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# GABA-A rhythmic inhibition in the claustrum drives network oscillation

Gilles Ouanounou, Ambre Ledoux and Thierry Bal

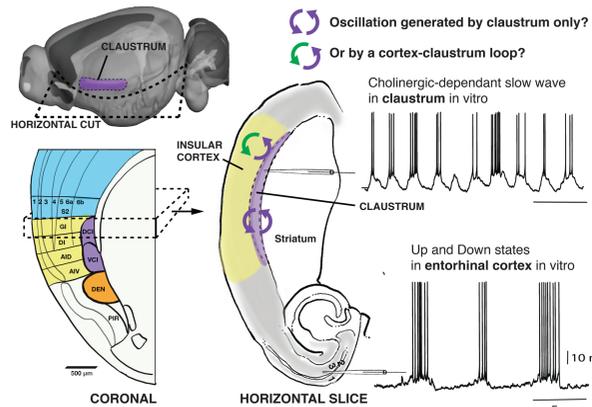
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## Introduction

The claustrum is phylogenetically highly preserved in amniotes, from reptiles and birds to mammals, including humans (Norimoto et al., 2020). It is a thin structure that lays deep between the insular cortex and the striatum. It contains a small proportion of interneurons of various types, and a majority of far-projecting excitatory neurons. It is a high-level structure holding the most central position in the brain hierarchy, being reciprocally connected to all cerebral cortical areas, to the amygdala, hippocampus, and thalamus. Its functional role is a recent focus of research in neurosciences as it remains largely unknown during both sleep and wakefulness. Due to its high connectivity it has been proposed to be a "seat of consciousness" and involved in multi-sensory binding (Crick & Koch, 2005), a hub for attention (Goll et al., 2015; Atlan et al., 2018; Fodouliau et al., 2020). Most importantly for the present work, the claustrum might orchestrate and integrate cortical activity by coordinating synchronized oscillations in cortical areas during sleep and quiet wakefulness (Narikiyo et al., 2020). The claustrum could act as a synchronizing relay between areas, activating their local GABAergic interneurons (Narikiyo et al., 2020). It could also act during sleep as a "conductor" (Crick & Koch, 2005), driving slow-wave sleep activity on its own rhythm.

Our recordings in the claustrum in vitro show the emergence of a highly regular oscillation in the 0.3-1 Hz range in the presence of low doses of the cholinergic agonist carbachol (CCH). Release of acetylcholine from channelrhodopsin-containing cholinergic afferents also boosts the oscillation (not shown). We found that the oscillation is a synchronized network phenomenon that relies on the occurrence of a rhythmic GABA-A inhibition in the excitatory claustral cells, suggesting the involvement of inhibitory interneurons in the claustrum. We also recorded a subset of claustral neurons with endogenous rhythmic behavior. Whether these (inter)neurons play a pacemaker role in the network oscillation, and what kinds of synaptic loops there are within the claustrum and/or between the claustrum and the adjacent insular cortex, remains to be examined.

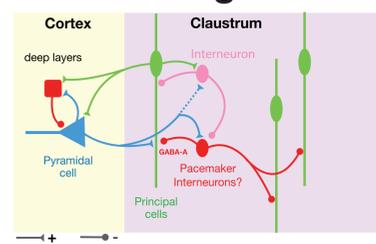
## Clastrum-cortex brain slice



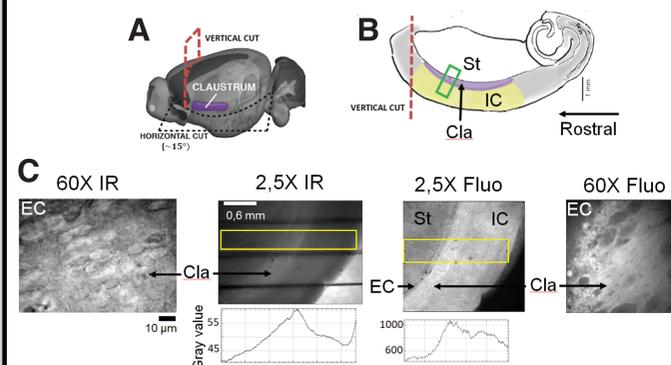
Submerged quasi horizontal slices from P12-P20 wild-type or transgenic mice maintained at 32-34°C generate slow oscillations of different types in the claustrum (purple babana shape) and the entorhinal cortex. Upper trace: Oscillations induced by cholinergic agonist carbachol in the claustrum range between 0.3-1 Hz (see Figs 2). Bottom trace: example of typical Up-and-Down oscillation generated through different mechanisms in the entorhinal cortex in the same horizontal slice in absence of Carbachol (0,13 +/- 0,02 Hz; Tahvilidari et al., 2012). Mouse brain: Allen Brain Explorer.

## Putative claustrum-cortex wiring circuit

Working synaptic diagram of the network present in the horizontal slice derived from Kim et al., 2016, and from our investigations. Present data suggest the existence of highly synchronized GABA-A inhibitions (generated by red interneurons) in claustrum principal cells (green). As suggested in the previous figure, the possible involvement of insular cortex (left) remains to be examined. GABAergic neurons (pink; red); Excitatory neurons (cortex: blue; claustrum: green).

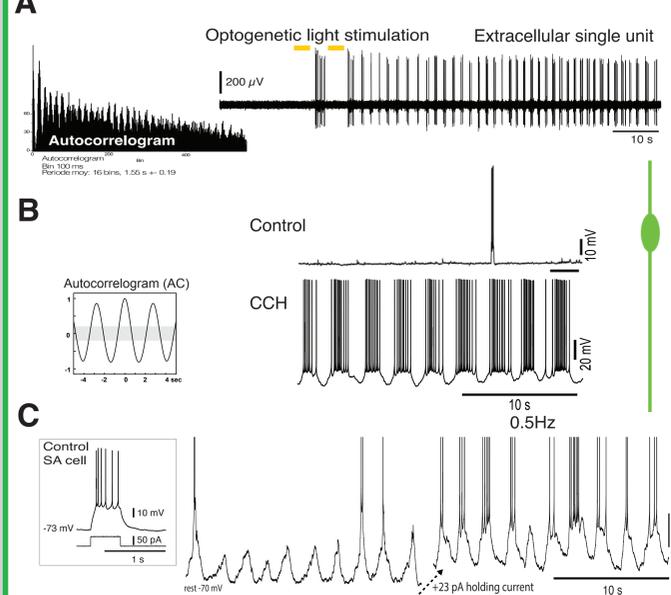


## 1 Anatomical identification of the claustrum in living brain slices



**A:** Mouse brain left hemisphere from Allen Brain Explorer, with our 2 dissection cuts and the claustrum in purple. **B:** Sketch of a horizontal slice obtained from the horizontal cut in (A). **C:** Horizontal slice pictures under infrared light (IR) and blue laser (Fluo). Yellow rectangles correspond to the green rectangle in (B), within which the mean gray values below are measured (sum of the gray values of all the pixels in the rectangle, divided by the number of pixels). Infrared light shines through the slice, revealing a clear band noticeable as a peak of mean gray value. This area has the same location and shape as the region with enhanced VGLUT2/EGFP-expression cells, which corresponds to the claustrum (Fodouliau, 2020). Cla: Claustrum; EC: External Capsule (myelinated dark curved line at the internal edge of the Cla); IC: Insular Cortex; St: Striatum; C: IR: n = 58, Fluo: n = 16.

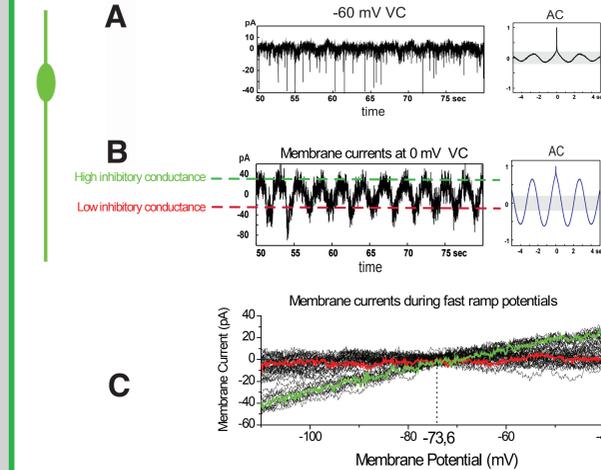
## 2 Cholinergic-induced oscillation in the claustrum



**A:** Extracellular unit during application of the cholinergic agonist carbachol. Full-field white light stimulation of the slice (yellow bars) in a *emx-creXAI35* P14 transgenic mice expressing Archaelrhodopsin kick starts the oscillation. **B:** Whole-cell recording of a claustrum principal cell. Cholinergic modulation (CCH) makes cla principal cells go from rare activity to rhythmic bursting activity. Autocorrelogram computed on spike counts for 200 ms bins. **C:** Strongly adapting (SA) claustrum principal cell (wild-type C57b16; P13; CCH). Hyperpolarized Vm reveals rhythmic synaptic drive.

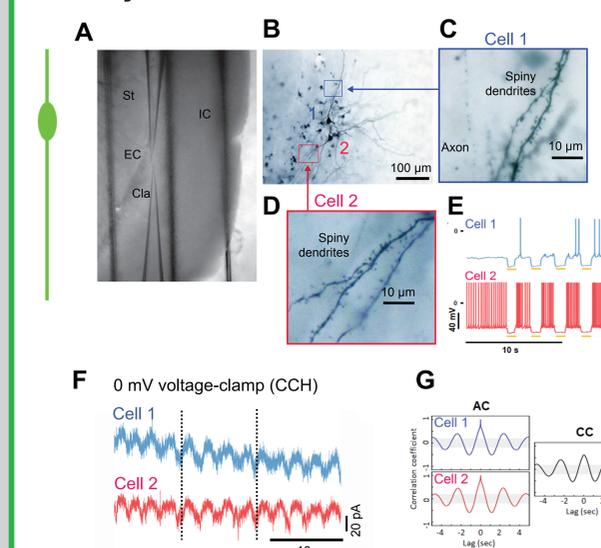
## A network oscillation

### 3 GABA-A inhibitions underly rhythmic oscillations



**A:** Voltage-clamp (VC) recording at -60 mV with CCH and corresponding autocorrelogram (AC). **B:** VC recording at 0 mV (CCH). Synaptic excitatory inputs are tonic but there is a periodic change in inhibitory conductances, which are GABA-A-related and could be responsible for the voltage oscillation (same cell as in Fig. 2B) as it occurs at the same frequency. **C:** IV curves resulting from voltage-clamp ramp protocol. Examples of IV curves obtained at high levels (green) and low levels (red) of inhibitory conductance as seen in (B). On average, the reversal potential was  $-73.1 \text{ mV} \pm 1.75 \text{SD}$ , n=6 cells.

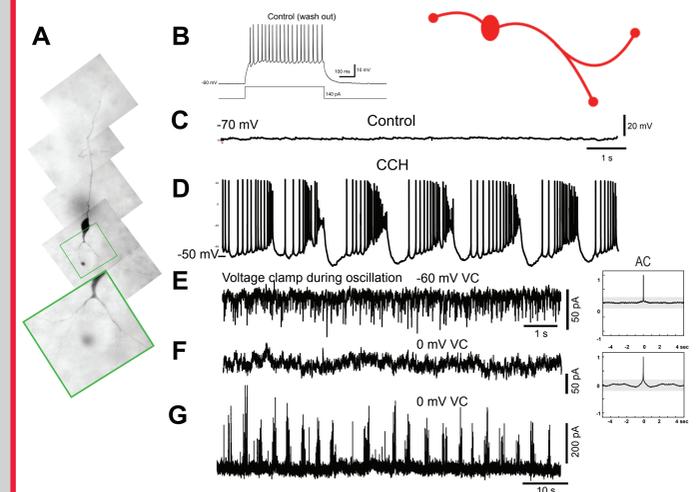
## 4 Dual voltage-clamp recordings reveal synchronized network oscillation



**A:** Recording of a pair of excitatory principal cells in the claustrum of a VGLUT2 mouse (Fodouliau, 2020) using two patch pipettes. **B:** Picture of the two biocytin-filled cells. Squares are details of the spiny dendrites of each cell shown in (C-D) at higher magnifications. **E:** Current clamp recording of these two archaelrhodopsin-containing cells showing hyperpolarizations subsequent to 575 nm light exposure, confirming that those are VGLUT2-expressing cells and thus principal neurons. **F:** Dual voltage-clamp recording of cells 1 and 2 at 0 mV under carbachol. Traces are so similar that the synchronized rhythmic inhibitory synaptic inputs we detect with auto- (G, left) and cross- (G, right) correlograms, must presumably come from one or a few interneurons only.

## A pacemaker hypothesis

### 5 A subset of neurons with intrinsic oscillatory membrane properties



**A:** Composite picture of a neuron presenting no dendritic spines, suggesting that it could be an interneuron subtype. **B:** Firing response to a current pulse. **C:** Current-clamp recording of the neuron. In absence of carbachol (CCH) the membrane potential is quiescent. **D:** CCH induces membrane oscillation which might be an endogenous membrane property. **E:** Voltage-clamp at -60 mV (left) with corresponding autocorrelogram (right) showing random tonic excitatory synaptic bombardment, as in principal cells. **F:** Voltage-clamp at 0 mV showing no rhythmic inhibitions. **G:** Voltage-clamp at 0 mV in another cell showing strong rhythmic inhibitions (notice different time scale).

## Conclusion

In a mouse brain slice containing portions of the claustrum and insular cortex, we previously found that claustral excitatory principal cells undergo a slow oscillation under light cholinergic modulation. Here transgenic mice were used to help identify a simple location criterion for the claustrum: its relative clarity under light shown through the living slices. Then we performed single and dual whole-cell patch-clamp recordings in order to characterize the oscillation as well as its synaptic underlying mechanisms.

We showed that the claustrum oscillation is a network phenomenon involving primarily excitatory principal cells simultaneously driven by a tonic excitatory drive and rhythmic GABA-A inhibitions from presumably local interneurons. In addition, we found a putative interneuron subtype that presents endogenous oscillatory membrane properties revealed only in the presence of the cholinergic agonist CCH and under the influence of a tonic excitatory synaptic drive. We propose that this intrinsic oscillator may play a crucial role in the network oscillation by driving it as a pacemaker. It remains to be examined whether the insular cortex, which is synaptically connected to the claustrum, could be involved in the oscillatory network mechanism via a simple excitatory drive or via a synaptic loop with the claustral network (see "putative claustrum-cortex wiring circuit").

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