

## MATERNALLY ACTIVATED CONNECTIONS OF THE VENTRAL LATERAL SEPTUM REVEAL INPUT FROM THE POSTERIOR INTRALAMINAR THALAMUS

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The lateral septum (LS) demonstrates activation in response to pup exposure in rat mothers, and its lesions eliminate maternal behaviors suggesting its involvement in maternal brain circuitry. This study shows that the density of pup-activated neurons in the ventral subdivision of the LS (LSv) is nearly equivalent to that in the medial preoptic area (MPOA), the major regulatory site of maternal behavior. When somatosensory inputs including suckling are not allowed, pup-induced activation was markedly reduced in the LSv. Retrograde tract tracing identified various brain regions potentially influencing LSv neuronal activation. Anterograde studies confirmed that the posterior intralaminar thalamic nucleus (PIL), implicated in processing touch-related stimuli, targets the pup-activated region of the LSv. Moreover, nerve terminals containing the maternally induced PIL neuropeptide parathyroid hormone 2 (PTH2), were found to synapse on the c-Fos activated LSv neurons by electron microscopy. Confirmation of PTH2+ PIL fibers projecting to LSv was achieved by retrograde tracing. Furthermore, double retrograde injections revealed that neurons within the PIL can project to both LSv and MPOA, suggesting their simultaneous regulation by PIL input. We also established that maternally activated septal neurons are GABAergic and send inhibitory projections to the MPOA. This implies that the LSv and MPOA form an interconnected subcircuit in the maternal brain network, which is primarily driven by somatosensory input from the pups via the PIL PTH2+ neurons.

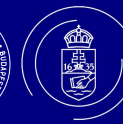
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# Septal-preoptic connectivity and their thalamic input in suckling rats

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## Introduction

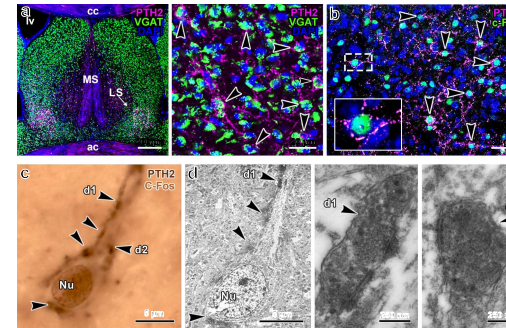
The **lateral septum (LS)** is a forebrain area suggested to be involved in the regulation of maternal care based on showed by previous c-Fos activation methods and lesion studies in rat. However, little is known about how LS is involved in maternal adaptation. Our research group has previously described 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. Moreover, neurons in the **medial preoptic area (MPOA)**, which is a central regulator of maternal care, receive prominent input from LS neurons. In this study, our second aim was to reveal the interaction between LS and MPOA neurons in the aspect of maternal care.

## Summary of the results

- The ventral subdivision of the lateral septum (LSv) is the second most activated region in mother rats
- The highest LSv neuronal activation in mother rats is attributed to the physical interaction with pups.
- Pup-activated septal neurons are GABAergic and innervated by PTH2+ terminals.
- PTH2+ fibers in the LSv originate from the maternally activated PIL neurons.
- The same neurons of the PIL project to both LSv and MPOA neurons.
- The most prominent target site of pup-activated LSv neurons is the MPOA.
- PTH2+ terminals are in close vicinity of MPOA-projecting LSv neurons.

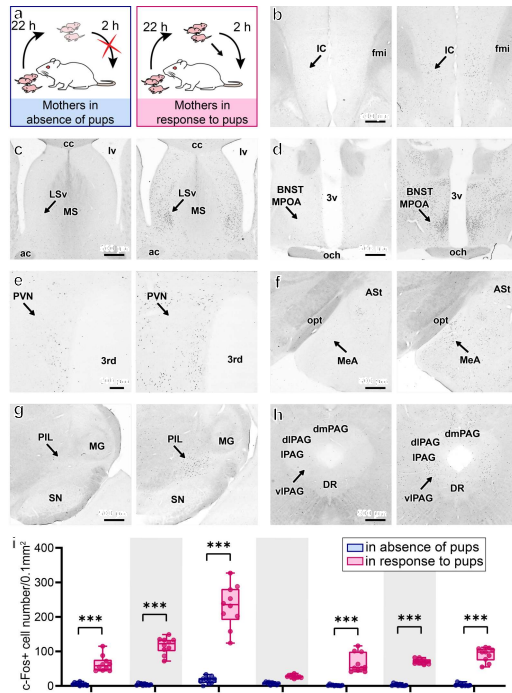
**IN CONCLUSION**, these data indicate that the LSv and MPOA form an interconnected subcircuit in maternal brain network, which is primarily driven by somatosensory input from the pups via the PIL PTH2+ neurons.

## PTH2+ terminals innervate pup-activated GABAergic LSv neurons



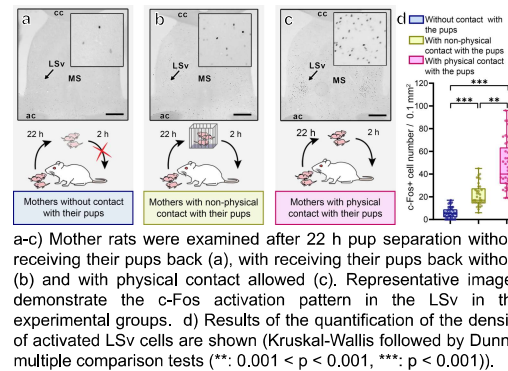
a) PTH2+ fibers are localized in the LSv of reporter mother mice expressing ZsGreen fluorescent protein in vesicular GABA transporter (VGAT) positive neurons. The high magnification image indicates VGAT+ neurons closely apposed by PTH2+ terminals (black arrowheads). b) Double fluorescent immunolabeling (PTH2/c-Fos) was performed on brain sections of mother mice after pup exposure. PTH2+ fibers are densely distributed close to maternally induced c-Fos activated neurons (white arrowheads) in the LSv. c) Peroxidase-based double immunolabeling of a brain slice of a dam after pup exposure demonstrates a neuron with c-Fos+ nucleus closely surrounded by PTH2+ terminals (arrowheads). d) The low magnification electron micrograph of the same neuron is demonstrated. PTH2+ fibers closely appose the neuronal soma and the proximal dendritic process performing synapses as shown in the high magnification microscopic images.

## Brain areas activated after pup exposure



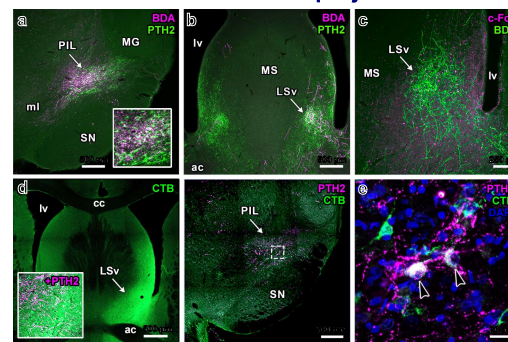
a) The experimental design of the pup-deprivation method used for the evaluation of maternally induced c-Fos activation. b-h) We compared c-Fos activation level in seven maternally implicated brain regions following peroxidase based c-Fos immunolabeling. The left panels demonstrate the low density of c-Fos+ neurons in the examined regions of totally pup-deprived mothers and the right panels show the high density of neurons expressing c-Fos in the same areas of pup-interacting mothers. Scale bars: 500 µm i) The diagram represents the number of activated c-Fos+ neurons of mothers in absence of pups and in response to their pups. N= 5 per group, two-way ANOVA followed by Šidák's multiple comparisons tests, \*\*\*\*: p < 0.001. Individual values are represented by dots. Whiskers indicate minimum and maximum values.

## Pup-induced activation of the LSv



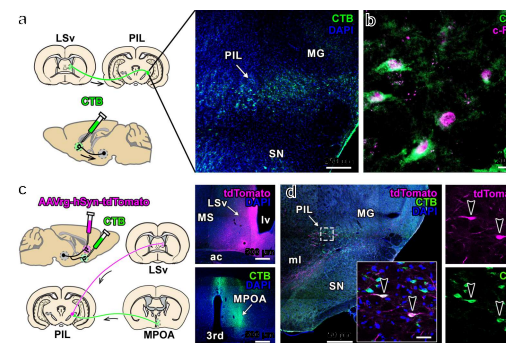
a-c) Mother rats were examined after 22 h pup separation without receiving their pups back (a), with receiving their pups back without (b) and with physical contact allowed (c). Representative images demonstrate the c-Fos activation pattern in the LSv in the experimental groups. d) Results of the quantification of the density of activated LSv cells are shown (Kruskal-Wallis followed by Dunn's multiple comparison tests (\*\*: 0.001 < p < 0.001, \*\*\*: p < 0.001)).

## PTH2+ neurons of the PIL project to the LSv



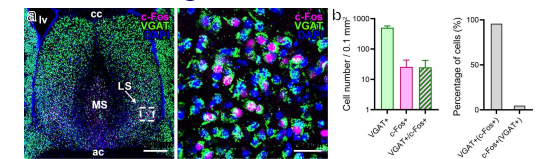
a) Anterogradely transporting biotinylated dextran amine tracer (BDA) injection into the PIL followed by pup exposure. demonstrates the injection site after BDA and PTH2 labeling in mother rat. Several BDA-containing neurons are PTH2+. b) LSv-projecting PTH2+ PIL neurons are demonstrated by BDA/PTH2+ double labeled fibers. c) BDA+ fibers are localized in the same area as c-Fos activated cells. d) Retrogradely transporting neuronal tracer, cholera toxin B (CTB) was injected into the LSv. Fluorescent image of the injection site with an inset shows PTH2+ fibers in the area. e) Retrogradely labeled LS-projecting PIL neurons show PTH2 positivity.

## Neurons of the PIL project both to the LSv and to the MPOA



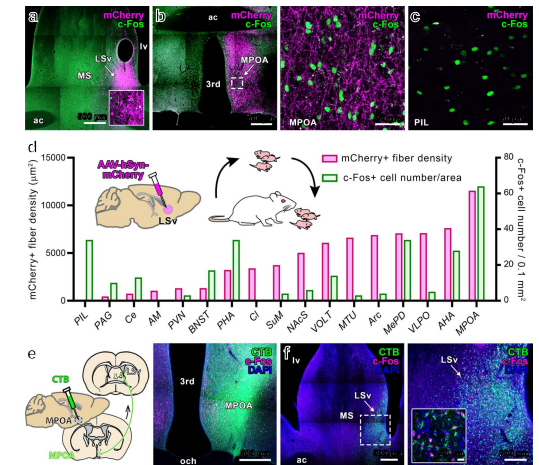
a) The retrogradely transporting neuronal tracer, cholera toxin B (CTB) was injected into the LSv. The fluorescent image shows retrogradely labeled neurons in the PIL. b) High magnification image shows LSv-projecting PIL neurons expressing pup-induced c-Fos. c) Double retrograde tracing was designed in the LSv and the MPOA meaning that an adeno-associated (AAV) virus tagged with tdTomato was introduced into the LSv and CTB into the MPOA of the same animal. Confocal images show the injection sites. d) The PIL contains retrogradely double-labelled neurons (arrowheads).

## LSv GABAergic cells are activated in dams



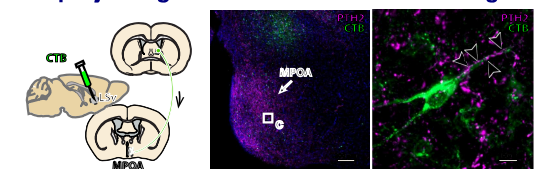
a) Activated neurons are GABAergic as it is shown by c-Fos immunolabeling of VGAT-ZsGreen reporter mice. b) The density of c-Fos+, VGAT+ and double labelled (VGAT+/c-Fos+) neurons and the quantification of the percentage of VGAT+ cells in the c-Fos+ population (VGAT+(c-Fos+)) and c-Fos+ cells in the VGAT+ population (c-Fos+(VGAT+)) in the LSv. Mother mice after 22 h of pup deprivation followed by 2 h of pup exposure were examined.

## Efferent connections of the LSv in mother rat



Anterogradely transporting AAV-hSyn-mCherry was injected into the LSv. a) A low magnification image of the injection site is shown after c-Fos immunolabeling. b) A low magnification image of the septal input in the MPOA of the same rat. The right panel shows higher magnified region. c) LSv neurons do not project to the PIL, that is also markedly activated in mothers. d) Relative fiber density and maternally activated c-Fos+ cell number were measured of the same rat. Among the projection sites of the LSv, the highest density of maternally-activated neurons and mCherry+ fibers were found in the MPOA. e) Retrogradely spreading cholera toxin B (CTB) was injected into the MPOA. The confocal image demonstrates the injection site of the mother rat. f) A high number of c-Fos+ neurons were retrogradely labeled in the LSv after CTB injection.

## LS-projecting MPOA neurons and PTH2 signal



a) CTB was injected into the LSv of lactating dam. b) PTH2 labelling was performed to examine the relation between PTH2+ terminals and LS-projecting MPOA neurons. c) Retrogradely labelled cells in the MPOA are surrounded by PTH2+ terminals (arrowheads).