Behavioural Brain Research 309 (2016) 44–50

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

# Neonatal finasteride administration decreases dopamine release in nucleus accumbens after alcohol and food presentation in adult male rats

Anna Llidó, Iris Bartolomé, Sònia Darbra, Marc Pallarès*

*Departament de Psicobiologia i Metodologia de les Ciències de la Salut, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*

## HIGHLIGHTS

- Neonatal finasteride decreases the accumbal dopaminergic response to alcohol intake.
- Neonatal finasteride increases alcohol intake in adulthood.
- Neonatal neurosteroids are important for ethanol rewarding properties.

## ARTICLE INFO

*Article history:*
Received 4 April 2016
Received in revised form 26 April 2016
Accepted 28 April 2016
Available online 29 April 2016

*Keywords:*
Allopregnanolone
Finasteride
Ethanol consumption
Dopamine
Nucleus accumbens
Microdialysis

## ABSTRACT

Endogenous levels of the neurosteroid (NS) allopregnanolone (AlloP) during neonatal stages are crucial for the correct development of the central nervous system (CNS). In a recent work we reported that the neonatal administration of AlloP or finasteride (Finas), an inhibitor of the enzyme 5α-reductase needed for AlloP synthesis, altered the voluntary consumption of ethanol and the ventrostriatal dopamine (DA) levels in adulthood, suggesting that neonatal NS manipulations can increase alcohol abuse vulnerability in adulthood. Moreover, other authors have associated neonatal NS alterations with diverse dopaminergic (DAergic) alterations. Thus, the aim of the present work is to analyse if manipulations of neonatal AlloP alter the DAergic response in the nucleus accumbens (NAcc) during alcohol intake in rats. We administered AlloP or Finas from postnatal day (PND) 5 to PND9. At PND98, we measured alcohol consumption using a two-bottle free-choice model (ethanol 10% (v/v) + glucose 3% (w/v), and glucose 3% (w/v)) for 12 days. On the last day of consumption, we measured the DA and 3,4-dihydroxyphenylacetic acid (DOPAC) release in NAcc in response to ethanol intake. The samples were obtained by means of *in vivo* microdialysis in freely moving rats, and DA and DOPAC levels were determined by means of high-performance liquid chromatography analysis (HPLC). The results revealed that neonatal Finas increased ethanol consumption in some days of the consumption phase, and decreased the DA release in the NAcc in response to solutions (ethanol + glucose) and food presentation. Taken together, these results suggest that neonatal NS alterations can affect alcohol rewarding properties.



## 1. Introduction

Allopregnanolone (AlloP) is a neurosteroid (NS) that acts as a positive allosteric modulator of GABA-A receptor ($GABA_AR$) [1]. The endogenous levels of this NS fluctuate greatly during development, presenting a significant increase in the second week of life [2] that has been related to brain maturation. Previous studies have shown that changes in early neonatal AlloP levels affect the development of the central nervous system, altering subsequent adolescent and adult behaviour [3–10]. Some of these behavioural alterations involve traits that can be related to vulnerability to initiate drug abuse [11], such as anxiety [5] and novelty-directed locomotion [3,4]. In a recent work, we reported that the subchronic neonatal administration (from PND5 to PND9) of 10 mg/kg of AlloP or 50 mg/kg of finasteride (Finas), an inhibitor of the enzyme 5α-reductase needed for the AlloP synthesis [12], alters the ethanol consumption in adulthood [13]. These results suggest that neonatal NS levels manipulations can increase alcohol abuse

* Corresponding author.
*E-mail address:* marc.pallares@uab.cat (M. Pallarès).

http://dx.doi.org/10.1016/j.bbr.2016.04.047
0166-4328/

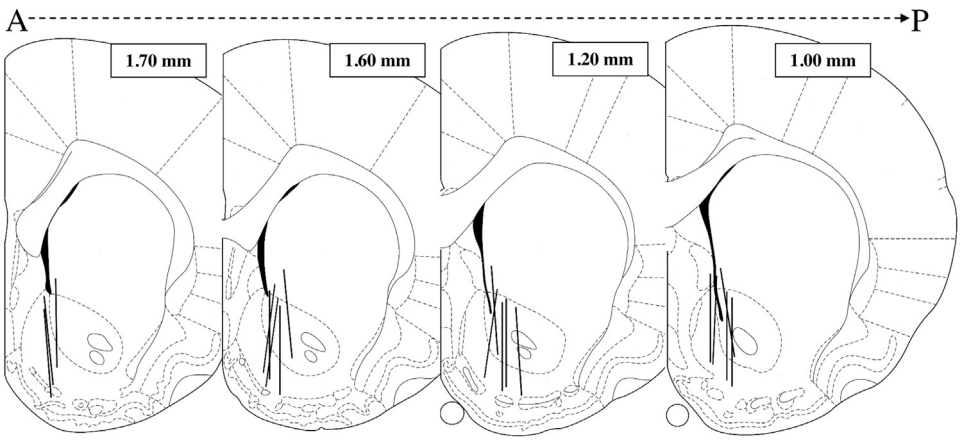


**Fig. 1.** Microdialysis probe placement within the Nucleus Accumbens. Schematic coronal sections of the rat brain's left hemisphere. Numbers indicate the anteroposterior (A-P) position of the slice relative to Bregma. Lines represent the membrane placements. Adapted from Paxinos and Watson [22].

vulnerability in adulthood. In addition, both neonatal Finas and AlloP administration decreased ventrostriatal dopaminergic (DAergic) and serotoninergic activity in rats after 15 days of ethanol consumption [13]. Furthermore, we have also observed that the neonatal administration of Finas reduced the sensitivity to locomotor stimulating effects of ethanol administration in adulthood, which could be indicating alterations in the reinforcing effects of ethanol [14].

It has been proposed that neonatal NS levels are a determining factor in the development of the mesolimbic, mesocortical and nigrostriatal DAergic systems [15,16]. Neurochemical studies have shown that the neonatal administration of the NS dehydroepiandrosterone increases dopamine (DA) transporter density in the nucleus accumbens (NAcc) and striatum in adolescence [16]. Moreover, the neonatal administration of AlloP [17], progesterone [18], or its precursor (pregnenolone) [19], alters the DA metabolism in adult striatum, as well as in frontal cortex, in the case of progesterone [18], and in the fronto-parietal cortex, in the case of pregnenolone [20].

Given that neonatal NS alterations could interfere with the development of the DAergic systems, and considering our previous results, we hypothesise that neonatal AlloP manipulations could affect the adult vulnerability to alcohol abuse by means of altering the rewarding ethanol effects. Thus, the aim of the present study was to evaluate possible changes in adult accumbal DA release in response to oral alcohol consumption in animals that were administered with AlloP or Finas during the neonatal period. For this purpose, we administered 10 mg/kg of AlloP or 50 mg/kg of Finas from postnatal day (PND) 5 to PND9, and we measured the adult alcohol intake using a two-bottle free-choice model for 12 consecutive days. On the last day of consumption, we determined the DA and 3,4-dihydroxyphenylacetic acid (DOPAC) release in the NAcc in response to ethanol intake. The samples were obtained by means of *in vivo* microdialysis in freely moving rats and the DA and DOPAC levels were determined by means of high-performance liquid chromatography analysis (HPLC). To our knowledge, this is the first study that investigates the possible alterations on the accumbal DAergic response to ethanol in animals with altered neonatal NS function.

## 2. Material and methods

### 2.1. Animals

18 male Wistar rats derived from 6 pairings raised in the Laboratori de Psicobiologia at Universitat Autònoma de Barcelona were used. Animals were housed in a temperature-controlled animal room (22–24 °C) on a 12 h light/dark cycle (light on from 8:00 to 20:00) and allowed with food and water *ad libitum*. Pregnant females were controlled twice a day to establish the exact date of birth of the offspring (called day 0). On day 0 the litter was reduced to 10 animals. Each litter was assigned to different neonatal treatment, and all animals within a litter received the same experimental manipulations. The subjects of each experimental group came from two different pairs of progenitors. Weaning took place at PND21, the males were separated and were housed into groups of brothers (2–4 subjects per cage), and females were sacrificed. This procedure has been followed in our previous experiments [3–5,8–10,13,21]. All animals were obtained, housed and sacrificed in accordance with protocols approved by the Animal Care and Use Committee of Autonomous University of Barcelona and the Department of Environment of the Generalitat de Catalunya (Regional Government), and with guidelines approved by the European Council Directive (2010/63/EU) for Care and Use of Laboratory Animals.

### 2.2. Neonatal neurosteroid administration

Pups were injected, subcutaneously, with AlloP (3α-hydroxy-5α-pregnan-20-one) (10 mg/kg, n = 6), Finas (*N-tert*-Butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide) (50 mg/kg, n = 6) or vehicle (Veh) (n = 6) from PND5 to PND9. The administration was performed once a day between 9:00 and 10:00 a.m. All pups, males and females, were injected in order to avoid possible effects on maternal care. After injection, pups were returned immediately to the home cage with their mother (they were never separated by more than 12 min). AlloP and Finas were dissolved in 10% β-cyclodextrin ((2-hydroxypropyl)-β-cyclodextrin) in 0.9% NaCl. 10% β-cyclodextrin dissolved in 0.9% NaCl was used as Veh. Injection volume was 0.1 mL/10 g body weight. The period of administration and the doses used were chosen based on previous experiments [3,4,7,8,10,13]. All products were obtained from SIGMA (Deisenhofen, Germany).

### 2.3. Stereotaxic surgery

At PND90 animals were anesthetized (i.p.) with ketamine (120 mg/kg) and xylazine (10 mg/kg), and placed in a stereotaxic apparatus (Stoelting, USA). A biocompatible polyurethane microdialysis guide cannula (CMA/11; CMA/Microdialysis AB, Sweden) was implanted into the NAcc of the left hemisphere at the following coordinates relative to Bregma, according to the Paxinos and