The hypothalamus predates the origin of vertebrates

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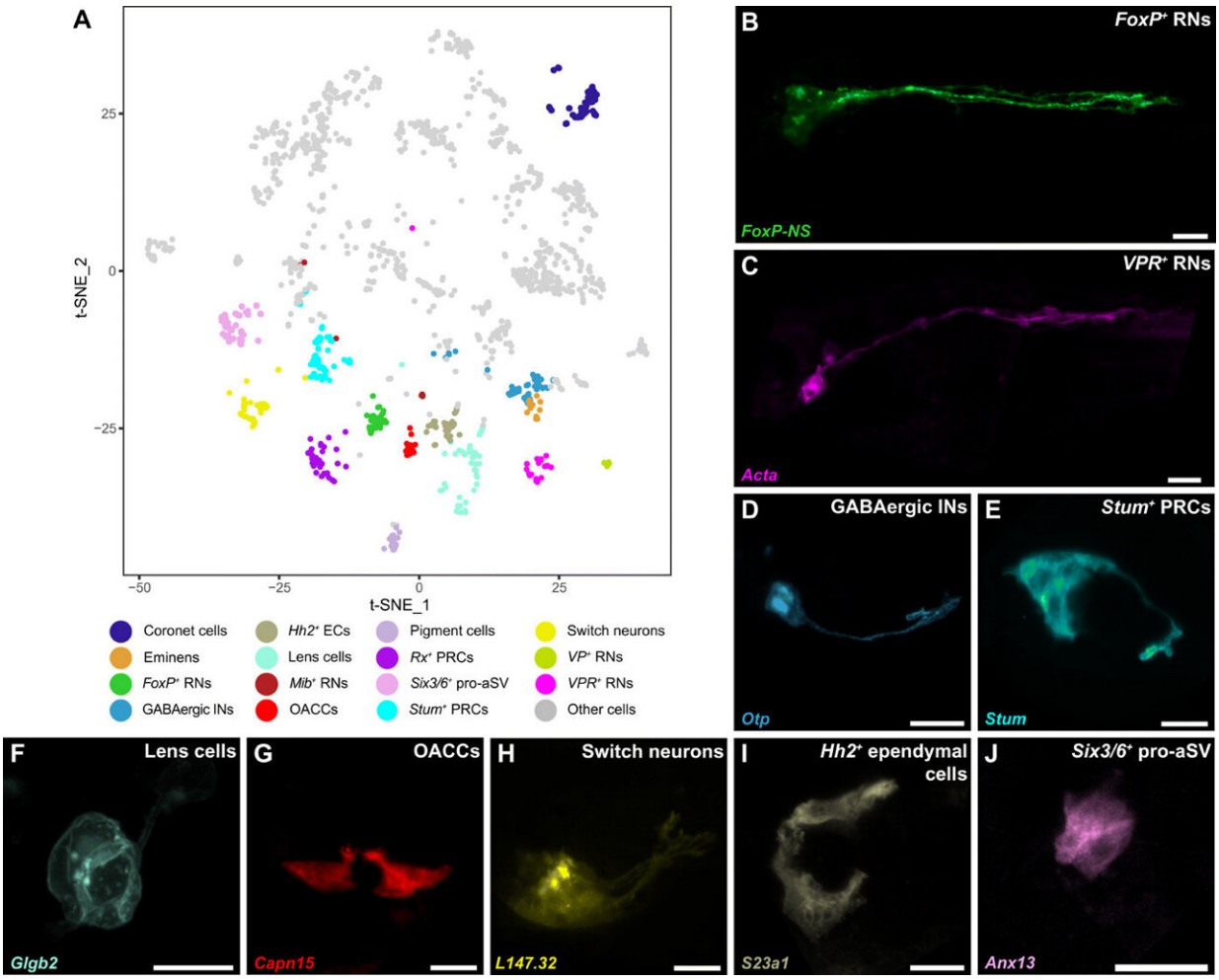


Brain map. (A) t-SNE plot of the Ciona nervous system cells in swimming tadpoles (n = 2021 cells). The color-coded cells belong to cell types in the sensory vesicle. Gray cells correspond to cells outside of the sensory vesicle. The identification of the different clusters was determined at the larva stage by expression of membrane fluorescent reporters under the control of regulatory

sequences for genes enriched in the cluster of interest: (B) FoxP+ RNs expressing FoxP regulatory sequences specifically active in the nervous system (FoxP-NS reporter; green), (C) VPR+ RNs (Acta reporter; magenta), (D) GABAergic INs (Otp reporter; blue), (E) Stum+ PRCs (Stum reporter; cyan), (F) lens cells (Glgb2 reporter; aqua), (G) the otolith associated ciliated cells (OACCs) (Capn15 reporter; red), (H) switch neurons (L147.32 reporter; yellow), (I) the Hh2+ ependymal cells (ECs) (S23a1 reporter; beige), and (J) the Six3/6+ pro-anterior sensory vesicle (aSV) (Anx13 reporter; pink). The description of the fusion genes used in the reporter assays and the number of replicates are provided in table S1. Scale bars, 10 μm. Credit: Science Advances, doi: 10.1126/sciadv.abf7452

The hypothalamus is involved during the coordination of neuroendocrine functions in vertebrates and their evolutionary origin can be described using integrated transcriptome or connectome brain maps of swimming tadpoles of Ciona intestinalis, also known as sea vase. These organisms serve as an approximation of their ancestral protovertebrate. The map included several cell types relative to different regions of the vertebrate hypothalamus, including the mammillary nucleus, arcuate nucleus and magnocellular neurons. These observations highlighted how the hypothalamus predates the evolution of the vertebrate brain. The neural crest and cranial placodes are key innovations that contributed to the evolution of the vertebrate head. However, less is known about the evolutionary origin of the crown and summit of the vertebrate brain. In a new study now on *Science Advances*, Laurence A. Lemaire and a research team in molecular biology and integrative genomics at the Princeton University, New Jersey, U.S., used an extensive single-cell transcriptome fate map of the Ciona tadpole to characterize the neural cell types comprising its brain also known as the sensory vehicle.

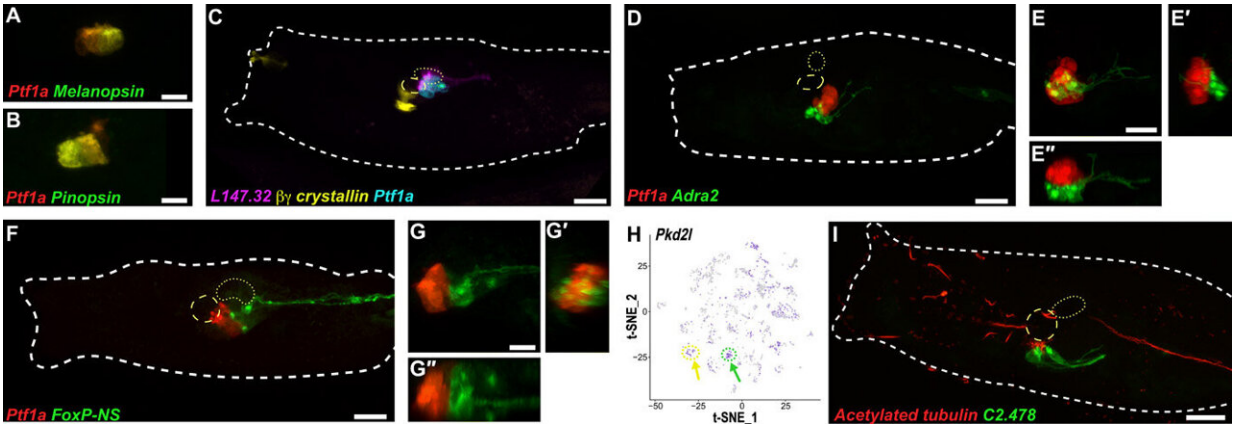
The hypothalamus

The sensory vesicle of the Ciona tadpole contains 215 neural cells including 143 neurons and is primarily responsible to relay sensory information including light, gravity and mechanical cues to the motor ganglion that controls the tadpole tail. The central nervous system (CNS) of the Ciona has facilitated lineage maps to allow the first comprehensive connectome of a chordate. In an attempt to include the synaptic connectome, Lemaire et al. studied the evolutionary origins of the vertebrate brain, specifically the hypothalamus. The hypothalamus has ancient origins and forms an ancient region of the vertebrate brain. The construct is found across all vertebrates including fish to humans, the hypothalamus controls homeostasis, metabolism and reproductive functions through intricate interconnecting neural circuits. In this work, Lemaire et al. propose the major function of the Ciona proto-hypothalamus to be to trigger the onset of metamorphosis of the tadpole species.

Brain map

The scientists conducted single-cell transcriptome profiling of the Ciona intestinalis embryogenesis from gastrulation to swimming larvae, to identify 40 t-distributed stochastic neighbor embedding (t-SNE) cell clusters of the CNS (central nervous system) and peripheral nervous system. Lemaire et al. mapped each of the neural cell types comprising the simple brain of the tadpoles also known as their sensory vesicles. Based on the studies, they identified 15 different neural cell types including previously identified coronet cells, Eminens neurons and pigment cells. Using neural-specific reporter genes, the team identified a range of relay neurons (RNs) including those that expressed vasopressin and tachykinin ($FoxP^+$), as well as others expressing the vasopressin receptor (VPR^+). The $FoxP^+$ relay neurons were cholinergic while the VPR^+ relay neurons were GABAergic. The researchers identified an underappreciated population of putative mechanosensory neurons, which they renamed 'switch neurons," corresponding to ciliated brain

interneurons in the connectome map of the CNS of tadpoles.

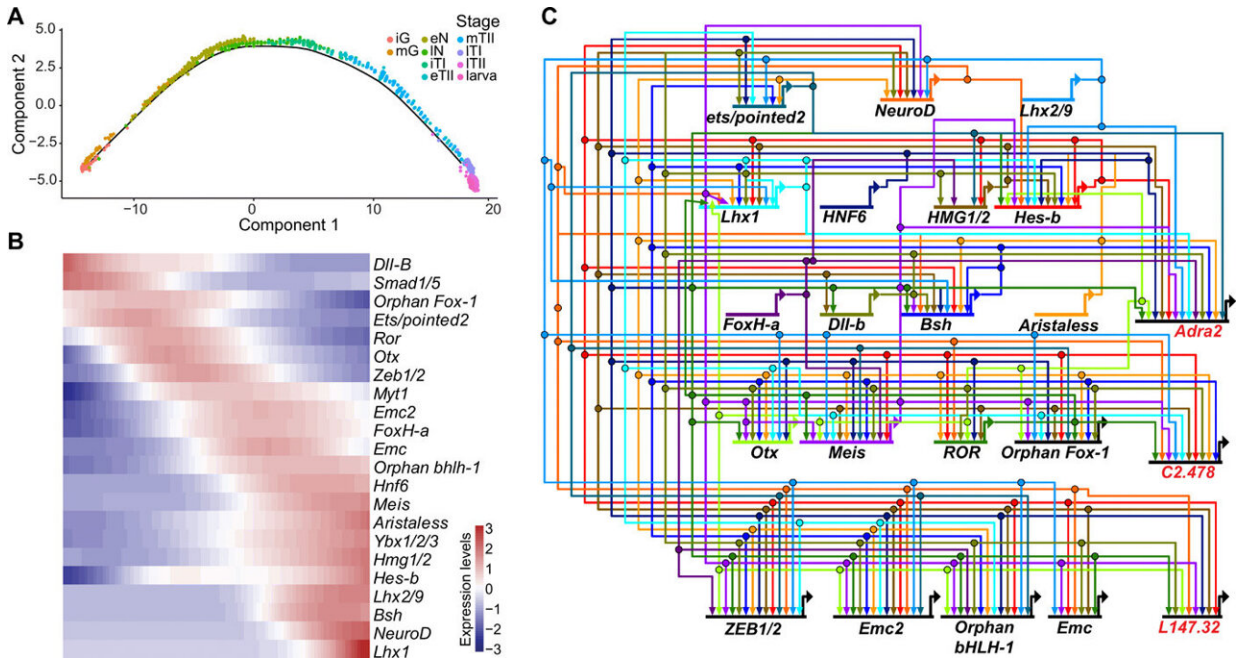


Coronet-associated circuit in swimming tadpoles. (A and B) Expression of reporter genes for melanopsin [(A), green] or pinopsin [(B), green] and a Ptf1a reporter gene (red) in coronet cells. (C) Switch neurons (L147.32 reporter; magenta) are closely associated with coronet cells (Ptf1a reporter; cyan) and the otolith (βγ-crystallin reporter; yellow) without touching the latter. (D) Coexpression of Ptf1a and Adra2 reporter genes (red and green, respectively) also show close associations of switch neurons and coronet cells. (E to E″) Higher-magnification views of the reporter genes shown in (D). (E) is a z-projection, (E′) y-projection, and (E″) x-projection, which highlights extensive cell-cell contacts between switch neurons and coronet cells. (F) Expression of a FoxP+ reporter gene (green) shows close contact of FoxP+ RNs with coronet cells (Ptf1a reporter; red). (G to G″) Higher-magnification views of the reporter genes shown in (F), corresponding to z-, y-, and x-projections, respectively. (H) t-SNE plot of Pkd2l expression across the nervous system (n = 2021 cells). The yellow dotted circle and arrow indicate the switch neurons, while the green dotted circle and arrow point to the FoxP+ RNs. (I) Immunostaining for acetylated tubulin (red) reveals cilia in switch neurons that were labeled with C2.478 reporter (green). All reporter assays were analyzed at the larva stage. Reporter genes code for membrane fluorescent proteins and their description as well as the number of replicates are provided in table S1. White dashed lines indicate the outline of the trunk regions of tadpoles, while yellow dotted and dashed lines identify the pigment of the ocellus and the otolith, respectively.

Scale bars, 10 μm (A, B, E, and G) and 20 μm (C, D, F, and I). Credit: Science Advances, doi: 10.1126/sciadv.abf7452

Neuronal circuit

Scientists had previously identified similarities of coronet cells with the vertebrate hypothalamus since they released dopamine to express diverse neuropeptides including neurotensin-like B and gonadotropin-releasing hormone (Gnrh). However, these cells were previously described to share morphological similarities with the coronet cells or a region of the hypothalamus present in non-tropical fish. The fish coronet cells also expressed melanopsin and detected a short wavelength light associated with seasonal lengthening of daylight to trigger reproduction by releasing a thyroid-stimulating hormone, followed by the secretion of Gnrh (gonadotropin-releasing hormone) as in other seasonal breeders. The work showed how coronet cells functioned as light-sensing sensory cells relative to their dopaminergic and neurosecretory activities. The coronet cells also interacted with adjacent neurons such as switch neurons and $FoxP^{+}$ relay neurons. On the basis of their anatomical position, the switch neurons corresponded to ciliated brain interneurons. On the basis of cell-cell associations, the VPR^{+} relay neurons also received inputs from switch neurons.

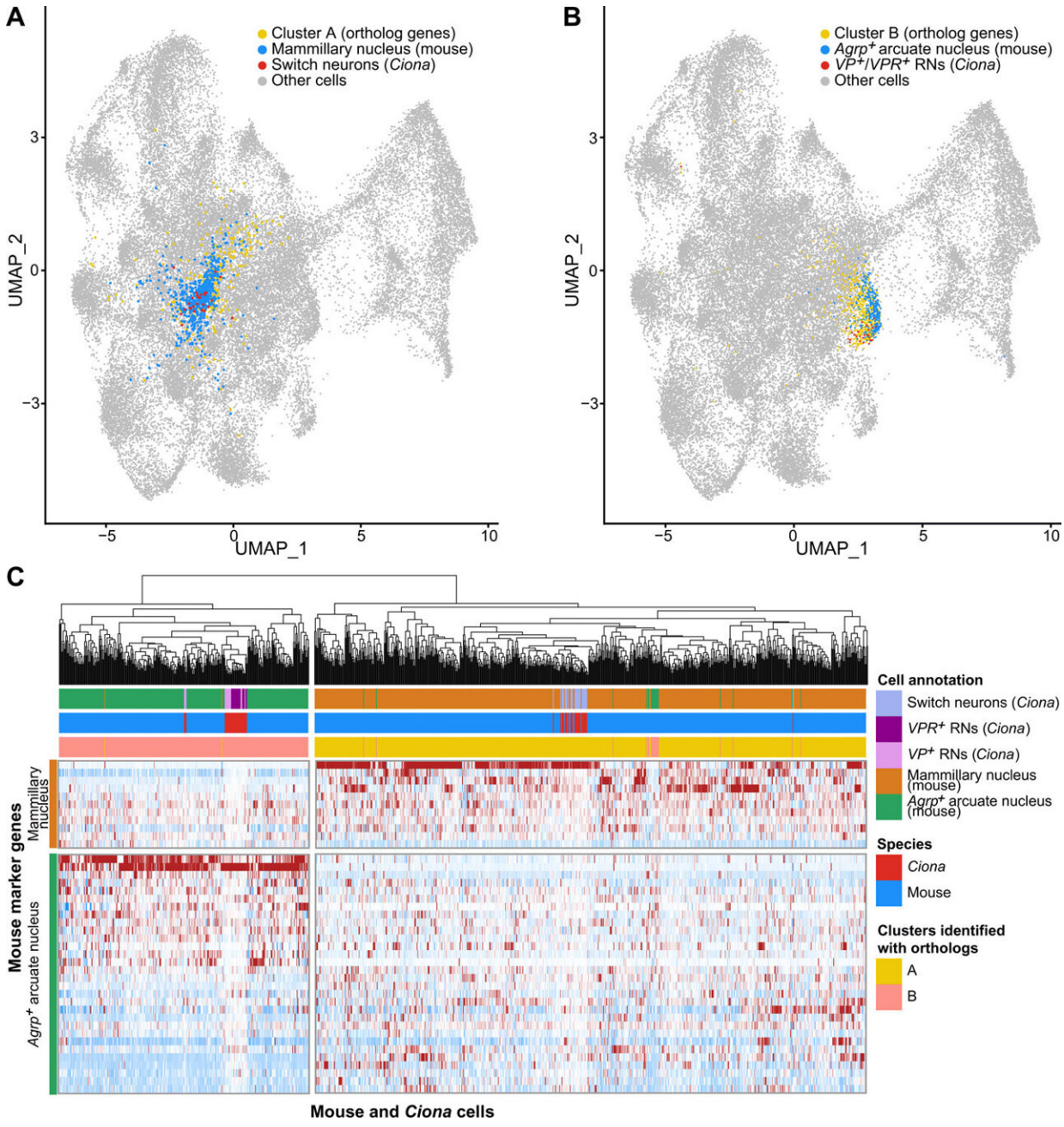


Switch neuron provisional gene regulatory network. (A) Single-cell transcriptome trajectory of the switch neuron during development (n = 1467 cells). The cells are ordered on the basis of pseudo-time, and the color code indicates their stage. iG, initial gastrula; mG, mid gastrula; eN, early neurula; lN, late neurula; iTI, initial tailbud I; eTII, early tailbud II; mTII, mid tailbud II; lTI, late tailbud I; lTII, late tailbud II. (B) Pseudo-temporal gene expression cascade of switch neurons. Expression of representative transcription factors and signaling components in reconstructed developmental trajectories. (C) Provisional gene regulatory network of switch neurons based on the regulatory cascade. Credit: Science Advances, doi: 10.1126/sciadv.abf7452

Mechanosensory switch neurons

Previous studies had shown coronet cells to be a central sensory node for associated neurons and FoxP^{+} relay neurons. While detailed information currently exists on the networks that underly the specifications of coronet cells, not much is known about the development of switch

neurons or $FoxP^{+}$ relay neurons. Lemaire et al. therefore focused on switch neurons due to their roles as specialized mechanosensory cell types in vertebrates, including the cerebrospinal fluid containing neurons present along the central canal and the ventricular cavities of the brain including the hypothalamus. Additionally, not much is also known about the development or function of vertebrate cerebrospinal fluid contacting neurons (CSF-cNs). To understand their ontogeny, Lemaire et al. created a provisional gene regulatory network for switch neurons, using previously published methods. The team identified transcriptome trajectories and temporal cascades of genes encoding transcription factors in the cell lineages to form switch neurons. They represented the resulting interconnections as a provisional gene network. As proof of concept, they used single-cell RNA sequencing (scRNA-seq) assays to understand the cell types that are transformed into switch neurons after the mis-expression of a gene, to test the authenticity of the network.

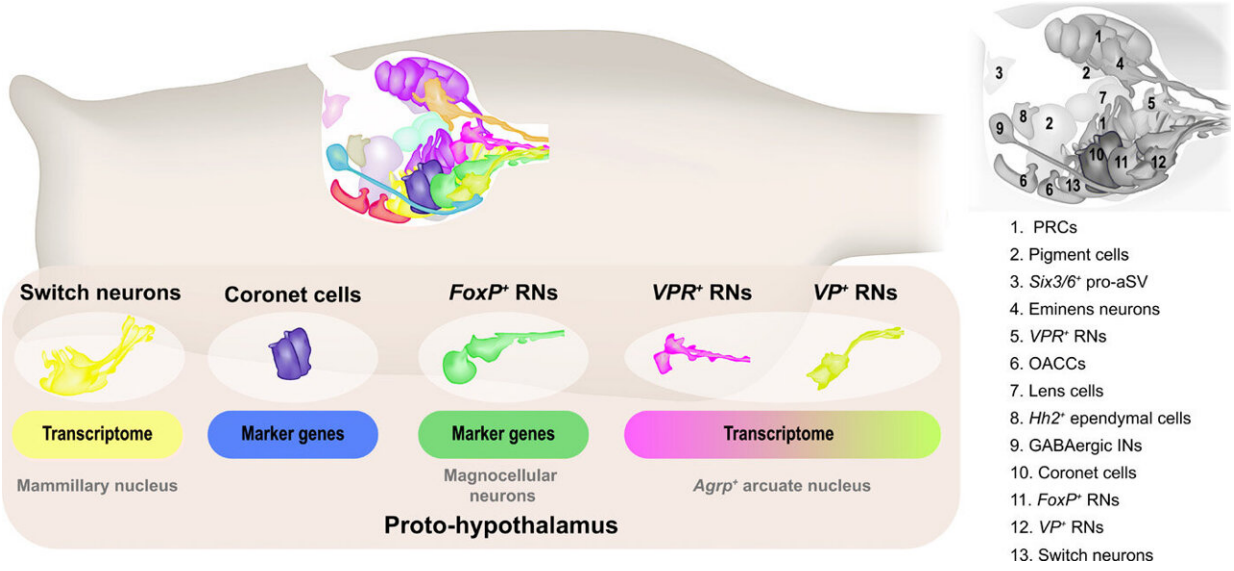


Integration of mouse hypothalamus single-cell transcriptome with Ciona nervous system. (A) Uniform manifold approximation and projection (UMAP) showing the integrated coclustering of single-cell transcriptome datasets of the Ciona nervous system (late tailbud II and larva stage; n = 4445 cells) and mouse hypothalamus (n = 33,893 cells) based on shared orthologous genes. Ciona switch neurons and the mouse mammillary nucleus are colocalized in the same cluster. (B) Colocalization of Ciona VP+ RNs, VPR+ RNs, and mouse Agrp+

arcuate nucleus cluster in the integrated data. (C) Heatmap of marker genes shared between mouse mammillary nucleus and Agrp+ arcuate nucleus clusters and Ciona switch neurons and VP+/VPR+ RNs, respectively. Unsupervised clustering is consistent with orthology of Ciona switch neurons and mouse mammillary nucleus, and Ciona VP+/VPR+ RNs and mouse Agrp+ arcuate nucleus. Credit: Science Advances, doi: 10.1126/sciadv.abf7452

Orthology maps of mouse hypothalamus and Ciona nervous system

The team formed a putative sensory circuit featuring coronet cells as a central node, in association with switch mechanosensory neurons and the relay neurons. Existing studies alongside the current demonstration of coronet cells that express melanopsin and pinopsin provided considerable evidence for the homology with the vertebrate hypothalamus. To test if the relay neurons and the associated switch might share homology with the hypothalamus, Lemaire et al. performed whole-transcriptome for each of the 40 neural cell types comprising the Ciona nervous system and compared them with the transcriptome maps of the mouse hypothalamus. The studies identified two Ciona lineages that matched two different clusters of mouse hypothalamic cells. The combined comparative transcriptome analyses suggested the coronet-associated neural circuit to contain multiple cell types relative to different regions of the mouse hypothalamus.



The hypothalamus predates the origin of vertebrates. Fifteen different clusters were identified within the Ciona sensory vesicle. Coronet cells have been considered as a "rudimentary" hypothalamus owing to their expression of dopamine pathway genes and neuropeptide such as Gnrh. Additional neurons within the "coronet-associated circuit" also share similarities with different regions of the hypothalamus. FoxP+ RNs express several genes that are evocative of magnocellular neurons of the supraoptic and periventricular nuclei of the hypothalamus. Comparative transcriptome analyses suggest orthology of switch neurons and the mouse mammillary nucleus, as well as VP+/VPR+ RNs and mouse Agrp+ neurons in the arcuate nucleus. These observations suggest that several different hypothalamic cell types predate the vertebrate brain. The numbers on the gray image on the right indicate the position of the different cell types. For clarity, Mib+ RNs and the Stum+ PRCs have been omitted, and the number of cells of each type has also been reduced. Credit: Science Advances, doi: 10.1126/sciadv.abf7452

Outlook

In this way, Laurence A. Lemaire and colleagues identified 15 different cell types in the sensory vesicle of Ciona larvae, while the connectome

map identified 31 cell types. The team credited this disparity to reflect the different methods of classification. For instance, they noted how a single cell type based on intrinsic genetic properties could acquire distinctive behaviors through associations with different neurons. The scientists described five different types of relay neurons based on the transcriptome trajectories and profiles, while the connectome map identified 11 such neurons relative to synaptic inputs. The outcomes suggested the simple brain morphology of Ciona to contain a complex proto-hypothalamus with a role during the onset of metamorphosis in the tadpoles. Regardless of the intended function, this work indicates the evidence of multiple hypothalamic cell types in Ciona to suggest an unexpectedly sophisticated blueprint for the evolution of the complex vertebrate brain.

More information: Lemaire A. L. et al. The hypothalamus predates the origin of vertebrates, *Science Advances*, 10.1126/sciadv.abf7452

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