Universitat Autònoma de Barcelona

Relationship between Neonatal Allopregnanolone and Neonatal Stress: Effects on Adolescent and Adult Behaviour

Anna Llidó León

Relationship between Neonatal Allopregnanolone and Neonatal Stress: Effects on Adolescent and Adult Behaviour

Academic dissertation to obtain the degree of PhD in Neuroscience of the Universitat Autònoma de Barcelona

- 2017 -

Presented by

Anna Llidó León

Supervised by

Dr. Marc Pallarès **Dr. Sònia Darbra**

Group of Neurosteroids and Behaviour

Departament de Psicobiologia i Metodologia de les Ciències de la Salut

Institut de Neurociències

Universitat Autònoma de Barcelona

The research described in this thesis was conducted at the department of Psychobiology and Methodology in Health Science, Institute of Neuroscience of the Universitat Autònoma de Barcelona in the group of Neurosteroids and Behaviour.

This work was supported by a grant from the Spanish Ministry of Science and Innovation (PSI 2009-13759) and Spanish Ministry of Economy and Competitiveness (PSI 2012-36646).

INDEX

ABBREVIATIONS

3α-diol: 3α-androstanediol

3α-HSD: 3α-hydroxysteroid dehydrogenase

3β-HSD: 3ß-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase

5-HIAA: 5-hydroxyindoleacetic acid

5-HT: Serotonin

5-HT2A: Serotonin type 2A

5-HT3: Serotonin type 3

5α-DHDOC: 5α-dihydrodeoxycorticosterone

5α-DHP: 5α-dihydroprogesterone

5α-DHT: 5α-dihydrotestosterone

ACTH: Adrenocorticotropic hormone

AlloP: Allopregnanolone

BDNF: Brain-derived neurotrophic factor

Cl-: Chloride ions

CNS: Central nervous system

CORT: Corticosterone

CRF: Corticotropin-releasing factor

Cytochrome P450scc: Cytochrome P450 cholesterol side chain cleavage

DA: Dopamine

DAergic: Dopaminergic

DHEA: Dehydroepiandrosterone

DHEAS: Dehydroepiandrosterone sulphate

DOPAC: 3,4-dihydroxyphenylacetic acid

DRD2: Dopamine D2 receptor

EMS: Early maternal separation

EPM: Elevated plus-maze

Finas: Finasteride

GABA: γ-aminobutyric acid

$GABA_AR$: γ-aminobutyric acid type A receptor

GR: Glucocorticosteroid receptor

HPA: Hypothalamic-pituitary-adrenal

HPLC: High-performance liquid chromatography

HVA: Homovanillic acid

IMM: Inner mitochondrial membrane

KCC2: Potassium chloride co-transporter 2

NA: Noradrenalin

NAcc: Nucleus accumbens

nAch: Nicotinic acetylcholine

NCAM: Neural cell adhesion molecule

NH: Non-handled

NKCC1: Sodium potassium chloride co-transporter 1

NMDA: N-methyl-D-aspartate

NOT: Novel object test

NS: Neurosteroid

NTS: Nucleus tractus solitarii

OCD: Obsessive compulsive disorder

OF: Open field

OMM: Outer mitochondrial membrane

PCP: Phosphate carrier protein

PND: Postnatal day

PNS: Peripheral nervous system

PPI: Prepulse inhibition of the acoustic startle response

PR: Progesterone receptor

PREGS: Pregnenolone sulphate

PTSD: Post-traumatic stress disorder

PVN: Paraventricular nucleus

PXR: pregnane xenobiotic receptor

SHRP: Stress hypo-responsive period

StAR: Steroidogenic acute regulatory protein

THDOC: 3α,5α-tetrahydrodeoxycorticosterone

TOM22: subunit 22 of the translocase of the outer mitochondrial membrane

TSPO: Translocator protein

TTX: Tetrodotoxin

VDAC: Voltage-dependent anion channel

Veh: Vehicle

VTA: Ventral tegmental area

INTRODUCTION

The interaction between genetic factors and environmental input during sensitive developmental periods determines the maturation of brain structure and function, and thus behaviour and later vulnerability or resilience to disorders.

Perinatal period appear a particularly critical time window during which the brain is highly sensitive to remodelling by environmental factors (both positive and negative). Several epidemiological and clinical studies have shown a link between adverse early life experiences and later neurodevelopmental disorders. Such is the case of schizophrenia, to which in utero exposure to infections (Brown, 2006; Buka et al., 2001a, 2001b), hypoxia (Cannon et al., 2000, 2002; Zornberg et al., 2000), starvations (Hoek et al., 1998; Susser et al., 2008), maternal stress (Khashan et al., 2008) and other adverse life events may represent vulnerability factors. Famine during the second and third trimester has also been related to major depression disorder (Brown et al., 1995, 2000), as well as child abuse and/or neglect (Bradley et al., 2008; Felitti et al., 1998), which have also been related to a higher vulnerability to post-traumatic stress disorder (PTSD) (Binder et al., 2008) and to drug addiction (see Enoch, 2011 for review). Animal models with controlled conditions have allowed to better study the cause-effect link between early-life adverse experiences (mostly pre and postnatal stress) and diverse neurobiological and behavioural alterations (see Maccari et al., 2014 for review).

Epigenetic mechanisms (e.g. DNA methylation, histone modifications, noncoding RNAs) seem to be the basis for the long-lasting consequences of environment on brain structure and function (see Roth and Sweatt, 2011 for review). But the fully comprehension of all the elements that intervene in brain development and all their possible disruptions that may represent a cause for later affectations, is far from be completely understood. Thereby, is highly significant to study the factors implicated in brain development, how they may be altered by environmental conditions, and how this alteration leads to disruptions in brain maturation, later behaviour and vulnerability to diseases.

1. Neurosteroids implications on development

In the last decades, the importance of steroids in multiple brain processes has become manifest. In 1981, after their observations on the steroid dehydroepiandrosterone sulphate (DHEAS), Baulieu and colleagues conclude that the brain was a steroidogenic organ (Corpéchot et al., 1981). This led to the term "neurosteroid" (NS), which is used to describe the subclass of steroids that share the particularity of being synthesized in the central nervous system (CNS) (Baulieu et al., 1981). Few years later, in 1986, Majewska and cools. found out that the NS allopregnanolone (3α-hydroxy-5α-pregnan-20-one or 3α-5α-tetrahydroprogesterone; AlloP) acted as a positive allosteric modulator of γ-aminobutyric acid (GABA) type A receptor ($GABA_AR$). This last discovery led to the term of "neuroactive steroid", which is used to describe all those steroids that, irrespective of their synthesis' place (CNS or peripheral steroidogenic organs), are capable of modify neuronal activity via the modulation of membrane receptors (Dubrovsky, 2005; Paul and Purdy, 1992). The discovery of steroids acting not only as endocrine messengers but also in a paracrine or autocrine manner, and their capacity to rapidly modulate neural excitability, granted remarkably importance to steroids given the huge array of brain activities in which they may be involved. Thus, in the last years NS have been reported to participate in several brain processes and alterations of their levels have been related to a variety of neurological/psychiatric disorders and pathologic behaviours, being proved their administration beneficial in a number of experimental models of certain diseases (see Dubrovsky, 2005; Melcangi and Panzica, 2014; Porcu et al., 2016 for reviews).

NS levels in the brain are not static but dynamically altered across life-time in response to physiological conditions. The concentration of AlloP oscillates during development (Grobin and Morrow, 2001), menstrual cycle (Monteleone et al., 2000), pregnancy and post-partum (Concas et al., 1998), as well as in response to stress (Purdy et al., 1991; Serra et al., 2000) or alcohol systemic administration (Barbaccia et al., 1999; Cook et al., 2014a, 2014b). Surprisingly, although the actions of NS on adult brain have been largely studied, their role on developing brain is still poorly understood. In the rat brain, enzymes responsible for steroidogenesis are present since early foetal stages (Compagnone et al., 1995; Lauber and Lichtensteiger, 1996; Lephart et al., 1990) and the ability of foetal CNS to synthesize NS has been proved (Pomata et al., 2000).

Furthermore, AlloP pre and postnatal fluctuations in rats' frontal cortex (Grobin and Morrow, 2001) are time-related to significant changes on brain development (see below), which may indicate an important role of AlloP in neural maturation. Thus, the study of the involvement of NS such as AlloP on brain development is of great interest.

1.1. Biosynthesis and brain distribution

Steroidogenesis begins in the inner mitochondrial membrane (IMM), with the conversion of cholesterol to pregnenolone, the first steroid formed, by the enzyme cytochrome P450 cholesterol side chain cleavage (cytochrome P450scc). Cholesterol can be *de novo* synthesized in the endoplasmic reticulum or imported from circulating lipoproteins, and its further translocation from the outer mitochondrial membrane (OMM) to the IMM is considered the rate-limiting step of steroidogenesis. The steroidogenic acute regulatory protein (StAR) initiates the intracellular cholesterol trafficking by transferring the free cholesterol from intracellular stores to the OMM. There, StAR interacts with other proteins that transfer the cholesterol into the IMM, where cytochrome P450scc is located (see Papadopoulos and Miller, 2012 and Rone et al., 2009 for review). For years the translocator protein (TSPO; formerly called mitochondrial-type benzodiazepine receptor or peripheral-type benzodiazepine receptor) has been considered the main responsible of this last movement, but new findings showing the viability of TSPO knockout mice without loss of steroid synthesis, have questioned its absolute requirement for steroidogenesis (Tu et al., 2014; discussed in Gut et al., 2015, in Selvaraj and Tu, 2016 and in Stocco et al., 2017). Thus, StAR and other OMM proteins such as the voltage-dependent anion channel (VDAC), the subunit 22 of the translocase of the OMM (TOM22) and the phosphate carrier protein (PCP), seem to be the essential proteins for the cholesterol transfer into the IMM and thus for steroidogenesis (Stocco et al., 2017).

Once synthesised, pregnenolone will act as itself or it will be converted to one or other steroid depending on which steroidogenic enzymes express the cell. AlloP synthesis begins with the conversion of pregnenolone to progesterone via the enzyme 3ß-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase (3ß-HSD). Then progesterone is reduced by 5α-reductase into 5α-dihydroprogesterone (5α-DHP), which will be further reduced into AlloP via 3α-hydroxysteroid dehydrogenase (3α-HSD) (also termed 3α-hydroxysteroid oxidoreductase, 3α-HSOR). 5α-reductase catalyses not only the reduction

of progesterone into 5α-DHP, but also the reduction of 11-deoxycorticosterone into 5α-dihydrodeoxycorticosterone (5α-DHDOC), and the reduction of testosterone into 5α-dihydrotestosterone (5α-DHT). Furthermore, some of the steroidogenic enzymes are involved in catalyse both the reduction and the oxidation of NS. Such is the case of 3α-HSD which catalyses the reversible conversion of 5α-DHP into AlloP, 5α-DHDOC into 3α,5α-tetrahydrodeoxycorticosterone (THDOC) and 5α-DHT into 3α-androstanediol (3α-diol).

The diverse steroidogenic enzymes have been found both in neurons, mainly GABAergic and glutamatergic, and in glial cells (i.e. astrocytes and oligodendrocytes) of numerous brain areas (see Do Rego et al., 2009 for review). For instance, initial studies in rats reported highly concentrations of 5α-reductase in glial cells of the hypothalamus, the thalamus, the hippocampus, the cerebral cortex and the circumventricular organs (Pelletier et al., 1994), and of 3α-HSD mainly in brain tissue of the olfactory bulb, with moderate levels also found in cerebral cortex, hypothalamus, cerebellum and pituitary (Khanna et al., 1995). More recent works with rats also reported 5α-reductase in glial cells of the olfactory bulb (Kiyokage et al., 2005). On the other hand, in mice, 5α-reductase and 3α-HSD are co-localized in several neuronal populations, such as cortical, hippocampal, and olfactory bulb glutamatergic principal neurons, in some output neurons of the amygdala and thalamus, and in principal GABAergic output neurons (striatal, medium spiny, reticular thalamic nucleus and cerebellar Purkinje neurons) (Agis-Balboa et al., 2006). The expression and activity of both enzymes has also been proved in the human brain, where diverse post-mortem studies have identified 5α-reductase mRNA in temporal cortex, subcortical white matter, hippocampus, cerebellum and pons; and 3α-HSD (type 2 and 3) mRNA in frontotemporal lobes, putamen, cerebellum, subcortical white matter, medulla and spinal cord (see Do Rego et al., 2009 and Stoffel-Wagner, 2003 for review).

Regarding synthesised NS, immunohistochemical assays of the cellular distribution of 3α-hydroxy, 5α-reduced pregnane steroids (i.e. AlloP and THDOC) from the forebrain to the brainstem of the adult rat, revealed their localization in the cell bodies and thick dendrites of neurons (mostly glutamatergic but also projecting GABAergic) of numerous brain structures, being the olfactory bulb, the striatum and the cerebral cortex the areas with highest density immunolabelling (Saalmann et al., 2007). In humans, a study that compared AlloP concentration in 17 regions of women's brains showed the highest levels in substantia nigra and basal hypothalamus (Bixo et al., 1997). While other work that

determined NS levels on 6 cerebral regions of old men also found the highest concentration of AlloP in the hypothalamus, followed (in decreasing order) by striatum, frontal cortex, cerebellum, hippocampus and amygdala (Weill-Engerer et al., 2002).

1.2. Mechanism of action: how neurosteroids may be implicated in CNS development

NS act both on intracellular receptors regulating gene expression, which are slow actions (minutes to days) limited by the rate of protein biosynthesis (Gronemeyer, 1992), and on membrane receptors modulating neural excitability and thus producing rapid changes (milliseconds to seconds) (Majewska et al., 1986). Therefore, the effects of NS on the developing CNS may be mediated either through the classical intracellular receptors or through membrane receptors. In this sense, it has been reported that progesterone and 5α-DHP stimulate myelination in the peripheral nervous system (PNS) by acting on the intracellular progesterone receptor (PR), while AlloP also stimulates myelination in the PNS but by acting on $GABA_AR$ (see Magnaghi et al., 2001 for review). Same results have been found in organotypic slice cultures of 7-day-old rat and mouse cerebellum: progesterone stimulates myelination by acting on PR but also by the action of its metabolite AlloP on $GABA_AR$ (positive modulation) (Ghoumari et al., 2003). Given that in rats the second postnatal week corresponds to a period of both intense myelination (Notterpek et al., 1993) and elevated levels of progesterone (Ukena et al., 1999), these results suggest that progesterone may play an important role in myelination during brain development (Ghoumari et al., 2003). Moreover, progesterone has been shown to promote both dendritic outgrowth and synaptogenesis in Purkinje cells through PR mediated mechanisms during cerebellar development (Sakamoto et al., 2001). Importantly, AlloP does not directly bind to PR, but it can act on it via its oxidation into 5α-DHP (Rupprech, 2003). Furthermore, AlloP directly activates the pregnane xenobiotic receptor (PXR) (Frye et al., 2012; Lamba et al., 2004; Langmade et al., 2006). PXR is a nuclear receptor that influences the transcription of several major families of genes, among them the cytochrome P450scc enzymes (Frye et al., 2012). Thus, PXR is involved in cholesterol homeostasis and its activation by AlloP, or other endogenous compounds, may serve to modulate NS biosynthesis (Frye et al., 2012; Lamba et al., 2004).

Regarding membrane receptors, although AlloP main effects are mediated though the positive modulation of $GABA_AR$, it also modulates other ionotropic receptors such as

neural nicotinic acetylcholine (nACh) receptor (negative modulation) (Bullock et al., 1997), and the serotonin type 3 (5-HT3) receptor (negative modulation) (Rupprecht, 2003; Wetzel et al., 1998). In the same way, THDOC and 3α-diol are also positive modulators of $GABA_AR$ (Lambert et al., 2009); and progesterone also acts as a negative modulator of nACh (Valera et al., 1992) and 5-HT3 (Wetzel et al., 1998) receptors, as well as of σ1 receptor (Monnet and Maurice, 2006). By contrast, dehydroepiandrosterone (DHEA), its sulphate form DHEAS and pregnenolone sulphate (PREGS) act as negative modulators of $GABA_AR$ and positive modulators of glutamate N-methyl-D-aspartate (NMDA) receptor (Akk et al., 2001; Majewska and Schwartz, 1987; Smith et al., 2014).

Actions of AlloP on $GABA_AR$ are especially relevant given the important role that GABA signalling plays on development (Owens and Kriegstein, 2002). $GABA_AR$ is an ionotropic receptor that allows the flux of chloride ions (Cl^-). In mature brain, Cl^- concentration is usually higher in the extracellular space; therefore the activation of $GABA_AR$ causes Cl^- influx and subsequent hyperpolarization of the membrane. $GABA_AR$ is a heteropentameric receptor, there are 19 variants of subunits (α1-6, ß1-3, γ1-3, δ, ε, θ, π and ρ1-3) and the most common arrangement consist on two α, two ß and usually one γ or one δ subunit (Olsen and Sieghart, 2009; Sieghart and Sperk, 2002). Those with γ subunit are usually in synaptic space mediating phasic inhibition (i.e. short and rapid inhibitory postsynaptic response), while those with δ subunit are extrasynaptic and thus mediate tonic inhibition (i.e. paracrine regulation of the neuron tone) (see Farrant and Nusser, 2005 for review). AlloP is able to modulate both, synaptic and extrasynaptic $GABA_AR$ (see Belelli and Lambert, 2005 and Carver and Reddy, 2013 for review), but it has preference for those that contain δ subunit, and especially for the combination α4ß2δ (reviewed in Shen and Smith, 2009). The action of AlloP on $GABA_AR$ depends on its concentration: at nanomolar concentrations AlloP works as an allosteric modulator and thus potentiates the actions of GABA; while at micromolar concentrations AlloP directly activates the $GABA_AR$ opening the Cl^- channel, permitting Cl^- influx and thus causing hyperpolarization of the cell membrane (GABA-mimetic effect) (Callachan et al., 1987; Shu et al., 2004).

In contrast to mature neurons, developing neurons have high intracellular Cl^- content, mainly due to an increased expression of the sodium potassium chloride co-transporter 1 (NKCC1; accumulate chloride) and a low expression of the potassium chloride co-transporter 2 (KCC2; chloride extruder) (Ben-Ari et al., 2007). In consequence, the activation of $GABA_AR$ causes an efflux of Cl^-, and thus the

depolarization of the membrane, instead of the Cl^- influx and subsequent hyperpolarization observed in mature neurons. This depolarization caused by $GABA_AR$ activation is enough to open voltage dependent L-type calcium channels as well as to relieve the magnesium block of glutamate NMDA receptors, and thus provoke a calcium influx that might activate downstream signalling pathways (e.g. increase expression of brain-derived neurotrophic factor (BDNF)) and regulate many processes of brain development. Thus, the activation of $GABA_AR$ early in development has been related to neuronal proliferation, migration and differentiation (see Owens and Kriegstein, 2002 for review), and so its modulation by NS such as AlloP may affect neural development. In this sense, several *in vitro* studies have shown that, besides myelination (see above), AlloP promotes neurogenesis in primary cultures of rat neural progenitors (Keller et al., 2004; Wang et al., 2005), effect that has also been related with the activation of $GABA_AR$.

As mentioned above, AlloP levels fluctuate during development. Grobin and Morrow (2001) characterized AlloP pre and postnatal fluctuations in the frontal cortex of rats and found out that during the last gestational days foetuses present elevated AlloP levels, fact that has been related to a protective role of AlloP against gestational stress (see Brunton et al., 2014 for review). These high levels decrease progressively during the first week of life, when rats present low levels that are similar to those found in adult brain. Then, during the second week of life a new elevation on AlloP levels occurs, reaching maximum values between postnatal day (PND) 10 and PND14, decreasing again to similar adult levels on PND15, and remaining low until puberty. This second peak in AlloP is time-related to the functional GABA shift from depolarization to hyperpolarization both in neocortex and hippocampus (reviewed in Dehorter et al., 2012). The primary signals of the polarity shift are the progressive up-regulation of KCC2 and down-regulation of NKCC1 (Ben-Ari et al., 2012). In a previous work, we showed that the alteration of physiological AlloP levels by means of the sub-chronic administration (from PND5 to PND9) of AlloP or finasteride (Finas), which in adult rats acts as an inhibitor of the enzyme 5α-reductase (Azzolina et al., 1997; Mukai et al., 2008), alters the developmental expression of KCC2 in the hippocampus (Mòdol et al., 2014b), which could be indicating the participation of AlloP in the GABA hyperpolarizing shift. Moreover, during the second week of life there are important changes in $GABA_AR$ subunit expression patterns (Laurie et al., 1992; Liu et al., 1997). In this sense, *in vitro* works show that the exposition of developing neurons to AlloP alters $GABA_AR$ function (Yu and Ticku, 1995) and α4 subunit gene expression (Grobin and Morrow, 2000) in a

concentration-dependent manner. Furthermore, *in vivo* studies have shown that the exogenous administration of Finas (50 mg/kg from PND5 to PND9) causes a neonatal overexpression of α4 and δ $GABA_AR$ subunits in the rat hippocampus that is still present in adulthood (Mòdol et al., 2014a). GABAergic alterations have also been found in other brain structures besides hippocampus. For instance, a single AlloP administration (10 mg/kg) on PND1 or PND5 alters the localization of parvalbumin-positive GABAergic interneurons in adult prefrontal cortex (Grobin et al., 2003). Therefore, all these results show that alterations in neonatal AlloP levels may directly affect the development of GABAergic neurotransmission. In addition, other studies have showed that neonatal AlloP administration produces a decrease of the total neuron number in adult medial dorsal thalamus, probably indicating a decrease in thalamocortical connectivity (Gizerian et al., 2004), and alters striatal dopaminergic (DAergic) activity in adulthood (Muneoka et al., 2009). This last result has also been found with the neonatal administration of progesterone (Muneoka et al., 2010), suggesting that neonatal progesterone may alter adult striatal DAergic activity via the action of its metabolite AlloP on $GABA_AR$.

1.3. Neonatal neurosteroids and postnatal development: effects on adolescent and adult behaviour

Several studies have shown that manipulations of the neonatal physiological NS levels in rats result in altered adolescent and adult behaviour. For instance, the neonatal administration of AlloP or Finas has been related to diverse changes in emotional behaviours. In this sense, an acute neonatal injection of AlloP (10 mg/kg) at PND5 increases novelty-directed locomotion measured in the open field (OF) in adulthood (Darbra and Pallarès, 2009). This effect has also been observed pre- and post-puberty with the administration of 10 μg/g of pregnenolone between PND3 and PND7 (Muneoka et al., 2002), and may indicate a reduction in the stress responses to novel environmental experiences. Furthermore, a single administration of 10 mg/kg of AlloP on PND2 or PND5 increases the locomotor response to amphetamine in adulthood, suggesting an altered DAergic mesocorticolimbic system (Gizerian et al., 2006). Alterations on anxiety-like behaviour have also been reported. Thus, sub-chronic neonatal administration of AlloP (5 mg/kg between PND2 and PND6) induces an anxiolytic-like profile in the elevated plus-maze (EPM) test in adulthood (Zimmerberg and Kajunski, 2004), effect that has also been found following the administration of a higher dose of AlloP (20 mg/kg

from PND5 to PND9) (Darbra and Pallarès, 2012). Moreover, an acute administration of AlloP (10 mg/kg) at PND5 alters the action of the benzodiazepine lorazepam, decreasing its anxiolytic effects (Darbra and Pallarès, 2009). On the other hand, the sub-chronic neonatal administration of Finas (50 mg/kg between PND5 and PND9) decreases the locomotor activity in the Boissier exploration test in adolescence (Darbra and Pallarès, 2010), as well as deteriorates passive avoidance and induces an anxiogenic-like profile in the EPM test in adulthood (Martin-García et al., 2008). Moreover, neonatal Finas administration (50 mg/kg from PND5 to PND9) has also been related to an anxiety-like profile in the EPM test in response to systemic progesterone administration in adulthood (Mòdol et al., 2014a). It has to be noted that both novelty-directed locomotion and anxiety responses are behavioural traits that can indicate an impulsive and risking behaviour and thus they could be related to an increased vulnerability to initiate drug abuse (Belin and Deroche-Gamonet, 2012).

Alterations of neonatal AlloP levels have also been related to an altered processing of sensory inputs to the brain. Both a single administration of 10 mg/kg of AlloP on PND2 or PND5 (Gizerian et al., 2006), and a daily administration of 10 mg/kg of AlloP between PND5 and PND9 (Darbra and Pallarès, 2010) decrease the prepulse inhibition of the acoustic startle response (PPI) in adulthood. A deficient PPI is an operative measure for disturbed sensorimotor gating, which is the ability of a weak sensory event to suppress - "gate"- the spontaneous motor response to an intense sensory stimulus (Swerdlow et al., 2001). Dysfunctions in this response have been associated to alterations in the mesocorticolimbic system (Gizerian et al., 2006; Swerdlow et al., 2001) but also in the hippocampus (Darbra et al., 2012; Zhang et al., 2002). In humans, PPI is impaired in several major psychiatric disorders, including schizophrenia and obsessive compulsive disorder (OCD) (Ahmari et al., 2012; Swerdlow et al., 2006; reviewed in Kohl et al., 2013). Thus, alterations of neonatal AlloP levels may be related to an increased vulnerability to suffer neurodevelopmental disorders such as schizophrenia. In this sense, the administration of the antipsychotic drug Clozapine (7.5 mg/kg) previous PPI test reversed the PPI deficit of those animals that were administered with AlloP on PND2 (Gizerian et al., 2006).

All these behavioural alterations may be reflecting multiple developmental changes in distinct systems and brain structures. For instance, given that the hippocampal formation seem to be involved in the mentioned behaviours (spatial exploration, anxiety-related behaviours) as well as in sensorimotor gating, behavioural alterations caused by

neonatal AlloP manipulations may be at least in part related to an altered hippocampal function (see Darbra et al., 2014 for review). Accordingly, as explained above, neonatal AlloP manipulations have been related with an altered expression of KCC2 (Mòdol et al., 2014b) and $GABA_AR$ subunits (Mòdol et al., 2014a) in hippocampus. Furthermore, manipulations of neonatal physiological NS levels alter the behavioural effects of intrahippocampal NS administration in adulthood. In this sense, the adult intrahippocampal infusion of AlloP decreases the locomotion on the OF test in control rats but has no effects in rats with sub-chronic neonatal administration of AlloP (10 mg/kg from PND5 to PND9) (Darbra and Pallarès, 2011). Moreover, sub-chronic neonatal injections of higher AlloP doses (20 mg/kg from PND5 to PND9) inhibit the anxiolytic-like effects (Mòdol et al., 2013) as well as the increase of PPI (Darbra et al., 2013) caused by adult intrahippocampal AlloP administration. The alteration of adult intrahippocampal AlloP infusion effects has also been found with the sub-chronic administration of Finas (50 mg/kg between PND5 and PND9) (Darbra and Pallarès, 2011; Darbra et al., 2013; Mòdol et al., 2013). Nevertheless, as previously explained, neonatal AlloP administration has also been related to GABAergic alterations in the prefrontal cortex (Grobin et al., 2003), to alterations on thalamocortical connectivity (Gizerian et al., 2004) and to an altered striatal DAergic activity (Muneoka et al., 2009). All these affectations could also underlie the explained effects on spatial exploration, anxiety-related behaviours and sensorimotor gating. Moreover, alterations of the DAergic activity may be especially relevant as they could suggest that neonatal AlloP manipulations could affect adolescent and adult behaviours related to dopamine (DA) function, such as drug seeking behaviour (Nutt et al., 2015).

Therefore, all these results indicate that the maintenance of physiological NS levels is critical for the correct development of several brain structures, the subsequent adolescent and adult behaviour and the vulnerability to psychopathological diseases.

2. Stress affects development: early maternal separation model

Stressful stimuli activate the hypothalamic-pituitary-adrenal (HPA) axis leading to the synthesis and release of glucocorticoids (mainly cortisol in humans and non-humans primates, and corticosterone (CORT) in rodents) from the adrenal cortex, which will promote diverse actions in response to stress (e.g. metabolic adaptations, facilitation of cardiovascular responses and modulation of immune and behavioural responses (see Sapolsky et al., 2000 for review)). Stress responses are needed to adapt the organism to the psychological and physiological challenges, but eventually they can have deleterious effects upon brain function. As mentioned above, during perinatal period brain is highly sensitive to environmental factors, thereby early exposure to adverse experiences as stress has been related to several developmental alterations and adult disorders (see Bale et al., 2010; Bock et al., 2014 and Chen and Baram, 2016 for review).

In neonatal animals, early stress arises mainly from disruptions on the maternal care, thus several models of neonatal stress in rodents consist on the manipulation of maternal-pup interactions. The two main methods used are the limitation of nesting and bedding material, which in turn alters the nurturing behaviours of the dams causing stress in the pups; and the deprivation of the mother, i.e. the separation for a certain time of the dam from her litter. This last method provokes hypothermia and starvation in the pups, thus physical stress, but also alters the nursing and nurturing behaviours of the mother when pups are returned to their litter. Thereby, both methods have proven to cause stress on the pups and to have consequences on the cognitive and emotional networks and functional outcomes (Chen and Baran, 2016).

Regarding maternal separation, several models have been used, differing on the extension of the separation period and its continuity (one or various intermittent periods), as well as on the age of the pups, both factors that have a significant impact on the outcomes consequences of the stress (for a review see Fumagalli et al., 2007). In this sense it is important to distinguish between short (<15 min) and long (>180 min) maternal separation models. Since the initial studies by Levine and colleagues (Levine et al., 1956; reviewed in Raineki et al., 2014), it has been well established that short periods (<15 min) of repeated maternal absence are usually related to positive behavioural consequences (Levine, 2005; Plotsky et al., 2005), as they simulate wildlife rearing conditions where the dam left the nest regularly (Grota and Ader, 1969 cited in

Nylander and Roman, 2012). In contrast, long absences (>180 min) disrupt normal maternal-pup interaction and are generally related with detrimental effects (see Faturi et al., 2010 for review).

The timing of the stress presentation is another important factor that determines its outcome and long-lasting consequences. Neonatally, rodents show very low basal levels of CORT as well as attenuated adrenocorticotropic hormone (ACTH) and CORT responses to environmental stressors (Rosenfeld et al., 1992; Sapolsky and Meaney, 1986). This period is named "stress hypo-responsive period" (SHRP) and extends between PND4 and PND14 in rats (Sapolsky and Meaney, 1986). The SHRP is a reflection of the still ongoing maturation of the HPA axis and it is thought to represent a protective mechanism to prevent the detrimental effects of increased levels of CORT on the developing brain (de Kloet et al., 1988; Ellenbroek and Cools, 2002). Even so, there are severe stressors that can overcome the SHRP, elicit increases in both ACTH and CORT and thus cause multiple neurodevelopmental alterations. Depending on when the stress occurs (pre-, during, or post- the SHRP), very different outcomes have been reported to the same kind of stressor (Bock et al., 2014; Chen and Baram, 2016). Moreover, the outcome of the stress exposition depends on the maturation status of each cerebral region, and given that the timing of brain development is not uniform but varies upon regions and systems (Giedd et al., 2009), distinct systems will be affected in function of the time of stress presentation.

A single period of 24 h of early maternal separation (EMS) at PND9, represents a potent stressor that has been related to multiple alterations. 24 h of EMS on PND9 overcomes the SHRP and causes an increase in basal CORT levels (Avishai-Eliner et al., 1995; Suchecki et al., 1995; Viveros et al., 2010), as well as an enhanced responsiveness of the HPA axis to further stressors, both neonatally (Avishai-Eliner et al., 1995; Suchecki et al., 1993, 1995) and in adulthood (Lehmann et al., 2002; Viveros et al., 2009). 24 h of EMS at PND9 has been proposed as a possible animal model to study specific aspects of schizophrenia (see Ellenbroek and Riva, 2003 for review) since it leads to a disruption of the PPI response after puberty (Ellenbroek et al., 1998, 2004), as well as to a disruption of adult latent inhibition (Ellenbroek and Cools, 1995), which is a phenomenon also found in certain schizophrenic patients (Gray et al., 1995). This EMS effect on PPI is reversed by typical and atypical antipsychotic drugs, which could suggest that EMS leads to a hyperactivity of the DAergic system (Ellenbroek et al., 1998). In this sense, adult DAergic activity after 24 h of EMS at PND9 has been found increased in

striatum, prefrontal cortex and amygdala (Rentesi et al., 2013). Moreover, animals submitted to 24 h of EMS at PND9 show a behavioural profile of higher impulsivity in adolescence and an increased locomotor response to novelty in adolescence (Marco et al., 2007) and adulthood (Rentesi et al., 2013); as well as an enhanced sensibility to the DA agonist apomorphine (Ellenbroek and Cools, 1995, 2000; Rentesi et al., 2013) and to amphetamine (Rentesi et al., 2013). Furthermore, in accordance with the neurodevelopmental hypothesis of schizophrenia, this EMS model leads to a developmental delay (i.e. a long lasting reduction in body weight, a delay in eye opening, in walking and in rearing), that includes a pre-weaning reduction of locomotor response to amphetamine, suggesting an altered development of the mesolimbic DAergic system (Ellenbroek et al., 2005).

24 hours of EMS on PND9 also relates to increased adult serotonergic function in the prefrontal cortex, amygdala (Rentesi et al., 2013) and hypothalamus (Rentesi et al., 2010), as well as to diverse alterations of the hippocampal endocannabinoid system (see Marco et al., 2015 for review). All these neurochemical disruptions may mediate some of the other behavioural effects reported, which include a depressive-like phenotype in the forced swim test in adolescence (Llorente et al., 2007) and in adulthood (Zamberletti et al., 2012), an adult anxiolytic-like profile in the EPM test (Llorente-Berzal et al., 2011), and a decrease in the discrimination index in the novel object test (NOT) in adulthood (Llorente et al., 2011). Furthermore, hippocampal alterations such as a reduction on the expression of neurotrophin BDNF in adolescence (Marco et al., 2013) and adulthood (Llorente et al., 2011; Roceri et al., 2002), and a reduction of two glutamate NMDA receptor subunits expression (Roceri et al., 2002) and of neuropeptide Y levels (Husum et al., 2002) in adulthood, have also been found in animals that have suffered EMS.

Thus, 24 h of EMS at PND9 represents a potent neonatal stressor that alters the development of several systems and the further adolescent and adult behaviours, being an interesting model for the study of a variety of disorders.

3. Relationship between neurosteroids and stress during neonatal stage

GABAergic inhibitory control plays an important role on the regulation of HPA axis activation (see Gunn et al., 2015 for review). In adult rats, numerous studies have shown that after acute stress the GABAergic transmission rapidly decreases (Biggio et al., 1990; Concas et al., 1998) while AlloP brain and plasma levels increase (Barbaccia et al., 1996; Purdy et al., 1991). Importantly, this increase in AlloP levels correlates with the restoration of the GABAergic transmission (Barbaccia et al., 1998). Additionally, several studies have shown that in rodents the increase in AlloP levels inhibits the corticotropin-releasing factor (CRF; also termed corticotropin-releasing hormone) production and release, vasopressin expression, ACTH release, and subsequent increase in CORT levels (Owens et al., 1992; Patchev et al., 1994, 1996). Thus, endogenous AlloP represents a homeostatic mechanism in the context of adaptation to stress by limiting the extent and duration of reduction in GABAergic inhibitory transmission and so the activation of the HPA axis (Girdler and Klatzkin, 2007). On the other hand, chronic stress induces important reductions in cerebrocortical and plasma concentrations of AlloP (Serra et al., 2000), as well as alterations in NS responses to acute stressors. Thus, it has been suggested that a disruption in this homeostatic mechanism may play a pathogenic role in some psychiatric disorders related to chronic stress (Girdler and Klatzkin, 2007). In this way, the administration of exogenous AlloP either during or following a period of chronic stress can prevent or normalize HPA axis dysfunction, precluding the establishment of depressive/anxiety-like behaviours in rats (Evans et al., 2012).

Changes in AlloP levels due to stress have also been observed during neonatal stages (Frye et al., 2006; Kehoe et al., 2000), but while several works have studied the relationship between AlloP and stress in adulthood, much less have studied how both factors interact in neonatal stages and how alterations of physiological AlloP levels may alter neonatal stress outcomes and *vice versa*. Given that adequate inhibitory GABAergic control is necessary for stress regulation, changes in physiological AlloP levels may imply changes in stress responses and thus distinct outcomes of neonatal stress. In this sense, previous works studying the effects of AlloP administration on neonatal stress showed that the administration of AlloP prior a brief maternal separation at PND7 decreases the ultrasonic vocalizations of rats (Zimmerberg et al., 1994; Zimmerberg and Kajunski, 2004). Ultrasonic vocalizations are emitted by neonatal rat pups in the range

of 20 to 50 kHz and are thought to contribute to the formation of the maternal-infant bond as infant mammals vocalize when separated from their dams to elicit protection, nourishment and warmth (Zimmerberg and Kajunski, 2004). Thus, the analysis of ultrasonic vocalizations is used as a measure of the pup's affective state (Zimmerberg et al., 2003). Other authors have showed that when applied concomitantly with the stressful challenge, the GABA-positive THDOC can attenuate the adult behavioural and neuroendocrine consequences of repeated maternal separation during early life (i.e. increased anxiety, enhanced HPA axis responses to stress and impaired glucocorticoid feedback) (Patchev et al., 1997). In the same way a single administration of 2 mg/kg of AlloP injected just prior 12 h of maternal separation on PND5 counteract the increased HPA axis response to subsequent stressors both in infants and adult rats (Mitev et al., 2003). Thus, the administration of NS such as AlloP that act as positive modulators of $GABA_AR$ may prevent or reverse some of the negative neuroendocrine and behavioural effects of neonatal stress. In fact, it has been suggested that a transient increase of NS biosynthesis may contribute to the SHRP (Mitev et al., 2003).

Besides the possible protective role of AlloP upon neonatal stress, it has to be considered that since alterations of neonatal AlloP levels have been related to several developmental disruptions (see above), some of the detrimental effects of neonatal stress may be in part elicited by an alteration of neonatal NS levels. In this sense, Zimmerberg and Kajunski (2004) showed that both pups submitted to 6 h per day of social isolation between PND2 and PND6 and pups not social isolated that were daily administered with AlloP (5 mg/kg, PND2-PND6) emitted fewer ultrasonic vocalizations than control rats during maternal separation on PND7. Authors proposed that social isolation effects on ultrasonic vocalizations may be mediated by an endogenous increase of AlloP, and therefore its exogenous administration provokes the same effect (Zimmerberg and Kajunski, 2004). Interestingly, when rats submitted to social isolation received a previous injection of AlloP, the rate of ultrasonic vocalizations on PND7 was unaffected, may be indicating that AlloP effects could be dose-dependent (Zimmerberg and Kajunski, 2004).

No previous work has study the possible relationship between the manipulation of neonatal AlloP levels between PND5 and PND9 and 24 h of EMS on PND9, but independent works have shown that both interventions affect similar systems, brain regions and adolescent and adult behaviours. For instance, as previously explained, both (neonatal AlloP levels alterations and 24 h of EMS on PND9) have being related to

hippocampal alterations (Darbra et al., 2014; Husum et al., 2002; Llorente et al., 2011; Marco et al., 2013; Mòdol et al., 2013, 2014a, 2014b; Roceri et al., 2002) and to alterations in adolescent and adult emotional behaviours, such as anxiety-like behaviour (Darbra and Pallarès, 2012; Llorente-Berzal et al., 2011; Martin-García et al., 2008) and novelty-directed locomotion (Darbra and Pallarès, 2009, 2010; Marco et al., 2007; Rentesi et al., 2013). Furthermore, both interventions alter PPI (Darbra and Pallarès, 2010; Ellenbroek and Riva, 2003) and seem to alter adult striatal and cortical DAergic function (Gizerian et al., 2006; Muneoka et al., 2009; Rentesi et al., 2013). Thus, all these similar results may indicate that the effects of EMS on systems' development and adult behaviour are, at least in part, mediated by the alteration of neonatal NS levels. Therefore, it is of great interest to study the effects of both interventions (manipulation of neonatal AlloP levels between PND5 and PND9 and 24 h of EMS on PND9) in these behaviours in order to establish their possible interactions and mutual influence.

It has to be taken into account that neonatal stress may alter not only neonatal NS levels but also NS biosynthesis and/or metabolism in adulthood. In this sense, adult male rats submitted to early stimulation (30 min of maternal separation on PND9 followed by 6 h of maternal separation on PND10) showed lower hippocampal AlloP levels than control rats (Frye et al, 2006) and adolescent rats exposed to prenatal stress presented decreased progesterone turnover into 5α-DHP and AlloP in the medial prefrontal cortex (Paris and Frye, 2011). Moreover, adult male rats that suffered prenatal stress presented decreased plasma 3α-diol levels and decreased hippocampal 5α-DHT levels (Walf and Frye, 2012), as well as greater plasma testosterone concentration and reduced 5α-reductase mRNA levels in the nucleus tractus solitarii (NTS) and in the paraventricular nucleus (PVN) (Brunton et al., 2015). Furthermore, neonatal stress also alters $GABA_AR$ subunits expression in adulthood (Caldji et al., 2000, 2003; Hsu et al., 2003), which may suppose an alteration in the ability of NS to exert their actions (Brunton, 2015). In the same way, changes on neonatal NS levels may not only imply altered GABAergic systems (Grobin and Morrow, 2000; Mòdol et al., 2014a; Yu and Ticku, 1995) but also an altered NS milieu in adulthood. For instance, a single administration of ß-estradiol 3-benzoate to female rats on the day of birth resulted in marked decreases of adult AlloP concentrations in the cerebral cortex and plasma (Calza et al., 2010), in the hypothalamus (Berretti et al., 2014) and in the hippocampus (Locci et al., 2017), as well as in an increased adult expression and function of $GABA_AR$ containing α4/δ in adulthood (Locci et al., 2017). Furthermore, the prenatal administration of Finas reduced hippocampal AlloP levels at PND30 in male and female

rats (Paris et al., 2011). It has to be taken in mind that in various animal models as well as in humans, reduced levels of AlloP in adulthood have been related to several pathophysiological conditions that may imply altered GABAergic tone such as major depression and anxiety disorders (Schüle et al., 2014), premenstrual dysphoric disorder (Backstrom et al., 2014), PTSD (Pibiri et al., 2008; Pinna et al., 2010; Rasmusson et al., 2006) and schizophrenia (Marx et al., 2006). Thus, alterations in adult NS milieu may underlie neonatal stress associated pathologies.

Besides alterations in emotional behaviours and pathologies such as schizophrenia, alterations of NS levels in adulthood have been related to the abuse and addiction of diverse drugs, being AlloP implication on ethanol effects one of the most studied relations. Both acting on $GABA_AR$, AlloP and ethanol share some of their effects (Morrow et al., 2001) and several works have shown that AlloP levels fluctuate in response to ethanol administration or intake. In adult male rats, an AlloP increase in response to ethanol administration has been found in a dose- and time-dependent manner in plasma (Barbaccia et al., 1999), as well as in several brain areas (i.e. cerebral cortex, hippocampus, bed nucleus of the stria terminalis and paraventricular nucleus) (Cook et al., 2014a). Ethanol-induced increase of AlloP is in part mediated by the activation of the HPA axis (Boyd et al., 2010; Khisti et al., 2003; O'Dell et al., 2004; Porcu et al., 2004), but ethanol is also able to locally increase brain synthesis of AlloP independently of adrenal secretion (Cook et al., 2014b). The AlloP rise is enough to enhance GABAergic transmission contributing then to several of the ethanol behavioural effects (Morrow et al., 2006). In this sense, it has been proved that in rats AlloP participates in the modulation of ethanol's anticonvulsant effects (VanDoren et al., 2000), sedation (Khisti et al., 2003), impairment of spatial memory (Matthews et al., 2002; Morrow et al., 2001) and anxiolytic-like actions (Hirani et al., 2005). On the other hand, ethanol consumption does not increase AlloP levels in dependent rats (Janis et al., 1998), fact that has been related to the loss of alcohol pharmacological effects (i.e. tolerance) after chronic ethanol use (Morrow et al., 2001). Moreover, in humans with alcohol withdrawal the levels of AlloP are decreased (Romeo et al., 1996), while AlloP administration in the hippocampus consistently reduces the withdrawal symptoms in rats (Martín-García and Pallarès, 2005). Furthermore, the administration of AlloP has proven to increase the consumption of alcohol in adult non-dependent rats (Janak et al., 1998) but decrease it in adult dependent rats (Morrow et al., 2001). This last effect is also found when AlloP is administered directly in the hippocampus (Martín-García et al., 2007), suggesting that this structure could play a role in the maintenance of ethanol

consumption. AlloP modulation of ethanol intake is dose-dependent: in rodents the administration of low AlloP doses increases ethanol intake but high AlloP doses supress ethanol intake (Ford et al., 2005; Janak et al., 1998; Sinnott et al., 2002). Thus, the fact that both alterations of neonatal NS levels and neonatal stress may produce changes in GABAergic systems and may alter brain's capacity for NS biosynthesis and/or metabolism, could directly affect the susceptibility to ethanol abuse. Furthermore, both neonatal stress and neonatal AlloP manipulations affect adult behavioural traits that have been related to vulnerability to initiate drug abuse, i.e. novelty-directed locomotion and anxiety responses (Belin and Deroche-Gamonet, 2012), and both interventions seem to alter adult striatal and cortical DAergic function (Gizerian et al., 2006; Muneoka et al., 2009; Rentesi et al., 2013), which can be related to drug reinforcing properties.

Taking into account all the previous results, it is of high relevance the study of the possible interactions between neonatal stress and neonatal physiological AlloP levels manipulations and their implications in brain's development and adolescent and adult behaviour.

OBJECTIVES & HYPOTHESIS

OBJECTIVES

The main objective of the present work is to assess the effects of neonatal AlloP manipulations and neonatal stress induced by EMS on adolescent and adult behaviour, in order to study their possible interactions and thus the possible relationship between both factors.

Experiment 1:

- Study the effects of neonatal AlloP administration and neonatal stress on:
  - ► Novelty-directed exploration in adolescent age.
  - ► Anxiety-like behaviour in adult age.
  - ► Processing of sensory inputs to the brain in adulthood.

Experiment 2:

- Study the effects of neonatal physiological AlloP levels manipulation and neonatal stress on the vulnerability to alcohol abuse by assessing:
  - ► Voluntary ethanol consumption in adulthood.
  - ► The activity of the mesolimbic DAergic pathways.

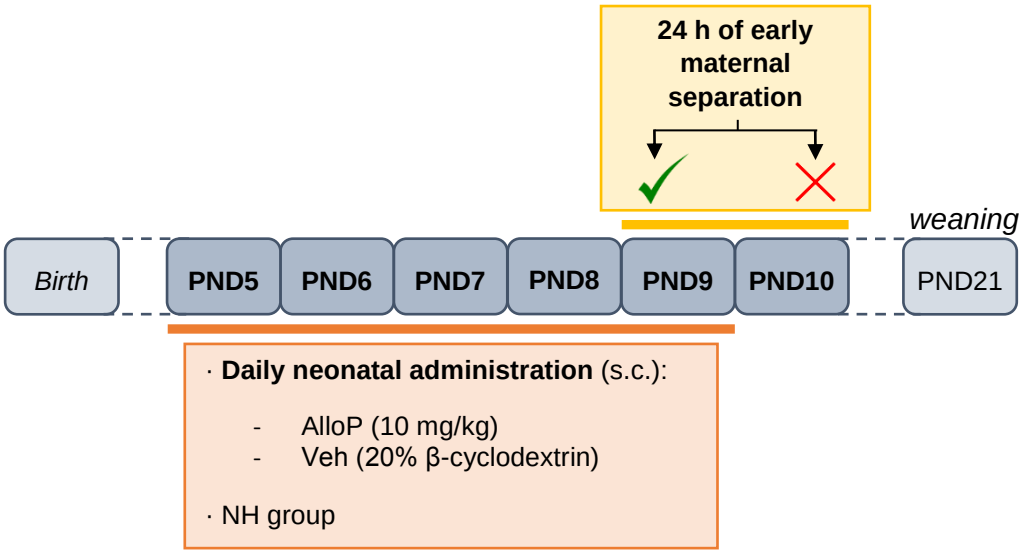
HYPOTHESIS

We hypothesize that the effects of neonatal stress induced by EMS on adolescent and adult behaviour can be related, at least in part, to changes on neonatal NS levels, including AlloP. Thus, the neonatal administration of a neuroactive steroid could interfere with the behavioural alterations caused by neonatal stress. In the same way, the effects of neonatal neuroactive steroid administration could be altered by subsequent EMS.

EXPERIMENT 1

The aim of the first experiment was to study the effects of neonatal AlloP administration, neonatal stress and their interactions on adolescent novelty-directed exploration and on adult anxiety-like behaviour and sensory brain inputs processing. For this purpose, we administered 10 mg/kg of AlloP between PND5 and PND9 to male Wistar rats. Two control groups were used: one administered with the vehicle (Veh) and the other one non-handled (NH). At PND9, half of the animals of each neonatal treatment group were submitted to 24 h of EMS. Novelty-directed exploration was analysed at PND40 (middle adolescence) and at PND60 (late adolescence) by means of the Boissier exploration test. The distance travelled throughout the area was recorded as a measure of novelty-induced motor activity, and the total number of holes explored by the animal was recorded as a measure of exploration directed to a stimulus. At adult age (PND85), anxiety-like behaviour was tested by means of the EPM test, and sensory brain inputs processing was evaluated by means of PPI test. In the EPM test the percentage of the open arm entries and the time spent on them were interpreted as measures of anxiety-like behaviour, the number of entries in closed arms was used as a measure of activity and the time spent in the central area was used as an independent measure of decision making. On the other hand, in the PPI test basal startle amplitude and the percentage of the degree of prepulse inhibition of the startle response were recorded and analysed.

Neonatal manipulations:



Final experimental groups					
NH	*NH + EMS*	*Veh*	*Veh + EMS*	*AlloP*	*AlloP + EMS*
N=10	*N=16*	*N=11*	*N=10*	*N=9*	*N=12*

Behavioural evaluation:

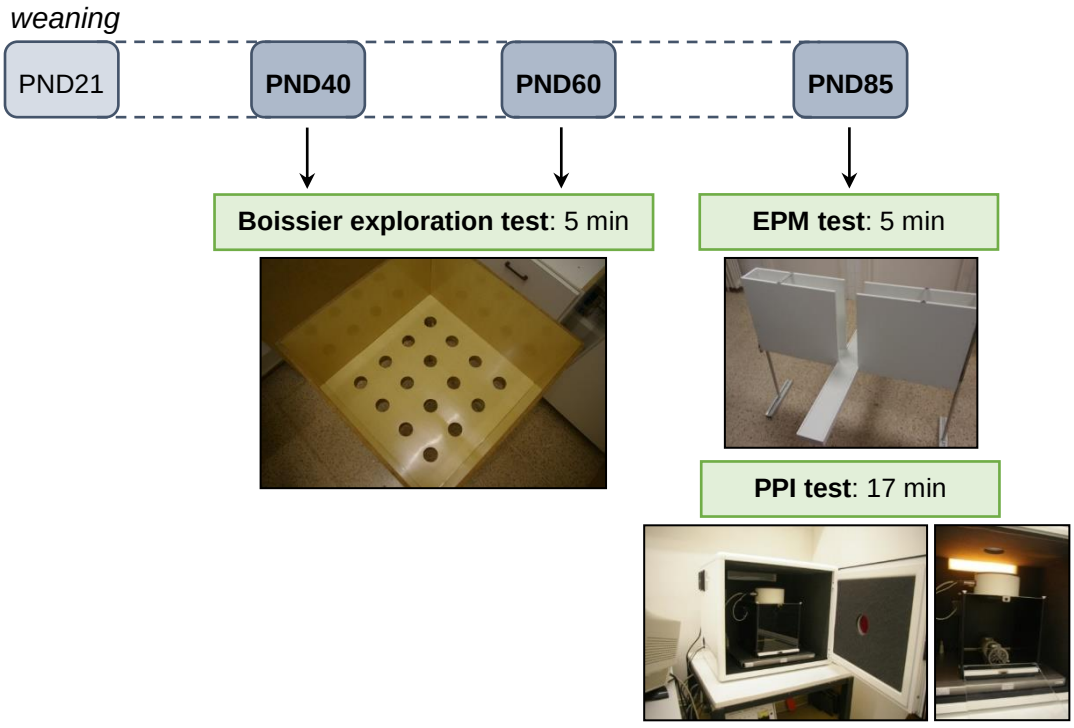


Figure 1. Experimental design of experiment 1.

EXPERIMENT 1 _ RESULTS

Research paper

LLidó, A., Mòdol, L., Darbra, S., Pallarès, M., 2013. Interaction between neonatal allopregnanolone administration and early maternal separation: Effects on adolescent and adult behaviors in male rat. Horm. Behav. 63 (4): 577-85.

Hormones and Behavior 63 (2013) 577–585

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

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

Interaction between neonatal allopregnanolone administration and early maternal separation: Effects on adolescent and adult behaviors in male rat

Anna Llidó, Laura Mòdol, 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

ARTICLE INFO

Article history:
Received 12 November 2012
Revised 28 January 2013
Accepted 1 February 2013
Available online 12 February 2013

Keywords:
Neurosteroids
Allopregnanolone
Early maternal separation
Neurodevelopment
Novelty exploration
Anxiety
Prepulse inhibition

ABSTRACT

Endogenous neurosteroid level fluctuations are related to several emotional and behavioral alterations. Neurosteroids also have important roles during neurodevelopment, with there being a relationship between modification of their levels in neurodevelopmental periods and behavioral alterations in adolescence and adulthood. Early maternal separation (EMS) is a stressful event that also alters neurodevelopment and adolescent and adult behaviors. The aim of the present study is to analyze the interaction between the effects of the neonatal alteration of allopregnanolone (AlloP), neurosteroid that increase its levels after acute stress presentation, and EMS on adolescent exploration and adult anxiety and sensorimotor gating in male rats. AlloP (10 mg/kg s.c.) was administrated between postnatal day 5 (PN5) and PN9, and a single 24-hour period of EMS was carried out on PN9. Exploration was analyzed at PN40 and PN60. At adult age (PN85), anxiety was tested by means of the elevated plus-maze test (EPM), and sensorimotor gating by means of prepulse inhibition test (PPI). PPI deterioration has been considered as a reliable indicator of diseases such as schizophrenia. Results showed that the previous neonatal AlloP administration neutralized the effects of EMS in the adolescent exploration (increase of traveled distance and decrease of head-dips). In adult age, an anxiolytic-like profile was observed as a consequence of EMS. Finally, EMS and neonatal AlloP disrupted PPI. Taken together, these data show the important role that physiological neonatal AlloP levels and stressful events play in neural development, adult behavior and vulnerability to neurodevelopmental disorders such as schizophrenia.

Introduction

Neurosteroids (NS) are a subclass of steroids that can be synthesized *de novo* in the brain from cholesterol, independent of peripheral sources (Baulieu et al., 1981). Allopregnanolone (AlloP), which is also produced by the endocrine glands and in particular by the adrenal glands, is a NS derived from 3α progesterone that acts as a positive modulator of GABAA receptor. In adult, showing a similar profile to that of benzodiazepines, the administration of AlloP has anxiolytic, anticonvulsant and sedative properties (Mellon and Griffin, 2002).

Endogenous levels of NS fluctuate in relation to stress, menopause, pregnancy or menstrual cycle (Mellon and Griffin, 2002). In the rat, the cortical levels of AlloP fluctuate greatly during development, showing a first prenatal peak followed by low levels during birth and first week of life (from postnatal day 0, PN0, to postnatal day 8, PN8), these levels are similar to those found in adult brain. During the second week of life (from PN10 to PN14) a second peak was observed (Grobin et al., 2006).

In the adult brain, GABA is the principal inhibitory neurotransmitter, but early in development GABAA receptor mediates excitatory signals. It has been demonstrated that GABA acts as a neurotrophic factor and it is involved in proliferation, migration, neural differentiation and synaptic maturation (Ben-Ari et al., 2012). As NS can act as positive GABAA receptor modulators, it has been suggested that they could be involved in brain maturation. In this regard, *in vitro* studies have shown that the exposure of neurons and glial cells to NS has trophic effects (Schumacher et al., 2000). Perinatal NS administration can also alter striatal and cortical dopaminergic activities (Muneoka and Takigawa, 2002), and the development of the hippocampus, given that AlloP induces a rise in intracellular calcium in embryonic hippocampal neurons that serves as the initiation mechanism that promotes neurogenesis (Wang and Brinton, 2008). In summary, neonatal AlloP levels are related to neural and glial proliferations, neuronal survival, and myelination (Mellon, 2007). Therefore, changes in neonatal AlloP levels could be related to diverse alterations in brain maturation. In fact, a single AlloP administration (10 mg/kg) at PN5 alters the localization of GABAergic interneurons causing a functional impairment of the prefrontal cortex and dorsal-thalamic pathway in adult rat brain (Grobin et al., 2006).

Several experiments carried out in our laboratory suggest that alterations in neonatal AlloP levels have long-lasting effects that are manifested by behavioral alterations during adolescence and adult age. In this way, the sub-chronic neonatal administration of finasteride (from PN5 to PN9), a substance that reduces AlloP synthesis through the inhibition of the 5α-reductase enzyme (Azzolina et al.,

* Corresponding author. Fax: +34 93 581 20 01.
E-mail address: marc.pallares@uab.cat (M. Pallarès).

0018-506X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2013.02.002

1997), deteriorates passive avoidance in adulthood and induces an anxiogenic-like profile in the elevated plus-maze test (EPM) (Martin-García et al., 2008). Neonatal finasteride administration also decreases the locomotor activity both in open field (OF) in adulthood and in the Boissier test in adolescence (Darbra and Pallarès, 2010). By contrast, sub-chronic neonatal administration of AlloP induces an anxiolytic-like profile in adulthood in the EPM (Darbra and Pallarès, 2012). An acute neonatal (PN5) administration of AlloP alters the action of the benzodiazepine lorazepam, decreasing its anxiolytic effects in the EPM, and increases novelty-directed locomotion in adulthood measured in the OF (Darbra and Pallarès, 2009). Moreover, results obtained by us and by other authors show that neonatal AlloP administration impairs the prepulse inhibition of the acoustic startle response (PPI) in adult age (Darbra and Pallarès, 2010; Grobin et al., 2006). Therefore changes in neonatal AlloP levels could be related with brain development alterations that are manifested by behavioral changes in learning and memory, response to anxiety, exploratory behavior and sensorimotor gating in adolescence and/or adulthood.

On the other hand, animal studies have clearly indicated that exposure to several types of stressors during development produces persistent behavioral disturbances that are associated with hormonal, neurotransmitter and functional changes and resemble an array of psychopathological conditions (Fumagalli et al., 2007). Pre- and postnatal stress conditions are widely embraced as paradigmatic examples of early life adversities (Fumagalli et al., 2007), in this sense, early maternal separation (EMS) has been characterized as a good model of neonatal stress. A variety of procedures have been described depending on the time of separation and the day/days that it is done (Lehmann et al., 2002; Slotten et al., 2006). In this study, we performed a single separation of 24 h in PN9, which has been shown to be enough to cause disruptions in adolescent and adult behaviors (Ellenbroek et al., 1998; Lehmann et al., 2002; Marco et al., 2007). This model, in addition to causing stress to the offspring, alters the behavior of the mother, providing less care and rejecting the litter when they meet again. It has been reported that this procedure of EMS causes a delay in neurodevelopment (Ellenbroek et al., 2005), and reduces the expression of brain-derived neurotrophic factor (BDNF) and glutamate N-methyl-D-aspartate (NMDA) receptors in the hippocampus (Roceri et al., 2002). Moreover, EMS affects the correct maturation of the hypothalamic–pituitary–adrenal axis (HPA) and determines its response to stress in adulthood (Ellenbroek and Cools, 2002; Lehmann et al., 2002).

Similarly to the alterations of neonatal AlloP levels, the neurodevelopment changes caused by the EMS have been related with adolescent and adult behavioral alterations. For instance, the reduced expression of NMDA glutamate receptors in the hippocampus has been related with less cerebral plasticity which results in less memory and learning ability (Roceri et al., 2002). EMS also has been associated with increased susceptibility to psychopathologies related with neurodevelopment disorders, such as schizophrenia (Ellenbroek et al., 1998; Fumagalli et al., 2007).

As it has been described that acute stress increases AlloP levels in brain and plasma (Barbaccia et al., 1998), it seems reasonable to study the relationship between neonatal stress and endogenous AlloP level alterations. Thus, the aim of present work is to study the effects of the previous neonatal AlloP administration to a single EMS episode on adolescent and adult behaviors, and the possible interactions between both interventions. Since the increase in AlloP levels due to acute stress could be related to a protective effect of AlloP (an increase of endogenous AlloP could serve as a biological mechanism to counteract environmental stress), we hypothesize that the neonatal AlloP administration prior to EMS, will neutralize or decrease the EMS effects on adolescent and adult behaviors. In order to study these possible relationships, we administrated 10 mg/kg of AlloP once daily between PN5 and PN9, and we performed one single 24 hour period of EMS on PN9–PN10. Adolescence is a stage in which the animals usually show a behavioral repertoire that includes high levels of exploration, seeking new sensations, high impulsivity, and risk taking (Laviola et al., 2003). An adolescent rodent has been classified by the use of three age-intervals, namely early adolescence (prepubescent or juvenile, PN21–PN34), middle adolescence (periadolescent, PN34–PN46), and late adolescence (young adult, PN46–60). The validity of such an animal model for the purpose of comparison or extrapolation to the human case has been recently endorsed by Spear (2000). These age-intervals had been previously used by us and other groups (Adriani and Laviola, 2004; Adriani et al., 2004; Darbra and Pallarès, 2009; Laviola et al., 2003) to extend the knowledge on the behavioral consequences of neonatal manipulations. At middle and late adolescence we focused on exploratory behavior, animals were evaluated using the Boissier test which involves exploration in a situation of novelty. Since neonatal AlloP administration alters both anxiety-like behavior and the sensorimotor gating in adulthood (see above), we study the possible interaction of AlloP administration and EMS effects on anxiety-like behavior by means of the EPM, and the sensorimotor gating by means of the PPI test at PN85. A deficient PPI is an operative measure for disturbed sensorimotor gating, which is the ability of a sensory event to suppress a spontaneous motor response; PPI is deficient in schizophrenia patients as well as other neuropsychiatric disorders (Swerdlow et al., 2006). PPI disruption in maternally-deprived rats occurs only after the puberty, i.e. the deficits in prepulse inhibition were not detectable before puberty at day 35 (Ellenbroek et al., 1998).

This study continues previous research of our group, but this experiment is the first in examining the effects of possible interactions between neonatal stress and neonatal AlloP administration on exploratory behavior induced by novelty in adolescence and anxiety and sensorimotor gating in adulthood.

Material & methods

Animals

68 male Wistar rats derived from 13 pairings raised in the Laboratori de Psicobiologia at Universitat Autònoma de Barcelona were used. 7 couples were assigned to the EMS group and the other 6 couples were assigned to the no EMS group (we obtained one litter from each couple). 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*. 48 hours after mating, males were separated from females. 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 administration groups (AlloP, vehicle and non-handled, see next section) and all animals within a litter were treated identically. Weaning took place at PN21, the males were separated and were housed in groups of 3 or 4 brothers, and females were sacrificed. 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 (86/609/EEC) for Care and Use of Laboratory Animals.

Neonatal neurosteroid administration

Pups were injected, subcutaneously, with AlloP (10 mg/kg, n = 21) or vehicle (Veh) (n = 21) from PN5 to PN9. 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). To control the possible handling effects, a "not handled"

group (NH) (n = 26) was included, which was neither injected nor manipulated rather than the weekly change of the bedding.

AlloP was dissolved in 0.9% NaCl by sonication for 10 min and suspended in 20% β-cyclodextrin. 20% β-cyclodextrin dissolved in 0.9% NaCl was used as Veh. Injection volume was 0.1 ml/10 g body weight. We used an AlloP concentration of 10 mg/kg based on previous studies that show that this dose causes behavioral alterations in the adult age (Darbra and Pallarès, 2009, 2010; Grobin et al., 2006). All products were obtained from SIGMA (Deisenhofen, Germany).

Early maternal separation

After the last NS administration in PN9, the litters from the 7 couples assigned to the EMS group were isolated from their mothers for a single period of 24 h (AlloP: n = 12; Veh: n = 10; NH: n = 16). Pups were returned to their cages after being injected, but mothers were housed in a different cage in the same room. 24 hours later, the mothers were returned to their respective litters and left undisturbed until weaning, at PN21. In PN9 after last NS administration, the subjects that did not suffer EMS (AlloP: n = 9; Veh: n = 11; NH: n = 10) were quickly reunited with their mothers as the previous days.

We have chosen this model because it allows us to perform the EMS once finished AlloP administration, as it has been documented that manipulations beyond the separation period may interfere with the effects of EMS (Ellenbroek and Cools, 2002).

Behavioral evaluation

The behavioral tests were conducted in adolescence (PN40 and PN60) and in adulthood (PN85). Only male rats were used in this experiment because it has been reported that the consequence of maternal separation on cognition shows sex–gender differences, since more pronounced effects were observed in males than in female animals (Fumagalli et al., 2007). During the middle and late adolescence (PN40 and PN60, respectively) animals conducted the Boissier exploration test and at adulthood they performed the EPM and the PPI test. Animals were weighed before testing.

Boissier exploration test

Boissier exploration test measures motor activity induced by novelty and provides a relatively reliable measurement of stimulus-directed exploratory behavior (File and Wardill, 1975). To perform this test, we used a square wooden arena (58 cm × 58 cm) with 16 equidistant holes of 5 cm in diameter, bounded by walls 40 cm high and the apparatus was elevated 60 cm above the ground. The apparatus was situated in a room lit by a bright light (300 lx mean). Rats were tested individually at PN40 (ranging between PN38 and PN41) and at PN60 (ranging between PN59 and PN62), between 9:00 and 11:00 a.m. The test duration was 5 min per subject, and behavior was evaluated by means of an activity monitoring system (SMART, Letica, Barcelona, Spain). This system is based on the automated analysis of real-time video-images, recorded by a video camera which is suspended from the ceiling over the apparatus and connected with a computer located in the enclosed room. The traveled distance throughout the area was recorded as a measure of novelty-induced motor activity and the number of holes explored by the animal was recorded as a measure of exploration directed to a stimulus (considered explored a hole when the animal introduced his head up to the eye line). After each trial, the apparatus was cleaned with a water solution containing ethanol at 20%, in order to prevent any olfactory-induced behavioral modifications.

Elevated plus maze test

The EPM has been validated as a test that allows to measure behavioral patterns related to anxiety (Pellow et al., 1985). The EPM was a white wooden apparatus shaped like a cross. It had two open arms and two closed arms (10 cm × 50 cm each one) perpendicular to each other, and was elevated 50 cm above the ground. The walls of the closed arms were 40 cm high. The arms were connected by a central square of 10 cm × 10 cm. The maze was placed in a room lit by a dim light (36 lx mean). All tests were videotaped and the behavior was analyzed using the recorded images. The test was performed individually at PN85 (ranging between PN83 and PN87) between 9:00 and 11:00 a.m. Each session lasted 5 min and started placing the animal at the center of the apparatus faced to an open arm. The number of open arm entries and the time spent on them were recorded, and the corresponding percentages were interpreted as measures of anxiety-like behavior. The number of entries in closed arms was used as a measure of activity. Finally, the time spent in the central area was also recorded and was used as an independent measure of decision making (Rodgers and Johnson, 1995). An entry was counted whenever the animal crossed with all 4 paws into an arm. After each trial, the apparatus was cleaned with a water solution containing ethanol at 20%, in order to prevent any olfactory-induced behavioral modifications. After the EPM, the animal performed immediately PPI session.

Prepulse inhibition of the acoustic startle response

PPI was tested in a StartFear system (Letica, Panlab, Barcelona, Spain) that records and analyzes the signal generated by the animal movement through a high sensitivity weight transducer, as previously described (Darbra and Pallarès, 2010). As already indicated, the animals were evaluated individually at PN85 (ranging between PN83 and PN87), between 9:00 and 11:30 a.m., the same day they were evaluated in EPM test and right after this. Each experimental session lasted about 17 min. Throughout the startle session a background level of 70 dB was maintained. Each session began with 5 min of habituation followed by 10 blocks of 5 trials to measure PPI. Each block consisted of one startle trial (pulse of 120 dB for 20 ms, which was delivered to measure basal startle responsiveness), one no-stimulus condition and 3 pairings of stimulus acoustic warning signal (prepulse) with acoustic startle (pulse) administered pseudo-randomly. In these pairings the prepulse was 3, 5 or 10 dB above background. These prepulses were always 20 ms broadband burst and given 100 ms before the startle pulse. The interval between two trials was between 10 and 20 s. The data recorded were basal startle amplitude, which was calculated as the mean of 10 delivered startle trials, and the percentage of the degree of prepulse inhibition of the startle response, which was calculated using the following formula: 100 − (mean of all startle amplitudes on prepulse trials/basal startle amplitude) × 100. After each trial, the apparatus was cleaned with a water solution containing ethanol at 20%, in order to prevent any olfactory-induces behavioral modifications.

Statistical analysis

For data analysis we used STATISTICA package (StatSoft, Tulsa, USA). Body weight data were analyzed using a mixed analysis of variance (ANOVA) with neonatal treatment (NEO, 3 levels: AlloP, Veh, NH) and EMS (2 levels: yes/no) as the between subject factors and AGE (3 levels: PN40, PN60 and PN85) as the within-subject factor. To analyze Boissier data across time, a mixed analysis of variance was used with NEO and EMS as the between subject factors and AGE (2 levels: PN40 and PN60) as the within-subject factor. Data from the EPM, the basal startle response in the PPI test and percentage of inhibition in the PPI were analyzed using a 2-way analysis of variance with NEO and EMS as the between subject factors. Moreover, the data of the prepulse inhibition of acoustic startle response were analyzed using a mixed analysis of variance with NEO and EMS as the between subject factors and prepulse intensity (PULSE, 3 levels: 3, 5 or 10 dB above the background) as the within-subject factor. Significance was set at $P<0.05$, and when they were necessary were carried

out subsequent partitions (the simple effects method) and the corresponding post-hoc Duncan's test. Data are shown in means ± SEM.

Results

Body weight

The analysis of the body weight evolution showed a significant effect of EMS [$F(1,62) = 19.21$, $P<0.001$] and AGE [$F(2,124) = 4234.58$, $P<0.001$], and a significant interaction of EMS × AGE [$F(2,124) = 3.99$, $P<0.05$]. The subsequent analyses split upper by AGE were performed: males that suffered EMS weighed less than males without EMS in all ages, that is at 40, 60 and 85 days old [$F(1,62) = 16.16$, $P<0.001$; $F(1,62) = 20.251$, $P<0.001$; $F(1,62) = 12.87$, $P<0.001$ respectively] (see Table 1). Neonatal AlloP administration had no effect on the weight of the animals.

Boissier exploration test

The ANOVA of traveled distance showed a significant effect of EMS [$F(1,62) = 5.08$, $P<0.05$] and AGE [$F(1,62) = 38.74$, $P<0.001$]. Significant interaction effects of EMS × NEO [$F(2,62) = 3.28$, $P<0.05$] and EMS × AGE [$F(1,62) = 4.80$, $P<0.05$] were also observed, indicating that EMS effects on locomotor activity were different depending on neonatal neurosteroid administration and age at which locomotor behavior was analyzed. In order to analyze these interactions, data from Boissier test were analyzed separately by AGE.

At 40 days, a significant main effect of EMS [$F(1,62) = 8.52$, $P<0.05$] along with an interaction effect of EMS × NEO that just failed to reach statistical signification was observed [$F(2,62) = 2.80$, $P = 0.068$]. Subsequent analyses showed that in control groups (Veh and NH) male rats that suffered EMS traveled significantly more distance than male rats that did not suffer EMS (Duncan: $P<0.05$ for both). However, this effect of EMS was not observed in those animals that previously had received AlloP, given that the traveled distance by the animals treated with AlloP was not statistically different between those that suffered and those that did not suffer EMS. In addition, animals that suffered EMS but had previously received AlloP, traveled the same distance of that animals of control groups (Veh and NH) which did not suffer EMS (see Fig. 1A for detailed post-hoc analysis).

At 60 days, however, no significant effects either of EMS or neonatal AlloP on locomotor activity were observed (see Fig. 1A).

In order to analyze the interaction effects between EMS and AGE on activity, a complementary analysis independently of neonatal treatment was performed. This ANOVA showed a significant effect of EMS [$F(1,66) = 5.06$, $P<0.05$], AGE [$F(1,66) = 38.13$, $P<0.001$], along with a significant interaction of EMS × AGE [$F(1,66) = 4.97$, $P<0.05$]. Post-hoc analysis revealed that the increase of traveled distance due to the EMS was only observed at 40 days old (Duncan: $P<0.001$). This analysis also revealed that at 60 days the distance traveled was lower than at 40 days in all animals (i.e. with and without EMS) (Duncan: $P<0.05$) (40 days: 1888.27 ± 83.73; 60 days: 1319.06 ± 68.09).

Regarding exploration, the ANOVA of head-dips scores showed a significant effect of NEO [$F(2,62) = 5.22$, $P<0.01$], EMS [$F(1,62) = 8.49$, $P<0.01$] and AGE [$F(1,62) = 33.24$, $P<0.001$]. A significant interaction effect of NEO × EMS was also found [$F(2,62) = 3.37$, $P<0.05$]. To analyze this interaction, data obtained at 40 days old and 60 days old were analyzed separately.

At 40 days, a significant effect of NEO [$F(2,62) = 4.11$, $P<0.05$] and EMS [$F(1,62) = 6.98$, $P<0.05$], along with a significant interaction effect of NEO × EMS [$F(2,62) = 3.05$, $P = 0.054$] was found. Post-hoc analysis showed that subjects of the control groups (Veh and NH) that suffered EMS explored significantly less holes than subjects that did not suffer EMS (Duncan: $P<0.05$ for both). This effect of EMS was not observed in those animals that had previously received AlloP, that is there were no differences between animals with AlloP administration and EMS and the groups that did not suffer EMS (see Fig. 1B for detailed post-hoc analysis).

Similar to what we had found on locomotor activity, neither EMS nor neonatal AlloP administration showed significant effects on head-dips scores at 60 days old (see Fig. 1B). Data also showed that head-dips scores decreased across time (from 40 to 60 days old) in all animals (40 days: 17 ± 0.86; 60 days: 11.18 ± 0.77).

Table 1
Body weight evolution.

		Age (in days)		
Group	n	40	60	85
EMS	38	155.52 ± 11.66*	275.92 ± 17.78*	355.29 ± 23.88*
No EMS	30	168.59 ± 16.41	299.61 ± 26.98	378.63 ± 33.04

Body weight (g) development (mean ± sem).
* $P<0.01$ vs. groups without EMS at each age.

Elevated plus maze test

The analysis of the EPM data showed a significant effect of EMS on the percentage of time spent [$F(1,62) = 0.32$, $P<0.01$] as well as an EMS effect on the percentage of entries in the open arms that just failed to reach statistical signification [$F(1,62) = 3.42$, $P = 0.059$]. Animals that suffered EMS entered and spent more time in the open arms than animals that did not suffer EMS, independently of the neonatal treatment (see Figs. 2A and B). No significant effects were found on closed arm entries or on the time spent in the center of apparatus (see Fig. 2C). No other significant main effect or interaction effects were observed.

Prepulse inhibition of the acoustic startle response

To analyze the effects of neonatal AlloP administration and EMS on the PPI test, first we characterized the basal startle amplitude and then we analyzed the percentage of PPI.

Basal startle amplitude

Regarding basal startle response, a NEO [$F(2,62) = 6.98$, $P<0.01$] and an EMS [$F(1,62) = 6.72$, $P<0.05$] effects were observed, without significant interaction of both factors. Post-hoc analysis showed that in AlloP group basal startle response was lower than in control groups (Veh and NH) (Duncan: $P<0.05$). On the other hand, male rats that suffered EMS showed a basal startle response significantly higher than male rats that did not suffer EMS (see Fig. 3).

Percentage of prepulse inhibition of the acoustic startle response

The ANOVA of the percentage of PPI showed a significant NEO effect [$F(2,62) = 4.13$, $P<0.05$], that is AlloP decreased the prepulse inhibition of the acoustic startle response (see Fig. 4); and a significant effect of PULSE [$F(2,124) = 6.72$, $P<0.01$], without significant interaction of both factors. Significant interaction effects of NEO × EMS [$F(2,62) = 2.79$, $P<0.065$] and EMS × PULSE [$F(2,124) = 4.84$, $P<0.01$] were also observed, indicating that the effects of NEO and PULSE depend on whether animals had suffered EMS or not.

In order to analyze these interactions, the analysis was split upper EMS. In the group that did not suffer EMS, a significant effect of NEO [$F(2,27) = 4.71$, $P<0.05$] and PULSE [$F(2,54) = 11.44$, $P<0.001$] was found, without significant interaction of both factors. This effect of the prepulse signal intensity reflects the expected gradual increase in the percentage of inhibition related to the increase in prepulse intensity (see Fig. 5A). Regarding NEO effect, post-hoc analysis showed that, regardless of the prepulse signal intensity, animals treated with AlloP showed a percentage of prepulse inhibition significantly lower than animals without neonatal manipulation (group NH) (Duncan: $P<0.01$) (see Fig. 4). The percentage of prepulse inhibition of AlloP

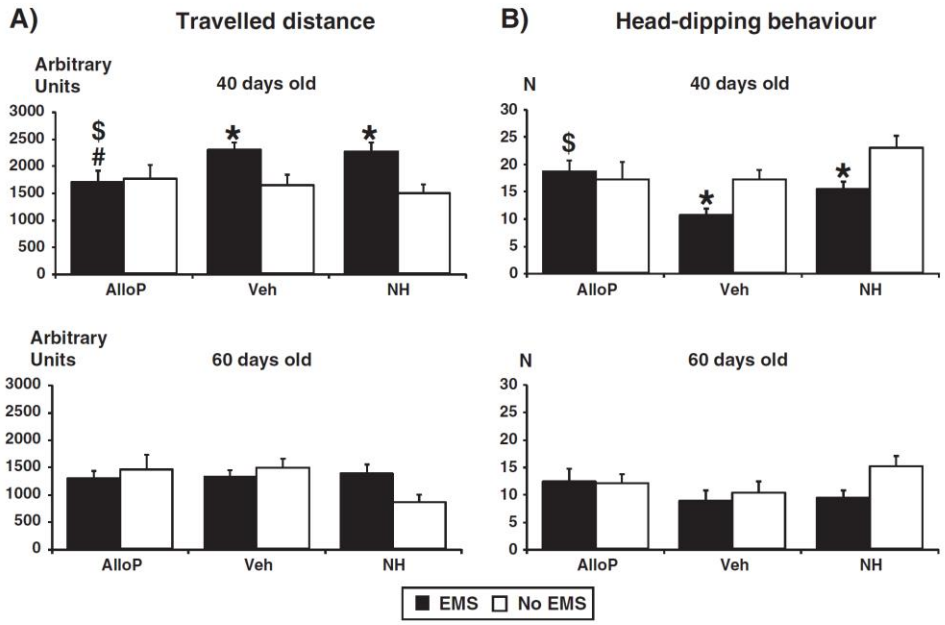


Fig. 1. Neonatal AlloP administration neutralizes EMS effects on exploratory behavior at 40 days old. A) Locomotor activity measured as traveled distance: *P<0.05 vs. group with same treatment but without EMS; $P<0.05 vs. Veh-EMS group; #P<0.05 vs. NH-EMS group. B) Head-dipping behavior: *P<0.05 vs. group with the same treatment but without EMS; $P<0.05 vs. Veh-EMS group. Exploratory behavior was evaluated in the Boissier test at 40 (upper panels) and 60 days old (lower panels). Data are shown in means ± SEM.

group was also lower than the percentage of Veh group, however these differences did not reach statistically significance. No differences were observed between NH and Veh groups.

In animals that suffered EMS, in contrast, no significant effects of NEO, PULSE or NEO × PULSE were observed, indicating that EMS impaired prepulse inhibition (see Fig. 5B).

Discussion

The results of the present study indicate that both adolescent and adult behaviors are altered by EMS and by neonatal AlloP administration, as well as by their interaction. Moreover, maternally-deprived males showed a reduced body weight in adolescence and in adulthood, as previously reported by other authors at similar ages (Ellenbroek et al., 2004; Gruss et al., 2008; Llorente et al., 2007; Rentesi et al., 2010). It has been suggested that reduced body weight of EMS rats is not only a result of the lack of milk intake during the 24 hour separation period, but it could be also attributed to the long-term modified interactions between the mother and their pups (Ellenbroek and Cools, 2002; Ellenbroek et al., 2005; Rentesi et al., 2010). The importance of mother–offspring interactions is supported by several studies that analyzed the effects of natural variation in maternal care (for review see Meaney and Szyf, 2005). In agreement with this, Ellenbroek and Cools (2002) showed that the long-term consequences of EMS are reduced when the maternally deprived rats are being fostered by non-deprived mothers.

Boissier exploration test

Our results show that EMS increases novelty-induced motor activity at 40 days old, and this increase is neutralized by the previous AlloP administration. The increase in locomotor activity caused by EMS is consistent with previous data that showed an increase in spontaneous locomotion in a new environment of adolescent rats that suffered 24 h of EMS at PN9 (Marco et al., 2007). It had been proposed that an increase in traveled distance could reflect the anxiety of the animal that tries to escape from the situation (Weiss et al., 1998), which could be more relevant when animals are not habituated to the apparatus. In order to check this interpretation, we decided to analyze the traveled distance in the virtual 29 cm × 29 cm center zone of the apparatus, since activity within the central zone is considered an anxiety relevant score (Prut and Belzung, 2003; Voikar et al., 2001). Data show that locomotor activity in center zone was lower in the EMS group than in the animals that did not suffer EMS [$F(1,62) = 8.94$, $P<0.01$]. Therefore, the increase in total traveled distance observed in EMS group is due to an increase of activity in the outermost portion of the apparatus (close to the wall).

Our results also show that EMS decreases head-dipping behavior, an effect that is also neutralized by prior AlloP administration. Since the number of explored holes is a traditional measure of exploration directed to a stimulus that reflects the attention given to new situations and the motivation to seek out new experiences (File and Wardill, 1975), a decrease in this is interpreted both as a decrease in the motivation to seek out new experiences and as an elevated level of anxiety in a new environment (Takeda et al., 1998). Taken together, our results could suggest that EMS causes an anxiogenic-like profile in adolescence (i.e. increase in traveled distance and decrease in head-dipping in the Boissier test at 40 days old). In this sense, other authors reported an increase of anxiety in adolescent mice after suffering 24 h of EMS at PN12 in the EPM (Martini and Valverde, 2011).

Our results indicate that the effects on novelty induced behavior caused by EMS could be neutralized by prior AlloP administration. Since there is no neutralization of the EMS effect in the Veh group, this effect is specific for AlloP and not due to the previous manipulations. In this sense, it would be plausible to relate this protective effect of AlloP administration on the novelty induced behavior caused by EMS to its well-established positive modulatory action on GABAA receptor. The previous increase in AlloP levels, produced by the exogenous administration, could cause desensitization or a down-regulation of GABAA receptor, interfering with the physiological response to the

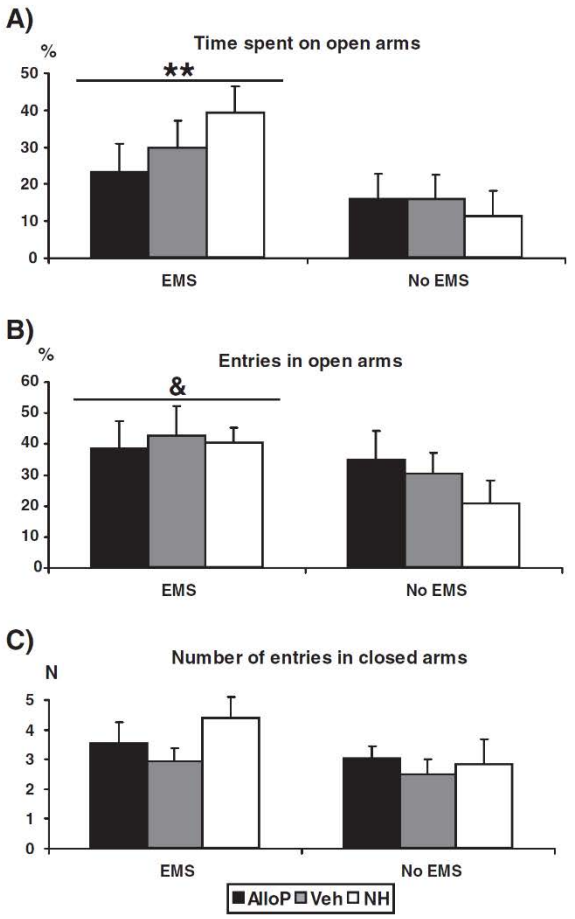


Fig. 2. EMS effects on anxiety-like behavior at 85 days old. A) Time spent on open arms: **P<0.01 vs. groups without EMS. B) Percentage of entries into open arms: $P = 0.059 vs. groups without EMS. C) Number of entries into closed arms. Anxiety-like behavior was evaluated in the elevated plus maze test. Data are shown in means ± SEM.

stress produced by EMS. In this regard, as mentioned in the Introduction section, an increase in AlloP levels after an acute stress has been reported in adulthood (Barbaccia et al., 1998). However, more studies are needed to confirm this hypothesis.

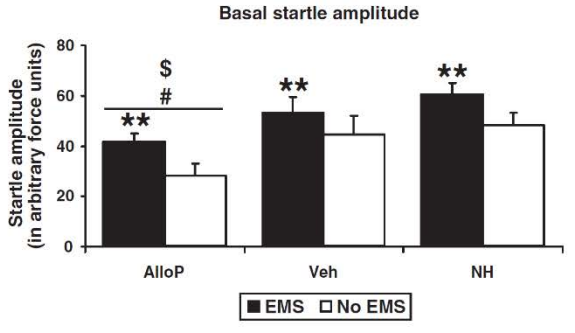


Fig. 3. Neonatal AlloP administration and EMS effects on the basal startle amplitude. Neonatal AlloP administration decreased the basal startle amplitude ($P<0.05 vs. Veh groups and #P<0.05 vs. NH groups). EMS increased the basal startle amplitude (**P<0.01 vs. group with same NS treatment but without EMS). Data are shown in means ± SEM.

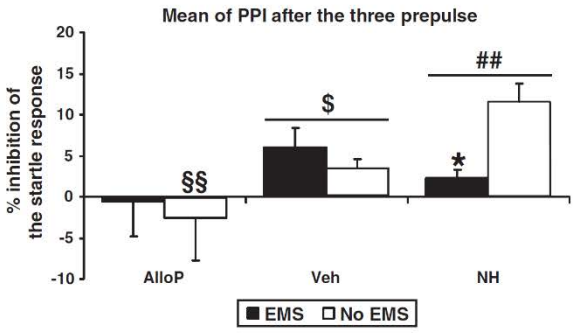


Fig. 4. Neonatal AlloP administration and EMS effects on the prepulse inhibition. AlloP administration impaired the prepulse inhibition (§§P<0.01 vs. NH-no EMS group; $P<0.05 vs. AlloP groups; ##P<0.01 vs. AlloP groups). EMS impaired the prepulse inhibition (*P<0.05 vs. NH-no EMS group). Prepulse inhibition is expressed as a collapsed mean of all prepulse intensities. Data are shown in means ± SEM.

At 60 days old there were no effects of EMS or AlloP. Since it was the second time that animals performed the same test (i.e. 40 and 60 days) habituation could have occurred (File, 2001). In fact, it has been reported that the repeated exposure of adult subjects to a novel hole-board apparatus greatly affects the behavioral response, and that the neophobic response experienced by subjects during the first exposure to an apparatus apparently declines with further exposures (Brown and Nemes, 2008). So, since the results can be altered by emotional states of fear and anxiety when faced with the new environment (Weiss et al., 1998), the fact that the apparatus no longer

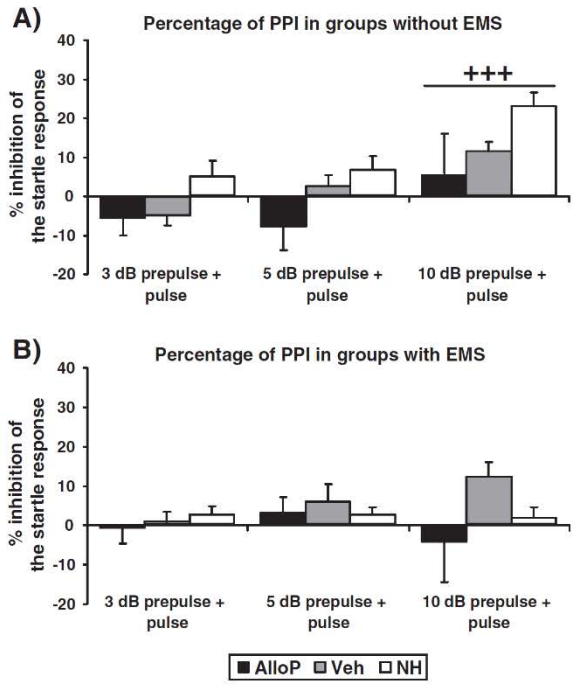


Fig. 5. Evolution of the percentage of prepulse inhibition of the acoustic startle response after the three prepulse signal intensities. A) The consequent improvement of PPI percentage related to the increase in prepulse intensity was observed in no EMS groups (+++P<0.001 vs. 3 dB prepulse trials and 5 dB prepulse trials). B) EMS impairs the expected progressive reduction of startle response after the increase in prepulse intensity. Data are shown in means ± SEM.

produces the same neophobia levels could also explain why EMS effects were not observed at 60 days old. On the other hand, all animals (i.e. regardless of neonatal treatment) traveled less distance and explored fewer holes at 60 days than at 40 days old. The decrease in traveled distance with age, which it has been already observed in previous studies (Darbra et al., 2003), is due to the specific behavioral profile that the animals had during adolescence. At 40 days old animals exhibit behaviors that involve novelty seeking and risk taking, with higher levels of impulsivity than those shown in adulthood, as well as elevated basal levels of locomotor and explorative activity (Laviola et al., 2003). In this sense, it has been described that adolescent male rats (PN33–35) under some previous circumstances can be more anxious in the EPM test than adults (PN70–75) (Doremus et al., 2004).

Elevated plus maze test

The EMS seems to cause an anxiolytic-like profile in the EPM performed in adulthood, since the animals that suffered EMS entered into and spend more time in the open arms, while there was no alteration in the number of closed arms entries. Previous studies already observed an anxiolytic-like profile in the EPM test of mice that suffered 24 h of EMS on PN9 (Fabricius et al., 2008). A decrease in anxiety was also observed as a consequence of 3 daily hours of separation during the first 2 weeks of life in female Wistar rats (Eklund and Arborelius, 2006). However, other studies reported an anxiogenic-like profile in EMS rats (Rentesi et al., 2010) or did not find any effect (Lehmann et al., 1999; Slotten et al., 2006). These discrepancies may be due to the use of a different strain of rats, since the EMS effects strongly depend on the strain of rats used (Ellenbroek and Cools, 2000), as well as on the different EMS procedures and the specific conditions under which it is performed, since the slightest variation in the deprivation model influences the results and makes it difficult to compare and contrast them (Ellenbroek and Cools, 2002). The test used to measure anxiety also has to be taken into account (Faturi et al., 2010). Furthermore, the characteristics of the EPM apparatus may vary (e.g. color or material), as well as the testing procedure and the environmental conditions, such as lighting in the room where the test is performed; all factors that can affect the results (García et al., 2005; Jones and King, 2001; Violle et al., 2009). Since there is no difference between animals in the time spent in the center of apparatus, we can say that the decision making in adulthood was not affected by the EMS (Rodgers and Johnson, 1995).

The apparently contrary profile of EMS in adolescence (anxiogenic-like profile) and adulthood (anxiolytic-like profile) may be due to the specific behavioral characteristics of adolescent age, as well as to the neurodevelopment that still takes place during adolescence, which suggest that the effects of neonatal interventions vary across the time. In fact, it has been described that some behavioral alterations caused by 24 h of EMS at PN9 do not develop until adulthood (Ellenbroek and Cools, 2002). However, it is also possible that such behavioral differences are related to the characteristics of the different tests used (Boissier exploration test and EMP).

We did not find any effects of neonatal AlloP administration on EPM behavior. These results replicate previous studies from our lab that showed that a daily dose of 10 mg/kg administered from PN5 to PN9, as well as a single administration of 10 mg/kg on PN5, had no effects on the EPM in adulthood (Darbra and Pallarès, 2009, 2012, respectively). In contrast, a daily dose of 20 mg/kg from PN5 to PN9 produced an anxiolytic-like profile in the EPM test in adulthood (Darbra and Pallarès, 2012).

Prepulse inhibition of the acoustic startle response

In the PPI test, neonatal AlloP administration causes a decrease in the basal startle response, as well as a deterioration in the prepulse inhibition of this response. Basal startle reflex is a cross-species, stereotyped response consequent to the presentation of a sudden and unexpected sensory stimulus (Grillon, 2008). It consists of a rapid sequential muscle contraction with the likely purpose of facilitating the flight reaction and/or to protect the body from a sudden attack (Grillon, 2008). The decrease in this response indicates that animals with neonatal AlloP administration are less reactive to auditory stimulus of high intensity. This result replicates our previous studies where we found that the same neonatal AlloP administration caused a decrease of basal startle response (Darbra and Pallarès, 2010). Animal, and more recently, human investigations have shown that the basic startle response can be increased by behavioral manipulations causing fear and anxiety (Grillon, 2008). Thus, the neonatal alterations in AlloP levels could have led to a decrease in the animals' fear levels (Darbra and Pallarès, 2010). In addition, neonatal AlloP administration impairs the prepulse inhibition of the startle response. Prepulse inhibition represents an index of sensorimotor gating (the ability of a weak sensory event to inhibit – "gate" – the motor response to an intense sensory stimulus) (Swerdlow et al., 2001). Animals with neonatal AlloP administration did not show the expected progressive reduction of startle response (and the consequent improvement of the PPI percentage) after the gradual increase in prepulse intensity (3, 5 and 10 dB above background), which indicates an alteration on the sensorimotor gating of these animals. We found this effect of neonatal AlloP administration in previous studies (Darbra and Pallarès, 2010). A deterioration of PPI in the adult rat was also observed with a single administration of AlloP in PN2 or PN5 (Grobin et al., 2006). Prepulse inhibition is impaired in several psychopathologies, including schizophrenia and post-traumatic stress (Ellenbroek et al., 1998; Swerdlow et al., 2006). Dysfunctions in this response have been associated with alterations in thalamocortical connections (Grobin et al., 2006), and in the hippocampus (Darbra et al., 2012).

As regards EMS effects, we found that the animals that suffered EMS exhibited an enhanced startle response and a disruption in prepulse inhibition, despite the previous neonatal treatment. Other studies found no effect of EMS on the basal startle response at 69 days old in Wistar rats (Ellenbroek and Cools, 2002; Ellenbroek et al., 1998). We hypothesize that these divergences are related to the specific conditions under which the test was performed. Ellenbroek et al. left the animals undisturbed in a room adjacent to the startle chamber room for at least 30 or 45 min before the PPI test, whereas the PPI test was carried out just after the EPM test in our experiment. Basal startle response can be potentiated by behavioral manipulations causing fear and anxiety (Grillon, 2008), so it could be that our animals were more stressful or more active at the time of testing. In this sense, stimulant drugs like methamphetamine also cause an increase of the basal startle response (Mizuno et al., 2010). Moreover, the behavior of animals that suffered EMS can be altered by the previous exposure to an acute stress (Estanislau and Morato, 2005).

Furthermore, the disruption of the prepulse inhibition found in those animals that had suffered EMS has been extensively studied and our results are consistent with previous studies (Ellenbroek and Cools, 2002; Ellenbroek et al., 1998). In fact, maternal deprivation has been proposed as a model of schizophrenia, leading to dopamine increases within the neostriatum (Ellenbroek and Cools, 2002).

Conclusions

In summary, the results obtained in the present study indicate that a daily administration of 10 mg/kg of AlloP from PN5 to PN9 does not affect the exploration behavior during adolescence or the anxiety-like behavior during adulthood in male rats. On the other hand, EMS causes an increase in locomotor activity, as well as a decrease in the head-dipping behavior in the exploration test of Boissier during adolescence, which could be indicating an increased anxiety when confronting to new environments in adolescence. Interestingly,

prior neonatal AlloP administration seems to have a protective effect against the effects of EMS on locomotor activity and exploration in adolescence. In adulthood, animals that suffered EMS showed an anxiolytic-like profile in EPM test, indicating that the effects of neonatal interventions vary across the time. In the PPI test, both neonatal AlloP administration and EMS cause a disruption in sensorimotor gating in adulthood. Although AlloP administration can neutralize some effects of EMS on novelty-exploration behaviors in the adolescence, it seems that neonatal AlloP administration is not protective against the deleterious effects of EMS on adult PPI behavior. Indeed, neonatal AlloP administration can deteriorate *per se* the PPI performance. This study points out the relevance of the neonatal AlloP levels for proper brain maturation and the importance of the stressful events during neurodevelopment on adolescent and adult behaviors. Results obtained also indicate the relevance of neonatal manipulations in the increased susceptibility to suffer neurodevelopmental disorders such as schizophrenia.

Acknowledgments

This work was supported by grants from the Spanish Ministry of Science and Innovation (PSI2009-13759).

References

Adriani, W., Laviola, G., 2004. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav. Pharmacol. 15 (5–6), 341–352.
Adriani, W., Granstrem, O., Macri, S., Izykenova, G., Dambinova, S., Laviola, G., 2004. Behavioral and neurochemical vulnerability during adolescence in mice: studies with nicotine. Neuropsychopharmacology 29 (5), 869–878.
Azzolina, B., Ellsworth, K., Andersson, S., Geissler, W., Bull, H.G., Harris, G.S., 1997. Inhibition of rat a-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J. Steroid Biochem. Mol. Biol. 61, 55–64.
Barbaccia, M.L., Concas, A., Serra, M., Biggio, G., 1998. Stress and neurosteroids in adult and aged rats. Exp. Gerontol. 33 (7–8), 697–712.
Baulieu, E.E., Fluxe, K., Gustafsson, J.A., Weterberg, L., 1981. Steroid Hormones in the Brain: Several Mecahnism? In Steroid Hormone Regulation of the Brain. Pergamon Press, Oxford, pp. 3–14.
Ben-Ari, Y., Khalilov, I., Kahle, K.T., Cherubini, E., 2012. The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. Neuroscientist 18 (5), 467–486.
Brown, G.R., Nemes, C., 2008. The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? Behav. Process. 78 (3), 442–448.
Darbra, S., Pallarès, M., 2009. Neonatal allopregnanolone increases novelty-directed locomotion and disrupts behavioural responses to GABAA receptor modulators in adulthood. Int. J. Dev. Neurosci. 27, 617–625.
Darbra, S., Pallarès, M., 2010. Alterations in neonatal neurosteroids affect exploration during adolescence and prepulse inhibition in adulthood. Psychoneuroendocrinology 35 (4), 525–535.
Darbra, S., Pallarès, M., 2012. Effects of early postnatal allopregnanolone administration on elevated plus maze anxiety scores in adult male Wistar rats. Neuropsychobiology 65 (1), 20–27.
Darbra, S., Garau, A., Balada, F., Sala, J., Martí-Carbonell, M.A., 2003. Perinatal hypothyroidism effects on neuromotor competence, novelty-directed exploratory and anxiety-related behaviour and learning in rats. Behav. Brain Res. 143, 209–215.
Darbra, S., Mòdol, L., Pallarès, M., 2012. Allopregnanolone infused into the dorsal (CA1) hippocampus increases prepulse inhibition of startle response in Wistar rats. Psychoneuroendocrinology 37 (4), 581–585.
Doremus, T.L., Varlinskaya, E.I., Spear, L.P., 2004. Age-related differences in elevated plus maze behaviour between adolescent and adult rats. Ann. N.Y. Acad. Sci. 1021, 427–430.
Eklund, M.B., Arborelius, L., 2006. Twice daily long maternal separations in Wistar rats decreases anxiety-like behaviour in females but does not affect males. Behav. Brain Res. 172 (2), 278–285.
Ellenbroek, B.A., Cools, A.R., 2000. The long-term effects of maternal deprivation depend on the genetic background. Neuropsychopharmacology 23 (1), 99–106.
Ellenbroek, B.A., Cools, A.R., 2002. Early maternal deprivation and prepulse inhibition. The role of the postdeprivation environment. Pharmacol. Biochem. Behav. 73, 177–184.
Ellenbroek, B.A., van-den-Kroonenberg, P.T.J.M., Cools, A.R., 1998. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr. Res. 30, 251–260.
Ellenbroek, B.A., de Bruin, N.M., van-den-Kroonenburg, P.T., van Luijtelaar, E.L., Cools, A.R., 2004. The effects of early maternal deprivation on auditory information processing in adult Wistar rats. Biol. Psychiatry 55, 701–707.
Ellenbroek, B.A., Derks, N., Park, H.J., 2005. Early maternal deprivation retards neurodevelopment in Wistar rats. Stress 8, 247–257.
Estanislau, C., Morato, S., 2005. Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. Behav. Brain Res. 163, 70–77.
Fabricius, K., Wörtwein, G., Pakkenberg, B., 2008. The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus. Brain Struct. Funct. 212, 403–416.
Faturi, C.B., Tiba, P.A., Kawakami, S.E., Catallani, B., Kerstens, M., Suchecki, D., 2010. Disruptions of the mother–infant relationship and stress-related behaviours: altered corticosterone secretion does not explain everything. Neurosci. Biobehav. Rev. 34, 821–834.
File, S.E., 2001. Factors controlling measures of anxiety and responses to novelty in the mouse. Behav. Brain Res. 125, 151–157.
File, S.E., Wardill, A.G., 1975. Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacology 44, 53–59.
Fumagalli, F., Molteni, R., Racagni, G., Riva, M.A., 2007. Stress during development: Impact on neuroplasticity and relevance to psychopathology. Prog. Neurobiol. 81 (4), 197–217.
García, A.M., Cárdenas, F.P., Morato, S., 2005. Effect of different illumination levels on rat behaviour in the elevated plus-maze. Physiol. Behav. 85, 265–270.
Grillon, C., 2008. Models and mechanisms of anxiety: evidence from startle studies. Psychopharmacology 199, 421–437.
Grobin, A.C., Gizerian, S., Lieberman, J.A., Morrow, A.L., 2006. Perinatal allopregnanolone influences prefrontal cortex structure, connectivity and behavior in adult rats. Neuroscience 138, 809–819.
Gruss, M., Braun, K., Frey, J.U., Korz, V., 2008. Maternal separation during a specific postnatal time window prevents reinforcement of hippocampal long-term potentiation in adolescent rats. Neuroscience 152, 1–7.
Jones, N., King, S.M., 2001. Influence of circadian phase and test illumination on preclinical models of anxiety. Physiol. Behav. 72, 99–106.
Laviola, G., Macrì, S., Morley-Fletcher, S., Adriani, W., 2003. Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. Neurosci. Biobehav. Rev. 27, 19–31.
Lehmann, J., Pryce, C.R., Bettschen, D., Feldon, J., 1999. The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. Pharmacol. Biochem. Behav. 64, 705–715.
Lehmann, J., Russig, H., Feldon, J., Pryce, C.R., 2002. Effect of a single maternal separation at different pup ages on the corticosterone stress response in adult and aged rats. Pharmacol. Biochem. Behav. 73, 141–145.
Llorente, R., Arranz, L., Marco, E.M., Moreno, E., Puerto, M., Guaza, C., De la Fuente, M., Viveros, M.P., 2007. Early maternal deprivation and neonatal single administration with a cannabinoid agonist induce long-term sex-dependent psychoimmunoendocrine effects in adolescent rats. Psychoneuroendocrinology 32, 636–650.
Marco, E.M., Adriani, W., Canese, R., Podo, F., Viveros, M.P., Laviola, G., 2007. Enhancement of endocannabinoid signalling during adolescence: modulation of impulsivity and long-term consequences on metabolic brain parameters in early maternally deprived rats. Pharmacol. Biochem. Behav. 86, 334–345.
Martin-García, E., Darbra, S., Pallarès, M., 2008. Neonatal finasteride induces anxiogenic-like profile and deteriorates passive avoidance in adulthood after intrahippocampal neurosteroid administration. Neuroscience 154, 1497–1505.
Martini, M., Valverde, O., 2011. A single episode of maternal deprivation impairs the motivation for cocaine in adolescent mice. Psychopharmacology 219 (1), 149–158.
Meaney, M.J., Szyf, M., 2005. Maternal care as a model for experience-dependent chromatin plasticity? Trends Neurosci. 28 (9), 456–463.
Mellon, S.H., 2007. Neurosteroid regulation of central nervous system development. Pharmacol. Ther. 116 (1), 107–124.
Mellon, S.H., Griffin, L.D., 2002. Neurosteroids: biochemistry and clinical significance. Trends Endocrinol. Metab. 13, 35–43.
Mizuno, M., Kawamura, H., Ishizuka, Y., Sotoyama, H., Nawa, H., 2010. The anthraquinone derivative emodin attenuates methamphetamine-induced hyperlocomotion and startle response in rats. Pharmacol. Biochem. Behav. 97, 392–398.
Muneoka, K.T., Takigawa, M., 2002. A neuroactive steroid, pregnenolone, alters the striatal dopaminergic tone before and after puberty. Neuroendocrinology 75, 288–295.
Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.
Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33.
Rentesi, G., Antoniou, K., Marselos, M., Fotopoulos, A., Alboycharali, J., Konstandi, M., 2010. Long-term consequences of early maternal deprivation in serotonergic activity and HPA function in adult rat. Neurosci. Lett. 480, 7–11.
Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B.A., Riva, M.A., 2002. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol. Psychiatry 7, 609–616.
Rodgers, R.J., Johnson, N.J.T., 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze. Pharmacol. Biochem. Behav. 52, 297–303.
Schumacher, M., Akwa, Y., Guennoun, R., Robert, F., Labombarda, F., Desarnaud, F., Robel, P., De Nicola, A.F., Baulieu, E.E., 2000. Steroid synthesis and metabolism in the nervous system: trophic and protective effects. J. Neurocytol. 29, 307–326.
Slotten, H.A., Kalinichev, M., Hagan, J.J., Marsden, C.A., Kevin, C.F., Fone, K.C.F., 2006. Long-lasting changes in behavioural and neuroendocrine indices in the rat following neonatal maternal separation: gender-dependent effects. Brain Res. 1097, 123–132.
Spear, L.P., 2000. The adolescent brain and age-related behavioural manifestations. Neurosci. Biobehav. Rev. 24, 417–463.
Swerdlow, N.R., Geyer, M.A., Braff, D.L., 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 156 (2–3), 194–215.
Swerdlow, N.R., Light, G.A., Cadenhead, K.S., Sprock, J., Hsieh, M.H., Braff, D.L., 2006. Startle gating deficits in a large cohort of patients with schizophrenia: relationship

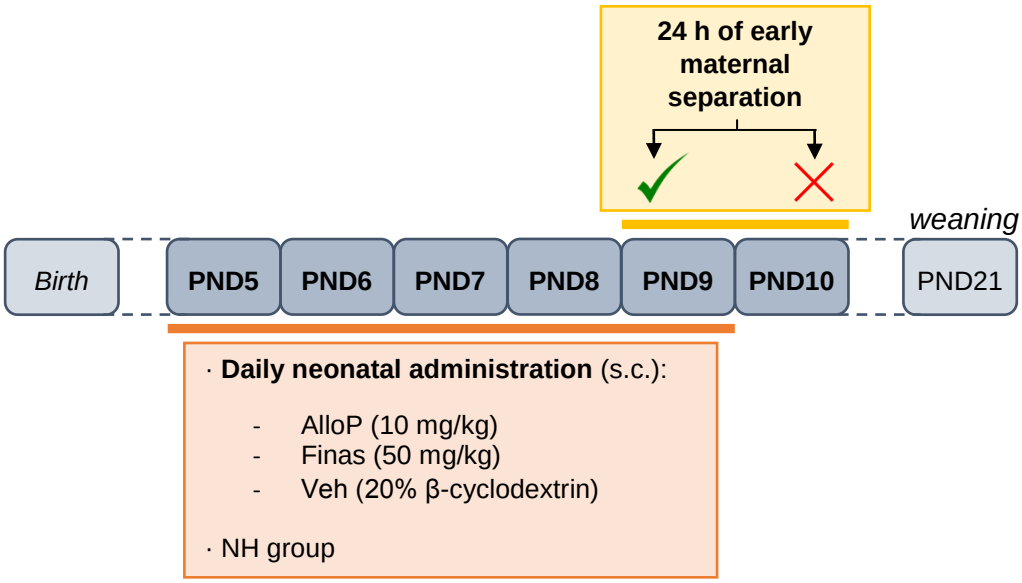
to medications, symptoms, neurocognition, and level of function. Arch. Gen. Psychiatry 63 (12), 1325–1335.
Takeda, H., Tsuji, M., Matsumiya, T., 1998. Changes in head-dipping behavior in the holeboard test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol. 350 (1), 21–29.
Violle, N., Balandras, F., Le Roux, Y., Desor, D., Schroeder, H., 2009. Variations in illumination, closed wall transparency and/or extramaze space influence both baseline anxiety and response to diazepam in the rat elevated plusmaze. Behav. Brain Res. 203, 35–42.
Voikar, V., Koks, S., Vasar, E., Rauvala, H., 2001. Strain and gender differences in the behavior of mouse lines commonly used in transgenic studies. Physiol. Behav. 72, 271–281.
Wang, J.M., Brinton, R.D., 2008. Allopregnanolone-induced rise in intracellular calcium in embryonic hippocampal neurons parallels their proliferative potential. BMC Neurosci. (Suppl. 2), S11.
Weiss, S.M., Wadsworth, G., Fletcher, A., Dourish, C.T., 1998. Utility of ethological analysis to overcome locomotor confounds in elevated plus maze. Neurosci. Biobehav. Rev. 23, 265–271.

EXPERIMENT 2

The second set of experiments was designed to study the effects of neonatal physiological AlloP levels manipulation and neonatal stress on the vulnerability to alcohol abuse. For this, we evaluated the effects of both neonatal interventions on adult voluntary ethanol consumption and ventrostriatal monoamine levels (experiment 2A). The neonatal NS levels were manipulated in male Wistar rats via the sub-chronic administration of AlloP (10 mg/kg) or of the 5α-reductase inhibitor, Finas (50 mg/ kg), between PND5 and PND9. There were also two control groups: one administered with Veh and the other one NH. At PND9 half of the subjects of each group were separated from the mother for 24 h. Voluntary ethanol consumption was measured in adulthood (PND70) using a two-bottle free-choice procedure for 15 consecutive days. Possible alterations on ventrostriatal monoamine levels were determined after the voluntary alcohol consumption procedure.

Given the results obtained in this experiment, we designed a second one in order to further evaluate the possible alterations on the activity of the rewarding DAergic pathways caused by neonatal NS levels manipulation (experiment 2B). Male Wistar rats received sub-chronic administration of AlloP (10 mg/kg), Finas (50 mg/kg) or Veh between PND5 and PND9. In adulthood, voluntary ethanol consumption was measured using a two-bottle free-choice procedure for 12 consecutive days. Last day of consumption, the DA and 3,4-dihydroxyphenylacetic acid (DOPAC) release in the nucleus accumbens (NAcc) in response to ethanol intake was evaluated by means of *in vivo* microdialysis.

Neonatal manipulations:



Final experimental groups							
NH	*NH + EMS*	*Veh*	*Veh + EMS*	*AlloP*	*AlloP + EMS*	*Finas*	*Finas + EMS*
N=18	*N=13*	*N=18*	*N=10*	*N=14*	*N=14*	*N=15*	*N=14*

Behavioural evaluation:

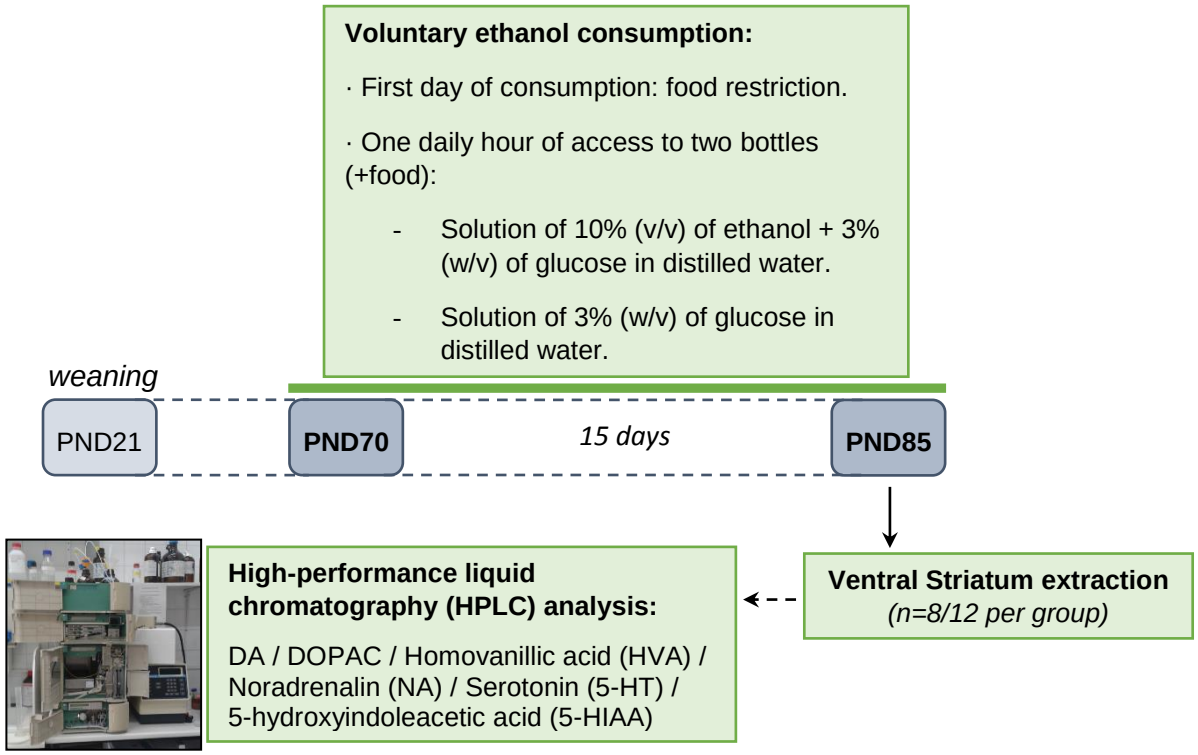


Figure 2. Experimental design of experiment 2A.

EXPERIMENT 2A _ RESULTS

Research paper

Llidó, A., Bartolomé, I., Darbra, S., Pallarès, M., 2016. Effects of neonatal allopregnanolone manipulations and early maternal separation on adult alcohol intake and monoamine levels in ventral striatum of male rats. Horm. Behav. 82: 11-20.

Hormones and Behavior 82 (2016) 11–20

Contents lists available at ScienceDirect

Hormones and Behavior

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

Effects of neonatal allopregnanolone manipulations and early maternal separation on adult alcohol intake and monoamine levels in ventral striatum of 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

ARTICLE INFO

Article history:
Received 21 December 2015
Revised 22 March 2016
Accepted 14 April 2016
Available online 16 April 2016

Keywords:
Allopregnanolone
Finasteride
Early maternal separation
Ethanol consumption
Dopamine
Serotonin
Ventral striatum

ABSTRACT

Changes in endogenous neonatal levels of the neurosteroid allopregnanolone (AlloP) as well as a single 24 h period of early maternal separation (EMS) on postnatal day (PND) 9 affect the development of the central nervous system (CNS), causing adolescent/adult alterations including systems and behavioural traits that could be related to vulnerability to drug abuse. In rats, some behavioural alterations caused by EMS can be neutralised by previous administration of AlloP. Thus, the aim of the present work is to analyse if manipulations of neonatal AlloP could increase adult alcohol consumption, and if EMS could change these effects. We administered AlloP or finasteride, a 5α-reductase inhibitor, from PND5 to PND9, followed by 24 h of EMS at PND9. At PND70 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 15 days. Ventral striatum samples were obtained to determine monoamine levels. Results revealed that neonatal finasteride increased both ethanol and glucose consumption, and AlloP increased alcohol intake compared with neonatal vehicle-injected animals. The differences between neonatal groups in alcohol consumption were not found in EMS animals. In accordance, both finasteride and AlloP animals that did not suffer EMS showed lower levels of dopamine and serotonin in ventral striatum. Taken together, these results reveal that neonatal neurosteroids alterations affect alcohol intake; an effect which can be modified by subsequent EMS. Thus, these data corroborate the importance of the relationship between neonatal neurosteroids and neonatal stress for the correct CNS development.

Introduction

During development, rat cortical levels of the neurosteroid (NS) allopregnanolone (AlloP) fluctuate greatly (Grobin and Morrow, 2001): rat pups present a first prenatal peak that lasts until birth followed by a progressive decrease during the first week of life; in the second week, a new elevation in AlloP levels occurs, reaching maximum values between postnatal day (PND) 10 and PND14. The alteration of this pattern has been related to several behavioural alterations in adolescence and/or adulthood (Darbra et al., 2014; Darbra and Pallarès, 2009, 2010, 2012; Grobin et al., 2006; Llidó et al., 2013; Mòdol et al., 2013, 2014), showing that neonatal AlloP levels might play an important role during development. Regarding anxiety and exploratory behaviour, we have observed that sub-chronic neonatal administration (from PND5 to PND9) of AlloP induces an anxiolytic-like profile in adulthood in the elevated plus-maze test (EPM) (Darbra and Pallarès, 2012), and an acute neonatal injection of AlloP in PND5 increases novelty-directed locomotion measured in the open field (OF) in adulthood (Darbra and Pallarès, 2009). By contrast, the sub-chronic neonatal administration of finasteride (Finas), an inhibitor of the enzyme 5α-reductase needed for the progesterone conversion to AlloP (Azzolina et al., 1997), decreases the locomotor activity in the Boissier exploration test in adolescence (Darbra and Pallarès, 2010). Moreover, manipulations of neonatal AlloP levels alter the behavioural responses to systemic progesterone or hippocampal AlloP administration in adulthood (Darbra et al., 2014; Mòdol et al., 2013, 2014).

On the other hand, alterations in the mesolimbic pathway have also been found. In this sense, neonatal administration of pregnenolone, the progesterone precursor, alters striatal and cortical dopaminergic activities in adulthood (Muneoka et al., 2009, 2002; Muneoka and Takigawa, 2002); and neonatal AlloP administration alters the striatal dopaminergic activity in adulthood (Muneoka et al., 2009). Thus, neonatal AlloP manipulations could affect adolescent and adult behaviours related to dopamine function, such as drug seeking behaviour (Nutt et al., 2015).

AlloP fluctuates with stress, specifically, AlloP brain and plasma levels increase after acute stress (Purdy et al., 1991), while they decrease after chronic stress (Serra et al., 2000). Changes in AlloP levels due to stress have also been reported during the neonatal period (Frye et al., 2006; Kehoe et al., 2000). Neonatal stress causes behavioural

* Corresponding author at: Departament de Psicobiologia i Metodologia de les Ciències de la Salut, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain.
E-mail address: marc.pallares@uab.cat (M. Pallarès).

http://dx.doi.org/10.1016/j.yhbeh.2016.04.003
0018-506X/

alterations in adolescence and adulthood related with several hormonal, neurotransmitter and functional disturbances (for a review see Fumagalli et al., 2007). Between PND4 and PND14 rats are in the stress hyporesponsive period (SHRP) (Sapolsky and Meaney, 1986). During SHRP rats show attenuated adrenocorticotropin (ACTH) and corticosterone (CORT) responses to environmental stressors (Rosenfeld et al., 1992; Sapolsky and Meaney, 1986), which is thought to represent a protective mechanism to prevent the detrimental effects of increased levels of CORT on the brain (de Kloet et al., 1988; Ellenbroek and Cools, 2002). Severe stressors during this period can overcome the SHRP and elicit increases in both ACTH and CORT, causing multiple neurodevelopmental alterations. A single period of 24 h of early maternal separation (EMS) in PND9 represents a potent stressor that increases basal CORT levels (Avishai-Eliner et al., 1995; Suchecki et al., 1995; Viveros et al., 2010) and enhances the responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to further stressors, both neonatally (Avishai-Eliner et al., 1995; Suchecki et al., 1993, 1995), and in adulthood (Lehmann et al., 2002; Viveros et al., 2009). In addition to affect the correct maturation of the HPA axis, 24 h of EMS on PND9 also causes a neurodevelopmental delay (Ellenbroek et al., 2005), alters serotonergic function in the prefrontal cortex and amygdala (Rentesi et al., 2013) and increases symptoms related to schizophrenia in animal models (Ellenbroek et al., 2005; Fumagalli et al., 2007). Moreover, EMS (in PND9) modifies the locomotor response to amphetamine, suggesting an altered development of the mesolimbic dopaminergic system (Ellenbroek et al., 2005); and it alters dopaminergic function in striatum, prefrontal cortex and amygdala in adulthood (Rentesi et al., 2013). In a previous work we observed that 24 h of EMS in PND9 increased novelty-induced locomotor activity and decreased head-dipping behaviour on Boissier exploration test in adolescence, and caused an anxiolytic-like profile in the EPM in adulthood (Llidó et al., 2013). Interestingly, neonatal administration of AlloP prior the EMS (10 mg/kg per day, between PND5 and PND9) neutralized the EMS effects on novelty-induced exploration in adolescence, but not the EMS effects on adult anxiety scores on the EPM (Llidó et al., 2013). Other authors have shown that, when applied concomitantly with the stressful challenge, GABA-positive NS such as THDOC can attenuate the behavioural and neuroendocrine consequences of repeated maternal separation during early life (Patchev et al., 1997); moreover, a single administration of 2 mg/kg of AlloP injected just prior 12 h of EMS in PND5 counteract the increased HPA response to subsequent stressors both in infants and adult rats (Mitev et al., 2003). In the same sense, early deprivation neutralizes the anxiolytic-like profile of neonatal AlloP (5 mg/kg) measured in the EPM in adult age (Zimmerberg and Kajunski, 2004). Thus, interactions between neonatal stress and AlloP alterations have been described, suggesting that the increase in brain AlloP levels could be related to a mechanism that counteract the deleterious effects of stress. It has also been suggested that transient increase of NS biosynthesis may contribute to stress hyporesponsiveness during early infancy (Mitev et al., 2003).

Thereby, considering that: (a) neonatal manipulation of NS such as AlloP alters adult behaviours related to anxiety, novelty seeking and pre-attentional processes (Gizerian et al., 2006; Darbra and Pallarès, 2009, 2010, 2012; Llidó et al., 2013); (b) neonatal AlloP levels in the second week of life seem to be important for the maturation of several brain structures, such as dopaminergic systems (Gizerian et al., 2006; Muneoka et al., 2009) and GABAergic thalamo-cortical connections (Grobin et al., 2003, 2006); (c) AlloP levels are normally increased after stress situations in order to neutralize their deleterious effects (Purdy et al., 1991; Girdler and Klatzkin, 2007); (d) the application of environmental stressors during the neonatal SHRP can overcome it and elicit increases in CORT levels (Avishai-Eliner et al., 1995; Suchecki et al., 1995; Viveros et al., 2010); and (e) previous AlloP manipulations can neutralize some of the neonatal stress effects (Llidó et al., 2013; Mitev et al., 2003); the experimental manipulation of neonatal NS, including AlloP, can be a good model to study neonatal disturbances that can affect brain maturation and subsequent individual responses to environmental stimuli in adult ages.

In adulthood, AlloP levels have been related with several aspects of alcohol abuse and addiction. Many studies have reported an increase in endogenous AlloP concentrations after an acute ethanol administration, even though inter-species response varies (see Porcu and Morrow, 2014 for review). In adult male rats the AlloP increase has been found in a dose- and time-dependent manner in the cerebral cortex, hippocampus, bed nucleus of the stria terminalis, and plasma (Barbaccia et al., 1999; Cook et al., 2014), and it seems to modulate some of the ethanol effects (Morrow et al., 2001). Moreover, ethanol consumption does not increase AlloP levels in the plasma or cerebral cortex of dependent rats, a fact which has been related to the loss of alcohol pharmacological effects (tolerance) after chronic alcohol use (Morrow et al., 2001). On the other hand, the administration of AlloP has proved to increase the consumption of alcohol in adult non-dependent rats but decrease it in adult dependent rats (Morrow et al., 2001). This last effect is also found when the AlloP is administered directly in the hippocampus (Martín-García et al., 2007), suggesting that this structure could play a role in the maintenance of ethanol consumption. Furthermore, AlloP levels decrease in subjects with alcohol withdrawal (Romeo et al., 1996), and AlloP administration in the hippocampus consistently decreases the withdrawal symptoms in rats (Martín-García and Pallarès, 2005).

Given that (1) changes in neonatal AlloP levels alter novelty-directed locomotion and anxiety responses, traits which have been related to vulnerability to initiate drug abuse (Belin and Deroche-Gamonet, 2012), and can also alter adult striatal and cortical dopaminergic function, which can be related to drug reinforcing properties; and that (2) adult endogenous AlloP levels seem important for alcohol intake, tolerance and withdrawal; we hypothesise that alterations in neonatal AlloP levels could increase drug abuse vulnerability in adulthood, and particularly alcohol abuse. Moreover, since EMS also causes behavioural and developmental alterations that can be related with vulnerability to drug abuse, and given our previous results showing that some of the adolescent behavioural alterations induced by EMS can be avoided by previous neonatal AlloP administration (Llidó et al., 2013); we hypothesise that the alterations on voluntary alcohol consumption related to neonatal AlloP manipulations could differ depending on whether animals have or have not suffered subsequent EMS. For this purpose, we administered 10 mg/kg of AlloP or 50 mg/kg of Finas from PND5 to PND9, and performed a single period of 24 h EMS on PND9. In adulthood (PND70) we measured the voluntary alcohol consumption using a two-bottle free-choice model for 15 consecutive days. After the alcohol consumption period, ventral striatum samples were obtained to determine monoamine levels. This is the first study that investigates the possible implications of altered neonatal AlloP levels in adult alcohol consumption and its possible relationship with neonatal stress.

Material and methods

Animals

116 Male Wistar rats derived from 25 pairings raised in the Laboratori de Psicobiologia at Universitat Autònoma de Barcelona were used. 14 couples were assigned to the no-EMS group and the other 11 couples were assigned to the EMS group, obtaining only one litter from each couple. 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*. 48 h after mating, males were separated from females. 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. Weaning

took place at PND21, the males were separated and were housed into groups of brothers (2 to 4 subjects per cage), and females were sacrificed. 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.

Neonatal neurosteroid administration

Pups were injected, subcutaneously, with AlloP (3α-hydroxy-5α-pregnan-20-one) (10 mg/kg, n = 28), Finas (*N-tert*-Butyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide) (50 mg/kg, n = 29) or vehicle (Veh) (n = 28) 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 >12 min). To control the possible handling effects, a non-handled group (NH) (n = 31) was included, which was neither injected nor manipulated rather than the weekly change of the bedding. No visible animal health alterations were detected during development as a consequence of the neonatal treatment. No rejection from the dam was observed, no cannibalism was registered, and pup mortality before weaning was absent. Nevertheless, it is not possible to rule out whether different neonatal treatments elicited differences in mothering style in the dam, which in turn could have affected adult behaviour (Weaver et al., 2004).

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 doses were chosen based on previous experiments (Darbra and Pallarès, 2009, 2010; Grobin et al., 2006; Llidó et al., 2013). Moreover, this dose has been proved to be non-sedative (Grobin et al., 2003) and a similar dose (8 mg/kg) administered to adult rats raises cortical AlloP levels to the range observed after environmental stress exposition (forced swim) (Vallée et al., 2000). All products were obtained from SIGMA (Deisenhofen, Germany).

Early maternal separation

After the last injection in PND9, litters assigned to the EMS groups were isolated from their mothers for a single period of 24 h (AlloP: n = 14; Finas: n = 14; Veh: n = 10; NH: n = 13). Pups were returned to their cages after being injected, but mothers were housed in a different cage in the same room. 24 h later, the mothers were returned to their respective litters and left undisturbed until weaning, at PND21. In PND9 after last NS administration, the subjects that did not suffer EMS (AlloP: n = 14; Finas: n = 15; Veh: n = 18; NH: n = 18) were quickly returned to their mothers as the previous days. This procedure has been previously used (Llidó et al., 2013).

Alcohol consumption

Animals were housed individually two days before starting the procedure in order to acclimate to the new condition, remaining with *ad libitum* access to water and food. Alcohol consumption took place for 15 consecutive days, starting at PND70 (ranging between PND68 and PND71). Rats had 1 h per day limited access to two bottles, one containing a solution of 10% (v/v) of ethanol plus 3% (w/v) glucose in distilled water, and the other one with a solution of 3% (w/v) of glucose in distilled water. Glucose was added to avoid taste aversion and ensure consumption, as used in previous experiments (Martín-García et al., 2007; Martín-García and Pallarès, 2005). Solutions were presented in plastic bottles with a safety valve that prevented spillage and evaporation. Bottles position was changed daily at random in order to avoid position bias. First day of alcohol consumption, the food was retired together with bottles after the hour of access and rats remained with food restriction for the rest of the procedure, receiving 5 g of food per day (this ration was adjusted when was needed in order to avoid that animals lost >15% of their initial weight). Their daily food ration was provided along with the solutions during the access hour. For the rest of the day, animals had *ad libitum* access to water.

Rats were weighted every day before the access to food and solutions in order to calculate their food ration and the ethanol dose consumed. Bottles were weighted every day before and after the access hour to them in order to determine the consumption. Ethanol and glucose doses daily consumed (g ethanol or g glucose/kg body weight) as well as daily ethanol solution preference (ethanol solution consumed/(ethanol solution consumed + glucose solution consumed)) were calculated. Ethanol solution was prepared using ethanol absolute (synthesis grade 99.9%) from Scharlab (Barcelona, Spain). The glucose used was D(+)-glucose anhydrous from Panreac (Barcelona, Spain).

Sample extraction and high-performance liquid chromatography analysis

Last day of consumption, part of the animals of each group (AlloP: n = 12; AlloP + EMS: n = 11; Finas: n = 8; Finas + EMS: n = 11; Veh: n = 12; Veh + EMS: n = 10; NH: n = 11; NH + EMS: n = 11) were sacrificed by decapitation during the hour of access to the solutions and food. Ventral Striatum samples were extracted, weighted and immediately frozen on dry ice and stored at −80 °C. L-3.4-dihydroxyphenylalanine (L-DOPA), Dopamine (DA), Noradrenalin (NA), 3.4-Dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA), Serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels were determined by high-performance liquid chromatography analysis (HPLC); and the corresponding turnover ratios of DA (DOPAC/DA, HVA/DA) and 5-HT (5-HIAA/5-HT) were calculated.

Ventral striatum samples were homogenised at proportion 1/10 according their weight (subsequent dilutions were made when needed), using a Polytron, into an acid buffer that contained perchloric acid 0.25 M, sodium metabisulphite 100 μM and ethylenediaminetetraacetic acid (EDTA) 250 μM. The mixture was kept at −80 °C for at least 24 h before centrifugation at 12000 rmp for 10 min, 4 °C. The corresponding supernatants were stored at −80 °C until the analysis day when they were unfrozen and injected (20 μl) into the HPLC system coupled to an ESA coulometric electrochemical detector. The mobile phase consisted of 50 mM citric acid, 210 μM sodium citrate, 50 μM EDTA and 1.21 mM octanesulfonic acid (SOS) in H_2O MilliQ; triethylamine (TEA) was added to adjust the pH to 2.75. Elution was performed at a flow rate of 0.8 ml/min. External standards were used at a concentration range of 2.5–200 pg/μl. The retention times for the standards were: NA (3.72 min), L-DOPA (4.03 min), DA (5.37 min), DOPAC (6.84 min), 5-HIAA (11.78 min), HVA (14.10 min), 5-HT (20.39 min). The HPLC system used was a Hitachi Elite LaChrom with L-2130 pump, L-2200 autosampler and L-2300 column oven. The reversed analytical column used was a Chromolith Performance RP-18e (4.6 mm diameter, 100 mm length); a guard column (Chromolith Guard Cartridge kit RP-18e (4.6 mm diameter, 5 mm length)) was also used. The electrochemical detector used was a Coulochem 5100 (ESA; Chelmsford, MA) with a high sensitivity dual-electrode analytical cell (Model 5011); the potential of electrode 1 was set at 5 mV and electrode 2 was set at 400 mV.

Statistical analysis

For data analysis we used STATISTICA package (StatSoft, Tulsa, USA). Alcohol consumption variables (ethanol dose, glucose dose and ethanol preference), were analysed using a mixed analysis of variance with neonatal treatment (NEO, 4 levels: AlloP, Finas, Veh, NH) and EMS (2 levels: yes/no) as the between subject factors, and DAYS (14 levels: from day 1 to 14; last day was not included in the analysis since animals were

sacrificed in the middle of the hour of access to the solutions and food) as the within-subject factor. To analyse the concentrations of L-DOPA, DA, NA, DOPAC, HVA, 5-HT and 5-HIAA in the ventral striatum samples, as well as the estimated DA (DOPAC/DA, HVA/DA) and 5-HT (5-HIAA/5-HT) turnover ratios, we use two-way ANOVAs with NEO and EMS as between subject factors. Correlation analyses between consumption variables (ethanol and glucose doses) and ventrostriatal monoamine and metabolite levels were performed. Effect sizes were estimated by calculating the partial η^2 (SSeffect / SSeffect + SSerror). Significance was set at $P < 0.05$, and subsequent partitions and the corresponding post hoc Duncan's test were used when necessary. Data are shown in means ± SEM.

Results

Solutions consumption

Ethanol dose

On the ethanol dose consumed throughout the 14 days of procedure we found a significant main effect of EMS [$F(1108) = 9.78$, $P < 0.001$, $\eta^2 = 0.91$] and DAYS [$F(13,1404) = 116.67$, $P < 0.001$, $\eta^2 = 0.99$], along with significant interactions EMS × DAYS [$F(13,1404) = 5.97$, $P < 0.001$, $\eta^2 = 0.86$] and NEO × EMS × DAYS [$F(39,1404) = 2.61$, $P < 0.001$, $\eta^2 = 0.72$]. For detailed main EMS effect see Fig. 1D (mean of ethanol intake (14 days)).

In order to study the NEO × EMS × DAYS interaction following our experimental hypothesis, the analysis was split upper EMS. In the group of animals that did not suffer EMS, we found a DAYS [$F(13,793) = 43.87$, $P < 0.001$, $\eta^2 = 0.98$] effect and a significant interaction NEO × DAYS [$F(39,793) = 2.89$, $P < 0.001$, $\eta^2 = 0.74$]. The post-hoc analysis showed that during the first 7 days of procedure there were no differences between groups on the ethanol dose consumed. However, from day 8 animals with neonatal Finas administration consumed higher doses of ethanol than AlloP and control animals (Veh and NH) (see Fig. 1A for detailed post-hoc analysis). In addition, we observed that animals that received neonatal Veh administration presented lower ethanol intake than the rest of the animals (that is: Finas, AlloP and NH) in the two last days of consumption, presenting AlloP animals statistically the same ethanol doses consumed as NH animals (see Fig. 1A). On the other hand, in the group of animals that suffered EMS we only found a significant main effect of DAYS [$F(13,611) = 73.86$, $P < 0.001$, $\eta^2 = 0.99$], showing that the increase in the amount of ethanol consumed among days was independent of the neonatal treatment (see Fig. 1B). Thus, the analysis indicated no differences in the ethanol dose consumed between the different NEO groups when animals had suffered EMS.

In order to analyse the effects of EMS on ethanol consumption, an additional ANOVA split upper NEO was performed. We found a DAYS effect on all the groups (AlloP: [$F(13,338) = 20.69$, $P < 0.001$, $\eta^2 = 0.95$]; Finas: [$F(13,351) = 35.20$, $P < 0.001$, $\eta^2 = 0.97$]; Veh: [$F(13,338) = 30.87$, $P < 0.001$, $\eta^2 = 0.97$]; NH: [$F(13,377) = 34.64$, $P < 0.001$, $\eta^2 = 0.97$]), indicating that alcohol intake was increasing among days in all groups. We

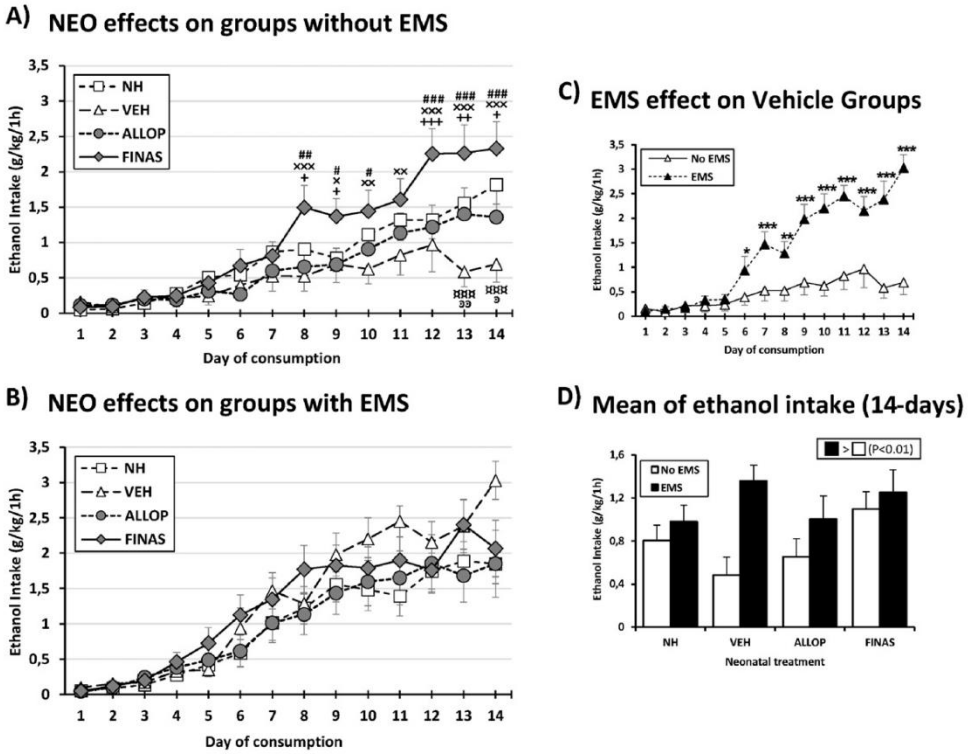


Fig. 1. Voluntary alcohol consumption (1 h limited access paradigm). A) NEO effects on groups without EMS. Animals that received neonatal Finas consumed higher doses of ethanol: $^{+}$indicates differences vs. NH group, $^{\times}$vs. Veh group and $^{\#}$vs. AlloP group. On days 13 and 14, animals that received Veh consumed lower doses of ethanol than the rest of the animals: $^{\alpha}$indicates differences vs. NH group, $^{\beta}$vs. AlloP group and $^{\varepsilon}$vs. Finas group. Post-hoc analysis showed an increase in ethanol intake across time (Day 1 vs. Day 14) in NH, AlloP and Finas groups ($P < 0.001$ for each comparison), but not in Veh rats (NS). B) NEO effects on groups with EMS. Neonatal treatment differences were not observed. Post-hoc analysis showed an increase in ethanol intake across time (Day 1 vs. Day 14) in all groups ($P < 0.001$ for each comparison). C) EMS effect on Vehicle groups. EMS increased ethanol consumption in those animals that previously received Veh: *indicates differences vs. No EMS group. D) Mean of ethanol intake (14 days). EMS increased the mean ethanol dose intake independently of NEO treatment. One sign: $P < 0.05$, two signs: $P < 0.01$, three signs: $P < 0.001$.

also observed a significant main EMS effect [$F(1,26) = 12.41$, $P < 0.01$, $\eta^2 = 0.93$] along with a significant interaction EMS × DAYS [$F(13,338) = 11.85$, $P < 0.001$, $\eta^2 = 0.92$] only in those animals that previously received Veh. That is, there were no differences between no-EMS and EMS rats in NH (EMS: [$F(1,29) = 0.67$, N.S.]; EMS × DAYS: [$F(13,377) = 1.27$, N.S.]), AlloP (EMS: [$F(1,26) = 1.68$, N.S.]; EMS × DAYS: [$F(13,338) = 1.08$, N.S.]), and Finas (EMS: [$F(1,27) = 0.36$, N.S.]; EMS × DAYS: [$F(13,351) = 1.15$, N.S.]). Thus the EMS effect on alcohol intake was only statistically significant in Veh group. Subsequent analyses showed that, from day 6 to 14, animals that had been administered with Veh and had not suffered EMS consumed lower doses of ethanol than those which had received Veh and had suffered EMS. The significant differences between no-EMS and EMS groups obtained in neonatal Veh animals can be observed in Fig. 1C.

Glucose dose

The analysis of the glucose dose consumed showed a significant main effect of NEO [$F(3,108) = 4.10$, $P < 0.01$, $\eta^2 = 0.80$], EMS [$F(1,108) = 5.19$, $P < 0.05$, $\eta^2 = 0.84$] and DAYS [$F(13,1404) = 16.86$, $P < 0.001$, $\eta^2 = 0.94$] (see Fig. 2A for main NEO and EMS effects), as well as significant interactions NEO × DAYS [$F(39,1404) = 2.74$, $P < 0.001$, $\eta^2 = 0.73$] and EMS × DAYS [$F(13,1404) = 2.10$, $P < 0.05$, $\eta^2 = 0.68$]. Thus, there was no significant interaction between NEO treatment and EMS. Subsequent analysis revealed that animals that suffered EMS consumed higher doses of glucose than animals that did not suffer EMS, from day 2 to day 8 (Duncan: $P < 0.001$ on days 4–6, $P < 0.01$ on days 2–3 and 8, $P < 0.05$ on day 7). On the other hand, animals administered with neonatal AlloP, consumed lower doses of glucose than the rest of the animals on days 3–5, while animals that received neonatal Finas globally consumed higher doses of glucose than the animals of the other groups between days 7 and 14 independently of EMS treatment. Given that there was no NEO × EMS interaction, in Fig. 2B has been represented the NEO × DAY effects on pooled EMS + no-EMS groups.

Ethanol preference

Results of alcohol preference parallel those reported for solutions consumption (ethanol and glucose doses). We found a significant main effect of DAYS [$F(13,1404) = 24.58$, $P < 0.001$, $\eta^2 = 0.96$], along with significant interactions EMS × DAYS [$F(13,1404) = 2.71$, $P < 0.001$, $\eta^2 = 0.73$] and NEO × EMS × DAYS [$F(39,1404) = 1.71$, $P < 0.01$, $\eta^2 = 0.63$]. In the group of animals that did not suffer EMS, we only found a significant main effect of DAYS [$F(13,793) = 6.35$, $P < 0.001$, $\eta^2 = 0.86$] showing no neonatal treatment differences on the ethanol preference progression (see Fig. 3A). Preference for alcohol in Finas subjects was not higher because neonatal Finas administration increased both alcohol and glucose consumption. By contrast, we found a significant main effect of NEO [$F(3,47) = 2.94$, $P < 0.05$, $\eta^2 = 0.75$] and DAYS [$F(13,611) = 21.50$, $P < 0.001$, $\eta^2 = 0.96$], along with a significant interaction of NEO × DAYS [$F(39,611) = 1.72$, $P < 0.01$, $\eta^2 = 0.63$] in the group of animals that suffered EMS. The subsequent analysis revealed that animals that received neonatal AlloP presented a higher ethanol preference than animals of the control groups during the firsts days of consumption (see Fig. 3B for detailed post-hoc analysis), related to the

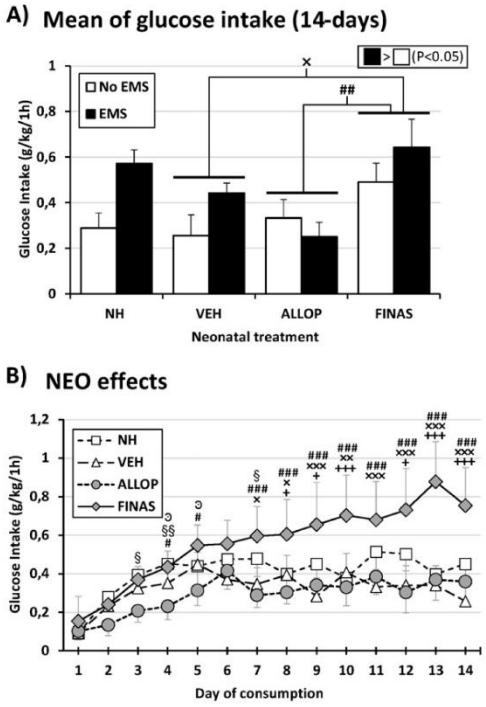


Fig. 2. Glucose consumption (1 h limited access paradigm). A) Mean of glucose intake (14-days). Independently of EMS condition, Finas increased glucose intake: ˣindicates differences vs. Veh group and #vs. AlloP group. All animals that suffered EMS consumed higher doses of glucose than animals that did not suffer EMS. B) NEO effects. Independently of EMS condition (pooled EMS + no-EMS groups), neonatal AlloP decreased glucose intake: $indicates differences vs. NH, ɔvs. Veh and #vs. Finas group; and Finas increased glucose intake: +indicates differences vs. NH group, ˣvs. Veh group and #vs. AlloP group. One sign: $P < 0.05$, two signs: $P < 0.01$, three signs: $P < 0.001$.

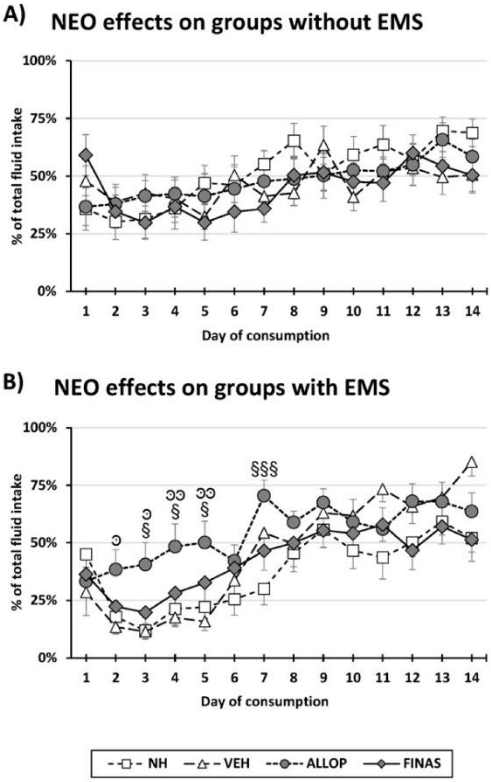


Fig. 3. Ethanol preference. A) NEO effects on groups without EMS. No neonatal treatment effects were observed on the ethanol preference progression. B) NEO effects on groups with EMS. Animals that received neonatal AlloP presented a higher ethanol preference than animals of the control groups during the firsts days of consumption: $indicates differences vs. NH ɔvs. Veh. One sign: $P < 0.05$, two signs: $P < 0.01$, three signs: $P < 0.001$.

decrease in glucose consumption that AlloP group present those days, as indicated above (see Glucose dose results).

Ventral striatum samples

The analysis of the DA levels on the ventral striatum samples revealed a significant main effect of EMS [$F(1,78) = 15.88$, $P < 0.001$, $\eta^2 = 0.94$] and a significant interaction NEO × EMS [$F(3,78) = 5.79$, $P < 0.01$, $\eta^2 = 0.85$]. The subsequent analysis showed that animals that received neonatal AlloP or Finas and did not suffer EMS had a lower concentration of DA than control animals (Veh and NH groups) as well as than animals that suffered EMS (see Fig. 4A for detailed post-hoc analysis). No effects of NEO, EMS or NEO × EMS were found on DOPAC and HVA levels (see Figs. 4B and 4C). In both turnover ratios of DA (DOPAC/DA and HVA/DA) EMS and NEO × EMS effects were found (DOPAC/DA: EMS [$F(1,78) = 20.53$, $P < 0.001$, $\eta^2 = 0.95$], NEO × EMS [$F(3,78) = 6.43$, $P < 0.001$, $\eta^2 = 0.87$]; HVA/DA: EMS [$F(1,78) = 10.71$, $P < 0.01$, $\eta^2 = 0.91$], NEO × EMS [$F(3,78) = 3.18$, $P < 0.05$, $\eta^2 = 0.76$]). Post-hoc analysis revealed that animals that received neonatal AlloP or Finas and did not suffer EMS, displayed a higher DOPAC/DA and HVA/DA ratios than NH animals and animals that suffered EMS (see Table 1). L-DOPA was not detectable in any of the ventral striatum samples.

On the other hand, on NA levels we found a significant main effect of NEO [$F(3,78) = 2.81$, $P < 0.05$, $\eta^2 = 0.74$], showing that animals that received neonatal AlloP (globally: with and without EMS) had a lower concentration of NA than those treated with Finas or those not-manipulated (Duncan: $P < 0.05$ for both) (see Fig. 4D).

The analysis of 5-HT levels showed a main significant effect of NEO [$F(3,78) = 9.92$, $P < 0.001$, $\eta^2 = 0.91$], EMS [$F(1,78) = 32.14$, $P < 0.001$, $\eta^2 = 0.97$] and a significant interaction NEO × EMS [$F(3,78) = 7.05$, $P < 0.001$, $\eta^2 = 0.88$]. Post-hoc analysis revealed that both animals that received neonatal AlloP or neonatal Finas and did not suffer EMS

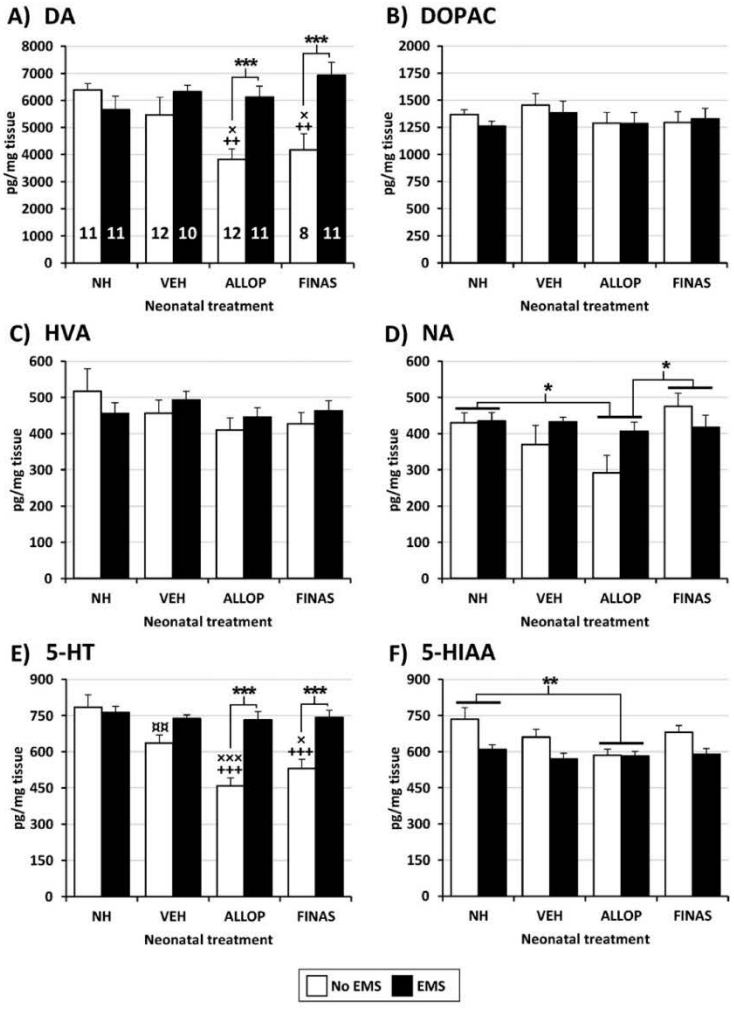


Fig. 4. Monoamines levels on ventral striatum samples. A) DA concentration. When animals did not suffer EMS, those that received neonatal AlloP or Finas had lower levels of DA than control animals ($^{\times}$vs. Veh, $^{+}$vs. NH). In AlloP or Finas groups, EMS increased the DA levels. B) DOPAC concentration. C) HVA concentration. D) NA concentration. Globally, animals that received neonatal AlloP had lower levels of NA than those administered with Finas or those non-handled. E) 5-HT concentration. When animals did not suffer EMS: those that received neonatal AlloP or Finas had lower levels than control animals ($^{\times}$vs. Veh, $^{+}$vs. NH); and those that received Veh had lower levels than NH animals ($^{\#}$vs. NH). In AlloP or Finas groups, EMS increased the 5-HT levels. F) 5-HIAA concentration. Globally, animals that received neonatal AlloP had lower levels of 5-HIAA than NH animals. And animals that suffered EMS had lower levels of 5-HIAA than those animals that did not suffer EMS ($P < 0.001$). One sign: $P < 0.05$, two signs: $P < 0.01$, three signs: $P < 0.001$. In panel A, numbers in bars indicate the number of samples per group: NH = 11; NH + EMS = 11; Veh = 12; Veh + EMS = 10; AlloP = 12; AlloP + EMS = 11; Finas = 8; Finas + EMS = 11.

Table 1
Turnover of DA and 5-HT in the ventral striatum samples (expressed as the ratio between neurotransmitter and metabolite; mean ± SEM). [a]indicates P < 0.001 vs. Veh + EMS; [b]indicates P < 0.001 vs. AlloP + EMS and vs. NH + NoEMS; [c]indicates P < 0.05 vs. AlloP + EMS and vs. NH + NoEMS; [d]indicates P < 0.001 vs. AlloP + EMS, vs. NH + NoEMS, and vs. Veh + NoEMS; [e]indicates P < 0.001 vs. Finas + EMS and P < 0.01 vs. NH + NoEMS; [f]indicates P < 0.01 vs. Finas + EMS and P < 0.05 vs. NH + NoEMS; [g]indicates P < 0.001 vs. Finas + EMS, vs. NH + NoEMS and vs.Veh + NoEMS.

	NH		VEH		ALLOP		FINAS	
	No EMS (n = 11)	EMS (n = 11)	No EMS (n = 12)	EMS (n = 10)	No EMS (n = 12)	EMS (n = 11)	No EMS (n = 8)	EMS (n = 11)
DOPAC/DA	0.216 ± 0.008	0.257 ± 0.043	0.286 ± 0.027	0.218 ± 0.015	0.354 ± 0.020[b]	0.215 ± 0.014	0.332 ± 0.032[e]	0.192 ± 0.006
HVA/DA	0.082 ± 0.011	0.090 ± 0.013	0.091 ± 0.008	0.078 ± 0.004	0.111 ± 0.006[c]	0.078 ± 0.009	0.110 ± 0.010[f]	0.068 ± 0.003
5-HIAA/5-HT	0.946 ± 0.034	0.812 ± 0.045	1.053 ± 0.042[a]	0.772 ± 0.028	1.325 ± 0.075[d]	0.808 ± 0.035	1.307 ± 0.069[g]	0.803 ± 0.036

had lower concentration of 5-HT than control groups (Veh and NH) as well as than animals that were submitted to EMS. Moreover, those animals that received the Veh and did not suffer EMS had lower 5-HT levels than NH animals (see Fig. 4E for detailed post-hoc analysis). On 5-HIAA levels we found a significant main effect of NEO [$F(3,78) = 3.41$, $P < 0.05$, $\eta^2 = 0.77$], showing that all animals that received neonatal AlloP (globally: with and without EMS) had lower concentration of 5-HIAA than NH animals. Moreover, a significant main EMS effect was found [$F(1,78) = 13.73$, $P < 0.001$, $\eta^2 = 0.93$], showing that those animals that suffered EMS had lower 5-HIAA levels than those that did not suffer EMS (see Fig. 4F). The analysis of the turnover ratio 5-HIAA/5-HT revealed a main significant effect of NEO [$F(3,78) = 7.99$, $P < 0.001$, $\eta^2 = 0.89$] and EMS [$F(1,78) = 109.61$, $P < 0.001$, $\eta^2 = 0.99$] along significant interaction NEO × EMS [$F(3,78) = 7.39$, $P < 0.001$, $\eta^2 = 0.88$]. The subsequent analysis showed that both, animals that received neonatal AlloP and did not suffer EMS, and animals that received neonatal Finas and did not suffer EMS, had higher 5-HIAA/5-HT turnover ratio than the rest of the animals (both control groups -NH and Veh- and animals that suffered EMS). Moreover those animals that received neonatal Veh and did not suffer EMS present a higher turnover ratio than EMS animals (see Table 1).

Finally, additional analyses showed no significant correlation between consumption variables (ethanol or glucose doses) and monoamine levels; neither in means nor day by day values [data not shown].

Discussion

Our results revealed that those animals which were administered with neonatal Finas consumed higher doses of both ethanol and glucose than the rest of the groups during the second week of consumption, and showed decreased ventrostriatal DA and 5-HT levels (with parallel increase in their respective turnover ratios) in comparison to controls (Veh and NH groups) at the end of the procedure. Interestingly, the differences in alcohol consumption and striatal monoamine levels between Finas and the rest of neonatal groups were not present when the animals suffered EMS. We observed that in no-EMS subjects not all neonatal groups (NH, Veh, AlloP and Finas) increased their ethanol intake among days in the same way, given that the intake increase was greater in Finas animals and Veh rats did not show an increased consumption at the end of the procedure (see discussion below). Instead, these effects were not maintained in EMS subjects, because in this situation there were no effects of the neonatal treatment and all neonatal groups increased their alcohol intake in the same way. Thus, our results indicate that the effects of neonatal NS manipulation on ethanol intake are different depending on whether animals have or have not suffered EMS. Moreover, these results indicate that our neonatal NS manipulation and the subsequent EMS exposure do not have an additive effect on alcohol consumption in adulthood, suggesting that the two manipulations could be affecting alcohol consumption via a similar mechanism. Regarding ventrostriatal monoamine levels, EMS altered the changes found in the Finas group: all animals that suffered EMS presented similar monoamines levels to those of the control groups, which are higher than those found in Finas administered animals not submitted to EMS. On the other hand, the effects of neonatal Finas administration on glucose solution intake were present in all animals independently of the EMS condition. Interestingly, the differences in ethanol intake do not appear until the second week of consumption, so neonatal Finas does not affect ethanol intake in the initial consumption phase, which has generally been considered the acquisition phase of the alcohol drinking behaviour (Spanagel, 2000; Vengeliene et al., 2005). This increase in ethanol consumption in the second week could suggest a main effect of neonatal Finas treatment in the period in which the pattern of drinking behaviour is being consolidated.

With regard to the increase in alcohol but also glucose intake in Finas animals, several studies have shown that a high intake of sweet solutions positively correlates with high ethanol consumption (Gosnell and Krahn, 1992; Koros et al., 1998), as well as diverse lines of selectively bred ethanol-preferring rats also present a high sweet preference and vice versa (Dess et al., 1998; Woods et al., 2003), indicating genetic overlapping between the preference for "sweet" solutions and high ethanol intake (Bachmanov et al., 2011).

The low DA and 5-HT levels and the increased turnover ratios (DOPAC/DA, HVA/DA and 5-HIAA/5-HT) found in the ventral striatum samples of Finas animals, possibly indicate a decrease in DA and 5-HT activity, since their corresponding metabolites (DOPAC, HVA, 5-HIAA) were not increased. Alterations in both DA and 5-HT activity have been related with altered ethanol consumption. For instance, decreased reward circuitry activation could lead to the consumption of more ethanol in order to get a higher DA release (Blum et al., 1996; Leyton, 2014), and deficits of 5-HT in the nucleus accumbens (NAcc) and other structures are also known to lead to a higher alcohol consumption (Müller and Homberg, 2015). Nevertheless, our results are difficult to interpret since the reported levels were obtained at the end of the consumption procedure. Although there were no significant correlations between ethanol intake and monoamine levels, we cannot discard any influence of the ethanol consumption for 15 days in the recorded monoamine levels. Thus, other studies to determine the basal monoamine levels of these animals are needed to clarify this issue.

Given that it has been recently reported that same neonatal administration of Finas (50 mg/kg from PND5 to PND9) causes an overexpression of α4 and δ GABA A receptor ($GABA_AR$) subunits in the hippocampus, which is still present in adulthood (Mòdol et al., 2014), it is plausible to think that the increase in alcohol consumption found in the rats that received neonatal Finas could be related, at least in part, to an altered α4 and δ expression. The extrasynaptic isoform α4βδ of $GABA_AR$ has proven to be sensitive to low-moderate ethanol doses in diverse *in vitro* studies (Sundstrom-Poromaa et al., 2002; Wei et al., 2004). Besides, *in vivo* studies have shown that the reduced expression of α4 subunit in the NAcc shell of rats decreased their free consumption of and preference for alcohol (Rewal et al., 2009), as well as their instrumental responding for oral ethanol (Rewal et al., 2012). In the same way, knockdown of the δ subunit in the medial shell region of the NAcc reduces alcohol intake (Nie et al., 2011) and δ knockout mice have a reduced ethanol preference and consumption (Mihalek et al., 2001). Moreover, it has been suggested that α4βδ $GABA_AR$ are present in GABAergic terminals on the ventral tegmental area (VTA) participating in the regulation of the excitability of dopaminergic neurons that project to the NAcc (Xiao et al., 2007), so alterations in the expression

of these receptors could also be related to the altered dopaminergic activity observed in Finas-treated animals.

On the other hand, we found that AlloP administration was also able to neutralize the decrease in ethanol consumption related to Veh injections. In fact, animals that received neonatal Veh administration consumed lower ethanol doses on the last two days of the procedure than the rest of the animals (that is: Finas, NH and AlloP groups). This effect could be due to the stress caused by the injection and/or to the brief periods of separation from the dam that pups suffer in order to be injected. Both, neonatal stress caused by saline injection (Frye et al., 2006) and short periods of maternal separation (Kehoe et al., 2000) have shown to alter endogenous neonatal AlloP levels, so it could be that AlloP administration neutralizes the Veh effects by means of restore physiological homeostasis. However, more studies are necessary in order to elucidate what concrete intervention is the responsible of the neonatal Veh administration effect and how AlloP manipulation neutralizes it. This is not the first time that we observed behavioural consequences of the neonatal manipulations that Veh administration implies prevented by AlloP administration (Mòdol et al., 2013). In addition to this effect, animals that received neonatal AlloP also presented lower DA and 5-HT levels in the ventral striatum than control animals (Veh and NH) and increased turnover ratios (DOPAC/DA, HVA/DA and 5-HIAA/5-HT) with lower 5-HIAA levels than NH animals; as well as low NA levels. As in Finas administered animals, the effects of neonatal AlloP on DA and 5-HT concentrations (decrease) were not found in animals which had suffered EMS.

Interestingly, although neonatal Finas increased ethanol intake in non-neonatal stressed rats (no-EMS), ethanol preference was not affected in these animals. The lack of differences probably responds to the fact that Finas increased the consumption of both ethanol and glucose (see above). In contrast, neonatal AlloP increased alcohol preference in stressed subjects during the first week of the consumption period. Nevertheless, given the lack of differences between injection groups on ethanol intake in the subgroup of EMS animals, this effect is probably related to the low glucose consumption that AlloP animals present during the first days (see Results). It can be hypothesized that the effects of exogenous AlloP administration between PND5-PND9 could have been potentiated by the putative increase in AlloP endogenous levels induced by neonatal stress. In this way, it has been described that acute stress increases brain AlloP production (Purdy et al., 1991). Anyway, an increase in the initial preference for the alcohol solution can be relevant for possible subsequent abusive consumption. Thus, it could be important to study more prolonged periods of ethanol consumption in future studies.

It has to be taken into account that neonatal Finas administration could be preventing not only the synthesis of AlloP but also of tetrahydrodeoxycorticosterone (THDOC), as well as the conversion of testosterone to dihydrotestosterone (DHT). Alterations in neonatal testosterone had been related to impulsivity (Bayless et al., 2013), a trait which is considered a risk factor for drug abuse (Belin and Deroche-Gamonet, 2012). In this sense, in previous works we reported that the same neonatal Finas administration caused an increase of hippocampal testosterone at PND9 (Darbra et al., 2013). Therefore, the effects of neonatal Finas administration may not necessarily be due to an inhibition of AlloP synthesis alone, but through affecting other neonatal NS levels. Thus, the fact that Finas can alter other steroids' pathways apart from AlloP could be related to the fact that some effects of neonatal Finas and AlloP administration go in the same direction (both neonatal AlloP and Finas decrease ventrostriatal DA and 5-HT levels). On the other hand, the fact that both Finas and AlloP effects are not present when animals suffer EMS could be related to further alterations of the neonatal NS milieu caused by the EMS. As mentioned in the Introduction, changes in AlloP levels due to the stress have been reported in the neonatal age (Frye et al., 2006; Kehoe et al., 2000), and it has been proposed that NS may provide a link between early-life stress and adverse programming of the brain and behaviour (Brunton, 2015). Moreover, the neonatal administration of AlloP or Finas, as well as EMS, could be causing alterations not only in the neonatal NS levels but also in the NS milieu in adulthood. This may directly affect the ethanol intake (see Introduction section for relationship between adult AlloP levels and ethanol intake) and monoamine levels. Thus, future studies measuring neonatal and adult NS levels are needed in order to characterize if neonatal treatments alter NS milieu and elucidate whether these alterations persist into adult animals.

In relation to the EMS effects, results indicate that this neonatal stress increased the average of alcohol consumption in adult age (mean of 14 days). However, detailed analysis of the ethanol dose consumed day per day revealed that the increase in alcohol intake induced by EMS was statistically significant only in the animals that were neonatally injected with Veh. That is, the amount of ethanol consumed was statistically the same in the no-EMS and EMS rats that were neonatally not-manipulated (NH group) or injected with Finas or AlloP. Thus, EMS seems to be neutralizing the effects of the previous manipulation and so increasing the ethanol consumption in Veh administered rats. In order to explain why neonatal manipulation (Veh injection) is decreasing alcohol consumption and why this effect is not found when animals suffered EMS, it is important to consider that different maternal separation models have shown distinct effects on adult ethanol consumption. Short periods of maternal absence (<15 min per day) have proven beneficial for the offspring (Nylander and Roman, 2013), and animals submitted to them usually present lower ethanol consumption than non-separated animals (Hilakivi-Clarke et al., 1991; Ploj et al., 2003). This effect is also found in ethanol-preferring rats (Roman et al., 2003), showing that short periods of maternal separation can even counteract genetic predisposition for high ethanol consumption (Nylander and Roman, 2013). As mentioned above, our animals are separated from their mother about 10 min per day between PND5 and PND9 in order to be injected, and data seem to indicate that the most sensible days to reach this protective effect are from PND5 to PND10 (Hilakivi-Clarke et al., 1991; Nylander and Roman, 2013). Thus, it could be that the low ethanol consumption observed in the Veh administered group is reflecting this protective effect of short maternal separation periods. On the other hand, longer periods of maternal absence (usually models of 180/360 min per day between PND1 and PND21) are considered detrimental and high ethanol consumption has been reported as a consequence, even though this increase in ethanol intake is usually only found when comparing with animals submitted to shorter periods of separation, but not when compared to NH animals (Hilakivi-Clarke et al., 1991; Nylander and Roman, 2013; Roman et al., 2003). Thus, our results seem in accordance with these observations, since EMS seems to be neutralizing the effects of the previous manipulation and so increasing the ethanol consumption in Veh administered rats. Moreover, is important to remark that 24 h of EMS have been able to increase neonatal plasma CORT levels during the SHRP (see Introduction), and that CORT levels could fluctuate depending on the neonatal AlloP manipulation, as it has been reported in prepubertal and adult age (Guo et al., 1995). Thus, the previous neonatal treatment (NH, Veh, AlloP or Finas) could affect differently the CORT levels and so be changing the subsequent CORT response to EMS, being this related to the fact that EMS effects were only found on Veh rats. However, future experiments are needed in order to elucidate the mechanism underlying these effects.

Furthermore, animals that were submitted to EMS consumed higher doses of glucose than the animals that did not suffer EMS during the first week of consumption, independently of the previous neonatal treatment. In this sense, and using diverse models of long periods of maternal separation, other studies have reported alterations in a sucrose preference test, specifically an increase in the adult consumption of a sucrose solution (Michaels and Holtzman, 2006; Mourlon et al., 2010) and an increased sucrose preference (Mourlon et al., 2010).

In summary, present results show that neonatal Finas administration increases alcohol consumption in adulthood in comparison with

the rest of neonatal groups (controls: Veh and NH groups; and AlloP group), reaching doses of 2.3 g/kg in one hour, which can be considered moderately high (Martín-García et al., 2007). Finas animals reached levels of ethanol intake that were similar to that observed after EMS, which increased the alcohol intake among days in the same way in all the neonatal groups. Thus, the effects of neonatal AlloP manipulations on adult alcohol consumption were different depending on subsequent EMS exposure. Moreover, neonatal AlloP administration increases alcohol intake compared to the neonatal Veh group. It seems that neonatal injections (Veh group) can decrease ethanol consumption in adulthood, and this effect can be prevented by neonatal AlloP manipulations or by subsequent EMS. Regarding monoamine levels, neonatal Finas and AlloP administration decreased ventrostriatal DA and 5-HT levels (increasing the corresponding turnover ratios), only in the animals that were not exposed to EMS. In addition to the ethanol consumption, neonatal Finas administration also increased the intake of reinforcing solutions (glucose), but the increase in glucose consumption in comparison to the rest of neonatal groups was maintained in the animals which had suffered EMS. Taken together, our results remark on the importance of both neonatal NS levels and neonatal stress events on neurodevelopment, showing for first time a possible implication of neonatal AlloP alterations in adult alcohol use disorders.

Acknowledgments

The authors want to thank Susana Benítez for her technical support on HPLC analysis.

This work was supported by a grant from the Spanish Ministry of Economy and Competitiveness (PSI2012-36646).

References

Avishai-Eliner, S., Yi, S.-J., Newth, C.L.J., Baram, T.Z., 1995. Effects of maternal and sibling deprivation on basal and stress induced hypothalamic-pituitary-adrenal components in the infant rat. Neurosci. Lett. 192, 49–52.

Azzolina, B., Ellsworth, K., Andersson, S., Geissler, W., Bull, H.G., Harris, G.S., 1997. Inhibition of rat a-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J. Steroid Biochem. Mol. Biol. 61, 55–64.

Bachmanov, A.A., Bosak, N.P., Floriano, W.B., Inoue, M., Li, X., Lin, C., Murovets, V.O., Reed, D.R., Zolotarev, V.A., Beauchamp, G.K., 2011. Genetics of sweet taste preferences. Flavour. Fragr. J. 26 (4), 286–294.

Barbaccia, M.L., Affricano, D., Trabucchi, M., Purdy, R.H., Colombo, G., Agabio, R., Gessa, G.L., 1999. Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats. Eur. J. Pharmacol. 384, R1–R2.

Bayless, D.W., Darling, J.S., Daniel, J.M., 2013. Mechanisms by which neonatal testosterone exposure mediates sex differences in impulsivity in prepubertal rats. Horm. Behav. 64, 764–769.

Belin, D., Deroche-Gamonet, V., 2012. Responses to Novelty and Vulnerability to Cocaine Addiction: Contribution of a Multi-Symptomatic Animal Model. Cold Spring Harb. Perspect. Med. 2 (11), pii: a011940.

Blum, K., Sheridan, P.J., Wood, R.C., Braverman, E.R., Chen, T.J., Cull, J.G., Comings, D.E., 1996. The D2 dopamine receptor gene as a determinant of reward deficiency syndrome. J. R. Soc. Med. 89 (7), 396–400.

Brunton, P.J., 2015. Programming the brain and behavior by early-life stress: a focus on neuroactive steroids. J. Neuroendocrinol. 27 (6), 468–480.

Cook, J.B., Dumitru, A.M.G., O'Buckley, T.K., Morrow, A.L., 2014. Ethanol administration produces divergent changes in GABAergic neuroactive steroid immunohistochemistry in the rat brain. Alcohol. Clin. Exp. Res. 38 (1), 90–99.

Darbra, S., Pallarès, M., 2009. Neonatal allopregnanolone increases novelty-directed locomotion and disrups behavioural responses to GABAA receptor modulators in adulthood. Int. J. Dev. Neurosci. 27, 617–625.

Darbra, S., Pallarès, M., 2010. Alterations in neonatal neurosteroids affect exploration during adolescence and prepulse inhibition in adulthood. Psychoneuroendocrinology 35 (4), 525–535.

Darbra, S., Pallarès, M., 2012. Effects of early postnatal allopregnanolone administration on elevated plus-maze anxiety scores in adult male Wistar rats. Neuropsychobiology 65 (1), 20–27.

Darbra, S., Mòdol, L., Vallée, M., Pallarès, M., 2013. Neonatal neurosteroid levels are determinant in shaping adult prepulse inhibition response to hippocampal allopregnanolone in rats. Psychoneuroendocrinology 38 (8), 1397–1406.

de Kloet, E.R., Rosenfeld, P., van Eekelen, J.A.M., Sutanto, W., Levine, S., 1988. Stress, glucocorticoids and development. Prog. Brain Res. 73, 101–120.

Dess, N.K., Badia-Elder, N.E., Thiele, T.E., Kiefer, S.W., Blizard, D.A., 1998. Ethanol consumption in rats selectively bred for differential saccharin intake. Alcohol 16, 275–278.

Ellenbroek, B.A., Cools, A.R., 2002. Early maternal deprivation and prepulse inhibition. The role of the postdeprivation environment. Pharmacol. Biochem. Behav. 73, 177–184.

Ellenbroek, B.A., Derks, N., Park, H.J., 2005. Early maternal deprivation retards neurodevelopment in Wistar rats. Stress 8, 247–257.

Frye, C.A., Rhodes, M.E., Raol, Y.H., Brooks-Kayal, A.R., 2006. Early postnatal stimulation alters pregnane neurosteroids in the hippocampus. Psychopharmacology 186, 343–350.

Fumagalli, F., Molteni, R., Racagni, G., Riva, M.A., 2007. Stress during development: impact on neuroplasticity and relevance to psychopathology. Prog. Neurobiol. 81 (4), 197–217.

Girdler, S.S., Klatzkin, R., 2007. Neurosteroids in the context of stress: implications for depressive disorders. Pharmacol. Ther. 116 (1), 125–139.

Gizerian, S.S., Moy, S.S., Lieberman, J.A., Grobin, A.C., 2006. Neonatal neurosteroid administration results in development-specific alterations in prepulse inhibition and locomotor activity: neurosteroids alter prepulse inhibition and locomotor activity. Psychopharmacology 186 (3), 334–342.

Gosnell, B.A., Krahn, D.D., 1992. The relationship between saccharin and alcohol intake in rats. Alcohol 9, 201–206.

Grobin, A.C., Morrow, A.L., 2001. 3Alpha-hydroxy-5alpha-pregnan-20-one levels and GABA(A) receptor-mediated 36Cl(−) flux across development in rat cerebral cortex. Brain Res. Rev. Brain Res. 131 (1–2), 31–39.

Grobin, A.C., Heenan, E.J., Lieberman, J.A., Morrow, A.L., 2003. Perinatal neurosteroid levels influence GABAergic interneuron localization in adult rat prefrontal cortex. J. Neurosci. 23, 1832–1839.

Grobin, A.C., Gizerian, S., Lieberman, J.A., Morrow, A.L., 2006. Perinatal allopregnanolone influences prefrontal cortex structure, connectivity and behavior in adult rats. Neuroscience 138, 809–819.

Guo, A.L., Petraglia, F., Criscuolo, M., Ficarra, G., Nappi, R.E., Palumbo, M.A., Trentini, G.P., Purdy, R.H., Genazzani, A.R., 1995. Evidence for a role of neurosteroids in modulation of diurnal changes and acute stress-induced corticosterone secretion in rats. Gynecol. Endocrinol. 9 (1), 1–7.

Hilakivi-Clarke, L.A., Turkka, J., Lister, R.G., Linnoila, M., 1991. Effects of early postnatal handling on brain beta-adrenoceptors and behavior in tests related to stress. Brain Res. 542, 286–292.

Kehoe, P., Mallinson, K., McCormick, C.M., Frye, C.A., 2000. Central allopregnanolone is increased in rat pups in response to repeated, short episodes of neonatal isolation. Brain Res. Dev. Brain Res. 124 (1–2), 133–136.

Koros, E., Piasecki, J., Kostowski, W., Bienkowski, P., 1998. Saccharin drinking rather than open field behavior predicts initial ethanol acceptance in Wistar rats. Alcohol 33, 131–140.

Lehmann, J., Russig, H., Feldon, J., Pryce, C.R., 2002. Effect of a single maternal separation at different pup ages on the corticosterone stress response in adult and aged rats. Pharmacol. Biochem. Behav. 73, 141–145.

Leyton, M., 2014. What's deficient in reward deficiency? J. Psychiatry Neurosci. 39 (5), 291–293.

Llidó, A., Mòdol, L., Darbra, S., Pallarès, M., 2013. Interaction between neonatal allopregnanolone administration and early maternal separation: Effects on adolescent and adult behaviors in male rat. Horm. Behav. 63 (4), 577–585.

Martín-García, E., Pallarès, M., 2005. Effects of intrahippocampal nicotine and neurosteroid administration on withdrawal in voluntary and chronic alcohol-drinking rats. Alcohol. Clin. Exp. Res. 29, 1654–1663.

Martín-García, E., Darbra, S., Pallarès, M., 2007. Intrahippocampal allopregnanolone decreases voluntary chronic alcohol consumption in non-selected rats. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 31, 823–831.

Michaels, C.C., Holtzman, S.G., 2006. Neonatal stress and litter composition alter sucrose intake in both rat dam and offspring. Physiol. Behav. 89, 735–741.

Mihalek, R.M., Bowers, B.J., Wehner, J.M., Kralic, J.E., VanDoren, M.J., Morrow, A.L., Homanics, G.E., 2001. GABA(A)-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. Alcohol. Clin. Exp. Res. 25, 1708–1718.

Mitev, Y.A., Darwish, Y.A.M., Wolf, S.S., Holsboer, F., Almeida, O.F.X., Patchev, V.K., 2003. Gender differences in the regulation of 3α-hydroxysteroid dehydrogenase in rat brain and sensitivity to neurosteroid-mediated stress protection. Neuroscience 120, 541–549.

Mòdol, L., Darbra, S., Vallée, M., Pallarès, M., 2013. Alteration of neonatal allopregnanolone levels affects exploration, anxiety, aversive learning and adult behavioural response to intrahippocampal neurosteroids. Behav. Brain Res. 241, 96–104.

Mòdol, L., Casas, C., Navarro, X., Llidó, A., Vallée, M., Pallarès, M., Darbra, S., 2014. Neonatal finasteride administration alters hippocampal α4 and δ GABAAR subunits expression and behavioural responses to progesterone in adult rats. Int. J. Neuropsychopharmacol. 17 (2), 259–273.

Morrow, A.L., VanDoren, M.J., Penland, S.N., Matthews, D.B., 2001. The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res. Brain Res. Rev. 37, 98–109.

Mourlon, V., Baudin, A., Blanc, O., Lauber, A., Giros, A., Naudon, L., Daugé, V., 2010. Maternal deprivation induces depressive-like behaviours only in female rats. Behav. Brain Res. 213, 278–287.

Müller, C.P., Homberg, J.R., 2015. The role of serotonin in drug use and addiction. Behav. Brain Res. 277, 146–192.

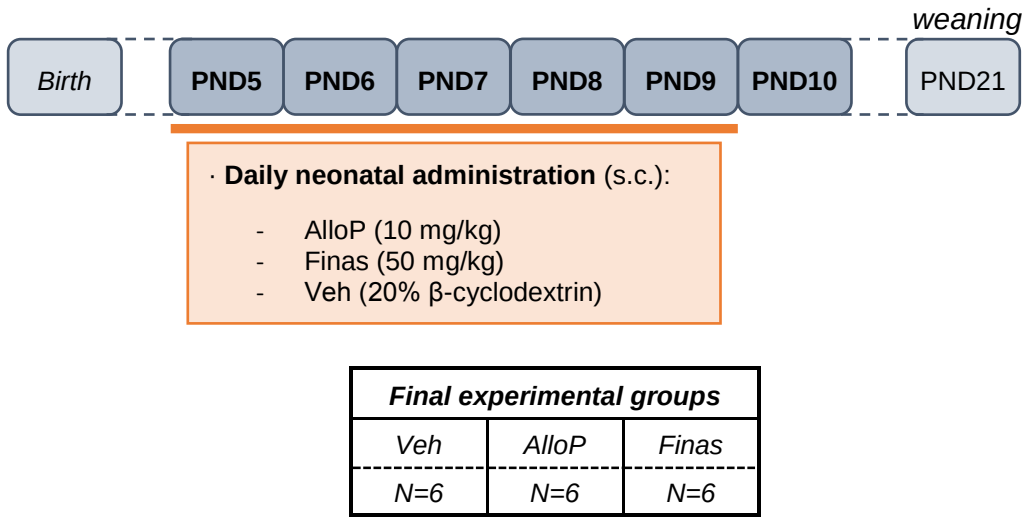
Muneoka, K.T., Takigawa, M., 2002. A neuroactive steroid, pregnenolone, alters the striatal dopaminergic tone before and after puberty. Neuroendocrinology 75, 288–295.

Muneoka, K.T., Shirayama, Y., Minabe, Y., Takigawa, M., 2002. Effects of a neurosteroid, pregnenolone, during the neonatal period on adenosine A1 receptor, dopamine metabolites in the fronto-parietal cortex and behavioral response in the open field. Brain Res. 956 (2), 332–338.

Muneoka, K.T., Kuwagata, M., Shirayama, Y., Ogawa, T., Shioda, S., 2009. Biphasic effects of neonatal allopregnanolone on striatal dopamine metabolism. Neuroreport 20 (9), 860–863.

Nie, H., Rewal, M., Gill, T.M., Ron, D., Janak, P.H., 2011. Extrasynaptic delta-containing GABAA receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. Proc. Natl. Acad. Sci. U. S. A. 108 (11), 4459–4464.
Nutt, D.J., Lingford-Hughes, A., Erritzoe, D., Stokes, P.R., 2015. The dopamine theory of addiction: 40 years of highs and lows. Nat. Rev. Neurosci. 16 (5), 305–312.
Nylander, I., Roman, E., 2013. Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? Psychopharmacology 229 (4), 555–569.
Patchev, V.K., Montkowski, A., Rouskova, D., Koranyi, L., Holsboer, F., Almeida, O.F.X., 1997. Neonatal treatment of rats with the neuroactive steroid tetrahydrodecorticosterone (THDOC) abolishes the behavioral and neuroendocrine consequences of adverse early life events. J. Clin. Invest. 99, 962–966.
Ploj, K., Roman, E., Nylander, I., 2003. Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. Neuroscience 121, 787–799.
Porcu, P., Morrow, A.L., 2014. Divergent neuroactive steroid responses to stress and ethanol in rat and mouse strains: relevance for human studies. Psychopharmacology 231 (17), 3257–3272.
Purdy, R.H., Morrow, A.L., Moore Jr., P.H., Paul, S.M., 1991. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc. Natl. Acad. Sci. U. S. A. 88 (10), 4553–4557.
Rentesi, G., Antoniou, K., Marselos, M., Syrrou, M., Papadopoulou-Daifoti, Z., Kon-standi, M., 2013. Early maternal deprivation-induced modifications in the neurobiological, neurochemical and behavioral profile of adult rats. Behav. Brain Res. 244, 29–37.
Rewal, M., Jurd, R., Gill, T.M., He, D.Y., Ron, D., Janak, P.H., 2009. Alpha4-containing GABAA receptors in the nucleus accumbens mediate moderate intake of alcohol. J. Neurosci. 29, 543–549.
Rewal, M., Donahue, R., Gill, T.M., Nie, H., Ron, D., Janak, P.H., 2012. Alph4 subunit-containing GABAA receptors in the accumbens shell contribute to the reinforcing effects of alcohol. Addict. Biol. 17 (2), 309–321.
Roman, E., Hyytiä, P., Nylander, I., 2003. Maternal separation alters acquisition of ethanol intake in male ethanol-preferring AA rats. Alcohol. Clin. Exp. Res. 27, 31–37.
Romeo, E., Brancati, A., De Lorenzo, A., Fucci, P., Furnari, C., Pompili, E., Sasso, G.F., Spalletta, G., Troisi, A., Pasini, A., 1996. Marked decrease of plasma neuroactive steroids during alcohol withdrawal. Clin. Neuropharmacol. 19, 366–369.
Rosenfeld, P., Suchecki, D., Levine, S., 1992. Multifactorial regulation of the hypothalamic–pituitary–adrenal axis during development. Neurosci. Biobehav. Rev. 16, 553–568.
Sapolsky, R.M., Meaney, M.J., 1986. Maturation of the adrenocotical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. Brain Res. Rev. 11, 65–76.
Serra, M., Pisu, M.G., Littera, M., Papi, G., Sanna, E., Tuveri, F., Usala, L., Purdy, R.H., Biggio, G., 2000. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J. Neurochem. 75, 732–740.
Spanagel, R., 2000. Recent animal models of alcoholism. Alcohol Res. Health 24 (2), 124–131.
Suchecki, D., Mozaffarian, D., Gross, G., Rosenfeld, P., Levine, S., 1993. Effects of maternal deprivation on the ACTH stress response in the infant rat. Neuroendocrinology 57, 204–212.
Suchecki, D., Nelson, D.Y., van Oers, H., Levine, S., 1995. Activation and inhibition of the hypothalamic-pituitary-adrenal axis of the neonatal rats: Effects of maternal deprivation. Psychoneuroendocrinology 20, 169–182.
Sundstrom-Poromaa, I., Smith, D.H., Gong, Q., Sabado, T.N., Li, X., Light, A., Wiedmann, M., Williams, K., Smith, S.S., 2002. Hormonally regulated α4β2δGABA-A receptors are a target for alcohol. Nat. Neurosci. 5, 721–722.
Vallée, M., Rivera, I.D., Koob, G.F., Purdy, R.H., Fitzgerald, R.L., 2000. Quantification of neurosteroids in rat plasma and brain following swim stress and allopregnanolone administration using negative chemical ionization gas chromatography/mass spectrometry. Anal. Biochem. 287, 153–166.
Vengeliene, V., Vollmayr, B., Henn, F.A., Spanagel, R., 2005. Voluntary alcohol intake in two rat lines selectively bred for learned helpless and non-helpless behavior. Psychopharmacology 178, 125–132.
Viveros, M.P., Llorente, R., López-Gallardo, M., Suarez, J., Bermúdez-Silva, F., De la Fuente, M., Rodriguez de Fonseca, F., Garcia-Segura, L.M., 2009. Sex-dependent alterations in response to maternal deprivation in rats. Psychoneuroendocrinology 34 (Suppl.1), S217–S226.
Viveros, M.P., Diaz, F., Mateos, B., Rodriguez, N., Chowen, J.A., 2010. Maternal depri-vation induces a rapid decline in circulating leptin levels and sexually dimorphicmodifications in hypothalamic trophic factors and cell turnover. Horm. Behav. 57, 405–414.
Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7 (8), 847–854.
Wei, W., Faria, L.C., Mody, I., 2004. Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing GABAA receptors in hippocampal neurons. J. Neurosci. 24, 8379–8382.
Woods, J.E., McKay, P.F., Masters, J., Seyoum, R., Chen, A., La Duff, L., Lewis, M.J., June, H.L., 2003. Differential responding for brain stimulation reward and sucrose in high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) rats. Alcohol. Clin. Exp. Res. 27, 926–936.
Xiao, C., Zhou, C., Li, K., Ye, J.H., 2007. Presynaptic GABAA receptors facilitate GABAergic transmission to dopaminergic neurons in the ventral tegmental area of young rats. J. Physiol. 580, 731–743.
Zimmerberg, B., Kajunski, E.W., 2004. Sexually dimorphic effects of postnatal allopregnanolone on the development of anxiety behavior after early deprivation. Pharmacol. Biochem. Behav. 78 (3), 465–471.

Neonatal manipulations:



Final experimental groups		
Veh	*AlloP*	*Finas*
N=6	*N=6*	*N=6*

Behavioural evaluation:

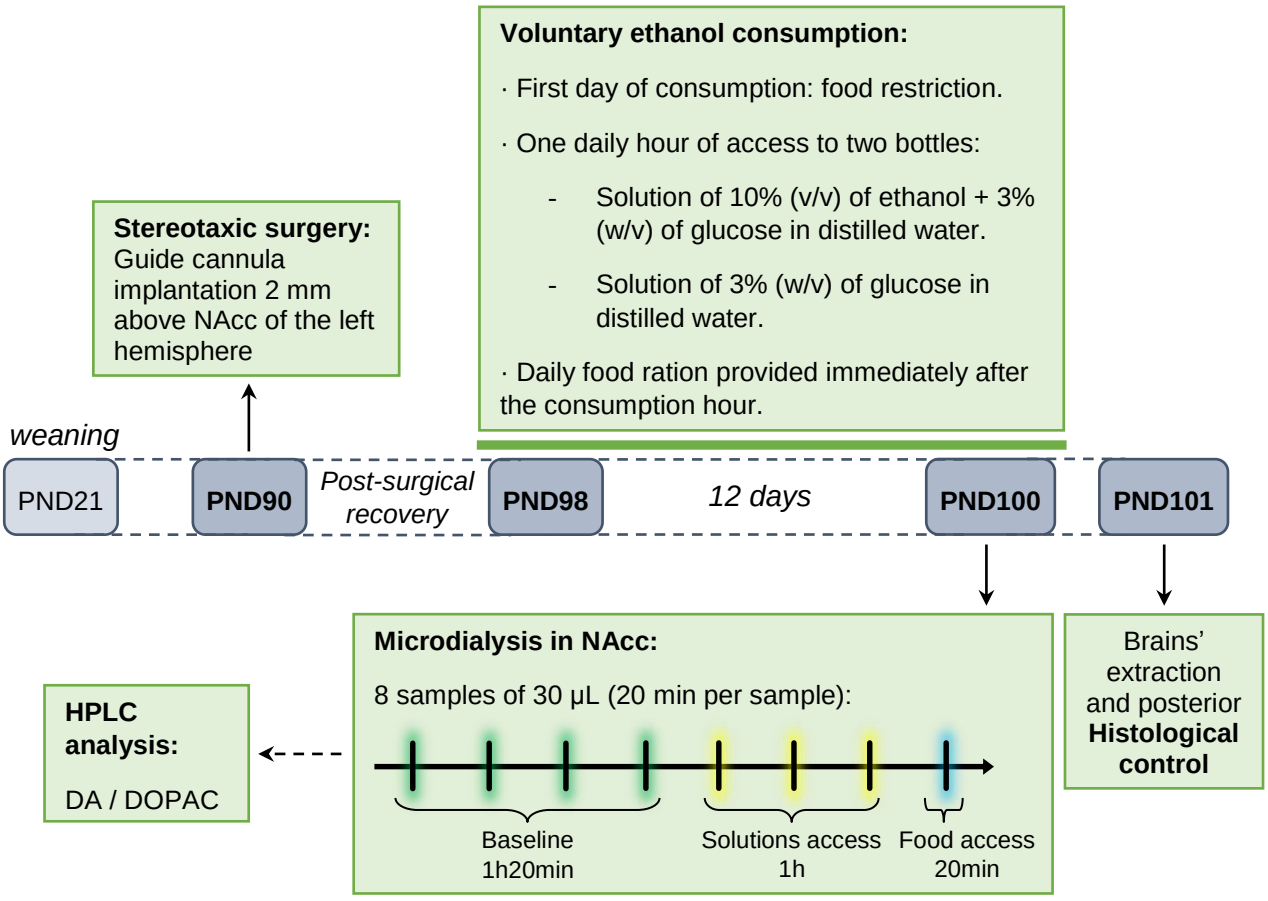


Figure 3. Experimental design of experiment 2B.

EXPERIMENT 2B _ RESULTS

Research paper

LLidó, A., Bartolomé, I., Darbra, S., Pallarès, M., 2016. Neonatal finasteride administration decreases dopamine release in nucleus accumbens after alcohol and food presentation in adult male rats. Behav. Brain Res. 309: 44-50.

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/

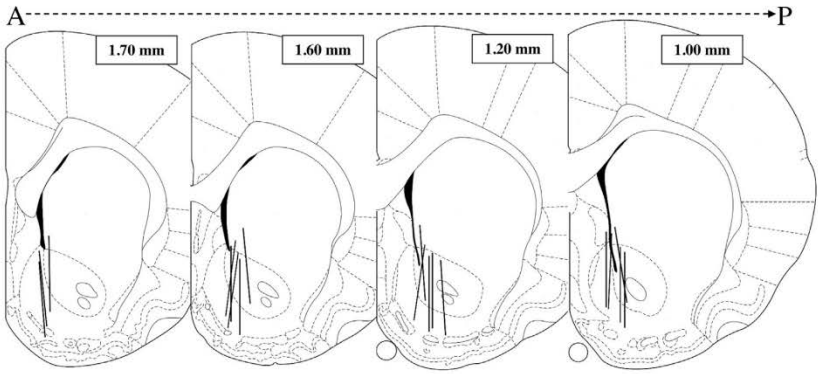


Fig. 1. Microdialysis probe placement within the Nucleus Accumbens. Schematic coronal sections of the rat brain's left hemisphere. Numbers indicate the anteroposterior (A-P) position of the slice relative to Bregma. Lines represent the membrane placements. Adapted from Paxinos and Watson [22].

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

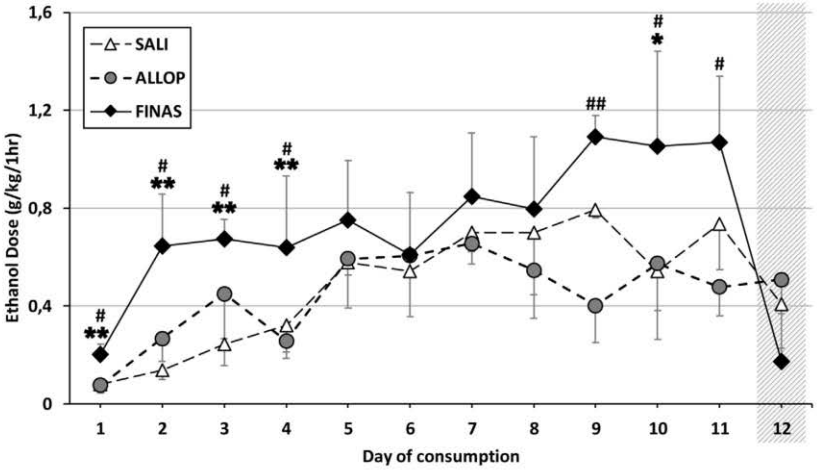


Fig. 2. Alcohol consumption (1 h limited access paradigm). Detailed analysis showed that animals receiving neonatal Finas consumed higher doses of ethanol than Veh and AlloP animals during several days of the consumption period: * indicates differences *vs.* Veh group and # *vs.* AlloP group. The grey column indicates the microdialysis day (day 12). One sign: $P < 0.05$, two signs: $P < 0.01$. For more details see text (Section 3.1).

Watson atlas [22]: anteroposterior: 1.6 mm; lateral: 1.1 mm; ventral: −6 mm from the skull surface. A previous pilot study with 3 animals was carried out to ensure that these coordinates correspond to the NAcc in our rats. The guide cannula was permanently fixed to the skull with four screws and dental cement.

2.4. Alcohol consumption

After surgery, rats were placed individually and allowed for eight days of post-surgical recovery, with food and water *ad libitum*, to regain their pre-operative body weight. During this period rats were weighted and manipulated daily in order to check the state of the guide cannula.

Once recovered from surgery, ethanol self-administration was assessed using an intermittent home-cage drinking procedure during 12 consecutive days. Adaptations of this procedure have been regularly used in our laboratory [13,23–25]. Rats had 1 h per day limited access to two bottles, one containing a solution of 10% (v/v) of ethanol plus 3% (w/v) glucose in distilled water, and the other one with a solution of 3% (w/v) of glucose in distilled water. For the rest of the day, animals had *ad libitum* access to water. Glucose was added to avoid taste aversion and ensure consumption, as used in previous experiments [13,25,26]. Solutions were presented in plastic bottles with a safety valve that prevented spillage and evaporation. Bottles position was changed daily at random in order to avoid position bias. The first day of alcohol consumption, food was retired together with bottles after the hour of access and rats remained with food restriction for the rest of the procedure, receiving 5 g of food per day (this ration was adjusted when was needed in order to avoid that animals lost more than 15% of their initial weight). Rats were food restricted in order to increase their ethanol intake, since it has been shown that restricted access to food increases the self-administration of drugs with rewarding proprieties, independently of their caloric value [27–29]. Their daily food ration was provided immediately after the 1 h of access to the ethanol solution in order to discriminate between the DA increase related to ethanol intake and the DA increase related to food intake in the day of microdialysis testing (last day of consumption).

During the drinking procedure, the state of the guide cannula was also daily checked and rats were weighted every day before the access to solutions and food in order to calculate their food ration and the ethanol dose consumed. Bottles were weighted every day before and after the access hour to them in order to determine the solutions consumption. Ethanol and glucose doses daily consumed (g ethanol or g glucose/kg body weight) were calculated. Ethanol solution was prepared using ethanol absolute (synthesis grade 99.9%) from Scharlab (Barcelona, Spain). The glucose used was D(+)-glucose anhydrous from Panreac (Barcelona, Spain).

2.5. Microdialysis procedure and high-performance liquid chromatography analysis

The microdialysis procedure took place on the twelfth day of consumption. The microdialysis probe (CMA/11; CMA/Microdialysis AB, Sweden) that extends 2 mm below the guide cannula was inserted, and rats were placed in Plexiglas test chambers (40 × 40 × 40 cm) provided with a counter-balanced lever arm (CMA/Microdialysis AB, Sweden) that allowed free-moving animals. The day before microdialysis, rats were placed in the Plexiglas chambers without subjection during the consumption hour in order to habituate to the new environment. The microdialysis probe was connected by polyethylene tubing to a syringe (500 μL; SGE Analytical Science, Australia) driven by the infusion pump (Harvard 22, Harvard Apparatus, USA). Artificial cerebrospinal fluid (Perfusion fluid CNS; CMA/Microdialysis AB, Sweden) with 185 mg/L of calcium chloride dihydrate (SIGMA, Deisenhofen, Germany) was used as a perfusion fluid. Micro eppendorfs were prepared with 5 μL of $HClO_4$ at 10 mM in order to prevent DA degradation. Pump was set to get a perfusion flow of 1.5 μL/min. We collected 8 samples of 30 μL (20 min per sample): 4 to get the baseline, 3 with the presence of the solutions and 1 with the presence of food. Samples were immediately stored at −20 °C; DA and DOPAC levels were determined by HPLC the following day. Dialysis results for each rat (DA and DOPAC) were calculated as percentages of baseline. In order to obtain a more stable baseline, it was defined as the average concentration value (fmol in 20 min) over the last two samples before ethanol access.

HPLC system consisted in a Waters 717plus autosampler (Waters Cromatografia, Cerdanyola, Spain), a Waters 515 pump, a Hichrom ultrasphere 3-um ODS column 7.5 × 0.46 cm (supplied

by Symta, Madrid) and a Waters 2465 amperometric detector set at an oxidation potential of 0.7 V. The mobile phase consisted of 0.15 M monosodium phosphate (NaH_2PO_4), 0.9 mM 1-octane sulfonic acid, 0.5 mM ethylenediaminetetraacetic acid (EDTA) (pH 2.8, adjusted with phosphoric acid) and 10% methanol and was pumped at 0.8 mL/min. The total sample analysis time was of 5 min and the DA and DOPAC retention times were 2.5 min and 2.8 min respectively. The detection limit for DA was of 3 fmol.

2.6. Histological control

After microdialysis procedure, animals were sacrificed by deep anaesthesia with i.p. sodium pentobarbital (200 mg/kg body weight at a concentration of 60 mg/ml) and brains were removed and stored in 10% formalin. Brains were sectioned in 100 μm coronal sections with a vibrating blade microtome (Leica VT 1000 S), mounted and stained with cresyl violet. The placement of the microdialysis probe membrane was confirmed histologically for each rat (see Fig. 1 for details on the microdialysis probe membrane placement).

2.7. Statistical analysis

For data analysis we used STATISTICA package (StatSoft, Tulsa, USA). In order to analyse alcohol consumption variables (ethanol and glucose doses), we used a mixed analysis of variance with NEO (3 levels: AlloP, Finas, Veh) as the between subject factor, and DAY as the within-subject factor. Treatment differences on the DA and DOPAC baseline were analysed using an analysis of the variance with NEO (3 levels: AlloP, Finas, Veh) as the between subject factor. DA and DOPAC (percentages of baseline) were analysed by means of a mixed analysis of variance with NEO (3 levels: AlloP, Finas, Veh) as the between subject factor and SAMPLE (4 levels: 3 samples obtained in presence of ethanol, and the last sample in presence of food) as the within-subject factor. Corresponding *post-hoc* Duncan's tests were performed when needed. Differences between DA baseline and DA after ethanol presentation (average of the three samples) were analysed by means of paired Student's *t* tests. Significance was set at $P < 0.05$.

3. Results

3.1. Ethanol and glucose consumption

The analysis of the ethanol doses consumed during the 12 days of the procedure showed a significant DAY effect [$F(11,165) = 5.68$, $P < 0.001$]. *Post-hoc* Duncan test indicated that alcohol intake significantly increased from day 1 to day 11 ($P < 0.001$) in all experimental groups. Also, ethanol consumption decreased ($P < 0.01$) from day 11 to day 12 (microdialysis test day), although alcohol consumption in day 12 remained higher than in the first day ($P = 0.05$). Moreover, ANOVA indicated no significant NEO [$F(2,15) = 1.75$, NS] nor NEOxDAY [$F(22,165) = 1.10$, NS] effects. Given that these results were not in accordance with previous data [13], we decided to perform a more detailed analysis, distinguishing between initial (days 1–4), intermediate (days 5–8), and final consumption (days 9–12).

On the ethanol dose consumed throughout the first 4 days of procedure we found a significant main effect of NEO [$F(2,15) = 6.33$, $P < 0.05$] and DAY [$F(3,45) = 4.15$, $P < 0.05$] without interaction between both factors [$F(6,45) = 0.61$, NS]. *Post-hoc* analyses revealed that animals that received neonatal Finas consumed higher doses of ethanol than animals with neonatal Veh or AlloP administration (see Fig. 2 for detailed *post-hoc* analysis). On the other hand, all animals consumed lower ethanol doses on the first day of consumption than on the following days ($P < 0.05$ *vs.* day 2 and day 4, $P < 0.01$ *vs.* day 3). In the intermediate phase of the consumption procedure (days 5–8) there were no significant effects of NEO [$F(2,15) = 0.27$, NS], DAY [$F(3,45) = 0.64$, NS] nor NEOxDAY [$F(6,45) = 0.20$, NS] (see Fig. 2). Finally, the analysis of the lasts days of consumption (days 9–12) revealed a significant DAY effect [$F(3,45) = 5.29$, $P < 0.01$] along with a significant interaction NEOxDAY [$F(6,45) = 2.95$, $P < 0.05$]. Subsequent analyses showed that those animals that received neonatal Finas consumed higher ethanol doses than AlloP animals on days 9, 10 and 11, and higher ethanol doses than Veh animals on day 10 (see Fig. 2). On the microdialysis day (day 12) there were no differences on the ethanol dose consumed between groups and Finas animals consumed a significantly lower ethanol dose than on the previous day ($P < 0.001$). Thus, the detailed analysis of the ethanol intake showed that Finas animals consumed higher ethanol doses than AlloP and Veh animals in several days of the consumption phase.

The analysis of the glucose consumption during the 12 days revealed a DAY [$F(11,165) = 2.59$, $P < 0.01$] effect. *Post-hoc* Duncan test indicated that glucose intake significantly decreased from initial days in comparison to the last days in all experimental groups (glucose consumption in days 2 and 3 compared to days 11 and 12; $P < 0.05$ for each comparison). Moreover, ANOVA indicated no significant NEO [$F(2,15) = 2.97$, NS] nor NEOxDAY [$F(22,165) = 0.98$, NS] effects.

3.2. DA and DOPAC release in the NAcc

The analysis of the baseline levels showed that neonatal treatment did not cause any effect on the basal levels of DA [$F(2,15) = 0.65$, NS] and DOPAC [$F(2,15) = 0.03$, NS].

The analysis of the DA levels (% of baseline) after solutions and food presentation revealed significant main effects of NEO [$F(2,15) = 4.04$, $P < 0.05$] and SAMPLE [$F(3,45) = 8.15$, $P < 0.001$], without significant interaction between both factors [$F(6,45) = 0.51$, NS]. Subsequent analysis showed that those animals that received neonatal Finas presented a lower DA increase in response to solutions and food presentation than Veh administered animals (see Fig. 3A). *Post-hoc* analysis of the SAMPLE effect revealed that in all animals the DA increase was higher in the last sample (20 min after food presentation) than in the previous three samples (20, 40 and 60 min after ethanol/glucose presentation) (see Fig. 3A). Regarding the analysis of DOPAC levels, no significant effects of NEO [$F(2,15) = 1.35$, NS], SAMPLE [$F(3,45) = 1.91$, NS] nor NEOxSAMPLE [$F(6,45) = 1.29$, NS] were found (see Fig. 3B).

In order to study the increase of DA after ethanol presentation, paired Student's *t* tests were performed. These analyses showed that the DA levels after solutions presentation (mean of 0–20, 20–40 and 40–60 min samples) increased in relation to baseline in those animals with neonatal AlloP [$t = -3.05$, $P < 0.05$] or Veh [$t = -5.19$, $P < 0.01$] administration, but not in Finas animals [$t = -1.03$, NS].

4. Discussion

The results showed that animals with neonatal administration of Finas consumed higher doses of ethanol than those administered with Veh or AlloP, during several days of the consumption period (see Fig. 2). Moreover, microdialysis results revealed that DA release in NAcc (increase of DA expressed as percentage of baseline) in response to solutions and food presentation was lower in neonatal Finas subjects than in control animals (Veh group). On the other hand, there were no significant differences between neonatal AlloP and Veh administration (neither on ethanol consumption nor on DA release).

In relation to the percentage of DA release in the NAcc (see Fig. 3A), the DA levels after presenting the solutions were lower in those animals that received neonatal Finas than in control group

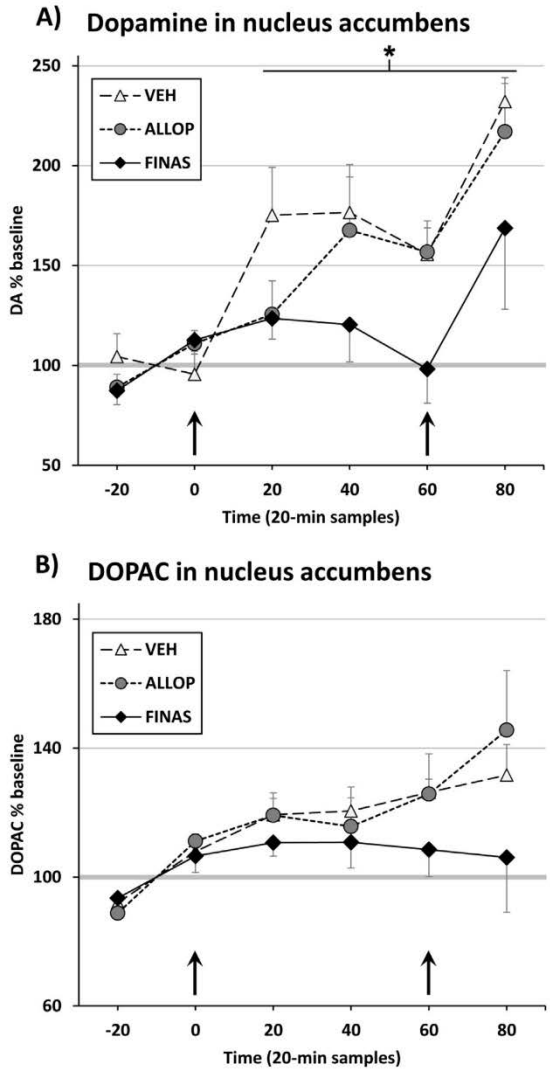


Fig. 3. Microdialysis results. (A) Dopamine release in NAcc (percentage of baseline). Animals that received neonatal Finas presented lower DA levels in response to ethanol/glucose and food presentation than Veh administered animals: *Finas *vs.* Veh. DA levels did not significantly increase after solutions presentation in Finas animals. Time-point 0, and the corresponding arrow, represents the moment of solutions presentation; arrow on time 60 represent the moment of food presentation. One sign: $P < 0.05$. (B) DOPAC release in NAcc (percentage of baseline). No significant effects of neonatal treatment were found.

(Veh). Moreover, Finas effect was maintained during the last sample that corresponded to the food access (60–80 min). In accordance, the increase in DA release after bottles and food presentation was observed in Veh and AlloP groups but not in Finas animals. On the other hand, Finas animals tended to have a reduction of DOPAC levels, although differences between groups were not significant, which seems to be in accordance with the reduced DA release. These data seem to indicate a hypo-DAergic activity in neonatal Finas treated rats that could be related to the increase in alcohol intake reported in these animals. In this sense, it has been described that a hypo-DAergic activity, caused either by a decrease in DA D2 receptors (DRD2) or by a decreased presynaptic DA release in the striatum, leads to an excessive craving and seeking for substances known to cause DA release in the NAcc [30–32]. Several studies in rats have shown that lines selective breed for high ethanol preference present decreased DRD2 density in NAcc [33], and lower DAergic innervation [34,35], with lower DA content [36,37], in the NAcc. Thus, our data could indicate that the higher ethanol consumption that neonatal Finas treated rats show, relates to a blunted DA response to the presentation of the solutions. However, we cannot assume a causal relationship between the hypo-DAergic activity and the increased ethanol consumption in Finas rats. Other studies determining the basal monoamine levels prior to the alcohol consumption phase are needed to test this hypothesis.

Elucidating how neonatal Finas administration can lead to this blunted DA response is complex, since several alterations could be taking place. Finas acts as an inhibitor of the 5α-reductase enzyme [12], which is responsible for the metabolism of progesterone to 5α-dihydroprogesterone which in turn reduces to AlloP, thus the neonatal administration of Finas alters the endogenous neonatal AlloP levels [9,10,21]. Given that AlloP mainly exerts its actions through the positive modulation of $GABA_AR$ (see Section 1), and that GABA acts as a neurotrophic factor during development [38], alterations in GABAergic transmission due to changes in $GABA_AR$ endogenous modulators, could affect the postnatal maturation of the mesocorticolimbic DAergic system [15,39,40]. Furthermore, in a previous work, we have found that neonatal Finas administration alters the expression of α4 and δ $GABA_AR$ subunits in the hippocampus [10], and we cannot rule out that this alteration could extend to other areas. The DAergic neurons of the ventral tegmental area (VTA) that project to NAcc are under GABAergic regulation by means of local interneurons [41] and GABAergic projections arising from the NAcc and ventral pallidum [42]. $GABA_AR$ in the VTA are present presynaptically in GABAergic axons terminals [43,44] and postsynaptically on the DAergic neurons [45,46]. Thus, alterations in the expression of $GABA_AR$ subunits on the VTA could lead to an altered GABAergic control that could explain the blunted DA activity.

As regards the alcohol intake results, an increase in ethanol consumption in neonatal Finas animals was previously reported [13], but we observed differences in the pattern of intake between the two studies. In the previous work, Finas animals consumed higher alcohol doses than controls during the second week of the procedure. By contrast, in the present study, the ethanol consumption increase in Finas animals was obtained at the beginning and the end of the procedure. These differences could be related to some protocol dissimilarities between both experiments. In the previous study, animals received their daily ration of food during the hour of access to the ethanol and glucose solutions, but here food rations were provided just after the drinking hour in order to distinguish between DA response to solutions (ethanol + glucose) access and DA response to food presentation. In this sense, previous works have shown that the timing of food presentation can influence ethanol drinking [47,48]. Moreover, the differences in the intake pattern could be also partially related to the fact that in the present work animals had stereotaxic surgery prior the consumption period, and were also daily manipulated in order to check the state of the guide cannula. On the other hand, the lack of effects of neonatal AlloP administration on ethanol consumption is also in agreement with our previous results [13]. In that work AlloP animals consumed higher ethanol doses than Veh animals, but this effect only appeared after a more prolonged period of alcohol intake. As regards ethanol intake, we can also observe that all groups showed a decrease in the doses consumed in the microdialysis session (day 12). This decrease is probably related to the change in environmental conditions, that is, the difference between microdialysis chamber habituation (consumption on day 11) and

microdialysis session (day 12) including probe and tubing connections.

In summary, present results show that animals with neonatal Finas administration have an increased adult ethanol consumption for several days of the alcohol consumption phase, and a decreased DA release in the NAcc in response to both ethanol and food. These data suggest that the blunted DA release in response to solutions presentation observed in neonatal Finas treated rats seems to be related to the higher ethanol consumption of those animals. Taken together, our results emphasise the importance of neonatal NS levels on neurodevelopment, showing for first time a possible implication of neonatal NS alterations (including AlloP) in adult alcohol rewarding properties.

Acknowledgments

The authors thank Leticia Campa for the HPLC analyses. This work was supported by a grant from the Spanish Ministry of Economy and Competitiveness (PSI2012-36646).

References

[1] M.D. Majewska, N.L. Harrison, R.D. Schwartz, J.L. Barker, S.M. Paul, Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor, Science 232 (1986) 1004–1007.
[2] A.C. Grobin, A.L. Morrow, 3Alpha-hydroxy-5alpha-pregnan-20-one levels and GABA(A) receptor-mediated 36Cl(−) flux across development in rat cerebral cortex, Brain Res. Rev. Brain Res. 131 (1–2) (2001) 31–39.
[3] S. Darbra, M. Pallarès, Neonatal allopregnanolone increases novelty-directed locomotion and disrups behavioural responses to GABAA receptor modulators in adulthood, Int. J. Devl. Neurosci. 27 (2009) 617–625.
[4] S. Darbra, M. Pallarès, Alterations in neonatal neurosteroids affect exploration during adolescence and prepulse inhibition in adulthood, Psychoneuroendocrinology 35 (4) (2010) 525–535.
[5] S. Darbra, M. Pallarès, Effects of early postnatal allopregnanolone administration on elevated plus-maze anxiety scores in adult male Wistar rats, Neuropsychobiology 65 (1) (2012) 20–27.
[6] S. Darbra, L. Mòdol, A. Llidó, C. Casas, M. Vallée, M. Pallarès, Neonatal allopregnanolone levels alteration: effects on behavior and role of the hippocampus, Prog. Neurobiol. 113 (2014) 95–105.
[7] A.C. Grobin, S. Gizerian, J.A. Lieberman, A.L. Morrow, Perinatal allopregnanolone influences prefrontal cortex structure, connectivity and behavior in adult rats, Neuroscience 138 (2006) 809–819.
[8] A. Llidó, L. Mòdol, S. Darbra, M. Pallarès, Interaction between neonatal allopregnanolone administration and early maternal separation: effects on adolescent and adult behaviors in male rat, Horm. Behav. 63 (4) (2013) 577–585.
[9] L. Mòdol, S. Darbra, M. Vallée, M. Pallarès, Alteration of neonatal Allopregnanolone levels affects exploration anxiety, aversive learning and adult behavioural response to intrahippocampal neurosteroids, Behav. Brain Res. 241 (2013) 96–104.
[10] L. Mòdol, C. Casas, X. Navarro, A. Llidó, M. Vallée, M. Pallarès, S. Darbra, Neonatal finasteride administration alters hippocampal α4 and δ GABAAR subunits expression and behavioural responses to progesterone in adult rats, Int. J. Neuropsychopharmacol. 17 (2) (2014) 259–273.
[11] D. Belin, V. Deroche-Gamonet, Responses to novelty and vulnerability to cocaine addiction: contribution of a multi-symptomatic animal model, Cold Spring Harb. Perspect. Med. 2 (11) (2012), pii: a011940.
[12] B. Azzolina, K. Ellsworth, S. Andersson, W. Geissler, H.G. Bull, G.S. Harris, Inhibition of rat a-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition, J. Steroid Biochem. Mol. Biol. 61 (1997) 55–64.
[13] A. Llidó, I. Bartolomé, D. Darbra, P. Pallarès, Effects of neonatal allopregnanolone manipulations and early maternal separation on adult vulnerability to alcohol intake and monoamine levels in ventral striatum of male rats, Horm. Behav. (2016), http://dx.doi.org/10.1016/j.yhbeh.2016.04.003.
[14] I. Bartolomé, A. Llidó, D. Darbra, P. Pallarès, Neonatal Allopregnanolone Manipulation Affects Sensitivity to Stimulating Effects of Alcohol in Adulthood: Possible Role in Drug Abuse Vulnerability, Poster presented at the EBBS & EBPS Joint Meeting, Verona, Italy, 2015.
[15] S.S. Gizerian, S.S. Moy, J.A. Lieberman, A.C. Grobin, Neonatal neurosteroid administration results in development-specific alterations in prepulse inhibition and locomotor activity: neurosteroids alter prepulse inhibition and locomotor activity, Psychopharmacology (Berl.) 186 (3) (2006) 334–342.
[16] K.T. Muneoka, M. Iwata, Y. Shirayama, Altered levels of synapsin I, dopamine transporter, dynorphin A, and neuropeptide Y in the nucleus accumbens and striatum at postpuberty in rats treated neonatally with pregnenolone or DHEA, Int. J. Dev. Neurosci. 27 (6) (2009) 575–581.
[17] K.T. Muneoka, M. Kuwagata, Y. Shirayama, T. Ogawa, S. Shioda, Biphasic effects of neonatal allopregnanolone on striatal dopamine metabolism, Neuroreport 20 (9) (2009) 860–863.
[18] K.T. Muneoka, M. Kuwagata, T. Ogawa, S. Shioda, Sex-specific effects of early neonatal progesterone treatment on dopamine and serotonin metabolism in rat striatum and frontal cortex, Life Sci. 87 (2010) 738–742.
[19] K.T. Muneoka, M. Takigawa, A neuroactive steroid, pregnenolone, alters the striatal dopaminergic tone before and after puberty, Neuroendocrinology 75 (2002) 288–295.
[20] K.T. Muneoka, Y. Shirayama, Y. Minabe, M. Takigawa, Effects of a neurosteroid, pregnenolone, during the neonatal period on adenosine A1 receptor, dopamine metabolites in the fronto-parietal cortex and behavioral response in the open field, Brain Res. 956 (2) (2002) 332–338.
[21] S. Darbra, L. Mòdol, M. Vallée, M. Pallarès, Neonatal neurosteroid levels are determinant in shaping adult prepulse inhibition response to hippocampal allopregnanolone in rats, Psychoneuroendocrinology 38 (8) (2013) 1397–1406.
[22] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press INC, San Diego, 1986.
[23] M. Pallarès, R. Nadal, N. Ferré, Effects of oral ethanol selfadministration on the inhibition of the lever-press response in rats, Pharmacol. Biochem. Behav. 43 (1992) 589–595.
[24] R.A. Nadal, M.A. Pallarès, N.S. Ferré, Oral intake of sweetened or sweetened alcoholic beverages and open-field behavior, Pharmacol. Biochem. Behav. 54 (1996) 739–743.
[25] E. Martín-García, M. Pallarès, Effects of intrahippocampal nicotine and neurosteroid administration on withdrawal in voluntary and chronic alcohol-drinking rats, Alcohol Clin. Exp. Res. 29 (2005) 1654–1663.
[26] E. Martín-García, S. Darbra, M. Pallarès, Intrahippocampal allopregnanolone decreases voluntary chronic alcohol consumption in non-selected rats, Prog. Neuropsychopharmacol. Biol. Psychiatry 31 (2007) 823–831.
[27] M.E. Carroll, D.C. Stotz, Oral D-amphetamine and ketamine selfadministration by rhesus monkeys: effects of food deprivation, J. Pharmacol. Exp. Ther. 227 (1983) 28–34.
[28] G.F. Koob, F.E. Bloom, Cellular and molecular mechanisms of drug dependence, Science 242 (1988) 715–723.
[29] R.A. Meisch, Oral drug self-administration: an overview of laboratory animal studies, Alcohol 24 (2001) 117–128.
[30] K. Blum, P.J. Sheridan, R.C. Wood, E.R. Braverman, T.J. Chen, J.G. Cull, D.E. Comings, The D2 dopamine receptor gene as a determinant of reward deficiency syndrome, J. R. Soc. Med. 89 (7) (1996) 396–400.
[31] K. Blum, P.K. Thanos, M.S. Gold, Dopamine and glucose, obesity, and reward deficiency syndrome, Front. Psychol. 17 (5) (2014) 919.
[32] P. Trifilieff, D. Martinez, Imaging addiction: D2 receptors and dopamine signaling in the striatum as biomarkers for impulsivity, Neuropharmacology 76 (2014) 498–509.
[33] W.J. McBride, E. Chernet, W. Dyr, L. Lumeng, T.K. Li, Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats, Alcohol 10 (1993) 387–390.
[34] M.A. Casu, G. Colombo, G.L. Gessa, L. Pani, Reduced TH-immunoreactive fibers in the limbic system of Sardinian alcohol-preferring rats, Brain Res. 924 (2) (2002) 242–251.
[35] F.C. Zhou, J.K. Zhang, L. Lumeng, T.K. Li, Mesolimbic dopamine system in alcohol-preferring rats, Alcohol 12 (1995) 403–412.
[36] W.J. McBride, B. Bodart, L. Lumeng, T.K. Li, Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference, Alcohol Clin. Exp. Res. 19 (6) (1995) 1420–1422.
[37] J.M. Murphy, W.J. McBride, L. Lumeng, T.K. Li, Regional brain levels of monoamines in alcohol-preferring and -nonpreferring lines of rats, Pharmacol. Biochem. Behav. 16 (1982) 145–149.
[38] Y. Ben-Ari, I. Khalilov, K.T. Kahle, E. Cherubini, The GABA excitatory/inhibitory shift in brain maturation and neurological disorders, Neuroscientist 18 (5) (2012) 467–486.
[39] J. Antonopoulos, I. Dori, A. Dinopoulos, M. Chiotelli, J.G. Parnavelas, Postnatal development of the dopaminergic system of the striatum in the rat, Neuroscience 110 (2) (2002) 245–256.
[40] M. Park, K. Kitahama, M. Geffard, T. Maeda, Postnatal development of the dopaminergic neurons in the rat mesencephalon, Brain Dev. 22 (Suppl. 1) (2000) S38–S44.
[41] S.W. Johnson, R.A. North, Opioids excite dopamine neurons by hyperpolarization of local interneurons, J. Neurosci. 12 (1992) 483–488.
[42] P.W. Kalivas, L. Churchill, M.A. Klitenick, GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area, Neuroscience 57 (1993) 1047–1060.
[43] S.R. Laviolette, D. van der Kooy, GABAA receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems, Eur. J. Neurosci. 13 (2001) 1009–1015.
[44] C. Xiao, C. Zhou, K. Li, J.H. Ye, Presynaptic GABAA receptors facilitate GABAergic transmission to dopaminergic neurons in the ventral tegmental area of young rats, J. Physiol. 580 (2007) 731–743.
[45] H. Okada, N. Matsushita, K. Kobayashi, K. Kobayashi, Identification of GABAA receptor subunit variants in midbrain dopaminergic neurons, J. Neurochem. 89 (2004) 7–14.
[46] B.H. Westerink, H.F. Kwint, J.B. deVries, The pharmacology of mesolimbic dopamine neurons: a dual probe microdialysis study in the ventral tegmental

area and nucleus accumbens of the rat brain, J. Neurosci. 16 (1996) 2605–2611.
[47] L.D. Middaugh, B.M. Kelley, A.L. Bandy, K.K. McGroarty, Ethanol consumption by C57BL/6 mice: influence of gender and procedural variables, Alcohol 17 (3) (1999) 175–183.
[48] H.H. Samson, G.A. Tolliver, A.O. Pfeffer, K. Sadeghi, M. Haraguchi, Relation of ethanol self-administration to feeding and drinking in a nonrestricted access situation in rats initiated to self-administer ethanol using the sucrose-fading technique, Alcohol 5 (5) (1988) 375–385.

SUMMARY OF RESULTS

Results of the present experiments reveal that both manipulation of neonatal NS levels and neonatal stress affect adolescent and adult behaviour. Some of the effects seem to go in the same direction, but importantly there were several interactions between both factors.

In experiment 1 we studied the effects of neonatal NS manipulation and EMS on novelty-directed exploration in adolescent age, and on anxiety-like behaviour and sensorimotor gating in adult age. Main results show that neonatal AlloP administration (10 mg/kg between PND5 and PND9) did not affect the behaviour in the Boissier exploration test at PND40 and PND60 nor in the EPM test at PND85, but it decreased the startle response and impaired the PPI of this response in adulthood. On the other hand, 24 h of EMS at PND9 increased novelty-induced motor activity and decreased head-dipping behaviour in the Boissier exploration test at 40 days old. In adulthood, EMS rats showed an anxiolytic-like profile in the EPM test, as well as an increase of the startle response and a disruption of the PPI. Remarkably, the neonatal administration of AlloP prevented EMS effects on novelty-directed exploration at PND40 but did not affect the EMS effects on adulthood.

On the other hand, in order to evaluate the effects of neonatal NS manipulation and neonatal stress on the vulnerability to alcohol abuse, in experiment 2A we analysed the voluntary ethanol consumption using a two-bottle free choice procedure during 15 consecutive days (starting at PND70). Results show that neonatal Finas administration (50 mg/kg between PND5 and PND9) related to an increase of alcohol consumption in adulthood during the second week of the procedure in comparison with the rest of neonatal groups (controls: Veh and NH groups; and AlloP group), but only in those animals that did not suffer EMS. When animals suffered EMS, there were no effects of previous neonatal treatment and all the animals progressively increased ethanol consumption in the same way. Neonatal Finas administration not only increased the ethanol intake but also the consumption of reinforcing solutions (glucose) during the second week of the procedure. Interestingly, the effect of neonatal Finas administration upon glucose intake was independent of the EMS condition, i.e. all the animals that received neonatal Finas consumed higher doses of glucose in adulthood. On the other hand, rats that received neonatal Veh administration presented a decreased ethanol intake in the last two days of the procedure. This effect was not observed in those animals administered with neonatal AlloP (10 mg/kg between PND5 and PND9) either

in those that suffered posterior EMS. Furthermore, EMS increased both the mean of the ethanol doses consumed during the whole procedure and the glucose consumption during the first week of procedure. Both EMS effects were independent of previous neonatal treatment. After the two weeks of the intake procedure, in experiment 2A we measured the ventrostriatal monoamine levels. Results show that both neonatal Finas and AlloP administration decreased ventrostriatal DA and serotonin (5-HT) levels, without affecting their corresponding metabolites and thus increasing DA and 5-HT turnover ratios. This effect was only present in the animals that were not exposed to EMS, and all the animals that suffered EMS presented unaltered ventrostriatal monoamine levels (i.e. similar levels to that of the control groups). Given these results, we performed experiment 2B in order to further evaluate possible alterations on the activity of the mesolimbic DAergic pathways related to neonatal NS manipulations. Results show that animals with neonatal Finas administration had a decreased DA release in the NAcc in response to both ethanol and food, thus suggesting that the higher ethanol consumption of these animals may be related to a blunted DA release in response to solutions presentation.

A detailed discussion of these results as well as a general discussion of the experiments is included in the next section.

DISCUSSION

1. Experiment 1

1.1. Effects of neonatal allopregnanolone administration and neonatal stress in novelty-directed exploration in adolescence

At PND40 control animals (i.e. Veh and NH groups) that have suffered EMS showed an increased novelty-induced motor activity and a decreased head-dipping behaviour. The increase of locomotion in a new environment is consistent with other works that have also reported it as a consequence of EMS both in adolescent (Marco et al., 2007) and in adult (Rentesi et al., 2013) rats. This increase in novelty-induced motor activity could be interpreted as a reduced stress response to novel environmental experiences, and may be related to an impulsive profile of EMS rats in adolescence (Marco et al., 2007). However, our results also show that EMS is causing a decrease of head-dipping behaviour. The number of explored holes in Boissier test is considered a measure of exploration directed to a stimulus that reflects the attention given to new situations and the motivation to seek out new experiences (File and Wardill, 1975). Thus, a decreased head-dipping behaviour is usually interpreted both as a decrease in the motivation to seek out new experiences and as an elevated level of anxiety in a new environment (Takeda et al., 1998). Given these apparently contradictory results (i.e. high locomotor activity but low head-dipping behaviour), we analysed the travelled distance in the virtual 29 cm × 29 cm centre zone of the apparatus, which is considered an anxiety relevant score (Prut and Belzung, 2003; Voikar et al., 2001), and found out that in the centre zone EMS rats travelled less distance than no EMS animals [$F(1,62)=8.94$, $P<0.01$]. Therefore, the reported increase in total travelled distance observed in EMS group relates to an increase of activity in the outermost portion of the apparatus (close to the walls), which may be reflecting the anxiety of the animal that tries to escape from the situation (Weiss et al., 1998). Taken together, our results could suggest that EMS causes an anxiogenic-like profile in adolescence. In this sense, previous works reported an increase of anxiety measured in the EPM test in adolescent mice after suffering 24 h of EMS at PND12, effect that authors interpreted as a reduced ability to cope with stressful situations and related with low BDNF protein levels in the hippocampus and amygdala (Martini and Valverde, 2011). Moreover, a more recent study has shown that adolescent rats that suffered 24 h of EMS on PND9 present a decrease in ambulation

and time expended in the centre portion of the OF, which is interpreted as an increased anxiety-like behaviour (Girard et al., 2014).

Although neonatal AlloP administration had no effects on Boissier exploration test in adolescence, which replicates previous results (Darbra and Pallarès, 2010), it presented a protective role over the EMS effects. That is, EMS animals that had been administered with AlloP travelled the same distance and explored the same number of holes than those animals that did not suffer EMS. As explained in the Introduction section, some preceding works already reported a protective role of previous AlloP administration on neonatal stress effects (Mitev et al., 2003; Zimmerberg and Kajunski, 2004). In the present work, it could be hypothesized that the previous increase in AlloP levels produced by its exogenous administration could be altering the expression of $GABA_AR$ or of its subunits, which in turn may interfere with the physiological response to the stress produced by EMS.

At PND60 neither EMS nor neonatal AlloP administration affected the travelled distance and the number of explored holes in Boissier exploration test. It has to be taken into account that during adolescence, maturation and rearrangement of major neurotransmitter pathways, such as mesocorticolimbic circuitry, are still taking place (see Spear, 2000 for review) and thus the effects of neonatal interventions may vary across the time. For instance, it has been reported that neonatal AlloP administration increased the locomotor response to amphetamine at PND20 (10 mg/kg of AlloP at PND2) and PND80 (10 mg/kg of AlloP at PND2 or at PND5) but not at PND40 or PND60 (Gizerian et al., 2006). On the other hand, given that at PND60 animals were exposed to the hole-board apparatus at the same conditions than at PND40, the lack of effects may be related to habituation (File, 2001). Previous works showed that the neophobic response experienced by subjects during the first exposure to a hole-board apparatus apparently declines with further exposures (Brown and Nemes, 2008). Thus, since results can be altered by emotional states of fear and anxiety elicited by the new environment (Weiss et al., 1998), the fact that the apparatus no longer produced the same neophobia levels could also explain why any effect was observed at 60 days old. Furthermore, it has to be taken into account that at PND40 animals exhibit behaviours that involve novelty seeking and risk taking, elevated basal levels of locomotor and explorative activity and higher levels of impulsivity than those shown in adulthood (Laviola et al., 2003). In this sense, it has been described that adolescent male rats (PND33–35) under some previous circumstances can be more anxious in the EPM test

than adults (PND70–75) (Doremus et al., 2004). In accordance, results of experiment 1 revealed that all animals (i.e. regardless of neonatal treatment) travelled less distance and explored fewer holes at 60 days than at 40 days old, replicating previous results (Darbra et al., 2003; Darbra and Pallarès, 2010).

1.2. Behavioural and cognitive effects of neonatal allopregnanolone administration and neonatal stress in adulthood

1.2.1. Anxiety-like behaviour

In contrast to adolescent results, in adulthood EMS related to an anxiolytic-like profile in the EPM test, i.e. all the animals that suffered EMS spent more time and realized more entries in the open arms than rats that did not suffer EMS. Moreover, these EMS effects were not affected by AlloP administration. This discrepancy between adolescent and adult effects shows again how neonatal interventions may cause different behavioural profiles depending on the animal's age and may be related both to the specific behavioural characteristics of adolescence as well as to the maturation and rearrangement of major neurotransmitter pathways (see above). In this sense, it has been described that some behavioural alterations caused by 24 h of EMS at PND9 do not develop until adulthood (Ellenbroek and Cools, 2002). Nevertheless, it is also possible that such behavioural differences between adolescent and adult effects relate to the distinct characteristics of the tests used, i.e. Boissier exploration test and EMP test. In any case, an anxiolytic-like profile in the EPM test in adulthood caused by 24 h of EMS on PND9 has also been reported by other authors in rats (Burke et al., 2013; Llorente-Berzal et al., 2011) and mice (Fabricius et al., 2008), as well as in female Wistar rats as a consequence of 3 daily hours of separation during the first 2 weeks of life (Eklund and Arborelius, 2006). However, discrepant results such as an anxiogenic-like profile (Rentesi et al., 2010) or no effects (Lehmann et al., 1999; Slotten et al., 2006) have also been reported. These variable results may be related to the use of a different rats' strain, since the EMS effects strongly depend on which strain is employed (Ellenbroek and Cools, 2000), as well as to the different EMS procedures and the specific conditions under which they are performed (Ellenbroek and Cools, 2002). Also, the use of different tests, the characteristics of the EPM apparatus or the testing conditions are factors that can dramatically affect the results (García et al., 2005; Jones and King, 2001; Violle et al., 2009). Nevertheless, it has to be taken into account that an increase in the

percentage of time spend in the open arms and of open arms entries in the EPM test could be not only interpreted as an anxiolytic-like profile but also as an enhanced risk-taking behaviour (Davies et al., 2009; Löfgren et al., 2006).

Neonatal AlloP administration had no effects in the EPM test, which is in accordance with previous studies from our lab showing that neither the dose used in the present work (10 mg/kg from PND5 to PND9) (Darbra and Pallarès, 2012), nor a single administration of 10 mg/kg on PND5 (Darbra and Pallarès, 2009), affected *per se* the measured variables in the EPM test. However, both higher AlloP doses (20 mg/kg from PND5 to PND9) (Darbra and Pallarès, 2012) and sub-chronic neonatal administration of lower AlloP doses (5 mg/kg between PND2 and PND6) (Zimmerberg and Kajunski, 2004) produced an anxiolytic-like profile in the EPM test in adulthood. Given that $GABA_AR$ sensitivity to AlloP binding depends on the subunit composition of the receptor (see Introduction section), these dose and day-dependent effects of neonatal AlloP administration could be related to the changes in $GABA_AR$ subunits expression during early development that previous studies have reported (Laurie et al., 1992).

Taken into account the EMS effects on novelty-directed exploration in adolescence and on anxiety-like behaviour in adulthood, it has to be considered that both represent emotional behaviours in which regulation participates the hippocampal formation (Bitran et al., 1999; Mineur et al., 2013; Mòdol et al., 2011; Xu et al., 1998), and thus the reported effects could be related to an altered hippocampal function in EMS animals. This area seems to be especially sensitive to EMS since its maturation extends into the first 3 weeks of postnatal life and it undergoes an experience-dependent development (Roceri et al., 2002). A previous work reported that mice that suffered 24 h of EMS at PND9 presented both an adult anxiolytic-like profile and a significant reduction in total neurons number in the dentate gyrus of the hippocampus (Fabricious et al., 2008). Accordingly to this data, other works have reported neonatal neurodegeneration in the hippocampal formation of rats as a consequence of 24 h of EMS at PND9 (Llorente et al., 2008, 2009; López-Gallardo et al., 2008). This neuronal loss is maintained until adolescence (Marco et al., 2013) and adulthood (López-Gallardo et al., 2012) and may be related to a decrease in neurogenesis or an increase in neuron apoptosis. Also, as explained in the Introduction section, 24 h of EMS on PND9 reduces the hippocampal expression of neurotrophin BDNF (Llorente et al., 2011; Marco et al., 2013; Roceri et al., 2002) and of synaptophysin and neural cell adhesion molecule (NCAM) (Llorente et al., 2011; Marco et al., 2013) in adolescence and adulthood. Given that the hippocampus

present high levels of glucocorticoid receptors (GR) that mediate negative feedback of HPA axis activation, developmental hippocampal alterations caused by EMS may be directly related to the increase of CORT levels during the critical SHRP (Lehmann et al., 2002). However, it has to be taken into account that hippocampal $GABA_AR$ have an increased sensitivity to AlloP during postnatal development (Mtchedlishvili et al., 2003). Thus, the possible alterations in neonatal AlloP levels related to EMS may in part underlie some of these hippocampal alterations found on EMS animals. In this sense, as explained in the Introduction section, neonatal NS manipulation though Finas administration has also been related to hippocampal alterations (Mòdol et al., 2014a, 2014b).

1.2.2. Sensorimotor gating

Both neonatal AlloP administration and EMS disrupted the PPI, without any interaction between them. Thus, neither animals that received AlloP nor animals that suffered EMS showed the expected progressive reduction of the startle response (and the consequent improvement of the PPI percentage) after the gradual increase in prepulse intensity (3, 5 and 10 dB above background), which indicates an alteration on the sensorimotor gating of these animals. These results were in accordance with previous studies showing a PPI disruption in adulthood after neonatal AlloP administration (Darbra and Pallarès, 2010; Gizerian et al., 2006), and as a consequence of 24 h of EMS at PND9 (Ellenbroek and Cools, 2002; Ellenbroek et al., 1998).

As explained in the Introduction section, sensorimotor gating is impaired in schizophrenia and other psychiatric disorders such as OCD, and PPI test is widely used both in humans and animal models as an operative measure of sensorimotor gating (Swerdlow et al., 2008). Multiple brain regions are involved in sensorimotor gating and thus, diverse neurological aberrations can underlie a deficient PPI. Hippocampal activity has proven necessary to maintain normal sensorimotor gating, since the temporary inactivation by tetrodotoxin (TTX, sodium-channel blocker) or the inhibition by muscimol ($GABA_AR$ agonist) of the dorsal or ventral hippocampus impairs PPI (Zhang et al., 2002). Authors propose that an altered hippocampal activity may decrease PPI by changing neuronal activity in the amygdala, the prefrontal cortex or the NAcc, all areas that have been related to sensorimotor gating (Zhang et al., 2002). Thus, hippocampal alterations on AlloP and EMS animals may underlie PPI disruption. In addition, previous studies

proposed that the deterioration of the PPI caused by neonatal AlloP administration (10 mg/kg on PND2 or on PND5) could be related to a possible decrease of both GABA-mediated and DA-mediated inhibition in prefrontal cortex (Gizerian et al., 2006). As hippocampal formation, prefrontal cortex laminates and forms its mature pattern of connections after birth and this confers it a special vulnerability to neonatal AlloP fluctuations and neonatal stress (Gizerian et al., 2006; Micheva and Beaulieu, 1997). In this sense, it has been reported that neonatal AlloP administration (10 mg/kg on PND1 or on PND5) alters GABAergic interneurons localization in adult prefrontal cortex of rats (Grobin et al., 2003).

On the other hand, results of experiment 1 show that neonatal AlloP administration and EMS not only caused a deterioration of PPI but also altered the basal startle response. Startle reflex is a cross-species, stereotyped response consequent to the presentation of a sudden and unexpected sensory stimulus. It consists of a rapid sequential muscle contraction with the likely purpose of facilitating the flight reaction and/or to protect the body from a sudden attack. Animal and, more recently, human investigations have shown that the basal startle response can be increased by behavioural manipulations causing fear and anxiety (see Grillon, 2008 for review). In agreement with previous results (Darbra and Pallarès, 2010), animals with neonatal AlloP administration presented a decrease of the basal startle response. Thus, these animals seem to be less reactive to an auditory stimulus of high intensity which may indicate that neonatal AlloP administration could lead to a decrease in the animals' fear levels (Darbra and Pallarès, 2010). In contrast, animals that suffered EMS exhibited an enhanced startle response. Previous studies found no effects of EMS on the basal startle response of PND65 Wistar rats (Ellenbroek and Cools, 2002; Ellenbroek et al., 1998). Nevertheless, divergences in the results may be related to the specific conditions under which the test was performed, as in our experiment animals performed PPI test just after the EPM test, while in previous studies animals were left undisturbed in a room adjacent to the startle chamber room for at least 30 or 45 min before the PPI test (Ellenbroek and Cools, 2002; Ellenbroek et al., 1998).

2. Experiment 2

2.1. Effects of neonatal physiological allopregnanolone levels manipulation and neonatal stress on adult voluntary ethanol consumption

In experiment 2A, the analysis of the ethanol doses consumed day by day during the 14 days of the procedure by no-EMS animals, showed that during the first 7 days there were not differences between groups in ethanol consumption. However, from day 8 animals with neonatal Finas administration consumed higher doses of ethanol than AlloP and control animals (Veh and NH). Thus, the escalation in alcohol drinking during the procedure was much more prominent in Finas rats. The fact that neonatal Finas increased ethanol intake during the second week but not during the first days of the procedure, could suggest a main effect of neonatal Finas treatment in the period in which the pattern of drinking behaviour is being consolidated (Spanagel, 2000; Vengeliene et al., 2005). On the other hand, we observed that animals that received neonatal AlloP consumed similar ethanol doses than NH animals, while those that were administered with Veh presented a lower ethanol intake than the rest of the animals in the last two days of consumption. Importantly, when animals suffered EMS, there were no effects of previous neonatal treatment and all the animals progressively increase ethanol consumption in the same way. Thus, EMS diluted the differences induced by previous NS manipulation.

Neonatal Finas administration not only increased ethanol but also glucose consumption during the second week of the procedure. Interestingly, the effects of Finas upon glucose intake were independent of the EMS condition, i.e. all the animals that received neonatal Finas consumed higher doses of glucose in adulthood. Given that neonatal Finas administration increased both ethanol and glucose intake, there were no differences on the ethanol preference of these animals. It has to be taken into account that as natural rewards, ethanol or other drugs, the ingestion of sweet substances activates the mesolimbic reward pathways and elicits DA release in the NAcc (Hajnal et al., 2004; Levine et al., 2003; Norgren et al., 2006). Furthermore, its hedonic responses involve opioidergic and serotonergic neurotransmitter systems which also participate in alcohol addiction (Bachmanov et al., 2011); thus the consumption of sweet solutions and ethanol activates similar reward mechanisms. In this sense, several studies have shown that a high intake of sweet solutions positively correlates with high ethanol consumption

(Gosnell and Krahn, 1992; Koros et al., 1998), as well as diverse lines of selectively bred ethanol-preferring rats also present a high sweet preference (Sinclair et al., 1992; Woods et al., 2003) and *vice versa* (Dess et al., 1998), indicating genetic overlapping between the preference for "sweet" solutions and high ethanol intake (Bachmanov et al., 2011).

When animals did not suffer EMS, those that received neonatal AlloP consumed similar amounts of ethanol than rats from NH group, i.e. lower doses than Finas treated rats, but higher ethanol doses than Veh group on the last two days of the procedure. In fact, animals that received neonatal Veh administration and did not suffer EMS did not show any increase in ethanol consumption at the end of the procedure, and on the last two days consumed lower ethanol doses than the rest of the animals (that is: Finas, NH and AlloP groups). It has to be taken into account that ethanol consumption is not stable from day to day, and the pattern of consumption may present ups and downs between days. Thus, the observed low ethanol intake in the last two days of the procedure in Veh group could correspond to a temporal decrease in the consumption but not to an established low ethanol intake. Protocols with longer periods of consumption would help to determine if this is a circumstantial effect or if it is maintained along the time with a stable low consumption. However, a Veh effect could be related to the neonatal manipulation that these animals received. In this sense, it has to be taken into account that animals were separated from their mother about 10 min per day between PND5 and PND9 in order to be injected. Importantly, short periods of maternal absence (< 15 min per day) have proven beneficial for the offspring (Nylander and Roman, 2013), and animals submitted to them usually present lower ethanol consumption than non-separated animals (Hilakivi-Clarke et al., 1991; Ploj et al., 2003). This effect is also found in ethanol-preferring rats (Roman et al., 2003), showing that short periods of maternal separation can even counteract genetic predisposition for high ethanol consumption (Nylander and Roman, 2013). Although these models usually imply more days of maternal separation (from PND1 to PND21), data seem to indicate that the most sensible days to reach this protective effect are from PND5 to PND10 (Hilakivi-Clarke et al., 1991; Nylander and Roman, 2013). Thus, it could be that the low ethanol consumption observed in the Veh administered group was reflecting the protective effect of short maternal separation periods. On the other hand, the differences between Veh and AlloP group could relate to the fact that both administrations could differently affect pups' behaviour, thus eliciting differences in dam's mothering style, which in turn could have affected adult behaviour (Weaver et al., 2004). In a previous study we already reported Veh effects altered by AlloP administration (Mòdol et al., 2013).

Results of experiment 2A showed that when animals suffered EMS there were no differences between neonatal treatments on ethanol consumption. Thus, EMS changed the effects of the previous NS manipulation. Moreover, there was a main EMS effect on the average dose of alcohol consumption in adult age (mean of 14 days), that showed that all the animals that suffered EMS consumed a high average ethanol dose than animals that did not suffer EMS. However, detailed analysis of the ethanol doses consumed day per day revealed that the increase in alcohol intake induced by EMS was statistically significant only in the animals that were neonatally injected with Veh. That is, the amount of ethanol consumed was statistically the same in the no-EMS and EMS rats that were neonatally not-manipulated (NH group) or injected with Finas or AlloP. In this sense, longer periods of maternal absence (usually models of 180/360 min per day between PND1 and PND21) are considered detrimental and high ethanol consumption has been reported as a consequence, even though this increase in ethanol intake is usually only found when comparing with animals submitted to shorter periods of separation, but not when compared to NH animals (Hilakivi-Clarke et al., 1991; Nylander and Roman, 2013; Roman et al., 2003). Thus, our results seem in accordance with these observations.

In addition to the effects on ethanol consumption, animals that were submitted to EMS consumed higher doses of glucose than the animals that did not suffer EMS during the first week of consumption, independently of the previous neonatal treatment. In this sense, and using diverse models of long periods of maternal separation, other studies have reported alterations in a sucrose preference test, specifically an increase in the adult consumption of a sucrose solution (Michaels and Holtzman, 2006; Mourlon et al., 2010) and an increased sucrose preference (Mourlon et al., 2010). Previous works have shown that diverse kinds of stressors with subsequent increase in CORT, increase the consumption of sweet food (Dallman et al., 2005; Ely et al., 1997; Machado et al., 2013), effect that does not relate to hunger states (Hagan et al., 2003; Machado et al., 2013) and in some cases neither to an increased anxiety-like behaviour (Silveira et al., 2005). Thus, given that rats submitted to 24 h of EMS at PND9 seem to present an enhanced ACTH and CORT response to stressful stimulus in adult age (Avishai-Eliner et al., 1995; Lehmann et al., 2002; Suchecki et al., 1993), the increase in glucose consumption may be related to an increased reactivity in front an environmental stimulus, e.g. food restriction or isolation. In this sense, neonatal stress caused by a limitation of nesting material from PND2 to PND9 leads to an increase in the consumption of palatable food

in adult female Wistar rats, effect that authors also related with a hyper-reactivity of the HPA axis in front of acute stress (Machado et al., 2013).

Finally, neonatal AlloP administration increased the alcohol preference during the first week of the consumption period but only in those animals that suffered EMS. Given the lack of differences between injection groups on ethanol intake in the subgroup of EMS animals, this effect is probably related to the low glucose consumption that AlloP animals presented during the first days of the procedure. Nevertheless, as this effect was only observed in EMS animals, it seems that there is a summative effect of exogenous AlloP administration and EMS.

2.2. Effects of neonatal physiological allopregnanolone levels manipulation and neonatal stress on ventrostriatal monoamine levels and on the activity of the mesolimbic dopaminergic pathways in adulthood

In experiment 2A we determined the monoamine levels in the ventral striatum after the 15 days of consumption. Results show that animals administered with neonatal Finas that did not suffer EMS, presented decreased ventrostriatal DA and 5-HT levels in comparison to controls (Veh and NH groups) without any alteration on their corresponding metabolites, i.e. DOPAC, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA). Thus, animals treated with Finas showed an increased turnover ratio of DA and 5-HT (DOPAC/DA, HVA/DA and 5-HIAA/5-HT), which possibly indicates a decrease in DAergic and serotonergic activity. Previous studies have related alterations in both DA and 5-HT activity with an altered ethanol consumption. For instance, decreased reward circuitry activation could lead to the consumption of higher amounts of ethanol in order to get a higher DA release (Blum et al., 1996; Leyton, 2014). Moreover, while an increase of 5-HT has been related to a reduced ethanol intake (Lu et al., 1994; Naranjo et al., 1994), deficits of 5-HT in the NAcc and other structures seem to lead to a higher alcohol consumption both in animals' models and in humans (Borg et al., 1985; Czachowski, 2005; Lovinger, 1999; Murphy et al., 1982; Sachs et al., 2014; see Müller and Homberg, 2015 for review). In the same way, ethanol preferring rats have a decreased overall serotonin function relative to ethanol-nonpreferring rats (McBride and Li, 1998; Müller and Homberg, 2015).

As observed in Finas animals, rats that received neonatal AlloP and did not suffer EMS also presented lower DA and 5-HT levels than control animals (Veh and NH) and

increased turnover ratios (DOPAC/DA, HVA/DA and 5-HIAA/5-HT) without affectation of DOPAC and HVA, but with lower 5-HIAA levels than NH animals. Moreover, all the animals of AlloP group presented low noradrenalin (NA) levels than NH animals. It has been suggested that noradrenergic system participates in ethanol-motivated behaviours (Verplaeste and Czachowski, 2015). In this sense, chemical lesions on noradrenergic system, blockade of NA synthesis and agents that reduce NA activity decrease voluntary ethanol intake (Froehlich et al., 2013; Weinsshenler and Schroeder, 2007). Since Finas and AlloP animals present a distinct pattern of ethanol intake, it may be surprising to find similar alterations on monoamine levels in both groups, but it must be considered that reported levels were obtained at the end of the procedure, after the two weeks of consumption. Thus, although there were no significant correlations between ethanol intake and monoamine levels, we cannot discard any influence of the ethanol consumed during 15 days in the recorded monoamine levels. Other studies to determine the basal monoamine levels of these animals are needed to clarify this issue and better interpret the effects.

In order to understand why both neonatal Finas and neonatal AlloP administration similarly alter DA and 5-HT levels, it also has to be taken into account that neonatal Finas administration could be preventing not only the synthesis of AlloP but also of THDOC, as well as the conversion of testosterone to 5α-DHT. In this sense, in previous works we reported that the same neonatal Finas administration caused an increase of hippocampal testosterone at PND9 (Darbra et al., 2013) and it has been reported that alterations in neonatal testosterone are related to impulsivity (Bayless et al., 2013), a trait which is considered a risk factor for drug abuse (Belin and Deroche-Gamonet, 2012). Therefore, the effects of neonatal Finas administration may not necessarily be due to an inhibition of AlloP synthesis alone, but through affecting other neonatal NS levels.

Interestingly, all the animals that suffered EMS presented similar ventrostriatal monoamines levels to those of the control groups, which are higher than those found in Finas or AlloP administered animals not submitted to EMS. Thus, EMS is somehow altering the effects of neonatal Finas and AlloP administration on adult monoamine levels, may be by causing further alterations on the neonatal NS milieu of these animals. Although we did not found altered ventrostriatal monoamine levels in EMS animals previously non-handled (NH+EMS group), other works have reported diverse DAergic and serotonergic alterations after 24 h of EMS at PND9. For instance, Rentesi and colleagues (2013) reported an increased DA turnover as well as increased DA D2

receptors (DRD2) expression in adult rat striatum (Rentesi et al., 2013), although other authors showed a decreased DRD2 expression in rat NAcc (Zamberletti et al., 2012). Regarding 5-HT, previous results showed no effects of EMS on 5-HT levels on the striatum, but a decrease of 5-HT type 2A (5-HT2A) receptor expression in adulthood (Rentesi et al., 2013).

In order to better characterize the effects of neonatal NS manipulations on DA response to ethanol, we performed experiment 2B, in which we measured DA and DOPAC release in NAcc during ethanol drinking in animals that were neonatally injected with Finas, AlloP or Veh and did not suffer EMS. In this second experiment, we also measured the ethanol and glucose consumption during the 11 days prior to the microdialysis procedure. In agreement with the results of experiment 2A, animals with neonatal administration of Finas consumed higher doses of ethanol than those administered with Veh or AlloP during several days of the consumption period. However, in experiment 2B the increase in ethanol intake was found at the beginning and at the end of the procedure, instead of during the second week. Moreover, neonatal Finas administration did not affect the glucose consumption, and there were not significant differences between AlloP and Veh groups on the ethanol intake. These distinct results of experiment 2A and 2B may be related to some protocol dissimilarities between both experiments. In experiment 2A animals received their daily ration of food during the hour of access to the ethanol and glucose solutions, but in experiment 2B food's rations were provided just after the drinking hour in order to distinguish between DA response to solutions (ethanol + glucose) access and DA response to food presentation during the microdialysis prove. Moreover, it has to be taken into account that in experiment 2B animals were submitted to stereotaxic surgery prior the consumption period and were daily manipulated in order to check the state of the guide cannula.

Microdialysis results revealed that DA release in NAcc increased in Veh and AlloP groups (increase of DA expressed as percentage of baseline) after solutions presentation, but not in those animals that received neonatal Finas. Thus, in Finas group, DA release in NAcc was not affected by solutions presentation. In accordance, the global increase of DA release respect the baseline in response to solutions (ethanol and glucose) and food presentation was lower in subjects that received neonatal Finas than in control animals (Veh group). Finas animals also tended to have a reduction of DOPAC levels, although differences between groups did not reach statistical significance. A reduction of DOPAC levels is in accordance with a reduced DA release. On the other hand, animals that

received neonatal AlloP released similar amounts of DA in NAcc in front of solutions (ethanol and glucose) and food than those of the animals that received Veh. Thus, neonatal AlloP administration did not affect DA release and neither DOPAC levels.

These data seem to indicate a hypo-DAergic activity in neonatal Finas treated rats that could be related to the increase in alcohol intake reported in these animals. In this sense, it has been described that a hypo-DAergic activity, caused either by a decrease in DRD2 or by a decreased presynaptic DA release in the striatum, leads to an excessive craving and seeking for substances known to cause DA release in the NAcc (Blum et al., 1996, 2014; Trifilieff and Martinez, 2014). Several studies in rats have shown that lines selective breed for high ethanol preference present decreased DRD2 density in NAcc (McBride et al., 1993), and lower DAergic innervation (Casu et al., 2002; Zhou et al., 1995), with lower DA content (McBride et al., 1995; Murphy et al., 1982), in the NAcc. In the same way, alcoholism in humans has been related with significant reductions in DR2D availability as well as reduced DA release in striatum (see Volkow et al., 2009 for review). Thus, our data could indicate that the higher ethanol consumption that neonatal Finas treated rats show, relates to a blunted DA response to the presentation of the solutions. However, we cannot assume a causal relationship between the hypo-DAergic activity and the increased ethanol consumption in Finas rats. As previously mentioned, other studies determining the basal monoamine levels prior to the alcohol consumption phase are needed to test this hypothesis. In accordance with these results, we have recently report that rats that received neonatal Finas presented a reduced sensibility to the stimulating motor effects of an acute intraperitoneal (i.p.) administration of 0.5 g/kg of ethanol (Bartolomé et al., 2017). Locomotor sensitivity to ethanol has been related with DAergic activity, and DA inhibition seems to lead to a reduced sensitivity to ethanol stimulating effects (see Brabant et al., 2014 for review).

Elucidating how neonatal Finas administration can lead to this blunted DA response and to the increased consumption of ethanol (experiment 2A and 2B) and reinforcing solutions (glucose) (experiment 2A) is complex, since several alterations could be taking place. As explained in the Introduction section, we have reported that neonatal administration of Finas at the same doses relates to an over-expression of α4 and δ $GABA_AR$ subunits in the hippocampus, which is still present in adulthood (Mòdol et al., 2014a). Thus, it is plausible to think that the increase in alcohol consumption found in the rats that received neonatal Finas could be related, at least in part, to an altered α4 and δ expression. The extrasynaptic isoform α4βδ of $GABA_AR$ has proven to be sensitive

to low-moderate ethanol doses in diverse *in vitro* studies (Sundstrom-Poromaa et al., 2002; Wei et al., 2004). Besides, *in vivo* studies have shown that the reduced expression of α4 subunit in the NAcc shell of rats decreased their free consumption and preference for alcohol (Rewal et al., 2009), as well as their instrumental responding for oral ethanol (Rewal et al., 2012). In the same way, knockdown of the δ subunit in the medial shell region of the NAcc reduces alcohol intake (Nie et al., 2011) and δ knockout mice have a reduced ethanol preference and consumption (Mihalek et al., 2001). Furthermore, it has to be taken into account that the DAergic neurons of the ventral tegmental area (VTA) that project to NAcc are under GABAergic regulation by means of local interneurons (Johnson and North, 1992) and GABAergic projections arising from the NAcc and ventral pallidum (Kalivas et al., 1993). $GABA_AR$ in the VTA are present presynaptically in GABAergic axons terminals (Laviolette and van der Kooy, 2001; Xiao et al., 2007) and postsynaptically on the DAergic neurons (Okada et al. 2004; Westerink et al., 1996). In this sense it has been suggested that α4βδ $GABA_AR$ are present in these GABAergic terminals participating in the regulation of the excitability of DAergic neurons that project to the NAcc (Xiao et al., 2007). Thus, alterations in the expression of $GABA_AR$ subunits on the VTA could lead to an altered GABAergic control that could explain the blunted DA activity. On the other hand, as explained in the Introduction section, given the important role of GABA during the development, neonatal alterations in GABAergic transmission due to changes in $GABA_AR$ endogenous modulators, could affect the postnatal maturation of the mesocorticolimbic DAergic system (Antonopoulos et al., 2002; Gizerian et al., 2006; Park et al., 2000).

In addition to the GABAergic control on DAergic activity, it has to be taken into account that serotonergic neurons from the dorsal raphe nucleus also modulate the DA release in the NAcc by acting on the DAergic and GABAergic neurons of the VTA and directly on the NAcc (see Sari et al., 2011 for review). In experiment 2A the analysis of the ventral striatum samples revealed that rats neonatally administered with Finas had a low concentration of 5-HT and an increased turnover ratio 5-HIAA/5-HT without alterations in 5-HIAA levels, therefore, an altered serotonergic function may also be contributing to the decreased DAergic activity observed in animals that received neonatal Finas. In this sense, 5-HT3 receptors may play an important role regulating the activity of VTA DAergic neurons and their projections to NAcc. Several studies have shown that ethanol increases 5-HT3 receptor-mediated ion currents *in vitro* (Lovinger and White, 1991; Lovinger et al., 2000; Machu and Harris, 1994; Zhou et al., 1998) and its activation in the posterior VTA seems to be involved on alcohol-induced DA increase

in ventral pallidum and medial prefrontal cortex (Ding et al., 2011). Furthermore, the administration of 5-HT3 receptor agonists increase extracellular DA in VTA and NAcc (Campbell et al., 1996; Campbell and McBride, 1995, Liu et al., 2006), while administration of 5-HT3 antagonists reduce the ethanol-induced extracellular DA increase in VTA and NAcc (Campbell et al., 1996; Campbell and McBride, 1995; Wozniak et al., 1990) and the voluntary alcohol intake in alcohol preferring rats (Fadda et al., 1991; McKinzie et al., 1998; Rodd-Henricks et al., 2000). Given this results, we are currently performing a study in order to elucidate if the administration of a 5-HT3 receptor antagonist alters ethanol consumption of neonatal Finas administered rats and to evaluate if this group present an altered ventrostriatal 5-HT3 expression.

3. General discussion

The objective of the present studies was to assess the consequences of neonatal NS alterations and of neonatal stress induced by EMS, in adolescent and adult behaviour in order to characterise their possible interactions and relationship. In adulthood, AlloP levels increase after an acute stress, which has been proposed to serve as a mechanism that helps to restore physiological homeostasis. However, the implications of AlloP on neonatal stress are much less clear. Thus, taken into account the importance of physiological neonatal AlloP levels in brain development and posterior adolescent and adult behaviour, we hypothesized that the behavioural effects of neonatal stress could be in part related to changes on neonatal AlloP levels. Thus, we expected to find that the manipulation of neonatal physiological NS, by means of the administration of AlloP or Finas, would interfere with the behavioural alterations caused by neonatal stress. In the same way, we presumed that the behavioural effects of AlloP or Finas administration could be altered by the pups' submission to posterior EMS.

Results seem to confirm this hypothesis since in experiment 1 we found that the neonatal administration of AlloP prevented the EMS effects on novelty-directed exploration in the middle adolescence. On the other hand, in experiment 2 EMS diluted the Finas effects on ethanol intake and change the effects of both AlloP and Finas administration on ventrostriatal monoamines levels. Even so, both interventions caused other effects in which no interaction was found. Thereby, EMS caused an anxiolytic-like profile in the EPM test, while AlloP did not alter this EMS effect, and at the tested doses neither affected the behaviour in the EMP *per se*. Remarkably, both interventions decreased the PPI response in adulthood. Although this might seem an expected effect since previous works already reported similar effects studying both factors independently, this was the first time that their possible interaction on the PPI test was studied. Thus, the administration of neonatal AlloP seems to be non-protective upon EMS effects on sensorimotor gating, also having in fact a negative outcome. Although similar effects do not imply equivalent mechanisms, it is tempting to speculate that in addition to the deleterious effects of increased glucocorticoids during the SHRP (Lehmann et al., 2002), the alteration of AlloP levels caused by EMS could be a putative mechanism that underlies its long-lasting effects, at least those related to sensorimotor gating. In this sense, it would be interesting to characterise which NS are altered as a

consequence of EMS, in which way and in which brain areas during the neonatal stage, as well as how the previous AlloP administration affects the HPA axis response to EMS in our protocol.

It is noteworthy that no previous work studied the possible effects of altered neonatal NS levels on the vulnerability to ethanol consumption in adulthood. Thus, present studies reveal important new data especially regarding the effects of neonatal Finas administration, which could be related to changes on neonatal AlloP levels but also to affectations on other NS synthesis pathways, such as the reduction of testosterone into 5α-DHT and of 11-deoxycorticosterone into 5α-DHDOC, which can consequently affect THDOC. Thus, given the observed effects of neonatal Finas administration on ethanol consumption (increase) and DAergic function in the NAcc in response to solutions and food presentation (decrease), it would be of high interest to better characterize its effects on other aspects of ethanol consumption, such as longer periods of consumption, abstinence, relapse, or its response to some agents used to treat alcoholism (e.g. naltrexone, topiramate, baclofen). The study of the neuroanatomical and neurochemical disruptions that may underlie these effects and the mechanism by which neonatal Finas administration alters neurodevelopment would also be of interest.

We decided to study the possible implications of altered neonatal NS levels and EMS on the vulnerability to drug abuse given previous results showing that neonatal AlloP administration reduced anxiety levels (Darbra and Pallarès, 2012; Zimmerberg and Kajunski, 2004) and increased exploration in novel environments (Dabra and Pallarès, 2009). As behavioural patterns of sensation seeking, impulsivity and novelty preference are related to increased vulnerability to drug abuse both in animals (Molander et al., 2011) and in humans (Bjork et al., 2004; Fernie et al., 2013; Kelly et al., 2006; Peterson and Smith, 2017), it could be hypothesized that neonatal AlloP-treated rats could present higher susceptibility to drug abuse. However, our results show that neonatal AlloP administration increased ethanol intake only respect Veh rats and only in the last two days of the procedure. These results are in agreement with the behavioural profile that rats showed in experiment 1, where the neonatal administration of AlloP at the present doses (10 mg/kg, PND5-PND9) did not alter the novelty-directed exploration behaviour on adolescent age and neither the anxiety-like behaviour on adult age. Thus, it would be interesting to study the effects of higher neonatal AlloP doses, known to alter behaviours that could relate to drug abuse vulnerability (Dabra and Pallarès, 2009, 2012), on ethanol consumption. Moreover, since in the present work AlloP effects not

appear until the last two days of the procedure, it could be considered the use of other ethanol consumption procedures, i.e. longer consumption period or non-limited access to the solutions. On the other hand, regarding EMS effects, results of experiment 1 are also in agreement with results of experiment 2, given that in adulthood EMS animals showed an anxiolytic-like profile in the EPM test, which could also be interpreted as an impulsive behaviour (Davies et al., 2009; Löfgren et al., 2006), and presented an increased ethanol intake.

Among all the possible affectations that may underlie the reported behavioural effects, it has to be taken into consideration the possible alterations in the GABAergic system. As already explained, both neonatal stress and neonatal NS manipulations resulted in alterations on the expression of $GABA_AR$ subunits, which may in turn affect $GABA_AR$ sensitivity to NS modulation. Moreover, previous studies also showed that both neonatal interventions affect NS milieu in adulthood. Thus, the better characterisation of these aspects would be of great interest given that alterations of both GABAergic function and adult NS biosynthesis and/or metabolism have been related to several of the studied behaviours (e.g. anxiety-like behaviour, sensorimotor gating and voluntary ethanol consumption), as well as to an altered DAergic activity.

Taken together, our results remark on the importance of both neonatal NS levels and neonatal stress events on critical neurodevelopmental periods, showing that the neonatal administration of AlloP may prevent some of the behavioural EMS effects on adolescence but also cause deleterious effects similar to those of EMS in adulthood (i.e. impaired sensorimotor gating). Moreover, it is shown for first time a possible implication of neonatal AlloP alterations in adult alcohol use disorders. Given that neonatal NS are highly sensitive to modulation by external factors such as stress, and this can dramatically affect neurodevelopment, study the effects of neonatal NS by means of its exogenous administration may represent a useful tool to understand their role on brain development and their implication on the effects of environmental circumstances that can alter them, such as neonatal stress.

CONCLUSIONS

EXPERIMENT 1

► EMS altered novelty-directed exploration, as reflected by an increase in locomotor activity and a decrease in head-dipping behaviour in the Boissier exploration test, but only in middle adolescence.

► The neonatal administration of AlloP prevented the EMS effects on novelty-directed exploration in middle adolescence.

► Animals that suffered EMS showed an anxiolytic-like profile in EPM test in adulthood, as reflected by the increase of time and entries in the open arms.

► Both interventions, neonatal AlloP administration and EMS, reduced PPI in adulthood, providing evidence of deficient sensorimotor gating.

EXPERIMENT 2

► The consumption of ethanol and reinforcing solutions (glucose) was increased by the neonatal administration of Finas.

► Neonatal AlloP administration increased ethanol consumption in the last two days of the procedure compared with the Veh group.

► EMS increased ethanol and glucose intake.

► The effects of neonatal administrations on alcohol intake were not present in the animals that suffered EMS.

► Neonatal Finas and AlloP administration decreased ventrostriatal DA and 5-HT levels, increasing their corresponding turnover ratios.

► The decrease of ventrostriatal DA and 5-HT levels observed in Finas and AlloP groups, was not found in those animals that suffered EMS.

► Neonatal Finas administration decreased DA release in the NAcc in response to both ethanol and food, suggesting a blunted DA release in response to solutions presentation.

REFERENCES

A

Agis-Balboa, R.C., Pinna, G., Zhubi, A., Maloku, E., Veldic, M., Costa, E., Guidotti, A., 2006. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc. Natl. Acad. Sci. U. S. A. 103: 14602-7.

Ahmari, S.E., Risbrough, V.B., Geyer, M.A., Simpson, H.B., 2012. Impaired sensorimotor gating in unmedicated adults with obsessive-compulsive disorder. Neuropsychopharmacology. 37 (5): 1216-23.

Akk, G., Bracamontes, J., Steinbach, J.H., 2001. Pregnenolone sulfate block of GABA(A) receptors: mechanism and involvement of a residue in the M2 region of the alpha subunit. J. Physiol. 532 (Pt 3): 673-84.

Antonopoulos, J., Dori, I., Dinopoulos, A., Chiotelli, M., Parnavelas, J.G., 2002. Postnatal development of the dopaminergic system of the striatum in the rat neuroscience. 110 (2): 245-256.

Avishai-Eliner, S., Yi, S-J., Newth, C.L.J., Baram, T.Z., 1995. Effects of maternal and sibling deprivation on basal and stress induced hypothalamic-pituitary-adrenal components in the infant rat. Neurosci. Lett. 192: 49-52.

Azzolina, B., Ellsworth, K., Andersson, S., Geissler, W., Bull, H.G., Harris, G.S., 1997. Inhibition of rat a-reductases by finasteride: evidence for isozyme differences in the mechanism of inhibition. J. Steroid Biochem. Mol. Biol. 61: 55–64.

B

Bachmanov, A.A., Bosak, N.P., Floriano, W.B., Inoue, M., Li, X., Lin, C., Murovets, V.O., Reed, D.R., Zolotarev, V.A., Beauchamp, G.K., 2011. Genetics of sweet taste preferences. Flavour. Fragr. J. 26 (4): 286-294.

Backstrom, T., Bixo, M., Johansson, M., Nyberg, S., Ossewaarde, L., Ragagnin, G., Savic, I., Stromberg, J., Timby, E., van Broekhoven, F., van Wingen, G., 2014. Allopregnanolone and mood disorders. Prog. Neurobiol. 113: 88-94.

Bale, T.L., Baram, T.Z., Brown, A.S., Goldstein, J.M., Insel, T.R., McCarthy, M.M., Nemeroff, C.B., Reyes, T.M., Simerly, R.B., Susser, E.S., Nestler, E.J., 2010. Early life programming and neurodevelopmental disorders. Biol. Psychiatry. 68 (4): 314-9.

Barbaccia, M.L., Affricano, D., Trabucchi, M., Purdy, R.H., Colombo, G., Agabio, R., Gessa, G.L., 1999. Ethanol markedly increases “GABAergic” neurosteroids in alcohol-preferring rats. Eur J Pharmacol. 384: R1–2.

Barbaccia, M.L., Concas, A., Serra, M., Biggio, G., 1998. Stress and neurosteroids in adult and aged rats. Exp. Gerontol. 33 (7-8): 697-712.

Barbaccia, M.L., Roscetti, G., Trabucchi, M., Mostallino, M.C., Concas, A., Purdy, R.H., Biggio, G., 1996. Time-dependent changes in rat brain neuroactive steroid concentrations and GABAA receptor function after acute stress. Neuroendocrinology 63 (2): 166-72.

Bartolomé, I., Llidó, A., Darbra, S., Pallarès, M., 2017. Effects of neonatal and adolescent neuroactive steroid manipulation on locomotor activity induced by ethanol in male wistar rats. Behav. Brain Res. 330: 68-74.

Baulieu, E.E., Fluxe, K., Gustafsson, J.A., Weterberg, L., 1981. Steroid Hormones in the Brain: Several Mecahnism? In Steroid Hormone Regulation of the Brain. Pergamon Press, Oxford. 3-14.

Bayless, D.W., Darling, J.S., Daniel, J.M., 2013. Mechanisms by which neonatal testosterone exposure mediates sex differences in impulsivity in prepubertal rats. Horm. Behav. 64: 764–769.

Belelli, D., Lambert, J.J., 2005. Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat. Rev. Neurosci. 6 (7): 565-75.

Belin, D., Deroche-Gamonet, V., 2012. Responses to Novelty and Vulnerability to Cocaine Addiction: Contribution of a Multi-Symptomatic Animal Model. Cold Spring Harb. Perspect. Med. 2 (11), pii: a011940.

Ben-Ari, Y., Gaiarsa, J.L., Tyzio, R., Khazipov, R., 2007. GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. Physiol. Rev. 87 (4): 1215-84.

Ben-Ari, Y., Khalilov, I., Kahle, K.T., Cherubini, E., 2012. The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. Neuroscientist. 18 (5): 467-86.

Berretti, R., Santoru, F., Locci, A., Sogliano, C., Calza, A., Choleris, E., Porcu, P., Concas, A., 2014. Neonatal exposure to estradiol decreases hypothalamic allopregnanolone concentrations and alters agonistic and sexual but not affective behavior in adult female rats. Horm. Behav. 65 (2): 142-53.

Biggio, G., Concas, A., Corda, M.G., Giorgi, O., Sanna, E., Serra, M., 1990. GABAergic and dopaminergic transmission in the rat cerebral cortex: effect of stress, anxiolytic and anxiogenic drugs. Pharmacol, Ther. 48 (2): 121-42.

Binder, E.B., Bradley, R.G., Liu, W., Epstein, M.P., Deveau, T.C., Mercer, K.B., Tang, Y., Gillespie, C.F., Heim, C.M., Nemeroff, C.B., Schwartz, A.C., Cubells, J.F., Ressler, K.J., 2008. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA. 299 (11): 1291-305.

Bitran, D., Dugan, M., Renda, P., Ellis, R., Foley, M., 1999. Anxiolytic effects of the neuroactive steroid pregnanolone (3 alpha-OH-5 beta-pregnan-20-one) after microinjection in the dorsal hippocampus and lateral septum. Brain Res. 850 (1-2): 217-24.

Bixo, M., Andersson, A., Winblad, B., Purdy, R.H., Bäckström, T., 1997. Progesterone, 5alpha-pregnane-3,20-dione and 3alpha-hydroxy-5alpha-pregnane-20-one in specific regions of the human female brain in different endocrine states. Brain Res. 764 (1-2): 173-8.

Bjork, J.M., Hommer, D.W., Grant, S.J., Danube, C., 2004. Impulsivity in abstinent alcohol-dependent patients: relation to control subjects and type 1-/type 2-like traits. Alcohol 34 (2-3): 133-50.

Blum, K., Sheridan, P.J., Wood, R.C., Braverman, E.R., Chen, T.J., Cull, J.G., Comings, D.E., 1996. The D2 dopamine receptor gene as a determinant of reward deficiency syndrome. J. R. Soc. Med., 89 (7): 396-400.

Blum, K., Thanos, P.K., Gold, M.S., 2014. Dopamine and glucose, obesity, and reward deficiency syndrome. Front. Psychol. 17 (5): 919.

Bock, J., Rether, K., Gröger, N., Xie, L., Braun, K., 2014. Perinatal programming of emotional brain circuits: an integrative view from systems to molecules. Front. Neurosci. 8: 11.

Borg, S., Kvande, H., Liljeberg, P., Mossberg, D., Valverius, P., 1985. 5-Hydroxyindoleacetic acid in cerebrospinal fluid in alcoholic patients under different clinical conditions. Alcohol. 2 (3): 415-8.

Boyd, K.N., Kumar, S., O'Buckley, T.K., Porcu, P., Morrow, A.L., 2010. Ethanol induction of steroidogenesis in rat adrenal and brain is dependent upon pituitary ACTH release and de novo adrenal StAR synthesis. J Neurochem 112: 784–796.

Brabant, C., Guarnieri, D.J., Quertemont, E., 2014. Stimulant and motivational effects of alcohol: lessons from rodent and primate models. Pharmacol, Biochem. Behav. 122: 37-52.

Bradley, R.G., Binder, E.B., Epstein, M.P., Tang, Y., Nair, H.P., Liu, W., Gillespie, C.F., Berg, T., Evces, M., Newport, D.J., Stowe, Z.N., Heim, C.M., Nemeroff, C.B., Schwartz, A., Cubells, J.F., Ressler, K.J., 2008. Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor gene. Arch. Gen. Psychiatry 65 (2): 190-200.

Brown, A.S., 2006. Prenatal infection as a risk factor for schizophrenia. Schizophr. Bull. 32 (2): 200–202.

Brown, A.S., Susser, E.S., Lin, S.P., Neugebauer, R., Gorman, J.M., 1995. Increased risk of affective disorders in males after second trimester prenatal exposure to the Dutch hunger winter of 1944-45. Br. J. Psychiatry 166 (5): 601-6.

Brown, A.S., van Os, J., Driessens, C., Hoek, H.W., Susser, E.S., 2000. Further evidence of relation between prenatal famine and major affective disorder. Am. J. Psychiatry 157 (2): 190-5.

Brown, G.R., Nemes, C., 2008. The exploratory behaviour of rats in the hole-board apparatus: is head-dipping a valid measure of neophilia? Behav. Process. 78 (3): 442-448.

Brunton, P.J., 2015. Programming the brain and behaviour by early-life stress: a focus on neuroactive steroids. J. Neuroendocrinol. 27 (6): 468-80.

Brunton, P.J., Donadio, M.V., Yao, S.T., Greenwood, M., Seckl, J.R., Murphy, D., Russell, J.A., 2015. 5α-Reduced neurosteroids sex-dependently reverse central prenatal programming of neuroendocrine stress responses in rats. J. Neurosci. 35 (2): 666-77.

Brunton, P.J., Russell, J.A., Hirst, J.J., 2014. Allopregnanolone in the brain: protecting pregnancy and birth outcomes. Prog. Neurobiol. 113: 106-36.

Buka, S.L., Tsuang, M.T., Torrey, E.F., Klebanoff, M.A., Bernstein, D., Yolken, R.H., 2001a. Maternal infections and subsequent psychosis among offspring. Arch. Gen. Psychiatry 58 (11): 1032–1037.

Buka, S.L., Tsuang, M.T., Torrey, E.F., Klebanoff, M.A., Wagner, R.L., Yolken, R.H., 2001b. Maternal cytokine levels during pregnancy and adult psychosis. Brain Behav. Immun. 215 (4): 411-20.

Bullock, A.E., Clark, A.L., Grady, S.R., Robinson, S.F., Slobe, B.S., Marks, M.J., Collins, A.C., 1997. Neurosteroids modulate nicotinic receptor function in mouse striatal and thalamic synaptosomes. J. Neurochem. 68 (6): 2412-23.

Burke, N.N., Llorente, R., Marco, E.M., Tong, K., Finn, D.P., Viveros, M.P., Roche, M., 2013. Maternal deprivation is associated with sex-dependent alterations in nociceptive behavior and neuroinflammatory mediators in the rat following peripheral nerve injury. J. Pain 14: 1173–1184.

Caldji, C., Diorio, J., Meaney, M.J., 2003. Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. Neuropsychopharmacology. 28 (11): 1950-9.

Caldji, C., Francis, D., Sharma, S., Plotsky, P.M., Meaney, M.J., 2000. The effects of early rearing environment on the development of GABAA and central benzodiazepine receptor levels and novelty-induced fearfulness in the rat. Neuropsychopharmacology. 22 (3): 219-29.

Callachan, H., Cottrell, G.A., Hather, N.Y., Lambert, J.J., Nooney, J.M., Peters, J.A., 1987. Modulation of the GABAA receptor by progesterone metabolites. Proc. R. Soc. Lond. B. Biol. Sci. 231 (1264): 359-69.

Calza, A., Sogliano, C., Santoru, F., Marra, C., Angioni, M.M., Mostallino, M.C., Biggio, G., Concas, A., 2010. Neonatal exposure to estradiol in rats influences neuroactive steroid concentrations, GABAA receptor expression, and behavioral sensitivity to anxiolytic drugs. J. Neurochem. 113 (5): 1285-95.

Campbell, A.D., Kohl, R.R., McBride, W.J., 1996. Serotonin-3 receptor and ethanol-stimulated somatodendritic dopamine release. Alcohol. 13: 569–74.

Campbell, A.D., McBride, W.J., 1995. Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens. Pharmacol. Biochem. Behav. 51: 835–42.

Cannon, T.D., Rosso, I.M., Hollister, J.M., Bearden, C.E., Sanchez, L.E., Hadley, T., 2000. A prospective cohort study of genetic and perinatal influences in the etiology of schizophrenia. Schizophr. Bull. 26 (2): 351-66.

Cannon, T.D., van Erp, T.G., Rosso, I.M., Huttunen, M., Lönnqvist, J., Pirkola, T., Salonen, O., Valanne, L., Poutanen, V.P., Standertskjöld-Nordenstam, C.G., 2002. Fetal hypoxia and structural brain abnormalities in schizophrenic patients, their siblings, and controls. Arch. Gen. Psychiatry 59 (1): 35-41.

Carver, C.M., Reddy, D.S., 2013. Neurosteroid interactions with synaptic and extrasynaptic GABA(A) receptors: regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability. Psychopharmacology (Berl). 230 (2): 151-88.

Casu, M.A., Colombo, G., Gessa, G.L., Pani, L., 2002. Reduced TH-immunoreactive fibers in the limbic system of Sardinian alcohol-preferring rats. Brain Res. 924 (2), 242-51.

Chen, Y., Baram, T.Z., 2016. Toward Understanding How Early-Life Stress Reprograms Cognitive and Emotional Brain Networks. Neuropsychopharmacology. 41 (1): 197-206.

Compagnone, N.A., Bulfone, A., Rubenstein, J.L., Mellon, S.H., 1995. Expression of the steroidogenic enzyme P450scc in the central and peripheral nervous systems during rodent embryogenesis. Endocrinology. 136 (6): 2689-96.

Concas, A., Mostallino, M.C., Porcu, P., Follesa, P., Barbaccia, M.L., Trabucchi, M., Purdy, R.H., Grisenti, P., Biggio, G., 1998. Role of brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. Proc. Natl. Acad. Sci. USA 95: 13284-9.

Cook, J.B., Dumitru, A.M.G., O'Buckley, T.K., Morrow, A.L., 2014a. Ethanol Administration Produces Divergent Changes in GABAergic Neuroactive Steroid Immunohistochemistry in the Rat Brain. Alcohol Clin. Exp. Res., Vol 38, No 1: 90–99.

Cook, J.B., Nelli, S.M., Neighbors, M.R., Morrow, D.H., O'Buckley, T.K., Maldonado-Devincci, A.M., Morrow, A.L., 2014b. Ethanol alters local cellular levels of (3α,5α)-3-hydroxypregnan-20-one (3α,5α-THP) independent of the adrenals in subcortical brain regions. Neuropsychopharmacology. 39 (8): 1978-87.

Corpéchot, C., Robel, P., Axelson, M., Sjövall, J., Baulieu, E.E., 1981. Characterization and measurement of dehydroepiandrosterone sulfate in rat brain. Proc. Natl. Acad. Sci. U S A. 78 (8): 4704-7.

Czachowski, C.L., 2005. Manipulations of serotonin function in the nucleus accumbens core produce differential effects on ethanol and sucrose seeking and intake. Alcohol Clin Exp Res. 29 (7): 1146-55.

Dallman, M.F., Pecoraro, N.C., la Fleur, S.E., 2005. Chronic stress and comfort foods: self-medication and abdominal obesity. Brain Behav. Immun. 19 (4): 275-80.

Darbra, S., Garau, A., Balada, F., Sala, J., Martí-Carbonell, M.A., 2003. Perinatal hypothyroidism effects on neuromotor competence, novelty-directed exploratory and anxiety-related behaviour and learning in rats. Behav. Brain. Res. 143: 209-215.

Darbra, S., Mòdol, L., Llidó, A., Casas, C., Vallée, M., Pallarès, M., 2014. Neonatal allopregnanolone levels alteration: effects on behavior and role of the hippocampus. Prog. Neurobiol. 113: 95-105.

Darbra, S., Mòdol, L., Pallarès, M., 2012. Allopregnanolone infused into the dorsal (CA1) hippocampus increases prepulse inhibition of startle response in Wistar rats. Psychoneuroendocrinology 37 (4): 581-5.

Darbra, S., Modol, L., Vallée, M., Pallarès, M., 2013. Neonatal neurosteroid levels are determinant in shaping adult prepulse inhibition response to hippocampal allopregnanolone in rats. Psychoneuroendocrinology. 38 (8): 1397-406.

Darbra, S., Pallarès, M., 2009. Neonatal allopregnanolone increases novelty-directed locomotion and disrupts behavioural responses to GABAA receptor modulators in adulthood. Int. J. Devl. Neuroscience 27: 617-625.

Darbra, S., Pallarès, M., 2010. Alterations in neonatal neurosteroids affect exploration during adolescence and prepulse inhibition in adulthood. Psychoneuroendocrinology 35 (4), 525-535.

Darbra, S., Pallarès, M., 2011. Interaction between early postnatal neurosteroid manipulations and adult infusion of neurosteroids into CA1 hippocampal region on the open field behavior. Behav. Brain Res. 216 (2): 705-711.

Darbra, S., Pallarès, M., 2012. Effects of early postnatal allopregnanolone administration on elevated plus-maze anxiety scores in adult male Wistar rats. Neuropsychobiology 65 (1): 20-27.

Davis, J.F., Krause, E.G., Melhorn, S.J., Sakai, R.R., Benoit, S.C., 2009. Dominant rats are natural risk takers and display increased motivation for food reward. Neuroscience 162: 23-30.

de Kloet, E.R., Rosenfeld, P., van Eekelen, J.A.M., Sutanto, W., Levine, S., 1988. Stress, glucocorticoids and development. Prog. Brain Res. 73: 101-20.

Dehorte, N., Vinay, L., Hammond, C., Ben-Ari, Y., 2012. Timing of developmental sequences in different brain structures: physiological and pathological implications. Eur. J. Neurosci. 35 (12): 1846-56.

Dess, N.K., Badia-Elder, N.E., Thiele, T.E., Kiefer, S.W., Blizard, D.A., 1998. Ethanol consumption in rats selectively bred for differential saccharin intake. Alcohol 16: 275-8.

Ding, Z.M., Oster, S.M., Hall, S.R., Engleman, E.A., Hauser, S.R., McBride, W.J., Rodd, Z.A., 2011. The stimulating effects of ethanol on ventral tegmental area dopamine neurons projecting to the ventral pallidum and medial prefrontal cortex in female Wistar rats: regional difference and involvement of serotonin-3 receptors. Psychopharmacology (Berl). 216 (2): 245-55.

Do Rego, J.L., Seong, J.Y., Burel, D., Leprince, J., Luu-The, V., Tsutsui, K., Tonon, M.C., Pelletier, G., Vaudry, H., 2009. Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. Front. Neuroendocrinol. 30 (3): 259-301.

Doremus, T.L., Varlinskaya, E.I., Spear, L.P., 2004. Age-related differences in elevated plus maze behaviour between adolescent and adult rats. Ann. N.Y. Acad. Sci. 1021: 427-430.

Dubrovsky, B.O., 2005. Steroids, neuroactive steroids and neurosteroids in psychopathology. Prog. Neuropsychopharmacol. Biol. Psychiatry 29 (2): 169-92.

Eklund, M.B., Arborelius, L., 2006. Twice daily long maternal separations in Wistar rats decreases anxiety-like behaviour in females but does not affect males. Behav. Brain. Res. 172 (2): 278-285.

Ellenbroek, B.A., Cools, A.R., 1995. Maternal separation reduces latent inhibition in the conditioned taste aversion paradigm. Neurosci. Res. Comm. 17 (1): 27–33.

Ellenbroek, B.A., Cools, A.R., 2000. The long-term effects of maternal deprivation depend on the genetic background. Neuropsychopharmacology 23: 99 –106.

Ellenbroek, B.A., Cools, A.R., 2002. Early maternal deprivation and prepulse inhibition. The role of the postdeprivation environment. Pharmacol. Biochem. Behav. 73: 177–184.

Ellenbroek, B.A., de Bruin, N.M., van-den-Kroonenburg, P.T., van Luijtelaar, E.L., Cools, A.R., 2004. The effects of early maternal deprivation on auditory information processing in adult Wistar rats. Biol. Psychiatry 55: 701-707.

Ellenbroek, B.A., Derks, N., Park, H.J., 2005. Early maternal deprivation retards neurodevelopment in Wistar rats. Stress. 8 (4): 247-57.

Ellenbroek, B.A., Riva, M.A., 2003. Early maternal deprivation as an animal model for schizophrenia. Clin. Neurosci. Res. 3 (4–5): 297–302.

Ellenbroek, B.A., van-den-Kroonenberg, P.T.J.M., Cools, A.R., 1998. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr. Res. 30: 251-260.

Ely, D.R., Dapper, V., Marasca, J., Corrêa, J.B., Gamaro, G.D., Xavier, M.H., Michalowski, M.B., Catelli, D., Rosat, R., Ferreira, M.B., Dalmaz, C., 1997. Effect of restraint stress on feeding behavior of rats. Physiol. Behav. 61 (3): 395-8.

Enoch, M.A., 2011. The role of early life stress as a predictor for alcohol and drug dependence. Psychopharmacology (Berl) 214 (1): 17-31.

Evans, J., Sun, Y., McGregor, A., Connor, B., 2012. Allopregnanolone regulates neurogenesis and depressive/anxiety-like behaviour in a social isolation rodent model of chronic stress. Neuropharmacology. 63 (8): 1315-26.

Fabricius, K., Wörtwein, G., Pakkenberg, B., 2008. The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus. Brain. Struct. Funct. 212: 403-416.

Fadda, F., Garau, B., Marchei, F., Colombo, G., Gessa, G.L., 1991. MDL 72222, a selective 5-HT3 receptor antagonist, suppresses voluntary ethanol consumption in alcohol-preferring rats. Alcohol Alcohol. 26: 107–10.

Farrant, M., Nusser, Z., 2005. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat. Rev. Neurosci. 6 (3): 215-29.

Faturi, C.B., Tiba, P.A., Kawakami, S.E., Catallani, B., Kerstens, M., Suchecki, D., 2010. Disruptions of the mother–infant relationship and stress-related behaviours: Altered corticosterone secretion does not explain everything. Neurosci. Biobehav. Rev. 34: 821-834.

Felitti, V.J., Anda, R.F., Nordenberg, D., Williamson, D.F., Spitz, A.M., Edwards, V., Koss, M.P., Marks, J.S., 1998. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. Am. J. Prev. Med. 14 (4): 245-58.

Fernie, G., Peeters, M., Gullo, M.J., Christiansen, P., Cole, J.C., Sumnall, H., Field, M., 2013. Multiple behavioural impulsivity tasks predict prospective alcohol involvement in adolescents. Addiction 108 (11): 1916-23.

File, S.E., 2001. Factors controlling measures of anxiety and responses to novelty in the mouse. Behav. Brain. Res. 125: 151-157.

File, S.E., Wardill, A.G., 1975. Validity of head-dipping as a measure of exploration in a modified hole-board. Psychopharmacology 44: 53-59.

Ford, M.M., Nickel, J.D., Phillips, T.J., Finn, D.A., 2005. Neurosteroid modulators of GABA(A) receptors differentially modulate Ethanol intake patterns in male C57BL/6J mice. Alcohol Clin. Exp. Res. 29 (9): 1630-40.

Froehlich, J.C., Hausauer, B.J., Federoff, D.L., Fischer, S.M., Rasmussen, D.D., 2013. Prazosin reduces alcohol drinking throughout prolonged treatment and blocks the initiation of drinking in rats selectively bred for high alcohol intake. Alcohol Clin. Exp. Res. 37 (9): 1552-60.

Frye, C.A., Paris, J.J., Walf, A.A., Rusconi, J.C., 2012. Effects and mechanisms of 3a,5a,-THP on emotion, motivation, and reward functions involving pregnane xenobiotic receptor. Front. Neurosci. 5: 136.

Frye, C.A., Rhodes, M.E., Raol, Y.H., Brooks-Kayal, A.R., 2006. Early postnatal stimulation alters pregnane neurosteroids in the hippocampus. Psychopharmacology 186: 343-350.

Fumagalli, F., Molteni, R., Racagni, G., Riva, M.A., 2007. Stress during development: Impact on neuroplasticity and relevance to psychopathology. Prog. Neurobiol. 81 (4): 197-217.

García, A.M., Cárdenas, F.P., Morato, S., 2005. Effect of different illumination levels on rat behaviour in the elevated plus-maze. Physiol. Behav. 85: 265-270.

Ghoumari, A.M., Ibanez, C., El-Etr, M., Leclerc, P., Eychenne, B., O'Malley, B.W., Baulieu, E.E., Schumacher, M., 2003. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. J. Neurochem. 86 (4): 848-59.

Giedd, J.N., Lalonde, F.M., Celano, M.J., White, S.L., Wallace, G.L., Lee, N.R., Lenroot, R.K., 2009. Anatomical brain magnetic resonance imaging of typically developing children and adolescents. J. Am. Acad. Child Adolesc. Psychiatry 48 (5): 465-70.

Girardi, C.E., Zanta, N.C., Suchecki, D., 2014. Neonatal stress-induced affective changes in adolescent Wistar rats: early signs of schizophrenia-like behavior. Front. Behav. Neurosci. 8: 319.

Girdler, S.S., Klatzkin, R., 2007. Neurosteroids in the context of stress: implications for depressive disorders. Pharmacol. Ther. 116 (1): 125-39.

Gizerian, S.S., Morrow, A.L., Lieberman, J.A., Grobin, A.C., 2004. Neonatal neurosteroid administration alters parvalbumin expression and neuron number in medial dorsal thalamus of adult rats. Brain Res. 1012: 66–74

Gizerian, S.S., Moy, S.S., Lieberman, J.A., Grobin, A.C., 2006. Neonatal neurosteroid administration results in development-specific alterations in prepulse inhibition and locomotor activity: neurosteroids alter prepulse inhibition and locomotor activity. Psychopharmacology 186 (3): 334-42.

Gosnell, B.A., Krahn, D.D., 1992. The relationship between saccharin and alcohol intake in rats. Alcohol 9: 201-206.

Gray, N.S., Pilowsky, L.S., Gray, J.A., Kerwin, R.W., 1995. Latent inhibition in drug naive schizophrenics: Relationship to duration of illness and dopamine D2 binding using SPET. Schizophr. Res. 17: 95–107.

Grillon, C., 2008. Models and mechanisms of anxiety: evidence from startle studies. Psychopharmacology 199: 421-437.

Grobin, A.C, Morrow, A.L, 2001. 3Alpha-hydroxy-5alpha-pregnan-20-one levels and GABA(A) receptor-mediated 36Cl(-) flux across development in rat cerebral cortex. Brain Res. Rev. Brain Res. 131 (1-2): 31-9.

Grobin, A.C., Heenan, E.J., Lieberman, J.A., Morrow, A.L., 2003. Perinatal neurosteroid levels influence GABAergic interneuron localization in adult rat prefrontal cortex. J. Neurosci. 23: 1832–1839.

Grobin, A.C., Morrow, A.L., 2000. 3alpha-hydroxy-5alpha-pregnan-20-one exposure reduces GABA(A) receptor alpha4 subunit mRNA levels. Eur. J. Pharmacol. 409 (2): R1-2.

Gronemeyer, H., 1992. Control of transcription activation by steroid hormone receptors. FASEB J. 6 (8): 2524-9.

Grota, L.J., Ader,R., 1969. Continuous recording of maternal behavior in *Rattus norvegicus*. Anim. Behav. 17: 722–729.

Gunn, B.G., Cunningham, L., Mitchell, S.G., Swinny, J.D., Lambert, J.J., Belelli, D., 2015. GABAA receptor-acting neurosteroids: a role in the development and regulation of the stress response. Front. Neuroendocrinol. 36: 28-48.

Gut, P., Zweckstetter, M., Banati, R.B., 2015. Lost in translocation: the functions of the 18-kD translocator protein. Trends Endocrinol. Metab. 26 (7): 349-56.

H

Hagan, M.M., Chandler, P.C., Wauford, P.K., Rybak, R.J., Oswald, K.D., 2003. The role of palatable food and hunger as trigger factors in an animal model of stress induced binge eating. Int J Eat Disord. 34 (2): 183-97.

Hajnal, A., Smith, G.P., Norgren, R., 2004. Oral sucrose stimulation increases accumbens dopamine in the rat. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286 (1): R31-7.

Hilakivi-Clarke, L.A., Turkka, J., Lister, R.G., Linnoila, M., 1991. Effects of early postnatal handling on brain beta-adrenoceptors and behavior in tests related to stress. Brain Res. 542: 286-292.

Hirani, K., Sharma, A.N., Jain, N.S., Ugale, R.R., Chopde, C.T., 2005. Evaluation of GABAergic neuroactive steroid 3alpha-hydroxy-5alpha-pregnane-20-one as a neurobiological substrate for the anti-anxiety effect of ethanol in rats. Psychopharmacology. 180: 267-278.

Hoek, H.W., Brown, A.S., Susser, E., 1998. The Dutch famine and schizophrenia spectrum disorders. Soc. Psychiatry Psychiatr. Epidemiol. 33 (8): 373-9.

Hsu, F.C., Zhang, G.J., Raol, Y.S., Valentino, R.J., Coulter, D.A., Brooks-Kayal, A.R., 2003. Repeated neonatal handling with maternal separation permanently alters hippocampal GABAA receptors and behavioral stress responses. Proc. Natl. Acad. Sci. U S A. 100 (21): 12213-8.

Husum, H., Termeer, E., Mathe, A.A., Bolwig, T.G., Ellenbroek, B.A., 2002. Early maternal deprivation alters hippocampal levels of neuropeptide Y and calcitonin-generelated peptide in adult rats. Neuropharmacology 42: 798–806.

J

Janak, P.H., Redfern, J.E., Samson, H.H., 1998. The reinforcing effects of ethanol are altered by the endogenous neurosteroid, allopregnanolone. Alcohol Clin. Exp. Res. 22 (5): 1106-12.

Janis, G.C., Devaud, L.L., Mitsuyama, H., Morrow, A.L., 1998. Effects of chronic ethanol consumption and withdrawal on the neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one in male and female rats. Alcohol Clin. Exp. Res. 22 (9): 2055-61.

Johnson, S.W., North, R.A., 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J. Neurosci. 12: 483-488.

Jones, N., King, S.M., 2001. Influence of circadian phase and test illumination on preclinical models of anxiety. Physiol. Behav. 72: 99-106.

K

Kalivas, P.W., Churchill, L., Klitenick, M.A., 1993. GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience 57: 1047-1060.

Kehoe, P., Mallinson, K., McCormick, C.M., Frye, C.A., 2000. Central allopregnanolone is increased in rat pups in response to repeated, short episodes of neonatal isolation. Brain Res. Dev. Brain Res. 124 (1-2): 133-6.

Keller, E.A., Zamparini, A., Borodinsky, L.N., Gravielle, M.C., Fiszman, M.L., 2004. Role of allopregnanolone on cerebellar granule cells neurogenesis. Brain Res. Dev. Brain Res. 153(1): 13-7.

Kelly, T.H., Robbins, G., Martin, C.A., Fillmore, M.T., Lane, S.D., Harrington, N.G., Rush, C.R., 2006. Individual differences in drug abuse vulnerability: d-amphetamine and sensation-seeking status. Psychopharmacology 189 (1): 17-25.

Khanna, M., Qin, K.N., Cheng, K.C., 1995. Distribution of 3α-hydroxysteroid dehydrogenase in rat brain and molecular cloning of multiple cDNAs encoding structurally related proteins in humans, J. Steroid Biochem. Mol. Biol. 53: 41-6.

Khashan, A.S., Abel, K.M., McNamee, R., Pedersen, M.G., Webb, R.T., Baker, P.N., Kenny, L.C., Mortensen, P.B., 2008. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. Arch. Gen. Psychiatry 65 (2): 146-52.

Khisti, R.T., VanDoren, M.J., O'Buckley, T.K., Morrow, A.L., 2003. Neuroactive steroid 3a-hydroxy-5a-pregnan-20-one modulates ethanol-induced loss of righting reflex in rats. Brain Res. 980: 255–265.

Kiyokage, E., Toida, K., Suzuki-Yamamoto, T., Ishimura, K., 2005. Localization of 5α-reductase in the rat main olfactory bulb. J. Comp. Neurol. 493: 38195.

Kohl, S., Heekeren, K., Klosterkötter, J., Kuhn, J., 2013. Prepulse inhibition in psychiatric disorders--apart from schizophrenia. J. Psychiatr. Res. 47 (4): 445-52.

Koros, E., Piasecki, J., Kostowski, W., Bienkowski, P., 1998. Saccharin drinking rather than open field behavior predicts initial ethanol acceptance in Wistar rats. Alcohol 33: 131–140.

Lamba, V., Yasuda, K., Lamba, J.K., Assem, M., Davila, J., Strom, S., Schuetz, E.G., 2004. PXR (NR1I2): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. Toxicol. Appl. Pharmacol. 199 (3): 251-65.

Lambert, J.J., Cooper, M.A., Simmons, R.D., Weir, C.J., Belelli, D., 2009. Neurosteroids: endogenous allosteric modulators of GABA(A) receptors. Psychoneuroendocrinology 34 Suppl 1: S48-58.

Langmade, S.J., Gale, S.E., Frolov, A., Mohri, I., Suzuki, K., Mellon, S.H., Walkley, S.U., Covey, D.F., Schaffer, J.E., Ory, D.S., 2006. Pregnane X receptor (PXR) activation: A mechanism for neuroprotection in a mouse model of Niemann–Pick C disease. Proc. Natl. Acad. Sci. U S A. 103 (37): 13807-12.

Lauber, M.E., Lichtensteiger, W., 1996. Ontogeny of 5 alpha-reductase (type 1) messenger ribonucleic acid expression in rat brain: early presence in germinal zones. Endocrinology 137(7): 2718-30.

Laurie, D.J., Wisden, W., Seeburg, P.H., 1992. The distribution of thirteen GABA, receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J. Neurosci. 12: 4151–4172.

Laviola, G., Macrì, S., Morley-Fletcher, S., Adriani, W., 2003. Risk-taking behavior in adolescent mice: Psychobiological determinants and early epigenetic influence. Neurosci. Biobeh. Rev. 27: 19-31.

Laviolette, S.R., van der Kooy, D., 2001. GABAA receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. Eur. J. Neurosci. 13: 1009-1015.

Lehmann, J., Pryce, C.R., Bettschen, D., Feldon, J., 1999. The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. Pharmacol. Biochem. Behav. 64: 705-715.

Lehmann, J., Russig, H., Feldon, J., Pryce, C.R., 2002. Effect of a single maternal separation at different pup ages on the corticosterone stress response in adult and aged rats. Pharmacol. Biochem. Behav. 73: 141–145.

Lephart, E.D., Andersson, S., Simpson, E.R., 1990. Expression of neural 5 alpha-reductase messenger ribonucleic acid: comparison to 5 alpha-reductase activity during prenatal development in the rat. Endocrinology 127 (3): 1121-8.

Levine, A.S., Kotz, C.M., Gosnell, B.A., 2003. Sugars: hedonic aspects, neuroregulation, and energy balance. Am. J. Clin. Nutr. 78 (4): 834S-842S.

Levine, S., 2005. Developmental determinants of sensitivity and resistance to stress. Psychoneuroendocrinology 30 (10): 939-46.

Levine, S., Chevalier, J.A., Korchin, S.J., 1956. The effects of early shock and handling in later avoidance learning. Journal of Personality 24: 475–493.

Leyton, M., 2014. What's deficient in reward deficiency?. J. Psychiatry Neurosci. 39 (5): 291-3.

Liu, J., Morrow, A.L., Devaud, L.L., Grayson, D.R., Lauder, J.M., 1997. Regulation of GABA(A) receptor subunit mRNA expression by the pesticide dieldrin in embryonic brainstem cultures: a quantitative, competitive reverse transcription-polymerase chain reaction study. J. Neurosci. Res. 49(5): 645-53.

Liu, W., Thielen, R.J., Rodd, Z.A., McBride, W.J., 2006. Activation of serotonin-3 receptors increases dopamine release within the ventral tegmental area of Wistar and alcohol-preferring (P) rats. Alcohol. 40: 167–76.

Llorente, R., Arranz, L., Marco, E.M., Moreno, E., Puerto, M., Guaza, C., De la Fuente, M., Viveros, M.P., 2007. Early maternal deprivation and neonatal single administration with a cannabinoid agonist induce long-term sex-dependent psychoimmunoendocrine effects in adolescent rats, Psychoneuroendocrinology 32: 636-650.

Llorente, R., Gallardo, M.L., Berzal, A.L., Prada, C., Garcia-Segura, L.M., Viveros, M.P., 2009. Early maternal deprivation in rats induces gender-dependent effects on developing hippocampal and cerebellar cells. Int. J. Dev. Neurosci. 27: 233–241.

Llorente, R., Llorente-Berzal, A., Petrosino, S., Marco, E.M., Guaza, C., Prada, C., López-Gallardo, M., Di Marzo, V., Viveros, M.P., 2008. Gender-dependent cellular and biochemical effects of maternal deprivation on the hippocampus of neonatal rats: a possible role for the endocannabinoid system. Dev. Neurobiol. 68: 1334–1347.

Llorente, R., Llorente-Berzal, A., Petrosino, S., Marco, E.M., Guaza, C., Prada, C., López-Gallardo, M., Di Marzo, V., Viveros, M.P., 2008. Gender-dependent cellular and biochemical effects of maternal deprivation on the hippocampus of neonatal rats: a possible role for the endocannabinoid system. Dev Neurobiol. 68 (11): 1334-47.

Llorente, R., Miguel-Blanco, C., Aisa, B., Lachize, S., Borcel, E., Meijer, O.C., Ramirez,M.J., De Kloet, E.R., Viveros, M.P., 2011. Long term sex-dependent

psychoneu-roendocrine effects of maternal deprivation and juvenile unpredictable stressin rats. J. Neuroendocrinol. 23: 329–344.

Llorente-Berzal, A., Fuentes, S., Gagliano, H., Lopez-Gallardo, M., Armario, A., Viveros,M.P., Nadal, R., 2011. Sex-dependent effects of maternal deprivation andadolescent cannabinoid treatment on adult rat behaviour. Addict. Biol. 16: 624–637.

Locci, A., Porcu, P., Talani, G., Santoru, F., Berretti, R., Giunti, E., Licheri, V., Sanna, E., Concas, A., 2017. Neonatal estradiol exposure to female rats changes $GABA_A$ receptor expression and function, and spatial learning during adulthood. Horm. Behav. 87: 35-46.

Löfgren, M., Johansson, I.M., Meyerson, B., Lundgren, P., Backstrom, T., 2006. Progesterone withdrawal effects in the open field test can be predicted by elevated plus maze performance. Horm. Behav. 50: 208–215.

López-Gallardo, M., López-Rodriguez, A.B., Llorente-Berzal, A., Rotllant, D., Mackie, K., Armario, A., Nadal, R., Viveros, M.P., 2012. Maternal deprivation and adoles-cent cannabinoid exposure impact hippocampal astrocytes, CB1 receptors andbrain-derived neurotrophic factor in a sexually dimorphic fashion. Neuroscience 204: 90–103.

Lovinger, D.M., 1999. 5-HT3 receptors and the neural actions of alcohols: an increasingly exciting topic. Neurochem. Int. 35 (2): 125-30.

Lovinger, D.M., Sung, K.W., Zhou, Q., 2000. Ethanol and trichloroethanol alter gating of 5-HT3 receptor-channels in NCB-20 neuroblastoma cells. Neuropharmacology 39 (4): 561-70.

Lovinger, D.M., White, G., 1991. Ethanol potentiation of 5-hydroxytryptamine3 receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. Mol. Pharmacol. 40: 263–70.

Lu, M.R., Wagner, G.C., Fisher, H., 1994. Ethanol consumption following acute treatment with methysergide, fluoxetine, fenfluramine, and their combination. Alcohol Clin. Exp. Res. 18: 60–3.

M

Maccari, S., Krugers, H.J., Morley-Fletcher, S., Szyf, M., Brunton, P.J., 2014. The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. J. Neuroendocrinol. 26 (10): 707-23.

Machado, T.D., Dalle Molle, R., Laureano, D.P., Portella, A.K., Werlang, I.C., Benetti, C. da S., Noschang, C., Silveira, P.P., 2013. Early life stress is associated with anxiety, increased stress responsivity and preference for "comfort foods" in adult female rats. Stress 16 (5): 549-56.

Machu, T.K., Harris, R.A., 1994. Alcohols and anesthetics enhance the function of 5-hydroxytryptamine3 receptors expressed in Xenopus laevis oocytes. J. Pharmacol. Exp. Ther. 271: 898–905.

Magnaghi, V., Cavarretta, I., Galbiati, M., Martini, L., Melcangi, R.C., 2001. Neuroactive steroids and peripheral myelin proteins. Brain Res Rev. 37 (1-3): 360-71.

Majewska, M.D, Schwartz, R.D., 1987. Pregnenolone-sulfate: an endogenous antagonist of the gamma-aminobutyric acid receptor complex in brain? Brain Res. 404 (1-2): 355-60.

Majewska, M.D., Harrison, N.L., Schwartz, R.D., Barker, J.L., Paul, S.M., 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232: 1004-1007.

Marco, E.M., Adriani, W., Canese, R., Podo, F., Viveros, M.P., Laviola, G., 2007. Enhancement of endocannabinoid signalling during adolescence: modulation of impulsivity and long-term consequences on metabolic brain parameters in early maternally deprived rats. Pharmacol. Biochem. Behav. 86: 334-345.

Marco, E.M., Llorente, R., López-Gallardo, M., Mela, V., Llorente-Berzal, Á., Prada, C., Viveros, M.P., 2015. The maternal deprivation animal model revisited. Neurosci. Biobehav. Rev. 51: 151-63.

Marco, E.M., Valero, M., de la Serna, O., Aisa, B., Borcel, E., Ramirez, M.J., Viveros, M.P., 2013. Maternal deprivation effects on brain plasticity and recognition memory in adolescent male and female rats. Neuropharmacology 68: 223–231.

Martín-García, E., Darbra, S., Pallarès, M., 2007. Intrahippocampal allopregnanolone decreases voluntary chronic alcohol consumption in non-selected rats. Prog. Neuropsychopharmacol. Biol. Psychiatry 31: 823-831.

Martin-García, E., Darbra, S., Pallarès, M., 2008. Neonatal finasteride induces anxiogenic-like profile and deteriorates passive avoidance in adulthood after intrahippocampal neurosteroid administration. Neuroscience 154: 1497-1505.

Martín-García, E., Pallarès, M., 2005. Effects of intrahippocampal nicotine and neurosteroid administration on withdrawal in voluntary and chronic alcohol-drinking rats. Alcohol Clin. Exp. Res. 29: 1654-63.

Martini, M., Valverde, O., 2011. A single episode of maternal deprivation impairs the motivation for cocaine in adolescent mice. Psychopharmacology 219 (1): 149-58.

Marx, C.E., Stevens, R.D., Shampine, L.J., Uzunova, V., Trost, W.T., Butterfield, M.I., Massing, M.W., Hamer, R.M., Morrow, A.L., Lieberman, J.A., 2006. Neuroactive steroids are altered in schizophrenia and bipolar disorder: relevance to pathophysiology and therapeutics. Neuropsychopharmacology 31: 1249-63.

Marx, C.E., VanDoren, M.J., Duncan, G.E., Lieberman, J.A., Morrow, A.L., 2003. Olanzapine and clozapine increase the GABAergic neuroactive steroid allopregnanolone in rodents. Neuropsychopharmacology 28(1): 1-13.

Matthews, D.B., Morrow, A.L., Tokunaga, S., McDaniel, J.R., 2002. Acute ethanol administration and acute allopregnanolone administration impair spatial memory in the Morris water task. Alcohol Clin. Exp. Res. 26: 1747-1751.

McBride, W.J., Bodart, B., Lumeng, L., Li, T.K., 1995. Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference. Alcohol Clin. Exp. Res. 19 (6): 1420-2.

McBride, W.J., Chernet, E., Dyr, W., Lumeng, L., Li, T.K., 1993. Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. Alcohol 10: 387-390.

McBride, W.J., Li, T.K., 1998. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Crit. Rev. Neurobiol. 12: 339–69.

McKinzie, D.L., Eha, R., Cox, R., Stewart, R.B., Dyr, W., Murphy, J.M., McBride, W.J., Lumeng L., Li, T.K., 1998. Serotonin3 receptor antagonism of alcohol intake: effects of drinking conditions. Alcohol. 15: 291–8.

Melcangi, R.C., Panzica, G.C., 2014. Allopregnanolone: state of the art. Prog. Neurobiol. 113: 1-5.

Michaels, C.C., Holtzman, S.G., 2006. Neonatal stress and litter composition alter sucrose intake in both rat dam and offspring. Physiol. Behav. 89: 735–741.

Micheva, K.D., Beaulieu, C., 1997. Development and plasticity of the inhibitory neocortical circuitry with an emphasis on the rodent barrel field cortex: a review. Can. J. Physiol. Pharmacol. 75 (5): 470-8.

Mihalek, R.M., Bowers, B.J., Wehner, J.M., Kralic, J.E., VanDoren, M.J., Morrow, A.L., Homanics, G.E., 2001. GABA(A)-receptor delta subunit knockout mice have multiple defects in behavioral responses to ethanol. Alcohol Clin. Exp. Res. 25: 1708-1718.

Mineur, Y.S., Obayemi, A., Wigestrand, M.B., Fote, G.M., Calarco, C.A., Li, A.M., Picciotto, M.R., 2013. Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. Proc. Natl. Acad. Sci. USA 110 (9): 3573-8.

Mitev, Y.A, Darwish, Y.A.M., Wolf, S.S., Holsboer, F., Almeida, O.F.X., Patchev, V.K., 2003. Gender differences in the regulation of 3α-hydroxysteroid dehydrogenase in rat brain and sensitivity to neurosteroid-mediated stress protection. Neuroscience 120: 541-549.

Mòdol, L., Casas, C., Llidó, A., Navarro, X., Pallarès, M., Darbra, S., 2014b. Neonatal allopregnanolone or finasteride administration modifies hippocampal K(+) Cl(-) co-transporter expression during early development in male rats. J. Steroid Biochem. Mol. Biol. 143: 343-7.

Mòdol, L., Casas, C., Navarro, X., Llidó, A., Vallée, M., Pallarès, M., Darbra, S., 2014a. Neonatal finasteride administration alters hippocampal α4 and δ GABAAR subunits expression and behavioural responses to progesterone in adult rats. Int. J. Neuropsychopharmacol. 17 (2): 259-73.

Mòdol, L., Darbra, S., Pallarès, M., 2011. Neurosteroids infusion into the CA1 hippocampal region on exploration, anxiety-like behaviour and aversive learning. Behav. Brain Res. 222 (1): 223-229.

Mòdol, L., Darbra, S., Vallée, M., Pallarès, M., 2013. Alteration of neonatal Allopregnanolone levels affects exploration, anxiety, aversive learning and adult behavioural response to intrahippocampal neurosteroids. Behav. Brain Res. 241: 96-104.

Molander, A.C., Mar, A., Norbury, A., Steventon, S., Moreno, M., Caprioli, D., Theobald, D.E., Belin, D., Everitt, B.J., Robbins, T.W., Dalley, J.W., 2011. High impulsivity predicting vulnerability to cocaine addiction in rats: some relationship with novelty preference but not novelty reactivity, anxiety or stress. Psychopharmacology 215 (4): 721-31.

Monnet, F.P., Maurice, T., 2006. The sigma1 protein as a target for the non-genomic effects of neuro(active)steroids: molecular, physiological, and behavioral aspects. J. Pharmacol. Sci. 100 (2): 93-118.

Monteleone, P., Luisi, S., Tonetti, A., Bernardi, F., Genazzani, A.D., Luisi, M., Petraglia, F., Genazzani, A.R., 2000. Allopregnanlone concentrations and premenstrual syndrome, Eur. J. Endocrinol. 142: 269-73.

Morrow, A.L., Porcu, P., Boyd, K.N., Grant, K.A., 2006. Hypothalamic-pituitary-adrenal axis modulation of GABAergic neuroactive steroids influences ethanol sensitivity and drinking behavior. Dialogues Clin. Neurosci. 8 (4): 463-77.

Morrow, A.L., VanDoren, M.J., Penland, S.N., Matthews, D.B., 2001. The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. Brain Res. Brain Res. Rev. 37: 98-109.

Mourlon, V., Baudin, A., Blanc, O., Lauber, A., Giros, A., Naudon, L., Daugé, V., 2010. Maternal deprivation induces depressive-like behaviours only in female rats. Behav. Brain Res. 213: 278–287.

Mtchedlishvili, Z., Sun, C.S., Harrison, M.B., Kapur, J., 2003. Increased neurosteroid sensitivity of hippocampal GABAA receptors during postnatal development. Neuroscience 118: 655-666.

Mukai, Y., Higashi, T., Nagura, Y., Shimada, K., 2008. Studies on neurosteroids XXV. Influence of a 5alpha-reductase inhibitor, finasteride, on rat brain neurosteroid levels and metabolism. Biol. Pharm. Bull. 31 (9): 1646–1650.

Müller, C.P., Homberg, J.R., 2015. The role of serotonin in drug use and addiction. Behav. Brain Res. 277: 146–192.

Muneoka, K.T., Kuwagata, M., Ogawa, T., Shioda, S., 2010. Sex-specific effects of early neonatal progesterone treatment on dopamine and serotonin metabolism in rat striatum and frontal cortex. Life Sci. 87: 738-742.

Muneoka, K.T., Kuwagata, M., Shirayama, Y., Ogawa, T., Shioda, S., 2009. Biphasic effects of neonatal allopregnanolone on striatal dopamine metabolism. Neuroreport 20 (9): 860–863.

Muneoka, K.T., Shirayama, Y., Minabe, Y., Takigawa, M., 2002. Effects of a neurosteroid, pregnenolone, during the neonatal period on adenosine A1 receptor, dopamine metabolites in the fronto-parietal cortex and behavioral response in the open field. Brain Res. 956 (2): 332-8.

Murphy, J.M., McBride, W.J., Lumeng, L., Li, T.K., 1982. Regional brain levels of monoaminesin alcohol-preferring and -nonpreferring lines of rats. Pharmacol. Biochem. Behav. 16: 145–9.

N

Naranjo, C.A., Poulos, C.X., Bremner, K.E., Lanctot, K.L., 1994. Fluoxetine attenuates alcohol intake and desire to drink. Int. Clin. Psychopharmacol. 9: 163–72.

Nie, H., Rewal, M., Gill, T.M., Ron, D., Janak, P.H., 2011. Extrasynaptic delta-containing GABAA receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. Proc. Natl. Acad. Sci. USA 108 (11): 4459-64.

Norgren, R., Hajnal, A., Mungarndee, S.S., 2006. Gustatory reward and the nucleus accumbens. Physiol. Behav. 89 (4): 531-5.

Notterpek, L.M., Bullock, P.N., Malek-Hedayat, S., Fisher, R., Rome, L.H., 1993. Myelination in cerebellar slice cultures: development of a system amenable to biochemical analysis. J. Neurosci. Res. 36 (6): 621-34.

Nutt, D.J., Lingford-Hughes, A., Erritzoe, D., Stokes, P.R., 2015. The dopamine theory of addiction: 40 years of highs and lows. Nat. Rev. Neurosci. 16 (5): 305-312.

Nylander, I., Roman, E., 2012. Neuropeptides as mediators of the early-life impact on the brain; implications for alcohol use disorders. Front. Mol. Neurosci. 5: 77.

Nylander, I., Roman, E., 2013. Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? Psychopharmacology (Berl). 229 (4): 555-69.

O

O'Dell, L.E., Alomary, A.A., Vallee, M., Koob, G.F., Fitzgerald, R.L., Purdy, R.H., 2004. Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats. Eur. J. Pharmacol. 484: 241–247.

Okada, H., Matsushita, N., Kobayashi, K., Kobayashi, K., 2004. Identification of GABAA receptor subunit variants in midbrain dopaminergic neurons. J. Neurochem. 89: 7-14.

Olsen, R.W., Sieghart, W., 2009. GABA A receptors: subtypes provide diversity of function and pharmacology. Neuropharmacology 56: 141-8.

Owens, D.F, Kriegstein, A.R., 2002. Is there more to GABA than synaptic inhibition? Nat. Rev. Neurosci. 3 (9): 715-27.

Owens, M.J., Ritchie, J.C., Nemeroff, C.B., 1992. 5 alpha-pregnane-3 alpha, 21-diol-20-one (THDOC) attenuates mild stress-induced increases in plasma corticosterone via a non-glucocorticoid mechanism: comparison with alprazolam. Brain Res. 573 (2): 353-5.

Papadopoulos, V., Miller, W.L., 2012. Role of mitochondria in steroidogenesis. Best Pract. Res. Clin. Endocrinol. Metab. 26 (6): 771-90.

Paris, J.J., Brunton, P.J., Russell, J.A., Walf, A.A., Frye, C.A., 2011. Inhibition of 5α-reductase activity in late pregnancy decreases gestational length and fecundity and impairs object memory and central progestogen milieu of juvenile rat offspring. J. Neuroendocrinol. 23(11): 1079-90.

Paris, J.J., Frye, C.A., 2011. Juvenile offspring of rats exposed to restraint stress in late gestation have impaired cognitive performance and dysregulated progestogen formation. Stress 14 (1): 23-32.

Park, M., Kitahama, K., Geffard, M., Maeda, T., 2000. Postnatal development of the dopaminergic neurons in the rat mesencephalon. Brain Dev. 22 (Suppl 1): S38-S44.

Patchev, V.K., Hassan, A.H., Holsboer, D.F., Almeida, O.F., 1996. The neurosteroid tetrahydroprogesterone attenuates the endocrine response to stress and exerts glucocorticoid-like effects on vasopressin gene transcription in the rat hypothalamus. Neuropsychopharmacology 15 (6): 533-40.

Patchev, V.K., Montkowski, A., Rouskova, D., Koranyi, L., Holsboer, F., Almeida, O.F.X., 1997. Neonatal treatment of rats with the neuroactive steroid tetrahydrodeoxycorticosterone (THDOC) abolishes the behavioral and

neuroendocrine consequences of adverse early life events. J. Clin. Invest. 99: 962-966.

Patchev, V.K., Shoaib, M., Holsboer, F., Almeida, O.F., 1994. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. Neuroscience 62 (1): 265-71.

Paul, S.M., Purdy, R.H., 1992. Neuroactive steroids. FASEB J. 6 (6): 2311-22.

Pelletier, G., Luu-The, V., Labrie, F., 1994. Immunocytochemical localization of 5α-reductase in rat brain. Mol. Cell. Neurosci. 5: 394–9.

Peterson, S.J., Smith, G.T., 2017. Association between Elementary School Personality and High School Smoking and Drinking. Addiction doi: 10.1111/add.13905.

Pibiri, F., Nelson, M., Guidotti, A., Costa, E., Pinna, G., 2008. Decreased corticolimbic allopregnanolone expression during social isolation enhances contextual fear: a model relevant for posttraumatic stress disorder. Proc. Natl. Acad. Sci. USA 105: 5567-72.

Pinna, G., 2010. In a mouse model relevant for post-traumatic stress disorder, selective brain steroidogenic stimulants (SBSS) improve behavioral deficits by normalizing allopregnanolone biosynthesis. Behav. Pharmacol. 21: 438-50.

Ploj, K., Roman, E., Nylander, I., 2003. Long-term effects of maternal separation on ethanol intake and brain opioid and dopamine receptors in male Wistar rats. Neuroscience 121: 787-99.

Plotsky, P.M., Thrivikraman, K.V., Nemeroff, C.B., Caldji, C., Sharma, S., Meaney, M.J., 2005. Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. Neuropsychopharmacology 30 (12): 2192-204.

Pomata, P.E., Colman-Lerner, A.A., Barañao, J.L., Fiszman, M.L., 2000. In vivo evidences of early neurosteroid synthesis in the developing rat central nervous system and placenta. Brain Res. Dev. Brain Res. 120 (1): 83-6.

Porcu, P., Barron, A.M., Frye, C.A., Walf, A.A., Yang, S.Y., He, X.Y., Morrow, A.L., Panzica, G.C., Melcangi, R.C., 2016. Neurosteroidogenesis Today: Novel Targets

for Neuroactive Steroid Synthesis and Action and Their Relevance for Translational Research. J. Neuroendocrinol. 28 (2): 12351.

Porcu, P., Sogliano, C., Ibba, C., Piredda, M., Tocco, S., Marra, C., Purdy, R.H., Biggio, G., Concas, A., 2004. Failure of g-hydroxybutyric acid both to increase neuroactive steroid concentrations in adrenalectomized-orchiectomized rats and to induce tolerance to its steroidogenic effect in intact animals. Brain Res. 1012: 160–168.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463: 3-33.

Purdy, R.H., Morrow, A.L., Moore, P.H. Jr., Paul, S.M., 1991. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. Proc. Natl. Acad. Sci. USA 88: 4553-7.

Raineki, C., Lucion, A.B., Weinberg, J., 2014. Neonatal handling: an overview of the positive and negative effects. Dev. Psychobiol. 56 (8): 1613-25.

Rasmusson, A.M., Pinna, G., Paliwal, P., Weisman, D., Gottschalk, C., Charney, D., Krystal, J., Guidotti, A., 2006. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. Biol. Psychiatry 60: 704-13.

Rentesi, G., Antoniou, K., Marselos, M., Fotopoulos, A., Alboycharali, J., Konstandi, M., 2010. Long-term consequences of early maternal deprivation in serotonergic activity and HPA function in adult rat. Neuroscience Letters 480 (1): 7–11.

Rentesi, G., Antoniou, K., Marselos, M., Syrrou, M., Papadopoulou-Daifoti, Z., Konstandi, M., 2013. Early maternal deprivation-induced modifications in the neurobiological, neurochemical and behavioral profile of adult rats. Behav. Brain Res. 244: 29–37.

Rewal, M., Donahue, R., Gill, T.M., Nie, H., Ron, D., Janak, P.H., 2012. Alph4 subunit-containing GABAA receptors in the accumbens shell contribute to the reinforcing effects of alcohol. Addict. Biol. 17 (2): 309-21.

Rewal, M., Jurd, R., Gill, T.M., He, D.Y., Ron, D., Janak, P.H., 2009. Alpha4-containing GABAA receptors in the nucleus accumbens mediate moderate intake of alcohol. J. Neurosci. 29: 543-549.

Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B.A., Riva, M.A., 2002. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol. Psychiatry 7: 609-616.

Rodd-Henricks, Z.A., McKinzie, D.L., Crile, R.S., Murphy, J.M., McBride, W.J., 2000. Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. Psychopharmacology (Berl) 149: 217–24.

Roman, E., Hyytiä, P., Nylander, I., 2003. Maternal separation alters acquisition of ethanol intake in male ethanol-preferring AA rats. Alcohol Clin. Exp. Res. 27: 31–37.

Romeo, E., Brancati, A., De Lorenzo, A., Fucci, P., Furnari, C., Pompili, E., Sasso, G.F., Spalletta, G., Troisi, A., Pasini, A., 1996. Marked decrease of plasma neuroactive steroids during alcohol withdrawal. Clin. Neuropharmacol. 19: 366-9.

Rone, M.B., Fan, J., Papadopoulos, V., 2009. Cholesterol transport in steroid biosynthesis: Role of protein–protein interactions and implications in disease states. Biochim. Biophys. Acta. 1791 (7): 646-58.

Rosenfeld, P., Suchecki, D., Levine, S., 1992. Multifactorial regulation of the hypothalamic–pituitary–adrenal axis during development. Neurosci. Biobehav. Rev. 16, 553-68.

Roth, T.L., Sweatt, J.D., 2011. Annual Research Review: Epigenetic mechanisms and environmental shaping of the brain during sensitive periods of development. J. Child Psychol. Psychiatry 52 (4): 398-408.

Rupprecht, R., 2003. Neuroactive steroids: mechanism of action and neuropsychopharmacological properties. Psychoneuroendocrinology. 28 (2): 139-68.

S

Saalmann, Y.B., Kirkcaldie, M.T., Waldron, S., Calford, M.B., 2007. Cellular distribution of the GABAA receptor-modulating 3alpha-hydroxy, 5alpha-reduced pregnane steroids in the adult rat brain. J. Neuroendocrinol. 19 (4): 272-84.

Sachs, B.D., Salahi, A.A., Caron, M.G., 2014. Congenital brain serotonin deficiency leads to reduced ethanol sensitivity and increased ethanol consumption in mice. Neuropharmacology 77: 177–84.

Sakamoto, H., Ukena, K., Tsutsui, K., 2001. Effects of progesterone synthesized de novo in the developing Purkinje cell on its dendritic growth and synaptogenesis. J. Neurosci. 21 (16): 6221-32.

Sapolsky, R.M., Meaney, M.J., 1986. Maturation of the adrenocotical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. Brain Res. Rev. 11, 65-76.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21 (1): 55-89.

Sari, Y., Johnson, V.R., Weedman, J.M., 2011. Role of the serotonergic system in alcohol dependence: from animal models to clinics. Prog. Mol. Biol. Transl. Sci. 98: 401-43.

Schüle, C., Nothdurfter, C., Rupprecht, R., 2014. The role of allopregnanolone in depression and anxiety. Prog. Neurobiol. 113: 79-87.

Selvaraj, V., Tu, L.N., 2016. Current status and future perspectives: TSPO in steroid neuroendocrinology. J. Endocrinol. 231(1): R1-R30.

Serra M., Pisu M.G., Littera M., Papi G., Sanna E., Tuveri F., Usala L., Purdy R.H. and Biggio G., 2000. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J. Neurochem. 75: 732–740.

Shen, H., Smith, S.S., 2009. Plasticity of the alpha4betadelta GABAA receptor. Biochem. Soc. Trans. 37 (Pt 6): 1378-84.

Shu, H.J., Eisenman, L.N., Jinadasa, D., Covey, D.F., Zorumski, C.F., Mennerick, S., 2004. Slow actions of neuroactive steroids at GABAA receptors. J. Neurosci. 24 (30): 6667-75.

Sieghart, W., Sperk, G., 2002. Subunit composition, distribution and function of GABAA receptor subtypes. Curr. Top. Med. Chem. 2: 795-816.

Silveira, P.P., Portella, A.K., Clemente, Z., Gamaro, G.D., Dalmaz, C., 2005. The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. Int. J. Dev. Neurosci. 23(1): 93-9.

Sinclair, J.D., Kampov-Polevoy, A., Stewart, R., Li, T.K., 1992. Taste preferences in rat lines selected for low and high alcohol consumption. Alcohol. 9 (2): 155-60.

Sinnott, R.S., Phillips, T.J., Finn, D.A., 2002. Alteration of voluntary ethanol and saccharin consumption by the neurosteroid allopregnanolone in mice. Psychopharmacology (Berl). 162 (4): 438-47.

Slotten, H.A., Kalinichev, M., Hagan, J.J., Marsden, C.A., Kevin C.F. Fone, K.C.F., 2006. Long-lasting changes in behavioural and neuroendocrine indices in the rat following neonatal maternal separation: Gender-dependent effects. Brain. Res. 1097: 123-132.

Smith, C.C., Gibbs, T.T., Farb, D.H., 2014. Pregnenolone sulfate as a modulator of synaptic plasticity. Psychopharmacology (Berl). 231 (17): 3537-56.

Spanagel, R., 2000. Recent animal models of alcoholism. Alcohol Res. Health 24 (2): 124-131.

Spear, L.P., 2000. The adolescent brain and age-related behavioural manifestations. Neurosci. Biobehav. Rev. 24: 417-463.

Stocco, D.M., Zhao, A.H., Tu, L.N., Morohaku, K., Selvaraj, V., 2017. A brief history of the search for the protein(s) involved in the acute regulation of steroidogenesis. Mol. Cell. Endocrinol. 441: 7-16.

Stoffel-Wagner, B., 2003. Neurosteroid biosynthesis in the human brain and its clinical implications. Ann. N. Y. Acad. Sci. 1007: 64-78.

Suchecki, D., Mozaffarian, D., Gross, G., Rosenfeld, P., Levine, S., 1993. Effects of maternal deprivation on the ACTH stress response in the infant rat. Neuroendocrinology 57: 204-12.

Suchecki, D., Nelson, D.Y., van Oers, H., Levine, S., 1995. Activation and inhibition of the hypothalamic-pituitary-adrenal axis of the neonatal rats: effects of maternal deprivation. Psychoneuroendocrinology 20: 169-82.

Sundstrom-Poromaa, I., Smith, D.H., Gong, Q., Sabado, T.N., Li, X., Light, A., Wiedmann, M., Williams, K., Smith, S.S., 2002. Hormonally regulated α4β2δGABA-A receptors are a target for alcohol. Nat. Neurosci. 5: 721-722.

Susser, E., St Clair, D., He, L., 2008. Latent effects of prenatal malnutrition on adult health: the example of schizophrenia. Ann. N Y Acad. Sci. 1136: 185-92.

Swerdlow, N.R., Geyer, M.A., Braff, D.L., 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl.) 156 (2-3): 194-215.

Swerdlow, N.R., Light, G.A., Cadenhead, K.S., Sprock, J., Hsieh, M.H., Braff, D.L., 2006. Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. Arch. Gen. Psychiatry 63 (12): 1325-1335.

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., Braff, D.L., 2008. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology 199 (3): 331–388.

T

Takeda, H., Tsuji, M., Matsumiya, T., 1998. Changes in head-dipping behavior in the holeboard test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol. 350 (1): 21-29.

Trifilieff, P., Martinez, D., 2014. Imaging addiction: D2 receptors and dopamine signaling in the striatum as biomarkers for impulsivity. Neuropharmacology 76: 498-509.

Tu, L.N., Morohaku, K., Manna, P.R., Pelton, S.H., Butler, W.R., Stocco, D.M., Selvaraj, V., 2014. Peripheral benzodiazepine receptor/translocator protein global knock-out mice are viable with no effects on steroid hormone biosynthesis. J. Biol. Chem. 289: 27444-54.

U

Ukena, K., Kohchi, C., Tsutsui, K., 1999. Expression and activity of 3b-hydroxysteroid dehydrogenase/D5-D4-isomerase in the rat Purkinje neuron during neonatal life. Endocrinology 140: 805–813.

V

Valera, S., Ballivet, M., Bertrand, D., 1992. Progesterone modulates a neuronal nicotinic acetylcholine receptor. Proc. Natl. Acad. Sci. U S A. 89(20): 9949-53.

VanDoren, M.J., Matthews, D.B., Janis, G.C., Grobin, A.C., Devaud, L.L., Morrow, A.L., 2000. Neuroactive steroid 3α-hydroxy-5α-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci. 20: 1982-1989.

Vengeliene, V., Vollmayr, B., Henn, F.A., Spanagel, R., 2005. Voluntary alcohol intake in two rat lines selectively bred for learned helpless and non-helpless behavior. Psychopharmacology (Berl) 178: 125-132.

Verplaetse, T.L., Czachowski, C.L., 2015. Low-dose prazosin alone and in combination with propranolol or naltrexone: effects on ethanol and sucrose seeking and self-administration in the P rat. Psychopharmacology (Berl) 232 (15): 2647-57.

Violle, N., Balandras, F., Le Roux, Y., Desor, D., Schroeder, H., 2009. Variations in illumination, closed wall transparency and/or extramaze space influence both baseline anxiety and response to diazepam in the rat elevated plusmaze. Behav. Brain Res. 203: 35-42.

Viveros, M.P., Diaz, F., Mateos, B., Rodriguez, N., Chowen, J.A., 2010. Maternal deprivation induces a rapid decline in circulating leptin levels and sexually dimorphicmodifications in hypothalamic trophic factors and cell turnover. Horm. Behav.57: 405–414.

Viveros, M.P., Llorente, R., López-Gallardo, M., Suarez, J., Bermúdez-Silva, F., De la Fuente, M., Rodriguez de Fonseca, F., Garcia-Segura, L.M., 2009. Sex-dependent alterations in response to maternal deprivation in rats. Psychoneuroendocrinology 34 (Suppl.1): S217-S226.

Voikar, V., Koks, S., Vasar, E., Rauvala, H., 2001. Strain and gender differences in the behavior of mouse lines commonly used in transgenic studies. Physiol. Behav. 72: 271-281.

Volkow, N.D., Fowler, J.S., Wang, G.J., Baler, R., Telang, F., 2009. Imaging dopamine's role in drug abuse and addiction. Neuropharmacology. 56 Suppl 1: 3-8.

W

Walf, A.A., Frye, C.A., 2012. Gestational or acute restraint in adulthood reduces levels of 5α-reduced testosterone metabolites in the hippocampus and produces behavioral inhibition of adult male rats. Front. Cell. Neurosci. 6: 40.

Wang, J.M., Johnston, P.B., Ball, B.G., Brinton, R.D., 2005. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. J. Neurosci. 25 (19): 4706-18.

Weaver, I.C., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., Meaney, M.J., 2004. Epigenetic programming by maternal behavior. Nat. Neurosci. 7 (8), 847–854.

Wei, W., Faria, L.C., Mody, I., 2004. Low ethanol concentrations selectively augment the tonic inhibition mediated by delta subunit-containing GABAA receptors in hippocampal neurons. J. Neurosci. 24: 8379-8382.

Weill-Engerer, S., David, J.P., Sazdovitch, V., Liere, P., Eychenne, B., Pianos, A., Schumacher, M., Delacourte, A., Baulieu, E.E., Akwa, Y., 2002. Neurosteroid quantification in human brain regions: comparison between Alzheimer's and nondemented patients. J. Clin. Endocrinol. Metab. 87 (11): 5138-43.

Weinshenker, D., Schroeder, J.P., 2007. There and back again: a tale of norepinephrine and drug addiction. Neuropsychopharmacology. 32 (7): 1433-51.

Weiss, S.M.,Wadsworth, G., Fletcher, A., Dourish, C.T., 1998. Utility of ethological analysis to overcome locomotor confounds in elevated plus maze. Neurosci. Biobehav. Rev. 23: 265-271.

Westerink, B.H., Kwint, H.F., deVries, J.B., 1996. The pharmacology of mesolimbic dopamine neurons: a dual probe microdialysis study in the ventral tegmental area and nucleus accumbens of the rat brain. J. Neurosci. 16: 2605-2611.

Wetzel, C.H.R., Hermann, B., Behl, C., Pestel, E., Rammes, G., Zieglgänsberger, W., Holsboer, F., Rupprecht, R., 1998. Functional antagonism of gonadal steroids at the 5-HT3 receptor. Mol. Endocrinol. 12: 1441-51.

Woods, J.E., McKay, P.F., Masters, J., Seyoum, R., Chen, A., La Duff, L., Lewis, M.J., June, H.L., 2003. Differential responding for brain stimulation reward and sucrose in high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) rats. Alcohol Clin. Exp. Res. 27: 926–36.

Wozniak, K.M., Pert, A., Linnoila, M., 1990. Antagonism of 5-HT3 receptors attenuates the effects of ethanol on extracellular dopamine. Eur. J. Pharmacol. 187 (2): 287-9.

Xiao, C., Zhou, C., Li, K., Ye, J.H., 2007. Presynaptic GABAA receptors facilitate GABAergic transmission to dopaminergic neurons in the ventral tegmental area of young rats. J. Physiol. 580: 731–743.

Xu, L., Anwyl, R., Rowan, M.J., 1998. Spatial exploration induces a persistent reversal of long-term potentiation in rat hippocampus. Nature 394 (6696): 891-4.

Y

Yu, R., Ticku, M.K., 1995. Chronic neurosteroid treatment decreases the efficacy of benzodiazepine ligands and neurosteroids at the gamma-aminobutyric acidA receptor complex in mammalian cortical neurons. J. Pharmacol. Exp. Ther. 275 (2): 784-9.

Z

Zamberletti, E., Prini, P., Speziali, S., Gabaglio, M., Solinas, M., Parolaro, D., Rubino, T., 2012. Gender-dependent behavioral and biochemical effects of adolescentdelta-9-tetrahydrocannabinol in adult maternally deprived rats. Neuroscience 204: 245–257.

Zhang, W.N., Bast, T., Feldon, J., 2002. Prepulse inhibition in rats with temporary inhibition/inactivation of ventral or dorsal hippocampus. Pharmacol. Biochem. Behav. 73 (4): 920–940.

Zhou, Q., Verdoorn, T.A., Lovinger, D.M., 1998. Alcohols potentiate the function of 5-HT3 receptor-channels on NCB-20 neuroblastoma cells by favouring and stabilizing the open channel state. J. Physiol. 507 (Pt 2): 335-52.

Zhou, F.C., Zhang, J.K., Lumeng, L., Li, T.K., 1995. Mesolimbic dopamine system in alcohol-preferring rats. Alcohol 12, 403-412.

Zimmerberg, B., Brunelli, S.A., Hofer, M.A., 1994. Reduction of rat pup ultrasonic vocalizations by the neurosteroid allopregnanolone. Pharmacol. Biochem. Behav. 47: 735– 8.

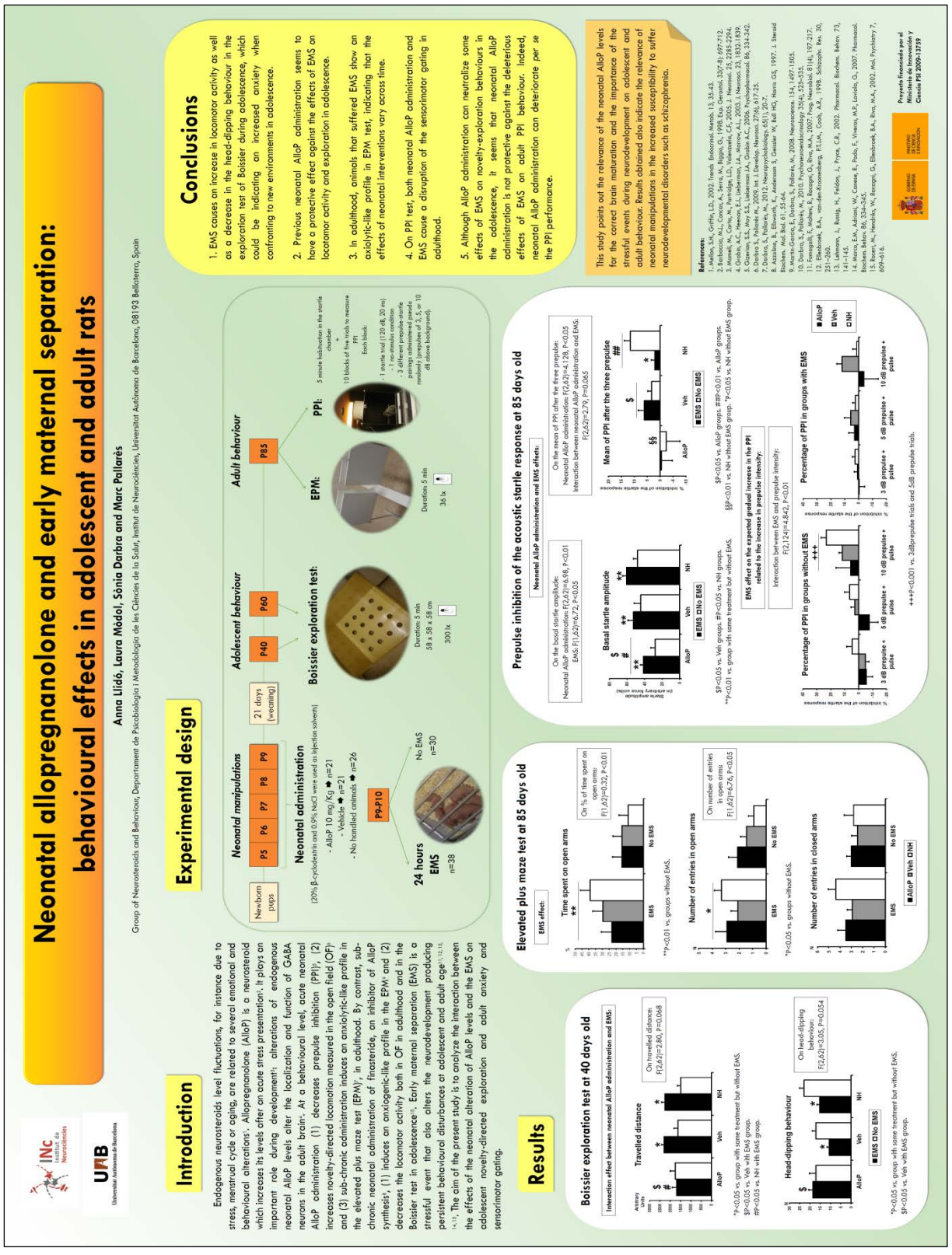
Zimmerberg, B., Rosenthal, A.J., Stark, A.C., 2003. Neonatal social isolation alters both maternal and pup behaviors in rats. Dev. Psychobiol. 42: 52–63.

Zimmerberg, B., Kajunski, E.W., 2004. Sexually dimorphic effects of postnatal allopregnanolone on the development of anxiety behavior after early deprivation. Pharmacol. Biochem. Behav. 78 (3): 465-471.

Zornberg, G.L., Buka, S.L., Tsuang, M.T., 2000. Hypoxic-ischemia-related fetal/neonatal complications and risk of schizophrenia and other nonaffective psychoses: a 19-year longitudinal study. Am. J. Psychiatry 157 (2): 196-202

ANNEX

► **Poster presentation at the 8th Federation of European Neuroscience Society Forum of Neuroscience (Barcelona, 2012)** ◄



► Poster presentation at the first edition of the CORE-seminars in Mental Health (Barcelona, 2014) ◄

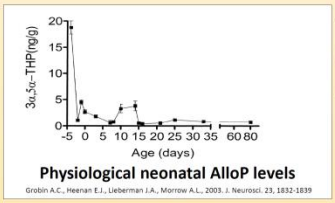
Neurosteroids, neurodevelopment and behavior

Anna Llidó, Iris Bartomolé, Laura Mòdol, Sònia Darbra and Marc Pallarès

Group of Neurosteroids and Behavior, Departament de Psicobiologia i Metodologia de les Ciències de la Salut, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Proyectos financiados por: Ministerio de Innovación y Ciencia PSI 2009-13759 Ministerio de Economía y Competitividad PS 12012-36646

1. Alterations in neonatal neurosteroid allopregnanolone (AlloP) levels and effects in adult behavior



Physiological neonatal AlloP levels

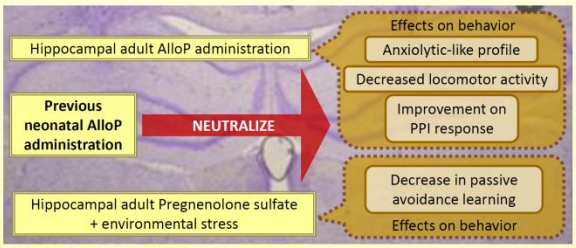
Grobin A.C., Heenan E.J., Lieberman J.A., Morrow A.L., 2003. J. Neurosci. 23, 1832-1839

Neonatal administration of AlloP → Behavioral effects → Increased novelty-directed locomotor activity → Low anxiety → Decreased PPI response

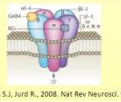
Neonatal stress relation: Previous AlloP administration neutralize early maternal separation effects on adolescent exploration

Neonatal administration of finasteride → Behavioral effects → Adolescence: Decreased novelty-exploration → Adulthood: Anxiogenic-like profile

2. Role of the hippocampus in behavioral effects

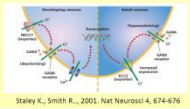


Changes in HIPPOCAMPAL:



Jacob T.C., Moss S.J, Jurd R., 2008. Nat Rev Neurosci. 9 (5), 331-43

GABA$_A$R subunits expression



Staley K., Smith R., 2001. Nat Neurosci 4, 674-676

K$^+$Cl$^-$ co-transporter 2 (KCC2) expression

Neonatal administration of AlloP → Neonatal: Decreased expression of α4 and δ GABA$_A$R subunits

Neonatal administration of AlloP → Neonatal: Altered expression of KCC2

Neonatal administration of finasteride → Neonatal and Adult: Over-expression of α4 and δ GABA$_A$R subunits

→ Changes in emotional behavior

3. Neonatal AlloP and vulnerability to drug abuse

Neonatal: Alteration of physiological AlloP levels + Neonatal stress → Effects on → Adulthood: Voluntary alcohol intake

Adulthood: Voluntary alcohol intake → Ventral Striatum samples → Determination of → - L-DOPA - Dopamine - Noradrenalin - DOPAC - Homovanilic Acid - Serotonin - 5-HIAA

Adulthood: Voluntary alcohol intake → Microdialysis *in vivo* in Nucleus Accumbens → Determination of → -Dopamine -DOPAC

Neonatal: Alteration of physiological AlloP levels → Adolescence: Increase of AlloP levels by means of progesterone administration → Adulthood: Administration of: - Vehicle - Ethanol 0.5 mg/kg - Ethanol 0.8 mg/kg

Adolescence: Increase of AlloP levels by means of progesterone administration → Behavioral evaluation: - Boissier exploration test - Elevated plus maze

Adulthood: Administration of: - Vehicle - Ethanol 0.5 mg/kg - Ethanol 0.8 mg/kg → Evaluation of stimulant ethanol effects in the open field

Selected publications:

Darbra S, Mòdol L, Llidó A, Casas C, Vallée M, Pallarès M. 2014. Prog Neurobiol. 113, 95-105.

Darbra S., Mòdol L., Pallarès M., 2012. Psychoneuroendocrinology. 37(4), 581-5.

Darbra S., Modol L., Vallée M., Pallarès M., 2013. Psychoneuroendocrinology. 2013. 38(8), 1397-406.

Darbra S., Pallarès M., 2009. Int. J. Develop. Neurosci. 27(6), 617-25.

Darbra S., Pallarès M., 2010. Psychoneuroendocrinology 35(4), 525-535.

Darbra S., Pallarès M., 2011. Behav. Brain. Res. 216(2), 705-11.

Darbra S., Pallarès M., 2012. Neuropsychobiology. 65(1), 20-7.

Llidó A., Mòdol L., Darbra S., Pallarès M., 2013. Horm. Behav. 63(4), 577-85.

Martin-Garcia E., Darbra S., Pallarès M., 2008. Neuroscience. 154, 1497-1505.

Martín-García E., Pallarés M., 2008. Exp. Brain. Res. 191(2), 123-31.

Mòdol L., Casas C., Llidó A., Navarro X., Pallarès M., Darbra S., 2014. J. Steroid. Biochem. Mol. Biol. 143, 343-7.

Modol L., Casas C., Navarro X., Llidó A., Vallée M., Pallarès M., Darbra S., 2014. Int. J. Neuropsychopharmacol. 17 (2), 259-73.

Mòdol L., Darbra S., Vallée M., Pallarès M., 2013. Behav. Brain. Res. 241, 96-104.

Mòdol, L., Llidó , A., Casas, C., Navarro, X., Pallarè s, M., Darbra, S., 2012. FENS Forum Abstr., A-471-0184-01765.

► Poster presentation at joint meeting of the European Brain and Behaviour Society and the European Behavioural Pharmacology Society (Verona, 2015) ◄

Effects of neonatal allopregnanolone manipulations and early maternal separation on adult vulnerability to drug abuse: ethanol preference and dopamine levels in ventral striatum

Anna Llidó, Iris Bartolomé, Sònia Darbra and Marc Pallarès

Group of Neurosteroids and Behaviour. Departament de Psicobiologia i Metodologia de les Ciències de la Salut. Institut de Neurociències. Universitat Autònoma de Barcelona. 08193 Bellaterra. Spain

Introduction

Previous studies have shown that changes in the endogenous neonatal levels of the neurosteroid (NS) allopregnanolone (AlloP) affect the development of the central nervous system altering posterior adolescent and adult behavior. Some of these behavioral alterations involve traits that can be related to vulnerability to initiate drug abuse1, such as anxiety2 and novelty-directed locomotion3,4. Furthermore, altered striatal and cortical dopaminergic activity has been also reported as a consequence of changes in neonatal AlloP levels5. Thus, the first objective of the present work is to analyze if manipulations of neonatal AlloP could lead to higher vulnerability to alcohol abuse in adult age. On the other hand, a single 24h period of early maternal separation (EMS) on postnatal day (PND) 9 causes multiple neurodevelopmental and behavioral alterations, also including systems and traits related to drug abuse6,7. Additionally, previous results showed that some of the adolescent behavioral alterations induced by EMS can be avoided by previous neonatal AlloP administration8. Thus, we hypothesize that the alterations on voluntary alcohol consumption related to neonatal AlloP manipulations could differ depending on whether animals have suffered or not posterior EMS.

Methodology

Male Wistar rats

Neonatal Manipulations

Between PND5 and PND9 → neonatal administration
- AlloP 10 mg/kg (n= 28).
- Finasteride 50 mg/kg (Finas; 5α-reductase inhibitor) (n=29).
- Vehicle (Veh; 10% ß-cyclodextrin) (n=28).
- Non-handled animals (NH) (n=31).

On PND9 → 24h early maternal separation
- 24h of EMS (n=51).
- No EMS (n=65).

Voluntary Alcohol Intake

Starting on PND70 → 15 consecutive days of consumption
- First day of consumption: food restriction.
- One daily hour of access to two bottles (+food):
- Solution of 10% (v/v) of ethanol + 3% (w/v) of glucose in distilled water.
- Solution of 3% (w/v) of glucose in distilled water.

Monoamine Levels Determination

Last day of consumption → ventral striatum extraction (n=8/12 per group).

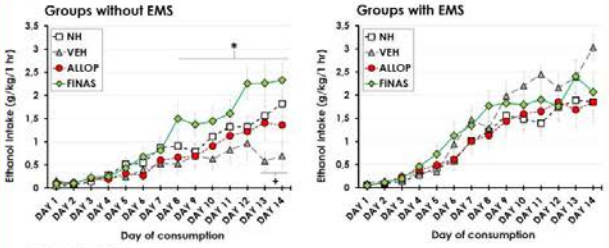
High-performance liquid chromatography analysis (HPLC):
- Dopamine (DA)
- Serotonin (5-HT)
- 3,4-Dihydroxyphenylacetic acid (DOPAC)
- Homovanillic acid (HVA)
- 5-hydroxyindoloucetic acid (5-HIAA)

Results

Voluntary alcohol intake:

Ethanol consumption:

Interaction effect between neonatal NS treatment, EMS condition and day of consumption [F(39,1404)=2.61, P<0.001]:



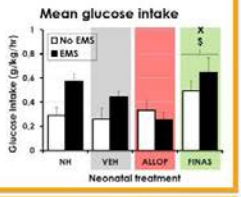
Interaction effect between neonatal treatment and day of consumption [F(39,793)=2.89, P<0.001]

* From day 8 **Finas** animals consumed higher doses of ethanol than the rest of the groups.

+ On days 13 and 14 **Veh** animals consumed lower ethanol doses than NH animals and **AlloP** administration prevent this effect.

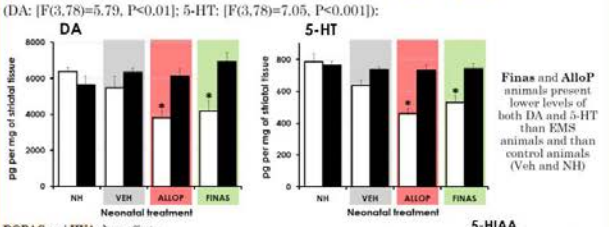
Glucose consumption:

Effect of neonatal NS treatment [F(3,108)=4.10, P<0.001]	**Finas** animals consume higher doses of glucose: **x** P<0.05 vs. Veh; **$** P<0.001 vs. AlloP. NSxDAY [F(39,1404)=2.74, P<0.001]: Finas ↑ than all groups between days 8 and 14.
Effect of EMS [F(1,108)=5.19, P<0.05]	**EMS** animals consume higher doses of glucose. EMSxDAY [F(13,1404)=2.10, P<0.05]: between days 2 and 8



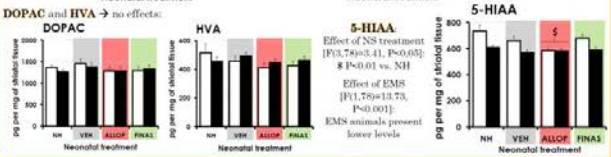
Striatal monoamine levels:

Interaction effect between neonatal NS treatment and EMS on **DA** and **5-HT** levels (DA: [F(3,78)=5.79, P<0.01]; 5-HT: [F(3,78)=7.05, P<0.001]):



Finas and **AlloP** animals present lower levels of both DA and 5-HT than EMS animals and than control animals (Veh and NH)

DOPAC and **HVA** → no effects:



5-HIAA: Effect of NS treatment [F(3,78)=3.41, P<0.05]: $ P<0.01 vs. NH. Effect of EMS [F(1,78)=13.73, P<0.001]: EMS animals present lower levels

Conclusions

1. Neonatal Finas administration increases both alcohol and glucose consumption in adult age in comparison with the rest of neonatal groups (controls: Veh and NH; and AlloP group).
2. Neonatal AlloP administration increases ethanol intake in comparison with the Veh group. Neonatal manipulation (Veh injections) seems to decrease adult ethanol consumption on the last days of the procedure, effect that AlloP administration prevent.
3. The effects of neonatal Finas or AlloP administration on ethanol consumption are not observed when animals suffer 24h of EMS on PND9.
4. EMS increases the glucose intake during the first days of procedure.
5. Both animals that received neonatal Finas and animals that received neonatal AlloP showed decreased ventrostriatal DA and 5-HT levels (with corresponding turnover ratios increased) at the end of the consumption period.
6. The differences in monoamine levels were not present when animals suffered EMS.

Proyecto financiado por: Ministerio de Economía y Competitividad PSI 2012-36846

References:

1. Belin, D., Deroche-Gamonet, V., 2012. Cold Spring Harb. Perspect. Med. 2(11), pii: a011940.
2. Darbra, S., Pallarès, M., 2012. Neuropsychobiology 65(1), 20-27.
3. Darbra, S., Pallarès, M., 2009. Int. J. Devl. Neuroscience 27, 617-625.
4. Darbra, S., Pallarès, M., 2010. Psychoneuroendocrinology 35(4), 525-535.
5. Muneoka, K.T., Kuwagata, M., Shimoyama, Y., Ogawa, T., Shioda, S., 2009. Neuroreport. 20(9), 860-3.
6. Ellenbroek, B.A., Derks, N., Park, H.J., 2005. Stress 8, 247-257.
7. Bentosi, G., Antoniou, K., Marselos, M., Syrrou, M., Papadopoulou-Daifoti, Z., Kostandi, M., 2013. Behav. Brain Res. 244, 29-37.
8. Llidó, A., Módol, L., Darbra, S., Pallarès, M., 2013. Horm. Behav. 63(4), 577-85.

Any question?
E-mail:
annallido33@gmail.com

► Poster presentation at the X Symposium of Neurobiology of the *Societat Catalana de Biologia* (Barcelona, 2016) ◄

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

Anna Llidó, Iris Bartolomé, Sònia Darbra and Marc Pallarès

Group of Neurosteroids and Behaviour, Departament de Psicobiologia i Metodologia de les Ciències de la Salut, Institut de Neurociències, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Introduction

Endogenous concentrations of the neurosteroid (NS) allopregnanolone (AlloP) during neonatal stages are crucial for the correct development of the central nervous system. Manipulations of its neonatal levels have been related with several behavioural alterations, including some traits that can be associated to vulnerability to initiate drug abuse[1] (i.e. anxiety[2] and novelty-directed locomotion[3,4]). In this sense, we have recently reported that the neonatal administration of AlloP or finasteride (Finas), an inhibitor of the enzyme 5α-reductase needed for the AlloP synthesis[5], alters the ethanol consumption in adulthood[6], suggesting that manipulations of neonatal NS levels can increase the vulnerability to alcohol abuse in adulthood. Moreover, after 15 days of ethanol consumption, these rats showed decreased dopaminergic (DAergic) and serotorinergic activity in the ventral striatum[6]. In addition, in another work we have observed that animals with neonatal administration of Finas present reduced adult sensitivity to locomotor stimulating effects of ethanol administration[7]. Given these results, and taking into account previous studies that have proposed neonatal NS levels as a determining factor in the development of DAergic systems[8,9], we hypothesise that neonatal AlloP manipulations could affect the vulnerability to alcohol abuse by means of altering its rewarding effects. Thus, the aim of the present study is to evaluate if manipulations of neonatal AlloP alter adult accumbal DA release in response to oral alcohol consumption.

Methodology

Neonatal Administration

Between PND5 and PND9:
- AlloP 10 mg/kg (n=6).
- Finas 50 mg/kg (n=6).
- Vehicle (Veh; 10% ß-cyclodextrin) (n=6).

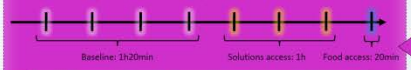
Stereotaxic Surgery

PND90 → Guide cannula implantation into the nucleus accumbens (NAcc) of the left hemisphere
Coordinates relative to Bregma: AP 1.6 mm; L 1.1 mm; V -6 mm from the skull surface.

Voluntary Alcohol Intake

Starting on PND98 → 12 consecutive days of consumption
- One daily hour of access to two bottles:
- Solution of 10% (v/v) of ethanol + 3% (w/v) of glucose in distilled water.
- Solution of 3% (w/v) of glucose in distilled water.
- Daily food ration provided immediately after the consumption hour.

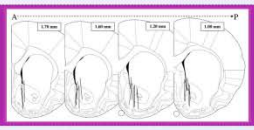
Last day of consumption:
8 samples of 30 µL (20 min per sample):



Microdialysis and DA determination

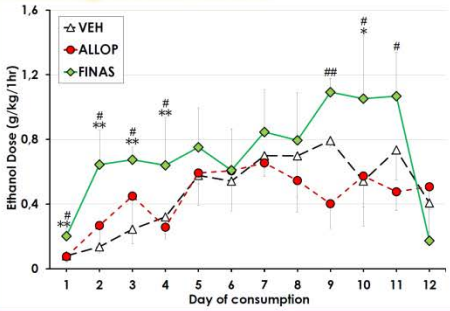
High-performance liquid chromatography (HPLC) analysis:
- Dopamine (DA).
- 3,4-Dihydroxyphenylacetic acid (DOPAC).

Histological Control



Results

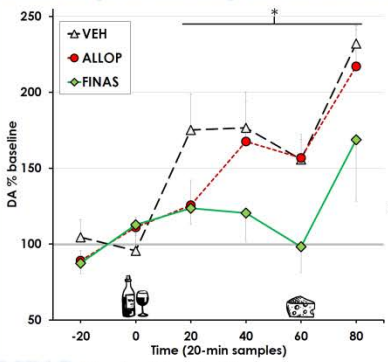
Voluntary alcohol intake: **Finas > Veh *, AlloP #**



Days 1-4:
Neonatal NS effect
[F(2,15)=6.33, P<0.05]
Days 9-12:
Neonatal NS x day effect
[F(6,45)=2.95, P<0.05]

DA and DOPAC release in the NAcc:

DA levels (% of baseline) after solutions and food access:



Neonatal NS effect
[F(2,15)=4.04, P<0.05]:
Finas < Veh *

DA levels increased after solutions presentation in Veh [t=−5.19, P<0.01] and AlloP [t=−3.05, P<0.05] groups, but not in Finas animals [t=−1.03, NS].

Sample effect [F(3,45)=8.15, P<0.001]: DA increase in the last sample (food presentation), in all animals.

DOPAC: No significant effects.

Conclusions

- Animals with neonatal Finas administration showed an increased ethanol intake for several days of the alcohol consumption phase in adulthood, and a decreased DA release in the NAcc in response to both ethanol and food.
- These data seem to indicate a hypo-DAergic activity in neonatal Finas treated rats that could be related to the increase in alcohol intake reported in these animals.

Proyecto financiado por: Ministerio de Economía y Competitividad PSI2012-36646

References:

1. Belin, D., Deroche-Gamonet, V., 2012. Cold Spring Harb. Perspect. Med. 2(11), pii: a011940.
2. Darbra, S., Pallarès, M., 2012. Neuropsychobiology 65 (1), 20-27.
3. Darbra, S., Pallarès, M., 2009. Int. J. Devl. Neuroscience 27, 617-625.
4. Darbra, S., Pallarès, M., 2010. Psychoneuroendocrinology 35 (4), 525-535.
5. Azzolina, B., Ellsworth, K., Andersson, S., Geissler, W., Bull, H.G., Harris, G.S., 1997. J. Steroid Biochem. Mol. Biol. 61, 55–64.
6. Llidó, A., Bartolomé, I., Darbra, D., Pallarès, P., 2016. Horm. Behav. 82, 11-20.
7. Bartolomé, I., Llidó, A., Darbra, D., Pallarès, P., 2015. Poster presented at the EBBS & EBPS Joint Meeting, Verona, Italy.
8. Gzieran, S.S., Moy, S.S., Lieberman, J.A., Grobin, A.C., 2006. Psychopharmacology 186 (3), 334-42.
9. Muneoka, K.T., Iwata, M., Shirayama, Y., 2009. Int. J. Dev. Neurosci. 27 (6), 575-81.

Any question?
E-mail:
annallido33@gmail.com