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Research report

Effect of prenatal exposure to ethanol on the pyramidal tract in developing rats

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ABSTRACT

Prenatal exposure to ethanol induces a relative increase in the numbers of pyramidal tract axons relative to the number of corticospinal projection neurons in somatosensory/motor cortices in the adult rat. The present study examines the effects of ethanol on the numbers of axons in the developing caudal pyramidal tract, i.e., corticospinal axons. Electron microscopic analyses of the pyramidal tracts of the offspring of pregnant rat dams fed a control diet *ad libitum*, pair-fed a liquid control diet, or fed an ethanol-containing diet *ad libitum* were performed. The pups were 5-, 15-, 30- and 90-days-old. The numbers of axons in control rats fell precipitously after postnatal day (P) 15 and the frequency of myelinated axons rose dramatically between P15 and P90. Ethanol exposure had no significant effect on the numbers of pyramidal tract axons at any age. Moreover, no ethanol-induced differences in the numbers of axons in different stages of myelination, i.e., axons that were "free" of glial associations, glia-engulfed, invested by 1–2 layers of myelin, or myelinated by 3+ layers of myelin, were detected on P15. Thus, it appears that (a) pyramidal tract axons are lost or pruned during the first two postnatal weeks and (b) postnatal development of pyramidal tract axons (e.g., pruning and myelination) is not affected by ethanol. The implications are that the ethanol-induced increase in the number of axons relative to the number of somata of corticospinal neurons detected in pups and adults results from the effects of ethanol on early stages (initiation) of axogenesis.

1. Introduction

Fetal alcohol spectrum disorder (FASD) is a complex condition that is often hallmarked by cognitive, sensory, and motor dysfunction (Coles, 2006; Fryer et al., 2006). Indeed, prenatal exposure to alcohol is the prime cause of cognitive impairments in the United States (Stratton et al., 1996; May et al., 2009). These clinical issues correlate with structural malformations in multiple CNS structures including the cerebral cortex, hippocampus, and cerebellum (e.g., Pentney and Miller, 1992; Lindsley, 2006; Siegenthaler and Miller, 2006). For example, prenatal exposure to ethanol can cause microencephaly, heterotopias and disorganized lamination, and dysmorphic neurons with hypo- or hypertrophic neurites.

Analyses of large fiber tracts have been used as models because of their clear delineation, relative homogeneity, and size. Tracts commonly examined include the corpus callosum and pyramidal tract. Imaging studies in the primate show that ethanol can cause callosal agenesis, dysgenesis (Clarren et al., 1978; Pratt and Doshi, 1984; Riley et al., 1995; Swayze et al., 1997; Sowell et al., 2001), malshapen, or even hypertrophic corpora callosa (Bookstein et al., 2002). Such findings have been replicated in the rat (Chernoff, 1977; Miller, 1987, 1997; Zimmerberg and Scalzi, 1989; Zimmerberg and Mickus, 1990; Livy and Elberger, 2001, 2008; Moreland et al., 2002) and non-human primates (Miller et al., 1999). In the pyramidal tract (which is principally composed of corticospinal axons), there is no effect of prenatal exposure to ethanol on the absolute number of axons in the adult (Miller and Al-Rabiai, 1994).

The lack of an ethanol-induced effect on the number of callosal and pyramidal tract axons in the adult rat must be interpreted in the context of the effect of ethanol on brain size. For example, prenatal ethanol causes smaller somatosensory cortices in mature rats exposed to ethanol during gestation (Miller and Potempa, 1990; Margret et al., 2005; Chappell et al., 2007). Somatosensory cortex is one third smaller in ethanol-treated rats than controls. This difference includes a decrease (>30%) in the numbers of neuronal

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somata in layers II/III and V, the sources of callosal and corticospinal projections. Thus, there is an imbalance in the proportion of cortical efferent axons to the number of neuronal somata of origin; i.e., prenatal exposure to ethanol induces an increase in axonal number relative to the numbers of neurons of origin (Miller, 1987, 1997; Livy and Elberger, 2008).

Dynamic events shaping a mature axonal projection are the generation of exuberant projections and the subsequent elimination of axons via a pruning process (Stanfield et al., 1982; Miller and Vogt, 1984; Stanfield and O'Leary, 1985; Shreyer and Jones, 1988). The density of cortical spinal projection neurons is greater and the zone of origin of these neurons is broader in ethanol-treated rats. Thus, it has been hypothesized that prenatal exposure to ethanol affects axonal pruning with the result being that exuberant axons are not eliminated (Miller, 1987, 1997; Miller et al., 1990). An alternative hypothesis is that the pruning process is not affected, but rather that the number of axons generated by an individual neurons is increased. To test these hypotheses, the present study examined the time-dependent effect on the number of pyramidal tract axons in rats during the period of maximal axonal number and through the pruning process.

2. Results

2.1. Control rats

The cross-sectional area of the pyramidal tract at the level of the medulla rostral to the pyramidal decussation was determined in the offspring of rats fed the chow and water diet (Ch) or pair-fed the liquid control diet (Ct) on postnatal day (P) 5, P15, P30, and P90 (Fig. 1). The cross-sectional area of the tract changed significantly over time ($F_{3,\ 31} = 22.884$, $p < 0.001$) between P5 and P90 (Fig. 2). It was stable on P5 and P15 and then doubled over the next 15 days. This increase continued between P30 and P90 when the size of the tract doubled again. No significant difference between the cross-sectional areas of the pyramidal tracts in Ch- and Ct-treated groups was detected.

The density of axons (the number of axons per unit space) was highest in the young control pups and fell steadily during the first month (Fig. 3). This time-dependent decline between P5 and P90 was statistically significant ($F_{3,\ 31} = 397.199$, $p < 0.001$). The greatest fall in the density was between P5 and P30 which was coincident with a marked increase in the size of the axons and in their investments. Axonal density in the two controls was not statistically distinguishable.

The number of axons was estimated as the product of the area of the pyramidal tract and the density of axons. The total numbers of axons was highest in control rats on P5 (Fig. 4A), and by P30, the number fell 61.5% and 66.2% in Ch- and Ct-treated rats, respectively. These drops contributed to a statistically significant change in axonal number between P5 and P90 ($F_{3,\ 31} = 11.026$, $p < 0.001$). No significant difference between the Ch- and Ct-treated rats was detected.

The greatest contribution to the decline in total axon number was the number of non-myelinated axons (Fig. 4B). The number of non-myelinated axons fell > 24-fold between P5 and P90; a fall that was statistically significant ($F_{3,\ 31} = 326.580$; $p < 0.001$). In contrast, the number of myelinated axons in the control rats rose slowly between P5 and P30 and then tripled between P30 and P90. The loss in non-myelinated axons was countered by a significant ($F_{3,\ 31} = 294.852$; $p < 0.001$) increase in the number of myelinated axons between P15 and P90. By P90, non-myelinated axons comprised only 8.5% and 11.1% of all pyramidal tract axons in Ch- and Ct-treated rats, respectively. Note, the number of myelinated axons on P90 was significantly different between Ch- and Ct-treated rats ($t = 4.759$; $p < 0.001$; Fig. 4C) which translates into a significant difference in the ratio of the number of myelinated axons to the number of non-myelinated axons (Fig. 4D). No such differences were detected on P30 in the present study or a previous study of the pyramidal tract (Miller and Al-Rabiai, 1994). As differences between controls are rare, it is unclear if these are meaningful or are Type II errors.

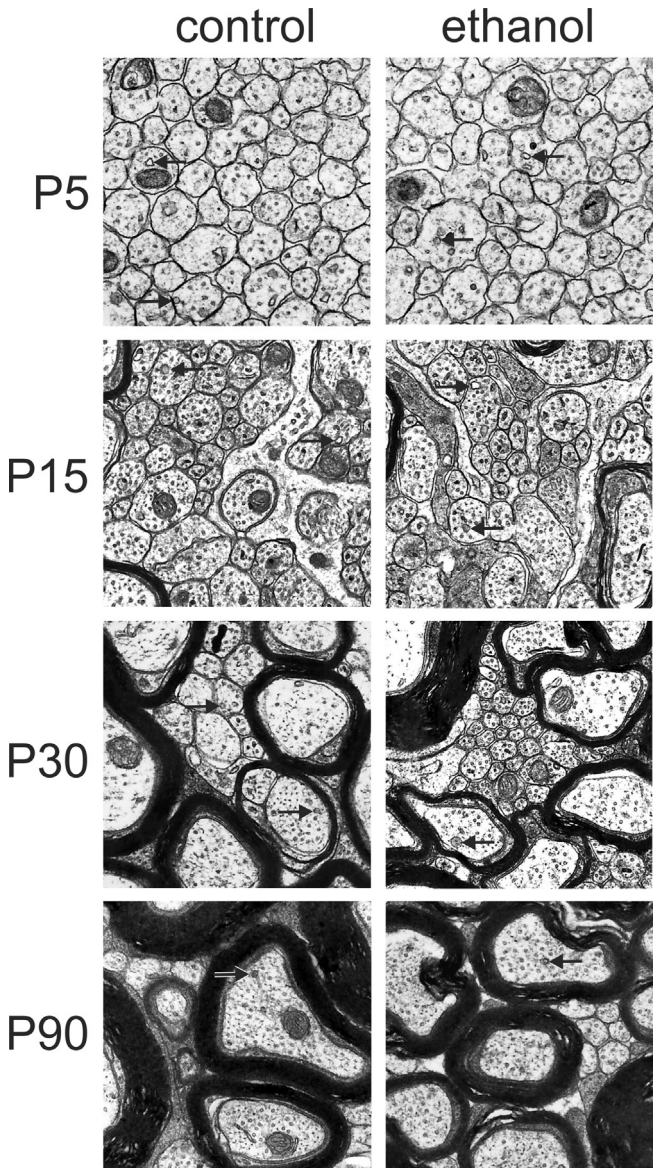

Fig. 1. Ultrastructure of developing pyramidal tract. The four pairs of electron micrographs depict cross-sections of the caudal pyramidal tract at postnatal day (P) 5, P15, P30, and P90 in control (left) and ethanol-treated (right) offspring. These are times when the axonal numbers are greatest (P5), at the beginning of myelination (P15), and at more mature times (P30 and P90). Arrows identify vesicles in axonal profiles. Each image is 1.15 μm × 1.15 μm.

2.2. Ethanol-treated rats

The same patterns in the change of the pyramidal tract described in control rats also were evident in Et-treated rats. This included significant increase in the cross-sectional area of the pyramidal tract above the pyramidal decussation ($F_{3,30} = 24.115$; $p < 0.001$; Fig. 2), decrease in the density of pyramidal tract axons ($F_{3,30} = 350.707$; $p < 0.001$; Fig. 3), and decrease in axonal number