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POSTER ABSTRACTS

Septal-preoptic connectivity involved in maternal adaptation

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The lateral septum (LS) is a forebrain area involved in the regulation of social behaviors including maternal care. However, the mechanisms how LS neurons control maternal behaviors are not well understood. Therefore, we addressed to characterize maternally activated LS neurons neurochemically and map their distribution pattern in mice. We established that LS neurons activated after pup exposure are GABAergic suggesting the importance of limbic inhibitory system in maternal care. As GABAergic calbindin (Cb) and calretinin cell groups localize in the LS, we determined their relation to pup induction. We showed that many c-Fos-activated neurons are positive for the marker Cb in the ventral part of LS (LSv). The ratio of Cb neurons in c-Fos activated neuron population was significantly higher in the presence of the pups compared with control mothers. We previously showed that LS contains a maternally induced neuropeptide, parathyroid hormone 2 (PTH2) positive fibers in high density. PTH2+ fibers closely apposed activated neurons suggesting synapses that was confirmed by electron microscopy in GABAergic neurons. Moreover, Cb neurons in the LS are also closely surrounded by PTH2+ terminals. As PTH2+ fibers arise from the posterior intralaminar nucleus of the thalamus (PIL), we examined the activation level of this region in the presence and absence of pups. We found that c-Fos expression was induced exclusively in PTH2+ neurons and all these PTH2+ cells were activated in response to pup exposure. PIL neurons are known to send PTH2+ projection to the medial preoptic area (MPOA), too. This phenomenon might be important in the aspect of maternal behavior as MPOA is the central regulatory area of parenting. We showed that the same neurons of PIL project to both LS and MPOA neurons which raises the possibility of simultaneous regulation of neurons in these 2 brain regions by PTH2+ PIL neurons. Based on our results of retrogradely transported tracer technique, we showed that a high number of MPOA-projecting LS neurons are Cb+ suggesting that these neurons may have an impact on maternal adaptation. We confirmed Cb+ projection by performing anterograde tract tracing in Cb-Cre mice too. Moreover, some LS-projecting neurons in the MPOA show synaptic-like vicinity to PTH2 terminals on dendritic process. In conclusion, the data suggest that PIL neurons convey stimulatory signal of pups to both LS and MPOA and the neurons of these areas may interact with each other.

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A konferencia linkje:

<https://mitt2023.mitt.hu/invitation>

Septal-preoptic connectivity involved in maternal adaptation

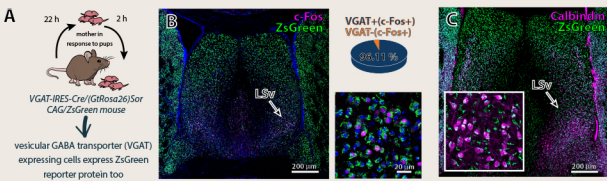
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Introduction

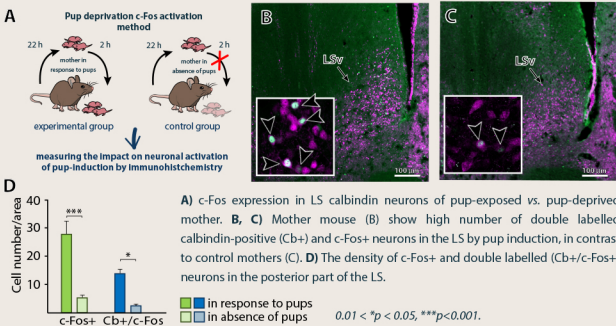
The **lateral septum (LS)** is a forebrain area suggested to be involved in the regulation of maternal care based on showed by previous c-Fos activation methods in lactating rodents. However, it is not known: 1) which LS neurons are activated by pups, and 2) how they control maternal behavior. Our research group has previously described that fibres containing a maternally induced neuropeptide, **parathyroid hormone 2 (PTH2)** localize in the LS. PTH2 expressing neurons are mainly located in the **posterior intralaminar nucleus of the thalamus (PIL)** in the maternal brain. Therefore, we addressed if PIL PTH2+ neurons activate subgroups of LS neurons in mothers. Neurons in the **medial preoptic area (MPOA)**, which is a central regulator of maternal care, receive prominent input from lateral septal neurons. In this study, our second aim was to reveal the interaction between lateral septal and medial preoptic neurons in the aspect of maternal care.

1) Maternally activated neurons are GABAergic in the LS



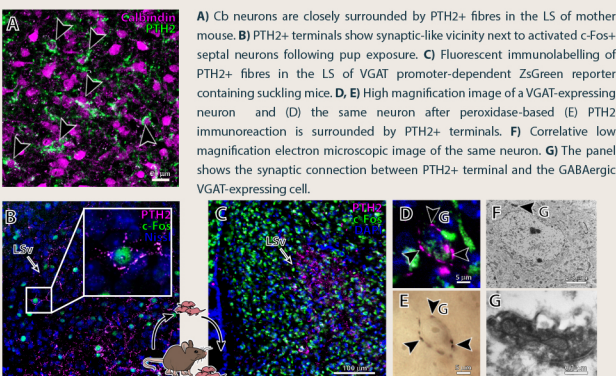
A, B) ZsGreen fluorescent reporter expressing mouse brain section shows that a high number of maternally activated neurons localize in the lateral septum (LS) especially in the ventral subdivision (vLS) following pup exposure. Nearly all pup-induced c-Fos+ neurons are GABAergic (VGAT+(c-Fos+)). **C)** A high number of calbindin (Cb) neurons are present in the same area. They are all GABAergic, suggesting that maternally activated neurons are Cb+.

2) Calbindin neurons in the maternal brain following pup exposure



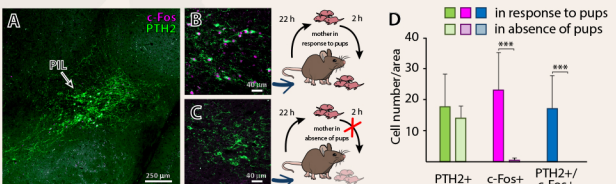
A) c-Fos expression in LS calbindin neurons of pup-exposed vs. pup-deprived mother. **B, C)** Mother mouse (B) show high number of double labelled calbindin-positive (Cb+) and c-Fos+ neurons in the LS by pup induction, in contrast to control mothers (C). **D)** The density of c-Fos+ and double labelled (Cb+/c-Fos+) neurons in the posterior part of the LS.

3) PTH2+ terminals in the LS of mothers



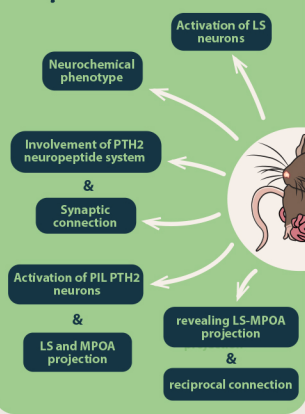
A) Cb neurons are closely surrounded by PTH2+ fibres in the LS of mother mouse. **B)** PTH2+ terminals show synaptic-like vicinity next to activated c-Fos+ septal neurons following pup exposure. **C)** Fluorescent immunolabelling of PTH2+ fibres in the LS of VGAT promoter-dependent ZsGreen reporter containing suckling mice. **D, E)** High magnification image of a VGAT-expressing neuron and (D) the same neuron after peroxidase-based (E) PTH2 immunoreaction is surrounded by PTH2+ terminals. **F)** Correlative low magnification electron microscopic image of the same neuron. **G)** The panel shows the synaptic connection between PTH2+ terminal and the GABAergic VGAT-expressing cell.

4) PTH2-expressing neurons are maternally activated in the PIL



A, B) Mother mouse deprived from their pups for 22 hours, which were then returned to them on the following day shows high number of double labelled PTH2+ and c-Fos+ neurons in the PIL. **C)** There is no c-Fos activation in control mother. **D)** The density of PTH2+ and c-Fos+ neurons in the PIL. ***p < 0.001.

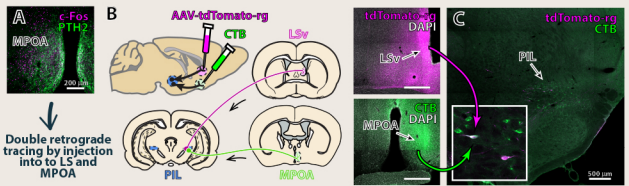
Objectives



Summary of results

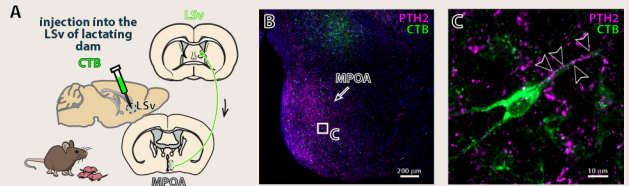
- 1) LS neurons activated in response to pup exposure are all GABAergic.
- 2) The ratio of calbindin neurons in c-Fos activated neuron population was significantly higher in the presence of the pups compared to control mothers.
- 3) PTH2+ fibers innervate GABAergic LSv and probably calbindin neurons.
- 4) In the PIL, c-Fos expression is induced exclusively in PTH2+ neurons. In turn, essentially all PTH2+ neurons were activated by exposure in the PIL.
- 5) Same neurons of PIL project to both LS and MPOA neurons.
- 6) Presumed LS-projecting MPOA neurons are innervated by PTH2+ terminals.
- 7) Calbindin neurons of the LSv project to the MPOA.

5) PTH2+ terminals in the LS of mothers



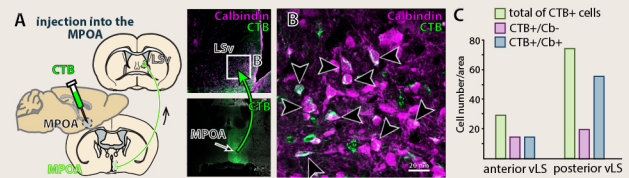
A) Dense PTH+ fibre network located in the LSv and the MPOA suggesting the same PIL neuronal input of both areas in mother dams. **B)** Thus, we injected retrogradely transporting adeno-associated viral vector (AAV) containing tdTomato reporter gene into the LS and cholera toxin B (CTB) into the MPOA. **C)** We confirmed that same PIL neurons project simultaneously to the LSv and MPOA as we found double retrogradely labelled neurons in the PIL.

6) LS-projecting MPOA neurons and PTH2+ network

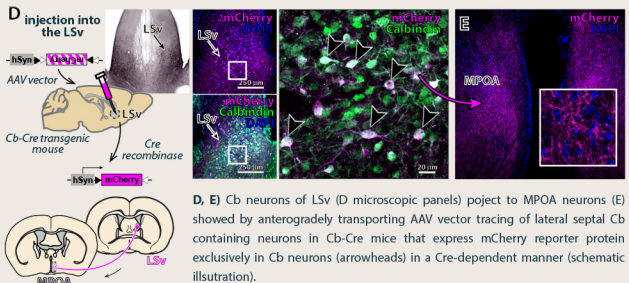


A) Retrogradely transporting cholera toxin B (CTB) was injected into the LSv of lactating dam as indicated in the schematic drawing. **B)** PTH2 immunolabelling was performed to examine the relation between PTH2+ terminals and LS-projecting preoptic neurons. **C)** Retrogradely labelled LS-projecting MPOA neuron is surrounded by PTH2+ terminals (pointed by arrowheads).

7) Calbindin LS neurons project to the medial preoptic area



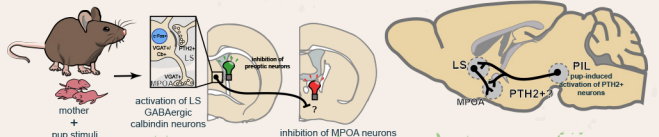
A) Cb neurons of the LSv project to the medial preoptic area (MPOA) confirmed by retrogradely transported cholera toxin B (CTB) injection into the preoptic area. **B)** High number of retrogradely labelled MPOA-projecting LSv neurons are Cb+ (arrowheads) which is prominent in the posterior part (C) of the LSv.



D, E) Cb neurons of LSv (D microscopic panels) project to MPOA neurons (E) showed by anterogradely transporting AAV vector tracing of lateral septal Cb containing neurons in Cb-Cre mice that express mCherry reporter protein exclusively in Cb neurons (arrowheads) in a Cre-dependent manner (schematic illustration).

Conclusions

Pup exposure activates GABAergic calbindin neurons in the LSv by PTH2+ projections ascending from the PIL, which are collaterals of the PIL neurons projecting to the major brain region controlling maternal behaviours, the medial preoptic area. In turn, the GABAergic calbindin neurons in the LSv also project to the medial preoptic area.



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