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# Investigating respiratory rhythm generation using endoscopic 2-photon and photoacoustic imaging

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**Abstract** (10 lines)

Breathing is a continuous oscillatory activity, driven by a complex neuronal network deep in the brain. Core to this network are intermingled neuronal microcircuits that generate the respiratory rhythm, yet we still lack fundamental understanding about how it works in adults *in vivo*. Our aim is to image the activity of these microcircuits with cellular resolution, to identify activity patterns that could underlie respiratory rhythm generation. Yet, the depth of these neurons prevents the use of conventional high resolution imaging techniques. We recently developed a flexible lensless endoscope that is compatible with non-linear imaging, therefore enabling 2-photon imaging deep in the brain of freely behaving animals. We are also developing a photoacoustic imaging setup, to image non-invasively in deep soft tissue, which will be tested in parallel. Using both systems, we will image the neurons of different respiratory rhythm generating microcircuits, based on their neurochemical phenotype and their projection patterns.

**Keywords**
Deep neuronal circuits imaging; respiratory rhythm generation; lensless 2-photon endoscope; functional micro-network dynamics; photoacoustic imaging.

**Objectives** (5 lines)

Our objective is to image with cellular resolution neuronal microcircuits involved in respiratory rhythm generation in freely moving animals. The successful applicant will (i) perform stereotaxic injections of viruses for expression of calcium indicators in respiratory neuronal groups in adult mice and rats, (ii) implant the lensless endoscope (for 2-photon imaging) or the Fabry-Perot polymer film (for photoacoustic imaging), and (iii) image neuronal activity simultaneously with recordings of respiratory activity.

**Expected profile** (5 lines)
We are looking for a highly motivated candidate with a PhD in neuroscience, biophysics, or similar. Independence and a strong interest in technological development are essential. Previous experience in rodent surgery, electrophysiology, signal processing and programming are required. Previous experience with stereotaxic injection of viruses, calcium imaging, optogenetics, and working in the field of central respiratory control would be a plus but is not compulsory.

**Is this project the continuation of an existing project or an entirely new one?**

**In the case of an existing project, please explain the links between the two projects (5 lines)**

It is not the continuation of an existing project.

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## 2 to 5 references related to the project

[1] Del Negro CA, Funk GD, Feldman JL, "Breathing matters", *Nat. Rev. Neurosci.,* 2018 Jun;19(6):351-367, DOI: 10.1038/s41583-018-0003-6.

[2] Ramirez JM and Baertsch NA, "The dynamic basis of respiratory rhythm generation: one breath at a time", *Annu. Rev. Neurosci.*, 41, 475-499, 2018, DOI: 10.1146/annurev-neuro-080317-061756.

[3] G. Meng *et al.*, "High-throughput synapse-resolving two-photon fluorescence microendoscopy for deep-brain volumetric imaging in vivo," *eLife*, vol. 8, p. e40805, Jan. 2019, DOI: 10.7554/eLife.40805.

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## 3 main publications from each PI over the last 5 years

Clément Menuet (lead supervisor)

[1] Menuet C, Connelly AA, Bassi JK, Melo MR, Le S, Kamar J, Kumar NN, McDougall SJ, McMullan S, Allen AM. PreBötzinger complex neurons drive respiratory modulation of blood pressure and heart rate. Elife 2020 Jun 15 ;9e57288. DOI: 10.7554/eLife.57288.

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[3] Ngo HB, Melo MR, Layfield S, Connelly AA, Bassi JK, Xie L, Menuet C, McDougall SJ, Bathgate RAD, Allen AM. A chemogenetic tool that enables functional neural circuit analysis. Cell Reports 2020 Sep 15;32(11):108139. DOI: 10.1016/j.celrep.2020.108139.

Hervé Rigneault (co-supervisor 1)

[1] V. Tsvirkun, S. Sivankutty, K. Baudelle, R. Habert, G. Bouwmans, O. Vanvincq, E. R. Andresen, and H. Rigneault, "Flexible lensless endoscope with a conformationally invariant multi-core fiber," Optica 6, 1185-1189 (2019). https://doi.org/10.1364/OPTICA.6.001185

[2] S. Sivankutty, V. Tsvirkun, O. Vanvincq, G. Bouwmans, E. R. Andresen, and H. Rigneault, "Nonlinear imaging through a Fermat's golden spiral multicore fiber," Optics Letters 43, 3638-3641 (2018). https://doi.org/10.1364/OL.43.003638

[3] Esben Ravn Andresen, Siddharth Sivankutty, Viktor Tsvirkun, Géraud Bouwmans, Hervé Rigneault, 'Ultrathin endoscopes based on multicore fibers and adaptive optics: a status review and perspectives,' J. Biomed. Opt. **21**(12), 121506 (2016). https://doi.org/10.1117/1.JBO.21.12.121506

Thomas Chaigne (co-supervisor 2)

[1] Schulze L, Henninger J, Kadobianskyi M, Chaigne T, Faustino AI, Hakiy N, Albadri S, Schuelke M, Maler L, Del Bene F, Judkewitz B. Transparent Danionella translucida as a genetically tractable vertebrate brain model. Nat Methods. 2018 Nov;15(11):977-983. doi: 10.1038/s41592-018-0144-6.

[2] Chaigne T, Arnal B, Vilov S, Bossy E, Katz O. Super-resolution photoacoustic imaging via flow-induced absorption fluctuations. Optica, 4(11), 1397-1404 (2017) doi: 10.1364/OPTICA.4.001397

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