

Research



Cite this article: Hoeksema N *et al.* 2021 Neuroanatomy of the grey seal brain: bringing pinnipeds into the neurobiological study of vocal learning. *Phil. Trans. R. Soc. B* **376**: 20200252.
<https://doi.org/10.1098/rstb.2020.0252>

Accepted: 4 February 2021

One contribution of 21 to a theme issue ‘Vocal learning in animals and humans’.

Subject Areas:

physiology, neuroscience, genetics, evolution, behaviour, cognition

Keywords:

comparative neuroanatomy, magnetic resonance imaging, FoxP2, language evolution, bioacoustics, *Halichoerus grypus*

Authors for correspondence:

Nienke Hoeksema
e-mail: nienke.hoeksema@mpi.nl
Andrea Ravnigani
e-mail: andrea.ravnigani@mpi.nl

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5527049>.

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-García⁵, Branislava Ćurčić-Blake⁶, Sonja C. Vernes^{1,7,8} and Andrea Ravnigani^{3,5}

¹Neurogenetics of Vocal Communication Group, ²Neurobiology of Language Department, and ³Comparative Bioacoustics Group, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands
⁴Faculty of Psychology and Neuroscience, Department NP&PP, Maastricht University, Maastricht, The Netherlands
⁵Research Department, Sealcentre Pieterburen, Pieterburen, The Netherlands
⁶Cognitive Neuroscience Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
⁷Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands
⁸School of Biology, University of St Andrews, St Andrews, UK

NH, 0000-0002-3454-1177; LV, 0000-0001-8957-5027; SCV, 0000-0003-0305-4584; AR, 0000-0002-1058-0024

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 under-studied: 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 three-dimensional model with volumetric measurements of brain regions, and histological cortical stainings. Four main features of the grey seal brain stand out: (i) it is relatively big and highly convoluted; (ii) it hosts a relatively large temporal lobe and cerebellum; (iii) the cortex is similar to that of humans in thickness and shows the expected six-layered mammalian structure; (iv) there is expression of FoxP2 present in deeper layers of the cortex; FoxP2 is a gene involved in motor learning, vocal learning, and spoken language. 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 relevant not only to vocal learning research but also to the study of mammalian neurobiology and cognition more in general.

This article is part of the theme issue ‘Vocal learning in animals and humans’.

1. 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 three-dimensional model of the weaned female grey seal brain. This outcome

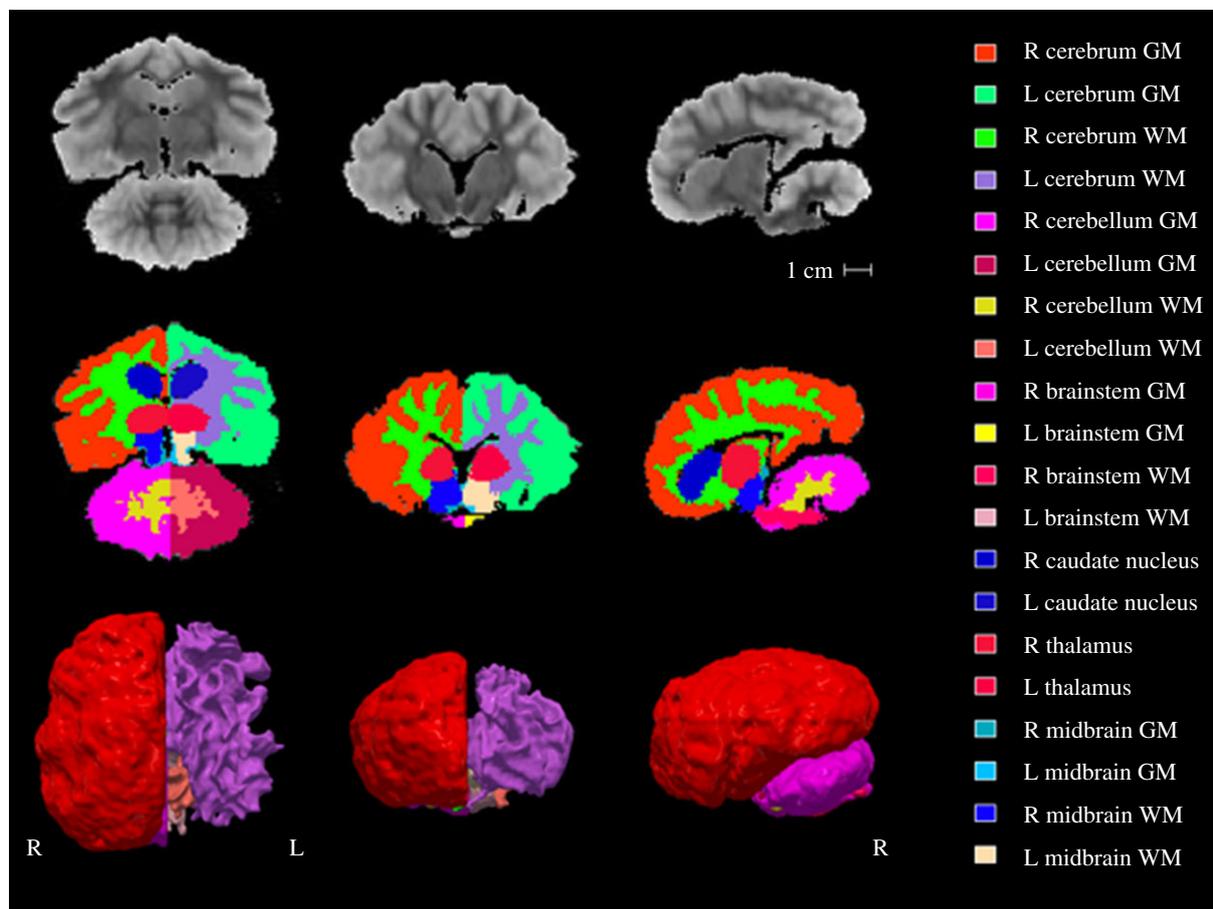


Figure 1. Overview of the grey seal brain template created from Grey Seal Brain 2. From left to right, the transverse (rostral portion on top), coronal and sagittal (rostral portion on the left) views are shown in radiological convention (right hemisphere on the left side). Top row: T2 FLAIR brain template. Middle row: segmented and labelled brain template. Bottom row: three-dimensional 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.

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.

2. Method

Two brains were retrieved during post-mortem examination from weaned (one month to 1 year old) female grey seals. Both animals died of natural causes during rehabilitation (electronic supplementary material, table S1). The brains were fixed in formalin and scanned in a 3T MRI scanner (electronic supplementary material, table S2). T1 and T2 weighted images were acquired, pre-processed, and segmented to develop three-dimensional models of the grey seal brains (figure 1) with volumetric measurements of brain regions (electronic supplementary material, tables S4 and S5). For one of the two brains, a labelled brain template and neuroanatomical atlas were generated based on the MRI images, dissected slices, and photographs (electronic supplementary material, figures S2–S8). Three cortical sections were taken from the same brain for histological examinations (electronic supplementary material, table S3 and figures S9 and S10). See the electronic supplement for the extended methodology.

3. Results and discussion

The measured average brain volume of the weaned female grey seals was 204 cm³ (electronic supplementary material, table S4).

The measured volumetric ratio of grey-to-white matter in the weaned female grey seal brains was 2.7 (electronic supplementary material, table S5). 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 seals' 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 [11]) and the harbour seal (*Phoca vitulina*, adult females 255 cm³ and adult males 271 cm³ [8]), but relatively big compared with those of other carnivores (e.g. polar bears (*Ursus maritimus*, adult 215 cm³ [12]) and dogs (*Canis lupus familiaris*, adult 72 cm³ [13])).

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 (electronic supplementary material, figure S5). A similar level of gyration has been observed in other pinnipeds, such as the harbour seal and the California sea lion, while lower levels of gyration are present in many terrestrial carnivores with smaller brain sizes, such as canids [11,14]. This complies with the fact that bigger brains are generally more convoluted [15]. The gyration patterns of the grey seal brain largely overlap with those described for harbour seal brains [16–19]. However, they both diverge significantly from the gyration

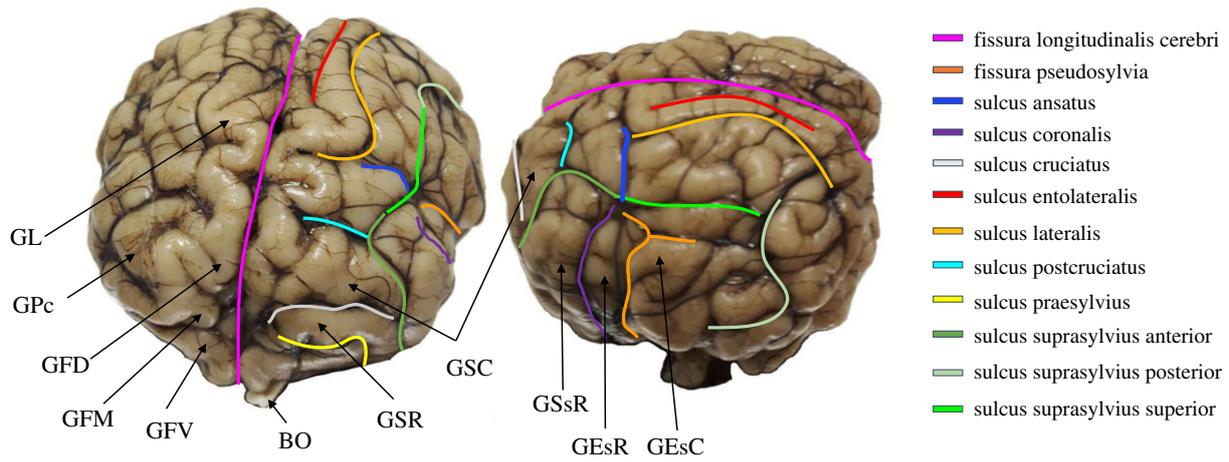


Figure 2. Gross anatomy of the grey seal brain. Left: dorso-frontal surface. Right: lateral surface. Each sulcus is marked in a different colour. Abbreviations: BO, bulbus olfactorius; GEsC, gyrus ectosylvius caudalis; GEsR, gyrus ectosylvius rostralis; GFD, gyrus frontalis dorsalis; GFM, gyrus frontalis medius; GFV, gyrus frontalis ventralis; GL, gyrus lateralis; GPc, gyrus postcruciatius; GSC, gyrus sigmoideus caudalis; GSR, gyrus sigmoideus rostralis; GSsR, gyrus suprasylvius rostralis.

patterns described in terrestrial carnivores, such as canids [14]. 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; figure 1). 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. [11,14]). 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 species) [20]. 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 [20]. 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 with their total brain size. The average cerebellum size in our current study was 31 cm³, which corresponded to 15% of the total brain volume (electronic supplementary material, table S4). The cerebellum is a key brain structure for motor learning and cognition [21]. It also plays an important role in vocal learning [22]. In humans [23], and in terrestrial carnivores such as canines [13], the cerebellum accounts for approximately 10% of the total brain volume. Interestingly, dolphins, which 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 [24]). Future studies could explore the functions of the cerebellum in grey seals to further elucidate the role of this structure in motor learning, vocal behaviour, 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, s.d. = 0.7 mm; electronic supplementary material, figures S2–S10). The mean cortical thickness was relatively close to that of harbour seals (2 mm range [19]),

canines (2–3 mm range [25]) and humans ($M = 2.5$ mm [15]). The grey seal brains observed here (electronic supplementary material, figures S2–S10) and the harbour 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 [19]. 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 (electronic supplementary material, figures S9 and S10). *FoxP2* is a gene involved in motor learning and established as important for human speech and songbird vocal learning [26,27]. 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.

4. Conclusion

The current paper provides a first investigation into the neuroanatomy of the grey seal brain. By using information from dissected brain sections and magnetic resonance images, we were able to create a neuroanatomical reference atlas, a standard brain template, and three-dimensional 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 bridge the gap from research in songbirds to research in humans and non-human primates [2].

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

Authors' contributions. N.H. and A.R. conceived and designed the study; N.H. drafted the manuscript; N.H. and L.V. prepared the neuroanatomical atlas and brain template; L.V. and A.R. coordinated the study and helped draft the manuscript; S.C.V. conceived and analysed the histological data and edited the manuscript; N.H., J.M., C.V.R. carried out the histology experiments and created the corresponding figures; S.V., A.S.-C. and A.R.-G. facilitated access to the seals and extraction of the brains and provided advice; B.C.-B. facilitated access to the scanning facilities and provided advice. All authors edited the manuscript, gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. N.H. is supported by funding from an International Max Planck Research School (IMPRS) for language sciences fellowship grant, and the work of N.H. and S.C.V. was supported by a Max Planck Research Group (MPRG) awarded to S.C.V. The work of L.V. and A.R. was supported by a Max Planck Research Group (MPRG) awarded to A.R. S.C.V. was also supported by a Human Frontiers Science Program (HFSP) Research grant (grant no. RGP0058/2016) and a UKRI Future Leaders Fellowship (grant no. MR/T021985/1).

Endnote

¹If volumetric information was not available, but weight measurements were, we converted the weight measurements to volume measurements based on the conversion rules found in [10].

References

- Stansbury AL, Janik VM. 2019 Formant modification through vocal production learning in gray seals. *Curr. Biol.* **29**, 2244–2249. (doi:10.1016/j.cub.2019.05.071)
- Ravnani A, Fitch WT, Hanke FD, Heinrich T, Hurgitsch B, Kotz SA, Scharff C, Stoeger AS, de Boer B. 2016 What pinnipeds have to say about human speech, music, and the evolution of rhythm. *Front. Neurosci.* **10**, 274. (doi:10.3389/fnins.2016.00274)
- Janik VM, Slater PJB. 1997 Vocal learning in mammals. *Adv. Stud. Behav.* **26**, 59–99.
- Lattenkamp EZ, Vernes SC. 2018 Vocal learning: a language-relevant trait in need of a broad cross-species approach. *Curr. Opin. Behav. Sci.* **21**, 209–215. (doi:10.1016/j.cobeha.2018.04.007)
- Mars RB, Eichert N, Jbabdi S, Verhagen L, Rushworth MFS. 2018 Connectivity and the search for specializations in the language-capable brain. *Curr. Opin. Behav. Sci.* **21**, 19–26. (doi:10.1016/j.cobeha.2017.11.001)
- Zhang K, Sejnowski TJ. 2000 A universal scaling law between gray matter and white matter of cerebral cortex. *Proc. Natl Acad. Sci. USA* **97**, 5621–5626. (doi:10.1073/pnas.090504197)
- Matsuzawa J, Matsui M, Konishi T. 2001 Age-related volumetric changes of brain gray and white matter in healthy infants and children. *Cereb. Cortex* **11**, 335–342. (doi:10.1093/cercor/11.4.335)
- Bininda-Emonds ORP. 2000 Pinniped brain sizes. *Mar. Mamm. Sci.* **16**, 469–481. (doi:10.1111/j.1748-7692.2000.tb00939.x)
- Berta A. 2005 Integumentary and sensory systems. In *Marine mammals evolutionary biology* (eds A Berta, JL Sumich, KM Kovacs), pp. 132–164. New York, NY: Academic Press.
- Stephan H, Frahm H, Baron G. 1981 New and revised data on volumes of brain structures in insectivores and primates. *Folia Primatol.* **35**, 1–29. (doi:10.1159/000155963)
- Montie EW, Pussini N, Schneider GE, Battey TWK, Dennison S, Barakos J, Gulland F. 2009 Neuroanatomy and volumes of brain structures of a live California sea lion (*Zalophus californianus*) from magnetic resonance images. *Anat. Rec.* **292**, 1523–1547. (doi:10.1002/ar.20937)
- Dong W. 2008 Virtual cranial endocast of the oldest giant panda (*Ailuropoda microta*) reveals great similarity to that of its extant relative. *Naturwissenschaften* **95**, 1079–1083. (doi:10.1007/s00114-008-0419-3)
- Thames RA, Robertson ID, Flegel T, Henke D, O'Brien DP, Coate JR, Olby NJ. 2010 Development of a morphometric magnetic resonance image parameter suitable for distinguishing between normal dogs and dogs with cerebellar atrophy. *Vet. Radiol. Ultrasound* **51**, 246–253. (doi:10.1111/j.1740-8261.2009.01655.x)
- Welker W, Johnson JI, Noe A. 2019 *Comparative mammalian brain collections*. See <http://neurosciencelibrary.org/>. (accessed 25 February 2019).
- Fischl B, Dale AM. 2000 Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl Acad. Sci. USA* **97**, 11 050–11 055. (doi:10.1073/pnas.200033797)
- Alderson AM, Diamantopoulos E, Downman CBB. 1960 Auditory cortex of the seal (*Phoca vitulina*). *J. Anat.* **94**, 506–511.
- Fish PA. 1895 A note on the cerebral fissuration of the seal. *J. Comp. Neurol.* **6**, 15–19. (doi:10.1002/cne.910060104)
- Langworthy OR, Hesser FH, Kolb LC, Rathbone HB, Rathbone JL. 1938 A physiological study of the cerebral cortex of the hair seal (*Phoca vitulina*). *J. Comp. Neurol.* **69**, 351–369. (doi:10.1002/cne.900690302)
- Rioch DM. 1937 A physiological and histological study of the frontal cortex of the seal (*Phoca vitulina*). *Biol. Bull.* **73**, 591–602. (doi:10.2307/1537617)
- Bryant KL, Preuss TM. 2018 A comparative perspective on the human temporal lobe. In *Digital endocasts* (eds E Bruner, N Ogihara, HC Tanabe), pp. 239–258. Tokyo, Japan: Springer.
- Stoodley CJ. 2012 The cerebellum and cognition: evidence from functional imaging studies. *Cerebellum* **11**, 352–365. (doi:10.1007/s12311-011-0260-7)
- Pidoux L, Le Blanc P, Levenes C, Leblois A. 2018 A subcortical circuit linking the cerebellum to the basal ganglia engaged in vocal learning. *eLife* **7**, 3–5. (doi:10.7554/eLife.32167)
- Swanson LW. 1995 Mapping the human brain: past, present, and future. *Trends Neurosci.* **18**, 471–474. (doi:10.1016/0166-2236(95)92766-J)
- Marino L, Rilling JK, Lin SK, Ridgway SH. 2000 Relative volume of the cerebellum. *Brain. Behav. Evol.* **30322**, 204–211. (doi:10.1159/000047205)
- Labounek R, Mai K, Mueller B, Ellinwood NM, Dickson P, Nestratil I. 2019 In-vivo cortical thickness estimation from high-resolution T_{1w} MRI scans in healthy and mucopolysaccharidosis affected dogs. In *Proc. 41st Annu. Int. Conf. IEEE Engineering in Medicine and Biology Society (EMBC), Berlin Germany*, pp. 2848–2851. New York, NY: Institute of Electrical and Electronics Engineers. (doi:10.1109/EMBC.2019.8856826)
- Fisher SE, Scharff C. 2009 FOXP2 as a molecular window into speech and language. *Trends Genet.* **25**, 166–177. (doi:10.1016/j.tig.2009.03.002)
- Vernes SC, Fisher SE. 2009 Unravelling neurogenetic networks implicated in developmental language disorders. *Biochem. Soc. Trans.* **37**, 1263–1269. (doi:10.1042/BST0371263)