

## PROTOCOL FOR ASSESSING FREEZING BEHAVIOUR IN HUMANS

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**Background:** Freezing is characterized by immobility or reduced movement (e.g. body sway) together with a simultaneous activation of the Autonomic Nervous System (ANS), with a parasympathetic dominance. In humans, freezing is a relevant topic in the context of cognition, emotion and behaviour, in health and disease. However, the internal and external parameters that modulate the decision of freezing remain largely unknown. Several experimental settings have been proposed, most of them using immobility, bradycardia, increasing galvanic skin response and pupil dilation as physiological markers of freezing. Nonetheless, the protocols used are mostly designed with tasks without ecological validity, focused on the study of anticipatory freezing, eliminating the possibility of choosing between escaping or freezing. There is a need for a standardized protocol that allows the study of probability of freezing for individuals.

**Aims:** To develop a protocol to assess freezing behaviour in humans based on movement and physiological markers of the ANS.

**Method:** We developed an experimental setting to record data on movement and physiological markers of the ANS – electrocardiogram (ECG), electrodermal activity (EDA) and pupil size – while subjects will participate on videogame presenting a dynamic scenario of an encounter with a threat. To assess movement we will use 6 inertial measurement units (IMUs) (Xsens Awinda; Netherlands) attached to the head, lumbar region, thighs and wrists. To record ECG and EDA, we will use disposable adhesive electrodes connected to Bionomadyx wireless amplifiers, connected to a data acquisition system MP160WSW (Biopac Systems, Inc.; USA). The electrodes will be attached on specific regions of the body as defined by the manufacturer. Eye-tracking glasses will be used to assess pupil size - Pupil Core (Pupil Labs GmbH; Germany). For the experimental task to induce a threat situation we developed a videogame in which subjects will have the option to escape, hide in a shelter or freeze in order to avoid contact with a fearful animated stimulus. In case of contact subjects will receive mild electric shocks on the wrist using a constant current stimulator (Digitimer DS7A; Digitimer Ltd.; UK). The intensity of the shock will be titrated with each participant for the stimulus to be considered by the participant as uncomfortable but not painful (max. intensity 9mA). All equipment will be synchronized using Arduino plaques signaling to the Biopac MP160WSW.

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