

Neuroanatomy of the grey seal brain: bringing pinnipeds into the neurobiological study of vocal learning

Nienke Hoeksema*^{1,2}, Laura Verga^{3,4}, Janine Mengede¹, Corné van Roessel¹, Stella Villanueva⁵, Anna Salazar-Casals⁵, Ana Rubio-Garcia⁵, Branislava Ćurčić-Blake⁶, Sonja Vernes^{1,7,8}, and Andrea Ravignani*^{4,5}

¹Neurogenetics of Vocal Communication Group, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands

²Neurobiology of Language Department, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands

³Faculty of Psychology and Neuroscience, Department NP&PP, Maastricht University, Maastricht, The Netherlands

⁴Comparative Bioacoustics Group, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands

⁵Research Department, Sealcentre Pieterburen, Pieterburen, The Netherlands

⁶Cognitive Neuroscience center, University Medical Center Groningen, Groningen, the Netherlands

⁷Donders Institute for Brain, Cognition & Behaviour, Nijmegen, The Netherlands

⁸School of Biology, University of St Andrews, UK

* Corresponding authors. Please email nienke.hoeksema@mpi.nl; andrea.ravignani@mpi.nl

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Abstract

Comparative animal studies of complex behavioural traits, and their neurobiological underpinnings, can increase our understanding of their evolution, including in humans. Vocal learning, a potential precursor to human speech, is one such trait. Mammalian vocal learning is understudied: most research has either focused on vocal learning in songbirds or its absence in non-human primates. Here we focus on a highly promising model species for the neurobiology of vocal learning: grey seals (*Halichoerus grypus*). We provide a neuroanatomical atlas (based on dissected brain slices and magnetic resonance images), a labelled MRI template, a 3D model with volumetric measurements of brain regions, and histological cortical stainings. Four main features of the grey seal brain stand out. (1) It is relatively big and highly convoluted. (2) It hosts a relatively large temporal lobe and cerebellum. (3) The cortex is similar to humans in thickness and shows the expected six-layered mammalian structure. (4) Expression of FoxP2 - a gene involved in motor learning, vocal learning, and spoken language - is present in deeper layers of the cortex. Our results could facilitate future studies targeting the neural and genetic underpinnings of mammalian vocal learning, thus bridging the research gap from songbirds to humans and non-human primates. Our findings are not only relevant to vocal learning research, but to the study of mammalian neurobiology and cognition more in general.

Introduction

Strong evidence for vocal learning in grey seals (*Halichoerus grypus* [1]) makes them an interesting target for comparative neurobiological research. Data from these pinnipeds could provide insights into neural and genetic networks involved in this complex ability in mammals [2–4]. However, cross-species investigation into the neurobiology of vocal learning of distantly related animals is complicated by the presence of large inherent brain differences, for example in morphology and size, unrelated to their vocal learning abilities [5]. To overcome this issue, we performed histological analyses, created a reference neuroanatomical atlas, a magnetic resonance imaging (MRI) brain template, and a volumetric 3D model of the weaned female grey seal brain. This outcome effectively facilitates comparative research by identifying and describing the similarities and differences between the brains of grey seals and other vocal learning and non-learning animals both within and outside the clade Pinnipedia, and the larger overarching suborder Caniformia.

Method

Two brains were retrieved during post-mortem examination from weaned (one month - one year old) female grey seals. Both animals died of natural causes during rehabilitation (Supplement, *Table S1*). The brains were fixed in formalin and scanned in a 3T MRI scanner. T1 and T2 weighted images were acquired, pre-processed, and segmented to develop 3D models of the grey seal brains (Figure 1) with volumetric measurements of brain regions (*Figure 1*; Supplement, *Table S4-S5*). For one of the two brains, a labelled brain template and neuroanatomical atlas was generated based on the MRI images, dissected slices, and photographs (Supplement, *Figures S2-S8*). Three cortical sections were taken from the same brain for histological examinations (Supplement, extended methodology and *Figure S9-S10*).

Results and discussion

The measured average brain volume of the weaned female grey seals was 204 cm³. The measured volumetric ratio of grey-to-white matter in the weaned female grey seal brains was 2.7. Total brain size and the relative proportion of grey and white matter volumes change across development; the values reported here fall within the expected range for the seal's developmental stage [6,7]. Previously, it was approximated that the adult male grey seal brain

was 330 cm³ and the adult female brain 262 cm³ [8,9]¹. This makes adult grey seal brains relatively similar in size to those of other species within Pinnipedia, such as the California sea lion (*Zalophus californianus*, adult 307 cm³ [10]) and the harbor seal (*Phoca vitulina*, adult females 255 cm³ and adult males 271 cm³ [8]), but relatively big compared to those of other carnivores (e.g., polar bears (*Ursus maritimus*, adult 215 cm³ [11]) and dogs (*Canis lupus familiaris*, adult 72 cm³ [12])).

The cortex of the weaned female grey seal brains was highly convoluted, with a large number of gyri and sulci (*Figure 2*). Moreover, a great deal of secondary gyration was present (sub-sulci and sub-gyri) in the grey seal brain (Supplement, *Figure S5*). A similar level of gyration has been observed in other pinnipeds, such as the harbor seal and the California sea lion, while lower levels of gyration are present in many terrestrial carnivores with smaller brain sizes, such as canids [10,13]. This complies with the fact that bigger brains are generally more convoluted [14]. The gyration patterns of the grey seal brain largely overlap with those described for harbor seal brains [15–18]. However, they both diverge significantly from the gyration patterns described in terrestrial carnivores, such as canids [13]. The most notable difference is the placement of the sulci delineating the temporal lobe (*Figure 2*).

The weaned female grey seal brains were fairly spherical (i.e., the width and length of the brain were almost identical). This is due to relatively small olfactory bulbs and an expanded temporal lobe. Other pinnipeds share this same brain shape, but other animals within Caniformia do not (e.g., [10,13]). The presence of a well-developed temporal lobe is relatively rare in mammals outside of primates but has previously been observed in a variety of cetaceans and pinnipeds (both vocal learning and vocal non-learning) [19]. The temporal lobe hosts among other areas the auditory cortex, which is involved in the processing of auditory stimuli, and in humans is involved in speech processing and production [19]. Future studies could further explore the function of the expanded temporal lobe, the auditory cortex and associated circuitry in pinnipeds.

The weaned female grey seal brains had a relatively large cerebellum compared to their total brain size. The average cerebellum size in our current study was 31 cm³, which corresponded to 15% of the total brain volume (Supplement, *Table S4-S5*). The cerebellum is a key brain structure for motor learning and cognition [20]. It also plays an important role in vocal learning [21]. In humans [22], and in terrestrial carnivores such as canines [12], the

¹ If volumetric information was not available, but weight measurements were, we converted the weight measurements to volume measurements based on the conversion rules found in [27].

cerebellum accounts for approximately 10% of the total brain volume. Interestingly, dolphins, who independently evolved for an aquatic lifestyle, have a similarly enlarged cerebellum taking up 15% of the total brain volume. This large cerebellum in dolphins has been hypothesized to allow for precise acoustic timing, acoustic processing, and echolocation abilities (e.g., for *Delphinus delphis*, *Tursiops truncatus*, see [23]). Future studies could explore the functions of the cerebellum in grey seals to further elucidate the role of this structure in motor learning, vocal behavior, and timing.

The weaned female grey seal brains showed the expected six-layer structure in the neocortex, which was of varying cortical thickness across the brain (min: 1 mm, max: 4.2 mm, $M = 2.6$ mm, $SD = 0.7$ mm; Supplement, *Figure S2-S10*). The mean cortical thickness was relatively close to that of harbor seals (2 mm range [18]), canines (2-3 mm range [24]), and humans ($M = 2.5$ mm [14]). The grey seal brains observed here (Supplement, *Figure S2-S10*) and the harbor seal brains examined in previous studies, showed high variation in both total cortical thickness across cortical areas (e.g., being very thick in gyri, very thin in sulci) and the thickness of specific layers across different cortical areas [18]. Given that grey seals have a neocortex characteristic of mammals, they could shed light on the role of different cortical areas and related laminar micro-circuits in mammalian vocal learning. This is particularly relevant since the most commonly studied vocal learners, songbirds, have a nuclear rather than laminar cortical organization making it more difficult to draw direct parallels to other vocal learning mammals such as humans.

Within the weaned female grey seal cortex, we found expression of FoxP2 in deeper layers (Supplement, *Figure S9-S10*). FoxP2 is a gene involved in motor learning and established as important for human speech and songbird vocal learning [25,26]. Future studies in grey seals could shed light on the role of FoxP2, and other key genes thought to be involved in vocal learning, in the cortex of mammalian vocal learners.

Conclusion

The current paper provides a first investigation into the neuroanatomy of the grey seal brain. By using information from dissected brain sections and MR images, we were able to create a neuroanatomical reference atlas, a standard brain template, 3D models of the weaned grey seal brain, and get a first glimpse of the neurogenetic properties of the grey seal brain. Based on the neuroanatomical information and brain templates provided here, future comparative studies of vocal learning in grey seals could employ techniques such as genetic mapping or diffusion tensor imaging to test hypotheses regarding the necessary and sufficient neural circuits

involved in mammalian vocal learning. We believe that the study of vocal-learning pinnipeds will be quintessential to a complete understanding of the neurobiology of vocal learning since, as mammals, they can help bridging the gap from songbirds to research in humans and non-human primates [2].

Data accessibility

The unlabeled brain template can be found here: <https://osf.io/hz3gc/>. The labeled brain template can be found here: <https://osf.io/xq9pb/>. Corresponding legends and labels can be downloaded here: <https://osf.io/4urbe/>. All raw MRI data can be found here: <https://osf.io/62ebs/>.

Authors' contributions

NH and AR conceived and designed the study; NH drafted the manuscript; NH and LV prepared the neuroanatomical atlas and brain template; LV and AR coordinated the study and helped draft the manuscript; SCV conceived and analysed the histological data and edited the manuscript; NH, JM, CVR carried out the histology experiments and created the corresponding figures; SV, ASC and ARG facilitated access to the seals and extraction of the brains and provided advice; BCB facilitated access to the scanning facilities and provided advice. All authors edited the manuscript and gave final approval for publication and agreed to be held accountable for the work performed therein.

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Figures

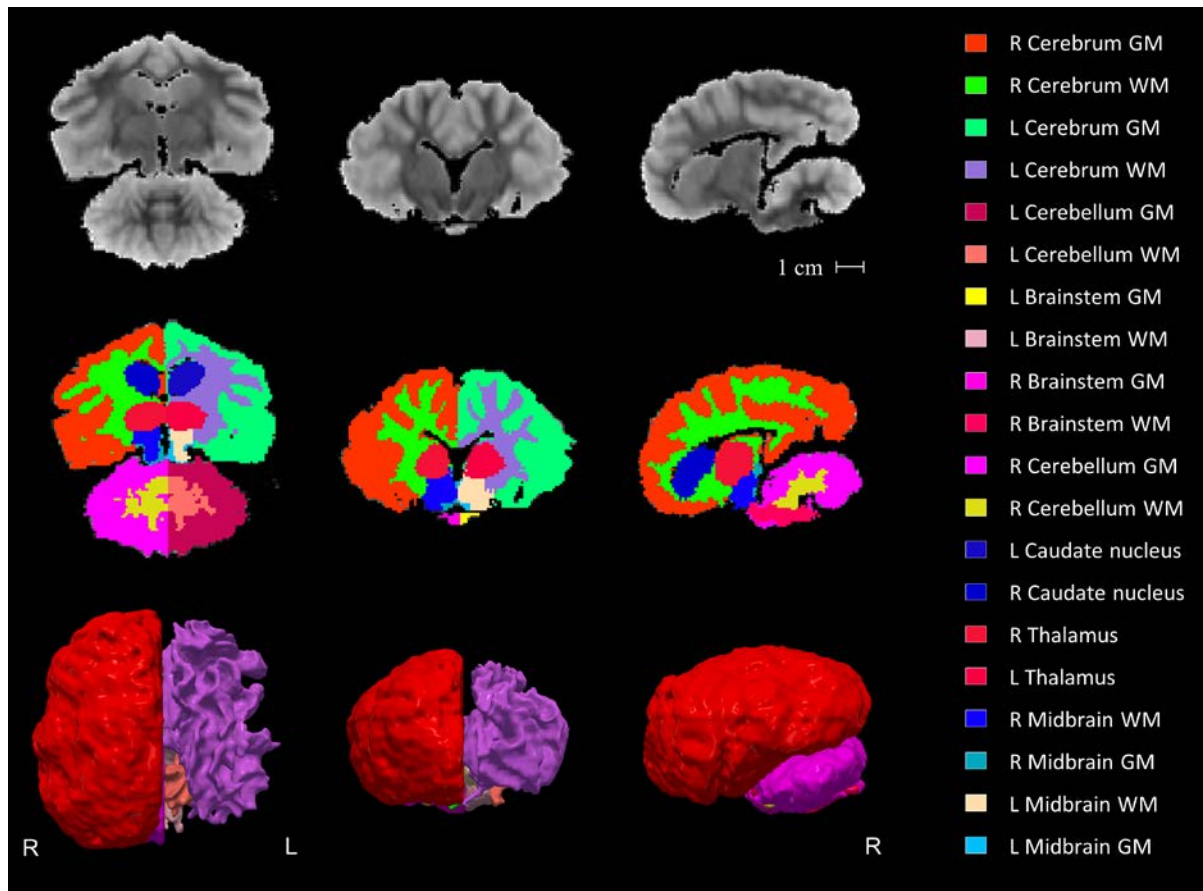


Figure 1. Overview of the grey seal brain template created from Grey Seal Brain 2. From left to right, the transverse (rostral portion on top), coronal, and sagittal (rostral portion on the left) views are shown in radiological convention (right hemisphere on the left side). Top row: T2 FLAIR brain template. Middle row: segmented and labeled brain template. Bottom row: 3D reconstruction of the brain surface from the dorsal (left), frontal (middle), and lateral (right) view showing the grey matter outer boundary on the right hemisphere and the white matter surface on the left. Abbreviations: L = Left, R = Right, WM = White Matter; GM = Grey Matter.

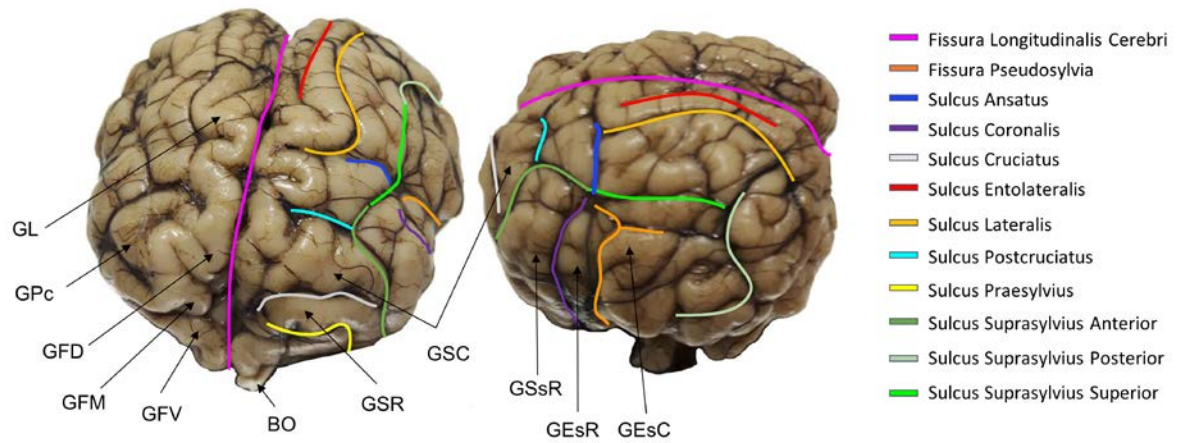


Figure 2. Gross anatomy of the grey seal brain. Left: dorso-frontal surface. Right: lateral surface. Each sulcus is marked in a different color. Abbreviations: BO = Bulbus olfactorius; GEsC = Gyrus Ectosylvius Caudalis; GEsR = Gyrus Ectosylvius Rostralis; GFD = Gyrus Frontalis Dorsalis; GFM = Gyrus Frontalis Medius; GFV = Gyrus Frontalis Ventralis; GL = Gyrus Lateralis; GPc = Gyrus Postcruciatius; GSC = Gyrus Sigmoides Caudalis; GSR = Gyrus Sigmoides Rostralis; GSsR = Gyrus Suprasylyvius Rostralis.

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