SOCIETÀ ITALIANA DI FARMACOLOGIA

MODELLO PER INVIO RELAZIONE DI METÀ E FINE PERIODO

NOME E COGNOME: Alice Ilari

UNIVERSITÀ: Università di Camerino

DIPARTIMENTO (in caso di borsa per soggiorno all'estero specificare l'ente presso cui si è svolta la ricerca): Department of Pharmacological Sciences – Icahn School of Medicine at Mount Sinai (NY)

TUTOR (in caso di borsa per soggiorno all'estero specificare il tutor dell'ente presso cui si è svolta la ricerca): Prof. Ming-Hu Han

TIPOLOGIA DI BORSA RICEVUTA: Borsa di ricerca SIF per brevi periodi all'estero

TIPOLOGIA DI RELAZIONE (es.: metà periodo o finale): Metà periodo – Interruzione causa emergenza Covid-19

TITOLO DELLA RELAZIONE: Voluntary ethanol consumption leads to adaptative changes in GABAergic transmission in a subset of VTA dopaminergic neurons

RELAZIONE:

Alcohol abuse is the most prevalent substance-use disorder worldwide. Dopaminergic projections from the ventral tegmental area (VTA) to multiple brain areas, such as nucleus accumbens (NAc) and prefrontal cortex (PFC), are strongly implicated in reward-related behaviours [Hyman et al., 2006; Russo and Nestler, 2013]. In particular, projections to NAc encode the rewarding sensation associated with drugs of abuse [Juarez et al., 2017]. Neuroadaptation in this system underlies the formation of compulsive drug-seeking behaviours. Conflicting reports are present in the literature on the neural alterations induced by voluntary EtOH consumption. Converging evidence indicates that prolonged stimulation of the dopaminergic reward system leads to a homeostatic response of the system ("hypodopaminergic state") [Weiss et al., 1996]. However, the details of the EtOH-induced adaptations are still unclear. On these premises, we studied the neurophysiological alterations induced by voluntary EtOH drinking and how this affects the response to acute EtOH perfusion of VTA dopaminergic (DA) neurons.

To study this process, we performed ex vivo electrophysiological experiments on mice expressing the Green Fluorescent Protein in dopamine neurons (TH-GFP) and previously exposed to a voluntary alcohol drinking paradigm ("drinking group") vs EtOH-naive mice ("control group"). At 6 weeks of age, mice were individually housed in cages with two bottle tubes: one filled with water and the other with increasing concentration of EtOH (3%, 6% for four days each, and 12% for ten days). For control group, naive mice had access to two bottles both filled with water.

After chronic EtOH exposure, electrophysiological recordings on acute brain slices were performed to determine the electrical activity of VTA DA neurons. Passive membrane properties, spontaneous firing rate and spontaneous excitatory/inhibitory synaptic activity were measured to study the

remodeling of intrinsic and network properties induced by voluntary EtOH intake. In addition, the response to 40 mM EtOH perfusion was also measured during recordings.
Our data show that there is no difference in passive membrane properties between groups, while we see that chronic EtOH causes a significant reduction in basal firing rate, and significantly increases the firing-enhancing effect of 40 mM EtOH bath perfusion. Moreover, chronic EtOH increases the frequency of GABAergic, but not glutamatergic, inputs onto VTA DA neurons, while reducing the response to acute EtOH perfusion. Interestingly, by restricting the analysis to neurons responding to acute EtOH, the difference in basal frequency of GABA inputs achieves statistical significance. Furthermore, voluntary EtOH consumption leads to the loss of the acute EtOH response in spontaneous inhibitory post-synaptic currents (sIPSCs) amplitude, but there is no difference in the basal amplitude between the two groups.
Altogether, these data show that chronic EtOH exposure, while not modifying the passive membrane properties of VTA DA neurons, leads to a permanent potentiation of GABA transmission, suggesting a prominent effect of EtOH on GABAergic neurons rather than on DA neurons. This is consistent with the evidence that addictive states are associated to a hypodopaminergic tone. Finally, the reduced GABA response may represent a compensatory mechanism aimed at potentiating the effect of single EtOH dose and relieving the negative affective state associated to the hypodopaminergic tone. For translational prospects, understanding GABA transmission alterations could become a new target for the investigation of putative mechanism involved in EtOH addiction.
Starting from these data, my original plan was to measure the firing rate of VTA GABAergic neurons after 18-day alcohol drinking paradigm and during acute 40 mM EtOH perfusion, performing ex vivo electrophysiological experiments, in order to verify that ethanol persistently increases the firing activity at least in a subset of these neurons.
To do that, I determined the genotype and infected mice expressing CRE recombinase in GABAergic neurons (GAD-CRE mice) with AAV5-EF1α-DIO-eYFP within VTA, in order to mark GABAergic neurons for patch-clamp recordings. This manipulation was necessary as there are no reliable electrophysiological markers for VTA GABA neurons.
One week after the surgery, mice started the 2-bottle choice paradigm (3%, 6% and 12% of EtOH) and I should have used them to perform ex vivo electrophysiological experiments. Unfortunately, because of COVID-19 emergency and the shutdown of all research activities at my host institution, the experiment was aborted.
Once research activity will resume, we will confirm the effect of EtOH on GABAergic transmission by direct recordings on GABA neurons. The following step will be the study of the activity of VTA GABAergic neurons in vivo by using fiber photometry. For this experiment, GAD-CRE mice injected in the VTA with AAVs carrying CRE-dependent GCAmp6 will be used.
Finally, if fiber photometry confirms that GABA neurons activity changes during specific stages of the drinking paradigm, we will attempt to modulate behavior by controlling the activity of VTA GABA neurons in the required direction. Following the hypothesis that GABA neurons are persistently activated by voluntary alcohol drinking, we will inject GAD-CRE mice with AAV-EF1a-DIO-eNpHR3.0-eYFP. Mice expressing the NpHR halorhodopsin in VTA GABA neurons will be used for in vivo photostimulation with green/yellow-light (590 nm) during all the phases associated to the drinking paradigm: acquisition, expression, withdrawal, re-exposure. This experiment will allow to clarify the link between changes in VTA GABA neurons activity and drinking behavior.

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Da inviare a: Società Italiana di Farmacologia – e-mail: sif.soci@segr.it; sifcese@comm2000.it