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ABSTRACT BOOK

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Functional characterisation of the lateral septal calbindin neurons

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The ventral subdivision of the lateral septum (LSv) is a forebrain region that has been linked to maternal care, but the specific cellular networks that are responsible remain unidentified. A high density of calbindin-containing (Cb+) neurons is present in the LSv, yet the properties, neuronal connections and functions of these cells remain unknown. In the present study, patch-clamp electrophysiological recordings were made of calbindin-containing neurons in coronal slices containing the lateral septum. Our findings revealed that these neurons exhibit regular firing patterns and possess a notably high membrane resistance. The mean amplitude and rise slope of spikes were found to be significantly lower in these neurons than in Cb- LSv neurons. To establish the projections of Cb+ LSv neurons, we applied viral-based, cell-type-specific anterograde tracing, and revealed that they send extensive projections to the medial preoptic area (MPOA), a region crucial for the regulation of maternal behaviour regulation. This projection was subsequently validated through retrograde tracing. To confirm whether information relevant to maternal behaviour is transmitted from the LSv Cb+ neurons to the MPOA, the pup-induced activation of these neurons was described using the c-Fos technique. To elucidate the pathway through which maternal signals reach the Cb+ LSv neurons, a retrograde tracer was injected into the LSv. This revealed input from the posterior intralaminar thalamus, which expresses the maternally activated parathyroid hormone 2 (PTH2) neuropeptide. Using double labelling, we identified PTH2 receptors on Cb+ LSv neurons. These receptors are likely activated by PTH2 released from nearby PTH2 terminals, which we also identified around the Cb+ neurons in the LSv. Using electron microscopy, we confirmed a synaptic connection between PTH2+ fibres and maternally activated inhibitory neurons in the LSv. Given that LSv Cb+ neurons have been demonstrated to be GABAergic, it seems plausible that they may be innervated by the PTH2+ terminals. Finally, functional studies using Cb-Cre mice revealed that the inhibition of LSv Cb+ neurons reduced pup-licking behaviour without affecting other maternal behaviours. In conclusion, our findings collectively suggest that activation of inhibitory Cb+ neurons in the LSv is essential for pup-licking behaviour, likely mediated through their projections to MPOA neurons.

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Introduction

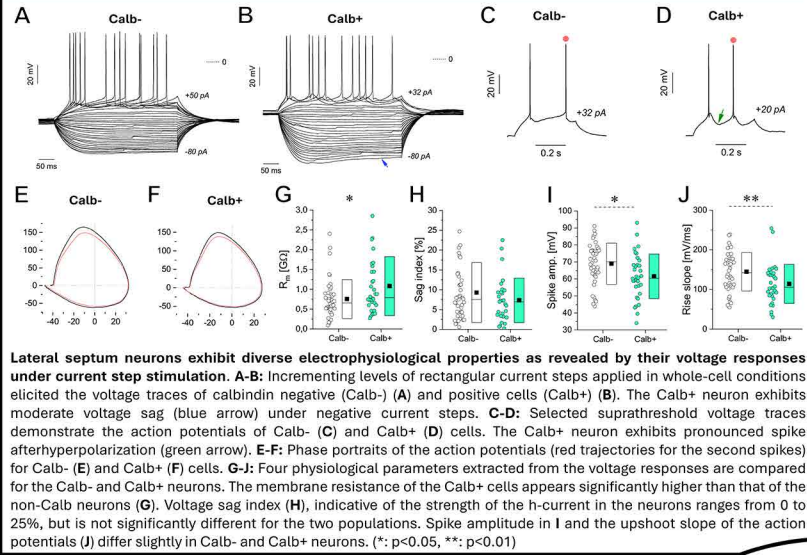
Maternal care is regulated by a complex neural network that includes the **lateral septum (LS)**. The LS has prominent connections with several brain regions involved maternal regulation including the **medial preoptic area (MPOA)**, a well-established brain site driving maternal motivation. The LS contains an elevated number of activated neurons in lactating rodents following pup exposure. However, the cell types of the LS participating in the control of maternal behaviours, and the inputs, which activate LS neurons by pup exposure are unknown. Our research group has previously described that neurons in the **posterior intralaminar thalamic nucleus (PIL)** express an excitatory neuropeptide, **parathyroid hormone 2 (PTH2)**, whose expression is induced in mothers. A dense network of PTH2+ fibres can be found in the LS, as well.

We hypothesize that the LS contains specific cell types involved in maternal adaptation via their projection to the MPOA driven by the PTH2+ pathway originated from the PIL.

Summary of results

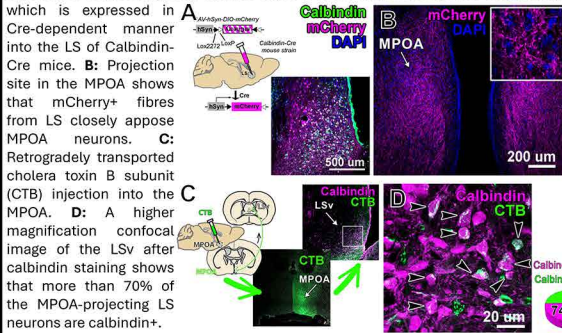
1. Calbindin+ LS neurons possess a notably high membrane resistance and the mean amplitude and rise slope of their spikes were found to be significantly lower than calbindin- cells.
2. Calbindin+ LS neurons are inhibitory neurons sending prominent projection to the MPOA.
3. Calbindin+ LS neurons are activated by pup-exposure.
4. PTH2R and PTH2+ fibres are located in the same area as pup-induced c-Fos+ LS calbindin neurons. PTH2+ terminals innervate LS inhibitory neurons. PTH2+ fibres probably arrive from the pup-induced c-Fos+ neurons of the PIL.
5. Inhibition of calbindin+ LS neurons reduced the time spent with licking of the pups in sensitized female mice.

1. Electrophysiological properties of ventral lateral septal neurons



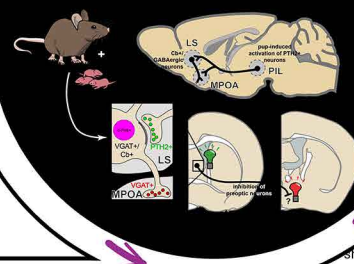
2. Lateral septal calbindin neurons project to the medial preoptic area

Calbindin neurons of the lateral septum project to the medial preoptic area. A: Injection of the anterogradely transporting AAV containing mCherry reporter protein gene, which is expressed in Cre-dependent manner into the LS of Calbindin-Cre mice. B: Projection site in the MPOA shows that mCherry+ fibres from LS closely appose MPOA neurons. C: Retrogradely transported cholera toxin B subunit (CTB) injection into the MPOA. D: A higher magnification confocal image of the LSv after calbindin staining shows that more than 70% of the MPOA-projecting LS neurons are calbindin+.

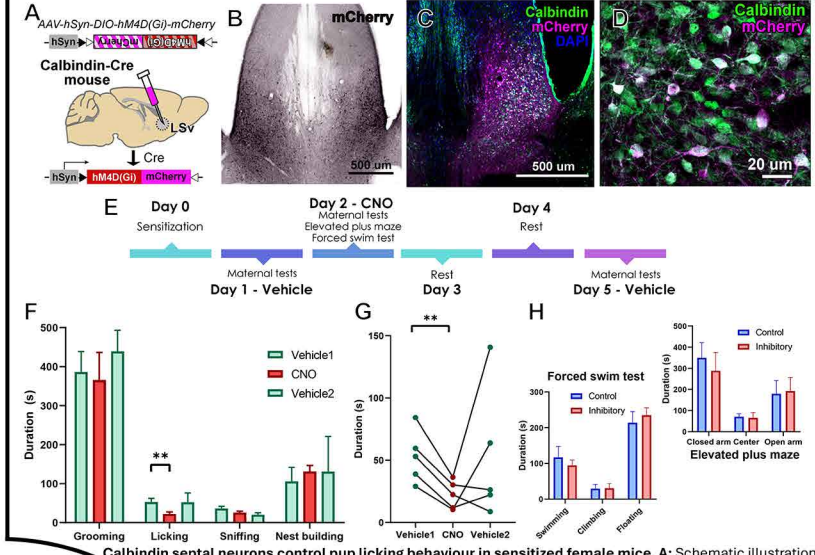


Conclusion

Pup exposure mediated by PTH2+ input activates GABAergic calbindin neurons of the lateral septum (LS), which regulate the pup licking behaviour in female mice. This modulation may occur via their projection to the medial preoptic area (MPOA), the major brain region controlling maternal behaviour.

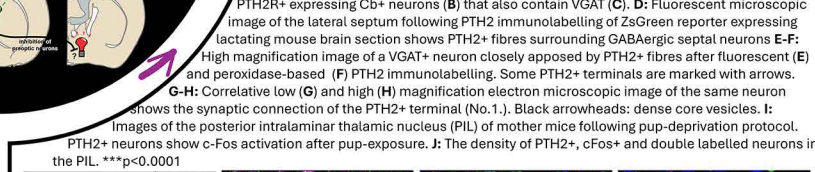


5. Inhibition of calbindin neurons reduces pup licking behaviour



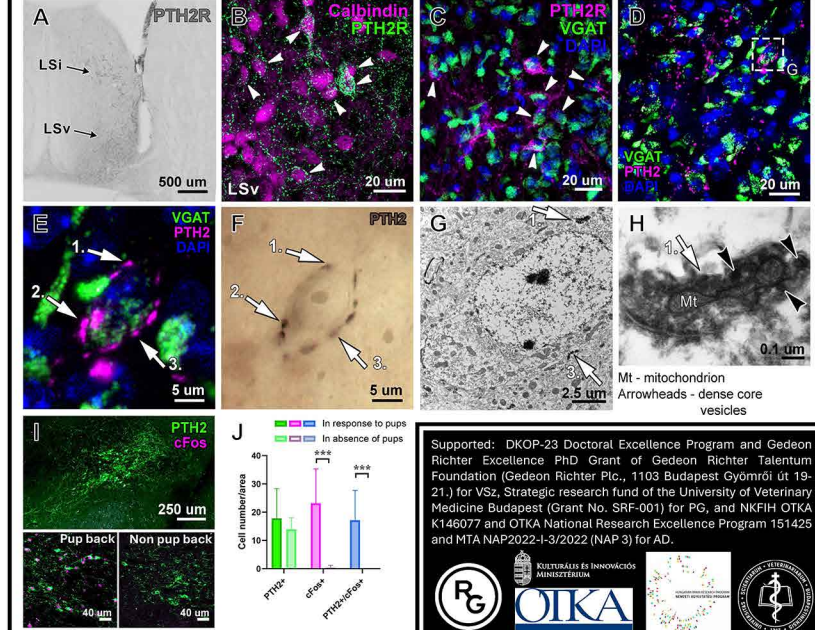
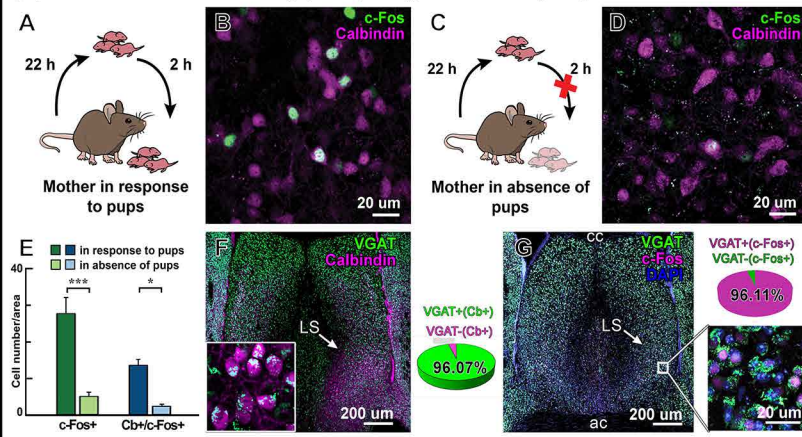
4. The localization of PTH2R and PTH2+ fibres in the lateral septum

Inhibitory neurons of the lateral septum receive PTH2+ input. A-C: Localization of PTH2 receptors (PTH2R) in the LS (A). Confocal images from the LSv with PTH2R+ terminals and PTH2R+ expressing Cb+ neurons (B) that also contain VGAT (C). D: Fluorescent microscopic image of the lateral septum following PTH2 immunolabelling of ZsGreen reporter expressing lactating mouse brain section shows PTH2+ fibres surrounding GABAergic septal neurons E-F: High magnification image of a VGAT+ neuron closely apposed by PTH2+ fibres after fluorescent (E) and peroxidase-based (F) PTH2 immunolabelling. Some PTH2+ terminals are marked with arrows. G-H: Correlative low (G) and high (H) magnification electron microscopic image of the same neuron shows the synaptic connection of the PTH2+ terminal (No.1.). Black arrowheads: dense core vesicles. I: Images of the posterior intralaminar thalamic nucleus (PIL) of mother mice following pup-deprivation protocol. PTH2+ neurons show c-Fos activation after pup-exposure. J: The density of PTH2+, cFos and double labelled neurons in the PIL. ***p<0.0001



3. Pup-induced activation of ventral lateral septal neurons

Calbindin neurons in the lateral septum are activated by pup exposure in mother mice and are shown to be GABAergic. A-D: Mother mice deprived from their pups for 22 hours, which were then returned to them on the following day (A) show higher number of double labelled calbindin-positive (Cb+) and c-Fos+ neurons in the LS (B), in contrast to the control maternal group in absence of their pups (C-D) showed by fluorescent immunohistochemistry. E: The density of c-Fos+ and double labelled (Cb+/c-Fos+) neurons in the LS. 51.12% of the Cb+ neurons are c-Fos+ following pup exposure. Area = 0.1 mm². All data are presented as means ± s.e.m. 0.01 < *p<0.05, ***p<0.0001. F: Immunolabelling of a ZsGreen reporter expressing mouse brain section shows that around 96% of lateral septal calbindin neurons contain the vesicular GABA transporter (VGAT) indicating that they are inhibitory GABAergic neurons. G: Confocal image demonstrates enhanced c-Fos positivity in the LS of a VGAT-ZsGreen mother mouse following pup exposure. Nearly all pup-induced c-Fos+ neurons are GABAergic (VGAT+(c-Fos+)), as shown in the high magnification inset.



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