Responses of ventral tegmental area neurons to stimulation of orexin cell fields *in vivo*

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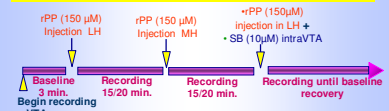
INTRODUCTION

- The orexins (hypocretins) are neuropeptide transmitters made exclusively in hypothalamic neurons.
- Recent work has shown that orexin in the ventral tegmental area (VTA) is critical for the induction of synaptic plasticity and the control of reward-driven behavior, including behaviors related to drugs of abuse.
- The orexin system can also activate dopaminergic (DA) and non-dopaminergic neurons in the VTA, in vitro. (Korotkova and al, 2003)
- Our lab reported evidence that the lateral hypothalamus (LH) and medial hypothalamus (MH) orexin cell fields are involved in different behavioral functions (Harris and Aston-Jones, TINS, 2006).
- In this study we investigated the influence of the LH and MH on neural activity in the VTA. The aim was to determine if LH and MH have different effects on VTA neurons.

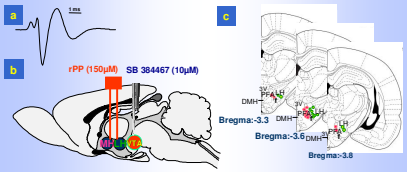
METHODS

- Male Sprague-Dawley rats (320-380g) were housed under a 12h/12h dark/light cycle. Animals were studied in their active (dark) period.
- Experiments were performed under 1.5 % isoflurane anesthesia.
- Chemical stimulation of orexin neurons in the medial hypothalamus (MH) and lateral hypothalamus (LH) was performed by microinfusion, via a double cannula, of the Y4 agonist rPP (rat pancreatic polypeptide) during single-unit extracellular recordings (0.5-5 kHz bandpass) of spontaneously active VTA neurons. rPP has previously been shown to preferentially stimulate orexin neurons (Campbell and al., 2003) and to drive reinstatement of an extinguished drug-seeking response (Harris and al., 2005).
- A double-barrel pipette was used for recording VTA spike activity with simultaneous local microinjection of the orexin-1 receptor antagonist, SB 384467 (SB) (10 µM, 120 nl).

❖Illustration showing the time course of the experimental protocol

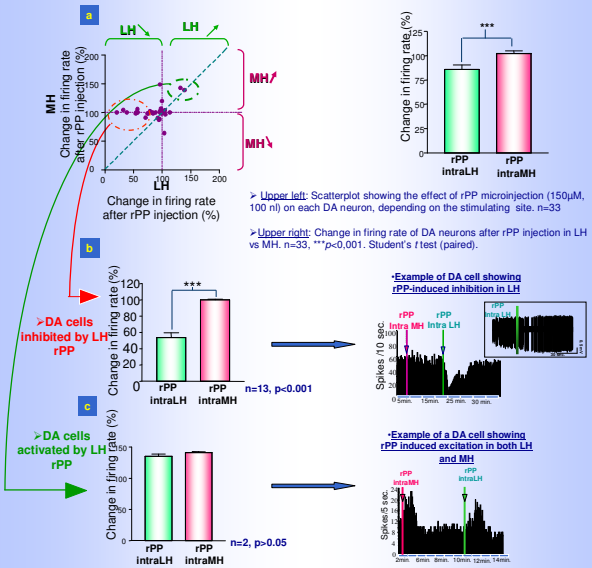


Stimulating and recording sites



a. Typical example of average extracellular waveform of DA neuron.
b. Stimulating (LH and MH) and recording sites (VTA).
c. Diagram of cannula placements within the LH (green/yellow circles) and MH (pink circles). LH, lateral hypothalamus; PFA, perifornical area; DMH, dorsomedial hypothalamus; 3V, third ventricle; and f, fornix

1.Activation of orexin cells by rPP, in LH and MH causes different responses in VTA DA neurons, *in vivo*

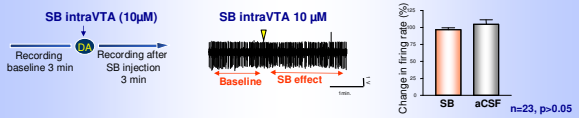


➢Upper left: Scatterplot showing the effect of rPP microinjection (150µM, 100 nl) on each DA neuron, depending on the stimulating site. n=33

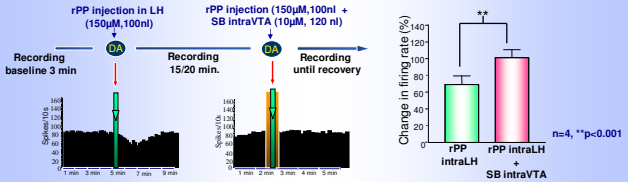
➢Upper right: Change in firing rate of DA neurons after rPP injection in LH vs MH. n=33, ***p<0,001. Student's t test (paired).

➢Summary: For DA cells inhibited by LH activation, MH stimulation has no effect. In contrast, cells activated by LH stimulation are also activated by MH stimulation.

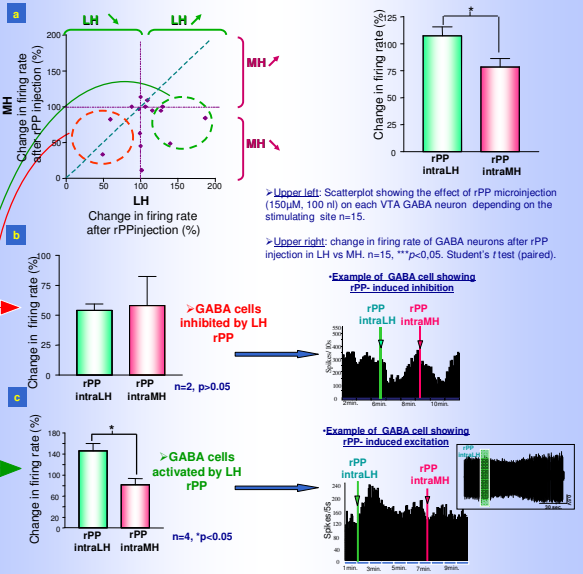
2. The orexin 1 receptor antagonist SB injected intraVTA has no effect on baseline firing rate of DA neurons.



3. SB attenuates inhibition of VTA DA neurons induced by LH rPP stimulation



4. Activation of orexin cells by rPP in LH and MH causes different responses in VTA GABA neurons *in vivo*

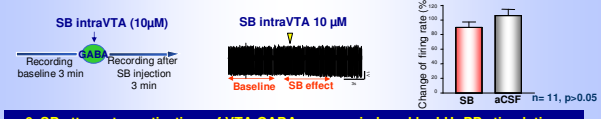


➢Upper left: Scatterplot showing the effect of rPP microinjection (150µM, 100 nl) on each VTA GABA neuron depending on the stimulating site n=15.

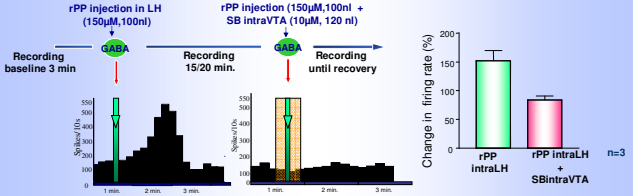
➢Upper right: change in firing rate of GABA neurons after rPP injection in LH vs MH. n=15, ***p<0.05. Student's t test (paired).

➢Summary:: GABA cells inhibited by LH stimulation are also inhibited by MH stimulation. In contrast, GABA cells activated by LH stimulation, are somewhat inhibited by MH stimulation.

5. The orexin 1 receptor antagonist SB injected intraVTA has no effect on baseline firing rate of GABA neurons.



6. SB attenuates activation of VTA GABA neurons induced by LH rPP stimulation.



SUMMARY

Activation of orexin neurons in LH vs MH produces a different pattern of responses in VTA neurons.

1.In the lateral hypothalamus:

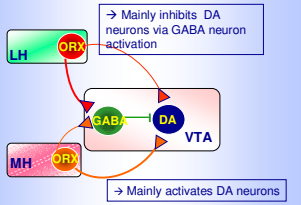
Activation of orexin neurons by rPP mainly produces:
→An inhibition of VTA DA cells
→An activation of VTA GABA cells

Blocked by injection of orexin-1 receptor antagonist into the VTA

2.In the medial hypothalamus:

Activation of orexin neurons by rPP mainly produces:
→An activation of VTA DA cells
→An inhibition of VTA GABA cells

Hypothetical theory:



Orexin neurons activate VTA DA cells directly while inhibition might be occurring indirectly via local inhibitory GABA inputs to DA cells. This study suggests that LH and MH may regulate the activity of VTA neurons in opposite ways. In fact, the LH may act mainly by indirectly inhibiting DA neurons via activation of local inhibitory GABA interneurons. On the other hand, the MH may act principally by directly activating VTA DA cells. Taken as a whole, our results support the view that different orexin cell fields are involved in different behavioral functions. (Harris and Aston-Jones, 2006).

ACKNOWLEDGEMENTS

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