The near-infrared autofluorescence fingerprint of the brain

José Lifante1,2 | Blanca del Rosal3 | Irene Chaves-Coira4 | Nuria Fernández1,2 | Daniel Jaque1,2* | Erving Ximendes1,2

1Fluorescence Imaging Group, Universidad Autonoma de Madrid, Madrid, Spain
2Nanobiology Group, Instituto Ramón y Cajal de Investigación Sanitaria, Madrid, Spain
3ARC Centre of Excellence for Nanoscale BioPhotonics, School of Science, RMIT University, Melbourne, Victoria, Australia
4Department of Anatomy, Histology and Neuroscience, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain

*Correspondence
Dr Daniel Jaque, Faculty of Sciences, Department of Physics of Materials, Universidad Autonoma de Madrid, Madrid 28049, Spain.
Email: daniel.jaque@uam.es

Funding information
Comunidad de Madrid, Grant/Award Number: B2017/BMD-3867RENIMCM; European Cooperation in Science and Technology, Grant/Award Number: CA17140; Fundación para la Investigación Biomédica del Hospital Universitario Ramón y Cajal, Grant/Award Number: IMP18_38/2018/0265; Horizon 2020 Framework Programme, Grant/Award Number: 801305; Instituto de Salud Carlos III, Grant/Award Number: PI16/00812; Ministerio de Ciencia, Innovación y Universidades, Grant/Award Numbers: MAT2016-75362-C3-1-R, MAT2017-85617-R

1o f1 0

Received: 23 April 2020 Revised: 15 July 2020 Accepted: 19 July 2020
DOI: 10.1002/jbio.202000154

J. Biophotonics. 2020;13:e202000154. www.biophotonics-journal.org © 2020 Wiley-VCH GmbH

1 INTRODUCTION

Central nervous system (CNS) disorders currently affect close to 2 billion people worldwide. This figure is expected to increase due to the progressive aging of the population, as the prevalence of these diseases increases steeply with age. CNS disorders are the leading cause of disability worldwide, imposing a large burden on healthcare systems [1]. Most of them are likely associated with subtle neuropathological changes, which are virtually undetectable until they reach a certain threshold of tissue damage [2]. Thus, being able to detect the very first pathological hallmarks of disease would allow developing new therapeutic strategies aimed to minimize the impact of CNS disorders. Several clinical imaging modalities such as single-photon emission computed tomography...
(SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI) or ultrasound imaging enable anatomical and functional imaging of the CNS. However, these techniques have some major drawbacks including the use of ionizing radiation (SPECT, PET), low acquisition speeds and high costs (MRI) and low spatial resolutions (ultrasound). Thus, implementing novel non-invasive, cost-effective, nonionizing techniques with a high spatial and temporal resolution for in vivo brain imaging would constitute a major breakthrough [3].

Near-infrared (NIR) fluorescence imaging could overcome the intrinsic limitations of conventional imaging modalities. It relies on the partial transparency of biological tissues to NIR radiation to achieve high-contrast, deep-tissue imaging [4, 5]. Recently, NIR-emitting organic molecules and nanoparticles (NPs) have been used for transcranial fluorescence brain imaging. As a consequence, noninvasive in vivo imaging of the brain vasculature, brain tumor detection and image-assisted surgery have been achieved [6]. Other applications, such as in vivo mesenchymal stem cell tracking or intracranial nanothermometry have also been demonstrated [7,8].

Despite the recent progress on NIR-II brain imaging (Table S1), this technique is still in its early stages and some key potential pitfalls remain unexplored. One of them is the possible interference of NIR-II brain autofluorescence that can hinder the contrast and resolution of NIR-II in vivo fluorescence images. Yet, brain autofluorescence has been systematically disregarded [9, 10]. As part of the development of NIR-II fluorescence imaging for preclinical applications, researchers have focused on developing strategies for autofluorescence background removal [11]. While the combination of long-lifetime NIR-II probes with time-gated image acquisition allows autofluorescence-free imaging [12–14], this approach is unsuitable for transcranial imaging due to the reduced fluorescence lifetimes (<1 μs) of most available NIR-II probes [15]. Alternatively, autofluorescence-free imaging is possible using contrast agents emitting well into the NIR-II, as autofluorescence intensity decreases at longer wavelengths. The combination of these probes with spectral filters is a cost-effective and reliable approach for whole-body autofluorescence-free in vivo imaging [9]. This approach calls for a rational selection of the NIR-II luminescence probes to minimize the spectral overlap between their emission and the tissue autofluorescence. This, in turn, requires full knowledge of the NIR-II autofluorescence of the tissues under study.

Fully characterizing NIR-II brain autofluorescence could not only allow for autofluorescence-free imaging, but it might also contribute to developing new diagnostic strategies. Tissue autofluorescence depends on the concentration and spatial distribution of endogenous fluorophores, so its corresponding spectrum contains information about the molecular composition of that tissue [16]. Histological and histochemical changes associated with disease development could then be tracked by changes in the autofluorescence. This has already been demonstrated for the diagnosis of unstable atheroma plaques [17]. In the case of the brain, observable changes in its characteristic autofluorescence could be useful indicators of CNS disorders. For instance, the brain has a high content of myelin, which is a known source of autofluorescence in the visible spectral range and increases during neuronal aging and in the development of some neurodegenerative disorders [18–21].

Despite its relevance for high contrast, fast and cost-effective imaging, the NIR-II brain autofluorescence remains unexplored. In this work, we report for the first time on the in vivo NIR-II autofluorescence of the mouse brain as obtained by hyperspectral imaging (HSI) under optical excitation at 808 nm. We have systematically compared the ex vivo NIR-II autofluorescence of the brain to that of other organs and explained the observed differences considering the particular composition of the brain. We have spatially mapped the NIR-II autofluorescence of the brain and observed a non-homogeneous signal that has been correlated with the presence of well-defined structures rich in neuromelanin (NM), thus identifying the first specific NIR-II endogenous brain fluorophore. This indicates that NIR-II autofluorescence has potential as a diagnostic tool in the context of specific CNS disorders, such as brain melanoma and Parkinson. We also propose the I_{1075nm}/I_{1015nm} ratio as a parameter sensitive enough to discriminate changes in myelin content of different areas of the brain. This could be useful in the identification of demyelinating diseases, such as multiple sclerosis or amyotrophic lateral sclerosis. Finally, we have analyzed the spectral overlap between the autofluorescence of the brain and the emission bands of NIR-II fluorescence probes used for transcranial imaging to identify the most suitable probes for autofluorescence-free NIR-II brain imaging.

2 | MATERIALS AND METHODS

2.1 | Hyperspectral imaging

The HSI setup used for NIR-II spectra acquisition is schematically represented in Figure S1. A fiber-coupled 808 nm laser diode (LIMO) was used for optical excitation. A short-pass filter (Thorlabs FES0850) was placed immediately in front of the laser fiber to minimize specular and diffuse reflection effects. The illumination density
was controlled by adjusting the fiber-to-tissue distance and fixed at 50 mW/cm² in all experiments. This low power density prevented excessive tissue heating and subsequent drying. The emitted and scattered light was spectrally filtered with two consecutive long-pass filters (Thorlabs FEL0850) that were used to block the 808 nm back-scattered signal and isolate the autofluorescence. A combination of shortwave infrared lenses was used to send the autofluorescence signal on to a Bragg tunable filter, which allows selecting a specific wavelength of the incoming light. A second tube lens focuses the filtered light on a NIR camera (ZephIR™ 1.7) to produce a monochromatic image. To cover a specific spectral range, each monochromatic image was recorded and then stored on a computer while the angle of the BTF rotation stage was continuously tuned. At the end of this process, the system built up a HSI cube, that is, a 3D spatial map of spectral variation: the first two dimensions providing spatial information and a third dimension accounting for the spectral information. The intensity values of a particular pixel in a HSI cube characterize its unique spectral fingerprint.

2.2 In vivo NIR-II autofluorescence imaging

For this study, two C57BL/6J male mice (20-22 weeks old) were used. Deep anesthesia was induced with 4.6% of inhaled isoflurane under a constant flow rate of 0.5 mL/min, and maintained between 2.5 and 3.0 during the surgical procedure to perform a cranial window to expose the left cerebral cortex. Briefly, the anesthetized mouse was placed in a stereotaxic frame. The scalp was cut so the skull was clearly exposed. Posterior craniotomy was performed using a dremmel so an ipsilateral window was open to expose the left hemisphere of the brain. The anesthetized animal was then placed into the experimental HSI chamber to collect the autofluorescence images as described above.

2.3 Ex vivo fluorescence imaging

For this study, three C57BL/6J male mice (20-22 weeks old) were used. Animals were euthanized by beheading after induction of deep anesthesia with isoflurane under a constant oxygen flow rate of 0.5 mL/min and a concentration of 4.6% of isoflurane. Prior to fluorescence imaging, the organs were immediately removed and washed thoroughly with cold 0.9% NaCl solution to slow down their degradation. The time between sacrifice and imaging was shorter than 5 minutes for all experiments. For brain imaging studies, we dissected the olfactory bulbs to expose the rostral part of the brain that is described to be enriched in NM. Freshly dissected tissues were analyzed with HSI using a spectral resolution of 5 nm and an exposure time of 20 seconds.

2.4 Animal experiments

Procedures involving animal experiments were approved by the regional authority for animal experimentation of the Comunidad de Madrid and were conducted in agreement with the Universidad Autónoma de Madrid Ethics Committee, in compliance with the European Union directives 63/2010UE and Spanish regulation RD 53/2013.

3 RESULTS AND DISCUSSION

3.1 NIR-II hyperspectral characterization of brain autofluorescence

HSI in the NIR-II (Section S2) allows for the acquisition of in vivo NIR autofluorescence spectra with a submillimetric spatial resolution. This, in turn, enables the discrimination between the autofluorescence spectra generated by different tissues and/or their substructures. To compare the in vivo autofluorescence of the brain to that of other organs, we performed HSI on a C57/BL6J mouse under illumination at 808 nm (Figure 1). To expose the brain cortex, a part of the skull on the left cerebral hemisphere was removed prior to imaging (Figure 1A). The narrowband autofluorescence images from the abdominal (Figure 1B) and cerebral (Figure 1C) regions were recorded in the 900 to 1600 nm wavelength range and the spectra corresponding to both regions were obtained after a careful analysis (Figure 1D). As observed in Figure 1C, the site where the cranial window was created presented a lower emission intensity than the regions covered by the skull. Though seemingly counter-intuitive, such results are expected in light of the transmission and autofluorescence properties of the skull (Section S3). Furthermore, in reasonable agreement with previously published data, the autofluorescence spectrum of the abdominal region was characterized by a peak centered at around 1015 nm and a long tail extending up to 1100 nm [10]. The in vivo autofluorescence of the brain, on the other hand, presented different spectral features, the most discernible being an extra peak centered at 1075 nm. To demonstrate that such characteristics could be observed in other individuals, the experiment was repeated under similar conditions (Section S4). The presence of the extra peak was once again confirmed.
To understand the origin of the peculiar NIR-II autofluorescence of the brain and rule out the presence of artifacts in the hyperspectral measurements, we recorded the autofluorescence spectra of different ex vivo organs under the same experimental conditions (Figure 2). Figure 2A shows an optical image of the different organs harvested from a healthy C57BL/6J mouse together with narrowband fluorescence images at some representative wavelengths. A detailed analysis of these monochromatic images reveals that the wavelength dependence of the autofluorescence differs from organ to organ. The autofluorescence spectra reconstructed from them allows splitting the analyzed tissues into three groups:

1. Type I: Tissues showing a NIR-II autofluorescence spectrum constituted by a broad fluorescence tail (Figure 2B).
2. Type II: Tissues with a NIR-II autofluorescence spectrum constituted by a single peak at 1015 nm superimposed to a broad fluorescence tail (Figure 2C).
3. Type III: Tissues showing a NIR-II autofluorescence spectrum constituted by two peaks at 1015 and 1075 nm superimposed to a broad fluorescence tail (Figure 2D).

All analyzed tissues fall into type I or type II groups except for the brain, which presents a peculiar autofluorescence profile. The tissues pertaining to type I show a NIR-II autofluorescence spectrum similar to that of the liver, as evidenced in Section S5. Consequently, the liver will hereafter be considered its representative. The type II group, on the other hand, could have the adipose tissue as its exemplary constituent. The fact that the adipose tissue shows the largest contribution of the 1015 nm peak to the overall autofluorescence spectrum seems to indicate that this particular peak arises from the presence of lipids. This would explain why this peak is also present in the brain autofluorescence spectrum, as the brain is enriched in lipids [20]. The contribution of the autofluorescence peak at 1075 nm, on the other hand, should be ascribed to a fluorophore that is specifically present in the brain. Independently of the fluorophore responsible for these spectral features, a proper analysis of autofluorescence images could be useful for anatomical imaging as the brain is the only tissue showing this 1075 nm emission band. A ratiometric analysis of the hyperspectral autofluorescence allows differentiating the brain from other tissues as shown in Section S6.

3.2 Imaging the heterogeneous composition of brain by NIR-II autofluorescence

To obtain further information about the origin of the peculiar NIR-II brain autofluorescence, a series of high magnification ex vivo autofluorescence images of a brain from C57BL6/J mice (n = 3) were recorded at different wavelengths (Figure 3). A representative example of the obtained results, together with an optical image of the brain under study, are shown in Figure 3A. As previously described, the autofluorescence of the brain...
decreases with increasing wavelength—with intensity maxima at around 1015 and 1075 nm. On top of this general observation, a detailed inspection of the autofluorescence images reveals a nonhomogeneous spatial behavior. In particular, the narrowband autofluorescence image at 1150 nm reveals that the signal is only generated from a very well localized area (indicated by a blue closed curve in both optical and

**FIGURE 2** Autofluorescence images of major organs at selected wavelengths in the near-infrared II (NIR-II) window. (A) Optical and ex vivo autofluorescence images (centered at 900, 950, 1015 and 1075 nm), obtained under 808 nm excitation, of selected organs harvested from a C57BL6/J mouse. (B) Autofluorescence spectra of organs showing an autofluorescence tail (type I), (C) an autofluorescence emission peak at 1015 nm (type II) and (D) two autofluorescence peaks at 1015 and 1075 nm (type III)

**FIGURE 3** Spatial distribution of near-infrared II (NIR-II) brain autofluorescence. (A) Autofluorescence images of a C57BL6/J mouse under 808 nm continuous excitation at selected wavelengths. The optical image of the analyzed brain is also included. The squares in the right image in the middle row indicate the location of the frontal brain (blue square) and the right cortex (green square). (B) Autofluorescence spectra as obtained at the locations indicated by the closed curves in (A). Again, the green spectrum is obtained from the right cortex, while the blue spectrum comes from the frontal cortex. The gray region highlights the third emission tail located approximately from 1150 to 1300 nm that is only observed in the frontal brain, where the dark neuromelanin enrichment can be observed by gross examination
fluorescence images in Figure 3A) whereas the rest of the brain shows no autofluorescence at this wavelength. Direct visual inspection reveals the presence of a well-demarcated black area (see green squares in the optical image of Figure 3A) that coincides with the anatomical location and shape of the fluorescence image in the 1150 to 1300 nm wavelength range (Section S7). According to previous studies related to the brains of C57BL/6J mice [22, 23], such structures can be identified as melanosomes found between the olfactory bulbs and cerebral hemispheres. Hence, as these vesicles are known to be enriched with a dark-neural pigment known as NM, we suggest that this characteristic autofluorescence 1150 to 1300 nm wavelength range might be caused by the presence of NM. The differences between the autofluorescence spectra of the right cortex and of the frontal brain (exposed after olfactory bulb resection in Figure 3A) are further evidenced in Figure 3B.

Though the peaks at 1015 and 1075 nm are present in both cases, only the rostral brain shows a third emission band (highlighted in gray in Figure 3B) in the 1150 to 1300 nm range. Based on the unique enrichment in NM of the rostral part of the brain, we propose NM as the first NIR-II brain endogenous fluorophore identified with a characteristic emission in the 1150 to 1300 nm range.

Results in Figure 3 reveal the capability of autofluorescence imaging for identifying different structures inside the brain. In particular, NIR-II brain autofluorescence emerges as a straightforward technique for the localization and monitoring of the local content of NM. This is of particular importance for clinical applications. NM is a brain pigment naturally expressed in melanocytes, a cell type that has been recently described to comprise a population capable of myelinate the axons of mouse neurons [24]. Furthermore, melanosomes are originated by the accumulation of the NM pigment in autophagic phagosomes inside the cells, which are enriched in lipids (such as dolichols and dolichols acids) [25]. Unlike non-neural melamins such as those present in the skin, the melanosomes in the brain are not synthesized under a controlled enzymatic pathway. They are, instead, spontaneously generated after free-radical oxidation of dopamine, norepinephrine and other catecholamines to semiquinones and quinones in the cytosol of the neuronal cells [26]. Contrary to what occurs in mice, NM is strongly expressed in humans in noradrenergic neurons of the substantia nigra pars compacta and locus coeruleus, leading to a black appearance in these regions of aged brains. Loss of NM and subsequent depigmentation of these brain regions is a hallmark feature of Parkinson’s disease (PD) [27]. Importantly, PD diagnosis occurs when motor symptoms are already apparent and 30% or more of the substantia nigra dopamine neurons are already dead revealing the importance of the development of early diagnosis techniques. NM detection by MRI has recently been proposed as a promising biomarker for PD [28] and this work opens the way toward early cost-effective and fast diagnosis of PD through the analysis of NIR-II brain autofluorescence.

Furthermore, an early detection of an increase of NM in the brain could be relevant in other scenarios, such as

**FIGURE 4** Distinguishing brain structures through luminescence intensity ratio. (A) Ratiometric autofluorescence image obtained after dividing the narrowband luminescence image corresponding to 1075 nm by the one centered at 1015 nm. A pseudocolor scale was utilized to better highlight the different ratio values. (B) Linear profile of the $I_{1075\text{ nm}}/I_{1015\text{ nm}}$ ratio as obtained from the dotted red line in (A). Three main ratio values were identified: $0.95 \pm 0.0.3$, $1.06 \pm 0.0.3$ and $1.15 \pm 0.0.3$ (indicated as gray, green and blue dotted lines)
Another interesting feature of the data in Figure 3 is that, in the right cortex, the 1075 nm peak seems to constitute a higher relative contribution to the total autofluorescence spectrum. This, in turn, suggests that tissue heterogeneity (specifically regarding the heterogeneous distribution of NIR-II fluorophores) inside the brain might play an important role in the relative contribution of the 1075 nm peak. To confirm this hypothesis, we performed a ratiometric analysis where we divided the narrowband fluorescence image corresponding to the 1075 nm emission by the one corresponding to 1015 nm (ie, the intensity of the second peak divided by the one of the first) (Figure 4). The result was a 2D spatial profile (known as ratiometric fluorescence image) of the $I_{1075\text{nm}}/I_{1015\text{nm}}$ ratio inside the brain (Figure 4A). As seen in the figure, the relative intensities between the 1075 and 1015 nm peaks are variable across the different parts of the brain. At least three zones can be visually identified in the image—two darker regions in the vertical extremes and a bright one between them. This spatial dependence of the ratiometric fluorescence was obtained by computing the profile of intensity through the indicated red line, as shown in Figure 4B. These data evidence the existence of three main ratio values: 0.95 ± 0.0.3, 1.06 ± 0.0.3 and 1.15 ± 0.0.3 (indicated as gray, green and blue dotted horizontal lines).

The clear difference between the areas corresponding to the forebrain and midbrain (center in Figure 4A) compared to that observed in distal areas (hindbrain) (bottom in Figure 4A) indicates that autofluorescence across the brain could be related to heterogeneities in NIR-II fluorophores specifically present in the brain. Superior structures in the brain such as the cortex concentrate a big proportion of the gray matter, which is comprised of the body of neurons called “soma” and in which myelin is absent. On the other hand, distal structures that connect the brain with the spinal cord such as the medulla and the pons are enriched in myelinated neuronal tracts. These results suggest that the $I_{1075\text{nm}}/I_{1015\text{nm}}$ ratio can not only be seen as a spectral fingerprint of the brain, but also as a parameter sensitive enough to the myelin content of different areas of the brain. This could be useful in the identification of demyelinating diseases, such as multiple sclerosis or amyotrophic lateral sclerosis, which are known to affect myelin and lipidic levels both in the CNS and in peripheral nervous system. Though the molecular origins of these differences are not explored in the present work, these results are highly relevant from a practical point of view.

### 3.3 Rational selection of NIR-II nanoprobes for in vivo transcranial imaging

As already mentioned, NIR-II autofluorescence could have a negative impact on the contrast of brain images when NIR-II fluorescence probes are used as contrast agents. Minimum overlap between the emission of these NIR-II probes and brain autofluorescence spectra is then desired, as it would enable the effective removal of autofluorescence background by straightforward spectral filtering. The knowledge of the NIR-II autofluorescence
of the brain allows evaluating the suitability of the different NIR-II probes for high contrast brain imaging. To quantify the overlap between the emission of NIR-II probes and the autofluorescence of the brain, we have used the dimensionless overlap parameter $\Gamma$, defined as:

$$\Gamma = \frac{\int \frac{I_{\text{Brain}}}{I_{\text{NP}}} \cdot d\lambda}{\int \frac{I_{\text{Brain}}}{\int I_{\text{NP}} \cdot d\lambda}}$$  \hspace{1cm} (1)$$

where $I_{\text{Brain}}$ and $I_{\text{NP}}$ are the emission intensity from the brain and the NIR-II fluorescent probe, respectively. The integrals extend along the full spectral range of the emissions. Under such a definition, the overlap parameter $\Gamma$ can range from 0 (no overlap, ideal case for background-free brain imaging) to 1 (equal shape of the two emission bands leading to poor contrast in brain imaging).

Figure 5 shows the brain autofluorescence together with the emission of the most widely used contrast agents in NIR-II imaging—organic dyes (Figure 5A), single-walled carbon nanotubes (Figure 5B), rare-earth-doped NPs (Figure 5C) and Ag$_2$S NPs (Figure 5D). The spectral overlap factor $\Gamma$ between the emission of each probe and the autofluorescence of the brain is also shown. As can be observed, $\Gamma$ ranges from high or moderate (Figure 5A, D) to low or negligible (Figure 5B,C). All NIR-II contrast agents evaluated here show non-negligible spectral overlaps with the autofluorescence of the brain except for erbium-doped NPs, which show an emission band at around 1500 nm that makes them ideal for autofluorescence-free NIR-II brain imaging. Other widely used probes, such as Ag$_2$S NPs, show a relevant overlap with autofluorescence that has so far been neglected. This fact indicates that brain images obtained using these NIR-II contrast agents contains, very likely, some background contribution from autofluorescence. Image contrast and spatial resolution could be easily improved in this case by using of band pass or long pass filters.

\section*{4 | CONCLUSION}

In summary, we have applied state of the art in NIR-II imaging technology (whole body HSI) to assess the in vivo infrared autofluorescence of the brain. We found that, when compared to other organs, the brain presents a peculiar infrared autofluorescence that can be attributed to its unique composition. In particular, we state that the dominant presence of lipids, melanin and NM in the brain is what causes its distinct autofluorescence fingerprint. The detailed characterization of the NIR brain autofluorescence provided here allows for the rational selection of NIR-II luminescent probes toward high contrast and high-resolution brain imaging. We have also demonstrated how the presence of autofluorescence in images obtained with widely used NIR-II contrast agents (such as carbon nanotubes or Ag$_2$S NPs) cannot be neglected, leading to a deterioration in image contrast. Finally, we have also provided experimental evidence of the nonhomogeneous distribution of brain autofluorescence, revealing the possibility of imaging brain structures based on their autofluorescence, that is, removing the need for contrast agents.

\section*{ACKNOWLEDGMENTS}

This work was supported by the Spanish Ministry of Economy and Competitiveness under projects MAT2016-75362-C3-1-R, MAT2017-83111R and MAT2017-85617-R, by the Instituto de Salud Carlos III (PI16/00812), by the Comunidad Autónoma de Madrid (B2017/BMD-3867RENIMCM), and co-financed by the European Structural and investment fund. Additional funding was provided by the European Union Horizon 2020 FET-Open project NanoTBTech (801305), the Fundación para la Investigación Biomédica del Hospital Universitario Ramón y Cajal project IMP18_38 (2018/0265), and also by COST action CA17140. E. X. is grateful for a Juan de la Cierva Formación scholarship (FJC2018-036734-I).

\section*{CONFLICT OF INTEREST}

The authors declare no conflict of interest.

\section*{AUTHOR CONTRIBUTIONS}

José Lifante and Erving Ximendes contributed to the imaging experiments and the writing of this manuscript. Irene Chaves-Coira contributed to the design and development of in vivo experiments. Nuria Fernández, Blanca del Rosal and Daniel Jaque contributed to the design and development of in vivo experiments and the writing of this manuscript.

\section*{DATA AVAILABILITY STATEMENT}

Data available on request from the authors.

\section*{ORCID}

Daniel Jaque https://orcid.org/0000-0002-3225-0667

\section*{REFERENCES}

[1] V. L. Feigin, A. A. Abajobir, K. H. Abate, F. Abd-Allah, A. M. Abdulle, S. F. Abraha, G. Y. Abyu, M. B. Ahmed, A. N. Aichour, I. Aichour, M. T. E. Aichour, R. O. Akinyemi, S. Alabed, R. Al-Raddadi, N. Alvis-Guzman, A. T. Amare, H. Ansari, P. Anwar, J. Arnlöv, H. Asayesh, S. W. Asgedom, T. M. Atey, L. Avila-Burgos, E. Frinel, G. A. Avokpah, M. R. Azarpazhooh, A. Barac, M. Barboza, S. L. Barker-Collo, T. Bärnighausen, N. Bedi, E. Beghi, D. A. Bennett, I. M. Bensenor, A. Berhane, B. D. Betsu, S. Bhaumik, S. M. Birlik, S. Biryukov, D. J. Boneya, L. N. B. Bulto, H. Carabin, D. Casey,
SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

[32] Y. Zhong, Z. Ma, S. Zhu, J. Yue, M. Zhang, A. L. Antaris, J. Yuan, R. Cui, H. Wan, Y. Zhou, W. Wang, N. F. Huang, J. Luo, Z. Hu, H. Dai Nat Commun. Theory 2017, 8, 737.

How to cite this article: Lifante J, del Rosal B, Chaves-Coira I, Fernández N, Jaque D, Ximendes E. The near-infrared autofluorescence fingerprint of the brain. J. Biophotonics. 2020;13: e202000154. https://doi.org/10.1002/jbio.202000154