

INHIBITORY CALBINDIN NEURONS OF THE LATERAL SEPTUM ARE INVOLVED IN MATERNAL CARE

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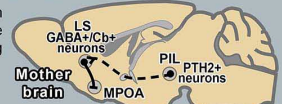
INTRODUCTION

The lateral septum (LS) is a forebrain area involved in the regulation of social behaviors including maternal care. Previous studies reported that LS show elevated number of activated neurons in lactating rodents following pup exposure. However, the mechanisms how LS neurons control maternal behavior are not well understood. 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 during the early postpartum period. Moreover, neurons in the medial preoptic area (MPOA), which is a central regulator of maternal care, receive prominent input from lateral septal neurons. We suppose that PTH2 innervated LS neurons are involved in maternal processes via its MPOA-projection. Thus, our aim was to examine the following issues:

- 1) Neurochemical characterization of LS neurons activated by pup-exposure in mice
- 2) Investigation whether PTH2 fibres in the LS represent synaptic input to neurochemically identified LS neurons
- 3) Determination of preoptic projections of neurochemically identified LS neurons

SUMMARY OF RESULTS

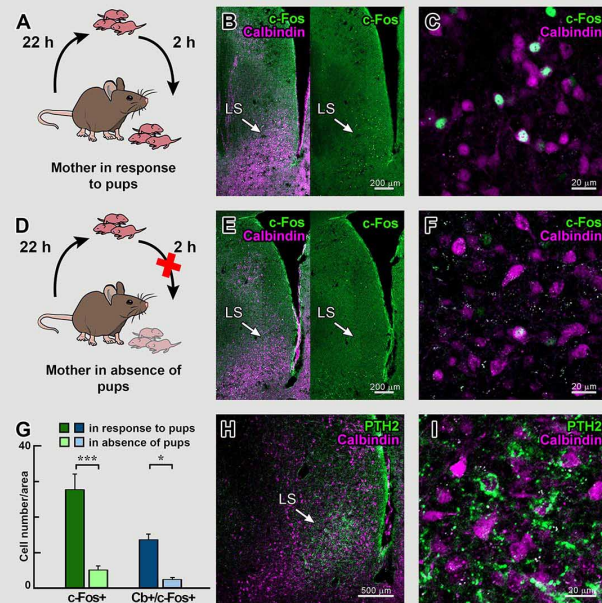
- 1) There is significantly elevated number of pup-induced c-Fos activated calbindin (Cb) neurons in the LS. Moreover, Cb+ neurons are surrounded by PTH2+ fibres. We showed that all maternally activated and Cb neurons are GABAergic. Therefore, activated Cb neurons are inhibitory cells.
- 2) We confirmed by correlative light and electron microscopy that VGAT-expressing GABAergic neurons are innervated by PTH2+ terminals in the LS.
- 3) GABAergic neurons send prominent projection to the MPOA. Further, we found by retrograde tracing that a high number of MPOA-projecting neurons are Cb+.



CONCLUSION

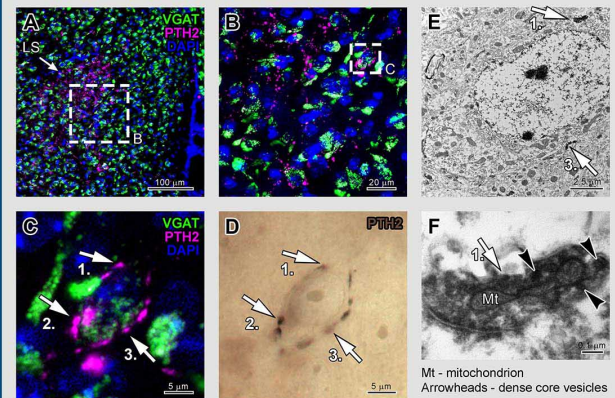
We conclude that pup exposure activated LS cells are GABAergic Cb neurons, which may control maternal behavior by transferring PTH2 input from the PIL towards the MPOA.

1a) CALBINDIN NEURONS IN THE MATERNAL BRAIN FOLLOWING PUP EXPOSURE



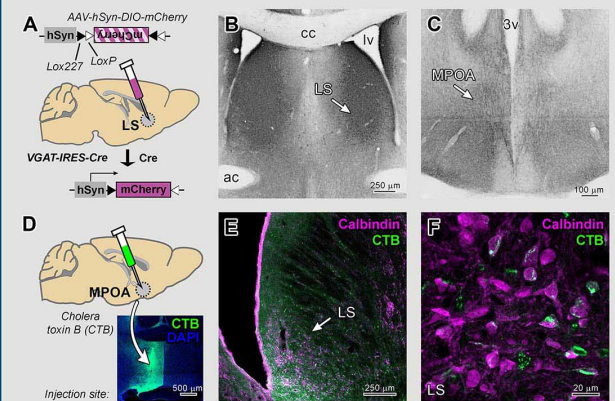
Calbindin neurons are activated by pup exposure and surrounded by PTH2+ terminals in the LS. A-C) Mother mice deprived from their pups for 22 hours, which were then returned to them on the following day (A) show high number of double labelled calbindin-positive (Cb+) and c-Fos+ neurons in the LS (B, C), in contrast to the control maternal group (D-F). G) The density of c-Fos+ and double labelled (Cb+/c-Fos+) neurons in the posterior part of the LS. $0.01 < *p < 0.05$, < 0.001 $p < 0.0001$. H) Prominent PTH2+ fibre localization is present in the LS of mother mice. I) High magnification image demonstrates that Cb+ neurons are closely surrounded by PTH2+fibres.

2) PTH2-EXPRESSING NEURONS INNERVATE GABAergic NEURONS IN THE LS OF MOTHER MICE



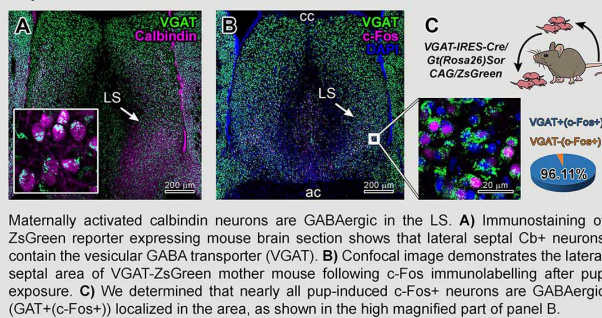
GABAergic septal neurons are innervated by PTH2-expressing cells. A, B) Fluorescent immunolabelling shows PTH2+ fibres surrounding GABAergic septal neurons in ZsGreen reporter containing suckling mice. C) High magnification image of a VGAT+ neuron. Some PTH2+ terminals are marked with white arrows. D) The same neuron in panel C is shown after peroxidase-based PTH2 immunolabelling. E) Correlative low magnification electron microscopic image of the same neuron. F) The panel shows the synaptic connection of the PTH2+ terminal (No.1) with the selected neuron.

3) GABAergic CALBINDIN SEPTAL NEURONS PROJECT TO THE MEDIAL PREOPTIC AREA



GABAergic and Cb neurons of the LS projects to the medial preoptic area (MPOA). A) Illustration of the anterograde vector tracing of lateral septal vesicular GABA transporter containing (VGAT+) neurons. B) Injection site in the LS. C) Dense VGAT+ fibres originated from the LS localize in the MPOA. D) Illustration of the retrogradely transported cholera toxin B (CTB) injection into the MPOA with a confocal microscopic image of the injection site. E) Retrogradely labelled neurons in the LS. F) Immunolabelling of septal neurons with Cb and CTB antibodies demonstrates that high number of MPOA-projecting neurons is Cb+.

1b) ACTIVATED NEURONS BY PUP EXPOSURE ARE GABAergic



Maternally activated calbindin neurons are GABAergic in the LS. A) Immunostaining of ZsGreen reporter expressing mouse brain section shows that lateral septal Cb+ neurons contain the vesicular GABA transporter (VGAT). B) Confocal image demonstrates the lateral septal area of VGAT-ZsGreen mother mouse following c-Fos immunolabelling after pup exposure. C) We determined that nearly all pup-induced c-Fos+ neurons are GABAergic (GAT+(c-Fos+)) localized in the area, as shown in the high magnified part of panel B.



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Poster session - Topic 5

Systems neuroscience

P5.26 Inhibitory calbindin neurons of the lateral septum are involved in maternal care

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The lateral septum (LS) is a forebrain area involved in the regulation of social behaviors including maternal care. Previous studies reported that LS show elevated number of activated neurons in lactating rats. However, the mechanisms how LS neurons control social behaviors are not well understood.

To determine the type of maternally activated neurons, we addressed their distribution in mouse dams in relation to calbindin neurons. We observed a discrete group of activated calbindin neurons throughout the intermediate and ventral part of the LS. The ratio of calbindin neurons in c-Fos activated neuron population was significantly higher in the presence of the pups compared with control mothers. By using VGAT-Cre-ZsGreen reporter mice, we showed that the neurons activated by pup-exposure are all inhibitory GABAergic cells. Moreover, both calbindin and GABAergic neurons are closely surrounded by terminals containing a maternally-induced neuropeptide, parathyroid hormone 2 (PTH2) supposing a synaptic connection, which was indeed confirmed in case of GABAergic neurons by electron microscopy. We also revealed that calbindin neurons are a part of the GABAergic inhibitory population in the LS. Finally, we found that neurons of the medial preoptic area (MPOA), the major regulatory region of maternal behaviour, receives prominent input from septal inhibitory neurons. We confirmed LS to MPOA projection by retrograde tracing and observed that a relatively high number of MPOA-projecting septal neurons show calbindin positivity.

In conclusion, the results suggest that GABAergic calbindin neurons of the LS innervated by PTH2+ terminals are involved in maternal adaptation processes through their projections to MPOA neurons.

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