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FORCED BUT NOT FREE-CHOICE NICOTINE DURING LACTATION ALTERS MATERNAL BEHAVIOR AND NORADRENERGIC SYSTEM OF PUPS: IMPACT ON SOCIAL BEHAVIOR OF ADOLESCENT ISOLATED MALE RATS

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Abstract—Adverse effects of nicotine during pregnancy have been greatly studied, while nowadays few works are focused on consequences of maternal tobacco smoking after birth. The present study investigated the behavioral and early neurochemical effects of nicotine treatment during first weeks of post-natal life in rats. We used ‘‘free choice” treatment (H_2O + NIC dams could drink from two bottles, containing 10 mg/L nicotine hydrogen tartrate salt, or water) versus ‘‘forced choice” (NIC + NIC mothers could drink from two bottles both containing nicotine hydrogen tartrate salt, range from 0.75 mg/L to 4.09 mg/L). We found that only ‘‘forced nicotine” had impact on maternal behavior, causing increased high-quality maternal care. This immediately impacted on neuro-chemical development, affecting NE levels (only males) in pup's striatum and prefrontal cortex (pFC) at PND 12. After weaning, animals were reared in normal conditions (two brother rats) or in *Social Isolation*. After two weeks, they were tested with *Social Interaction Test* (isolated rats met non-isolated opponents, siblings vs. non-siblings). As expected, isolated rats displayed an aggressive form of soliciting behavior: when facing an isolated unknown partner, the non-isolated rat tried to escape. Interestingly, if their dams were exposed to forced nicotine, both rats sooner behaved very affiliative (possibly empathic) between non-sibling partners. As expected, being exposed to post-natal nicotine could alter neuro-chemical development, but with important interactions between both maternal care and adolescent social behavior. **© 2017 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: breast feeding, tobacco smoking, neurodevelopmental sequelae, prefrontal cortex, affiliative behavior, juvenile rat.

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INTRODUCTION

According to World Health Organization (WHO), around 30% of women is unable to abstain from smoking during pregnancy. Even among those who do, too many among those mothers start again with the habit during lactation period. Damages for the fetus due to nicotine and smoke are well recognized (Fried et al., 1998; Romano et al., 2006; Steyn et al., 2006; Jacobsen et al., 2007), yet not so much is known about consequences of maternal smoking after birth of the baby. Negative effects of nicotine are likely to take place during all the span of postnatal development. Vulnerability characterizes the ‘‘brain growth spurt”, associated with various biochemical changes, that converts feto-neonatal brain into that of the mature adult (Eriksson et al., 2000). For this reason, an important priority for biomedical research is to focus on negative consequences of tobacco exposure during the critical periods of postnatal phase.

In particular, adverse effects of nicotine administration during prenatal life are well known to be due to its action on nicotine receptor NACh-R α7. This subunit involves regulation of apoptosis, and also of synaptic plasticity in hippocampus and prefrontal cortex (Belluardo et al., 2000; Ferdman et al., 2007). Thus, changes in normal development of acetylcholine neurotransmitter system are reported (Fone and Porkess, 2008; Hall and Perona, 2012; Ago et al., 2013). Nicotine anticipates critical period closing, prematurely triggering GABA's role inversion (Hensch, 2004; Dwyer et al., 2009). On contrary, the effects of nicotine post-natal exposure have not been investigated yet. This despite it's likely that the newborn can assume, through breast-milk, some toxic substances of smoke: a baby is exposed to second-hand nicotine not only when the mother is still smoking tobacco, but also if using e-cig or nicotine patches, which maybe paradoxically perceived as ‘‘safe”.

Animal models of nicotine exposure usually have been performed by administering nicotine, to the dams during pregnancy, through osmotic minipumps (with a concentration level of 6 mg/kg/day). However, the oral administration through drinking water (consisting of bottles with nicotine concentrations around 0.06 mg/mL) is preferable, since it's possible investigating consequences of voluntary vs. inescapable intake (as described by Maehler et al., 2000). Indeed, nicotine exposure can be modeled using a ‘‘free choice” nicotine intake, to mimic voluntary smoking of mothers, versus “forced choice” treatment with only nicotine available, somewhat mimicking passive second-hand exposure. These two possibilities were offered to lactating dams in our study. Direct exposure to pups, via subcutaneous injections or gastrostomy tubes, affords the direct effect of nicotine on the offspring, but these methods are stressful and require an artificial rearing. Conversely, exposure through milk suckling appeared to be a “natural route” of nicotine administration to the offspring that closely mimics the nicotine intake in a human baby.

Adolescence is an important and critical period during which social interaction and the possibility of social play are fundamental to establish social dominance in future (Panksepp and Beatty, 1980; Hall and Perona, 2012). Extended periods of *social isolation* produce deep alterations of social and emotional behavior (Matsuda et al., 2001). After weaning, adolescents interact with peers of the same sex (Panksepp and Beatty, 1980): during this period, the most distinctive behavior is *rough and tumble play*, which consists of a movements’ constellation, like punching and wrestling, that mimic a fight and/or represent a hunt simulation. From this point of view, animals socially isolated after weaning grow into conditions not allowing them to learn how to interact with a partner. When adolescents are deprived of the possibility to play with peers, upon social reunion they will assume much more this behavior, which is resulting strongly motivated (Ikemoto and Panksepp, 1992; Douglas et al., 2004). Our goal was to ascertain whether nicotine exposure would interact with enhanced social behavior elicited by isolation.

Whereas the dangerous effects of nicotine during pregnancy have been extensively depicted, it is not clear yet if and how nicotine affects neonatal development when exposure occurs during lactation, in particular during first two weeks of post-natal life. First aim of this study was to investigate if nicotine could pass from the mother to the offspring through breast milk. Second aim was to study if this treatment could alter maternal behavior and, in this case, if there are differences between “free choice” or “forced choice” nicotine intake. The ultimate goal was to investigate the effects on the offspring: we assessed neurochemical effects on amines’ system in striatum and prefrontal cortex (pFC) immediately after indirect nicotine treatment through lactation, namely in two-week-old pups. We also characterized behavioral effects on playful repertoire of adolescent rats after a period of *social isolation*.

EXPERIMENTAL PROCEDURES

All procedures were formally approved by Ministry of Health (formal license to Giovanni Laviola; veterinary surveillance by Gianluca Panzini, years 2014–2017), and conducted according to the Italian National Law (D. Lgs. 26/14) and the European Communities Council Directive (2010/63/EEC) on the use of animals for experimental research.

Subjects and sacrifices

We used 64 male Wistar rats obtained as offspring of 16 pregnant females. After birth (which will be considered post natal day 0, PND = 0), pups for each mother were reduced to 5 males and 3 females. At PND 12 ± 1, a total of 48 rats (32 male and 16 female pups, three per litter) were sacrificed to allow raising of hematic and cerebral samples (analyzed by HPLC). At PND 27 ± 1, the remaining 3 males per litter were separated from mothers and housed in couple with a brother (rats “A” and “B”, *N* = 32) or in social isolation (rat “C”, *N* = 16) in policarbonate cages (48 × 26.5 × 21 cm). After behavioral test during their adolescence, these subjects were sacrificed to take further samples used for another study (Faure et al., manuscript in preparation).

Nicotine treatment

During lactation, specifically from PND 3 ± 1 to PND 12 ± 1, mothers were randomly assigned to one of three experimental groups.

In H_2O + NIC group (*N* = 6), the mother could freely choose to drink from either of two bottles, containing respectively water or a solution of nicotine (10 mg/L, concentration refers to the nicotine hydrogen tartrate salt), chosen to simulate a moderate to heavy smoker (depending on individual choice). Bottle position was alternated once daily and counter-balanced (right vs. left); measure of drunk fluid from each bottle was taken every 24 h by weighting bottles; fluid leakage due to evaporation was measured on two identical bottles on an empty cage and subtracted; this method has been validated previously (Adriani et al., 2002).

In NIC + NIC group (*N* = 5), the mother was forced to nicotine treatment: she could drink from either of two bottles containing both a nicotine solution with a lower concentration (chosen to simulate a passive smoker with low to moderate exposure). In fact, everyday we prepared a new solution with the purpose to make these mothers drink same concentration of nicotine already drunk by mothers of the free-choice group on the day before (since free-choice preference for NIC bottles varied among dams and across days, ranging between 15.0% and 81.8%, the nicotine range was from 0.75 mg/L to 4.09 mg/L, concentration refers to the nicotine hydrogen tartrate salt). The H_2O + H_2O group (*N* = 5) represented the control: mothers could choose to drink from either of two bottles both containing water.

Therefore, pups from these mothers were passively exposed to nicotine treatment by means of mother’s milk from PND 3 ± 1 to PND 12 ± 1. They were divided into three groups as well, according to the neonatal exposure received: H_2O + NIC (*N* = 18), NIC + NIC (*N* = 15) and H_2O + H_2O (*N* = 15).

Social isolation procedure

Animals were also socially manipulated. When pups were separated from their mothers at weaning on PND 27 ± 1 two further experimental groups were formed: some subjects (2 out of 3 males per mother: *n* = 12 H_2O

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