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Identifying SUM projections to claustrum is about knowing your limits

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Abstract

Barbier and colleagues confirm a projection from the supramammillary nucleus to the claustrum using immunohistochemistry to validate the structural boundaries of the claustrum. This refines earlier conclusions made by Vertes and colleagues and highlights the importance of properly anatomically characterizing the claustrum for future structural and functional studies.

Keywords

anatomy; function; hypothalamus; endopiriform nucleus; insular cortex; supramammillary nucleus

The claustrum in the human brain is anatomically defined as the island of gray matter that lies between the external and extreme white matter capsules [1]. Due to the lack of an extreme capsule in such popularly studied species as the mouse and rat, the precise anatomical boundaries of the claustrum in these animals is debated [2]. In a recent article in the journal *Neuroscience*, Barbier and colleagues circumvented this issue to accurately identify a subcortical projection to the claustrum [3].

Early connectivity studies performed in rodents revealed widespread cortico-claustral connectivity [4–7]. While these reciprocal connections with cortex are common, and therefore easy to identify, the subcortical inputs to claustrum are relatively scarce and more difficult to study. Pioneering anatomical work in rat performed by Vertes and colleagues identified a projection from the supramammillary nucleus (SUM) that terminates in the general area of the claustrum [8]. The SUM lies dorsal to the mammillary body in the caudal hypothalamus. SUM neurons fire synchronously with hippocampal theta rhythms in rat and lesions of SUM in children are associated with disruptions in rapid eye movement (REM) sleep [9,10]. Vertes and colleagues [8] injected an anterograde anatomical tracer into the SUM and observed SUM fibers in the general region occupied by claustrum, the endopiriform nucleus, and the insular cortex. As methods to accurately define the claustrum borders were not in practice at the time, it remained unclear as to whether SUM projects to claustrum, endopiriform nucleus, insular cortex, or some combination thereof.

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Historically, to identify claustrum boundaries it was common practice to use a reference brain atlas [6–8]. While this method is the gold standard for identifying anatomical boundaries of other brain regions, the lack of consensus regarding claustrum boundaries across various atlases renders reliance on this method untenable. However, markers specific to the claustrum are emerging. For example, data now identifies G protein gamma 2 subunit (Gng2) as a claustrum specific neuroanatomical marker [11]. In addition, immunohistochemical staining for parvalbumin (PV) is isomorphic with Gng2 expression [11,12]. The application of these anatomical markers to accurately label the claustrum border represents a major advantage for correctly determining claustrum connectivity. In the Barbier et al. 2019 paper, Barbier's group utilizes these revised methods to reassess the subcortical projection from SUM to claustrum that was initially identified by Vertes and co-authors [8].

Barbier's group tested this SUM-to-claustrum projection while defining the claustrum based on empirical evidence: PV immunostaining. Dense labeling of axons were observed in the claustrum following injections of the anterograde tracer, *Phaseolus vulgaris* Leurcoagglutinin (PHAL) into the lateral SUM [3]. These PHAL labeled axons were confirmed to reside within the PV-defined anatomical borders of the claustrum. Using high magnification imaging, Barbier and colleagues showed labeled SUM boutons and putative synaptic contacts with claustral cell bodies. They found no labeled fibers from the SUM outside the PV-defined claustrum. They therefore concluded that SUM afferents only innervate the claustrum and not neighboring structures. This refines the conclusion by Vertes and colleagues [8] who, without defining claustrum boundaries, concluded that SUM projections target the claustrum and the adjacent endopiriform nucleus.

To verify the origins of this projection, the authors injected the retrograde neuronal tract tracer Fluoro-Gold into the claustrum and observed labeled cell bodies in the SUM. In this experiment, a PHAL injection in the SUM was used as a control to label axons in the claustrum to confirm if the injection site for the retrograde tracer did or did not exceed SUM afferent-defined claustrum boundaries. Some of the cases of Fluoro-Gold injections involved surrounding regions such as insular cortex. Nonetheless, at least one case had little-to-no contamination. This successful case was compared to contaminated cases to determine any differences in Fluoro-Gold retrograde transport in the SUM and surrounding regions. This comparison revealed that the contaminated injection site cases resulted in Fluoro-Gold retrograde transport in the SUM and regions outside SUM, such as the parasubthalamic nucleus, which were absent in the accurately injected case. These results demonstrate that the SUM indeed innervates claustrum and projections to the claustrum do not arise from structures neighboring the SUM. In addition to validating the SUM projection to the claustrum, Barbier's group also confirmed dense projections from the SUM to the medial septum and the dentate gyrus of the hippocampus. However, it remains unknown whether the SUM neurons projecting to the dentate gyrus and the medial septum are the same neurons that project to the claustrum.

Taken together, these results confirm a subcortical projection to the claustrum that may bear importantly on claustrum functional relevance. Due to strong projections from the SUM into the claustrum and dentate gyrus, Barbier supported Vertes' hypothesis that the claustrum

may aid in facilitating the spatial encoding of sensory inputs by the hippocampus [8]. The SUM and the medial septum play a pacemaker role to synchronize hippocampal neurons in a theta oscillatory pattern [13,14]. These theta oscillations are linked to spatial learning and memory retrieval [15,16]. In a spatial working-memory task in rats, medial prefrontal cortex neurons are more phase-locked to hippocampal theta rhythms during correct trials versus incorrect trials, which suggests a link between theta coupling and spatial memory task performance [16]. Based on the connectivity of the claustrum with cortex and the SUM, the claustrum may work in tandem with the SUM and the medial septum to modulate hippocampal theta rhythms. Although the claustrum is weakly connected to the hippocampus in rodents, Barbier describes its strong bidirectional connection with the entorhinal area as a means for the claustrum to feed information to the hippocampus [17–19].

Beyond the awake state, SUM and claustrum may play a role in REM sleep [20, 21]. During REM sleep in rats, increased expression of brain-derived neurotrophic factor (BDNF) and the immediate early gene c-fos, a marker for neuronal activation, were observed in the dentate gyrus, claustrum, SUM, and several cortical regions [21]. In addition, chemical lesioning of the lateral SUM decreases c-fos expression within the dentate gyrus during REM sleep [21]. These findings altogether suggest that the SUM and claustrum may activate the hippocampus and cortex during REM sleep.

Ultimately, the extensive work done on claustrum connectivity must translate to a determination of claustrum function. In awake states, the claustrum is implicated in several processes including integrating sensory information to generate conscious percepts or top-down cognitive processing [2, 22–23]. In both cases, these hypotheses are largely based on claustrum connectivity. Further developing these or new hypotheses will rely upon known and newly discovered connections such as the SUM-to-claustrum projection defined by Barbier and colleagues [3]. Functional hypotheses based on inaccurate connectivity data inherently hold little value. Thus, when rightfully subscribing to the “structure leads to function” axiom that Crick so effectively championed, it should be stressed that claustrum function will only be revealed if our structural knowledge is sound.

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