



# On the Accuracy of *In Vivo* Ethanol and Acetaldehyde Monitoring, a Key Tile in the Puzzle of Acetaldehyde as a Neuroactive Agent

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Over the last 20 years researchers have explored the postulated role of acetaldehyde (ACD) as a mediator of some of the actions of ethanol (EtOH) in the central nervous system (CNS). However, efforts have been hampered mainly by the difficulty of directly measuring *in vivo* EtOH and ACD levels in the CNS and thus, our knowledge is based on indirect evidences. Although technically challenging, the development of reliable methods for *in vivo* measurement of ACD and EtOH is of paramount importance to solve the “puzzle of acetaldehyde as a neuroactive agent.” In this short review we discuss the recent advances on brain EtOH pharmacokinetic and state-of-the-art available techniques that could be used for *in vivo* detect EtOH and ACD both non-invasively (magnetic resonance spectroscopy), and invasively (microdialysis and biosensors). Among the different *in vivo* sampling techniques described, particular emphasis is paid to the field of enzyme-based amperometric biosensors. Biosensors have gained much attention in recent years for their ability to online monitor biological signals *in vivo*, and several micro- and nano-structured devices have been successfully used for *in vivo* studies. Owing to their high temporal and spatial resolution, biosensors could provide the adequate technology for studying *in vivo* EtOH pharmacokinetic.

**Keywords:** ethanol, acetaldehyde, magnetic resonance spectroscopy, microdialysis, biosensors

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## INTRODUCTION

Acetaldehyde (ACD) is a naturally occurring compound, found in several fruits and vegetables as well as in tobacco smoke and fermented alcoholic beverages (Cao et al., 2007).

In the last decades many attempts have been made to quantify brain EtOH and ACD, in order to correlate their concentrations with behavior (Correa et al., 2012; Israel et al., 2015). So far this line of research has yielded conflicting results, mostly due to discrepancy and controversy with quantitative measures of brain EtOH and ACD.

In this brief review we offer an overview of the recent advances on brain EtOH pharmacokinetic and discuss the state-of-the-art of available techniques for *in vivo* EtOH and ACD study.

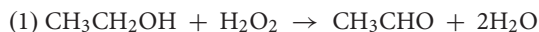
## EtOH METABOLISM IN THE BRAIN

Since EtOH readily enters the brain, *in situ* synthesis has been long postulated as a plausible source of brain ACD (Cohen et al., 1980). It is now demonstrated that the brain tissue contains all of the main EtOH metabolizing enzymes: alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1), and catalase; however, their relative role in metabolizing EtOH into ACD is still debated.

Alcohol dehydrogenase is a zinc-containing enzyme localized in the cytosol, it has broad substrate specificity (many primary or secondary alcohols) and is found in highest amount in the liver. However, since ADH is not uniformly expressed in the brain tissue, its real contribution to local ACD levels in discrete brain areas could have been underestimated and may deserve more detailed evaluation (Bühler et al., 1983; Kerr et al., 1989; Mori et al., 2000).

Cytochrome P450s are a family of heme enzymes mainly located in the endoplasmic reticulum and in mitochondria. CYP2E1 is the P450 family with the highest activity for oxidizing EtOH to ACD, and is widely expressed in the human and rodent brain (Tindberg and Ingelman-Sundberg, 1996; Sánchez-Catalán et al., 2008; Ferguson and Tyndale, 2011). CYP2E1 has been shown to metabolize EtOH in both neurons and astrocytes at a rate of 0.00051  $\mu\text{mol}/\text{min}/\text{g}$ , and CYP2E1 pharmacological inhibition significantly reduces ACD formation in rat brain homogenates incubated with EtOH (Hansson et al., 1990; Gill et al., 1992; Warner and Gustafsson, 1994; Zimatkin et al., 2006). Further, reduced ACD brain levels have been shown in transgenic KO CYP2E1 mice after incubation with EtOH, relative to their wild-type counterparts (Ziegler et al., 2006). CYP2E1 activity has been accounted for a 20% fraction of brain EtOH oxidation, and it may represent a major adaptive response to chronic EtOH consumption as shown in a recent *in vivo* study on EtOH-induced locomotion (Hansson et al., 1990; Heit et al., 2013; Ledesma et al., 2014). Further, *in vitro* evidences in KO CYP2E1, acatalasemic and double mutants (KO CYP2E1 and acatalasemic) mice, suggest that CYP2E1 function may be linked to catalase-mediated EtOH oxidation by increasing the availability of  $\text{H}_2\text{O}_2$  (Halliwell, 2006; Zimatkin et al., 2006; Deng and Ra, 2008).

Catalase, a heme containing enzyme, is found in the peroxisomal fraction of the cell and can oxidize EtOH as shown in reaction 1.



Recent results also show that 3-amino-1,2,4-triazole (3AT) administration impairs the acquisition of operant EtOH self-administration in the rat (Peana et al., 2015). However, 3AT has been shown to cause non-specific effects on behavior and therefore other procedures have been used to inhibit catalase-mediated ACD formation (Rotzinger et al., 1994; Tampier et al., 1995).

A valuable method for *in vivo* studying the involvement of catalase in brain ACD formation is based on the use of lentiviral

vectors coding for an anticatalase shRNA (RNAi precursor), which allows for efficient (up to 75%) inhibition of catalase activity (Karahanian et al., 2011). This technique appears also of particular interest because by allowing localized inhibition of catalase activity, it may be used to precisely pinpoint those brain areas involved in the psychopharmacological effects of EtOH (Israel et al., 2015). In fact, it has been shown that administration of an anticatalase vector into the ventral tegmental area (area which plays a key role in the neurobiological basis of addiction, VTA), significantly reduced EtOH consumption and EtOH stimulated dopamine release in its projection fields (in particular the nucleus accumbens shell) (Karahanian et al., 2011; Israel et al., 2012; Quintanilla et al., 2012). Another study shows that anticatalase vector administration in the VTA can efficiently inhibit EtOH intake following deprivation (Tampier et al., 2013).

## IN VIVO EtOH AND ACD DETECTION: NON-INVASIVE APPROACHES

### Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a non-invasive analytical technique used to provide a measure of *in vivo* brain biochemistry (Soares and Law, 2009; Strózik-Kotlorz, 2014; Buonocore and Maddock, 2015). *In vivo* MRS can be performed with common clinical magnetic resonance imaging equipments and since EtOH methyl protons can be detected (Sammi et al., 2000), MRS has been largely used to measure *in vivo* brain EtOH levels in both humans and laboratory animals (Hanstock et al., 1990; Kaufman et al., 1994; Rooney et al., 2000; Zahr et al., 2010). However, magnetic transfer evidences have clearly shown the *in vivo* presence of a free, observable EtOH pool and a membrane-associated EtOH pool that escapes direct detection (Fein and Meyerhoff, 2000; Nagel and Kroenke, 2008). Therefore, since a (possibly significant) fraction of brain EtOH content cannot be measured by MRS, this technique must be considered only for qualitative measurements.

Ethanol oxidative metabolism has been studied with MRS, after  $^{13}\text{C}$ -labeled EtOH administration (Xiang and Shen, 2011; Wang et al., 2013a,b). The results show that  $^{13}\text{C}$  nuclei from  $^{13}\text{C}$ -labeled EtOH are incorporated into multiple metabolites including glutamate, glutamine, and aspartate, but no significant conversion of EtOH into ACD in the brain could be evidenced.

Despite the low sensitivity and temporal resolution, MRS still provides an opportunity for *in vivo* qualitative study of the effects of EtOH in the brain (Nagel and Kroenke, 2008; Niciu and Mason, 2014). MRS is fundamental for human studies allowing the dynamic evaluation of EtOH effects, and providing an important framework for comparing experimental results in humans and animal models (Cifuentes Castro et al., 2014). Further, since magnetic resonance images can be obtained concurrently with spectroscopic data, MRS also provides valuable structural information (Alger, 2010; Befroy and Shulman, 2011).

## **IN VIVO EtOH AND ACD DETECTION: INVASIVE APPROACHES**

### **Microdialysis**

Microdialysis is *de facto* the gold-standard *in vivo* sampling technique for the central nervous system (CNS), allowing the analysis of several molecules in cerebro spinal fluid (CSF) based on their diffusion across a semi-permeable membrane (Chefer et al., 2009; Kennedy, 2013). Despite its popularity, microdialysis is not free of limitations (Westerink, 1995), some of which are particularly relevant for EtOH and ACD *in vivo* measurement. In particular, due to low probe recovery, the concentrations of substances in the dialysate only partially reflect true tissue concentrations and thus, analytes present at very low concentrations are difficult to detect. This issue could be also worsened by the fact that some compounds may be adsorbed by the dialysis membrane further decreasing probe recovery (Buttler et al., 1996). It is well-known that microdialysis has poor time resolution and therefore is not suitable for studying events that change in short time intervals. Another problem is the effect of tissue damage secondary to probe implantation; although microdialysis probes have been miniaturized in time, alterations in tissue metabolism cannot be neglected (Borland et al., 2005; Carson et al., 2015).

Despite all technical shortcomings, the advantages of using microdialysis for *in vivo* monitoring of brain neurochemistry are clear. Microdialysis is a well-known and widely reproduced technique, sampling can be performed on freely moving subjects, and long-term studies can be carried out with minimal influence on the brain tissue physiology (Westerink, 1995; Chefer et al., 2009; Kennedy, 2013). On these bases, several authors used brain microdialysis to study EtOH and ACD *in vivo* (Yoshimoto and Komura, 1993; Jamal et al., 2003, 2007, 2015). However, this approach yielded only limited results, mostly due to the various technical and analytical issues which specifically impair the usefulness of microdialysis for EtOH and (especially) ACD *in vivo* monitoring.

### **High Performance Liquid Chromatography (HPLC)**

High performance liquid chromatography (HPLC)-based analytical methods are largely employed in microdialysis studies (Cheng et al., 2009; Guihen and O'Connor, 2009). With regard to EtOH and ACD determination HPLC-based methods appear particularly suitable, since samples are not heated during analysis and thus heat sensitive or volatile compounds (such as EtOH and ACD) can be efficiently separated. Several protocols for EtOH analysis with HPLC have been developed using flame ionization detection (Yarita et al., 2002), ultraviolet detection after conversion to acetaldehyde-phenylhydrazone (Pellegrino et al., 1999), indirect photometric detection (Takeuchi et al., 1988), and enzymatic assay (Kristoffersen and Smith-Kielland, 2005; Peris et al., 2006). An optimized HPLC-based protocol for ACD determination in biological samples after derivatization with dinitrophenylhydrazine (Vogel et al., 2000) and diode array detector is also available (Guan et al., 2011).

### **Gas Chromatography (GC)**

Gas chromatography (GC) is an efficient analytical technique for separating volatile species in complex samples, and several GC-based protocols have been developed for the detection of EtOH and ACD in biological matrices.

Several detector types can be used in conjunction with GC for EtOH and ACD detection; the most efficient protocols available have been developed mainly using mass spectrometry (GC-MS) (Heit et al., 2016) or flame ionization (GC-FID) (Chun et al., 2016), alone or in combination (Tiscione et al., 2011).

Gas chromatography with headspace extraction and mass spectrometry or flame ionization detection is the most reliable and sensitive technique available for EtOH and ACD detection in microdialysates (Xiao et al., 2014; Heit et al., 2016). Indeed, owing to their robustness and reliability, GC-based EtOH analysis are the gold standard technique for blood alcohol concentration measurement in forensic and toxicological laboratories (Cordell et al., 2013; Xiao et al., 2014; Goullé and Guerbet, 2015).

### **Fluorimetry**

A new fluorimetry-based analytical method for EtOH and ACD has been recently published (Zachut et al., 2016). Although not specifically developed for EtOH and ACD detection in brain dialysates, this technique appears quite compatible with microdialysis; in particular: small sample volume, no sample pre-processing, simple methodology, relatively inexpensive laboratory equipment. Further, the limits of detection of the technique are reported to be comparable with the performance of GC methods.

### **Biosensors**

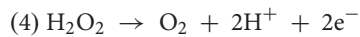
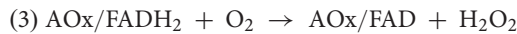
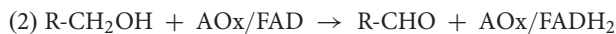
A biosensor can be defined as “a self-contained analytical device that combines a biological component with a physicochemical device for the detection of an analyte of biological importance” (Hasan et al., 2014). Biosensors typically consist of two key components: (1) a biological recognition element to detect the analyte; (2) a transducer to convert the biological response into a convenient output signal.

Among the different devices available, amperometric enzyme-based biosensors (AEBs) are increasingly employed in *in vivo* brain monitoring (Thévenot et al., 2001; Weltin et al., 2016). In fact, miniature (active surface – 1 mm, diameter – 150  $\mu$ m) AEBs implantation induces reduced tissue damage, allows for real-time monitoring, with high sensitivity and specificity for analytes which cannot be studied with microdialysis (Timmerman and Westerink, 1997; Sirca et al., 2014). Another important feature of biosensors is the possibility of associating the implanted device to a telemetric system, allowing experiments in freely moving subjects (Olivo et al., 2011).

Amperometric enzyme-based biosensors are mainly based on enzymes that belong to two classes: oxidases and dehydrogenases; in their most common implementation the enzyme is linked on the transducer surface and the output signal is generated by measuring the electroactive by-products of enzymatic reaction.

In recent years several AEBs for EtOH detection have been developed, based on both alcohol oxidase (AOx) or dehydrogenase (ADH).

Alcohol oxidase catalyzes the oxidation of aliphatic, low molecular weight alcohols to their respective aldehydes using molecular oxygen (O<sub>2</sub>) as the electron acceptor and flavin-adenine dinucleotide (FAD) as cofactor (reactions 2 and 3).

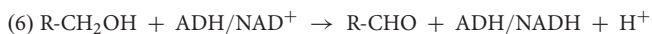


The hydrogen peroxide produced by reaction 3 can be directly detected at the transducer surface of AOx-based AEBs (reaction 4). However, the high anodic potential needed to oxidize H<sub>2</sub>O<sub>2</sub> poses a problem of Faradaic interference due to the presence of other compounds (such as ascorbic acid and uric acid) physiologically present in high concentrations in the CSF, which are also oxidized at the same potential (Belluzo et al., 2008). The use of a bi-enzyme AEB is a common way to circumvent this problem. In fact, by coupling a peroxidase [usually horseradish peroxidase (HRP)] to AOx it is possible to indirectly monitor EtOH-derived H<sub>2</sub>O<sub>2</sub> at low working potentials reducing interfering signals (Vijayakumar et al., 1996; Azevedo et al., 2005).



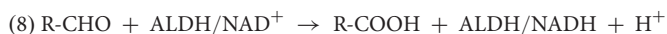
The HRP<sup>+</sup>/HRP<sup>-</sup> redox couple (reaction 5) is used as the sensing scheme at the transducer surface of AOx/HRP-based AEBs.

Alcohol dehydrogenase catalyzes the reversible oxidation of primary aliphatic and aromatic alcohols using nicotinamide-adenine dinucleotide (NAD) as cofactor (reaction 6).



The most common way to monitor an ADH-catalyzed reaction is by using the NAD<sup>+</sup>/NADH redox couple (reaction 7) as the sensing scheme at the transducer surface of ADH-based AEBs (Lorenzo et al., 1998).

Acetaldehyde biosensors developed so far are based on ALDH, which catalyzes the oxidation of biogenic and xenobiotic aldehydes (including ACD) into acetate using NAD as cofactor (reaction 8).



The ALDH-catalyzed reaction is monitored by using the NAD<sup>+</sup>/NADH redox couple (reaction 9) as the sensing scheme at the transducer surface of ALDH-based AEBs (Lorenzo et al., 1998).

Acetaldehyde biosensors have been mostly developed for toxicological and industrial purposes and therefore their biological applicative potential is much less characterized, when compared with EtOH AEBs. However the available evidence

show that these devices can efficiently detect ACD in the μM range *in vitro*, with high time resolution and substrate specificity (Noguer and Marty, 1997; Noguer et al., 2001; Avramescu et al., 2002; Yao and Handa, 2003; Ghica et al., 2007).

Although the development of an adequate biosensor technology for *in vivo* EtOH and ACD detection is still in its infancy, the available evidence clearly show that this approach holds tremendous technological potential. In fact the prototypical properties of biosensors including high spatial and temporal resolution together with high sensitivity and specificity, render these devices the best candidates for *in vivo* accurate EtOH and ACD detection.

Several AEBs for *in vivo* EtOH determination are already commercially available; however since *in vivo* biosensors use is not deprived of drawbacks, the fundamentals of this technology are to be well-understood in order to obtain reproducible results (Vigneshvar et al., 2015; Weltin et al., 2016). In particular, the interactions of the implanted AEB with the biological environment may severely affect its bioanalytical performances via metabolic biofouling, electrode passivation, or biodegradation. Metabolic biofouling is probably the most important problem being able to quickly alter sensitivity, limit of detection, and linear response of the implanted device (Gifford et al., 2006; Kotanen et al., 2012). Unfortunately, biocompatibility-based issues cannot be easily circumvented and adequate pre- and post-calibration procedures are needed in order to properly evaluate *in vivo* AEBs measurements (Wilson and Gifford, 2004; Wahono et al., 2012). However it is expected that the forthcoming generation of biosensors, either based on nanoscale or polymeric materials, will greatly help reducing biocompatibility issues (Nichols et al., 2013; Weltin et al., 2014; Saxena and Das, 2016).

## CONCLUSION

The many attempts to quantify ACD in the brain have yielded conflicting results, mainly because of the inadequacy of the available analytical techniques. Thus, it is clear that in order to solve the puzzle of ACD as a neuroactive agent we need to use adequate analytical tools, fostering their improvement, while discarding the most problematic approaches.

Spectroscopic techniques have proved to be useful for studying *in vivo* brain EtOH kinetics, in both humans and experimental animals, but ACD measurement remains outside MRS analytical scope. Nevertheless, owing to its absolute non-invasive nature MRS provides a great opportunity for *in vivo* qualitative study of the effects of EtOH in the intact brain.

Brain microdialysis is a well-known sampling technique for *in vivo* applications. However, it is now clear that many of the features that made microdialysis so successful for *in vivo* monitoring of several neurochemicals, are of limited use when coming to *in vivo* EtOH and (especially) ACD analysis. Its invasiveness together with the low temporal resolution, and the necessity of complex analytical procedures, represent the most important problems.

Biosensors are the emerging tool for the preclinical *in vivo* study of neurochemistry. When compared to microdialysis the main advantages of AEBs are represented by their reduced invasiveness, high time resolution, and the possibility to detect analytes which cannot be studied with microdialysis. In the case of EtOH monitoring, AOX-based AEBs have proved to be capable of *in vitro* and *in vivo* detecting concentrations of EtOH in the  $\mu\text{M}$  range. ALDH-based ACD AEBs have not been applied for *in vivo* ACD detection yet, however *in vitro* data strongly suggest that these devices may represent

the most promising opportunity for *in vivo* brain ACD detection.

## AUTHOR CONTRIBUTIONS

PE: performed literature analysis and data collection, wrote manuscript and acted as corresponding author. MD: supervised development of work, helped in data interpretation and manuscript evaluation and editing.

## REFERENCES

- Alger, J. R. (2010). Quantitative proton magnetic resonance spectroscopy and spectroscopic imaging of the brain: a didactic review. *Top. Magn. Reson. Imaging* 21, 115–128. doi: 10.1097/RMR.0b013e31821e568f
- Avramescu, A., Noguier, T., Avramescu, M., and Marty, J.-L. (2002). Screen-printed biosensors for the control of wine quality based on lactate and acetaldehyde determination. *Anal. Chim. Acta* 458, 203–213. doi: 10.1016/S0003-2670(01)01580-X
- Azevedo, A. M., Prazeres, D. M. F., Cabral, J. M. S., and Fonseca, L. P. (2005). Ethanol biosensors based on alcohol oxidase. *Biosens. Bioelectron.* 21, 235–247. doi: 10.1016/j.bios.2004.09.030
- Befroy, D. E., and Shulman, G. I. (2011). Magnetic resonance spectroscopy studies of human metabolism. *Diabetes Metab. Res. Rev.* 60, 1361–1369. doi: 10.2337/db09-0916
- Belluzo, M. S., Ribone, M. E., and Lagier, C. M. (2008). Assembling amperometric biosensors for clinical diagnostics. *Sensors* 8, 1366–1399. doi: 10.3390/s8031366
- Borland, L. M., Shi, G., Yang, H., and Michael, A. C. (2005). Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of the anesthetized rat. *J. Neurosci. Methods* 146, 149–158. doi: 10.1016/j.jneumeth.2005.02.002
- Bühler, R., Pestalozzi, D., Hess, M., and Von Wartburg, J. P. (1983). Immunohistochemical localization of alcohol dehydrogenase in human kidney, endocrine organs and brain. *Pharmacol. Biochem. Behav.* 18(Suppl. 1), 55–59. doi: 10.1016/0091-3057(83)90147-8
- Buonocore, M. H., and Maddock, R. J. (2015). Magnetic resonance spectroscopy of the brain: a review of physical principles and technical methods. *Rev. Neurosci.* 26, 609–632. doi: 10.1515/revneuro-2015-0010
- Buttler, T., Nilsson, C., Gorton, L., Marko-Varga, G., and Laurell, T. (1996). Membrane characterisation and performance of microdialysis probes intended for use as bioprocess sampling units. *J. Chromatogr. A* 725, 41–56. doi: 10.1016/0021-9673(95)01120-X
- Cao, J., Belluzzi, J. D., Loughlin, S. E., Keyler, D. E., Pentel, P. R., and Leslie, F. M. (2007). Acetaldehyde, a major constituent of tobacco smoke, enhances behavioral, endocrine, and neuronal responses to nicotine in adolescent and adult rats. *Neuropsychopharmacology* 32, 2025–2035. doi: 10.1038/sj.npp.1301327
- Carson, B. P., McCormack, W. G., Conway, C., Cooke, J., Saunders, J., O'Connor, W. T., et al. (2015). *In vivo* microdialysis characterization of the transient changes in the interstitial dialysate concentration of metabolites and cytokines in human skeletal muscle in response to insertion of a microdialysis probe. *Cytokine* 71, 327–333. doi: 10.1016/j.cyto.2014.10.022
- Chefer, V. I., Thompson, A. C., Zapata, A., and Shippenberg, T. S. (2009). Overview of brain microdialysis. *Curr. Protoc. Neurosci.* Chapter 7:Unit7.1. doi: 10.1002/0471142301.ns0701s47
- Cheng, G.-W., Hsu, K.-C., Lee, C.-F., Wu, H.-L., and Huang, Y.-L. (2009). On-line microdialysis coupled with liquid chromatography for biomedical analysis. *J. Chromatogr. Sci.* 47, 624–630. doi: 10.1093/chromsci/47.8.624
- Chun, H.-J., Poklis, J. L., Poklis, A., and Wolf, C. E. (2016). Development and validation of a method for alcohol analysis in brain tissue by headspace gas chromatography with flame ionization detector. *J. Anal. Toxicol.* 40, 653–658. doi: 10.1093/jat/bkw075
- Cifuentes Castro, V. H., López Valenzuela, C. L., Salazar Sánchez, J. C., Peña, K. P., López Pérez, S. J., Ibarra, J. O., et al. (2014). An update of the classical and novel methods used for measuring fast neurotransmitters during normal and brain altered function. *Curr. Neuropharmacol.* 12, 490–508. doi: 10.2174/1570159X13666141223223657
- Cohen, G., Sinet, P. M., and Heikkilä, R. (1980). Ethanol oxidation by rat brain *in vivo*. *Alcohol. Clin. Exp. Res.* 4, 366–370. doi: 10.1111/j.1530-0277.1980.tb04833.x
- Cordell, R. L., Pandya, H., Hubbard, M., Turner, M. A., and Monks, P. S. (2013). GC-MS analysis of ethanol and other volatile compounds in micro-volume blood samples—quantifying neonatal exposure. *Anal. Bioanal. Chem.* 405, 4139–4147. doi: 10.1007/s00216-013-6809-1
- Correa, M., Salamone, J. D., Segovia, K. N., Pardo, M., Longoni R., Spina L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neurosci. Biobehav. Rev.* 36, 404–430. doi: 10.1016/j.neubiorev.2011.07.009
- Deng, X.-S., and Ra, D. (2008). Putative role of brain acetaldehyde in ethanol addiction. *Curr. Drug Abuse Rev.* 1, 3–8. doi: 10.2174/1874473710801010003
- Fein, G., and Meyerhoff, D. J. (2000). Ethanol in human brain by magnetic resonance spectroscopy: correlation with blood and breath levels, relaxation, and magnetization transfer. *Alcohol. Clin. Exp. Res.* 24, 1227–1235. doi: 10.1111/j.1530-0277.2000.tb02088.x
- Ferguson, C. S., and Tyndale, R. F. (2011). Cytochrome P450 enzymes in the brain: emerging evidence of biological significance. *Trends Pharmacol. Sci.* 32, 708–714. doi: 10.1016/j.tips.2011.08.005
- Ghica, M. E., Pauliukaite, R., Marchand, N., Devic, E., and Brett, C. M. A. (2007). An improved biosensor for acetaldehyde determination using a bienzymatic strategy at poly(neutral red) modified carbon film electrodes. *Anal. Chim. Acta* 591, 80–86. doi: 10.1016/j.aca.2007.03.047
- Gifford, R., Kehoe, J. J., Barnes, S. L., Kornilayev, B. A., Alterman, M. A., and Wilson, G. S. (2006). Protein interactions with subcutaneously implanted biosensors. *Biomaterials* 27, 2587–2598. doi: 10.1016/j.biomaterials.2005.11.033
- Gill, K., Menez, J. F., Lucas, D., and Deitrich, R. A. (1992). Enzymatic production of acetaldehyde from ethanol in rat brain tissue. *Alcohol. Clin. Exp. Res.* 16, 910–915. doi: 10.1111/j.1530-0277.1992.tb01892.x
- Goullé, J.-P., and Guerbet, M. (2015). [Pharmacokinetics, metabolism, and analytical methods of ethanol]. *Ann. Pharm. Fr.* 73, 313–322. doi: 10.1016/j.pharma.2015.03.003
- Guan, X., Rubin, E., and Anni, H. (2011). An optimized method for the measurement of acetaldehyde by high-performance liquid chromatography. *Alcohol. Clin. Exp. Res.* 36, 398–405. doi: 10.1111/j.1530-0277.2011.01612.x
- Guihen, E., and O'Connor, W. T. (2009). Current separation and detection methods in microdialysis the drive towards sensitivity and speed. *Electrophoresis* 30, 2062–2075. doi: 10.1002/elps.200900039
- Halliwel, B. (2006). Oxidative stress and neurodegeneration: where are we now? *J. Neurochem.* 97, 1634–1658. doi: 10.1111/j.1471-4159.2006.03907.x
- Hansson, T., Tindberg, N., Ingelman-Sundberg, M., and Köhler, C. (1990). Regional distribution of ethanol-inducible cytochrome P450 IIE1 in the rat central nervous system. *Neuroscience* 34, 451–463. doi: 10.1016/0306-4522(90)90154-V
- Hanstock, C. C., Rothman, D. L., Shulman, R. G., Novotny, E. J., Petroff, O. A., and Prichard, J. W. (1990). Measurement of ethanol in the human brain using NMR spectroscopy. *J. Stud. Alcohol* 51, 104–107. doi: 10.15288/jsa.1990.51.104
- Hasan, A., Nurunnabi, M., Morshed, M., Paul, A., Polini, A., Kuila, T., et al. (2014). Recent advances in application of biosensors in tissue engineering. *Biomed Res. Int.* 2014:307519. doi: 10.1155/2014/307519

- Heit, C., Dong, H., Chen, Y., Thompson, D. C., Deitrich, R. A., and Vasiliou, V. K. (2013). "The role of CYP2E1 in alcohol metabolism and sensitivity in the central nervous system," in *Cytochrome P450 2E1: Its Role in Disease and Drug Metabolism Subcellular Biochemistry*, ed. A. Dey (Dordrecht: Springer), 235–247. doi: 10.1007/978-94-007-5881-0\_8
- Heit, C., Eriksson, P., Thompson, D. C., Charkoftaki, G., and Fritz, K. S. (2016). Quantification of neural ethanol and acetaldehyde using headspace GC-MS. *Alcohol. Clin. Exp. Res.* 40, 1825–1831. doi: 10.1111/acer.13156
- Israel, Y., Quintanilla, M. E., Karahanian, E., Rivera-Meza, M., and Herrera-Marschitz, M. (2015). The "first hit" toward alcohol reinforcement: role of ethanol metabolites. *Alcohol. Clin. Exp. Res.* 39, 776–786. doi: 10.1111/acer.12709
- Israel, Y., Rivera-Meza, M., Karahanian, E., Quintanilla, M. E., Tampier, L., Morales, P., et al. (2012). Gene specific modifications unravel ethanol and acetaldehyde actions. *Front. Behav. Neurosci.* 7:80. doi: 10.3389/fnbeh.2013.00080
- Jamal, M., Ameno, K., Kumihashi, M., Ameno, S., Kubota, T., Wang, W., et al. (2003). Microdialysis for the determination of acetaldehyde and ethanol concentrations in the striatum of freely moving rats. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 798, 155–158. doi: 10.1016/j.jchromb.2003.09.015
- Jamal, M., Ameno, K., Tanaka, N., Ito, A., Takakura, A., Kumihashi, M., et al. (2015). Ethanol and acetaldehyde after intraperitoneal administration to Aldh2-knockout mice-reflection in blood and brain levels. *Neurochem. Res.* 41, 1029–1034. doi: 10.1007/s11064-015-1788-6
- Jamal, M., Ameno, K., Uekita, I., Kumihashi, M., Wang, W., and Ijiri, I. (2007). Catalase mediates acetaldehyde formation in the striatum of free-moving rats. *Neurotoxicology* 28, 1245–1248. doi: 10.1016/j.neuro.2007.05.002
- Karahanian, E., Quintanilla, M. E., Tampier, L., Rivera-Meza, M., Bustamante, D., Gonzalez-Lira, V., et al. (2011). Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. *Alcohol. Clin. Exp. Res.* 35, 606–612. doi: 10.1111/j.1530-0277.2011.01439.x
- Kaufman, M. J., Chiu, T.-M., Mendelson, J. H., Woods, B. T., Mello, N. K., Lukas, S. E., et al. (1994). *In vivo* proton magnetic resonance spectroscopy of alcohol in rhesus monkey brain. *Magn. Resonan. Imaging* 12, 1245–1253. doi: 10.1016/0730-725X(94)90089-A
- Kennedy, R. T. (2013). Emerging trends in *in vivo* neurochemical monitoring by microdialysis. *Curr. Opin. Chem. Biol.* 17, 860–867. doi: 10.1016/j.cbpa.2013.06.012
- Kerr, J. T., Maxwell, D. S., and Crabb, D. W. (1989). Immunocytochemistry of alcohol dehydrogenase in the rat central nervous system. *Alcohol. Clin. Exp. Res.* 13, 730–736. doi: 10.1111/j.1530-0277.1989.tb00412.x
- Kotanen, C. N., Moussy, F. G., Carrara, S., and Guiseppi-Elie, A. (2012). Implantable enzyme amperometric biosensors. *Biosens. Bioelectron.* 35, 14–26. doi: 10.1016/j.bios.2012.03.016
- Kristoffersen, L., and Smith-Kielland, A. (2005). An automated alcohol dehydrogenase method for ethanol quantification in urine and whole blood. *J. Anal. Toxicol.* 29, 387–389. doi: 10.1093/jat/29.5.387
- Ledesma, J. C., Miquel, M., Pascual, M., Guerri, C., and Aragon, C. M. G. (2014). Induction of brain cytochrome P450 2E1 boosts the locomotor-stimulating effects of ethanol in mice. *Neuropharmacology* 85, 36–44. doi: 10.1016/j.neuropharm.2014.05.018
- Lorenzo, E., Pariente, F., Hernández, L., Tobalina, F., Darder, M., Wu, Q., et al. (1998). Analytical strategies for amperometric biosensors based on chemically modified electrodes. *Biosens. Bioelectron.* 13, 319–332. doi: 10.1016/S0956-5663(97)00138-3
- Mori, O., Haseba, T., Kameyama, K., Shimizu, H., Kudoh, M., Ohaki, O., et al. (2000). Histological distribution of class III alcohol dehydrogenase in human brain. *Brain Res.* 852, 186–190. doi: 10.1016/S0006-8993(99)02201-5
- Nagel, B. J., and Kroenke, C. D. (2008). The use of magnetic resonance spectroscopy and magnetic resonance imaging in alcohol research. *Alcohol Res. Health* 31, 243–246.
- Nichols, S. P., Koh, A., Storm, W. L., Shin, J. H., and Schoenfish, M. H. (2013). Biocompatible materials for continuous glucose monitoring devices. *Chem. Rev.* 113, 2528–2549. doi: 10.1021/cr300387j
- Niciu, M. J., and Mason, G. F. (2014). Neuroimaging in alcohol and drug dependence. *Curr. Behav. Neurosci. Rep.* 1, 45–54. doi: 10.1007/s40473-013-0005-7
- Noguer, T., and Marty, J. L. (1997). Reagentless sensors for acetaldehyde. *Anal. Lett.* 30, 1069–1080. doi: 10.1016/j.talanta.2008.04.040
- Noguer, T., Tencaliec, A., Calas-Blanchard, C., Avramescu, A., and Marty, J.-L. (2001). Interference-free biosensor based on screen-printing technology and sol-gel immobilization for determination of acetaldehyde in wine. *J. AOAC Int.* 85, 1382–1389.
- Olivo, J., Carrara, S., and De Micheli, G. (2011). Energy harvesting and remote powering for implantable biosensors. *IEEE Sens. J.* 11, 1573–1586. doi: 10.1109/JSEN.2010.2085042
- Peana, A. T., Porcheddu, V., Bennardini, F., Carta, A., Rosas, M., and Acquas, E. (2015). Role of ethanol-derived acetaldehyde in operant oral self-administration of ethanol in rats. *Psychopharmacology* 232, 4269–4276. doi: 10.1007/s00213-015-4049-0
- Pellegrino, S., Bruno, F. S., and Petrarulo, M. (1999). Liquid chromatographic determination of ethyl alcohol in body fluids. *J. Chromatogr. B Biomed. Sci. Appl.* 729, 103–110. doi: 10.1016/S0378-4347(99)00131-0
- Peris, J., Zharikova, A., Li, Z., Lingis, M., MacNeill, M., Wu, M. T., et al. (2006). Brain ethanol levels in rats after voluntary ethanol consumption using a sweetened gelatin vehicle. *Pharmacol. Biochem. Behav.* 85, 562–568. doi: 10.1016/j.pbb.2006.10.010
- Quintanilla, M. E., Tampier, L., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2012). Reward and relapse: complete gene-induced dissociation in an animal model of alcohol dependence. *Alcohol. Clin. Exp. Res.* 36, 517–522. doi: 10.1111/j.1530-0277.2011.01606.x
- Rooney, W. D., Lee, J. H., Li, X., Wang, G. J., Franceschi, D., Springer, C. S., et al. (2000). 4.0 T water proton T1 relaxation times in normal human brain and during acute ethanol intoxication. *Alcohol. Clin. Exp. Res.* 24, 830–836. doi: 10.1111/j.1530-0277.2000.tb02062.x
- Rotzinger, S., Smith, B. R., and Amit, Z. (1994). Catalase inhibition attenuates the acquisition of ethanol and saccharin-quinine consumption in laboratory rats. *Behav. Pharmacol.* 5, 203–209. doi: 10.1097/00008877-199404000-00012
- Sammi, M. K., Pan, J. W., Telang, F. W., Schuhlein, D., Molina, P. E., Volkow, N. D., et al. (2000). Measurements of human brain ethanol T2 by spectroscopic imaging at 4T. *Magn. Reson. Med.* 44, 35–40. doi: 10.1002/1522-2594(200007)44:1<35::AID-MRM7<3.0.CO;2-G
- Sánchez-Catalán, M. J., Hipólito, L., Guerri, C., Granero, L., and Polache, A. (2008). Distribution and differential induction of CYP2E1 by ethanol and acetone in the mesocorticolimbic system of rat. *Alcohol Alcohol.* 43, 401–407. doi: 10.1093/alcalc/agn012
- Saxena, U., and Das, A. B. (2016). Nanomaterials towards fabrication of cholesterol biosensors: Key roles and design approaches. *Biosens. Bioelectron.* 75, 196–205. doi: 10.1016/j.bios.2015.08.042
- Sirca, D., Vardeu, A., Pinna, M., Diana, M., and Enrico, P. (2014). A robust, state-of-the-art amperometric microbiosensor for glutamate detection. *Biosens. Bioelectron.* 61C, 526–531. doi: 10.1016/j.bios.2014.04.054
- Soares, D. P., and Law, M. (2009). Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. *Clin. Radiol.* 64, 12–21. doi: 10.1016/j.crad.2008.07.002
- Strózik-Kotlorz, D. (2014). Magnetic resonance spectroscopy of the human brain. *Phys. Part. Nuclei* 45, 347–348. doi: 10.1134/S1063779614010997
- Takeuchi, T., Murase, K., and Ishii, D. (1988). Determination of alcohols in alcoholic beverages by micro high-performance liquid chromatography with indirect photometric detection. *J. Chromatogr.* 445, 139–144. doi: 10.1016/S0021-9673(01)84515-5
- Tampier, L., Quintanilla, M. E., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., and Israel, Y. (2013). The alcohol deprivation effect: marked inhibition by anticalase gene administration into the ventral tegmental area in rats. *Alcohol. Clin. Exp. Res.* 37, 1278–1285. doi: 10.1111/acer.12101
- Tampier, L., Quintanilla, M. E., and Mardones, J. (1995). Effects of aminotriazole on ethanol, water, and food intake and on brain catalase in UChA and UChB rats. *Alcohol* 12, 341–344. doi: 10.1016/0741-8329(95)00014-I
- Thévenot, D. R., Toth, K., Durst, R. A., and Wilson, G. S. (2001). Electrochemical biosensors: recommended definitions and classification. *Biosens. Bioelectron.* 16, 121–131. doi: 10.1016/S0956-5663(01)00115-4
- Timmerman, W., and Westerink, B. H. (1997). Brain microdialysis of GABA and glutamate: what does it signify? *Synapse* 27, 242–261.
- Tindberg, N., and Ingelman-Sundberg, M. (1996). Expression, catalytic activity, and inducibility of cytochrome P450 2E1 (CYP2E1) in the rat central nervous

- system. *J. Neurochem.* 67, 2066–2073. doi: 10.1046/j.1471-4159.1996.67052066.x
- Tiscione, N. B., Alford, I., Yeatman, D. T., and Shan, X. (2011). Ethanol analysis by headspace gas chromatography with simultaneous flame-ionization and mass spectrometry detection. *J. Anal. Toxicol.* 35, 501–511. doi: 10.1093/anatox/35.7.501
- Vigneshvar, S., Sudhakumari, C. C., Senthilkumaran, B., and Prakash, H. (2015). Recent advances in biosensor technology for potential applications - An overview. *Front. Bioeng. Biotechnol.* 4:11. doi: 10.3389/fbioe.2016.00011
- Vijayakumar, A. R., Csöregi, E., Heller, A., and Gorton, L. (1996). Alcohol biosensors based on coupled oxidase-peroxidase systems. *Anal. Chim. Acta* 327, 223–234. doi: 10.1016/0003-2670(96)00093-1
- Vogel, M., Büldt, A., and Karst, U. (2000). Hydrazine reagents as derivatizing agents in environmental analysis—a critical review. *Fresenius J. Anal. Chem.* 366, 781–791. doi: 10.1007/s002160051572
- Wahono, N., Qin, S., Oomen, P., Cremers, T. I. F., de Vries, M. G., and Westerink, B. H. C. (2012). Evaluation of permselective membranes for optimization of intracerebral amperometric glutamate biosensors. *Biosens. Bioelectron.* 33, 260–266. doi: 10.1016/j.bios.2012.01.019
- Wang, J., Du, H., Jiang, L., Ma, X., de Graaf, R. A., Behar, K. L., et al. (2013a). Oxidation of ethanol in the rat brain and effects associated with chronic ethanol exposure. *Proc. Natl. Acad. Sci. U.S.A.* 110, 14444–14449. doi: 10.1073/pnas.1306011110
- Wang, J., Du, H., Ma, X., Pittman, B., Castracane, L., Li, T.-K., et al. (2013b). Metabolic products of [2-(13)C]ethanol in the rat brain after chronic ethanol exposure. *J. Neurochem.* 127, 353–364. doi: 10.1111/jnc.12405
- Warner, M., and Gustafsson, J. A. (1994). Effect of ethanol on cytochrome P450 in the rat brain. *Proc. Natl. Acad. Sci. U.S.A.* 91, 1019–1023. doi: 10.1073/pnas.91.3.1019
- Weltin, A., Kieninger, J., Enderle, B., Gellner, A.-K., Fritsch, B., and Urban, G. A. (2014). Polymer-based, flexible glutamate and lactate microsensors for in vivo applications. *Biosens. Bioelectron.* 61, 192–199. doi: 10.1016/j.bios.2014.05.014
- Weltin, A., Kieninger, J., and Urban, G. A. (2016). Microfabricated, amperometric, enzyme-based biosensors for in vivo applications. *Anal. Bioanal. Chem.* 408, 4503–4521. doi: 10.1007/s00216-016-9420-4
- Westerink, B. H. (1995). Brain microdialysis and its application for the study of animal behaviour. *Behav. Brain Res.* 70, 103–124. doi: 10.1016/0166-4328(95)80001-8
- Wilson, G. S., and Gifford, R. (2004). Biosensors for real-time in vivo measurements. *Biosens. Bioelectron.* 20, 2388–2403. doi: 10.1016/j.bios.2004.12.003
- Xiang, Y., and Shen, J. (2011). *In vivo* detection of intermediate metabolic products of [1-13C]ethanol in the brain using 13C MRS. *NMR Biomed.* 24, 1054–1062. doi: 10.1002/nbm.1653
- Xiao, H.-T., He, L., Tong, R.-S., Yu, J.-Y., Chen, L., Zou, J., et al. (2014). Rapid and sensitive headspace gas chromatography-mass spectrometry method for the analysis of ethanol in the whole blood. *J. Clin. Lab. Anal.* 28, 386–390. doi: 10.1002/jcla.21698
- Yao, T., and Handa, S. (2003). Electroanalytical properties of aldehyde biosensors with a hybrid-membrane composed of an enzyme film and a redox Os-polymer film. *Anal. Sci.* 19, 767–770. doi: 10.2116/analsci.19.767
- Yarita, T., Nakajima, R., Otsuka, S., Ihara, T. A., Takatsu, A., and Shibukawa, M. (2002). Determination of ethanol in alcoholic beverages by high-performance liquid chromatography-flame ionization detection using pure water as mobile phase. *J. Chromatogr. A* 976, 387–391. doi: 10.1016/S0021-9673(02)00942-1
- Yoshimoto, K., and Komura, S. (1993). Monitoring of ethanol levels in the rat nucleus accumbens by brain microdialysis. *Alcohol Alcohol.* 28, 171–174.
- Zachut, M., Shapiro, F., and Silanikove, N. (2016). Detecting ethanol and acetaldehyde by simple and ultrasensitive fluorimetric methods in compound foods. *Food Chem.* 201, 270–274. doi: 10.1016/j.foodchem.2016.01.079
- Zahr, N. M., Mayer, D., Rohlfing, T., Hasak, M. P., Hsu, O., Vinco, S., et al. (2010). Brain injury and recovery following binge ethanol: evidence from in vivo magnetic resonance spectroscopy. *Biol. Psychiatry* 67, 846–854. doi: 10.1016/j.biopsych.2009.10.028
- Ziegler, T. L., Bludeau, P., Petersen, D. R., Gonzalez, F. J., and Deitrich, R. A. (2006). CYP2E1 and catalase influence ethanol sensitivity in the central nervous system. *Pharmacogenet. Genomics* 16, 51–58. doi: 10.1097/01.fpc.0000182777.95555.56
- Zimatkin, S. M., Pronko, S. P., Gonzalez, F. J., and Deitrich, R. A. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol. Clin. Exp. Res.* 30, 1500–1505. doi: 10.1111/j.1530-0277.2006.00181.x

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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