MAPPING OF THE NEURONAL POPULATIONS ACTIVE DURING WAKING AND PARADOXICAL (REM) SLEEP IN THE HYPOTHALAMUS: A STUDY USING THE TRAP2 TRANSGENIC MICE

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Introduction

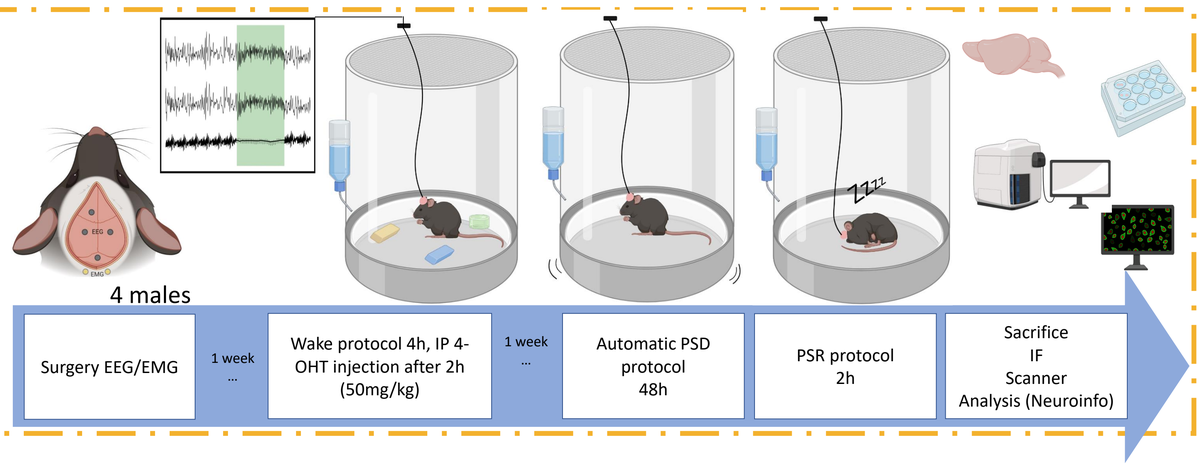
The hypothalamus (hyp) regulates many physiological functions like vigilance states. Our team has shown there are many cFos-immunopositive cells within the Lateral hypothalamus area (LH), zona incerta (ZI), after a homeostatic paradoxical sleep rebound (PSR). We further showed that a large number of these neurons express either melanin-concentrating hormone (MCH), GAD2 or Lim homeobox 6 (Lhx6).

Aims

We will determine the percentage of neurons activated during W and PS which are expressing MCH, Lhx6 and Orex. It will allow us to determine the proportion of neurons activated during W and PS in the different hypothalamic nuclei which are not chemo-genetically identified.

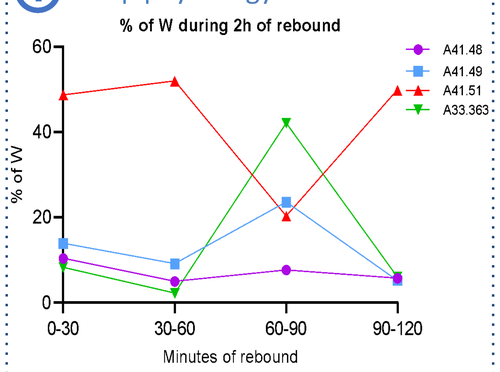
Methods

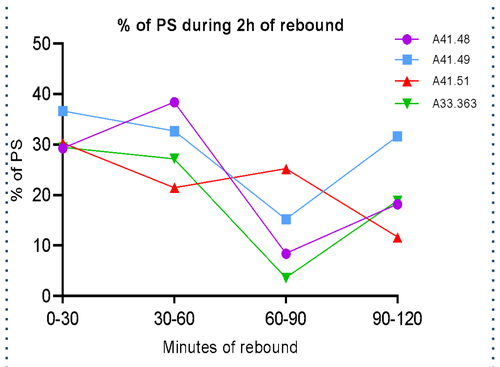
Thanks to the transgenic TRAP2-red mouse, we map in the same animal the neurons activated during W (tdtomato) and those during PSR (cFos), which allows to show differential topography within hyp.



Results

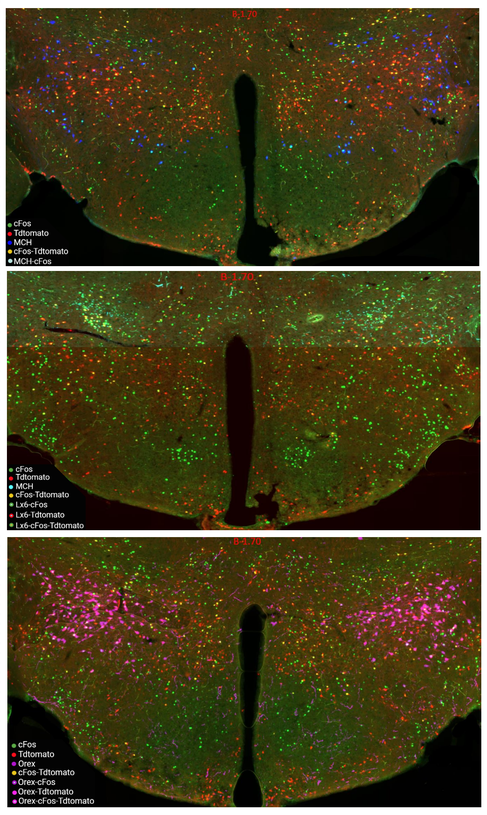
1 Sleep physiology





The quantity of PS was of 31,5% for the 1st 30 minutes, 30% for the 2nd and then decreased to 13,1% and 20,1%. It must be noted that the mice also displayed W during the PS rebound.

2 Comparison of MCH and Orex populations topography across the hypothalamus

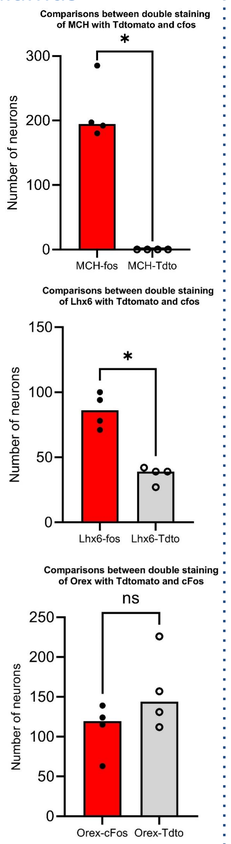


Lhx6 neurons are above the MCH neurons, which encircle the Orex neurons between B-1.20/-1.95.

In the rostral part of the hyp, MCH neurons are in the ZI, DMH, LH. At B-1.70, two LH subpopulations can be distinguished, one is dorso-lateral, one ventro-medial and contained more cFos/MCH neurons. MCH neurons express neither Tdt, nor Lhx6, and most of the cFos/MCH neurons aren't in the LH.

Most of the Lhx6 neurons are localised in the ZI, and doesn't mix with MCH and Orex neurons. Around B-1,70 there are numerous Lhx6 neurons cFos+. They also express Tdt.

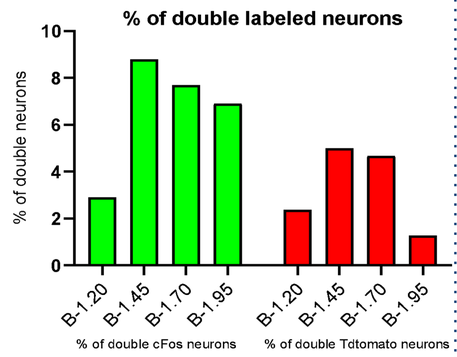
Many Orex neurons are Tdt+ in the caudal and lateral part of the LH, and many were also labelled for cFos.



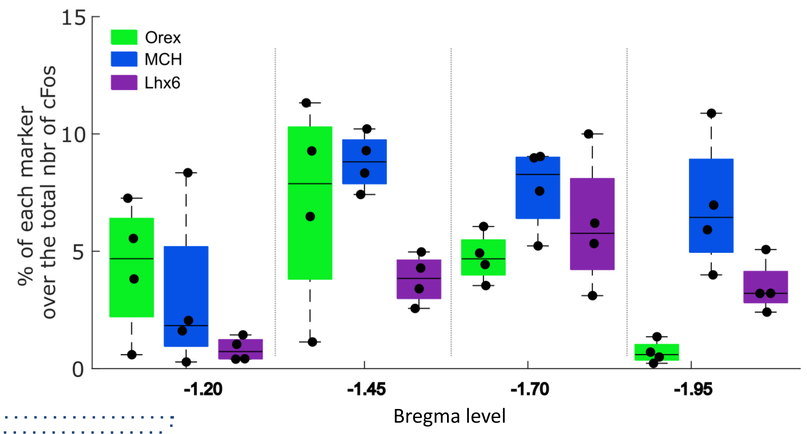
We can hypothesize that part of the Orex neurons are active during PS, or during the wake periods occurring during the rebound.

3 What is the percentage of neurons activated during W and PS rebound not yet characterized neurochemically?

Many Tdt and cFos neurons aren't Orex+ and MCH+ : between B-1.20 and B-1.95, 6.6% cFos neurons are double labelled with MCH, and 3.4% Tdt neurons are double labelled with Orex in the hyp. For MCH neurons 31,9% are cFos+ and for Orex neurons, 38.5% are Tdt+.

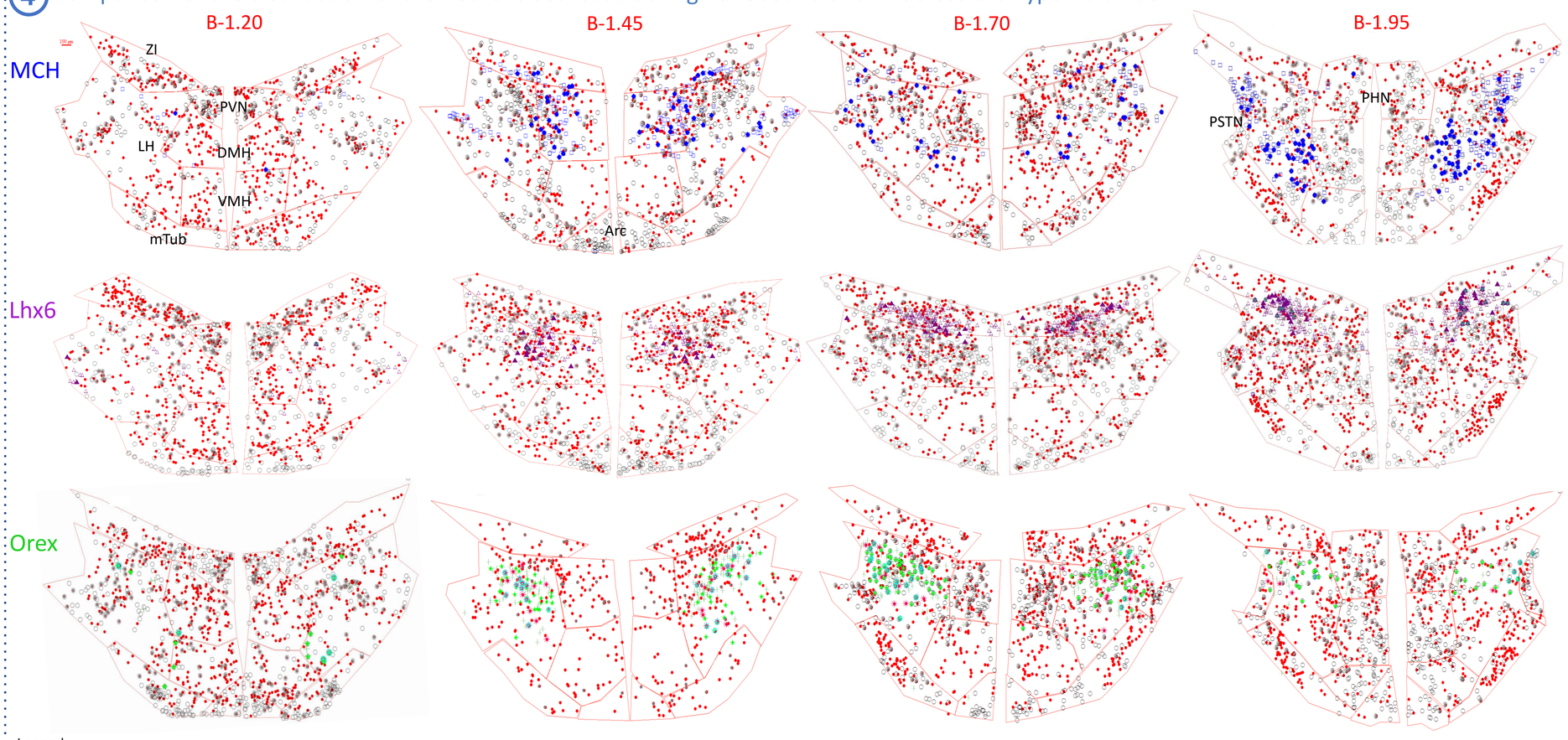


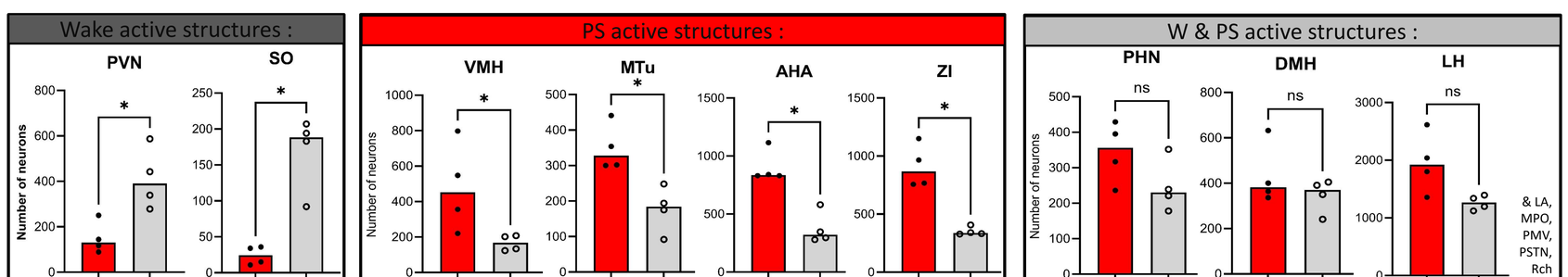
The neurochemical identity of most W and PS active neurons remains to be identified.



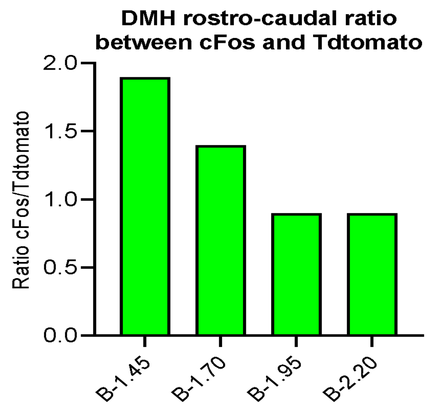
The MCH, Lhx6 and Orex neurons constitute a large part of the neurons activated during PSR only in the central part of the LH. For the rostral and caudal LH as well as for the DMH, MTu, VMH, AHA and ZI, the neurochemical identify of the neurons activated during PS rebound remains to be identified.

4 Comparison of the distribution of the neurons activated during PS rebound and W across the hypothalamus





5 Focus on DMH



In the DMH, the cFos/Tdt ratio decreases rostro-caudally whereas the ratio Tdt/cFos increase.
We can hypothesize that the rostral DMH contains subpopulations playing a role in PS, while the caudal DMH is implicated in W.
For the LH, there is no rostro-caudal difference. It seems that a dorso/ventral organization exist (to be confirmed).

4-OHT: 4-hydroxytamoxifen; **AHA**: Anterior hypothalamic area; **Arc**: Arcuate hypothalamic nucleus; **DMH**: Dorsomedial hypothalamus; **LH**: Lateral hypothalamic area; **Mtu**: Medial tuberal nucleus; **PHN**: Posterior hypothalamic area; **PSTh**: Parasubthalamic nucleus; **PVN**: Paraventricular hypothalamic nucleus; **RCh** : Retrochiasmatic area; **Sch**: Suprachiasmatic nucleus; **SO**: Supraoptic nucleus; **VMH**: Ventromedial hypothalamic nucleus; **ZI**: Zona incerta

Conclusion

In summary, MCH neurons are activated during PSR but not W, while surprisingly Orex neurons are activated during both. In the caudal LH, two MCH subpopulations might be distinguished, one is ventro-medial and strongly activated during PSR while the other is dorsal and seems less activated during PSR. This need to be confirmed. In addition, our results indicate that in many hypothalamic structures most of the neurons activated during W and PS rebound aren't containing Orex and MCH indicating that most of the hyp neurons activated during PS and W have not yet been studied. Finally, certain structures are specifically implicated in W or PS while others like the LH or DMH, seem to play a role in both states.

To go further

Our aim is to identify specific markers for the uncharacterized hypothalamic PS and W active neurons to allow the study of their function using optogenetic, chemogenetic, calcium imaging and retrograde tracing. It will also help to better understand sleep-related pathologies.

References

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