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# Cholinergic-induced slow-wave oscillation in the claustrum *in vitro*

Thierry Bal, Gilles Ouanounou and Cyril Monier

Neuro-Psi, CNRS/Université Paris-Sud, UMR 9197, Integrative and Computational Neuroscience department (ICN), Gif-sur-Yvette, France

## Introduction

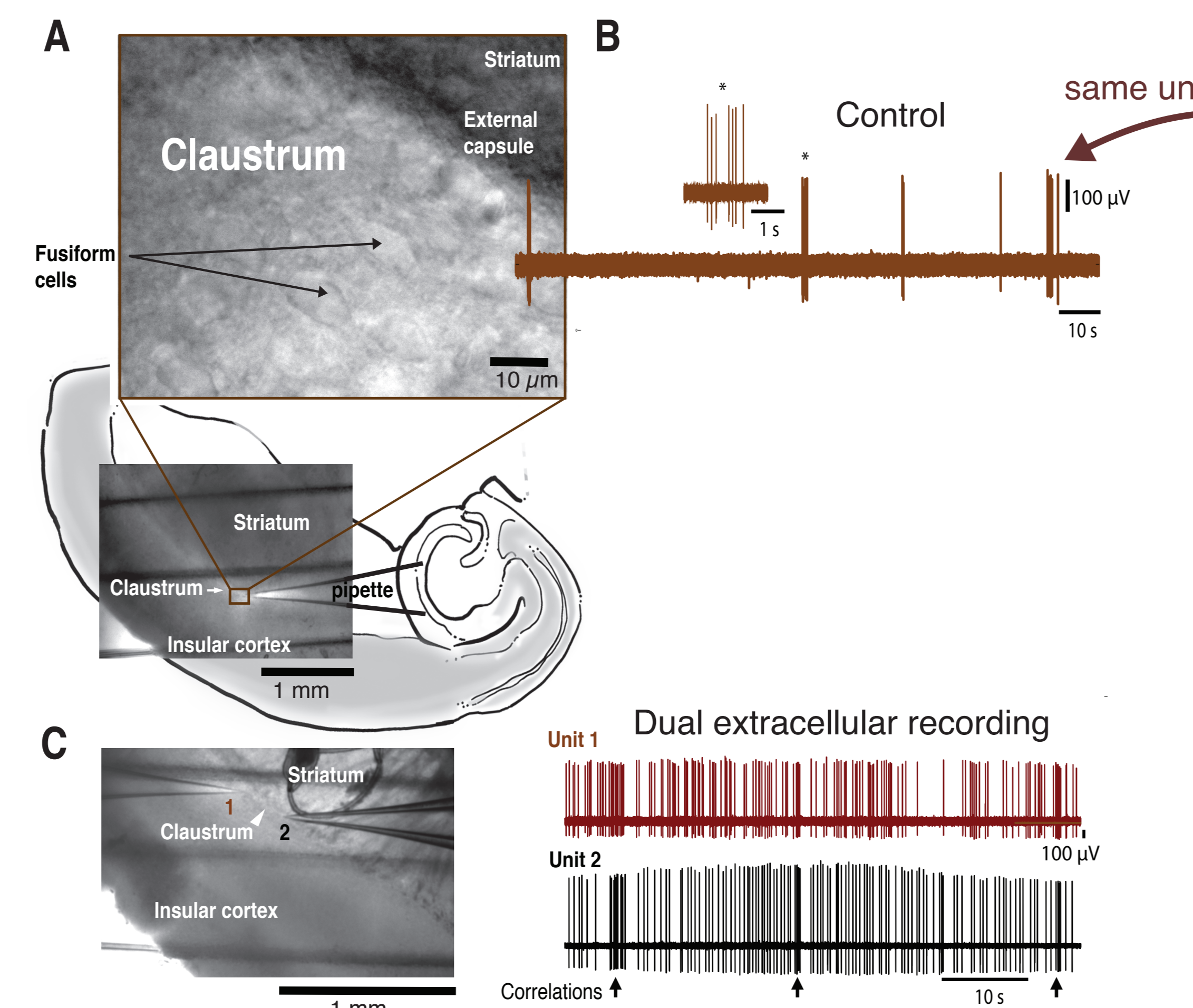
The claustrum contains interneurons and excitatory claustrum-cortical principal neurons that lay deep below the insular cortex. It is the most reciprocally connected structure in the brain and receives cholinergic and a variety of neuromodulatory inputs from subcortical structures (Goll et al., 2015). The functional role of the claustrum remains largely unknown. Often compared to the thalamus, it has been proposed to be a central integrator for the unified sense of cognition (Crick & Koch, 2005), a hub for attention (Atlan et al., 2018), for multi-sensory binding, and may modulate the synchronization of neocortical slow-wave activity (Narikiyo et al., 2018) or enhance synchronized oscillations between cortical areas (Vidyasagar & Levichkina, 2019). Little is known on the claustrum circuit properties or on its cell-types: the membrane and synaptic properties, the sensitivity to neuromodulation, and spontaneous states of activities.

In a mouse brain slice in which portions of the claustrum and cortex remain synaptically connected, we found that the claustrum generates two different types of activities: i) A spontaneous and moderate spiking discharge in principal neurons and interneurons generated by background synaptic activity appears randomly, can last hundreds of milliseconds to seconds, and returns to quiescent periods. ii) Application of low concentrations of the cholinergic agonist carbachol induces in about half of the experiments a robust 0,5 - 0,7 Hz rhythmic recurrent network oscillation characterized by the occurrence of compound excitatory and inhibitory postsynaptic potentials and spike discharge.

We are exploring the mechanisms of this claustrum slow-wave activity: Does it result solely from the interactions of neurons within the claustrum (green arrows in fig. below), although there are few direct connections between its principal cells (Kim et al., 2016), and/or is it due to synaptic loops between the claustrum and neocortex, such as the adjacent insular cortex, or the entorhinal cortex that spontaneously generates Up & down states in the same slice (orange arrows)?

## Claustrum activity in absence of neuromodulation

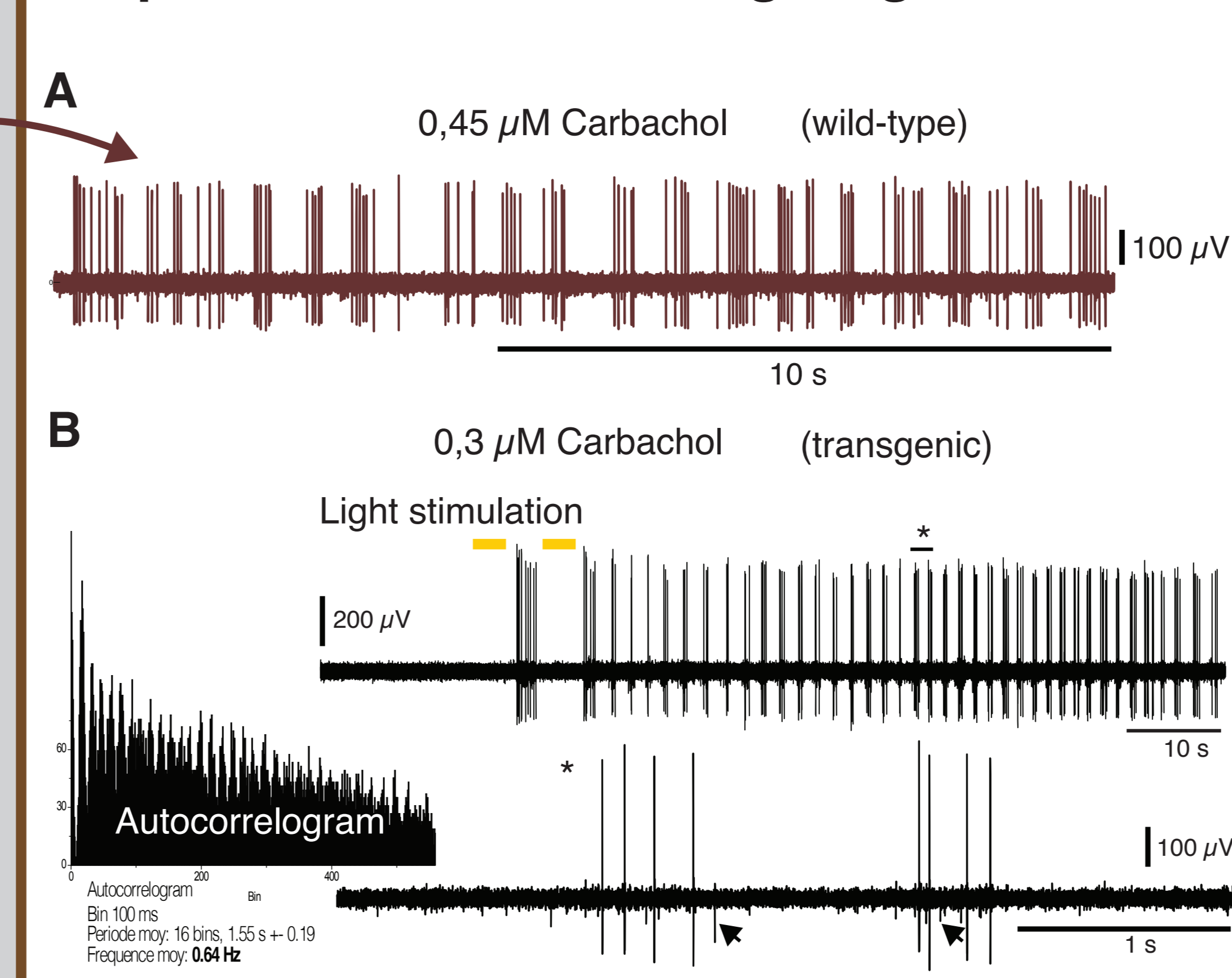
### 1 Claustrum units generate irregular tonic and burst firing in absence of neuromodulation



**A.** Overlay of a picture of the recording site on a brain slice drawing. **B.** Single unit recorded in (A). **C.** In another slice, examples of spontaneous tonic and bursts (arrows) activities. Note that burst activities are synchronized between the two units, suggesting a correlated synaptic drive.

## Cholinergic modulation induces slow oscillation

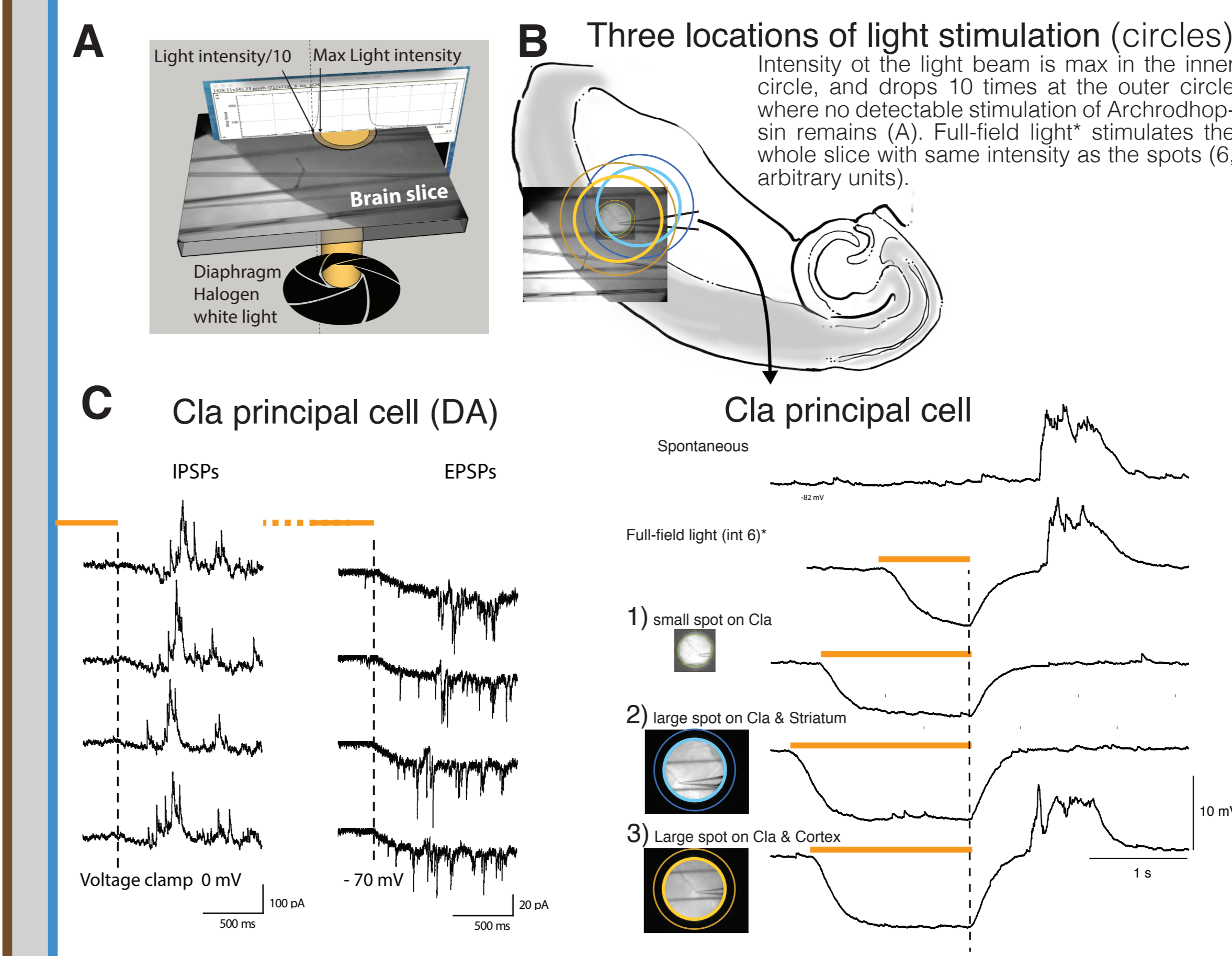
### 3 Claustrum units oscillate at 0,5-0,7 Hz in presence of a cholinergic agonist



**A.** Extracellular units in the claustrum of a wild-type C57bl6 P12 mice during bath application of the cholinergic agonist carbachol. **B.** Full-field white light stimulation of the slice (yellow bars) in a *emx-creXAI35* P14 transgenic mice (expressing *ArchaeRodhopsin*) kick starts the oscillation in an otherwise quiescent network. Arrows: correlated units during bursts.

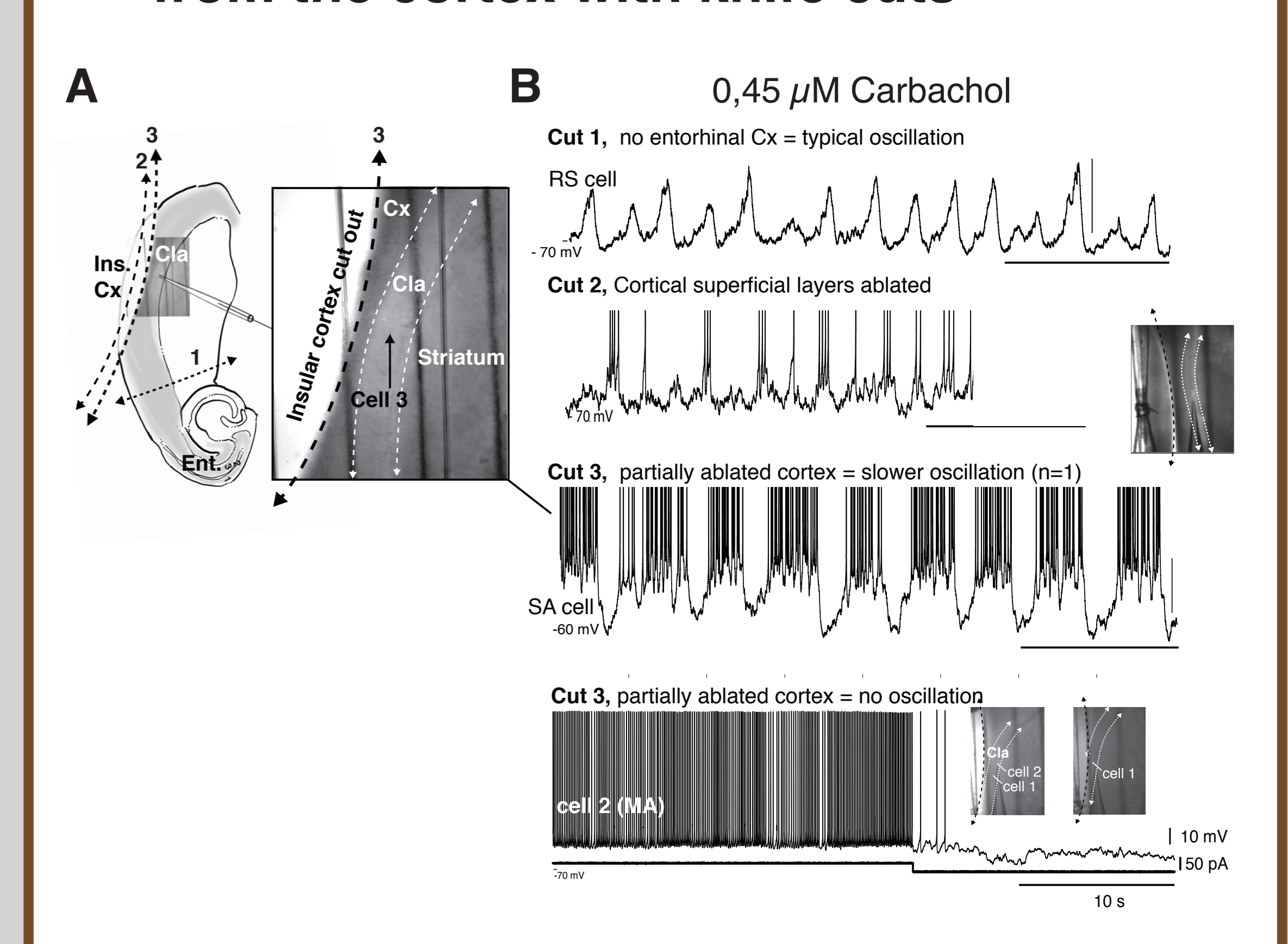
## Is the oscillation emerging within the claustrum, or from an interaction with the cortex? (Preliminary experiments)

### 5 Probing connectivity with optogenetics



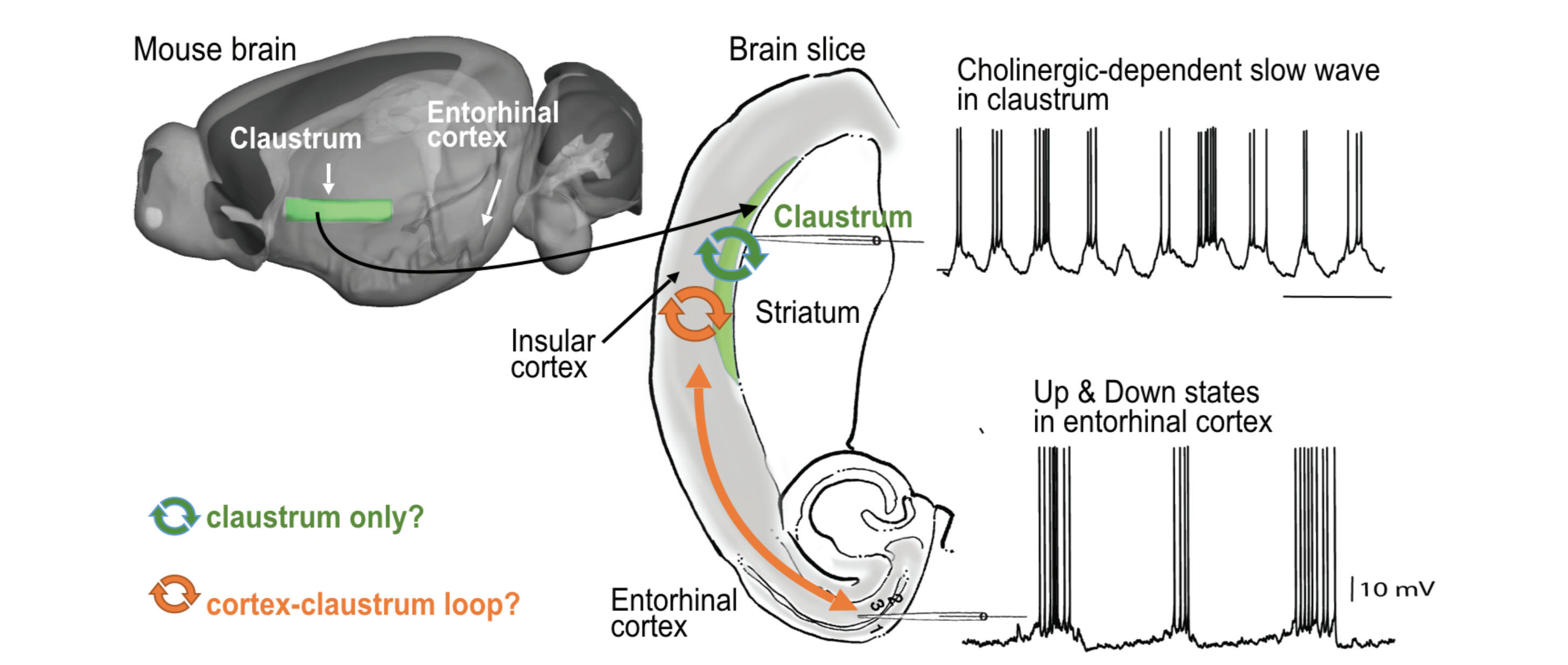
**Light spots inhibit neurons expressing *ArchaeRodhopsin* (*EMX\*cre-Ai35* in claustrum (Cla) and cortex).** **A.** A beam of white light of various diameter is focused at different locations from below the slice. **B.** Rebounds of synaptic activity at the offset of light stimulations in a principal cell. **C.** A DA (doublet amplitude) neuron receives compound IPSPs & EPSPs at the offset of light stimulation (dashed line).

### 7 Partial disconnection of the claustrum from the cortex with knife cuts



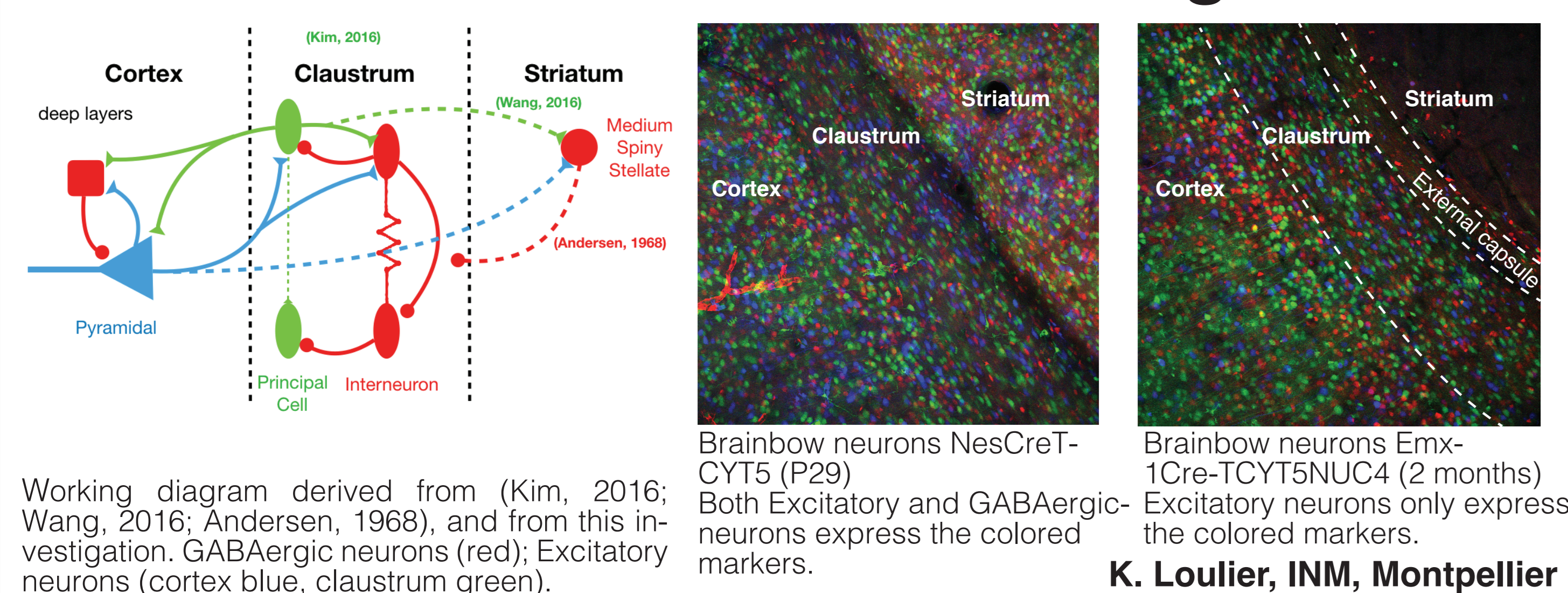
Preliminary experiments suggesting that the typical 0,5 - 0,7 Hz oscillation is altered after partial disconnection from cortex. **A.** positions of cuts in different experiments (black dashed lines). **B.** Ablation of the entorhinal cortex (1); Partial ablations of the insular cortex (2,3) (cell 1, same result, not shown). More experiments needed.

## Cortical-claustrum brain slice



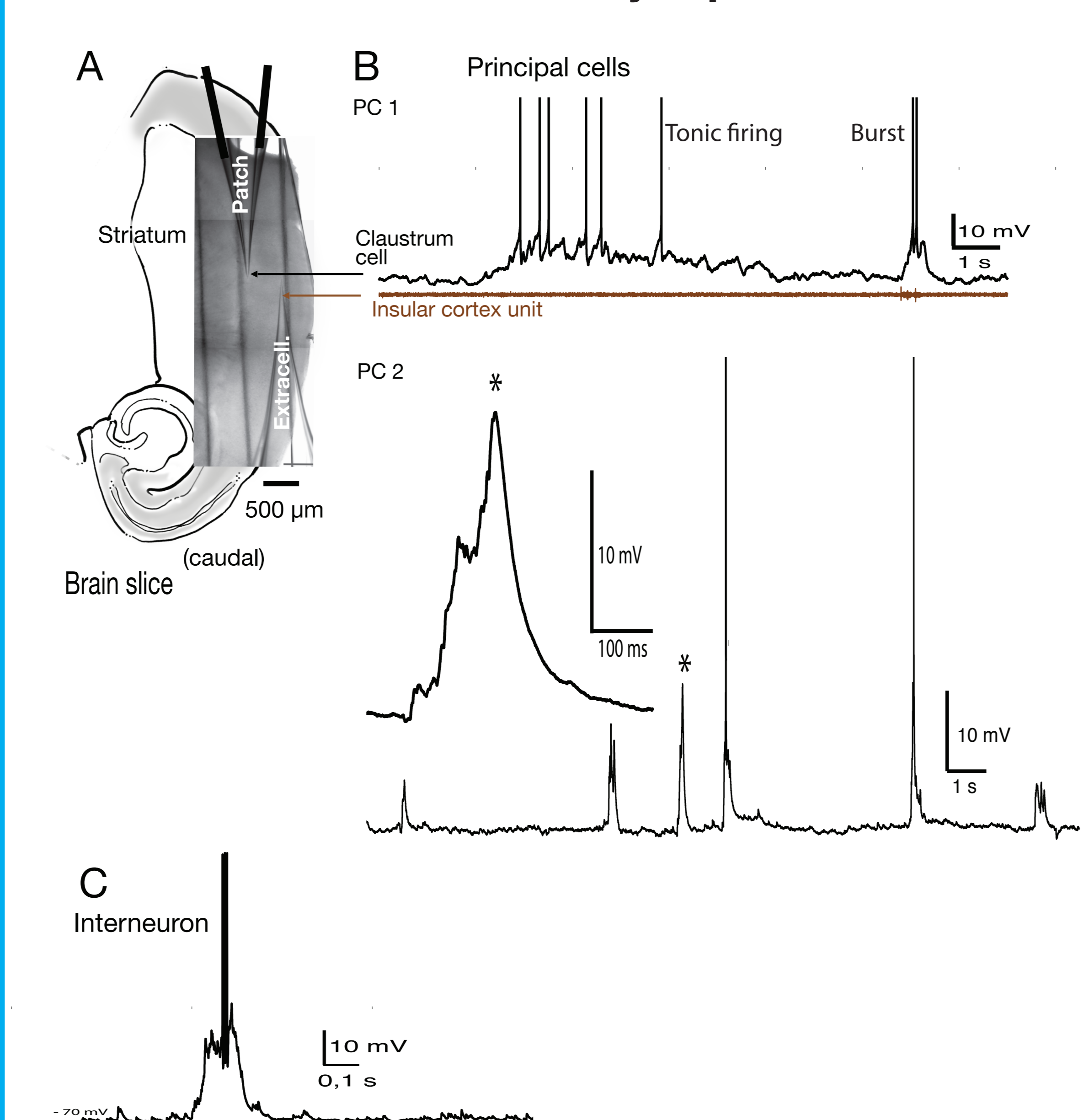
Submerged quasi horizontal slices from P12-P20 wild-type or transgenic mice maintained at 34°C generate slow oscillations of different types in the entorhinal cortex and the claustrum (green tabana shape). Oscillations in the entorhinal cortex have a significant lower frequency under 0,5 μM carbachol (0,2 ± 0,03Hz, data from Tomas Gorski (Nghiem et al., BioRxiv 2018), than in the claustrum (0,5-0,8 Hz, Figs 3 & 4). Bottom trace no Carbachol (0, 13 ± 0,02 Hz). Mouse brain: Allen Brain Explorer.

## Putative claustrum-cortex wiring circuit



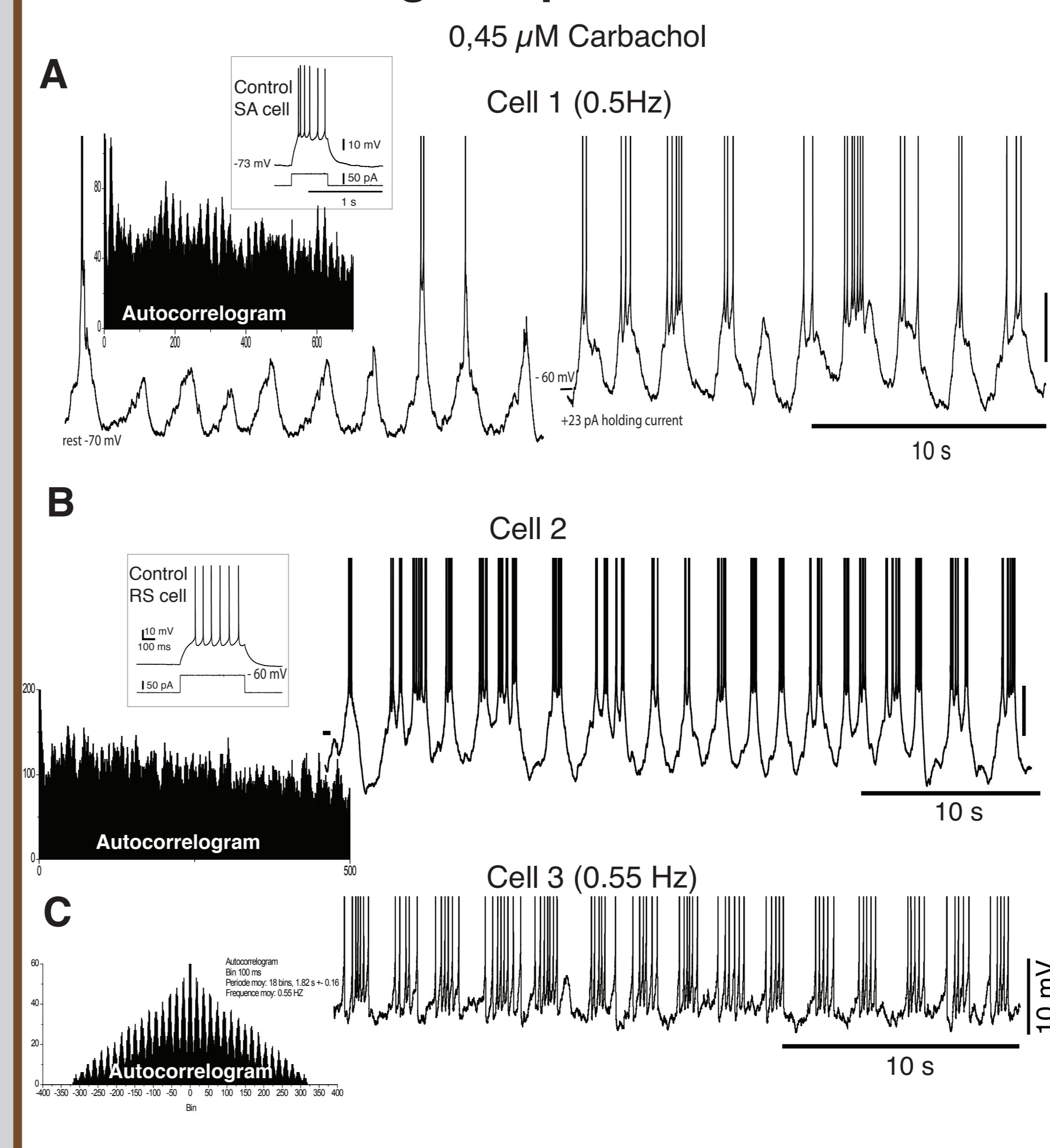
Working diagram derived from (Kim, 2016; Wang, 2016; Andersen, 1968), and from this investigation. GABAergic neurons (red); Excitatory neurons (cortex blue, claustrum green). **K. Loulier, INM, Montpellier**

## 2 Tonic and irregular burst firing in claustrum neurons results from a synaptic drive



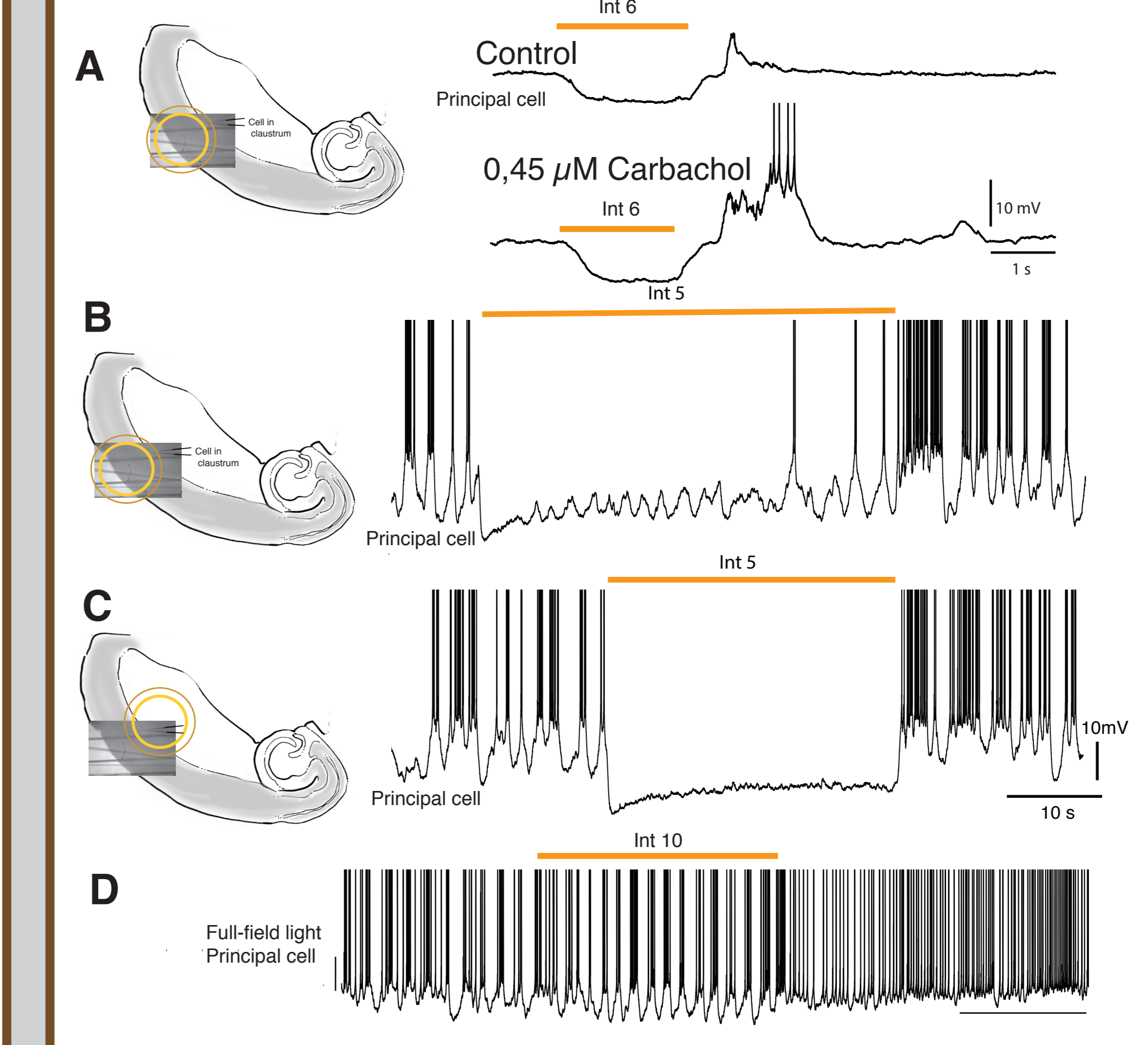
**In absence of neuromodulation.** **A.** Overlay of recording sites on a brain slice drawing. **B.** Examples of spontaneous synaptic activities (tonic or sharp synaptic drives) recorded in claustrum principal neurons (PC) in two different slices (PC1 is cell 3 in fig 4C). **C.** A sharp synaptic drive in a Cla interneuron.

## 4 Rhythmic synaptic bombardment drives the cholinergic-dependent oscillation



**A.** Whole-cell patch recording of a strongly adapting (SA) claustrum principal cell (wild-type C57bl6 P13 mouse). **B.** Regular spiking (RS) neuron (*EMX-creXAI35* P14 transgenic mice) **C.** PC1 in Fig. 2B. Autocorrelograms reveal the regularity of the oscillation for each cell. Mean frequency from autocorrelograms = 0,58 ± 0,06 Hz (n = 2 intracell. + 3 extracell.)

## 6 Optogenetic dissection of the oscillation



**Inhibiting parts of the network with *ArchaeRodhopsin* activation.** **A.** CCH enhance the network rebound activity at the offset of light stimulation centered on cortex (intensity 6, arbitrary scale, white light). **B.** Light spot over cortical layers, while Cla is in dimmer light, preserves the oscillation suggesting that cortical layer under the spot are not sufficient for the oscillation genesis, despite their connections with Cla (see A & Fig 5). **C.** Moving the spot on Cla blocks the oscillation, indicating that either the claustrum is necessary for the oscillation and/or that *archaeRodhopsin* activation in cla cells shunts the inputs coming from outside the shunted area. **D.** Inhibition of Parvalbumin-containing cells (full field illumination of slices, PVcreXai35 mice, n=4).

## Conclusion

We found that, in the absence of neuromodulation, different types of claustrum principal neurons and interneurons receive erratic and robust compound synaptic inputs (up to 30mV amplitude) that drive their spike response. This pattern contrasts with the regular Up & Down rhythm in the entorhinal cortex (EC) located in the same slice.

In contrast, cholinergic modulation results in the occurrence of a synaptically driven (mixing EPSPs and IPSPs) periodic oscillation in claustrum principal neurons of presumably different origin than in the EC, and three times faster.

At present, we have not fully identified the nature of the synaptic drive. We found that it relies on a mixture of excitation and inhibition which origin, balance and timing remain to be identified. The synaptic drive is independent of the EC's Up & down oscillation, but could follow, or interact with, the periodic oscillations from another cortex such as the nearby insular cortex. We have not yet tested the putative implication of the striatum that is reported to receive claustral inputs (Wang et al., 2016).

Due to its unique widespread connectivity in the brain, the functional implications of the claustrum as a "conductor of slow oscillation" would relate to sleep and wake states. During sleep it could entrain the rhythm of slow-waves (Narikiyo, 2018). During wake it may relate to slow waves in attentional selection (Lakatos, 2008).

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