# Poster Abstracts for
# Society for Claustrum Research Symposium 2016

(1)
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Characterization and functional analysis of claustral VIP interneurons

The claustrum is a little-understood structure that is embedded within the deep layers of the insular cortex. As in all other brain regions, it is likely that interneurons play important roles in information processing within the claustrum. We have defined the morphology, intrinsic electrical properties and functional connectivity of one type of claustral interneuron, VIP expressing interneurons (VIP+ INs). The electrical properties of these interneurons were determined via patch clamp recordings in acute brain slices. VIP+ INs from transgenic mice expressing Channelrhodopsin-2 exclusively in VIP neurons could be identified by photostimulation when exposed to blue light. VIP+ INs differed from other claustral INs in their somatic shape, which were either multipolar or elongated/ bipolar. Their neurites projected mainly to the shell or edge of the claustrum core, which contrasts from the enrichment of processes from PV INs within the core. VIP+ IN had the highest input resistance, broadest AP half-width, the shallowest AHP, and lowest maximum firing rate of any claustral INs. High-speed optogenetic circuit mapping (PNAS 104: 8143) revealed that VIP+ INs are connected to projection neurons as well as to other IN populations. Remarkably, this inhibitory input was much stronger and more efficient for INs than for projection neurons. Thus, it appears that claustral VIP+ INs specifically target other local IN populations and might relieve projection neurons from local inhibition imposed by PV+ and SST+interneurons. In general, VIP+ INs are strategically situated to increase signaling of claustral projection neurons via disinhibition, well-positioning these INs to regulate information flow through the claustrum during attention allocation (TINS 38: 486), consciousness (Philos Trans R Soc Lond B Biol Sci. 360: 1271), or other functions proposed for this highly interconnected brain region.

(2)
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The claustrum activates the cortex during paradoxical (REM) sleep

Recent studies strongly support a role of the paradoxical sleep (PS) in learning and memory consolidation (Dudai et al. 2015). However, the mechanisms underlying the beneficial effect of PS on learning and memory have not yet been identified. To this aim, we recently identified at

cellular level the populations of cortical neurons activated and displaying plasticity during PS hypersomnia by means of functional neuroanatomy (Renouard et al. 2015). Our mapping clearly shows for the first time that only a small number of limbic structures are activated during PS in contrast to waking. These structures are the cortical amygdaloid nucleus, the anterior cingulate, retrosplenial and medial entorhinal cortices, the claustrum and the dentate gyrus (DG) (Renouard et al. 2015). Further, combining retrograde tracing, neurotoxic lesion and FOS immunostaining, we showed that neurons of the claustrum and from the lateral part of the supramammillary nucleus (SuML) are responsible for the activation of the cortical structures and the DG during PS (Renouard et al. 2015). These surprising results pointed out for the first time that the claustrum and the SuML activate a subset of limbic cortical neurons specifically during PS. We propose that such activation might play a key role in the previously reported beneficial effect of PS on learning and memory. Indeed, many studies clearly indicate that PS is instrumental for memory consolidation (Maquet et al. 2000). Further, it has recently been shown that PS deprivation in rats impairs consolidation of contextual fear conditioning (Ravassard et al. 2016). Our results also point out a key role of claustrum neurons in cortical activation taking place during PS. In summary, original new converging data strongly suggest that the claustrum is activated during PS and plays a key role in activating neurons located in selected cortical limbic structures. Dudai Y, Karni A, Born J (2015) The Consolidation and Transformation of Memory Neuron 88:20-32 doi:10.1016/j.neuron.2015.09.004 Maquet P et al. (2000) Experience-dependent changes in cerebral activation during human REM sleep Nat Neurosci 3:831-836 Ravassard P et al. (2016) REM Sleep-Dependent Bidirectional Regulation of Hippocampal-Based Emotional Memory and LTP Cereb Cortex 26:1488-1500 doi:10.1093/cercor/bhu310 Renouard L et al. (2015) The supramammillary nucleus and the claustrum activate the cortex during REM sleep Science Advances 1 doi:10.1126/sciadv.1400177

(3)

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Computer models of claustrum subnetworks

The dynamical properties of a brain area are determined by its connectivity and by the activation properties of its cells. We utilized data obtained from electrophysiological characterization of newly identified cell types in the claustrum to create a model with 4 classes of excitatory (E) and 3 classes of inhibitory (I) neurons. E cells were classified by degree of adaptation in response to current clamp spike patterns (strongly adapting SA; mildly adapting MA). I cells were classified by adaptation and by peptide markers -- somatostatin (SST) and parvalbumin (PV). Network circuitry between classes was estimated based on functional mapping experiments using optogenetic stimulus to identify locations and, in some cases, cell-type identity of neurons synapsing on a recorded postsynaptic neuron. A simplified spiking neuron model was used in

order to allow running of many thousands of network simulations efficiently. Simulations were run in NEURON on the San Diego Supercomputer via the Neuroscience Gateway. Driving the model with continuous subthreshold background white-noise input induced a persistent excitatory state. In the setting of low background drive, single activation of any one of the 4 E-neuron types would also lead to persistent activation. Stimulation of the MA2 subpopulation gave immediate network activation. Network activation was slightly delayed with stimulation of SA3 or SA4, and delayed >1 s with stimulation of the SA2 population. These delays represented the time required to secondarily activate the MA2 population. The initial activity transient was generally followed by a sustained oscillation whose frequency and amplitude varied depending on precise connection strength parameters. Ongoing activity depended on strong reciprocal connectivity within the MA2 population, which sustained low-level activity in the network during periods between the recurring phases of widespread network activation. Increasing the MA2-MA2 connection strength generally increased oscillatory frequency. Our results suggest that the MA2 neuronal population's strong reciprocal connections would make this a critical population for controlling activation of the claustrum. This suggests this cell population as a possible entry point for claustrum activation which would provide the most rapid activation.

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Secondary Autism Spectrum Disorder following a bilateral lesion of the claustrum: a case report

Despite a growing interest in the role of the claustrum, its function in normal neurological and psychological processes remains largely enigmatic. Nevertheless, there is recent evidence that suggests the claustrum is structurally and functionally compromised in disorders such as epilepsy, autism, schizophrenia, and Alzheimer's disease (for details and references see Smythies J, Edelstein L, Ramachandran V., 2014 and Patru M.C. and Reser D.H., 2015). We present the case of a 5-year-old boy diagnosed with febrile infection-related epilepsy syndrome (FIRES). Approximately three weeks after the onset of this illness, characterized by daily refractory multiple focal seizures, isolated bilateral claustral lesions were observed on a repeated brain MRI. The T2 hypersignal was not visible during the acute phase (i.e., Day 2 and Day 4) and was no longer evident at 3 months after onset. On clinical follow-up, this boy, with previous normal psychological development, progressively developed autistic symptoms that persist to this day; he is now 11- years-old and exhibits in addition drug-resistant epilepsy. Brain MRI coupled with PET-scan at age 7 revealed respectively cortical atrophy and hypometabolism of the frontal, parietal and temporal cortical association areas. Our observation is one of the very few describing isolated acquired lesion of the claustrum (e.g., Ishii et al., 2011; Meletti et al., 2015).

Typically, lesions of the claustrum are associated with concurrent and overt subcortical and cortical damage, thus complicating efforts to establish specific and reproducible relationships between damage to the claustrum and psychiatric or neurological diseases. To the best of our knowledge, this is the first case describing a secondary acquired autistic features in the setting of bilateral lesion of the claustrum. The late onset of autistic symptoms is intriguing and, as suggested by the latest MRI, could be related to damage to corticoclaustral connections, which play a critical role in sensory integration. References Ishii K, Tsuji H, Tamaoka A. Mumps virus encephalitis with symmetric claustrum lesions. AJNR Am J Neuroradiol (2011) 32(7):E139 Meletti S. Slonkova J., Mareckova I. Monti G., Specchio N., Hon P., Giovannini G., Marcian V., Chiari A., Krupa P., Pietrafusa N., Berankova D., Bar M. Claustrum damage and refractory status epilepticus following febrile illness. Neurology 2015;85;1224-1232 Patru M.C. and Reser D.H., A new perspective on delusional states—evidence for claustrum involvement. Front. Psychiatry, 6 (2015), p. 158 Smythies J, Edelstein L, Ramachandran V. The Claustrum: Structural, Functional, and Clinical Neuroscience. San Diego, CA: Elsevier: Academic Press; 2014. 393 pp.

(5)
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Functional connectivity of the claustrum in humans and rats at 7T MRI

The claustrum has reciprocal connectivity with multiple cortical networks and potentially has an important role in orchestrating activity across these function networks. However, the claustrum's sheet-like shape and close apposition to medially lying putamen and laterally lying insular cortex have hindered such assessments with functional MRI. To surpass the confounds associated with imaging of claustrum, in both rats and humans we used 7- Tesla fMRI datasets in to examine the whole-brain resting state connectivity of the claustrum compared to its neighboring regions, the insula and striatum. Adult female Sprague–Dawley rats (200–250 g) were scanned on a Bruker BioSpec 70/30USR Avance III 7-Tesla scanner (Bruker Biospin MRI GmbH, Germany) including a high resolution T1-weighted scan (RARE, TR = 2000 ms, TE = 14 ms, 256 x 256, in plane resolution = 100 μm, 24 axial slices, 1 mm slice thickness) and resting state scans (TR = 1500 ms, TE = 35.0966 ms, 75 x 75, in plane resolution = 0.4 x 0.4 x 1 mm, 24 axial slices). We also analyzed a publicly shared dataset of 17 human subjects (Gorgolewski et al. Sci Data doi: 10.1038) scanned on a 7T MR scanner (MAGNETOM 7T, Siemens Healthcare, Erlangen, Germany) that included a structural 3D MP2RAGE with resolution of 0.7mm isotropic voxels and a resting state functional EPI scan with resolution of 1.5mm isotropic voxels. Preprocessing of both datasets included slice timing correction, realignment, normalization, smoothing, physiological and scanner noise reduction, and band-pass filtering. We performed seed-based connectivity analyses by delineating the striatum (putamen in humans, caudate-putamen in rats), insula, and claustrum for each subject individually. A cluster-forming threshold

of $p<0.001$ was used for all analyses and significant clusters based on FWE correction are reported. In both rats and humans, our preliminary findings suggest that the claustrum is functionally connected to widespread cortical and subcortical brain areas. In rats, the connectivity of the claustrum with multiple cortical regions was significantly greater than insula or striatum. Furthermore, there were no regions in the brain that had greater connectivity to the insula or striatum than to the claustrum. In humans, insula connectivity to widespread cortical areas was greater than claustrum, while claustrum had greater connectivity than striatum to sensorimotor and visual cortices. Our results suggest that while it is possible to examine claustrum connectivity in rodents and humans at high-field MRI, determining specific connectivity of the human claustrum is highly confounded by its three dimensional structure. Our ongoing studies in rats use optogenetic drive of known corticoclaustral pathways to disambiguate claustrum-specific activation signals from neighboring structures.

(6)
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Bilateral claustro-cingulate connections in the origin of the corpus callosum

The claustrum is a region of the pallial subplate highly interconnected with several cortical regions. It is present in all mammalian lineages, located deeply at the temporal boundary of the allocortex and isocortex. A remarkable feature of claustral circuits is their wide range of connections both within and between hemispheres. Interestingly, while interhemispheric connections course through both the corpus callosum and the anterior commissure in placental mammals, they only use the anterior commissure in monotremes and marsupials. Here I highlight the interhemispheric circuitry between the claustrum and cingulate cortices across mammals to understand the origin of the corpus callosum. I will argue that claustro-cingulate bilateral circuits arose in early mammals possibly related to multimodal integration, and that functional conservation of developmental circuits was critical for axon re-routing and the evolution of the corpus callosum in placental ancestors.

(7)
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On similarity of claustral and cortical neurons

Gene expression is a window through which we can take a glimpse of the nature of the cells of interest. It reflects cell types, some functional properties, as well as developmental and evolutionary background. During the course of my study to find universal marker genes for cortical projection neuron subtypes, I noticed that a set of genes that are enriched in the rodent claustrum (latexin, nurr1, cux2, and netrinG2) are co-expressed in a select population of deep

cortical neurons in the lateral cortex. This observation raised the possibility for common developmental and evolutionary origins for claustral and cortical neurons. To gain more insight to this problem, I performed comparative analyses of these and other genes in macaque monkeys and marmosets and found the followings. (1) Claustral gene expression appears to be overall conserved across rodents and monkeys. (2) On the other hand, cortical expression of these genes can differ across species. For example, latexin was not expressed in the cortical neurons in macaques or marmosets. Tmem163 gene was expressed in layer 3 and not in deep layers. (3) A subpopulation of layer 6 neurons, which likely project to other cortical neurons, co-express nurr1 and netrinG2. The neuronal subtype characterized by these genes exists only in the lateral cortex in the rodents, but seem to scatter across entire cortical areas in macaques and marmosets. Based on these data and nurr1 expression in embryonic monkeys, we propose common origin of claustral and cortical neurons which migrate and acquire differential properties in the adult monkey brain.

(8)
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Micro- and macrocircuit components of a putative attention filter

The claustrum has broad cortical interconnectivity and transiently responds to salient stimuli. This enigmatic forebrain structure is therefore proposed to enable the intercortical communication necessary for attentional allocation (Mathur 2014). Using neuronal tract tracers we explore the connectivity profile of the mouse claustrum with cortical areas implicated in attention. We find that the claustrum displays dense interconnectivity with the anterior cingulate cortex (ACC) in particular, which is functionally implicated in attentional control in rodents. To examine the responsivity of claustrum neurons to ACC input, we use whole-cell patch clamp electrophysiology and optogenetics in acute mouse brain slices. We find that claustral spiny projection neurons faithfully fire in response to ACC afferent stimulation and that inhibitory claustral microcircuits provide feedforward and feedback inhibition, thus sculpting the ACC-mediated drive of claustrum projection neurons. Our results suggest that the claustrum is organized to filter and propagate incoming frontal cortical signals.

(9)
Juhyun Kim, Chanel J. Matney, Richard H. Roth, Solange P. Brown
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Synaptic Organization of the Neuronal Circuits of the Claustrum

The claustrum is a poorly understood subcortical structure located bilaterally in mammals that forms widespread connections with almost all cortical areas. However, the cellular organization of claustral circuits remains largely unknown. Based primarily on anatomical data, it has been

proposed that the claustrum plays a role in multimodal sensory integration. However, the extent to which the synaptic organization of claustral circuits supports integration from cortical areas is unclear. Here, we used whole-cell recordings of unitary synaptic connections and optogenetic activation of corticoclaustral axons to determine the cellular organization of the claustrum in the mouse. Recordings in mouse brain slices showed that unitary synaptic connections among claustrocortical neurons were rare. In contrast, we found that parvalbumin-positive (PV) inhibitory interneurons were highly interconnected with both chemical and electrical synapses. In addition, we found that claustrocortical neurons and PV interneurons formed frequent chemical synaptic connections. Using optogenetic approaches, we found that corticoclaustral afferents formed monosynaptic connections onto both claustrocortical neurons and PV interneurons, consistent with data from anatomical studies. By comparing the cortical input to these two cell types, we found that cortical responses were comparatively stronger in PV interneurons relative to claustrocortical cells. Consistent with the overall circuit organization that we elucidated, activation of corticoclaustral afferents generated monosynaptic excitatory responses as well as disynaptic inhibitory responses in claustrocortical neurons. Taken together, these data indicate that recurrent excitatory circuits within the claustrum alone are unlikely to integrate across sensory modalities. Rather, the cellular organization of the claustrum is typical of circuits sensitive to correlated inputs. Although single claustrocortical neurons may integrate corticoclaustral input from different cortical regions, our results are consistent with more recent proposals implicating the claustrum in detecting sensory novelty or in amplifying correlated cortical inputs to coordinate the activity of functionally related cortical regions. More details of this work can be found in: Kim J, Matney CJ, Roth RH, Brown SP. Synaptic organization of the neuronal circuits of the claustrum. *Journal of Neuroscience.* 36:773-784.

(10)
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The Claustrum related to the formation of the temporal lobe

The Claustrum was originally conceived as a layer of gray matter enclosed between fibrous laminae of the external and extreme capsules (Vicq d'Azyr, 1786) It has for too long been reputed to be a dependency of overlying insular cerebral cortex.

Rather than any functional or anatomical connections, study has now shown (Heimer, 2007) that the inferior edge of the Claustrum is simply juxtaposed to
1) the inferior edge of the Insula (the Limen insulae),
2) the posteriormost reach of the medial surface of the Frontal Lobe, and
3) the proximalmost portion of the Temporal Lobe.
Together these regions make up the Piriform Cortex, the central terminal region of the Lateral Olfactory Tract, giving rise to its designation as "primary olfactory cortex".

Depending on one's criteria of primarihood, this folded region could as well be thought of as a secondary olfactory cortex, receiving input connections from the true primary olfactory cortex located in the glomerular layer of the peripheral olfactory bulb, where the olfactory receptor cells make their first contact with the cortical layers that have been shaped into layers of dendrites of mitral and tufted cells of the olfactory bulb.

Besides the integrity of the Lateral Olfactory Tract, another tie that keeps these diverse regions together is their common substantial connection with the Amygdala. A popular ontogenetic possibility, the Claustro-Amygdalar Hypothesis, suggests that Claustrum and Amygdala both derive from common, or at least closely neighboring, lateral pallial primordiums (Puelles, 2014; Butler, 2010).

For years the Claustrum was considered to have Dorsal and Ventral parts, the Dorsal underlying insular meso- and neo-cortex, then after a narrowing, spreading out into a Ventral part underlying the piriform olfactory paleocortex. The notion that the Ventral portion is actually a distinct cellular region, named the Endopiriform, is supported by its proteomic profile different from that of the Dorsal portion (Druga, 1993).

However the Claustrum and Endopiriform, often and perhaps always, maintain continuity with one another that we call the Root of the Claustrum. Whatever it is that Claustrum does for all reaches of meso- and neo-cortex, then the Endopiriform might perform a similar function for the paleocortical piriform cortex. The boundary between Claustrum and Endopiriform is directly internal to the fundus of the rhinal sulcus that separates paleo- from meso- and neo-cortex.

In the evolution of several large mammalian brains, the rhinal sulcus deepens, and expands, separating the temporal and frontal lobes. The temporal lobe expands laterally rather than in some other direction, and then folds to fit inside the skull. The crease of the fold holds the Piriform laterally and includes the cortical amygdalar nuclei always located near the temporal pole. Internal to this is the Root of the Claustrum, with the paleocortical Endopiriform disto-laterally and the "true Claustrum" proximo-superiorly internal to the meso- and neo-cortical regions of the insula. The temporal expansion can be ascribed to additions of cascades of connections that form the information pathways, to and from the bulk of neocortex and the processing factories of amygdala and hippocampus, used by all neocortical regions.

(11)

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Claustrum neurons projecting to the anterior cingulate cortex are topologically organized and exhibit sexual dimorphism

Among the reciprocal anatomical connections the claustrum shares with cortical regions, the connection with the Anterior Cingulate Cortex (ACC) appears the densest and most widespread. However, the function of this connection is unknown. To address this question, we have characterized the neurons projecting from the claustrum to the ACC. Claustrum cells that project to the ACC were identified by injecting into the ACC fluorescent beads that were retrogradely transported to the sites of ACC input. To identify the claustrum in live slices, we used a transgenic mouse line that expresses YFP-tagged Volvox channelrhodopsin-1 at high levels within the claustrum (Front. Neural Circ. 7:160). Many bead-labelled neurons were found in the claustrum, while few were found in the insular cortex; this indicates a preferential and monosynaptic connection between claustrum and ACC. The majority of ACC-projecting

claustrum neurons were found ipsilateral to the bead injection site, with a greater density of labelled cells observed in the claustrum lateral to the preoptic area. Whole-cell patch clamp recordings from bead-labelled claustrum neurons were used to characterize the intrinsic properties of these neurons. These properties varied in different regions of the claustrum: labelled projection cells were predominantly (44%) type 4 Strongly Adapting cells (SA4) in the most anterior part of the claustrum, while type 2 Mildly Adapting cells (MA2) were predominant (77%) in claustrum lateral to the preoptic area, and SA4 cells were the majority (63%) of neurons found in the posterior part of the claustrum. When sorted by sex, the male claustrum showed a significant bias toward SA3 type cells (53%) within the anterior claustrum and no SA4 type cells were found, while in female mice there was a bias for SA4 cells in the anterior claustrum (76%) and no SA3 cells. Our results show that there is ipsilateral dominance for ACC-projecting claustrum neurons, with topological selectivity varying along the anterior-posterior axis. We have also found, for the first time, sexual dimorphism in ACC-projecting claustrum cells. These results demonstrate a direct pathway from claustrum to ACC, consistent with the hypothesis that the claustrum serves as a link for information flow between the insular cortex and the ACC, a circuit that may play an important role in the salience network.