Study of age-dependent effects of ethanol on glutamate dynamics in prefrontal cortex of awake rats using microelectrode amperometry

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Conclusions

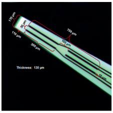
- Basal extracellular levels of cortical glutamate are significantly higher in adolescent animals compared to adults
- Ethanol has a prominent biphasic effect on glutamate release in adolescent animals but not in adults
- Ethanol modulates the clearance rate of glutamate from the extracellular space

Introduction

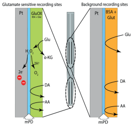
Alcohol addiction is associated with dysfunctional glutamatergic neurotransmission in the prefrontal cortex (PFC)[1,2]. Long-term psychological and short-term pharmacological effects of alcohol inhibit PFC function causing abnormal maturation of the PFC if consumed during adolescence and thereby increasing the risk of alcohol addiction[3,4,5]. We investigated the effects of systemic alcohol on glutamate dynamics in the rat PFC using enzyme based microelectrode amperometry[6].

Methodology

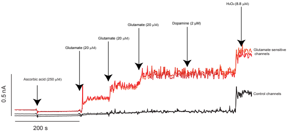
a) Microelectrode tip with four platinum recording sites



b) Coating schedule



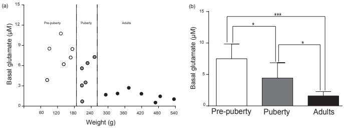
c) *In vitro* calibration prior to microelectrode implantation in PFC



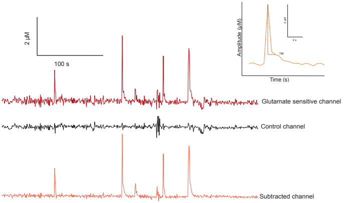
Microelectrodes: Studies were conducted in adult and adolescent, male Sprague Dawley rats. The tip of the mpD-plated microelectrode consists of 2 pairs of Pt recording sites (15 x 333 µm; see Fig. a). One pair, the glutamate-sensitive sites, was coated with glutamate oxidase (GluOx), cross-linked to the electrode with BSA/glutaraldehyde. The remaining pair, the sentinel (background) sites, was coated with BSA/glutaraldehyde (see Fig. b). The current generated exclusively by the oxidation of glutamate at the surface of the electrode is isolated by self-referencing the current at the GluOx-positive site from the sentinel site. Microelectrodes were calibrated using the FAST-16 Quanteon recording system prior to implantation in the PFC. Constant voltage amperometry was conducted using an applied potential of +0.7 V vs a Ag/AgCl reference electrode (2 Hz). Calibrations were performed using known concentrations of glutamate, potential interferents (dopamine and ascorbic acid), and the reporter molecule H_2O_2 in a stirred solution of PBS (37°C; see Fig. c). All microelectrodes exhibited the following calibration criteria: (i) similar background current on GluOx and sentinel channels, (ii) linear response to increasing glutamate concentrations (R^2 > 0.998), (iii) sensitivity > -300 pA/µM glutamate, (iv) L.O.D. < 0.5 µM), and (v) selectivity to glutamate over interferents = 50:1. Recordings were conducted in freely-moving rats on Days 2 (saline i.p. 6 ml/kg) and 3 (alcohol i.p. 1 g/kg) after surgery.

Results

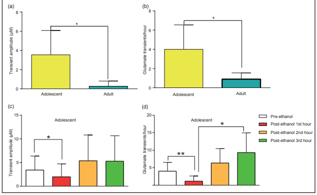
I. Cortical glutamate levels decrease with age



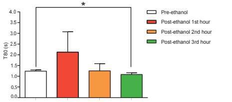
II. Spontaneous glutamate release or glutamate transients during *in vivo* recordings



III. Glutamate transients and biphasic effects of ethanol on glutamate transients in adolescent animals



IV. Ethanol modulates clearance rate of glutamate from extracellular space



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