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Impact of combined prenatal ethanol and prenatal stress exposures on markers of activity-dependent synaptic plasticity in rat dentate gyrus

Miranda C. Staples, Morgan W. Porch, Daniel D. Savage*

Department of Neurosciences, School of Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, USA

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ABSTRACT

Prenatal ethanol exposure and prenatal stress can each cause long-lasting deficits in hippocampal synaptic plasticity and disrupt learning and memory processes. However, the mechanisms underlying these perturbations following a learning event are still poorly understood. We examined the effects of prenatal ethanol exposure and prenatal stress exposure, either alone or in combination, on the cytosolic expression of activity-regulated cytoskeletal (ARC) protein and the synaptosomal expression of AMPA-glutamate receptor subunits (GluA1 and GluA2) in dentate gyrus of female adult offspring under baseline conditions and after 2-trial trace conditioning (TTTC). Surprisingly, baseline cytoplasmic ARC expression was significantly elevated in both prenatal treatment groups. In contrast, synaptosomal GluA1 receptor subunit expression was decreased in both prenatal treatment groups. GluA2 subunit expression was elevated in the prenatal stress group. TTTC did not alter ARC levels compared to an unpaired behavioral control (UPC) group in any of the 4 prenatal treatment groups. In contrast, TTTC significantly elevated both synaptosomal GluA1 and GluA2 subunit expression relative to the UPC group in control offspring, an effect that was not observed in any of the other 3 prenatal treatment groups. Given ARC's role in regulating synaptosomal AMPA receptors, these results suggest that prenatal ethanol-induced or prenatal stress exposure-induced increases in baseline ARC levels could contribute to reductions in both baseline and activity-dependent changes in AMPA receptors in a manner that diminishes the role of AMPA receptors in dentate gyrus synaptic plasticity and hippocampal-sensitive learning.

Introduction

Prenatal exposure to ethanol results in long-lasting deleterious effects on IQ, behavior, attention and, in cases of more severe exposures, physical dysmorphology. The severity of ethanol's impact on the developing fetus is related to the quantity of ethanol consumed, the pattern of ethanol consumption (Bailey et al., 2004), and the gestational timing of exposure (Kelly, Day, & Streissguth, 2000; Sood et al., 2001). The resulting range of outcomes has been termed fetal alcohol spectrum disorder (FASD). In the US, an estimated 2–5% of school-aged children suffer from FASD (May et al., 2009), the majority of which have no physical characteristics, but demonstrate significant reductions in IQ and cognitive deficits (Green et al., 2009; Kodituwakku, 2009; Willoughby, Sheard, Nash, & Rovet, 2008). These reductions in cognition persist into adulthood (Barr et al., 2006), signifying a long-lasting role of ethanol exposure during development on learning and memory.

Various animal models of prenatal ethanol exposure have been employed to elucidate the teratogenic mechanisms of ethanol in the developing fetus (Cudd, 2005). These models allow for precise experimental control, as well as providing for potential cellular and molecular analysis of animals demonstrating behavioral deficits, which parallel those observed in humans. Studies of adult animals exposed to moderate amounts of ethanol during gestation have reported reductions in hippocampus-sensitive learning and memory tasks (Berman & Hannigan, 2000; Brady, Allan, & Caldwell, 2012; Savage, Becher, de la Torre, & Sutherland, 2002; Savage et al., 2010; Staples, Rosenberg, Allen, Porch, & Savage, 2013; Sutherland, McDonald, & Savage, 2000; Weeber, Savage, Sutherland, & Caldwell, 2001), as well as deficits in measures of hippocampal synaptic plasticity, an important process associated with learning (Brady et al., 2013; Savage et al., 2002; Sutherland, McDonald, & Savage, 1997; Titterness & Christie, 2012; Varaschin, Akers, Rosenberg, Hamilton, & Savage, 2010).

* Corresponding author. Department of Neurosciences, MSC08 4740, 1 University of New Mexico, Albuquerque, NM 87131-0001, USA. Tel.: +1 505 272 8808; fax: +1 505 272 8082.
E-mail address: DSavage@salud.unm.edu (D.D. Savage).

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Similar to ethanol, maternal stress during pregnancy can also cause adverse neurodevelopment in the fetus. In human populations, children born to women who have survived acute trauma (severe weather events, terrorist attacks, etc.) or have adverse living circumstances (chronic stress) have been reported to suffer from significant cognitive impairments (Laplante et al., 2004; Martini, Knappe, Beesdo-Baum, Lieb, & Wittchen, 2010; O'Connor, Heron, Golding, Glover, & ALSPAC Study Team, 2003; Rodriguez & Bohlin, 2005). However, identifying and characterizing stress in human populations is challenging due to the high number of variables associated with individual perceptions of stress. Thus, most knowledge regarding the effects of prenatal stress on offspring behavior has come from animal model studies. Animals born to mothers who were exposed to stressors during gestation are typically smaller (Rice et al., 2010), demonstrate heightened anxiety and stress reactivity (Vallée et al., 1997), and display cognitive deficits, particularly on hippocampal-sensitive tasks, as well as long-term potentiation (Yang, Han, Cao, Li, & Xu, 2006). The severity of these characteristics is related to the type and duration of the stress the mother was subjected to during her pregnancy (Mychasiuk, Ilnytskyy, Kovalchuk, Kolb, & Gibb, 2011; O'Connor et al., 2003), as well as her predisposition to stress reactivity.

While a great deal is known regarding the neurochemical changes associated with synaptic strengthening in long-term potentiation (see review by Shepherd & Huganir, 2007), the neurochemical mechanisms underlying the deficits in activity-dependent synaptic plasticity as a consequence of either prenatal ethanol exposure or prenatal stress are not well understood. The quantity, activity, and/or translocation of a number of proteins are critical to the expression or maintenance of long-term potentiation (LTP), thought to be one cellular mechanism underlying learning (see review by Citri & Malenka, 2008). In the present study, we examined the impact of either prenatal ethanol exposure and/or prenatal stress exposure on the expression of 3 proteins involved in activity-dependent changes in synaptic plasticity, namely, activity-regulated cytosolic protein (ARC) and 2 AMPA-glutamate receptor subunits (GluA1 and GluR2). ARC protein is up-regulated within 4 h following maximal electroconvulsive shock (MECS) (Lyford et al., 1995), immediately following classical eye-blink conditioning (Mokin, Lindahl, & Keifer, 2005), 1 h following auditory-cued fear conditioning (Lonergan, Gafford, Jarome, & Helmstetter, 2010), and 1 h following the final training in a lever-press task (Kelly & Deadwyler, 2003). ARC has direct roles in expanding the cytoskeletal network within a postsynaptic cell (Huang, Chotiner, & Steward, 2007). Likewise, previous work has demonstrated activity-dependent increases in AMPA receptors following the induction of LTP (Shi, Hayashi, Esteban, & Malinow, 2001; Takahashi, Svoboda, & Malinow, 2003), with specific increases observed in GluA1 and GluA2 expression following various forms of fear-conditioned learning (Matsuo, Reijmers, & Mayford, 2008; Yeh, Mao, Lin, & Gean, 2006). Interestingly, the interaction of ARC protein expression and the AMPA receptor has implications of various forms of synaptic plasticity. While ARC is known to play a role in expanding the actin cytoskeleton network, it also has been demonstrated to play a role in AMPA receptor endocytosis from the synaptic membrane (Chowdhury et al., 2006), implicating a role in long-term depression (LTD) (Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008).

As described above, either prenatal ethanol or prenatal stress exposure can separately induce deficits in LTP and learning. ARC expression and AMPA receptor expression are required for proficient performance on learning tasks, with recent evidence demonstrating an elevation in ARC expression following various models of trace fear conditioning (Chau, Prakapenka, Fleming, Davis, & Galvez, 2013; Chia & Otto, 2013) as well as the expression of LTP (Plath et al., 2006). In models of prenatal ethanol exposure, basal quantities of AMPA receptor subunits are differentially expressed as compared to non-exposed controls (Dettmer et al., 2003), and ARC mRNA expression is reduced as compared to controls following a novel social experience (Hamilton et al., 2010). However, basal ARC protein expression has not been evaluated in animals prenatally exposed to ethanol, stress, or a combination of the two. Similarly, changes in the expression of ARC protein or AMPA receptor subunits following behavioral activation has not been examined in animals prenatally exposed to ethanol, stress, or the combination of these 2 prenatal exposure paradigms.

Using a novel model of combined prenatal ethanol and prenatal stress exposure, we recently reported that moderate prenatal ethanol exposure decreased learning of a behaviorally challenging TTTC paradigm (Staples et al., 2013). Employing the training portion of TTTC as a behavioral activation paradigm, we tested the hypothesis that prenatal ethanol exposure and/or prenatal stress exposure would diminish TTTC-induced elevations in ARC- and AMPA-receptor subunit expression.

Materials and methods

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) unless noted otherwise. All animal care and usage complied with the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

Prenatal ethanol and prenatal stress paradigms

Prenatal ethanol-exposed and prenatal stress-exposed rat offspring were generated as previously described by Staples et al. (2013). Briefly, Long-Evans females and breeder males (Harlan Industries, Indianapolis, IN) were single-housed and maintained on a reverse light/dark cycle. Prior to mating, females were gradually acclimated to voluntary drinking of 5% ethanol in 0.066% saccharin (Sacc) for 4 h during the awake phase of the light/dark cycle. Tap water and rat chow were available *ad libitum*. Following 2 weeks of 5% ethanol consumption, females drinking within 1 standard deviation of the group mean were paired with a proven breeder male until pregnancy was confirmed via the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Pregnant rat dams were then returned to their single-housed condition and voluntarily consumed either 0% or 5% ethanol in 0.066% saccharin until delivery.

Prenatal stress was elicited by mimicking exposure to a predator scent. Accordingly, rat dams assigned to the prenatal stress group were exposed to 3% 2,3,5-trimethyl-3-thiazoline (TMT, Contech, Victoria, BC, Canada) for 20 min at 1600 h (4 h following the removal of the ethanol or saccharin drinking tube) on gestational days (GD) 13, 15, 17, and 19 (see Fig. 1), because evidence has demonstrated that developing rodent fetuses are only susceptible to stress during the third week of gestation (Diaz, Brown, & Seckl, 1998). This paradigm has been shown to elevate maternal serum corticosterone levels 3-fold over non-stressed controls, and the rat dams do not acclimate to this stressor with repeated TMT exposure episodes (Staples et al., 2013). Additionally, this prenatal stress paradigm does not elicit significant growth deficits in the offspring, as noted in Staples et al. (2013), thereby reducing the number of potential confounding factors. Following delivery, dams and litters were left undisturbed until weaning on postnatal day 25 when the offspring were moved into sex-matched group housing with littermates. Except for routine cage bedding changes, offspring were left undisturbed until experimental use. Only female offspring