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Research Report

Prenatal opiate exposure impairs radial arm maze performance and reduces levels of BDNF precursor following training

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ABSTRACT

Prenatal exposure to opiates, which is invariably followed by postnatal withdrawal, can affect cognitive performance. To further characterize these effects, we examined radial 8-arm maze performance and expression of brain derived neurotrophic factor (BDNF) in male rats prenatally exposed to the opiate l-α-acetylmethadol (LAAM). Female rats received 1.0 mg/kg/day LAAM or water via daily oral gavage for 28 days prior to breeding, during breeding, and throughout pregnancy. Pups were fostered to non-treated lactating dams at birth and underwent neonatal opiate withdrawal. At 5–6 months, prenatal water- and LAAM-exposed males (n=6 each; non-littermates) received radial arm maze training consisting of ten trials a day for five days and three retention trials on day six. Rats prenatally exposed to LAAM had poorer maze performance, decreased percent correct responding and more reference and working memory errors than prenatal water-treated controls. However, they were able to acquire the task by the end of training. There were no differences between the groups on retention 24 h after testing. Following retention testing, hippocampi were removed and protein extracted from cytosol and synaptic fractions. Western blots were used to measure levels of mature and precursor BDNF protein, as well as the BDNF receptor TrkB. BDNF precursor protein was significantly decreased in the synaptic fraction of trained prenatal LAAM-treated rats compared to prenatal water-treated trained controls. No effects were found for the full-length or truncated TrkB receptor. In untrained rats, prenatal treatment did not affect any of the measures. These data suggest that prenatal opiate exposure and/or postnatal withdrawal compromise expression of proteins involved in the neural plasticity underlying learning.

1. Introduction

Methadone-maintenance remains the only recommended treatment option for opiate-dependent pregnant women (Jarvis and Schnoll, 1994; SAMHSA, 2003). In addition to the heroin and methadone-exposed population, there is growing concern about prenatal opiate exposure in offspring of women dependent on prescriptive opiates. Studies from animal models suggest that

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Abbreviations: BCA, bicinchoninic acid; BCIP, 5-bromo-4-chloro-3′-indolyphosphate p-toluidine salt; BDNF, Brain derived neurotrophic factor; LAAM, l-α-acetylmethadol; PND, postnatal day; TrkB, tyrosine kinase receptor B

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prenatal exposure to opiates may compromise aspects of cognitive function and synaptic plasticity.

Morphine administered in mid to late gestation increased the latency to complete a non-delay version of the radial arm maze (Slamberová et al., 2001). Sex specific changes in the hippocampus in mu-opioid receptors and endogenous opioids were suggested as mechanisms for the poorer learning (Schindler et al., 2004; Slamberová et al., 2003). Other groups have demonstrated poorer performance in the Morris water maze and lower levels of long-term depression in juvenile rats exposed prenatally and postnatally to morphine (Yang et al., 2003, 2006). In these studies morphine was administered throughout pregnancy and for the first 30 days post-partum. The rats were assessed while on morphine exposure or shortly after treatment ended. The detrimental effects of morphine could be attenuated by coadministration of dextromethorphan during pregnancy, suggesting that NMDA receptor changes were involved in the effect (Tao et al., 2001; Yang et al., 2006). The effects of prenatal heroin exposure on neural plasticity have also been noted. Rats exposed to heroin during mid to late gestation (days 9–18) had impaired radial arm maze performance and both an increase in the number of cholinergic transporter binding sites and a different pattern of transporter localization in the hippocampus (Steingart et al., 2000; Vatury et al., 2004).

The aim of the present study was two-fold, to replicate the prior studies using an opiate with pharmacological properties that differ from those of morphine and heroin and to extend the previous research by examining the role of neurotrophins in the cognitive deficits. We exposed rats *in utero* to the long-acting opiate *l*-α-acetylmethadol (LAAM) and allowed the pups to undergo spontaneous withdrawal after birth. LAAM is a full mu-opioid receptor agonist, but differs from other commonly studied opiates in its long duration of action. Morphine has a short half-life in the rat, such that even with twice-daily injections withdrawal signs are present (Gellert and Sparber, 1979). The half-life of heroin is even shorter (Cohn et al., 1973). Drugs used in opiate substitution therapy are generally longer acting; however, methadone has a half-life of less than 3 h in the rat (Misra et al., 1973). This means that in rat studies, with daily administration of heroin, morphine, or methadone cycles of exposure and withdrawal are likely to occur. This cycling may have greater detrimental effects than opiate exposure alone. The presence of active metabolites lengthens the duration of LAAM's action, such that with daily administration, withdrawal signs are not present in rats, unless antagonist-challenged (Henderson et al., 1977; Lichtblau and Sparber, 1982, 1983; York et al., 2002). After birth, opiate withdrawal emerges in the pups as the remaining drug is metabolized (e.g., Lichtblau et al., 1982) and this is manifest as poorer body weight gain (e.g., Hamilton et al., 2005; Lichtblau and Sparber, 1981), behavioral activation and increased concentrations of stress hormones such as corticosterone (unpublished data). Thus, we examined whether continuous exposure to the opiate LAAM during gestation followed by postnatal withdrawal affected acquisition or retention of a complex learning task, the eight-arm radial maze.

The second goal of the study was to examine potential mechanisms for changes obtained in the acquisition of the task. We investigated the role of BDNF because it has been implicated in the development of synaptic architecture throughout development (i.e., Chan et al., 2006; Bao et al., 1999; Liou et al; 1997; Martinez et al., 1998; Wang et al., 1995) and thus has the potential to be affected by drug insults. In addition, in the adult animal, BDNF has been implicated in changes in plasticity associated with drug exposure, escalation, and abuse (reviewed by Bolanos and Nestler (2004); see also Berglind et al. (2007); Cheng et al. (2005); and Tsai (2007)). Finally, there is a substantial literature documenting the role of BDNF in maintaining synaptic plasticity and modulating learning and memory in adult rats (i.e., Bekinschtein et al., 2007; Hall et al., 2000; Linnarsson et al., 1997; Ma et al., 1998; Mu et al., 1999). The convergence of these factors makes BDNF an attractive target for changes in learning and memory that follow prenatal drug exposure.

While most studies that have implicated BDNF in the above areas have examined BDNF mRNA or the mature or secreted form of BDNF, there is growing interest in the precursor form of the protein. BDNF mRNA is translated into a pre-proprotein that is packaged in secretory vesicles before cleavage to the mature, secreted protein (Mowla et al., 2001). The precursor form of BDNF may actually play a different role in the brain than the mature form (Teng et al., 2005), including affecting learning and memory and drug abuse (Cheng et al., 2005; Hariri et al., 2003; Silhol et al., 2007). We also examined changes in BDNF's high affinity receptor, TrkB. There are two forms of this receptor, a full-length signal transducing receptor with a tyrosine kinase

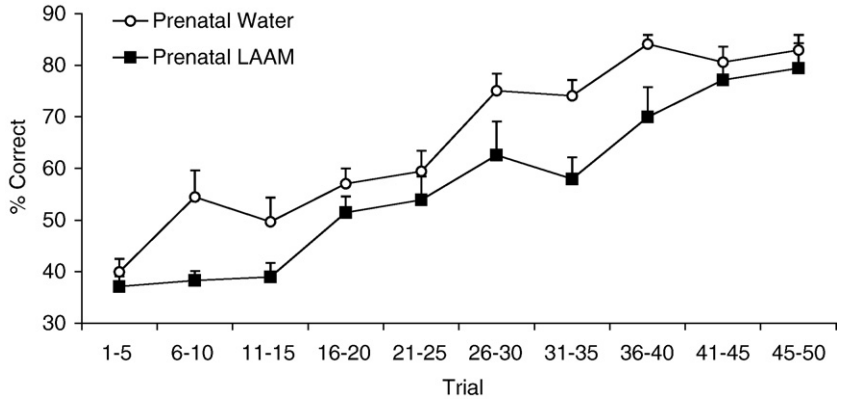

Fig. 1 – Prenatal LAAM-treated male rats (dark squares) had reduced performance in the radial arm maze compared with prenatal water-treated rats (open circles) as assessed by the percent of correct responses. However by the final trial blocks, there was no difference between the groups. Data depict mean ± SEM for trial blocks of 5 trials. ($n = 6$ per group).