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Distinct effects of perinatal exposure to fluoxetine or methylmercury on parvalbumin and perineuronal nets, the markers of critical periods in brain development

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ABSTRACT

The *in utero* exposure to common chemical stressors, environmental pollutant methylmercury and antidepressant fluoxetine, results in behavioral impairments persistent into adulthood. Modulation of critical periods in brain development may alter proper network formation and lastingly impair brain function. To investigate whether early-life stressors can modulate critical periods, we analyzed the development of parvalbumin (PV) and perineuronal nets (PNNs) in the dentate gyrus and CA1 area of the hippocampus and the basolateral amygdala in mice perinatally exposed to either fluoxetine or methylmercury. The number of PV and PNN neurons, and PV intensity, were analyzed by fluorescent immunohistochemistry at the postnatal ages P17 (ongoing critical period) and P24 (closing critical period). The exposure to fluoxetine did not affect the number of PV cells and PV intensity but decreased PNN formation around the cells at P17 and P24 in all tissues. In contrast, perinatal methylmercury inhibited the development of PV interneurons and PV expression at P17 only, but at P24 these parameters were restored. Methylmercury strongly increased PNN formation from P17 to P24 in the amygdala only. We suggest that perinatal fluoxetine and methylmercury might delay the closure and the onset, respectively, of the critical periods in the amygdala and hippocampus.

1. Introduction

The pathophysiology of many neuropsychiatric disorders, including schizophrenia and depression, is associated with prenatal stress (Onishchenko et al., 2008; Weinberger, 1987). The formation of neuronal circuits starts in the first half of gestation in humans, and the majority of functional synapses actively develop in the second half of gestation and the neonatal period (Kostovic and Jovanov-Milosevic, 2006). In comparison with humans, the brain development in laboratory rodent is delayed and its status at the day of parturition (age P0) matches with the early premature human brain (Pressler and Auvin, 2013). The active synaptogenesis in rodent brain takes place from the end of gestational phase and continues for the first 2–3 postnatal weeks (Levitt, 2003; Pressler and Auvin, 2013; Zagon and McLaughlin, 1977). Both in humans and rodents, the *in utero* and early postnatal period of brain development is especially sensitive to stress factors including medications and environmental pollutants, to which the exposure may lead to altered postnatal brain functioning and predispose the offspring to brain disorders for life (Ceccatelli et al., 2013). However, the cellular and molecular mechanisms of these effects are not fully understood.

Of particular interest are the chemical compounds to which the exposure is common among pregnant women, thus affecting a significant part of human population. Methylmercury (MeHg) is a well-known environmental contaminant present in sea food. Growing evidence report detrimental effects of MeHg – exposure on brain development in humans and rodents: the *in utero* exposure to low-dose of MeHg (with concentrations similar to that found in sea food) induces anxiety and depression, and has a long-term pathological effect on learning, memory and development of motor skills (Ceccatelli et al., 2013; Fujimura et al., 2012; Grandjean and Landrigan, 2006; Karpova et al., 2014; Onishchenko et al., 2008).

Abbreviations: BLA, basolateral amygdala; BLc, basolateral complex of amygdala; Cntl, control; DG, dentate gyrus; Flx, fluoxetine; G7, gestational day 7; IDA, iron deficiency anemia; LA, lateral amygdala; MeHg, methylmercury; O.D., optical density; P0–24, postnatal day 0–24; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PNN, perineuronal nets; PV, parvalbumin; vs, vers; w/o, without; WFA, lectin from *Wisteria floribunda*.

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Fluoxetine (Flx) is a widely prescribed antidepressant approved for use during pregnancy and lactation; however, for the developing fetus Flx exposure can represent a chemical stress, which is not investigated in details. In contrast to MeHg-exposure, different studies report about both beneficial and pathological effects of prenatal exposure to Flx. *In utero* Flx treatment has been shown to protect the offspring against other types of maternal stress (Rayen et al., 2011) and decrease the anxiety-like behavior in female offspring (McAllister et al., 2012). Other studies, however, report that prenatal Flx increases anxiety and decreases social behavior (Olivier et al., 2011; Smit-Rigter et al., 2012), while the neonatal Flx exposure has a long-term effect on reduced activity, impaired prepulse inhibition performance, abnormal emotional and social behaviors, and morphological changes of dendritic architectures in the prefrontal cortex and basolateral amygdala (Ko et al., 2014).

Among the central mechanisms, through which the altered *in utero* environment may influence the development of brain disorders later in life, could be the modulation of the postnatal critical periods during development. The critical period is a specific time window of heightened neural plasticity during which the environmental (*e.g.*, sensory) input is required for correct experience-dependent development of a particular brain structure or circuit (Hensch and Bilimoria, 2012). During postnatal development, the critical periods for different brain circuits and functions open and close in a highly ordered manner, from the development of sensory functions to the motor and language skills, and then complex cognitive functions. After closing the windows of heightened plasticity, or critical periods, the juvenile ability to shape brain circuits in response to environmental stimuli is weakened (Hensch and Bilimoria, 2012), although could be pharmacologically enhanced in adulthood (Gogolla et al., 2009; Karpova et al., 2011; Maya Vetencourt et al., 2008).

Although a number of different markers for critical period have been evaluated, the significance of parvalbumin (PV) and perineuronal nets (PNNs) is the most validated. The onset of the critical period has been connected to the emergence of PV-expressing neurons, whereas its closure coincided with neuronal maturation and formation of PNNs (Pizzorusso et al., 2002), as was shown for the visual cortex (Hensch, 2005) and barrel cortex (McRae et al., 2007) in rodents and for the song nucleus HVC in birds (Balmer et al., 2009). PV is a calcium-binding protein specifically expressed in a subset of GABAergic fast-spiking interneurons (Kosaka et al., 1987). PNNs, the reticular structures composed of extracellular matrix proteoglycans and link proteins, are especially enriched around GABAergic interneurons (Carulli et al., 2010) and critically involved in the maturation of inhibitory circuits (Balmer et al., 2009). The number of PNNs developmentally increases and reaches the adult levels by the end of the critical periods coinciding with decreased experience-dependent plasticity (Pizzorusso et al., 2002). The significant changes in the markers of critical periods, PV expression and/or PNN formation, were also found in the hippocampus (Fretham et al., 2012) and the basolateral amygdala (Gogolla et al., 2009), which play an important role in learning, anxiety and emotional memory, spatial memory, and depression. The shift in the onset or closure of critical periods may underline impaired brain functioning (Morales et al., 2002; Weikum et al., 2012). For example, the environmental stimuli, such as the iron deficiency anemia (IDA) early in life, altered PV and PNN expression and the time window of the critical period of hippocampal development which were proposed to be a cellular basis for electrophysiological and behavioral pathologies induced by early-life IDA (Callahan et al., 2013; Fretham et al., 2012). However, the effect of other prenatal stressors on PV and PNN markers of critical periods was poorly studied so far. Moreover, the analysis of PV and PNN neurons extended to several brain regions would be important for better understanding the general mechanisms of early-life stress-induced pathological influence on development of brain disorders.

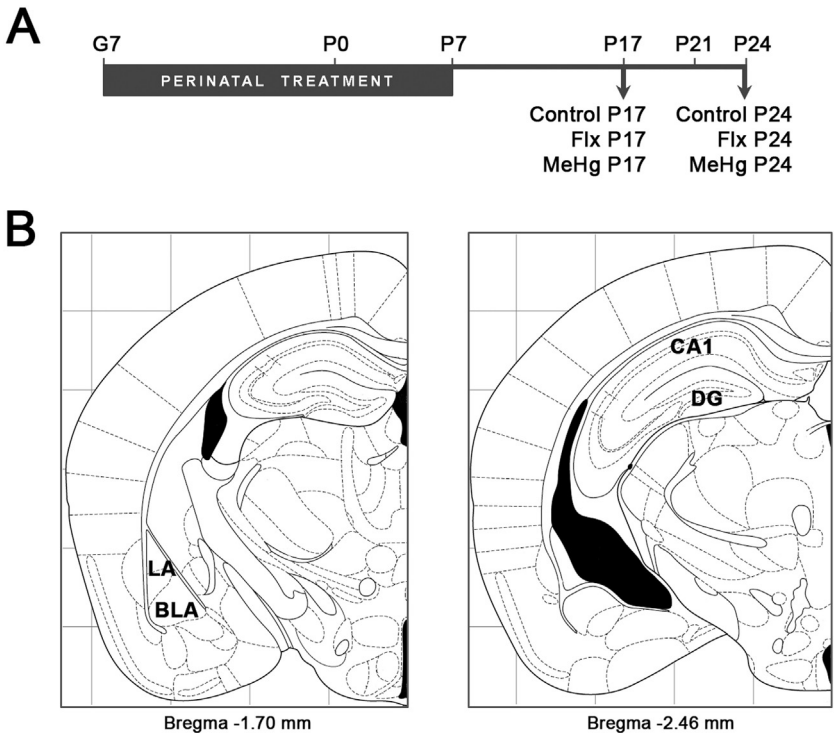

Fig. 1. (A) Experimental protocol. The dams received tap water (control), water with fluoxetine (Flx) or methylmercury (MeHg) from gestational day G7 to the day after delivery P7. The offspring brains were collected at P17 and P24. P0 and P21 are the day of birth and the day of weaning, respectively. (B) The lateral (LA) and basolateral (BLA) nuclei of the amygdala basolateral complex, and the CA1 and the dentate gyrus (DG) of the hippocampus were analyzed. Brain structure were identified according to (Paxinos and Franklin, 2001).