

# Python-based automatization of fear memory using DeepLabCut

*Júlia Pinho<sup>1</sup>, Carla Ramon-Duaso<sup>1</sup>, Arnau Busquets-Garcia<sup>1</sup>*

<sup>1</sup> **IMIM-Hospital del Mar Medical Research Institute**

**PRBB Building, Barcelona, Spain. *IMIM-Hospital del Mar Research Institute*  
SPAIN**

Higher-order conditioning, also known as mediated learning, plays a significant role in shaping our daily decision-making, where our decisions are usually influenced by stimuli that are not directly associated with reinforcers (previous appetitive or aversive experiences). Sensory preconditioning tasks can be used to measure this process in mice, which involves several simultaneous pairings between two low-intensity stimuli, such as light and tone, followed by a devaluation phase where one of the stimulus is paired with an unconditioned stimulus, such as a mild foot shock. The outcome is a conditioned response (i.e. freezing response) to both the conditioned stimulus (direct learning) and the non-conditioned stimulus (mediated learning).

In our study, we developed a python based automatic analysis of the freezing response using the tracking output of DeepLabCut. The DeepLabCut is an open-source software that allows the pose estimation of several animals and multiple bodyparts. Using python based scripts we developed automatic freezing quantification with up to 90 % correlation with both, manual scoring and EzTrack® (another automatic software for freezing analysis). Thus, we are able to 1) measure different parameters (speed, time), 2) explore the temporal resolution of the behavior and 3) quantify pose differences of the body. This allows us to have an automatized way to study, not only higher-order conditioning in mice, but also classical fear conditioning paradigms.

Overall, we implemented an automatic way to accurately explore fear responses and, in the future, we expect to contribute to the better characterization of the active and passive fear responses.