and structures, and to b) bridge between *post mortem* histology, *post mortem* MRI and *in vivo* MRI to optimise multiple contrast mechanisms with regard to capturing specific biological properties in the brainstem (akin to a 'rosetta stone' for translating biological properties into MRI contrasts). The Action will **establish an iterative exchange between three typically separately operating methodological expertises in brainstem MRI** necessary for supporting these developments: Neuroanatomists (WG1), MR physicists (WG2), and analysis experts working with brainstem MRI data (WG3). Intriguingly, this multidisciplinary set-up will also allow to foster three methodological innovations of great relevance for brainstem MRI, namely a) to co-develop automatic segmentation algorithms for newly mapped structures in *post mortem* as well as in *in vivo* data which will increase the reliability and speed of atlas generation also in diverse populations, b) to base *in vivo* maps and atlases on highest-resolution *post mortem* assessments (between 50-200 micrometers[17]), which will allow for mapping also smaller brainstem structures and for 'future-proofing' brainstem mapping with regard to expected higher *in vivo* field strengths, as well as c) to facilitate a translation into quantitative *in vivo* imaging approaches which capture specific microstructural properties (i.e. tissue iron content, fibre and cell densities) in tissue-specific quantitative maps and offer a more site- and scanner independent anatomical assessment ideal for creating big and longitudinal datasets. As some structures will lack visible boundaries and contrast for being captured in anatomical imaging, a related important endeavour will be **to establish functional localizers based on standardised experimental paradigms** (WG4). Finally, reducing the unknown also means using advances in high resolution functional brainstem MRI and high-angular resolution diffusion MRI to advance our knowledge on the **function of brainstem structures as contributors within a network spanning the brainstem and connected cortical or downstream areas**[18]. Recent studies show that it is possible to address the study of the brainstem with graph-analysis and hierarchical models similar to those employed for the cortex[19]. Fostering network analyses including brainstem structures will enable connectivity-based parcellations and identifying joint contributions of brainstem structures (WG2, 4, 5). (e.g. Is there an equivalent to the resting state network in the brainstem?)

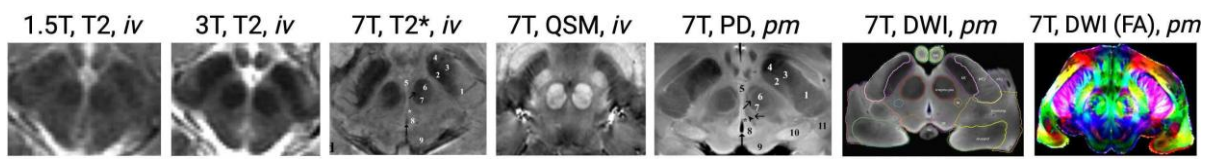

Figure 4: Examples of imaging RN and SN across different field strengths (1.5, 3T, 7T), *in vivo* (*iv*) or *post mortem* (*pm*) and with different imaging sequences. Only the two images on the left are acquired in the same person, numbers and outlines indicate labelled structures. Unpublished or reproduced with permission from Zhang et al. 2007, Donatelli et al. 2023, Adil et al. 2021.

Response to Challenge 4: Integration of methods development and clinical application: A particular focus of our Action will be on linking methods experts in brainstem MRI and experts in a wide range of relevant clinical conditions from the start of the Action (cf. Kick-off meeting in Figure 5). The aim is to **evaluate and develop brainstem MRI methods not in isolation but in a user-oriented and user-guided manner for clinical applications**. This means that best practices in data acquisition and analyses methods will be established for the main currently available field strengths (1.5T, 3T, 7T) (cf. Figure 4), including lower field strengths more commonly used in clinical applications. Using the established best practices, the Action will acquire data at different field strengths in the same sample, which will allow for assessing whether AI-based segmentation approaches on lower resolutions profit from training on various, also higher resolutions[15]. Imaging and analyses methods will be selected with a particular focus on shorter acquisition durations, automatization and ease of applicability. Moreover,

the Action is in a unique position to **orient methods development towards clinically relevant targets,** which is indispensable for a) accelerating our understanding of the brainstem's contribution to clinical conditions and b) ensuring that imaging and analyses methods are suitable for detecting atrophies and altered functional states typical for clinical conditions. As part of this endeavour, experts on genetic analyses (WG2), clinical conditions (WG5) and brainstem mapping (WG1) will work together to **establish genetic architectures of individual brainstem structures** to identify genetic underpinnings of brainstem regions and their roles in common brain disorders.

Response to Challenge 5: Reliability of brainstem MRI markers: A specific challenge in brainstem MRI is the comparatively low signal as well as higher exposure to physiological noise due to the brainstems position in the middle of the brain, far from receive coil array elements and its proximity to ventricles, large vessels, and respiratory organs. The Action will therefore bring together experts on physiological noise correction as well as recordings of sources of physiological noise in order to **establish best practices and explore new approaches for noise reduction in brainstem MRI** (e.g. through dedicated sequences such as multi-echo functional MRI for reducing the influence of cerebral spinal fluid (CSF) and vascular pulsations). Related to this, a special focus in our Action will be on **establishing reliabilities of anatomical and functional brainstem MRI approaches** through test-retest acquisitions performed as part of establishing best practices, and through comparing parallel and sufficiently powered datasets acquired within the Action. Moreover, reliabilities will be enhanced through fostering automatic over manual segmentation approaches, and a list of recommended experimental paradigms for eliciting reliable brainstem activations.

Response to Challenge 6: Joint creation of Big Data: In order to provide the necessary amount of parallel and sufficiently powered datasets, our Action will create a freely accessible growing database of *in vivo* brainstem MRI data in humans. Legal and formal requirements in establishing this database will be supported by the **'Brainstem Bank (BB) task force'** which includes external collaborators at online data repositories. Methodological experts in the network will support multi-site methodological harmonizations based on best practice approaches which will result in a growing number of state-of-the-art brainstem MRI datasets in diverse populations in this Action.

1.2.2. OBJECTIVES

1.2.2.1. Research Coordination Objectives

Research coordination objectives (RCOs) aim to coalesce the dispersed expertise in brainstem MRI research present in particular in Europe to create a worldwide unique transnational and multidisciplinary research infrastructure for human brainstem MRI. Fulfilling these objectives will ensure a lasting impact of our Action in shaping human brainstem MRI well beyond the four-year funding period.

RCO1 'Establishing and disseminating best practices & state of the art': The current state of the art of brainstem function in health and disease as well as current methodological standards in brainstem-specific anatomical atlases, imaging and analyses methods will be established by a network of internationally leading experts. Knowledge will be most importantly disseminated through 7 training schools, freely available videos and methods resources on the Action website and YouTube channel. **Tasks** 1,2,5,7,11, C1-C3; **Deliverables** 1-6, 9-12, 15,16, 22, 35-38; **Months** 4-48.

RCO2 'Multidisciplinary development': An important focus of our Action will be to build the multidisciplinary networks necessary to foster the development of new methods and insights for human brainstem MRI and facilitate their application in clinical research. This will be achieved in particular through interdisciplinary STSMs (Short-Term Scientific Missions), multidisciplinary meetings, and joint publications. **Tasks** M1-M6; **Deliverables** 26-34; **Months** 2-38.

RCO3 'Harmonized research infrastructures and joint data repositories: To assure a lasting impact of our Action on the future of human brainstem research, the Action will create harmonized research infrastructures, including best practices in brainstem MRI and analyses methods, resulting in a European network of imaging sites for multi-site studies. Members commit to sharing brainstem MRI data in EU-based repositories and search engines, to create a 'super dataset' of human brainstem data and to collaborate on multi-site cohorts to combat insufficient sample sizes in clinical research (about 13,160 shareable brainstem MRI datasets in 16 different clinical conditions and controls are already present or expected during the Action). This objective will be supported in particular through the 'Brainstem Bank' (BB) task force supporting legal and (data) administrative aspects of data sharing,

WG5 for multi-site cohort coordination, as well as experts in WG2 & 3 supporting methods implementation. **Tasks** 3, 12, B1, B2; **Deliverables** 7, 24, 39,40; **Months** 18-48.

1.2.2.2. Capacity-building Objectives

Capacity-building objectives (CBOs) aim at extending our network and building up a growing community of in particular YRIs from ITCs educated in brainstem MRI. Moreover, CBOs aim to increase the relevance of brainstem MRI in clinical research as well as to facilitate the adoption of results from our Action by industry partners as well as policy stakeholders.

CBO1: '**Building up a growing community of brainstem MRI experts'**: The Action will develop a growing community of in particular YRIs and clinicians with methodological knowledge and skills in brainstem MRI. This aim will be supported by an active recruitment of in particular YRIs in the Action through the Communication, Dissemination and Training (CDT) task force as well as through biannual training schools 'Brainstem Tutorials'. The Action is expected to grow in this manner by about 20 researchers and clinicians every 6 months. **Task** C3; **Deliverable** 37; **Months** 12-48.

CBO2: 'Dissemination and integration of stakeholders': The Action will integrate a network of stakeholders, including health professionals, patient organizations, and policymakers. The Action will approach 2 new stakeholder contacts (Policy or industry), and 5 new patient or health care professional organization contacts on average per year per site. **Tasks** 13, C4; **Deliverables** 25, 38; **Months** 12-48.

2. NETWORKING EXCELLENCE

2.1. ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Our Action will establish a unique network which brings together typically fragmented lines of research and disconnected stakeholders. By approaching the topic of brainstem MRI with this unprecedented scope, the Action will be able to support currently hindered innovations and breakthroughs in brainstem MRI as detailed below:

a) Creating an infrastructure for exchange between methods development, stakeholders, and experts applying the methods, in particular in the clinical domain. This exchange will stimulate a user- and stakeholder-oriented development of new delineation, imaging and analyses methods, both in terms of feasibility and applicability in clinical research (e.g. duration of sequences, costs of acquisition) as well as in terms of closing gaps in *in vivo* imaging methods needed in clinical assessments (i.e. Brainstem MRI might have the answer, but might not be aware of the question from clinical research or stakeholders). Moreover, existing research into (human) brainstem function typically focuses either on understanding a particular function (e.g. motor control or memory) or on understanding a particular anatomical structure (e.g. dopaminergic nuclei). Similarly, in the clinical domain, researchers typically focus on a particular condition (e.g. depression) or on the vulnerability of a particular structure. By bringing together cognitive, behavioural as well as neurological and psychiatric experts, the network will be able to **b) provide a comprehensive platform to understand the brainstem in its full complexity.** This means that the Action will be able to identify **transdiagnostic patterns in vulnerabilities** in brainstem structures (e.g. catecholaminergic nuclei in neurodegeneration and psychiatric conditions) and to foster an understanding of brainstem function based on how **networks of structures** act together to support a specific range of functions. The Action will also **c) support and accelerate the development of urgently needed new methods for mapping the brainstem and investigating it *in vivo***, which can only be achieved in this unique multidisciplinary set-up.

Moreover, a particular focus of our Action is on **d) Creating lasting knowledge-bases and databases to establish an infrastructure for human brainstem education and research in Europe and worldwide, which currently do not exist.** This includes establishing best practices in imaging and analysis methods, and supporting their implementation in training schools and national teaching curricula. Through a harmonization of methods around best practices and a commitment of participating researchers in the network to share data in central repositories, our network will establish a database of brainstem MRI data across the lifespan and across clinical populations. In order to assure the durability of these infrastructures, the digital homes of the associated websites and databases are with

participating universities as well in EU-based data sharing initiatives.

A network with relevant multidisciplinary expertise in human brainstem MRI as outlined above is currently missing at the European or international level. Existing research initiatives investigating the brainstem in Europe focus in their majority on animal models such as mice and zebrafish and are restricted to investigate specific functions (motor functions in MoThal, InterAct, ReticulOme, LocomotorIntegration, StartAct, EXPLORATOME, sleep regulation: SleepCirc, Reflexes: CoreInstincts, and memory: DREAM). Similarly, there are EU initiatives for improving MR imaging methods, however, with the notable exception of IronSleep, which investigates imaging approaches of the brainstem in sleep disturbances in PD, these typically focus on developing a particular imaging method (e.g. European Network on NMR Relaxometry (EURELAX)) or investigating a particular brain phenomenon (e.g. brain tumors in: Glioma MR Imaging 2.0 (GliMR)).

2.2. ADDED VALUE OF NETWORKING IN IMPACT

2.2.1. SECURING THE CRITICAL MASS, EXPERTISE AND GEOGRAPHICAL BALANCE WITHIN THE COST MEMBERS AND BEYOND

The proposers of the Action cover all relevant scientific areas required for leading a pan-European effort to advance brainstem MRI and its application in clinical research and practice as suggested in the objectives and challenges outlined above (cf. Figure 1, 3): **a) Brainstem neuroanatomists** (WG1) which use ultra-high field imaging and *post mortem* data to delineate brainstem structures and to build 3D probabilistic atlases of normal and pathologically affected brainstems; **b) MRI physicists and neuroscientists** (WG2) with expertise in biophysical modelling of MRI contrast mechanisms based on biological tissue properties, expertise in developing and using cutting-edge multi-contrast imaging relevant for characterising the diverse neurobiological anatomy of the brainstem *in vivo* across different field strengths (expertise in the Action includes: diffusion MRI, iron- and neuromelanin-sensitive MRI, QSM, quantitative mapping (R1, R2*, MT, PD), spectroscopy, PET, concurrent MR-PET), expertise in high-resolution task-related and resting-state functional MRI of the brainstem at various field strengths, and expertise in assessing physiological processes related to brainstem function (perfusion, cerebrovascular reactivity, CSF dynamics); **c) MRI analyses experts** (WG3) who develop toolboxes tailored for the analysis of brainstem neuroimaging data (including physiological noise correction, high precision spatial transformations, automatic segmentations of brainstem structures); **d) neuroscientists working on humans and animal models** (WG4) investigating brainstem-related functions across the lifespan with expertise in paradigms and experimental set-ups which allow to link functional MRI recordings of the brainstem to cognitive and emotional functions, sleep, muscular control, gut control, vascular control, vestibular control, and intraneural recordings of the sympathetic nervous system; **e) clinicians, clinical researchers and neurosurgeons** (WG5) with expertise in using MRI or brainstem MRI in the following clinical conditions: Alzheimer‘s disease, Parkinson‘s disease, Lewy-Body dementia, Wilson‘s disease, amyotrophic lateral sclerosis, epilepsy, migraine, chronic pain, brain tumours, ADHD, autism, essential tremor, movement disorders, dystonia, glioma, schizophrenia, Hunter-Cheyne-Stokes respiration disease, sleep disorders, chronic fatigue and long-term COVID, as well as brainstem genetics; and **f) cognitive and clinical researchers with expertise in pertinent intervention approaches for the brainstem** (WG4&5) (non-invasive vagus nerve stimulation, pharmacological interventions, neurosurgery and deep-brain stimulation (DBS)). The scientific background of the proposers for this Action is well-balanced across a wide range of disciplines including Engineering, Natural, Medical, Clinical, Health and Basic Sciences. The Action is also already suitably balanced with regards to expertises, geographical distribution, age and gender, which will facilitate a balanced growth of Action memberships within Europe. In **extending the Action**, a particular focus will be on adding more expertise on relevant clinical conditions (e.g. multiple sclerosis) as well as on including researchers working in relevant industries for applying brainstem MRI (scanner manufacturers, or software developers) as well as medical or pharma companies. Existing collaborations of proposers of this Action with relevant industry partners and employees in medical companies will facilitate this targeted expansion. As the Action **aims to involve in particular YRIs from ITC countries as our Action grows**, the leads of the CDT task force, which includes the lead for scientific communication management and junior researcher coordination, will aim to preferentially include ITC YRIs. Finally, given their multidisciplinary backgrounds, our network of proposers will provide access to relevant research resources for our Action including hospital patients, patient networks, collections of biological samples and imaging databases.

2.2.2. INVOLVEMENT OF STAKEHOLDERS

As our Action will generate relevant knowledge for various patient groups and stakeholders in the health industry, an involvement of stakeholders at all levels and throughout the Action will be continuously supported. Engagement of stakeholders already linked to the Action or proposers of the Action as well as the **acquisition of new stakeholders and their integration in the Action will be coordinated and supported by the CDT Task force** (cf. Figure 3). The following stakeholders will be included:

1. Researchers: Action members are well-connected to relevant scientific communities, serving as regular members, board members, and mentors in European and other international scientific societies with relevance for brainstem MRI as well as coordinators in local PhD and MD programs. These contacts will be used and extended to inform and recruit in particular YRIs in ITCs. Freely accessible online educational resources and regular training schools will democratize knowledge on human brainstem MRI and related clinical conditions (for more details on activities supporting knowledge transfer and integration of researchers see 3.1.2). **2. Healthcare professionals:** An early involvement of healthcare professionals in developing innovative research approaches is crucial in order to facilitate the application of brainstem MRI into clinical practice. Indeed, several of the Action network members are healthcare professionals as well as researchers. Throughout the Action, the CDT task force will coordinate and further extend the involvement of healthcare professionals. This will take place through dedicated multidisciplinary webinars, involvement in WG and Action meetings, lectures via open education resources as well as contributions to national educational curricula of healthcare professionals. **3. Patients and carers:** The Action will maintain existing and develop new contacts with national and European patient and carer networks. Patients and carers will play a crucial role as 'Patient researchers' in generating new ideas, improving research approaches and disseminating results. Regular meetings, (online) surveys, workshops, focus groups, and dedicated patient-researcher symposia in yearly Action meetings will ensure their involvement in research in the Action. **4. Industry Partners:** Collaborations with relevant industry partners (pharmaceutical industry, medical device manufacturers (e.g. for brain stimulation), manufacturers of MRI scanners and developers of MRI analyses toolboxes) already exist within the Action. The Action is therefore in an excellent position to foster methods development also in collaboration with industry partners and to work towards implementing new methods standards into standard industrial releases and products (e.g. establishing a ‘brainstem MRI suite’ in scanner software packages and a ‘brainstem analysis toolbox’, in prevalent analyses softwares. New contacts with further industry partners will be sought continuously during the Action. Industry partners will actively participate in network activities, including WG meetings, research visits, training schools, and workshops. Contacts with stakeholders may also lead to academia-industry spin-off research and the development of research projects on a local and pan-European level. **5. Government agencies and policy makers:** Early involvement of policy makers, including government and healthcare system representatives, is crucial for brainstem MRI implementation and dissemination in clinical applications. The CDT task force will coordinate personalized and consistent contact with relevant policy stakeholders at each site/country.

3. IMPACT

3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAKTHROUGHS

3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

Impact to science: The following contributions of our Action will ensure a significant impact on science: **In the short term**, a) the evaluation and harmonization of brainstem MRI methods will result in best practice recommendations including quality controls. This will enable b) the accumulation of larger less heterogeneous datasets, c) establishing harmonized multi-site research infrastructures and shared data repositories in diverse samples across the lifespan and across clinical conditions, and d) developing and assessing innovative AI-based methods for data analyses. As a result of these innovations and through establishing a multidisciplinary research network, the following breakthroughs in brainstem MRI will be facilitated **in the mid and long term**: a) reliable anatomical and functional biomarkers for earlier diagnosis, personalised interventions and disease monitoring, b) an extension of the amount of brainstem structures accessible for *in vivo* clinical research, and c) the identification of new intervention targets and approaches.

Impact for society: MRI is successfully and routinely used to diagnose and monitor a number of

prevalent health conditions like cancer, stroke, or multiple sclerosis. **In the short term**, our Action will raise awareness of the relevance of pathological alterations in the brainstem amongst policy makers, healthcare workers and the general public (for details on specific activities see 2.2.2). **In the mid and long term**, **making the brainstem more accessible for medical investigations through brainstem MRI will have immense societal impact** given the often particular vulnerability of the brainstem in neurological and psychiatric conditions. Among the many health conditions in which brainstem structures appear to be intricately involved are neurodegenerative conditions, expected to affect 153 Million people worldwide in 2050[20]; anxiety and affective disorders affecting 586 Million people worldwide[21]; sleep disturbances affecting about 30% of older adults[22]; and chronic pain affecting 19% of Europeans[23]. If successful, work in our Action will for example allow a) to determine whether anatomical and functional changes in brainstem structures can serve as earliest indicators of dementia risk, b) to identify new potential DBS targets for therapeutic interventions, c) to delineate whether a corrective regulation of the pain network in the brainstem can alleviate chronic pain, or d) to assess whether functional brainstem MRI can inform risk factors as well as personalized treatments in affective and anxiety disorders. Moreover, earlier diagnoses and improved treatments in these diseases and disorders will contribute substantially to reducing health care costs.

MEASURES TO MAXIMISE IMPACT

3.1.2. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

Knowledge creation: Through regular meetings within work groups but also through multidisciplinary meetings and interdisciplinary STSMs, our Action will provide the multidisciplinary network necessary to create vital new knowledge on the human brainstem. **In the short term**, this will result in: a) Identifying optimal approaches for functional and anatomical brainstem MRI at various field strengths; b) Advancing our knowledge on normal and pathologically-altered human brainstem function, in a wide range of clinical conditions; c) Identifying new targets for disease-modifying interventions and disease prevention in the brainstem. **In the mid and long term**, this will allow us to: a) Integrate best practice methods in standard industry release of MRI machines ('brainstem MRI suite') and prevalent analyses softwares for MRI data (e.g. SPM, FSL, FreeSurfer, etc.); b) Improve and extend current atlases of the human brainstem, including a better representation of diverse healthy as well as clinical populations across the whole lifespan; c) Render more brainstem structures accessible for *in vivo* investigations.

Knowledge transfer: The CDT task force, which includes the 'education lead', coordinates knowledge transfer in the action and is responsible for collecting and making accessible online and training materials for the **'Brainstem Tutorials' training schools**. Schools will be held twice a year, predominantly in ITC countries, will feature inter- and multidisciplinary sessions, and will include in-person expert tutorials, hands-on workshops and video lectures. The aim is to enable participants to implement brainstem MRI at the current state of the art. An online **'Brainstem Tutorials User Group'**, which is supported once per month by experts from the methods WGs, will provide continuous personal support for trainees. An important feature of our Training schools is furthermore the involvement of relevant stakeholders from patient organisations and industry partners in 'expert chats' with the aim of providing attendees with background information on the relevance of brainstem MRI. A further important instrument of knowledge transfer in our Action is the **Action website** which will be developed to serve as a 'one stop shop' and knowledge repository for Action members (in a private section) but also interested external researchers, patients or other stakeholders (in a public section). The Action website will contain a) information about Action members and the aims of our Action, b) links to brainstem atlases and instructions on how to use them, c) Information on and download links for brainstem MRI sequences including example datasets, d) tutorials and links for brainstem analyses toolboxes, e) information about Activities within the Action (training schools, work group meetings, Action meetings, scientific findings, public lectures, etc.), f) information for current (and potential future) Action members on how to participate in the Action /join the Action, g) a 'Market for STSMs' which will allow labs to advertise open research visit positions, upcoming meetings and ongoing research projects. The Action will also establish a **YouTube channel** and **Podcast** to provide lectures and knowledge clips on brainstem structures and their relevance in clinical conditions. The YouTube channel will feature hour-long lectures (e.g. 'Challenges in histological mapping of the brainstem') and shorter 'knowledge clips' of about 5 minutes with introductory information on individual brainstem structures and the relevance of the brainstem in specific clinical conditions. The podcast will feature weekly episodes highlighting new findings, researchers, and ongoing research questions. The podcast will encourage exchanges between disciplines, members, and stakeholders. Video and podcast creation will be supported by external

dissemination experts. Apart from knowledge transfer instruments within the Action, a continuous effort will be made to include newest insights into the human brainstem as well as knowledge captured in training materials into **local University curricula** of members of the Action. This transfer will be facilitated through various already existing neuroscientific teaching engagements by Action members.

Career development: YRIs are an important part of the leading team (5 leads and 2 co-leads), and a crucial focus in the Actions professional development efforts through training schools, STSMs, participation in third-party conferences and the Actions dissemination activities. They contribute to research outputs on equal footing and will form a **'YRIs network'** to communicate needs and suggestions to the AMC (Action Management Committee) and as an information hub for opportunities within the Action for YRIs. Through a **mentoring scheme**, experienced researchers and YRIs will be paired up, with a preference for interdisciplinary pairings, to support individual career developments via STSMs, research collaborations, virtual and in person exchange, and career counselling. A particular focus on supporting grant applications will allow YRIs to establish research groups and engage in longer-term research visits. The network's unique multidisciplinary expertise ensures YRIs are competitive in developing unique and relevant lines of research and leveraging industry contacts.

3.1.3. PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

Given the considerable societal relevance of brainstem health, a **continuous dialogue with the public and stakeholders is a moral obligation and already common practice amongst Action members**. **Scientific results** of the Action will be disseminated through conferences, symposia and publications in high impact scientific journals as well as on preprint servers in order to ensure public accessibility. New research results across the Action will be collected once per month by the CDT Task Force and disseminated via the Action website, updated '*Brainstem Tutorials'*, as well as communicated to Action members, associated patient organisations, press outlets and stakeholders, via social media accounts of the Action, email newsletters, and where appropriate, the Action podcast and YouTube channel. To ensure **accessibility, user-orientation, and impact of the communication**, the task force will be supported through an external dissemination expert partner (to help with e.g. infographics and animations). The Action will additionally communicate brainstem research through **public lectures and lectures for patient organisations** by network members in each member state (at least one lecture per year /per member). The CDT task force is furthermore in charge of creating a yearly research report which will be circulated to stakeholders and Action members. (see also 2.2.2 for more details on integration of and dissemination to stakeholders). A plan for **exploiting results of the Action** will be formulated during the kick-off meeting. It will include a) establishing contacts with local transfer offices for translating research results into patents, spin-offs and business ideas, b) formation of an 'Exploitation network' in the Action through the CDT task force which will link researchers experienced in exploitation with inexperienced interested researchers, and c) dedicated mixed industry-research workshops during the yearly Action meetings for identifying exploitation targets.

4. IMPLEMENTATION

4.1. COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES

Implementation will proceed in two general phases (cf. Figure 3). In **Phase I**, WGs will assess current best practices and states of the art. Methods WGs (WG1-3) will exchange in particular with the clinical WG (5) already at the kick-off meeting in order to **orient best practice assessments towards ease of application in clinical research and practice** (cf. Figure 5: violet colouring at beginning of Task 1, 2, 5, M1, M2, M5). **Phase II** will be dominated by multidisciplinary tasks to support new developments in brainstem MRI. **An overview of implementation tasks and activities can be found in Figure 5.** The aims of the WGs and TFs in the Action are as follows:

WG1, Brainstem Mapping: a) provide an overview of existing probabilistic atlases for the brainstem, b) improve access to specialized neuroanatomical knowledge of the brainstem, c) establish a research platform supporting the development of new *post mortem* validated submillimeter resolution 3D probabilistic atlases of brainstem structures in diverse populations *in vivo*.

WG2, Brainstem imaging: a) establish current best practices and reliabilities in brainstem MRI

protocols through multi-site evaluations, b) facilitate the translation of advanced neuroimaging protocols from ultrahigh-field imaging to clinical settings and from development to clinical application, c) establish a platform for identifying, jointly developing, and promoting new brainstem characterization tools that integrate pulse sequence development with anatomical priors and image segmentation tools, d) establish a research infrastructure which will allow to increase the specificity of brainstem neuroimaging by bridging histology and neuroimaging.

WG3, Brainstem Analyses: a) establish best practice pipelines for automatic brainstem segmentations in anatomical brainstem data and for analysing functional brainstem data; integrate these into standard analyses toolboxes, b) establish a research platform for extending available automatic segmentations, improving denoising approaches and integrating additional physiological measurements, c) establish multivariate genetic architectures of brainstem structures based on more precise segmentations.

WG4, Brainstem Function: a) provide an overview of the current insights into human brainstem function, including underexplored functions (e.g. autonomic-central nervous system interactions), also based on meta-analyses, b) assess the reliability of functional brainstem activations in large and multi-site datasets, explore functional networks of brainstem structures, c) establish a consensus on experimental set-ups for investigating brainstem functions including functional localizers, d) establish cross-species paradigms.

WG5, Brainstem Clinic: a) provide an overview of our current knowledge on the brainstem in clinical conditions, b) identify new targets for methods development as well as interventions to improve brainstem-related clinical conditions, c) establish a network of researchers and cohorts for multi-site brainstem MRI and brainstem interventions, d) foster contacts with policymakers to promote the relevance of brainstem MRI in clinical assessments and therapies.

CDT (Communication, Dissemination, Training) Task Force: support communication within the Action and between the Action and external parties. Support the dissemination of research results and integration of stakeholders as well as establish, update and support training schools. **Tasks: C1 – C5.**

BB (Brainstem Bank) Task Force: establish a data repository with brainstem MRI data from patients and controls. Support legal and formal aspects of data sharing throughout the Action (**Tasks B1 & B2**).

Tasks and activities in Phase I: Best practice / State of the Art /Harmonization: Task1: Overview of available atlas information of subcortical and brainstem structures *in vivo:* a) manual delineations including detailed instructions, b) automatic algorithmic approaches, c) probabilistic atlases. Distinction of approaches suitable for imaging in clinical settings.(**WGs 1, 5**). **Task2: Define best practices in current brainstem MRI sequences:** Travelling head study across 3 sites and 9 scanners to compare current functional and anatomical brainstem MRI protocols, establish test- retest reliabilities and quality control standards at 1.5T, 3T and 7T with a particular focus on imaging methods suitable for clinical research and application; consideration of relevant differences between scanner manufacturers.(**WGs 2, 5**) **Task3: Industry implementation and site harmonization:** Promote adoption of harmonized brainstem MRI sequences by industry partners and supporting implementation within the Action.(**WG2**) **Task4: Quantitative brainstem MRI:** Establish best practices in quantitative neuroimaging protocols for brainstem anatomical imaging across field strengths.(**WG2, WG5**) **Task5: Define best practices in analyses methods:** a) physiological noise correction and spatial precision in post-processing pipelines for functional MRI (quality control: e.g. minimum SNR), b) automatic segmentation approaches to estimate volume/integrity in anatomical MRI (quality control: e.g. permissible partial voluming). Emphasis on automated analyses approaches suitable for clinical research and application.(**WGs 3, 5**) **Task6: Characterize multivariate genetic architecture of brainstem structures:** Employ brainstem structure-specific segmentation in large imaging genetics repositories, (e.g. UK Biobank). Conduct a multivariate genome-wide-association-study (GWAS) of brainstem structures, taking into account the phenotypic correlation structure of different structures to boost discovery of genes associated with brainstem structure and function and establish their roles in common brain disorders.(**WGs 2, 3, 5**). **Task7: Overview of brainstem structures in functional *in vivo* assessments:** Review of brainstem structures that can be reliably studied with current functional imaging protocols and mapping approaches; current developments in automatic segmentations and high-resolution functional imaging; consensus on desirable new targets which are methodologically within reach.(**WG4**) **Task8: Capitalize on existing data to extend our knowledge of human brainstem function and connectivity:** Reanalyse suitable existing datasets (e.g. ultra-high-resolution datasets) with brainstem-specific approaches and masks to add to current knowledge on brainstem

function and functional networks in the brainstem and beyond.(**WG4**) **Task9: Overview of study paradigms to investigate brainstem function:** Recommendations for standardized study paradigms to assess brainstem function, including multimethod experimental set-ups based on the current understanding of regulatory pathways of brainstem structures (e.g. autonomic functions, muscular assessments, etc.), recommendations for functional localizers.(**WG4**) **Task10: Protocols for cross-species research:** Develop parallel study protocols for human and animal models to improve cross-species comparability in brainstem research, focusing on functional similarities and differences in several species.(**WG4**) **Task11: Assess current knowledge on the role of the brainstem in clinical conditions.** Overview of typical brainstem alterations in clinical conditions, identification of transdiagnostic vulnerabilities.(**WG5**) **Task12: Establish a network of patient cohorts as a basis for future multi-site studies**. Available patient cohorts for brainstem MRI will be registered early in the Action. Methods harmonization and recommended standard paradigms will facilitate the implementation of multi-site studies.(**WG5**) **Task13: Establish contact with policy makers to promote integration of brainstem MRI in clinical assessments**.(**WG5**)

Tasks and activities in Phase II: Multidisciplinary Development: In Phase II, the Action will build on the established interdisciplinary exchanges and best practices to foster the development of new methods and the accumulation of diverse brainstem MRI data: **TaskM1: Establish interdisciplinary exchanges to increase clinical applicability of new brainstem MRI protocols:** Identify relevant new targets for brainstem MRI protocols, as well as ensuring their applicability in clinical practice in joint meetings of clinical and cognitive neuroscientists with MR Physicists.(**WGs 2, 4, 5**). **TaskM2: Establishing exchange between methods development and application:** Determine for which structures atlas generation should be prioritized based on clinical relevance; provide anatomists and basic researchers with information and easier access to data on typical deformations and dysfunctions of brainstem structures in clinical conditions through an exchange between anatomists, cognitive researchers and clinical researchers.(**WGs 1, 4, 5**). **TaskM3: Setting up an interdisciplinary expert network to extend brainstem mapping:** Exchange between brainstem anatomists, brainstem imaging and analyses experts to support joint methods development to a) validate *in vivo* parcellations with *post mortem* histology as well as to b) develop automatic segmentation approaches for *post mortem* as well as *in vivo* data to increase available maps for brainstem structures and render segmentations on *in vivo* data more reliable.(**WGs 1, 2, 3**). **TaskM4: Integration between *in vivo* MRI and histology for new sequence development:** Advance the understanding and development of MRI contrast mechanisms in the brainstem through bridging post-mortem and in vivo approaches ('rosetta stone' for biological properties and MRI contrasts).(**WGs 1, 2**). **TaskM5: New brainstem-specific analyses methods:** Identify unmet needs in brainstem MRI for new user-friendly analyses approaches (e.g. automatic noise corrections, automatic segmentations, etc.)(**WGs 2, 3, 4, 5**). **TaskM6: Identify new targets for interventions in the brainstem**: Exchanges between basic and clinical brainstem MRI researchers familiar with intervention approaches (i.e. pharmacological, invasive or non-invasive stimulation) to identify new targets for interventions to improve brainstem function.(**WGs 4, 5**)

4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

Deliverables (D) (grouped by Tasks, cf. Figure 5)	Month(s)
Task1: D1 Linked atlas resources on Action website with instructions for users / **D2** Training school materials 'Mapping the brainstem' / **D3** Overview paper 'Brainstem mapping'.	**6** / **8** / **12**
Task2: D4 Consensus paper: 'Recommended sequences for brainstem MRI' / **D5** Training school materials 'Human brainstem MRI protocols' / **D6** Downloadable list of recommended imaging protocols, standard operating procedures and quality assurance tools (including also vendor-independent implementations using Pulseq).	**8** / **10** / **14c**
Task3: D7 Installation of harmonized imaging protocols across Action sites / **D8** Promote adoption of 'brainstem MRI suite' by manufacturers (Siemens, Philips, GE).	**18c** /**20c**

Task4: D9 Reference dataset of current quantitative brainstem imaging protocols.	**30**
Task5: D10 Best practice paper 'Brainstem analyses' / **D11** Training school materials 'Analysing brainstem data' / **D12** Download links for methods toolboxes on Action website / **D13** Integrate brainstem analysis tools in well-established neuroimaging softwares (SPM, FSL, freesurfer, MRtrix3, ANTs, fMRIprep, Neurodesk etc.).	**8** / **10** / **12c** /**16c**
Task6: D14: Report 'Genetic architecture of individual brainstem structures'. **D15**: Scripts and tutorials for GWAS brainstem analysis freely available on Action website; GWAS summary statistics shared in open repositories.	**36** / **40**
Task7: D16 Consensus paper 'Current and future targets in functional brainstem MRI' / **D17** Training school materials 'Human brainstem function'.	**8** / **10**
Task8: D18 min. 4 papers: Reanalyses and meta-analyses of functional brainstem activations and connectivity in existing datasets with suitable resolution and tSNR.	**18**
Task9: D19 2-3 overview papers 'Paradigms for assessing brainstem function/Functional localizers' / **D20** Downloadable study protocols and paradigms.	**22** / **26**
Task10: D22 Consensus paper 'Recommendations for cross-species assessments of brainstem function' / **D23** Study protocols including downloadable task paradigms for conducting brainstem MRI in humans and different species.	**42** / **48**
Task11: D24 Overview paper 'Brainstem alterations in clinical conditions' / **D24** Training school materials 'Brainstem alterations'.	**8** / **10**
Task12: D25 Multi-site cohort coordination: Description and documentation of available clinical cohorts at Action member sites.	**4c**
Task13: D26 Exchange with policy makers (1 new contact per site/country per year)	**2c**
TaskM1: D27 Methods paper 'Brainstem MRI in the clinic' / **D28** Consensus paper 'New avenues for *in vivo* brainstem MRI in clinical conditions'.	**18** / **24**
TaskM2: D29 Establish subsection in online data repositories for *post mortem* anatomical brainstem data from different clinical cohorts for data and image sharing between clinicians and anatomists / **D30** Roadmap paper 'New targets for mapping the brainstem in clinical conditions'.	**2** / **18**
TaskM3: D31 Roadmap paper 'Towards easier, more precise and more reliable brainstem mapping'.	**30**
TaskM4: D32 Roadmap paper: 'Histology-informed mechanisms of MRI contrast mechanisms in human brainstem in health and pathology'.	**36**
TaskM5: D33 Pilot dataset: SNR achieved using different denoising approaches / **D34** Methods paper 'Reducing noise in brainstem recordings' / **D35** Opinion paper: 'Towards easier and more reliable brainstem MRI analyses.	**20** / **24** / **30**
TaskM6: D36 Consensus papers 'High priority targets for brainstem interventions', 'The future of interventions targeting the brainstem'.	**38**
TaskC1: D37 Action website set-up / **TaskC2: D38** YouTube channel setup & support creation of information videos from all WGs (4-6 videos /month) / **TaskC3: D39** Collecting training school materials from all WGs / **TaskC4: D40** Stakeholder integration in WG activities.	**6** / **4c** / **8c** / **4c**
TaskB1: D41 Establish site- and dataset specific data sharing agreements, apply BIDS transformations / **Task B2: D42** Continuous support and advertisement of data sharing.	**12c** / **14c**

4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

Risk1 (low): Misunderstandings across disciplines. **Mitigation:** The Action initiates interdisciplinary exchanges at the kick-off meeting which will help establish a common nomenclature.

Risk2 (low): Differences in prior knowledge levels render training efforts challenging. **Mitigation:** Each learning module in the training school will be tailored to different entry levels of expertise, starting with basic concepts and advancing to more complex topics.

Risk3 (mid): Difficulties to translate imaging sequences to lower field strengths. **Mitigation:** It is expected that not all, in particular functional, imaging approaches can be translated to lower field strengths. Nonetheless, anatomical imaging of brainstem structures is already successfully employed e.g. at 1.5T by Action members. The Action will ensure that the range of quality-assured and clinically suitable brainstem imaging approaches also on lower field strengths is maximised.

Risk4 (high): Smallest brainstem structures are out of reach for the current resolution of imaging and analyses methods. **Mitigation:** Current technical possibilities limit possible resolutions in data acquisitions with suitable acquisition time constraints. Nonetheless, methods developments enabled by this Action (i.e. noise reduction, biologically targeted sequence development, etc.) will optimize resolutions within the current 'hardware restrictions' and provide the basis to improve possible resolutions beyond the current state of the art. In addition, given our focus on highest resolution atlas and sequence validations in *post mortem* data, our Action is in an excellent position for preparing methods development in anticipation of increasing field strengths.

Risk5 (low): Adherence to harmonized analyses and imaging standards will be slow or reluctant. **Mitigation:** Thresholds to adopting harmonization standards will be lowered by a) prioritizing automatized and easy to use methods, b) assuring free access to methods resources, c) support networks which are regularly monitored by methods experts, d) sequence installations supported by experts in the networks experienced in multi-site set-ups.

Risk6 (mid): Researchers will be reluctant to share data in the brainstem bank database. **Mitigation:** Thresholds to data sharing will be lowered through a) the use of established data sharing platforms well versed in different requirements for data sharing and GDPR regulations across different countries in Europe/worldwide, b) administrative support in preparing legal and formal aspects of data sharing.

4.1.4. GANTT DIAGRAM

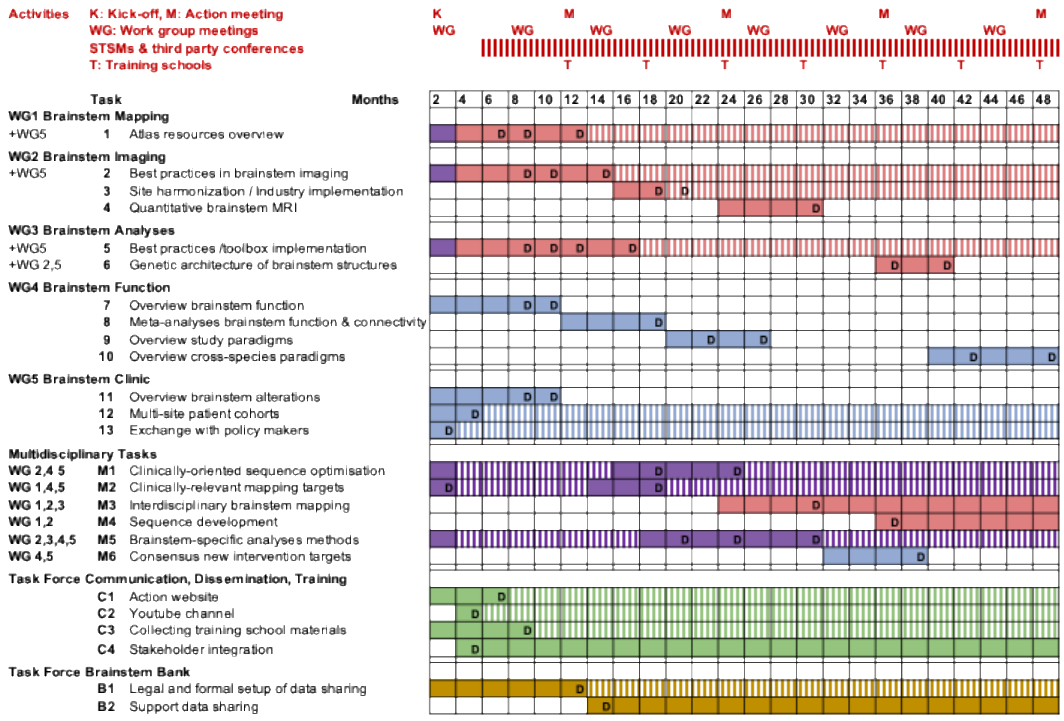

Figure 5: Overview of Activities and Tasks. Methods-oriented (also multidisciplinary) tasks in red, Function and clinically-oriented (also multidisciplinary) tasks in blue; multidisciplinary collaborations between methods, basic science and/or clinical experts in violet. Time points of deliverables: D; striped areas: periods of continued task pursuit based on established consensus or infrastructures (e.g. updating website, multi-site cohorts, methods developments etc.).

References

1. Akeret, K. et al. Topographic volume-standardization atlas of the human brain. Brain Struct Funct 226, 1699–1711 (2021).

2. Anatomica, T. Federative committee on anatomical terminology. (1998).

3. Ehrenberg, A. J. et al. Priorities for research on neuromodulatory subcortical systems in Alzheimer's disease: Position paper from the NSS PIA of ISTAART. Alzheimer's & Dementia (2023).

4. Cai, J., Wang, Y. & McKeown, M. J. Advances in functional and structural imaging of the brainstem: implications for disease. Curr Opin Neurol 37, 361–368 (2024).

5. Braak, H., Thal, D. R., Ghebremedhin, E. & Del Tredici, K. Stages of the Pathologic Process in Alzheimer Disease: Age Categories From 1 to 100 Years. Journal of Neuropathology & Experimental Neurology 70, 960–969 (2011).

6. Kelberman, M. A. et al. Age-dependent dysregulation of locus coeruleus firing in a transgenic rat model of Alzheimer's disease. Neurobiology of Aging 125, 98–108 (2023).

7. Damier, P., Hirsch, E. C., Agid, Y. & Graybiel, A. M. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. Brain 122 (Pt 8), 1437–1448 (1999).

8. Sasaki, M. et al. Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. Neuroreport 17, 1215–1218 (2006).

9. Suárez-Pereira, I. et al. The Role of the Locus Coeruleus in Pain and Associated Stress-Related Disorders. Biological Psychiatry91, 786–797 (2022).

10. Maia, T. V. & Frank, M. J. From reinforcement learning models to psychiatric and neurological disorders. Nature Neuroscience14, 154–162 (2011).

11. Perna, G., Alciati, A., Sangiorgio, E., Caldirola, D. & Nemeroff, C. B. Personalized Clinical Approaches to Anxiety Disorders. Adv Exp Med Biol 1191, 489–521 (2020).

12. Mills, E. P. et al. Altered Brainstem Pain Modulating Circuitry Functional Connectivity in Chronic Painful Temporomandibular Disorder. The Journal of Pain 22, 219–232 (2021).

13. Ossipov, M. H., Morimura, K. & Porreca, F. Descending pain modulation and chronification of pain. Current Opinion in Supportive and Palliative Care 8, 143 (2014).

14. Kan, S. et al. Three weeks of exercise therapy altered brain functional connectivity in fibromyalgia inpatients. Neurobiology of Pain 14, 100132 (2023).

15. Billot, B. et al. SynthSeg: Segmentation of brain MRI scans of any contrast and resolution without retraining. Medical Image Analysis 86, 102789 (2023).

16. Dickie, D. A. et al. Whole Brain Magnetic Resonance Image Atlases: A Systematic Review of Existing Atlases and Caveats for Use in Population Imaging. Front. Neuroinform. 11, (2017).

17. Adil, S. M. et al. A high-resolution interactive atlas of the human brainstem using magnetic resonance imaging. Neuroimage 237, 118135 (2021).

18. Meola, A., Yeh, F.-C., Fellows-Mayle, W., Weed, J. & Fernandez-Miranda, J. C. Human Connectome-Based Tractographic Atlas of the Brainstem Connections and Surgical Approaches. Neurosurgery 79, 437 (2016).

19. Wang, M. et al. Brainstem Functional Parcellation Based on Spatial Connectivity Features Using Functional Magnetic Resonance Imaging. in Artificial Intelligence (eds. Fang, L., Pei, J.,

Zhai, G. & Wang, R.) 452–460 (Springer Nature, Singapore, 2024). doi:10.1007/978-981-99-9119-8_41.

20. Livingston, G. et al. Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. The Lancet404, 572–628 (2024).

21. WHO. Depression and Other Common Mental Disorders: Global Health Estimates. (2017).

22. Du, M., Liu, M., Wang, Y., Qin, C. & Liu, J. Global burden of sleep disturbances among older adults and the disparities by geographical regions and pandemic periods. SSM - Population Health 25, 101588 (2024).

23. Breivik, H., Collett, B., Ventafridda, V., Cohen, R. & Gallacher, D. Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. European Journal of Pain 10, 287–333 (2006).