Real-time detection of ethanol in the nucleus accumbens shell of freely moving rats

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Ethanol consumption is a serious health issue in terms of the amount consumed and the behavior related to its consumption. There is controversy regarding the active agent responsible for alcohol addiction (Peana et al., 2017). The theory that ethanol itself was the agent in alcohol drinking behavior was widely accepted until acetaldehyde or salsolinol were found in the brain. The importance of metabolites formation in the brain is still subject to speculation due to the lack of a method to accurately assay the metabolites levels directly. Even the most common used technique has been microdialysis since now, recently, a valid alternative has been found in amperometric biosensors. In this study, we describe the implementation and characterization of an implantable enzyme-based biosensor for the amperometric detection of brain (nucleus accumbens shell) ethanol in real time by optimizing a previous implantable design (Rocchitta et al., 2012 and 2013). We have selected the most efficient enzyme, among three different alcohol oxidase strains, in order to be able to detect ethanol concentration changes in the nucleus accumbens. The developed biosensors have proven to be the most sensitive analytical devices for real time ethanol detection in vivo which can be found in literature. Moreover, the biosensors have been coupled with a telemetric system for transmitting data, in order to allow freely movement to the animals. We implanted the biosensors in the shell of the nucleus accumbens of rats (A/P +1.7 from the bregma, −0.9 M/L, and −7.6 D/V from the dura) by means of stereotaxic surgery. Experiments started after a period of recovery from surgery. The animals were a) allowed access to 10% ethanol voluntarily or b) passively (by an intragastric 1g/kg ethanol administration). The biosensor was connected via the telemetric system and the amperometric signal from ethanol was recorded up to its stabilization: the rats were allowed to drink, or treated with intragastric administration, only after having reached a stabile baseline current. In these experiments we were able to detect the arrival of ethanol in the nucleus accumbens shell in real time, monitoring a significant increase of the current derived from ethanol when the rats consumed (voluntarily or passively) the ethanol solution, and evaluating a decrease of the signal at the end of ethanol administration. In conclusion, by allowing quantification of very low ethanol concentrations, this method may be used to detect ethanol kinetic in murine brain tissue in alcohol research during self-administration experiments.

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