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Early postnatal allopregnanolone levels alteration and adult behavioral disruption in rats: Implication for drug abuse

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ABSTRACT

Several studies have highlighted the role that early postnatal levels of allopregnanolone play in the development of the CNS and adult behavior. Changes in allopregnanolone levels related to stress have been observed during early postnatal periods, and perinatal stress has been linked to neuropsychiatric disorders. The alteration of early postnatal allopregnanolone levels in the first weeks of life has been proven to affect adult behaviors, such as anxiety-related behaviors and the processing of sensory inputs. This review focuses on the first studies about the possible relationship between the early postnatal allopregnanolone levels and the vulnerability to abuse of drugs such as alcohol in adulthood, given that (1) changes in neonatal allopregnanolone levels affect novelty exploration and novelty seeking has been linked to vulnerability to drug abuse; (2) early postnatal administration of progesterone, the main allopregnanolone precursor, affects the maturation of dopaminergic meso-striatal systems, which have been related to novelty seeking and drug abuse; and (3) alcohol consumption increases plasma and brain allopregnanolone levels in animals and humans. Manipulating neonatal allopregnanolone by administering finasteride, an inhibitor of the 5α-reductase enzyme that participates in allopregnanolone synthesis, increases alcohol consumption and decreases the locomotor stimulant effects of low alcohol doses. At a molecular level, finasteride decreases dopamine and serotonin in ventral striatum and dopamine release in nucleus accumbens. Preliminary results suggest that serotonin 5HT3 receptors could also be affected. Although an in-depth study is necessary, evidence suggests that there is a relation between early postnatal allopregnanolone and vulnerability to drug use/abuse.

1. Introduction

In decades, it has been shown that steroids are important in many brain processes. In 1981, Baulieu and colleagues studied the steroid dehydroepiandrosterone sulfate, and concluded that the brain was a steroidogenic organ (Corpéchot et al., 1981). This led to the term 'neurosteroid', which is used to describe the subclass of steroids that are synthesized in the central nervous system (Baulieu et al., 1981). A few years later, in 1986, Majewska and cols. found that the neurosteroid (NS) allopregnanolone (AlloP) acted as a positive allosteric modulator of γ-aminobutyric acid type A (GABAA) receptors (Majewska et al., 1986). This last discovery led to the term 'neuroactive steroids' (NS), which is used to describe all those steroids that, whether they are synthesized in the brain, the adrenal gland or gonads, are capable of modifying neuronal activity via membrane receptors modulation (Dubrovsky, 2005; Paul and Purdy, 1992). The discovery of steroids that not only act as endocrine messengers but also in a paracrine or autocrine manner, and their capacity to rapidly modulate neural excitability, revealed that they are extremely important, given the huge array of brain activities in which they may be involved. Thus, in recent years, NS have been reported to participate in several brain processes and changes in their physiological levels have been related to a variety of neurological/psychiatric disorders and pathologic behaviors. It has also been shown that administering NS is beneficial in a number of experimental models of certain disorders (see Dubrovsky, 2005; Melcangi and Panzica, 2014; Porcu et al., 2016 for reviews).

NS levels in the brain are not static but rather naturally fluctuate over the life-time in response to different physiological and environmental conditions. For example, the concentration of AlloP oscillates during development (Grobin and Morrow, 2001), the menstrual cycle (Monteleone et al., 2000), pregnancy and post-partum (Concas et al., 1998), as well as faster changes occur in response to adverse events, such as stress (Purdy et al., 1991; Serra et al., 2000), or drugs, such as the systemic administration of alcohol (Barbaccia et al., 1999; Cook et al., 2014a, 2014b). Surprisingly, although the actions of NS in the adult brain have been studied extensively, their role in the developing

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brain and their long-term effects are still poorly understood. The enzymes responsible for steroidogenesis are present in the brains of rats from early fetal stages (Compagnone et al., 1995; Lauber and Lichtensteiger, 1996; Lephart et al., 1990) and the ability of the fetal brain to synthesize NS has been proven (Pomata et al., 2000). Furthermore, pre and postnatal fluctuations of AlloP in the frontal cortex of rats are time-related to significant changes in brain development (Grobin and Morrow, 2001). Thus, studying the involvement of NS, such as AlloP, in brain development and the possible effects of changes in their levels on vulnerability to neurological/psychiatric disorders is of great interest.

2. Neonatal allopregnanolone levels and stress

GABAergic inhibitory control plays an important role in regulation hypothalamic-pituitary-adrenal (HPA) axis activation (see Gunn et al., 2015 for a review). Several studies have shown that 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) in adult rats after acute stress, such as the exposure to CO2 or swimming. Moreover, this increase in AlloP levels correlates with the restoration of the GABAergic transmission (Barbaccia et al., 1998). Therefore, it has been suggested that endogenous AlloP represents a homeostatic mechanism in the context of adaptation to stress by limiting the extent and duration of the reduction in GABAergic inhibitory transmission and activation of the HPA axis (Girdler and Klatzkin, 2007). In this line, it has been observed in humans that a pretreatment with 50 mg of progesterone prior to psychosocial stress diminished stress-induced cortisol increase (Childs et al., 2010), showing the possible action of progesterone-derived metabolites, such as AlloP, on the HPA axis. However, it has been reported that chronic stress due to social isolation induces large reductions in AlloP cerebrocortical and plasma concentrations (Serra et al., 2000), as well as alterations in brain NS levels, such as AlloP or tetrahydrodeoxycorticosterone (THDOC), in response to acute stressors. Thus, it has been proposed that a disruption in this homeostatic mechanism may play a pathogenic role in some psychiatric disorders related to chronic stress, for instance, depressive disorders (Girdler and Klatzkin, 2007). Therefore, the administration of exogenous AlloP either during or following a period of chronic stress, such as social isolation, can prevent or normalize HPA axis dysfunction, precluding the establishment of depressive/anxiety-like behaviors in the rat (Evans et al., 2012). On the other hand, it seems that there are some individuals more sensitive to these NS levels fluctuations. In this sense, it has been observed that NS level changes are relevant to the induction of the altered affective state in women with premenstrual dysphoric disorder (for a review see Wei et al., 2018), which are more vulnerable to increased anxiety (Kim et al., 2004) and alcohol abuse (Stout et al., 1986; Caan et al., 1993; Chuong and Burgos, 1995).The possibility that these women have suffered NS levels alterations during neurodevelopment that could have made them more behaviorally sensitive to these fluctuations in adulthood is a question that opens new lines of research.

During the perinatal period the brain is highly sensitive to environmental factors, and thus early exposure to adverse experiences like stress have been related to several developmental alterations, such as impoverished dendritic trees and reduced hippocampal volume, and adult disorders, such as long-lasting memory deficits (see Bale et al., 2010; Bock et al., 2014; Chen and Baram, 2016 for a review). In neonatal animals, early stress arises mainly from disruptions in maternal care. For this reason, several models of neonatal stress in rodents consist in manipulating maternal-pup interactions. One of the main methods used to cause stress for the pups which has consequences for their cognitive and emotional networks and functional outcomes, is early maternal separation (EMS) (Chen and Baram, 2016), in its many procedure variants (see Fumagalli et al., 2007 for a review).

Changes in AlloP levels related to stress have also been observed during early postnatal stages (Frye et al., 2006; Kehoe et al., 2000). Given that adequate inhibitory GABAergic control is necessary for stress regulation, variations in physiological AlloP levels may imply changes in stress responses, and thus different outcomes of neonatal stress. Previous works studying the effects of administering AlloP on neonatal stress have shown that administering AlloP prior to a brief EMS at postnatal day 7 decreased 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 20–50 kHz and are thought to contribute to the formation of the maternal-infant bond as infant mammals vocalize when separated from their mothers to elicit protection, nourishment and warmth (Zimmerberg and Kajunski, 2004). Therefore, the analysis of ultrasonic vocalizations is used to measure the pup's affective state (Zimmerberg et al., 2003). Other authors have shown that when the neurosteroid THDOC, which is a GABAA-positive modulator, is administered together with EMS, it can attenuate the adult behavioral and neuroendocrine consequences of this repeated stress during early life (i.e. increased anxiety, enhanced HPA axis responses to stress, such as an exaggerated adrenocortical secretion, and impaired glucocorticoid feedback). (Patchev et al., 1997). Similarly, a single administration of 2 mg/kg of AlloP injected just prior to 12h of EMS on postnatal day 5 counteracted the increased HPA axis response to subsequent stressors in both infants and adult rats (Mitev et al., 2003). Thus, administering NS, such as AlloP, which acts as a positive modulator of GABAA receptors, might prevent or reverse some of the negative neuroendocrine and behavioral effects of neonatal stress. In fact, it has been suggested that a transient increase in NS biosynthesis may contribute to the stress hyporesponsive period (SHRP) (Mitev et al., 2003). The early postnatal period between postnatal days 2 and 14 is known as SHRP and is a phase of high plasticity within the CNS with neuronal migration, synaptogenesis and apoptosis (Gunn et al., 2015). During this period, animals have low corticosterone (CORT) and adrenocorticotropic hormone (ACTH) levels in response to stress (Sapolsky and Meaney, 1986; Sapolsky et al., 2000; Rosenfeld et al., 1992), which reflects the activity of the HPA axis that is still developing (Ellenbroek and Cools, 2002). The hypoactivity of this axis in response to stress is key during early life since it protects the developmental nervous system of stressful events (Brunton et al., 2014; deKloet et al., 1988; Ellenbroek and Cools, 2002). If a stressor is able to raise the CORT and ACTH levels during SHRP it can cause multiple alterations in neurodevelopment (Bock et al., 2014; Chen and Baram, 2016), such as, a reduction in the density of GABAergic interneurons in the medial prefrontal cortex and the hippocampus (Fride et al., 1986; Barros et al., 2006), which have been linked to several neuropsychiatric disorders, such as schizophrenia, autism, and anxiety (Fine et al., 2014).

Besides the possible protective role of AlloP for neonatal stress, it has to be considered that since alterations in neonatal AlloP levels have been related to several developmental disruptions, some of the detrimental effects of neonatal stress may be in part elicited by an alteration in neonatal NS levels. Zimmerberg and Kajunski (2004) showed that both pups submitted to 6h per day of social isolation between postnatal days 2 and 6, and pups that were not socially isolated and that were administered AlloP (5 mg/kg) daily emitted fewer ultrasonic vocalizations than control rats during EMS at postnatal day 7. These authors proposed that the effects of social isolation on ultrasonic vocalizations could be mediated by an endogenous increase in AlloP; and therefore, administering it exogenously leads to 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 was unaffected. This may again indicate a protective effect of AlloP against stress due to EMS when AlloP is administered prior to stress, as well as the endogenous AlloP increase that occurs as an adaptive response. In a set of experiments carried out in our laboratory we observed that early postnatal AlloP administration (from postnatal

days 5–9) played a protective role against posterior effects of neonatal stress (maternal deprivation for 24 h at postnatal day 10). For instance, and consistent with previous works in adolescent (Marco et al., 2007) and adult rats (Rentesi et al., 2013), EMS increased locomotion and decreased exploration of holes in a new environment in adolescent age (postnatal day 40), and these effects were prevented by previous neonatal AlloP administration (Llidó et al., 2013). These data suggest that the previous increase in AlloP levels produced by administering it exogenously could alter the function of GABAA receptors, which in turn could interfere with the physiological response produced by EMS. However, the recorded anxiolytic effect of EMS measured in the elevated plus-maze test at adult age, which has also been reported by other authors who studied rats (Burke et al., 2013; Llorente-Berzal et al., 2011) and mice (Fabricius et al., 2008), was not prevented or neutralized by administering AlloP previously to EMS (Llidó et al., 2013). Thus, it seems that the kind of behavior and the developmental period are key factors for obtaining the protective effects of AlloP on neonatal stress.

3. The effects of changes in neonatal allopregnanolone levels on

3.1. Brain maturation

As mentioned above, the brain in the fetal stage already acts as a steroidogenic organ (Pomata et al., 2000) and has NS levels similar to those found in the adult brain (Kellogg et al., 2006). AlloP levels fluctuate during neurodevelopment, and are higher in fetal nervous tissue during late gestation (Kellogg and Frye, 1999; Grobin and Morrow, 2001). According to Hirst and his collaborators (Hirst et al., 2016), the high fetal levels of AlloP during late gestation would play a key role in regulating the activity of the fetal brain. In addition, stressful events during late pregnancy stimulate the release of AlloP; thus, this NS may play a neuroprotective role during pregnancy (Brunton et al., 2014; Hirst et al., 2014).

Grobin and Morrow (2001) characterized AlloP pre and postnatal fluctuations in the frontal cortex of rats. These authors reported that fetuses have elevated AlloP levels during the last gestational days, which has been related to the mentioned protective role played by AlloP against gestational stress (see Brunton et al., 2014 for review). These high levels decrease progressively during the first week of life, when rats have low levels similar to those found in the adult brain. Then, during the second week of life there is another rise in AlloP levels, and maximum values are reached between postnatal day 10 and 14, decreasing again to similar adult levels on postnatal day 15 and remaining low until puberty. This second peak in AlloP is time-related to the functional GABA shift from depolarization to hyperpolarization in both the neocortex and hippocampus (reviewed in Dehorter et al., 2012). Unlike the adult brain, during early neurodevelopment, GABA has a depolarizing profile caused by the activation of GABAA to trigger Cl- (Ben-Ari et al. 2007, 2012). The depolarization induced by GABA eliminates the voltage-dependent blockade exerted by magnesium in the NMDA-channel receptors, which generates a large influx of calcium and activates various neurotrophic mechanisms that mediate neuronal proliferation, migration and differentiation processes (see Owens and Kriegstein, 2002 for a review). The depolarizing profile of GABA is promoted by the higher concentration of intracellular Cl-contained in immature neurons, since the sodium-potassium-chloride cotransporter type 1 (NKCC1), which transports Cl-within the cell, is expressed much more than the KCC2 potassium-chloride cotransporter (which extracts the Cl-out of the cell). This causes the intracellular accumulation of this anion. In contrast, an increase in KCC2 expression is observed to the detriment of NKCC1 in the adult brain, providing the hyperpolarizing profile of the GABAergic system (Ben-Ari et al. 2007, 2012). Even so, it has to be kept in mind that this change in the response of the GABAA receptor depends on the age and sex of the subject, in addition to the area of the brain (Ben-Ari et al., 2007). However, it has been observed in animal models that this change in GABAergic polarity would occur from the second postnatal week in several brain areas, such as the striatum and the spinal cord (Dehorter et al. 2011, 2012) or the hippocampus (Sipilä et al., 2006), and would be able to temporarily coincide with the increase in AlloP levels during postnatal day 10. In a previous work, we reported that the alteration of physiological AlloP levels by means of sub-chronic administration (from postnatal day 5–9) of AlloP or finasteride, which acts as an inhibitor of the 5α-reductase enzyme and decreases plasmatic AlloP levels in adult rats (Azzolina et al., 1997; Mukai et al., 2008), alters the developmental expression of KCC2 in the hippocampus (Modol et al., 2014). These results may indicate that AlloP participates in the GABA hyperpolarizing shift.

As discussed in the previous sections, NS can play an important role in regulating early neurodevelopment. Thus, when the endogenous NS levels are altered, for example, through the neonatal administration of AlloP, then the number and location of GABAergic interneurons in the adult prefrontal cortex are modified (Grobin et al., 2003), the total number of neurons in the dorsomedial nucleus of the thalamus decreases (Gizerian et al., 2004) and the basal levels of striatal dopamine decrease (Muneoka et al., 2009). The neonatal administration of progesterone, the main precursor of AlloP, also induces a decrease in basal dopamine levels, together with a decrease in serotonin levels in the striatum (Muneoka et al., 2010). Furthermore, it has been documented that neonatal treatment with estradiol, which induces a persistent decrease in cerebral and plasma concentrations of progesterone and AlloP (McCarthy, 2008; Berretti et al., 2014; Calza et al., 2010), increases production of extracellular dopamine in the medial prefrontal cortex, an effect that can be reversed through subsequent treatment with progesterone (Porcu et al., 2017). However, the neonatal administration of the 5α-reductase inhibitor finasteride, which has been observed to tend to increase the AlloP levels in the neonatal hippocampus (Mòdol et al., 2013; Darbra et al., 2014), causes an increase in the expression of the α4δ subunits of the GABAA receptor in this same structure (Modol et al., 2014). This data set highlights the importance of basal levels of NS during early neurodevelopment because manipulating it alters the maturation of diverse brain structures.

3.2. Adolescent and adult behavior

At the behavioral level, it has been observed that the manipulation of neonatal levels of NS such as AlloP, can modify adolescent and adult behavior. It has been shown that administrating AlloP during the early postnatal stage in rats causes a decrease in responses related to anxiety in the elevated plus-maze (EPM) in adult age, because animals spent more time and entered the open arms of the apparatus more often without showing differences in locomotor activity (Zimmerberg and Kajunski, 2004; Darbra and Pallarès, 2012). It has also been shown that the early postnatal administration of AlloP causes a decrease in the anxiolytic effects of lorazepam in the EPM in adult animals, suggesting that an increase in neonatal levels of this NS could decrease sensitivity to the effects of this benzodiazepine (Darbra and Pallarès, 2009). However, it has been observed that the neonatal administration of finasteride causes a decrease in the time and number of entries in the open arms of the EPM in adult animals that have undergone stereotactic surgery (Martín-García et al., 2008). Concerning the behavior induced by novelty, in our laboratory we have observed that neonatal treatment with finasteride can induce a decrease in hole exploration in the Boissier test during adolescence (Darbra and Pallarès, 2010). In contrast, a single administration of AlloP in the early postnatal stage can cause an increase in the exploratory behavior induced by a new environment in an open field (OF) in adulthood (Darbra and Pallarès, 2009). Regarding locomotor activity, other studies have shown that the neonatal administration of AlloP causes an increase in the activity induced by amphetamine during adulthood (Gizerian et al., 2006). This suggests that this manipulation during the early postnatal period would increase the sensitivity to the stimulant effects of this drug. Our group has also

Table 1
Effects of neonatal neuroactive steroid levels alteration on adult and adolescent behavior (Accioly and Guedes, 2019; Bayless et al., 2013; Bonansco et al., 2018; Dib et al., 2018; Jorge et al., 2005; Komine et al., 2017; Li et al., 2019; Locci et al., 2017; Muneoka et al., 2002; Shiga et al., 2016; Velásquez et al., 2019).

Abbreviations: PN, postnatal day; PN0, day of birth; s.c., subcutaneous injection; i.p., intraperitoneal injection; EMS, early maternal stress; PREGS, pregnenolone sulfate; THDOC, tetrahydrodeoxycorticosterone; EPM, elevated plus maze; OF, open field; PPI, prepulse inhibition; ORT, object recognition test; CPP, conditioned place preference; NOP, novel object preference.

	NEONATAL ADMINISTRATION	BEHAVIORAL RESPONSE	BEHAVIORAL TEST	ADOLESCENT/ADULT AGE	REFERENCES
ALLOPREGNANOLONE	PN2-6 (2mg/kg; s.c.)	↓ Anxiety-like behavior	EPM	PN125-135	Zimmerberg and Kajunski, 2004
	PN5-9 (10mg/kg; s.c.)	↓ Anxiety-like behavior		PN80	Darbra and Pallarès, 2012
	PN5 (10mg/kg; s.c.)	↓ Anxiolytic-like effect of Lorazepam		PN80	Darbra and Pallarès, 2009
	PN5 (10mg/kg; s.c.)	↑ Novel environment exploration	OF	PN80	Darbra and Pallarès, 2009
	PN2 / PN5 (10mg/kg; s.c.)	↑ Locomotor activity induced by amphetamine		PN20 / PN80	Gizerian et al., 2006
	PN5-9 (10mg/kg; s.c.)	Neutralizes high locomotor activity induced by EMS	Boissier test	PN40	Llidó et al., 2013
	PN5-9 (10mg/kg; s.c.)	Neutralizes PREGS impairment on learning	Passive Avoidance	PN90	Martín-Garcia et al., 2008
	PN2 or 5 (10mg/kg; s.c.)	↓ Prepulse inhibition	PPI	PN20 / PN80	Gizerian et al., 2006;
	PN5-9 (10mg/kg; s.c.)			PN85	Darbra and Pallarès, 2010; Llidó et al., 2013
PROGESTERONE	PN7-21 (50μg/kg; i.p.)	↑ Recognition memory	ORT	PN84	Accioly and Guedes, 2019
TESTOSTERONE	PN0-1 (150μg/kg; s.c.)	↑ Impulsivity	delay-based impulsive choice task	PN24-35	Bayless et al., 2013
	PN1 (1mg/50μL of sesame oil; s.c.)	↓ Methylphenidate-induced locomotor activity	OF	PN60-62	Dib et al., 2018
THDOC	PN2-10 (20μg/rat; s.c.)	Neutralizes anxiety-like behavior induced by EMS	EPM	PN75-80	Patchev et al., 1997
PREGNENOLONE	PN3-7 (10μg/g)	↑ Locomotor activity	OF	PN28 / PN77	Muneoka et al., 2002
PREGNENOLONE SULFATE	PN1-14 (1mg/kg; s.c.)	↓ Anxiety-like behavior	EPM	PN69	Jorge et al., 2005
	PN1-14 (1mg/kg; s.c.)	↓ Locomotor activity	automated activity monitors	PN70	Jorge et al., 2005

(*continued on next page*)

Table 1 (*continued*)

	PN0 (10μg/50μL of sesame oil; s.c.)	↑ Anxiolytic-like effect of Diazepam	EPM	PN60-90	Calza et al., 2010
	PN0 (10μg/50μL of sesame oil; s.c.)	↑ Reduction of locomotor activity induced by Diazepam	OF	PN60-90	Calza et al., 2010
	PN1 (0.1mg/50μL of sesame oil; s.c.)	↑ Morphine-induced locomotor activity		PN60-62	Velásquez et al., 2019
β-ESTRADIOL	PN7-21 (50μg/kg; i.p.)	↑ Recognition memory	ORT	PN84	Accioly and Guedes, 2019
	PN0 (10μg/50μL of sesame oil; s.c.)	↑ Spatial learning and memory	Morris water maze test	PN90	Locci et al., 2017
	PN7 (100 ug/kg; i.p.)	↑ Ketamine-induced decline of learning and memory		PN42-47	Li et al., 2019
	PN1 (0.02mg/kg; s.c.)	↓ Aversive learning	Passive Avoidance	PN42 / PN105-119	Shiga et al., 2016
	PN0-1 (150μg/kg; s.c.)	↑ Impulsivity	delay-based impulsive choice task	PN24-35	Bayless et al., 2013
	PN1 (0.1mg/50μL of sesame oil; s.c.)	↑ Morphine-induced place preference	CPP	PN60-62	Bonansco et al., 2018; Velásquez et al., 2019
	PN0 (10μg/50μL of sesame oil; s.c.)	↑ Agonistic behaviors delivered	Resident–intruder test	PN60-110	Berretti et al., 2014
	PN0 (10μg/50μL of sesame oil; s.c.)	↓ Sexual activity	female-paced mating situation	PN60-110	Berretti et al., 2014
	PN1 (20mg/kg; s.c.)	Impaired sexual behaviours		PN77-105	Komine et al., 2017
	PN5-9 (50mg/kg; s.c.)	↑ Anxiety-like behavior	EPM	PN90	Martín-García et al., 2008
	PN5-9 (50mg/kg)	↓ Anxiolytic effects induced by Progesterone		PN90	Modol et al., 2014
	PN5-9 (50mg/kg; s.c.)	↓ Locomotor activity induced by alcohol	OF	PN70	Bartolomé et al., 2017
	PN5-9 (50mg/kg; s.c.)	↓ Holes exploration and locomotion	Boissier test	PN40 / PN60	Darbra and Pallarès, 2010
5a-reductase inhibitor (FINASTERIDE)					

(*continued on next page*)

Table 1 (*continued*)

PN5-9 (50mg/kg; s.c.)	↑ Preference for exploring the novel object in front a familiar one	NOP	PN70	Bartolomé et al., 2018
PN5-9 (50mg/kg; s.c.)	↓ Learning retention	Passive Avoidance	PN90	Martín-García et al., 2008
PN5-9 (50mg/kg; s.c.)	↑ Initial consumption	Alcohol intake	PN70	Llidó et al., 2016a; Llidó et al., 2016b
			PN77	Bartolomé et al., 2018
PN5-9 (50mg/kg; s.c.)	↑ Sensitivity to ondansetron effects (↓ consumption)		PN77	Bartolomé et al., 2018

conducted studies on prepulse inhibition (PPI). This is a pre-attentional task consisting of a weak stimulus (prepulse) preceding a stronger stimulus (pulse) that generates a startle reaction. Under normal conditions the prepulse produces an attenuation of the startle response reflex caused by the pulse. The neonatal administration of AlloP both injected acutely (Gizerian et al., 2006), or during several consecutive days (Darbra and Pallarès, 2010; Llidó et al., 2013), has been observed to decrease PPI in adults. Altering the PPI has been linked to affecting the dopaminergic system and mesocortical and prefrontal cortex GABAergic systems (Swerdlow et al., 2001; Gizerian et al., 2006) in addition to the dorsal and ventral hippocampus (Zhang et al., 2002). Thus, the behavioral results obtained in the PPI test are consistent with some of the structural alterations observed after the manipulation of neonatal NS levels (see section 3.1).

Other studies have focused on observing behavioral effects in adverse situations, such as stress, after manipulating neonatal NS levels. As previously mentioned, acute stress increases the cerebral and plasma concentrations of AlloP in rodents (Purdy et al., 1991; Barbaccia et al. 1996, 1997; Cozzoli et al., 2014). This kind of response has also been observed in experimental studies in humans, since some types of stress, such as psychosocial stress, can increase circulating levels in healthy humans which do not present a lower basal AlloP concentrations (Girdler et al., 2001; Droogleever Fortuyn et al., 2004; Crowley and Girdler, 2014). In this way, it has to be kept in mind that the nature of the stressor may influence the magnitude of AlloP response in humans, as well as the moment when AlloP is sampled and because of inter-individual characteristics. Moreover, periods of neurosteroid fluctuation, such as during the ovarian cycle, can influence that reactivity of AlloP in acute stress situations and it may be linked to alterations in the sensitivity of GABAA receptors to NS, resulting in mood instability in vulnerable women, such as those with premenstrual dysphoric disorder (Crowley and Girdler, 2014). Interestingly, in our laboratory, we have observed that male wistar rats subjected to forced swimming during adulthood show an increase in plasma levels of AlloP, which are not observed if finasteride is previously administered (Pallarès et al., 2015). One way to mimic the increase in AlloP levels observed after acute stress is by administering its precursor, progesterone (Moran and Smith, 1998; Pisu et al., 2016). In this way, it has been previously reported that the administration of progesterone during early adolescence induces an anxiolytic-like behavior in the EPM regardless of previous neonatal NS levels manipulations (Bartolomé et al., 2017). Nevertheless, our group has documented that administering progesterone during adulthood causes an anxiogenic profile in EPM in those adult animals neonatally treated with finasteride (Modol et al., 2014). Therefore, altering neonatal levels of NS by inhibiting the enzyme 5α-reductase may modify sensitivity to the anxiolytic effects caused by administering this AlloP precursor during adulthood. These differences between the results of both experiments could have been due to the stages of development (postnatal day 30 vs. postnatal day 90) in which progesterone was administered as well as the behavioral test was carried out. In this sense, we had already observed an effect of age on the behavioral evaluation, regardless of neonatal treatment, in our laboratory. Specifically, the hole exploration levels in the Boissier test were higher in postnatal day 40 than in postnatal day 60 (Darbra and Pallarès, 2010). These results show that the specific behavioral effects observed during a period of development do not necessarily have to manifest in other maturation stages (Doremus-Fitzwater et al., 2012; Barbayannis et al., 2017; Blume et al., 2018). However and as explained in section 2, animals subjected to EMS stress had low exploration scores in the hole-board exploration test (Boissier test) during adolescence, which was neutralized or prevented by the neonatal administration of AlloP prior to separation (Llidó et al., 2013). In summary, quite a lot of evidence shows that manipulating neonatal levels of NS, such as AlloP, modifies the maturation of the nervous system at a structural and functional level, which is reflected at the behavioral level. A systematic review of the main studies modifying neonatal NS levels and the corresponding behavioral effects is presented in the Table 1.

4. Why to study the alteration of neonatal allopregnanolone and its possible effect on alcohol abuse vulnerability?

First of all, it is important to highlight that if applying early postnatal stress can alter plasmatic and cerebral AlloP levels (Frye et al., 2006; Kehoe et al., 2000) (see section 3.1), and neonatal stress has been considered to be a key factor that increases the vulnerability to developmental alterations and adult neuropsychiatric disorders (see Bale et al., 2010; Bock et al., 2014; Chen and Baram, 2016 for a review), it is reasonable to consider that early postnatal alterations in AlloP levels are an important factor to be considered for studying the development of behavioral disorders.

4.1. What has previously been observed at a behavioral level?

In relation to drug abuse, as mentioned above (see section 3.2), altering early postnatal AlloP levels can affect novelty exploration and anxiety. Interestingly, an increase in novelty exploration has been related to a novelty or sensation seeking pattern, which has been linked to vulnerability to drug use and/or abuse (Belin and Deroche-Gamonet, 2012; Deroche-Gamonet and Piazza, 2014; Manzo et al., 2014). It has been proposed that novelty seeking (tendency to explore new environments) and sensation seeking (tendency to search intense emotional experiences) are two related (Arnett, 1994; Belin and Deroche-Gamonet, 2012; Belin et al., 2016) but dissociable traits (Cain et al., 2005; Belin et al., 2001; Flagel et al., 2014). Sensation seeking is usually

inferred by measuring locomotor activity in a new environment (Dellu et al., 1996; Piazza et al., 1989), whereas novelty seeking is studied by determining the preference for new versus familiar environments using the conditioned place preference or the new object preference tests (Belin et al., 2016; Ennaceur et al., 2010; Parkitna et al., 2013; Montagud-Romero et al., 2014; delaPeña et al., 2015). These two behavioral traits have been related to different aspects of drug abuse. It seems that a sensation seeking pattern could be related to the acquisition of drug intake (Piazza et al., 1989; Klebaur et al., 2001; Belin et al., 2008), whereas novelty seeking has proven to be important for drug abuse in addition to the severity of addiction behavior (Belin et al., 2011; Belin et al., 2016; Flagel et al., 2014; Foulds et al., 2017). A possible link between sensation/novelty seeking and drug use/abuse can be related to dopamine release in the nucleus accumbens. It has been documented that the animals who showed more locomotion in response to novelty also showed higher levels of mesolimbic dopamine release (Flagel et al., 2010; Hooks et al., 1991; Marinelli and White, 2000), and similar results have been obtained in Roman High Avoidance rats (Tournier et al., 2013).

4.2. What has previously been observed at a neurochemical level?

It is important to note that early-postnatal manipulations of neuroactive steroid levels by administering progesterone, the main precursor of AlloP, affect the normal development and maturation of the dopaminergic meso-striatal systems (Muneoka and Takigawa, 2002; Muneoka et al., 2010). These results are similar to those previously reported in neonatally AlloP-treated rats (Muneoka et al., 2009b). It has been observed that the administration of progesterone increases cortical and plasmatic AlloP levels in animals (Pisu et al., 2016) as well as in the brain of progesterone-treated neonates (Palliser et al., 2015). The developing brains can synthesize AlloP from progesterone (Pomata et al., 2000) and, furthermore, the action of progesterone on many neuronal receptors, such as GABAA, seems to be mediated by its reduced metabolite AlloP (Melcangi et al., 2014; Schumacher et al., 2014). Thus, it is conceivable that at least part of the dopaminergic alterations observed in those progesterone-treated animals were induced by AlloP. Thus, if neonatal AlloP manipulation affects the maturation of dopamine systems, then it is conceivable that these animals have alterations in drug seeking behavior.

On the other hand, GABAA receptors participate in regulating excitability of the dopaminergic neurons in the ventral tegmental area (Xiao et al., 2007). Regarding this receptor, previous experiments carried out in our laboratory showed that early postnatal AlloP manipulation produced an over-expression of hippocampal GABAA receptors containing α4/δ subunits, and these changes were maintained in adult age (Modol et al., 2014). This result is relevant for drug abuse vulnerability because this kind of GABAA receptor has been related to alcohol use. Several studies have shown that a low expression of α4/δ GABAA receptors in the mesolimbic system seems to be related to low alcohol consumption (Mihalek et al., 2001; Nie et al., 2011; Rewal et al., 2009, 2012).

4.3. The interaction between allopregnanolone levels and alcohol

Another important factor to take into consideration is that alcohol consumption or its administration (i.p.) increases brain cortical and hippocampal AlloP levels in adult animals (Barbaccia et al., 1999; VanDoren et al., 2000; Morrow et al. 2001, 2006). Moreover, the development of tolerance to depressing alcohol effects has been linked to a reduction in AlloP production (Janis et al., 1998; Morrow et al., 2001). Regarding human studies, although AlloP levels increase in the plasma of adolescent men and women after alcohol intoxication (Torres and Ortega, 2003, 2004), it has been observed that they don't increase or even decrease when the doses of alcohol consumption are moderate (Holdstock et al., 2006) and/or when the subjects have reached adulthood (Holdstock et al., 2006; Pierucci-Lagha et al., 2006). Thus, making a comparative study between different ages and alcohol consumption levels would be interesting.

In parallel, the systemic administration of AlloP leads to an increase in alcohol intake in rats that had low levels of basal consumption, and decreases alcohol ingestion in rats with high levels of alcohol consumption (Janak et al., 1998; Morrow et al., 2001), even when AlloP was injected directly into the hippocampus (Martín-García et al., 2007). In other brain structures, such as the ventral tegmental area, the overexpression of the steroidogenic enzyme cytochrome P450 side chain cleavage increased AlloP and reduced long-term operant ethanol self-administration in rats (Cook et al., 2014b). In mice, although differences in the two sexes have been detected, a dose-response relation has been proposed in several alcohol intake paradigms: low AlloP doses (3.2 mg/kg i.p.; 50 ng i.c.v.) increased EtOH ingestion, and high doses (17 or 24 mg/kg i.p.; 400 ng i.c.v.) decreased it (Ford et al., 2005, 2007, 2008, 2015). These results suggest that changes in central AlloP production as a consequence of alcohol ingestion may be involved in some aspects of the alcohol abuse spectrum, such as the development of tolerance. Therefore, it has also been proposed that the anxiolytic effects of alcohol are, at least in part, mediated by the increase in central AlloP levels induced by the drug (Hirani et al., 2005).

In conclusion, taking these results together, it seems conceivable that alterations in AlloP levels during development can affect drug intake or drug seeking behaviors, and alcohol abuse is a reasonable study subject.

5. The effects of altering neonatal allopregnanolone levels on alcohol abuse

The developing brain shows a variety of neuroplasticity responses that are not normally shown in adult age. Several mechanisms contribute to the equilibrium between neuronal plasticity and homeostasis in the developing brain, which allow synaptic stability and flexibility (Johnston, 2004). Adverse events or pathological states can alter the normal homeostasis in the developing brain and induce an aberrant neuroplastic effect that can contribute to a neurophysiologic or behavioral abnormal phenotype (Ismail et al., 2017). Recent works carried out in our laboratory have shown that neonatal manipulation of AlloP levels by administering finasteride increased the consumption of a sweetened alcohol solution (10% EtOH + 3% glucose) in non-selected adult male rats (Bartolomé et al., 2018; Llidó et al., 2016a, 2016b). This increase in alcohol intake was reported in the initial period of consumption, specifically in a two-week procedure of EtOH access. Thus, it seems that early postnatal AlloP levels could be important for determining the initial sensitivity to alcohol seeking behavior in adulthood. Furthermore, when monoamine levels are analyzed in ventral striatum by means of HPLC, we observed that the neonatal finasteride group consumed a higher amount of alcohol and had lower levels of dopamine and serotonin in this brain structure compared to control groups when they were sacrificed at the end of the two-week consumption period (Llidó et al., 2016a). Moreover, the levels of the main metabolites of dopamine (3,4-dihydroxyphenylacetic and homovanillic acid) and serotonin (5-hydroxyindoleacetic acid) were not affected. This indicates that animals neonatally treated with finasteride had an increased turnover ratio of dopamine and serotonin, which possibly indicates a decrease in dopaminergic and serotoninergic activities (Llidó et al., 2016a). Interestingly, the effects of neonatal finasteride administration (postnatal days 5–9) on ethanol intake and ventrostriatal dopamine and serotonin levels described above were neutralized by later EMS (24 h of maternal separation at postnatal day 10) (Llidó et al., 2016a). This last result suggests that in the same way that the effects of neonatal stress can be neutralized or prevented by the previous application of AlloP (see section 2), the effects of an alteration in AlloP levels produced by finasteride administration can also be modulated by the posterior application of postnatal stress and the consequent

increase in endogenous AlloP levels induced by it.

In a parallel experiment, we measured dopamine release in the nucleus accumbens during alcohol drinking in animals submitted to early postnatal manipulation of AlloP levels. Microdialysis results revealed that dopamine release in the nucleus accumbens (expressed as a baseline percentage), which was increased in controls after alcohol solution presentation, was not affected in neonatal finasteride treated animals (Llidó et al., 2016b). Accordingly, the overall increase in dopamine release with respect to the baseline in response to solutions (ethanol and glucose) and food presentation was lower in finasteride subjects than in control subjects (Llidó et al., 2016b). Taken together, the ventral striatum HPLC and nucleus accumbens microdialysis results seem to indicate a hypo-dopaminergic activity in early postnatal finasteride treated rats, which could be related to the increase in alcohol drinking reported in these animals.

In this way, it has been described that a hypo-dopaminergic activity, induced either by a decrease in dopamine D2 receptors or by a decreased presynaptic dopamine release in the striatum, leads to an excessive craving and seeking for substances known to cause dopamine release in nucleus accumbens (Blum et al., 1996, 2014; Trifilieff and Martínez, 2014). Several studies in rats have shown that lines selectively bred for high ethanol preference had a decreased dopamine D2 receptor density in the nucleus accumbens (McBride at al. 1993), and lower dopaminergic innervation (Casu et al., 2002; Zhou et al., 1995), with lower dopamine content in the nucleus accumbens (McBride et al., 1995; Murphy et al., 1982). Similarly, alcohol addiction in humans has been related to significant reductions in D2 receptor availability as well as reduced dopamine release in the striatum (see Volkow et al., 2009 for a review). Therefore, our data could indicate that the higher ethanol consumption in neonatal finasteride treated rats might be related to a blunted dopamine response in ventral striatum and nucleus accumbens.

Precisely, early postnatal finasteride administration decreased the locomotor activity induced by a low ethanol dose (0.5 g/kg), thus reducing the sensitivity to the stimulant motor effects of alcohol (Bartolomé et al., 2017). Reduced sensitivity to ethanol stimulating effects has been related to dopamine inhibition (see Brabant et al., 2014 for a review). In accordance whit this, our results indicate that neonatally finasteride treated rats: (1) have lower dopamine levels in the ventral striatum and release in nucleus accumbens; (2) exhibit lower sensitivity to the motor stimulant effects of a low alcohol dose; and (3) show higher alcohol consumption in the two-week access procedure. Thus, following the hypo-dopaminergic hypothesis (Blum et al., 1996, 2000; Febo et al., 2017), it is reasonable to think that finasteride animals consumed greater amounts of ethanol in order to compensate the deficiency of mesolimbic dopamine; however, more experiments are needed to confirm this (see section 6).

Moreover, in order to characterize the effects of early postnatal administration of finasteride on drug use/abuse behavior, we evaluated the novelty object preference, since the trait of novelty seeking has been related to both consumption and substance abuse (Deroche-Gamonet and Piazza, 2014, Manzo et al., 2014; Belin et al., 2016) (see section 4). The results indicated that animals neonatally treated with finasteride had a higher preference index for exploring the new object versus the familiar one (Bartolomé et al., 2018). Consequently, this increase in the preference for novelty could indicate that these subjects are more vulnerable to consuming addictive substances, which would be related to the increase in EtOH consumption exhibited by these animals. However, as we have already mentioned, in previous studies we have observed that animals neonatally treated with finasteride have low levels of both exploratory behavior and locomotor activity with respect to the control group (Darbra and Pallarés, 2010; Mòdol et al., 2013). This suggests that the neonatal administration of finasteride can produce a novelty but not sensation seeking profile. This result would be in accordance with the hypothesis that proposes that these two traits are dissociable (Cain et al., 2005; Belin et al., 2012; Flagel et al., 2014). However, it would be interesting to carry out other behavioral tests in order to better define a profile of vulnerability (searching for sensations and/or novelty) together with evidence of drug use and abuse.

However, as we also observed decreased ventro-striatal levels of serotonin in the early postnatal finasteride treated rats, and serotonin together with dopamine has been proposed to modulate the motivational properties of alcohol (Deehan et al., 2016) and participate in alcohol abuse vulnerability (Barker et al., 2014; Yaakob et al., 2011). In addition, 5HT3 receptors could regulate the brain function and network formation during early development (Engel et al., 2013). Thus, we initiated a set of experiments focusing on the serotoninergic system. Taking into account that AlloP, like the rest of neuroactive steroids, binds with high affinity to ionotropic receptors (for a review see Rupprecht, 2003), we decided to study possible alterations in the 5HT3 receptor function, the unique ionotropic subtype of serotonin receptors. Previous experiments have shown that AlloP at high doses negatively modulates 5HT3 receptors (Wetzel et al., 1998), and it has also been described that mesolimbic dopamine release can be promoted by activating central 5HT3 receptors (for a review see Barnes and Sharp, 1999). In a first experiment, we analyzed the effects of ondansetron on alcohol consumption (in the previously mentioned two-week free access procedure) in animals submitted to the manipulation of AlloP levels in the early postnatal period. Ondansetron is a 5HT3 antagonist that has been shown to effectively reduce alcohol intake in rats in free choice conditions, i.e., when the animals can choose between drinking a sweetened alcohol solution or drinking a sweetened control solution (Heiling and Egli, 2006; McKinzie et al., 1998; Sari et al., 2011). Although its efficacy varies depending on drinking conditions after stabilization of ethanol intake, no studies have focused on their possible effects in the initial period of ethanol intake (for instance the first two weeks of consumption), which is related to the initial alcohol seeking behavior. In our experiment, the highest dose of ondansetron (0.1 mg/kg) tested in male wistar rats decreased the consumption of alcohol, but only in the animals that were administered finasteride postnatally, which were the ones that consumed the highest amount of alcohol (Bartolomé et al., 2018). In control animals, the initial alcohol intake was not affected by ondansetron administration. As previously proposed, it seems that 5HT3 antagonists could be effective or ineffective depending on several variables such as the dose, the ethanol drinking conditions (initial versus stabilized), the duration of the treatment, or the sex or strain of the animals (McKinzie et al., 1998; Knapp et al., 1992; Moore et al., 2014; Pei et al., 1993). Nevertheless, the fact that ondansetron was effective in our finasteride animals but not in controls suggests that 5HT3 receptors work differently in these animals as a consequence of the neonatal treatment, making them more sensitive to the effects of ondansetron. Thus, alterations in early postnatal serotoninergic pathways including 5HT3 receptors, together with those previously described that affect dopaminergic systems (Muneoka and Takigawa, 2002; Muneoka et al., 2009), could be related to the increase in ethanol intake in initial periods and the corresponding higher alcohol seeking behavior observed in the neonatal finasteride treated animals.

The results of the ondansetron experiment suggesting that 5HT3 receptors could be more sensitive to antagonists in neonatal finasteride animals, together with the possible role of these receptors in brain maturation, led us to study the expression of 5HT3 receptors in the early postnatal period. Therefore, we analyzed the density of 5HT3 receptors by means of Western Blot analyses in the midbrain of rats submitted to neonatal manipulation of AlloP. Preliminary results of our group (Bartolomé, 2018) indicate that early postnatal administration of either AlloP or finasteride alters the pattern of 5HT3 expression in male Wistar rat offspring. Specifically, neonatal AlloP administration anticipated the normal peak of 5HT3 expression (shown in controls) from postnatal day 12 to postnatal day 9, whereas the 5HT3 receptor densities in the finasteride treated group did not show statistical differences between the different days compared to control subjects. These results can be seen in Fig. 1.

Therefore, these preliminary results seem to highlight the possible

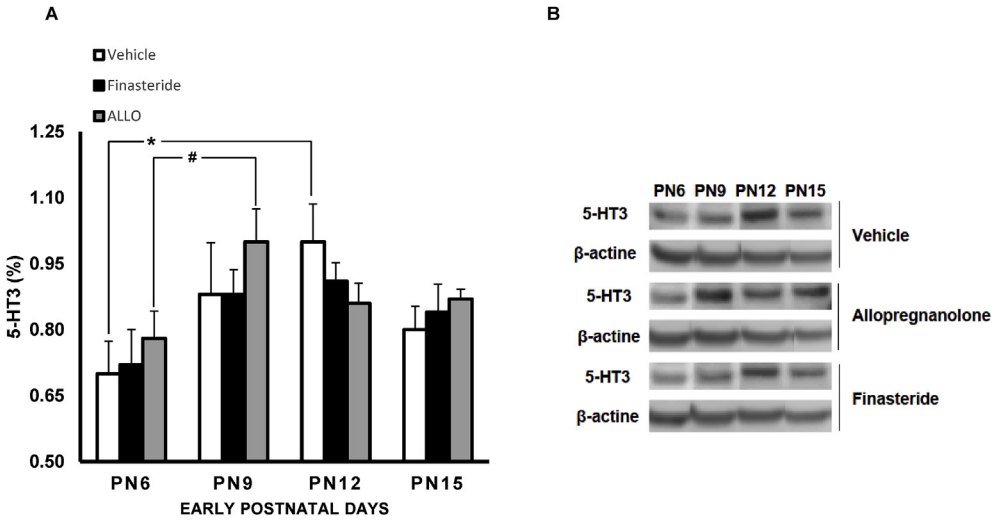

Fig. 1. Percentage of 5HT3 protein receptor expression. **A)** 5-HT3 expression is different between PN6 and PN12 in control vehicle group ($t = -2.73$; $p < 0.05$; the difference is indicated by the symbol: *). Instead, 5-HT3 expression is different between PN6 and PN9 in Allopregnanolone group ($t = -2.3$; $p < 0.05$; the difference is indicated by the symbol: #). On the other hand, there were no statistically significant differences between the different days of samples extraction in Finasteride group. **B)** Western Blot images.

role played by serotonin systems containing 5HT3 receptors in the effects of early postnatal manipulation of AlloP levels on adult alcohol intake in initial periods of consumption, and thus on initial ethanol seeking behavior.

However, to our knowledge, there is very little information in the literature about the role of early postnatal manipulation of AlloP levels on the consumption or effects of the abuse of drugs other than alcohol. The only exception is a study that showed that AlloP administration at postnatal day 2 or 5 increased locomotor response to amphetamine (28% and 20% respectively) in adulthood (postnatal day 80) (Gizerian et al., 2006). This suggests that possible variations in neonatal AlloP levels can be important for determining individual differences in the response to psycho-stimulant drugs, such as amphetamines, and the subsequent vulnerability to their abuse or addiction.

6. Perspectives

Based on the above evidence it is clear that a set of new experiments are needed to determine the role of early postnatal neurosteroids, particularly AlloP, on drug seeking behavior and vulnerability to drug use or abuse in adult ages. For instance, it is very important to: (1) evaluate the effects of manipulating neonatal AlloP levels on chronic alcohol consumption, thus increasing the period of alcohol access; (2) have basal ventro-striatal DA/serotonin or accumbic DA levels previous to the alcohol access phase in order to establish causal relations; (3) analyze the different effects of ondansetron depending on neonatal manipulations in long-term stabilized alcohol consumption; (4) study 5HT3 expression in different brain structures and in different developmental stages (i.e. early postnatal, adolescent and adult ages); and (5) study the role that neonatal NS levels play in the vulnerability to abuse of drugs other than alcohol. Therefore, there is a long way to go to be able to accurately explain the role that AlloP plays during neurodevelopment in the vulnerability to drug abuse. These first exploratory experiments open the path towards designing new interesting studies. We have no doubt that this is a vast, but very exciting, field of research.

7. Conclusions

At crucial stages of development, the physiological levels of NS, like AlloP, which fluctuate over a life-time in response to different physiological and environmental conditions, such as stress, seem to play an important role in the maturation of diverse areas of the brain as well as in the behavior related to these areas in different periods of life. Therefore, altering of early postnatal AlloP levels in the first weeks of life has been proven to affect the adult response to novel environmental stimuli, anxiety-like behaviors and the processing of sensory inputs. Moreover, recent studies indicate that early postnatal AlloP could play an important role in vulnerability to alcohol consumption. As evidence of this, it has been observed that inhibiting the enzyme 5α-reductase, which participates in AlloP synthesis, by administering finasteride in the early postnatal period: (1) increases initial alcohol consumption and the preference for novelty in adult age; (2) decreases the locomotor stimulant effects of low alcohol doses in adults; (3) promotes a hypodopaminergic profile in adulthood that is characterized by a decrease in dopamine levels in the ventral striatum and in dopamine release in the nucleus accumbens in response to alcohol and/or food presentation; and (4) increases the 5HT3 receptors' sensitivity to their antagonist ondansetron for reducing alcohol consumption, probably by altering early postnatal 5HT3 receptor expression. Thus, taken together, these results highlight the important role played by fluctuations in AlloP levels during the first two weeks of life for modulating adequate neurodevelopmental processes to prevent behavioral alterations related to neuropsychiatric and neurologic diseases.

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