A prefronto-habenular circuit involved in stress-induced reinstatement

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INTRODUCTION:

Addiction is defined as a chronically relapsing disorder, characterized by compulsion to seek and take the drug, loss of control in limiting intake, and emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented[1,2]. Nevertheless, the neurobiology underlying relapse remains unknown, although different risk factors have been described such as the drug itself or stress, even after long periods of abstinence[2–6]. Drugs of abuse also have profound influence on lateral habenula (LHb activity)[7–10]. Cocaine specifically induces a biphasic neuronal response in the LHb which may plays a likely role in the rewarding but also the aversive effects of cocaine. Moreover, LHb hyperactivity is found in rodents undergoing protracted withdrawal from cocaine. This hyperactivity may be one of the mechanisms responsible of the aversive states experienced by abstinent drug users[8,11]. Moreover, the LHb is also activated after application of stressful stimuli, such as tail pinch, electrical shock, or placement in a novel environment[12–15]. We propose that the LHb participates in the phenomenon of stress-induced reinstatement, likely via its connection with the medial prefrontal cortex (mPFC), well-known to be involved in stress integration[16], through descending inputs to the midbrain dopaminergic system[17].

Hypothesis: We hypothesize mPFC inputs to the LHb are responsible for integrating the negative valence of psychological stress and thus contributes to the phenomenon of stress-induced relapse in cocaine abstinent rodents.

Aims: We suggest examining, in rodents, the network, including the LHb and the mPFC, involved in the integration of stress in the context of addiction as well as the behavioral disorders which may result from an alteration of the activity of this network. We will characterize the LHb network potentially implicated in a model of stress-induced relapse in rodent. Then, we will study how an alteration of this network may lead to the reinstatement of addictive behaviors after a stressful event.

The mPFC-LHb network

1 *mPFC projections to the LHb and the VTA*

Method: retrograde tracing

A combination of two retro viruses infused within the LHb (retroAAV-mCherry, 0.25uL) and the VTA (retroAAV-GFP, 0.3uL) was used in mice (n=8).

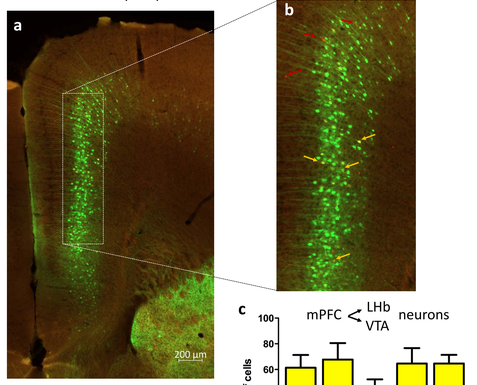

Figure 1 : (a) Picture of mPFC neurons projecting to the LHb or the VTA. (b) Zoom showing 3 different populations of neurons, 1) mPFC neurons projecting to the LHb only ($mPFC_{\rightarrow LHb}$), 2) mPFC neurons projecting to the VTA only ($mPFC_{\rightarrow VTA}$) and 3) mPFC neurons projecting to both LHb and VTA. (c) About 70% of the $mPFC_{\rightarrow LHb}$ neurons also project to the VTA ($mPFC_{\rightarrow LHb/VTA}$).

2 *mPFC-LHb-VTA network*

Method: transynaptic retrograde tracing

Three weeks after infusing a retroAAV-cre virus within the VTA (0.3uL) and a mix of DIO-TVA-mCherry and DIO-Gprotein (1:1, 0.25uL) within the LHb, we infused the EnvA-RVΔG-GFP virus to map the inputs of the LHb neurons projecting to the VTA.

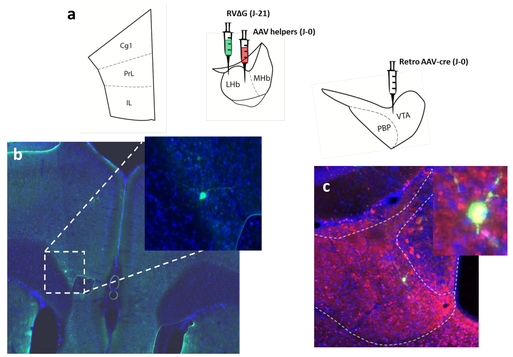

Figure 2 : (a) Schema of the injection design. (b) Zoom on a GFP positive neurons within the mPFC, suggesting an indirect pathway from the mPFC to the VTA through the LHb. (c) Zoom on a "starter cell" within the LHb. This yellow cell expresses both the red fluorescence from the DIO-TVA-mCherry virus (allowed by the presence of cre coming from the VTA) and the EnvA-RVΔG-GFP.

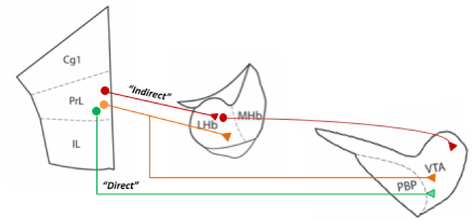

Figure 3 : Schema of the network comprising direct and indirect projections from the mPFC to the LHb and VTA.

Results

In mice, the mPFC projects to both the LHb and VTA. Furthermore, a large population (~ 67%) of mPFC neurons projects directly and simultaneously to both structures. Moreover, we observed mPFC neurons sending inputs to LHb neurons projecting to the VTA. These tracing experiments revealed a complex network composed by direct and indirect pathways connecting the mPFC to the VTA through the LHb, likely to control dopamine release.

The mPFC-LHb role in stress-induced reinstatement

3 *Inactivation of the mPFC-LHb pathway prevents stress-induced reinstatement of cocaine conditioned place preference*

Method: Wild-type mice were injected with a retro-cre-GFP virus into the LHb (0.25uL/side) and a DIO-hM4-mCherry AAV or DIO-mCherry AAV into the mPFC (0.3uL/side), allowing the pharmacogenetic manipulation of the $mPFC_{\rightarrow LHb}$ pathway. Those animals were subjected to a conditioned-place preference protocol followed by an extinction phase and a stress-induced reinstatement session (CPP-SIR). Another cohort was used to assess the chemogenetic inactivation of the $mPFC_{\rightarrow LHb}$ pathway in "control" behaviors such as exploration and anxiety.

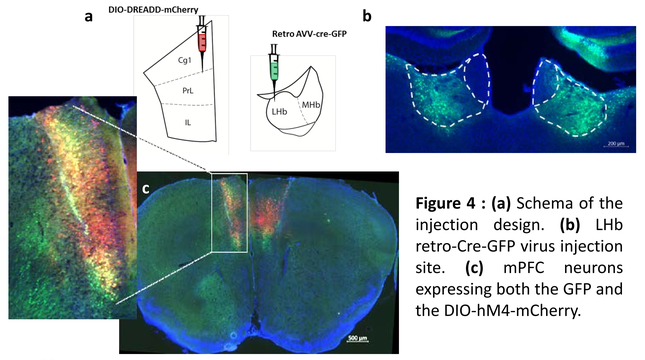

Figure 4 : (a) Schema of the injection design. **(b)** LHb retro-Cre-GFP virus injection site. **(c)** mPFC neurons expressing both the GFP and the DIO-hM4-mCherry.

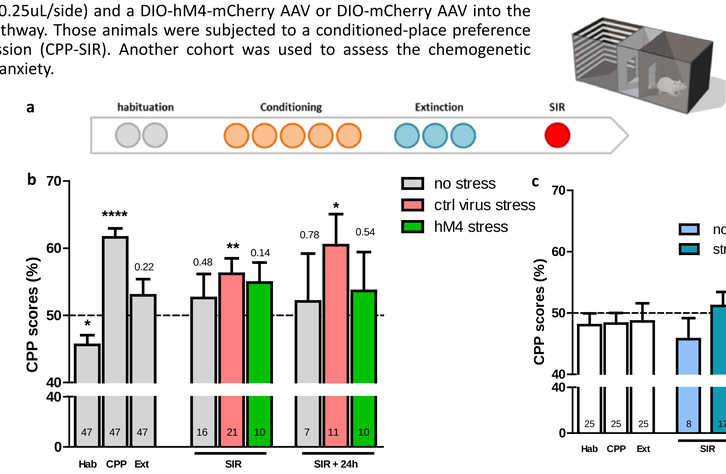

Figure 5 : (a) Schema of the behavioral design. **(b)** After 2 days of habituation (hab) and 5 days of conditioning, mice treated with cocaine (left panel) spent more than 50% of their time in the cocaine-paired side, suggesting a preference. **(c)** Control animals treated with saline didn't show any preference . This preference is abolished after a period of extinction (ext). Twenty-four hours after the last day of extinction, mice were injected or not with yohimbine (SIR). Only yohimbine-injected animals (ctrl virus stress, in red) expressed again a preference for the cocaine-paired side. In the contrary, mPFC-LHb chemogenetic silencing (hM4 PFC-LHb stress, in green) prevented the reinstatement. This effect lasts at least 24h after SIR (postSIR), suggesting for the first time a role of this pathway in stress-induced reinstatement. Statistics: * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$

4 *Control behaviors*

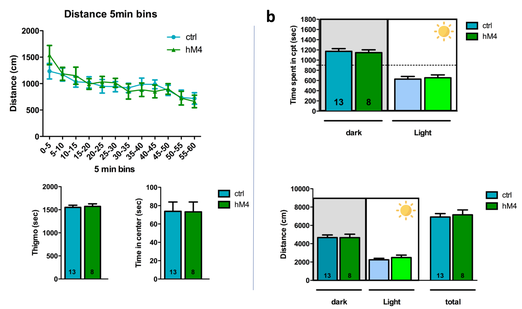

Figure 6 :

(a) Locomotor behavior: Fifteen minutes after CNO injection (3mg/kg), mice were placed in an open-field for 60 min. No differences in the distance travelled by the mice nor the time spent in thigmotaxis or in the center of the field were observed, suggesting that the $mPFC_{\rightarrow LHB}$ manipulation didn't disturb exploratory/locomotor behaviors.

(b) Anxiety-like behavior: Fifteen minutes after CNO injection (3mg/kg), mice were placed in a light dark box for 30 min. No differences in the time spent in the lit or dark side of the box, or the distance travelled were observed, suggesting that the $mPFC_{\rightarrow LHB}$ manipulation didn't disturb anxiety-like behaviors.

Results:

$mPFC_{\rightarrow LHB}$ chemogenetic inhibition prevented stress-induced reinstatement for cocaine in a cpp paradigm. The manipulation of this pathway didn't disturb exploratory, locomotor and anxiety-related behaviors. Together these results suggest a novel role of cortical inputs to the LHb in addictive behaviors

CONCLUSIONS:

1. Tracing experiments :

In mice, mPFC sends dense direct projections to both LHb and VTA. Furthermore, a large population (~ 67%) of mPFC neurons project directly and simultaneously to both structures. Furthermore, mPFC neurons also project to the VTA via the LHb, suggesting a control of the dopamine release from the mPFC via direct and indirect pathways involving the LHb.

2. Behavioral experiments:

Chemogenetic inhibition of the $mPFC_{\rightarrow}$LHb pathway prevents cocaine stress-induced reinstatement in a CPP protocol, indicating the necessity of cortical inputs to the LHb for mediating stress-induced relapse of drug seeking behavior.

Together these results suggest a complex network involving direct and indirect connections between mPFC, LHb and VTA, likely influencing dopamine release. Moreover, our results suggest for the first time a key role of cortical inputs to LHb in addictive behaviors.

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